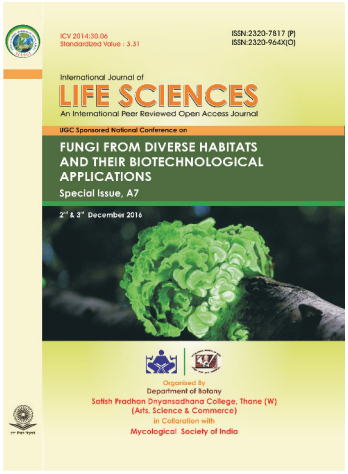
ICV 2014: 30.06
Standardized Value: 3.31

ISSN: 2320-7817 | e-ISSN: 2320-964X
Special Issue A7, December, 2016

INTERNATIONAL JOURNAL OF LIFE SCIENCES

AN INTERNATIONAL PEER REVIEWED OPEN ACCESS JOURNAL



National Conference on

FUNGI FROM DIVERSE HABITATS AND THEIR BIOTECHNOLOGICAL APPLICATIONS

STATUTORY WARNING

All enquiries and manuscript should be directed to

The Managing editor IJLSCI
46, Guruvandan, Jawahar Nagar,
VMV Road, Amravati -444604,
(MS) India

Contact: +91-942 077 5527
+91-7249 600 511
Email: editor@ijlsci.in
editorijlsci@gmail.com
web:www.ijlsci.in

Department of Botany
Satish Pradhan Dnyansadhana College, Thane (W)
(Arts, Science & Commerce)
in Collaration with
Mycological Society of India

| IJLSCI

About IJLSCI

International Journal of Life Sciences (IJLSCI) is a peer-reviewed, open access journal; it publishes original articles in all areas of Biology and Life Sciences. Authors are encouraged to submit complete unpublished and original works, which are not under review in any other journals. The scopes of the journal include, but limited to the following topic areas: Biology, Anatomy, Botany, Cytology, Genetics, Microbiology, Physiology, Zoology, Ecology, Environmental Science, Hydrobiology, Neurobiology, Developmental Biology, Immunology, Molecular Biology, Biochemistry, Biophysics, and Biotechnology, Genomics and Proteomics. It is an open access journal, which publishes original research articles, reviews and short communication in all areas of life sciences and at the interface of related disciplines.

TYPES OF RESEARCH PAPER

Original Articles:
These should describe new and carefully confirmed findings, and experimental methods should be given in sufficient detail for others to verify the work. The length of an original article is the minimum required to describe and interpret the work clearly.

Short Communications:
Short communication also contain abstract. The list of references should not exceed 15. The presentation of Short Communications should contain headings such as Introduction, Materials and Methods, Results and Discussion, etc.

Reviews Articles:
Submissions of review articles and perspectives covering topics of current interest are welcome and encouraged. Publication Frequency: Quarterly (published in March, June, September & December).

The journal is published in print as well as online version which is free access and downloads.

This Journal is circulated / Distributed Through Subscription only

Submission of Paper: Submission of paper is encouraged by e-mail to editorijlsci@gmail.com
Subscription Plan: Rs. 2500 /- or 150 US$ (Individual Annual); Rs. 3500/- or 250 US$ (Institutional)
Life Membership: Rs. 10000/- or 550 US$ (Individual) Rs. 15000/- or 850 US$ (Institutional)
Fellow Membership: Rs. 15000/- or 850 US$ (Individual) Rs. 25000/- or 1700 US$ (Institutional)

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

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

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

Indexing:

Index Copernicus, OCLCWorldCat, Directory of Science INNOSPACE (SJIF), ROAD, OAJI, AcademicKeys, CiteFactor, J-index.net, Scholar Steer, ResearchBible, Google Scholar, DAIJ , DRIJ, DIIF, SIS, ISI, ASI.

All Articles are freely accessible on the website of the International Journal of Life Sciences http://www.ijlsci.in As well as some of the Indexing agencies website via., oaji.net, research bible, Cite factors, ISI, etc.

MESSAGE FROM CHAIRPERSON MYCOLOGICAL SOCIETY OF INDIA -MUMBAI UNIT

Fungi are ubiquitous, found in varied habitats. Performing different roles in nature as saprophytes, parasites, predators fungi have proven to be the most important link in sustainance of life on the Earth. Not only that, enzymes, bioactive compounds and biomaterials produced by them are also useful in a variety of industrial applications, medicine, food and breweries, dyes, bioremediation etc. It's very interesting to see the diverse structures of fungi. Fungi like *Chanterelles*, *Laetiporus sulphureus*, red *Russula*s, and bright colored *Amanita muscaria* have no dyeing ability at all. Some fungi that are popular for dyeing wool are *Cortinarius (Dermocybe) semisanguineus*, *Hapalopilus nidulans*, and *Hydnellum* species, to name a few. *Pisolithus tinctorius* imparts a reddish brown to black sort of color to the wool. All of them are found in the tropical and temperate forests.

Fungi such as *Trichoderma reesei* and *Aspergillus niger* produce large amounts of extracellular cellulolytic enzymes, whereas bacterial and a few anaerobic fungal strains mostly produce cellulolytic enzymes in a complex called cellulosome, which is associated with the cell wall. In filamentous fungi, cellulolytic enzymes including endoglucanases, cellobiohydrolases (exoglucanases) and â-glucosidases work efficiently on cellulolytic residues in a synergistic manner. In addition to cellulolytic/hemicellulolytic activities, higher fungi such as basidiomycetes (e.g. *Phanerochaete chrysosporium*) have unique oxidative systems which together with ligninolytic enzymes are responsible for lignocellulose degradation.

It has been a practice of Mycological Society of India Mumbai unit to highlight the interesting mycological research by organizing seminars and conferences in collaboration with different colleges in Mumbai covering varied topics of fungal diversity and biotechnological applications, thus providing a platform for the young researchers to present their work.

I am grateful to the Principal and Management of Satish Pradhan Dnyanasadhana College for collaborating this year for the National Conference on “Fungi from Diverse habitats and their biotechnological applications”.

This conference is designed for introducing the necessity of mycological research among students, research scholars and teachers who can undertake an intense general survey of the fungi in India. I believe that a general knowledge of the morphology and taxonomy of the fungi is fundamental before students are plunged into the more glamorous phases of experimental mycology.

My Best wishes for the success and meaningful deliberations through the conference.

Dr. Sashirekha Suresh Kumar
Mycological Society of India Mumbai unit

FROM THE DESK OF PRICIPAL

It gives us immense pleasure and honour to host a National Conference on "Fungi from diverse habitats and their biotechnological applications" in our college in association with the Mycological Society of India, Mumbai Unit under the aegis of University of Mumbai on 2nd and 3rd December 2016.

Dnyanasadhana, a Premiere Education Institute, located in the Thane city, Maharashtra, India is registered under the Societies Registration Act, 1960 and is permanently affiliated to the University of Mumbai. The Mission of the college is to strengthen the students academically, socially and economically.

Fungi, the major decomposers of ecosystem play a pivotal role in the recycling of nutrients.They are cosmopolitan organisms with diverse industrial, biotechnological and environmental applications.

I believe this two day conference will surely provide value based education in the field of mycological research. I congratulate the entire organizing committee for putting their efforts to make the event successful. I wish all the participants and research scholars for their future endeavour.

Dr. C.D.Marathe
Principal

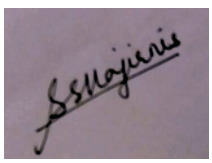
FROM THE DESK OF CONVENOR

It is my great privilege to host this National Conference on, "Fungi from Diverse Habitats and their Biotechnological Applications" in the premises of this prestigious institute.

As a hard core Mycologist myself, I took this opportunity to organise the seminar with an intention of making these little known, greatly misunderstood but extremely valuable fungi know to all and the vast benefits they offer in the form of their unique ability as decomposers, reserves of secondary metabolites, tools for Genetic engineering and applications in Biotechnology, to name a few.

Through this forum I wish to spread the knowledge of fungi, their vastness, opportunities in the field of research and industry through participation of eminent scientists and researchers. I hope the young generation takes this torch of knowledge and carries it ahead for the betterment of the society.

No great task is achieved singularly. I am therefore thankful for the efforts of all the colleagues for their share of work, the Principal, for the dynamic person that he is and his leadership and guidance and also the Management for their inspiration and fatherly support they offer.



Sarita Hajirnis
Convenor

IJLSCI

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

INTERNATIONAL JOURNAL OF LIFE SCIENCES

National conference on

FUNGI FROM DIVERSE HABITATS AND THEIR BIOTECHNOLOGICAL APPLICATIONS

2nd and 3rd December 2016

PATRONS

Shri. Satish Pradhan
President, Dnyanasadhana Society

Shri. Kamlesh Pradhan
Secretary

Dr.Sanjay Deshmukh
Vice chancellor, Mumbai University

NATIONAL ADVISORY COMMITTEE

Prof. B. N. Johri, President, MSI
Dr. D. J. Bhat, Former Dean and Adjunct Prof., Dept of Botany, Goa
Dr. N. Raman, Secretary, MSI, Chennai
Prof. M. Sudhakara Reddy,Treasurer, MSI
Dr. S. Raghukumar, MD, Mykotech, Goa
Dr. M. C. Srinivasan, *Retd. Scientist, NCL,* Pune
Dr. M.V. Deshpande, Vice-President (SCi.G), Biochemical Sciences Div.NCL, Pune
Dr. S. K. Deshmukh, Fellow and Area Convenor, TERI, Delhi
Dr. R. G. Bagool, Ad-junct Res. Guide, Dapoli
Dr. C. Manoharachary, Hyderabad.
Prof. T. Satyanarayana, Past President MSI, Delhi University
Dr.B.F.Rodriques, Head,Dept.of Botany , Goa University
Dr. N. S. Atri, Prof. Head,Dept.of Botany, Punjabi University Patiala
Dr.Mahavir Gosavi, Assisstant Prof. Dept. of Botany, S.I.E.S. college
Dr.Vishnu Vaze, Head,Dept.of Biology, Mithibai college.

LOCAL ADVISORY COMMITTEE

Dr.Ambika *Joshi* *(Ad hoc ,Chair person, Board of Studies-Botany)*
Dr.Usha Mukundan (*Principal, R.J. College ,Ghatkopar)*
Dr. R.L Mishra *(Principal, LSPM College Chondi)*
Dr. Shilpa A. Verekar, (Assistant Prof. Dept of Microbolgy, St. Xavier's College)
Dr. Vikas Golatkar *(Retd. Head, Dept. of Botany D.G. Ruparel Colleges, Mumbai)*

EXECUTIVE COMMITTEE MSI (Mumbai Unit)

Dr. Sashirekha Sureshkumar, Chairperson ,Head,Dept.of Botany , Mithibai College
Dr.Suhasini Samant,Secretary Dept.of Microbiology, Bhavan,s College.
Dr.Behnaz Patel, Treasurer Former Head, Dept. of Botany, Ruia College

MEMBERS

Dr. Moses Kolet, Principal, G.M.Momins college, Bhiwandi.
Dr.P.G.Kale, Vice-Prin.& Head,Dept.of zoology,R.J. college
Dr.Kavita Rambal, Head,Dept.of Botany,M. D. college
Dr.Sunita Chahar,Head,Dept.of Botany,Ratnam college
Dr. Charuta Vaidya,(Head,Dept.of Botany, Gurunanak college,)
Dr.Sharda Vaidya,Associate Prof.,,Dept.of Botany,C.H.M. college

LOCAL ORGANIZING COMMITTEE

CHAIRMAN

Dr. C. D. Marathe
(Principal, Satish Pradhan Dnyanasadhana college)

CONVENOR

Mrs. S. S. Hajirnis
(Associate Professor, Vice Principal & Head, Dept. Of Botany, Satish Pradhan Dnyanasadhana college)

ORGANIZING SECRETARY

Mrs. M. R. Ingle
(Assistant Professor Satish Pradhan Dnyanasadhana college)

MEMBERS

Mr.Vinodkumar Kushwaha Mr.Sunil Swami
Mrs.Suman Yadav Mr.Deepak Patil

IJLSCI

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

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Editorial Board

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)
Special Issue A7, December 2016

Special Issue, A7 December, 2016 INTERNATIONAL JOURNAL OF LIFE SCIENCES

Contents

KEYNOTE ADDRESS

&

INVITED TALK

KEYNOTE ADDRESS

Fungal Biodiversity, Germplasm Conservation and Mycotechnology : an Overview

Srinivasan MC

Head (Retd.), Biochemical Sciences Division, National Chemical Laboratory, Pune, 411 008, India
Email srinivasanmc@gmail.com

In the twenty first century the three "Big B's" of Biology are Biodiversity, Biotechnology and Business.Natural fungal biodiversity is recognised on the basis of the morphology of spore forms but their classification in addition may also be on physiological grounds (e.g. Extremophiles) Induced biodiversity through human intervention includes high productivity industrial mutants as well as recombinant strains.

Isolation in pure culture and conservation "in vitro" ensuring morphological and genetic stability of fungal strains is an essential pre-requisite for Mycotechnology,i.e. technology development based on fungal strains.

Exploring fungal biodiversity from diverse natural habitats necessitates employment of selective isolation techniques developed on the basis of a sound knowledge of the biochemistry and nutritional aspects of diverse fungi in artificial pure cultures.

Conservation techniques for long term preservation of fungi in germplasm banks require thorough understanding of their "in vitro" physiological behaviour.

A wide variety of valuable bioactive metabolites ranging from antibiotics and hormones to industrial enzymes are manufactured from fungi worldwide in a well established multi-million dollar fermentation industry.

Mycology and fungal biotechnology are in a progressive state of development and offer tremendous scope for the discovery of novel fungal strains as well as bio-products.

It is essential for our younger generation to understand and appreciate this immense potential and gear up their expertise to levels of global competitiveness..

***

PLENARY LECTURES

Litter Fungi from Dharwad

Dr. Ch. Ramesh

Dept. of Botany, Karnatak University, Dharwad

Fungi play an important role in plant litter decomposition in forest ecosystems through nutrient recycling and humus formation in soil (Swift, 1979) because they attack the lignocellulose matrix in litter that other organism are unable to assimilate. Living plants provide a wide variety of microbial habitats and there is a large amount of detailed information about fungi that occur in and around healthy plant organs (Cook and Rayner 1984). As a specific habitat for a specific association of fungi the litter mycoflora is important in ecological studies.

Litter can include fallen leaves, dead herbaceous material parts of flowers and fruits, twigs, branches and logs including bark and for some even the roots which penetrates shallowly or even deeply into the soil. The fungal population of the litter can be derived from previous generations of litter materials the soil populations, or the phyllosphere and related areas of plant surfaces which have become colonized from their initiation by one fungus or another (Dickinson 1974)

Plant litter is composed of six main categories of chemical constituents 1. Cellulose 2. Hemi-cellulose 3. Lignin 4. Water soluble sugars, amino acids and aliphatic acids 5. ether and alcohol soluble constituents including fats, oils, waxes, resins and many pigments and proteins. The breakdown of these constituents is effected as a sequence of specific reactions with the enzyme systems of specific organisms, because many species have broadly similar enzyme compliments they can be classified in physiological groups which follows a succession as the litter decomposes (Webster and Dix 1957)

Soil moisture and temperature are the two important parameters for breakdown of leaf litter. Before leaves and other plant structures fall from the plant producing them to form litter their surfaces become populated with a number of kind of organisms which may react with each other and the leaf. Beyond maturity the leaves go through a period of senescence before falling as a response to certain physiological and morphological factors (Preece and Dickinson 1971). Fungal species composition and successional changes during the decomposition process have been quantitatively investigated on several litter types (Hudson 1968), but the frequency of occurance of individual species is a poor guide to their importance in the decomposition process. Pure culture decomposition tests have been therefore carried out to assess the decomposing abilities and the substrate utilization patterns of fungi. It is important to evaluate the lignin and cellulose decomposing ability of fungi occurring on litter in order to understand their role in decomposition process. (Osono and Takeda 1999, Miya-moto et al 2000).

Soil inhabiting organisms in general and micro fauna in particular play profound role in litter degradation process. Fungal communities played a dominant role in litter decay. The loss of the leaf litter during decomposition is accompanied by marked changes in chemical composition of the litter. These changes are due to leeching, feeding and digestion by sapropagus soil animals and degradation of leaf litter compounds through activity of microbial enzymes. Nature of substrates characteristics of environment and soil inhabiting microflora are important parameters that influence the rate of decomposition.

Litter and other dead organic substrates include a wide range of material from dead herbaceous leaves that decay in a matter of months to large, rotting logs that persists for several decades. A variety of techniques have been developed to isolate fungi from dead organic matter with varieng results (Frankland, 1990). The starting diversity of fungi isolated by Bills and Polishook (1994) suggests that particle filtration is one method that yields a maximal number of fungal species with the least amount of effort in a relative short time.

PLENARY LECTURES

Study of Wood Rotting Fungi

Dr. R. L. Mishra

Professor & Principal LSPM College, Chondhi-Kihim, Alibag

What are wood rotting fungi?

A **wood-rotting fungus** is a species of fungus that digests moist wood, causing it to rot or decay. Most of these fungal forms are saprophytes; however, some are parasites such as honey fungus, e.g. *Armillaria* colonizing living trees. Fungi that not only grow on wood but actually cause it to decay are called **lignicolous** fungi. Various lignicolous fungi consume wood in various ways; for example, some attack the carbohydrates in wood and some others decay lignin.

Types of wood rot and wood rotting fungi:

The criterion for classification of wood-decay fungi is usually the type of decay that they cause. The best-known types are **brown rot**, **soft rot**, and **white rot.** The decay of the wood is due to the various kinds of enzymes they produce that can degrade different plant materials, and can colonize different environmental niches. The residual products of decomposition from fungal action have variable pH, solubility, reduction and oxidation potentials. Over time this residue will become incorporated in the soil and sediment, so can have a noticeable effect on the environment of that area.

Brown rot and brown rot fungi:

Brown-rot fungi primarily decay the cellulose and hemicelluloses in wood, leaving a brown residue of lignin, the substance which holds the cells together. These fungi breakdown hemicellulose to release hydrogen peroxide that helps in breaking down of cellulose is broken down by hydrogen peroxide (H_2O_2) that is produced during the breakdown of hemicellulose. H_2O_2 can diffuse rapidly through the wood, leading to a decay that is not confined to the direct surroundings of the fungal hyphae. As a result of this type of decay, the wood shrinks, shows a brown discoloration **(spalting)** and cracks into roughly cubical pieces; hence the names **brown rot** or **cubical brown rot**. Brown rot in a dry, crumbly condition is sometimes incorrectly referred to as dry rot in general. Dry rot is a **generic name** for certain species of brown-rot fungus. Some brown rot fungus like species of *Poria*, specifically *P. incrassata* develop specialized root-like water-conducting tubes, called rhizomorphs, which allow it to transport water from the soil to the wood decaying the wood very fast hence also called water conducting fungi.

Brown rot **Soft rot** **White rot**

Fomitopsis pinicola ***Pycnoporus cinnabarinus*** ***Antrodia sp***

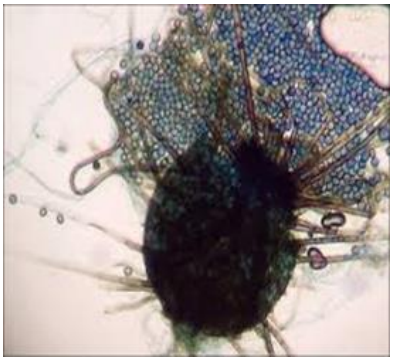
Brown-rot fungi are predominantly members of the Basidiomycetes and of particular economic importance include *Serpula lacrymans*, *Fibroporia vaillantii*, and *Coniophora puteana* which may attack timber in buildings. Other brown-rot fungi include the *Phaeolus schweinitzii*, *Fomitopsis pinicola*, species of *Pycnoporus*, *Daedalea*, *Laetiporus, Antrodia, Fomes fomentarius* etc.

Soft rot and soft-rot fungi:

Soft-rot fungi secrete the enzyme cellulase that breaks down cellulose in the wood forming microscopic cavities inside them, and sometimes to a discoloration and cracking pattern similar to brown rot. They can also decompose woods with high levels of compounds that are resistant to biological attack such as tannin and suberin. These fungi typically occur in wood of high water content and high nitrogen content as nitrogen is essential to synthesize the hormone and can colonize in quite adverse conditions of environments that can be too hot, cold or wet where other wood rot fungi cannot.

The fungi that cause soft rots include several Ascomycetous and mitosporic fungal species, such as *Chaetomium* and *Ceratocystis* in terrestrial environments and species of *Lulworthia, Halosphaeria* and *Pleospora* in marine and estuarine environments

Pleospora herbarum



Chaetomium globosum

Ganoderma lucidum

Polyporus tsugae

Phellinus discipes

White rot and white rot fungi:

White rot fungi break down lignin and cellulose and commonly cause rotted wood to feel moist, soft, spongy, or stringy and appear white or yellow. Wood affected by white rot normally does not crack across the grain and will only shrink and collapse when severely degraded. These fungi secrete enzyme laccase which is required to break down lignin and other complex organic molecules, hence got application in mycoremediation.

White-rot fungi include both Ascomycetes such as *Xylaria spp*., and Basidiomycetes such as species of *Pleurotus*, *Tyromyces, Polyporus, Ganoderma, Innotus, Rigidoporus,* and *Phellinus* etc. These fungi have been found more common than brown rot fungi in nature.

How to study these fungi?

Since these fungi are predominant in rainy season, are collected during the season at regular intervals i.e. from June to October. Microphotographs are to be taken with the help of high pixel camera to get clear

pictures, as many forms are delicate such as agarics and get distorted during collection. Many fungal forms are stiff and sturdy such as polypores, can be removed along with substratum with the help of knife or scalpel. A field note book is to be maintained to record the habitat, colour of the sporophore etc. in the field as many of these alter their original colour after collection.

The collected specimens are placed in a suitable container and brought to the laboratory for further study. The sporophores of some fungi are flaccid e.g. Agarics and undergo autolysis process or attacked by other microbes or pests and undergo decay or destruction, are preserved in suitable preservatives (wet preservation) or dried in an oven set to a temperature of 125ºC for half an hour or more depending on its softness or hardness, while the sturdy fungi like polyporales members are air dried or dried in an oven at the same temperature, and then stored in suitable containers using moisture absorbent like chemicals like silica gel bags and any sublimating chemicals like naphthalene ball so as to avoid destruction of specimens during storage by pests, for further investigation.

Spore print of certain forms like Agarics, becomes important taxonomical tool and therefore spore print of these forms are to be taken. It characterises the gill pattern of the specific form that eases the identification.

Similarly, certain specific chemical tests are performed for identification of wood rotting fungi such as Lugol's Iodine and KOH test. Lugol's Iodine is used for distinguishing amyloid, dextrinoid and non-amyloid fungal forms for example - Blue-black reaction ascertains amyloid spores, reddish or purplish brown reaction indicates dextrinoid spores and no colour change for non-amyloid spores (http://www.britmycolsoc.org.uk/).

Study of macroscopic and microscopic characters:

The sporophore i.e. fruiting body is considered as the most important criterion in taxonomical studies of wood rotting fungi. The macroscopical characters of various parts of it like, presence or absence of stipe, its length, width, colour, shape, texture; presence or absence of veil, its position on the stipe and point of attachment of the stipe to the pileus; presence or absence of pileus, its shape (top view as well as horizontal view), size, colour, shininess and texture of the pileus, nature of the margin, wetness and nature of the flesh; hymenial structure, its colour, edge, nature of attachment to the stipe, branching if any and their closeness with respect to each other, and microscopical characters like the nature of the hyphal system, whether monomitic, dimitic, trimitic, presence or absence of clamp connections and /or septa, setae and hyphal pegs if present, structure of the sub-hymenium, basidia, basidiospores, cystidia and tramal pattern in Agarics and size of the micro – pores etc. in polypores, are studied for proper identification of the species.

The sporophores of most wood rotting fungi (such as *Armillaria, Bjerkandera, Cerrena, Collybia, Daedaleopsis, Hericium, Hypsizygus, Laetiporus, Lentinus, Lenzites, Pholiota, Piptoporus, Pleurotus, Polyporus, Schizophyllum, Trametes,* and *Trichaptum*) are formed annually and do not produce spores for over a year, while those of *Fomes, Oxyporus*, and *Phellinus* are usually perennial and add a new layer of spore-producing tissue each year.

The sporophores produce basidiospores at the hymenial surface of gills or pores during part or most of the year, and the spores are carried by air currents, rain, insects, birds and other animals, or other agents to nearby tree wounds. A single large conk may shed up to 100 billion basidiospores in a single day.

For identification of species, various literatures can be used such as Bakshi (1971), Kirk et al. (2008), Largent et al. (1973, 1977), Pegler (1977) Ryvarden (1980), Roy (1996), Sharma (1995), Singer (1986) and Stuntz (1977).

Name corrections, authorities and taxonomic assignments of all can be checked by surfing the websites like (<Mycobank.org>), (< index fungorum.org>) and (<species fungorum.org>).
Classification can be achieved through any recent systematic scheme of fungal classification.

Conditions for Fungal Growth:

Growth and development of wood rotting fungi depend on many factors or conditions. However, there are five essential conditions for germination and growth viz. source of infection, suitable substrate (food), moisture, oxygen and suitable temperature.

Source of Infection:
The normal healthy wood is infected when it comes in contact with any fungal propagule such as hypha, spores etc. Infection may also spread directly from soil to wood in contact with it, because most soils contain quantities of organic matter in which fungal organisms are growing. Even when there is no contact between non-infected wood and infected materials, the space between can be bridged by airborne spores.

Substrate:
Wood provides a suitable substrate for fungus growth, and the cellulose, lignin, and other components of the cell walls and wood tissues provide suitable food. Some species of wood are more naturally durable because they contain substances toxic to fungi e.g. Heart wood. However, no wood is entirely immune to attack if placed in conditions favourable to fungal growth.

Moisture:
Moisture is the most important factor for wood rotting fungi. However, moderate amount of water is basic requirement for fungal growth. The fungal spores germinate when the substratum is quite moist and the humidity of the surrounding atmosphere is high. About 35 to 50 per cent moisture is required for wood rotting fungi to flourish, the actual moisture content depending on the species of fungi and the kind of wood.

Oxygen:
Being aerobes, all wood-rotting fungi require some air for growth. Many fungal forms die quickly if they are deprived of air. If there is no interchange of air, the fungus will die from suffocation by carbon dioxide.

Temperature:
The growth of wood-rotting fungi is faster in warm weather than in cold. All fungi show little or no growth at freezing temperatures or slightly above. Growth becomes less rapid as temperatures are increased above 95°F and ceases for most fungi at temperatures slightly in excess of 100°F. Prolonged exposure to temperatures slightly above the maximum for growth, or even short exposure to temperatures much above the maximum, can kill fungus completely. The actual death point is influenced by temperature, length of time and moisture content.

Other Factors:
Most species grow more vigorously in subdued light, but some do not grow normally in total darkness. Usually these forms flourish well on materials that are slightly acid however; very few can tolerate alkaline conditions.

Why to study wood rotting fungi?
Wood rotting fungi are ecologically as well as economically important.

i) As decomposer:
Wood is high in carbon and generally nutrient deficient; wood rarely contains more than .3% nitrogen by weight however, wood decay fungi manage to decompose a nitrogen deficient substrate by three possible mechanism, i) preferential allocation of nitrogen to metabolically active substances and pathways highly efficient in the use of wood constituents; ii) utilization of their own old mycelium as the sources of nitrogen through a continuos process of autolysis and reuse; and iii) utilization of nitrogen sources outside the wood itself.

Majority of the wood rotting fungi are saprophytes that feed on the contents of the wood such as cellulose, hemicellulose, lignin etc. and convert them into simple organic matters that is added in the forest soil. Thus decomposition of organic matter present in the wood is considered as the major ecological role of these fungi.

ii) Role in Forest soil development:
Decay fungi have three major roles in the forest soil development process viz. breaking down plant residues and recycle carbon to the soil or the atmosphere, releasing mineral nutrients from plant residues and make the nutrients available to living organisms and producing the physical character of the soil organic matrix. The outcomes of these processes promote soil water infiltration rates, soil water-holding capacity, cation exchange capacity, nutrient availability, nitrogen fixing activity, and habitat for mycorrhizal associations, to name a few.

iii) Role in Nutrient cycling and nutrient availability:
Wood decomposers make nutrients available to plants and other microbes and retain nutrients that might otherwise be leached from the root zone.

iv) Plant – Fungi Symbiosis:
Many wood rotting fungi such as species of *Amanita, Calvatia, Pholiota, Boletus, Cantharellus, Lacterius, Russula,* etc. show symbiotic association with the roots of vascular plants called mycorrhizal association that helps in absorption of water and mineral to the plants especially phosphates.

v) Plant-Fungi Parasitism:
The wood rotting fungi like *Armillaria, Heterobasidium,* and *Laetiporus* etc. show parasitic relationship with the living plants causing death thereby bringing loss to forest ecosystem.

vi) As Food:
Some of the agarics like Agaricus, *Boletus, Leccinum, Pleurotus, Coprinellus, Gomphidius, Hydnum,* etc. are highly nutritious food not only for human being but also other animals like squirrels, rodents, bears, beavers etc. However, some of these like *Amanita, Gyromitra, Cortinarius,* etc. are poisonous that can be identified by wild animals and mostly are inedible.

vii) As biological control agent:
Pioneer colonizing white rot fungi are also being used as biological control agents to prevent stain in wood used for pulp and paper production. Fungi such as *Phlebiopsis gigantea* (previously called *Peniophora gigantea*) have been used to treat pulpwood during shipping and storage.

viii) Wood and wood product destruction: Wood is used in the construction of building, making furniture, sport goods etc. There is huge economic loss to the country as wood and wood products are destroyed by wood rotting fungi especially in the rainy season as all optimal conditions prevail for growth and development of these fungi in this season. Decay results in weight loss, strength loss, increased permeability, increased electrical conductivity, reduction in volume, changes in pulping quality, discoloration and reduction in caloric value of the wood.

ix) As medicines:
Agaricus subrufescens contains a high level of alpha and beta glucans, compounds known for stimulating the immune system, the fungus is used in oncological therapy in Japan and Brazil.

Ganoderma lucidum is the well known wood rotting fungi having various types of chemical compounds, comprises anti-allergic/anti-inflammatory, anti-convulsant, anti-cancer, anti-oxidant, cardio protective, hepato protective, neuro protective, immune stimulant and glycemic regulatory effects thus used in treatment of various disorders in the human being. The shiitake mushroom i.e. *Lentinus edodes* is a source of lentinan, a clinical drug approved for use in cancer treatments in several countries, including Japan.

In Europe and Japan, polysaccharide-K (brand name Krestin), a chemical derived from *Trametes versicolor*, is an approved adjuvant for cancer therapy. *Grifola*, a polypore has antibiotic activities due to the compound, grifolin, which is found to be bactericidal. Antitumor substances called D-fractions have been obtained from extracts of the basidiocarp that reduces the side effects of chemotherapy. It is also effective defense against HIV. A serious skin cancer that is one manifestation of AIDS may be treated with its extracts.

Fomes fomentarius has been used to cauterize wounds, as a styptic to stop bleeding, as a diuretic and laxative, and as a primitive antibiotic. Recent studies have shown that compounds isolated from it exhibit antibacterial, antiviral, and antitumor properties.

Fomitopsis officinalis is used as a poultice for infections, swelling, and bleeding and extract to treat cough and asthma.

x) Biotechnological aspect of wood rotting fungi:
a) A number of white-rot fungi like *Pleurotus ostreatus, Trametes versicolor, Bjerkandera adusta, Lentinula edodes, Irpex lacteus, Agaricus bisporus, Pleurotus tuber-regium*, *Pleurotus pulmonarius* etc. can degrade persistent xenobiotic compounds such as polycyclic aromatic hydrocarbons, halo-organics, nitro aromatic compounds, pesticides, synthetic dyes etc.

b) To reduce the use of chemicals, and thus to minimize cost and risks to human and environmental health, wood rotting/degrading fungi like *Ceriporiopsis subvermispora, Trametes versicolor, Bjerkandera adusta* etc. have been utilized.

c) A large number of enzymes are extracted from wood rotting fungi such as cellulase, lignocellulose, laccase etc.
***

PLENARY LECTURES

Bio-prospecting of insect pest controlling fungi

Value addition to high volume low cost product

Deshpande MV

mv.deshpande@ncl.res.in

The pest control in the field using fungi such as *Metarhizium, Beauveria, Paecilomyces*, to name a few has indeed gone beyond 'proof of concept'. However, in view of the performances of these organisms in the fields and moreover, acceptability by the end users regarding cost-effectiveness, shelf life, intellectual property rights (IPR), the additional roles and possible applications can be explored. Dual pathogenicity of both insect - as well as fungal- pathogens has added advantage of wide spectrum of biocontrol activity. In addition to plant protection the insect controlling fungi can promote plant growth (PG Promoting activity) too. Other potential areas for value addition to insect pathogenic fungi, such as bioremediation of pesticides, use of proteases and lipases in food and dairy, detergent, leather, pharmaceutical/healthcare and textile industries, use of biomass for nano-material synthesis, chitin /chitosan production, so on and so forth, will be discussed.

PLENARY LECTURES

Mangrove Mycology: Opportunities and Challenges

Kandikere R. Sridhar*
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka, India

Mangroves are unique tidal forests comparable to tropical rainforests in terms of biomass production. They embrace several habitats throughout the tidal range and offer a variety of detritus for decomposer community. As buffer zones, mangroves link terrestrial and marine habitats with unique biotic factors, abiotic factors and ecological niches. Fungal resources of mangroves are of special significance as they involve in detritus turnover, mineralization and energy transfer. Wide range of fungi have adapted to mangrove habitats and their substrata. Two types of coarse particulate organic matter occur in mangroves: i) fragile, short-lived and fast degrading; ii) sturdy, stable and long-lasting. Both of them are differently processed by fungi to unlock nutrients and to enrich by their biomass (enzymes, proteins and fatty acids), which is nutritious to detritus dependent fauna. Several ecological services by fungi have been recognized such as enrichment of detritus, decrease in C/N ratio, conversion of coarse particulate matter into fine/dissolved organic matter and accumulation of minerals (N, P and K). In addition, fungi are the major source of rare metabolites valuable to treat human diseases. Some fungal metabolites serve as insecticides, helpful in bioremediation and possess industrial applications. There are several gaps in our knowledge on mangrove fungi which needs more focused attention.

Keywords: Mangroves, Niches, Detritus, Fungi, Diversity, Ecological functions, Metabolites, Bioactive compounds

INTRODUCTION

Mangroves constitute unique wetland forests (shrub- or wood-land) confined to the tropical and subtropical coastal regions of immense biological, ecological and social relevance (Kathiresan and Bingham, 2001; Bandaranayake, 2002; Gopal and Chauhan, 2006). They are established at the intertidal zones like estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats. Mangrove forests (between latitudes 25ºN and 25ºS) dominate up to 25% of coastline in 112 countries (Saplding *et al*., 1997). In terms of productivity and tertiary yield, mangroves stand next to the coral reefs (Qasim and Wafar, 1990; Alongi, 2002). Mangroves, salt marshes and sea grasses are known to capture high amount of biological carbon (up to 55%). They are of special interest owing to unique abiotic factors like wide range of salinity, large tidal amplitudes, severe wind velocity, high temperature and anaerobic sediments (Chapman, 1976). Being major primary producers and detritus-based ecosystems, mangroves accumulate high quantity of autochthonous and allochthonous detritus such as leaf, inflorescence and woody (bark, twigs and wood) debris. In addition, live, senescent and standing dead stem, roots and pneumatophores provide suitable niches for colonization of flora, fauna and microbiota. Mangrove forests are comparable to tropical rainforests as they contribute standing biomass up 700 t/ha (Clough, 1992; Twilley *et al*., 1992). Mangroves are unique as they accommodate diverse plant species: i) true mangroves (80 trees and shrub species: e.g. *Avicennia, Kandelia, Rhizophora* and *Sonneratia*); ii) minor plant species (rarely form pure stands: e.g. *Aegiceras* and *Excoecaria*); iii) mangrove associates (salt-tolerant plant species associated with true mangrove vegetation: e.g. *Acanthus, Dalbergia* and *Derris*) (Field, 1995; Tomlinson, 1986). Mepham and Mepham (1985) gave a wide outlook of flora by listing up to 900 plant species including epiphytes in mangroves.

Mangrove forests are focus of attraction of researchers as they serve as buffer zones between terrestrial and marine habitats in tropical and sub-tropical coastal regions. They are hotspots of fungi encompassing mosaic of marine, facultative and terrestrial fungi (Shearer *et al*., 2007). The main function of these fungi is decomposition of organic matter by their potential enzymatic capabilities and gear up energy flow to higher trophic levels. Compared to other areas of

mycology, marine mycology is very recent area. The first monograph of mangrove fungi "*A Treatise on Fungi and Oceans and Estuaries*" was published by Johnson and Sparrow in 1961. Being distributed in Atlantic, Pacific and Indian Oceans, mangrove fungi are the second largest ecological group of marine fungi (Hyde and Jones, 1988). Some of the updated illustrated monographs useful for identification of mangrove fungi are authored by Jones *et al*. (2009, 2015), Alias and Jones (2009) and Pang *et al*. (2011). Although most of the marine fungi have been described on morphological basis by light microscopic studies, further advances have taken place by ultrastructural studies (transmission and scanning electron microscopic). In addition, molecular phylogeny also enhanced precision of taxonomy of marine fungi to a greater extent (Jones *et al*., 2009). A dichotomous key for the genera of marine fungi occurring on the major plant species *Rhizophora* deals with 139 species (Sarma, 2015). The definition of marine fungi was recently debated and the term 'marine-derived fungi' has been proposed to embrace wide range fungi recovered repeatedly from the marine habitats (marine, facultative and terrestrial fungi) as well as a wide variety of natural products: i) grow and or sporulate on substrata; ii) symbiotically associated with organisms; iii) adapted and evolved at the genetic level or metabolically active (Pang *et al*., 2016). Mangrove mycology is a fast emerging field providing ample opportunities and challenges like exploration of diversity, role in ecosystem functioning, ecosystem services and harness value-added metabolites. This article highlights importance of filamentous fungi in mangroves, gaps in our knowledge and future perspectives with emphasis on the Indian Subcontinent.

ECOLOGICAL NICHES

Mangroves are production-based (high quantity of living plant tissue) as well as detritus-based (fine and coarse particulate organic matters: FPOM, CPOM), which ultimately result in accumulation of easily degradable (e.g. leaf, twig, bark, inflorescence and seed) and recalcitrant (branch, wood and large roots) detritus (Wafar *et al*., 1997; Ellison, 2008). The global estimate of litter accumulation in mangrove stands ranges from 130–1870 g/m^2, which is mangrove-dependent (geographic location) as well as host-dependent (plant species) (Clugh, 1992; Kathiresan and Bingham, 2001). Mangroves provide many macro- and micro-ecological niches in different substrata and sites (e.g. woody litter, leaf litter, seagrasses, sediments, soil, sand, algae, corals and calcareous materials) (Kohlmeyer and Kohlmeyer, 1979). In addition, pneumatophores, seedlings, sedges, proproots and animal remains also serve as potential source for colonization and perpetuation of filamentous fungi.

In broader context, mangroves embrace a wide spectrum of habitats like estuaries, backwaters, deltas, tidal creeks, lagoons, marshes, mudflats and near shore islands. Even though some locations are devoid of typical vegetation or having sparse vegetation, some regions support valuable flora like sedges (e.g. *Cyperus*), ferns (e.g. *Acrostichum*), seagrasses (e.g. *Cymodocea* and *Halophila*) and palms (e.g. *Nypa*). Each one of these habitats is unique in their biotic as well as abiotic conditions under the influence of fresh, brackish and marine waters. They also have unique features regarding substrate diversity, salinity gradient and pattern of inundation. Salinity of mangroves show a wide range of variations depending on the season, for instance Deep Bay mangrove (Hong Kong) ranges from 1–30‰, while 39.9–46‰ in Red Sea mangroves (Egypt) (El-Sharouney *et al*., 1998; Abdel-Wahab and El-Sharouney, 2002).

Zonation of detritus accumulation is very distinctive in the mangroves (e.g. horizontal and vertical). Detritus tends to accumulate beyond high tide region, below high tide region and locations continuously inundated (low tide and below low tide regions). However, depending on the size and topography of location these distinct zones may differ and in turn influence the fungal colonization and diversity. Several factors control the detritus distribution, which depends on the type and structure of mangroves. Detritus shift from one region to another depends on the mass, size, quantity and extent of floatation of detritus. Likewise, obstructions differ from moderate to severe impediments due to the nature of root system of mangrove vegetation. Some root systems have obstructions for easy movement of detritus include stilt (*Rhizophora* and *Pendanus*), knee (*Bruguiera*), pneumatophours (*Avicennia*), cone (*Sonneratia* and *Xylocarpus*) and buttress (*Heritiera* and *Pelliciera*) roots. Duration of retention of detritus amidst root system is dependent on the size and extent of its fragileness. Great variations also prevail in vertical zonation of detritus in mangroves. Senescent leaves and twigs detach from canopy trap above the high tide

level without influence of tidal waters (but receives spray), entrap in canopy at high tide level or below high tide level (partially or fully inundated), some reach water or sediment surface in fresh or dry state and some may get buried in sediments. All these conditions in mangrove ecosystem provide unique niches for colonization, perpetuation and dissemination of fungi.

FUNGAL DIVERSITY

Mangroves being hub of fungal diversity embraces a wide variety of fungi such as typical marine fungi, lignocellulose degrading fungi, endophytic fungi, arbuscular mycorrhizal fungi, phosphate-solubilizing fungi, freshwater fungi, macrofungi and pathogenic fungi. The estimate of marine fungi was about 1500 species in 1996 and it has been revised up to 10,000 species in 2011(Jones, 2011). So far, up to 1112 marine fungi have been documented from marine habitats including yeasts (Jones *et al*., 2015). The global estimate of mangrove filamentous fungi include meiosporic ascomycetes, anamorphic ascomycetes and basidiomycetes (Schmit and Shearer, 2003; Shearer *et al*., 2007). From 10 biogeographic mangrove regions (north temperate, tropics, Asian temperate, tropical Africa, Madagascar, temperate Africa, Middle East, tropical Asia, Australasia and Pacific Islands) these fungi range from 0–225 meiosporic ascomycetes, 1–190 anamorphic ascomycetes and 0–33 basidiomycetes. The Madagascar represented least (0/1/1) and the tropical Asia possess the highest (225/190/33) mangrove fungi. The highest fungi were seen in tropical Asia owing to more studies as well as diverse mangrove plant species. For comparison, mangroves of the west coast of India comprise 127 fungi (ascomycetes, 78; anamorphs, 48; basidiomycete, 1) (Sridhar, 2013), while the east coast represents 131 species (ascomycetes, 102; anamorphs, 27; basidiomycete, 2) (Vittal and Sarma, 2006; Sridhar, 2013). Based on published literature, nearly 40 species of mangrove fungi have been identified as core-group in mangroves of the world (Ascomycetes, 28; anamorphs, 10; basidiomycetes, 2) (see Sridhar *et al*., 2012). Mangroves of the Indian subcontinent consist of 35 core-group fungi (ascomycetes, 23; anamorphs, 11; basidiomycete, 1) (Sridhar, 2009).

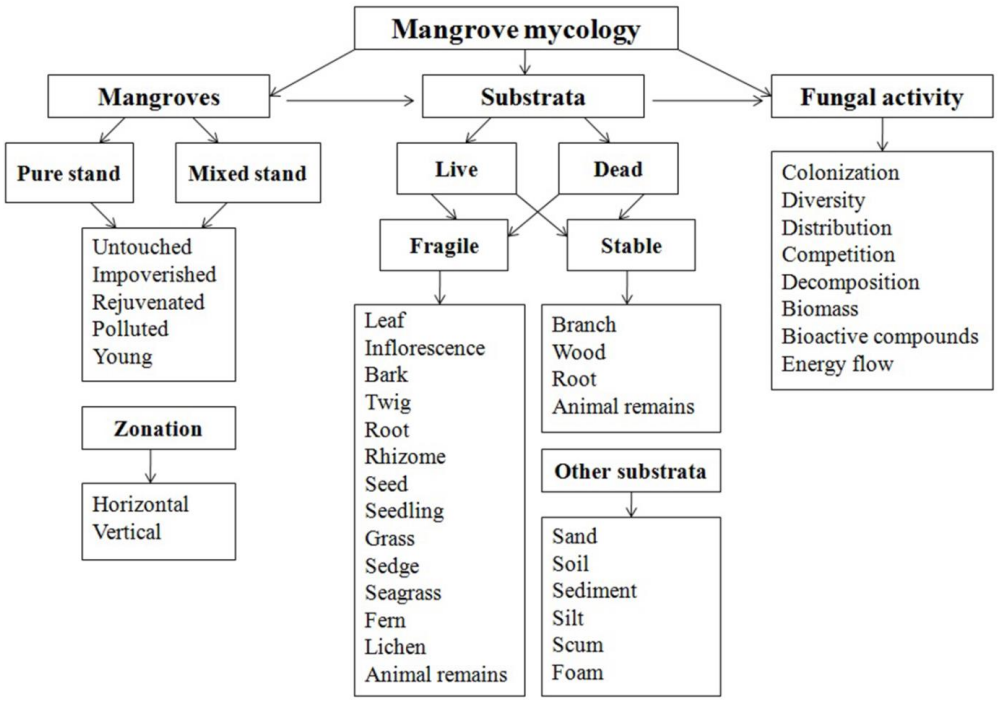


Figure 1. Outline on approaches to pursue mangrove mycology.

Aerial part of mangrove plants is endowed with several groups of fungi (phylloplane, endophytes and saprophytic fungi). Foliar endophytes consists of pathogens, toxigenic and entomopathogenic fungi (e.g. *Alternaria, Aspergillus, Curvularia, Drechslera, Paecilomyces* and *Trichoderma*) (Anita and Sridhar, 2009; Anita *et al*., 2009; Ananda and Sridhar, 2002; Maria and Sridhar, 2003a). *Acremonium, phomopsis,*

Phyllosticta and *Sporormiella* were also common foliar endophytic fungi (Suryanarayanan and Kumaresan, 2000). Many endophytic fungi have dual role as saprophytes or opportunistic pathogens (e.g. *Chaetomium* and *Paecilomyces*) (Ananda and Sridhar, 2002; Vega *et al*., 2008). On fallen leaves, besides terrestrial fungi (e.g. *Aspergillus, Cladosporium* and *Phoma*) occurrence of typical marine fungi was common (*Hydea, Periconia, Trichocladium* and *Lulworthia*) (Nakagiri *et al*., 1997; Ananda *et al*., 2008).

Fungi are integral part of woody litter in mangroves (e.g. drift wood, immersed wood, standing dead wood and wood jammed/buried in sediment) (Sridhar *et al*., 2012). Unlike, standing and buried dead wood, drift wood shift its location often due to turbulence. The quantity and location of detritus deposition also show variation in fungal communities (e.g. different tidal regions) (Hyde, 1988, 1990). Interestingly, even though immersed woody materials provide information on the pattern of colonization of fungi, randomly sampled driftwood supports higher diversity possibly due to diversity of driftwood (Maria and Sridhar, 2004; Sridhar *et al*., 2012). However, the pattern of driftwood colonization by fungi varies between nature of detritus as well as seasons (wet and dry) (Maria and Sridhar, 2003b). Woody material initially colonized by a few fungi, the diversity will be highest during intermediate stage, a few species will be represented during late stage and some persistent throughout exposure time (Sridhar *et al*., 2012). *Bruguiera gymnorrhiza* and *Rhizophora mucronata* were the most promising tree species each harbored up to a maximum of 58 species of fungi in the west coast of India (Maria and Sridhar, 2003b), while *Rhizophora apiculata* is the most potential mangrove tree species harbored up to 61 species in the east coast (Sarma and Vittal, 2000). Vertical zonation in mangrove fungi is common in several locations as they have adapted to different zones (see Sridhar *et al*., 2012). Usually the mean tidal level shows high diversity, those fungi having carbonaceous ascomata and possesses colored/ornamented ascospores restricted to above mean tide level. Those fungi have immersed ascomata and adapted for passive spore dispersal with membranous wall distributed throughout the tidal levels.

It is likely colonization of filamentous fungi varies between pure stand and mixed stand. In a co-habiting mangrove plant species (*Acanthus ilicifolius, Avicennia marina* and *Rhizophora apiculata*), 22 species of filamentous fungi were recovered with only five common species (*Halocyphina villosa*, *Lignincola laevis*, *Periconia prolifica*, *Verruculina enalia* and *Zopfiella latipes*) in southwest India (Manimohan *et al*., 2011). Interestingly, all common species except for *Z. latipes* were identified as core-group fungi in mangroves (Sridhar *et al*., 2012). Co-occurrence of fungi ranges from 2–9 on *Rhizophora mucronata* (Sridhar and Maria, 2006). Higher co-occurrence was seen during monsoon season than summer season in naturally deposited wood (72 vs. 36%) as well as immersed wood (53 vs. 45%) of *R. mucronata*. Such co-occurrence of fungi might be valuable in degradation different components of woody litter as well as production stress dependent or competition dependent secondary metabolites.

Investigations on filamentous fungi in mangroves have also been extended to mangrove palms (e.g. *Nypa*) (Loilong *et al*., 2012). Up to 139 species were reported in *Nypa fruticans* especially from the Southeast Asia. Seagrasses are also potential source of fungi in mangroves, which are common in Bermuda, Hong Kong, India, Puerto Rico and Thailand (Sakayaroj *et al*., 2012). Spread of seagrasses up to 360 km^2 across has been reported in the Indian coast with main distribution in the east coast (Andaman and Nicobar Islands, Chilka lagoon, Gulf of Mannar and Palk Bay) than the west coast (Gulf of Kutch and Lakshadweep). *Cyperus malaccensis* is one of the dominant sedges in Southwest mangroves of India was colonized by a variety of filamentous fungi (Karamchand *et al*., 2009; Sridhar *et al*., 2010). Fungal association (saprophytes and endophytes) in *C. malaccensis* has been studied in different tissue classes (rhizome, leaf, stem and bract) and age classes (mature, senescent and standing dead). Colonization of fungi showed zonation in *C. malaccensis* and its dead part and rhizome provide nutrition and shelter for several fauna including the giant snail *Telescopium telescopium* (Sridhar, 2012a). Recently, inventories have been further extended to assess macrofungi in mangroves: arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi and mushrooms (Sengupta and Chaudhuri, 2002; Wang *et al*., 2010; D'Souza and Rodrigues, 2013; Dutta *et al*., 2013; Ghate and Sridhar, 2015). Although mangroves are rich in nutrients, mangrove plant species are associated with several AM and EM fungi probably to overcome stress conditions (Sridhar *et al*., 2011; Ghate and Sridhar, 2015). In addition to EM

fungi, edible (9 species) and medicinal (2 species) mushrooms were also recovered from the mangroves of Southwest India. Macrofungal distribution was higher in woody litter (22 species) as well as soil (22 species) than to leaf litter (7 species).

ECOLOGICAL FUNCTIONS

Mangroves as buffer zones dependent on autochthonous and allochthonous lignocellulosic organic matter distributed in whole range of horizontal and vertical zones. Processing, mineralization and export of such organic matter is the major function of mangrove ecosystem. Fragile detritus (e.g. leaf litter, twigs, bark, sedges and seagrasses) in mangroves serve as immediate source of energy for the food web owing to their fast rate of degradation by fungal colonization, while the recalcitrant debris (woody litter, branches, trunks and logs) support fungi, other detritus dependent organisms and mineralize nutrients (N, P and K) for longer duration (Maria *et al*., 2006; Ananda *et al*., 2008). Substantial quantities of animal remains also recycled in mangrove habitats. Nitrogen poor detritus (C/N ratio, >100) in mangrove habitats turn in to nitrogen rich source by lowered C/N ratio, which are highly attractive to mangrove fauna.

Three important segments of decomposition of organic matter include: i) leaching; ii) colonization of microbiota; iii) fragmentation (Gessner *et al*., 1999). Such sequential events are connected to: i) loss of proteins, carbohydrates, phenolics and cellulose; ii) increased fungal biomass and decreased C/N ratio; iii) decline in microbial biomass followed by decreased cellulose and lignin (Raghukumar *et al*., 1995). The efficiency of mangroves is dependent on the turnover and extent of mass loss of CPOM and its transformation into FPOM and dissolved organic matter (DOM). The half-life (t_{50}) or 50% mass loss of detritus in different habitats is one of the major yardsticks of efficiency of mangroves in nutrient turnover. In addition, the decay coefficient (k) of detritus denotes slow, medium and fast recycling patterns (see Sridhar, 2012b). The mass loss, half-life and decay coefficient will be useful parameters to assess the mangrove habitats, which is dependent on the mangrove location (latitude) as several biotic and abiotic factors involved. Such parameters also serve to decide the nature of mangroves as virgin, impoverished and rejuvenated.

Decomposition of detritus in mangroves increases invertebrate activities (e.g. crabs and amphipods) (Middleton and McKee, 2001). The giant snail *Telescopium* is a potential detritivore in swards of *Cyperus malaccensis* of Southwest mangroves of India (Sridhar, 2012a). The detritivore fish *Liza macrolepsis* in Taiwan also dependent on *C. malaccensis* as potential food source. Sridhar (2012b) suggested a model representing detritus processes in mangrove habitats. To derive benefit of this model in a specific mangrove, additional efforts are necessary: i) qualitative and quantitative assessment of CPOM in different habits of mangroves; ii) assessment of abiotic factors; iii) survey of microbes and fauna involved in processing of CPOM.

BIOACTIVE COMPOUNDS

The major function of mangrove fungi is decomposition of organic matter by their potential enzymes. Mangrove fungal enzymes utilize carbohydrates in wide range of salinity (0–34‰) (Rohrmann and Molitoris, 1992; Pointing *et al*., 1998). Similarly, wide pH-tolerant thermostable xylanases are useful in paper and textile industries (Raghukumar *et al*., 1999). Marine-derived fungi are also known for production of enzymes at wide range of temperature (35–70ºC) and pH (3–11) (Bonugli-Santos *et al*., 2015). Mangrove fungi have been divided into three categories based on enzyme functions: i) producers of endoglucanase, xylanase and laccase; ii) unable to utilize xylan; iii) lacking laccase activity (Luo *et al*., 2005). Enzymes of mangrove fungi especially lignocellulases are useful in bioleaching, biopulping and bioremediation (Raghukumar, 2002). Decolorization of dyes (e.g. poly R-478, azure B) is associated with lignin-degrading ability of fungi (Pointing *et al*., 1998; Raghukumar, 2002; Bucher *et al*., 2004).

The long awaited expectation 'drugs from the sea' was realized from 1970 onwards by description of more than 1000 new natural products derived from fungi occurring on different substrata in marine habitats (Overy *et al*., 2014). Potential of marine fungi in biotechnology has been reviewed by Raghukumar (2008). Thatoi *et al*. (2013) reviewed bioactive potential of mangrove fungi useful in biotechnological, pharmaceutical and nutraceutical importance. Raghukumar (2005) reviewed mangrove fungal lignocellulolytic activities in degradation of colored paper and pulp effluents. Several mangrove-derived

fungi are effective as antimicrobial, antioxidant, antidiabetic and anticancer agents. Bioprospecting of some mangrove fungal endophytes have been reviewed by Suryanarayanan *et al*. (2009). Mangrove fungi are important source of many enzymes, biosurfactants and essential fatty acids, while several of them involve in hydrocarbon degradation and sequestering heavy metals (Damare *et al*., 2012). *Aspergillus fumigatus, A. parasiticus* and *Chrysonilia sitophila* from hydrocarbon contaminated South Sumatra mangroves showed total petroleum hydrocarbon degradation *in vitro*, the latter fungus showed highest degradation capability (5–20% petroleum residue) (Gofar, 2011). In addition to inhibition of routine pathogens, endophytic fungi derived from seagrasses in Thailand could inhibit potential pathogens like *Cryptococcus neoformans* and *Microsporium gypseum* (Supaphon *et al*., 2013). Four new compounds were isolated from endophytes of Chinese mangrove possess antimalarial activity (Calcul *et al*., 2013). Ethyl acetate extracts of different strains of mangrove fungus *Emericella nidulans* showed larvicidal activity against the potent agricultural pest leaf worm moth *Spodoptera litura* (Abraham *et al*., 2015).

OUTLOOK

Mangrove mycology offers excellent opportunities as well as challenges to evaluate fungal resources, fungal interactions, fungal diversity and ecosystem functioning. Mangroves supply food (fishes, legumes and ferns), possess diverse organisms (plants, animals and microbiota), provide shelter (aquatic and terrestrial) and source of bioactive compounds (medicinal, therapeutic and bioremediation). Nearly 80 species of filamentous fungi have been recognized as core-group fungi in mangrove habitats of the world (Sarma and Hyde, 2001; Sridhar *et al*., 2012), which are likely drivers of mangrove ecosystem under optimum as well as harsh conditions due to their adaptability. Several fungi might have adapted to mangrove habitats and helpful in heavy metal bioremediation and plastic litter degradation. Indian subcontinent with long coastline provides ample opportunities to investigate mangrove fungi. However, pattern of detritus production, extent of detritus accumulation, turnover of detritus, comparison of fungal resource between west and east coast of India are lacking. The Figure 1 gives an outline on approaches to be followed on mangrove mycology considering mangroves, substrata and fungal activity towards possible research efforts in future. In addition, several ecological niches of mangroves could be considered, however, some segments may differ depending the geographic location.

There are several threats for mangroves such as habitat loss, habitat alteration, pollution, climate change and diseases (Osorio *et al*., 2014). The rehabilitation of mangroves although practiced, it is not really matching with the rate of destruction. In many cities human habitation is right on the mangrove habitats and some cities possess traces of mangroves and studies of such remnants in the name 'urban ecology' might provide astonishing results. Urban localities in mangrove locations should maintain part of their land as 'mangrove parks' with different biota for demonstration and education purposes. Investigations on mangrove ecosystems will be rewarding if designed to study one mangrove several concepts or several mangroves one concept, likewise one plant several substrata or several plants one substrate for more precision.

Acknowledgements

The author is grateful to Mangalore University for facilities to carry out studies on marine fungi in the Department of Biosciences and indebted to the UGC, New Delhi for UGC-BSR Faculty Fellowship.

REFERENCES

Abdel-Wahab MA and El-Sharouny HM (2002) Ecology of subtropical mangrove fungi with emphasis on *Kandelia candel* mycota. *Fungal Diversity Research Series*, 7: 247–265.

Abraham S, Basukriadi A, Pawiroharsono S and Sjamsuridzal W (2015) Insecticidal activities of ethyl acetate extract of Indonesian mangrove fungus *Emericella nidulans* BPPTCC 6038 on *Spodoptera litura*. *Microbiology Indonesia*, 9, 97–105.

Alias SA, Jones EBG (2009) *Marine Fungi from Mangroves of Malaysia*. IOES Monograph Series # 8, Kuala Lumpur: University of Malaya.

Alongi DM (2002) Present state and future of the world's mangrove forests. *Environmental Conservation*, 29: 331–349.

Ananda K and Sridhar KR (2002) Diversity of endophytic fungi in the roots of mangrove species on west coast of India. *Canadian Journal of Microbiology*, 48: 871–878.

Ananda K, Sridhar KR, Raviraja NS, Bärlocher F (2008) Breakdown of fresh and dried *Rhizophora mucronata* leaves in a mangrove of Southwest India. *Wetlands Ecol Management*, 16: 1–9.

Anita DD and Sridhar KR (2009) Assemblage and diversity of fungi associated with mangrove wild legume *Canavalia cathartica*. *Tropical and Subtropical Agroecosystems*, 10: 225–235.

Anita DD, Sridhar KR and Bhat R (2009) Diversity of fungi associated with mangrove legume *Sesbania bispinosa* (Jacq.) W. Wight (Fabaceae). *Livestock Research for Rural Development*, 21, Article # 67: http://www.lrrd.org/lrrd21/5/cont2105.htm

Bandaranayake WM (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetland Ecology and Management*, 10: 421–452.

Bonugli-Santos RC, Vasconcelos MRS, Passarini MRZ, Vieira GAL, Lopes VCP, Mainardi PH, Santos JA, Duarte LA, Otero IVR, Yoshida AMS, Feitosa VA, Pessoa A and Sette LD (2015) Marine-derived fungi: diversity of enzymes and biotechnological applications. *Frontiers in Microbiology*, 6: 269; doi: 10.3389/fmicb.2015.00269

Bucher VVC, Hyde KD, Pointing SB, Reddy CA (2004) Production of wood decay enzyme s, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. *Fungal Diversity*, 15: 1–14.

Calcul L, Waterman C, Ma WS, Lebar MD, Harter C, Mutka T, Morton L, Maignan P, Olphen AV, Kyle DE, Vrijmoed L, Pang K-L, Pearce C and Baker BJ (2013) Screening mangrove endophytic fungi for antimalarial natural products. *Marine Drugs*, 11: 5036–5050.

Chapman VJ (1976) *Mangrove Vegetation*. Liechtenstein, Germany: Cramer Vadyz.

Clough BF (1992) Primary productivity and growth of mangrove forests. In *Tropical Mangrove Ecosystems*, Eds., Robertson AI, Alongi DM. Washington DC: American Geophysical Union, pp: 225–249.

Damare SR, Singh P and Raghukumar C (2012). Biotechnology of marine fungi. In *Biology of Marine Fungi, Progress in Molecular and Subcellular Biology* # 53, Ed., Raghukumar C. Berlin Heidelberg: Springer-Verlag, pp: 279–297.

D'Souza J, Rodrigues BF (2013) Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India. *Journal of Forestry Research*, 24: 515–523.

Dutta AK, Pradhan A, Basu SK, Acharya K (2013) Macrofungal diversity and ecology of the mangrove ecosystem in the Indian part of Sundarbans. *Biodiversity*, 14: 196–206.

Ellison AM (2008) Managing mangroves with benthic biodiversity in mind: Moving beyond roving banditry. *Journal of Sea Research*, 59: 2–15.

El-Sharouny HM, Raheem AM and Abdel-Wahab MA (1998). Manglicolous fungi of the Red Sea in Upper Egypt. *Microbiological Research*, 153: 81–96.

Field C (1995) *Journey Amongst Mangroves*. Okinawa, Japan: International Society for Mangrove Ecosystems.

Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. *Oikos*, 85: 377–384.

Ghate SD, Sridhar KR (2015) Contribution to the knowledge on macrofungi in mangroves of the Southwest India. *Plant Biosystems*; doi: 10.1080/11263504.2014.994578

Gofar N (2011) Characterization of petroleum hydrocarbon decomposing fungi isolated from Mangrove rhizosphere. *Journal of Tropical Soils*, 16: 39–45.

Gopal B and Chauhan M (2006) Biodiversity and its conservation in the Sundarban mangrove ecosystem. *Aquatic Science*, 68: 338–354.

Hyde KD (1990) A study of vertical zonation of intertidal fungi on *Rhizophora apiculata* at Kampong Kapok mangrove, Brunei. *Aquatic Botany*, 36: 255–262.

Hyde KD (1998) Studies on the tropical marine fungi of Brunei. II. Notes on five interesting species. *Transactions of the Mycological Society of Japan*, 29: 161–171.

Johnson TW and Sparrow FK (1961) *Fungi in Oceans and Estuaries*. Weinheim, Germany: J. Cramer.

Jones EBG (2011) Fifty years of marine mycology. *Fungal Diversity*, 50: 73–112.

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity*, 35: 1–203.

Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, Boekhout T, Pang K-L (2015) Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. *Fungal Diversity*, 73: 1–72.

Karamchand KS, Sridhar KR, Bhat R (2009) Diversity of fungi associated with estuarine sedge *Cyperus malaccensis* Lam. *Journal of Agricultural Technology*, 5: 111–227.

Kathiresan K, Bingham BL (2001) Biology of mangrove ecosystem. *Advances in Marine Biology*, 40: 81–251.

Kohlmeyer J, Kohlmeyer E (1979) *Marine mycology: The Higher Fungi*. New York: Academic press.

Loilong A, Sakayaroj J, Rungjindamai N, Choeyklin R and Jones EBG (2012) Biodiversity of fungi on the palm *Nypa fruticans*. In *Marine Fungi and Fungal-like Organisms*, Eds., Jones EBG and Pang K-L. Berlin/Boston: Walter de Gruyter, pp: 273–290.

Luo W, Vrijmoed LLP, Jones EBG (2005) Screening of marine fungi for lignocellulose -degrading enzyme activities. *Botanica Marina*, 48: 379–386.

Manimohan P, Amritha M, Sairabanu NK (2011) A comparison of diversity of marine fungi on three co-habiting mangrove plants. *Mycosphere*, 2: 533–538.

Maria GL, Sridhar KR (2003a) Endophytic fungal assemblage of two halophytes from west coast mangrove habitats, India. *Czech Mycology*, 55: 241–251.

Maria GL, Sridhar KR (2003b) Diversity of filamentous fungi on woody litter of five mangrove plant species from the southwest coast of India. *Fungal Diversity*, 14:109–126.

Maria GL, Sridhar KR (2004) Fungal colonization of immersed wood in mangroves of the southwest coast of India. *Canadian Journal of Botany*, 82: 1409–1418.

Maria GL, Sridhar KR, Bärlocher F (2006) Decomposition of dead twigs of *Avicennia officinalis* and *Rhizophora mucronata* in a mangrove in southwest India. *Botanica Marina*, 49: 450–455.

Mepham RH, Mepham JS (1985) The flora of tidal forest – a rationalization of the use of the term "mangrove". *South African Journal of Botany*, 51: 77 – 99.

Middleton BA and McKee KL (2001) Degradation of mangrove tissues and implications for peat formation in Belizean island forests. *Journal of Ecology*, 89: 818–828.

Nakagiri A, Okane I, Ito T, Katumoto K (1997) *Lanceispora amphibia* gen *et* sp. nov., a new amphisphaeriaceous ascomycete inhabiting senescent and fallen leaves of mangrove. *Mycoscience*, 38: 207–213.

Osorio JA, Wingfield MJ and Roux J (2014) A review of factors associated with decline and death of mangroves, with particular reference to fungal pathogens. *South African Journal of Botany*; http://dx.doi.org/10.1016/j.sajb.2014.08.010

Overy DP, Bayman P, Kerr RG, Bills GF (2014) An assessment of natural product discovery from marine (*sensu strictu*) and marine-derived fungi. *Mycology* 5, 145–167.

Pang K, Overy DP, Jones EBG, Calado ML, Burgaud G, Walker AK, Johnson JA, Keer RG, Cha H-J and Bills GF (2016)

'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: Toward a new consensual definition. *Fungal Biology Reviews*; http://dx.doi.org/10.1016/j.fbr.2016.08.001

Pang KL, Jheng JS, Jones EBG (2011) *Marine Mangrove Fungi of Taiwan*. Keelung, Taiwan: National Taiwan Ocean University Press.

Pointing SB, Vrijmoed LLP, Jones EBG (1998) A qualitative assessment of lignocellulose biodegrading enzyme activity in marine fungi. *Botanica Marina*, 41: 293–298.

Qasim SZ, Wafar MVM (1990) Marine resources in the tropics. *Resource Management & Optimization* 7:141–169.

Raghukumar C (2002) Bioremediation of coloured pollutants by terrestrial versus facultative marine fungi. In *Fungi in Marine Environment*, Ed., Hyde KD. Hong Kong: Fungal Diversity Press, pp: 317–344.

Raghukumar C (2005). Marine fungi and their enzymes for decolorization of colored effluents. In *Marine Microbiology: Facets and Opportunities*, Ed., Ramaiah N. Goa: National Institute of Oceanography, pp: 145–158.

Raghukumar C (2008) Marine fungal biotechnology: an ecological perspective. *Fungal Diversity*, 31: 19–35.

Raghukumar C, D'Souza TM, Thorn RG, Reddy CA (1999) Lignin-modifying enzyme s of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. *Applied Envi. Microbiology*, 65: 2103–2111.

Raghukumar S, Sathe-Pathak V, Sharma S, Raghukumar C (1995) Thraustochytrid and fungal component of marine detritus III. Field studies on decomposition of leaves of the mangrove *Rhizophora apiculata*. *Aquatic Microbial Ecology*, 9: 117–125.

Rohrmann S and Molitoris HP (1992) Screening for wood decay enzymes in marine fungi. *Canadian Journal of Botany*, 70: 2116–2123.

Sakayaroj J, Preedanon S, Phongpaichit S, Buatong J, Chaowalit P and Rukachaisirikul V (2012). Diversity of endophytic and marine-derived fungi associated with marine plants and animals. In *Marine Fungi and Fungal-like Organisms*, Eds., Jones EBG and Pang K-L. Berlin/Boston: Walter de Gruyter, pp: 291–328.

Sarma VV (2015) A dichotomous key to the genera of marine fungi recorded on *Rhizophora* spp. *Indian Journal of Geo-Marine Sciences*, 44: 573–579.

Sarma VV and Vittal BPR (2000) Biodiversity of mangrove fungi on different substrata of *Rhizophora apiculata* and *Avicennia* spp. from Godavari and Krishna deltas, east coast of India. In *Aquatic Mycology Across the Millennium*, Eds., Hyde KD, Ho WH and Pointing SB. *Fungal Diversity*, 5: 23–41.

Sarma VV, Hyde KD (2001) A review on frequently occurring fungi in mangroves. *Fungal Diversity*, 8: 1–34.

Schmit JP, Shearer CA (2003) A checklist of mangrove-associated fungi, their geographical distribution and known host plants. *Mycotaxon*, 53: 423–477.

Sengupta A and Chaudhuri S (2002) Arbuscularmycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza*, 12: 169–174.

Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett DE, Porter D, Raja HA, Schmit JP, Thorton HA, Voglymayr H (2007) Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*, 16: 49–67.

Spalding M, Blasco F, Field C (1997) *World Mangrove Atlas*. Cambridge: Cambridge Samara Pub. Co.

Sridhar KR (2009) Mangrove fungi of the Indian Peninsula. In *Frontiers in Fungal Ecology, Diversity and Metabolites*, Ed., Sridhar KR. New Delhi: IK International Publishing House, pp: 28–50.

Sridhar KR (2012a) On the sedge *Cyperus malaccensis* in mangroves and estuaries in tropical habitats. In *Aquatic Plants and Plant Diseases: Types, Characteristics and Management*, Ed., Sridhar KR. New York: Nova Science Publishers, pp: 227–247.

Sridhar KR (2012b) Decomposition of materials in the sea. In *Marine Fungi and Fungal-like Organisms*, Eds., Jones EBG and Pang K-L. Berlin and Boston: Walter de Gruyter, pp: 475–500.

Sridhar KR (2013) Mangrove fungal diversity of west coast of India. In *Mangroves of India: Their Biology and Uses*, Eds., Bhatt JR, Ramakrishna, Sanjappa M, Remadevi OK, Nilaratna BP and Venkataraman K. Kolkota: Zoological Survey of India, pp: 161–182

Sridhar KR, Alias SA and Pang K-L (2012) Mangrove fungi. In *Marine Fungi and Fungal-like Organisms*, Eds., Jones EBG and Pang K-L. Berlin and Boston: Walter de Gruyter, pp: 253–271.

Sridhar KR, Karamchand KS, Sumathi P (2010) Fungal colonization and breakdown of sedge (*Cyperus malaccensis* Lam.) in a southwest mangrove, India. *Botanica Marina*, 53: 525–533.

Sridhar KR, Roy S, Sudheep NM (2011) Assemblage and diversity of arbuscular mycorrhizal fungi in mangrove plant species of the southwest coast of India. In *Mangroves: Ecology, Biology and Taxonomy*, Ed., Metras JN. New York: Nova Science Publishers, pp: 257–274.

Supaphon P, Phongpaichit S, Rukachaisirikul V, Sakayaroj J (2013) Antimicrobial potential of endophytic fungi derived from three seagrass species: *Cymodocea serrulata*, *Halophila ovalis* and *Thalassia hemprichii*. *PLoS ONE*, 8: e72520; doi:10.1371/journal.pone.0072520

Suryanarayanan TS, Kumaresan V (2000) Endophytic fungi of some halophytes from an estuarine mangrove forest. *Mycological Research*, 104: 1465–1467.

Suryanarayanan TS, Thirunvukkarasu N, Govindarajulu MB, Sasse F, Jansen R and Murali TS (2009) Fungal endophytes & bioprospecting. *Fungal Ecology*, 23: 9–19.

Thatoi H, Behera BC and Mishra RR (2013). Ecological role and biotechnological potential of mangrove fungi: a review. *Mycology*, 4: 54–71.

Tomlinson PB (1986) *The Botany of Mangroves*. Cambridge: Cambridge University Press.

Twilley RR, Chen R, Hargis T (1992) Carbon sinks in mangroves and their implications to carbon budget of tropical ecosystems. *Water Air & Soil Pollution*, 64:265–288.

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. *Biological Control*, 46: 72–82.

Vittal RPR and Sarma VV (2006) Diversity and ecology of fungi on mangroves of Bay of Bengal region - An overview. *Indian Journal of Marine Sciences*, 35: 308–317.

Wafar S, Untawale AG, Wafar M (1997) Litterfall and energy flux in a mangrove ecosystem. *Estuarine, Coastal Shelf Science*, 44: 111–124.

Wang Y, Qui Q, Yang Z, Hu Z, tam NF and Xin G (2010) Arbuscular mycorrhizal fungi in two mangroves in South China. *Plant and Soil*, 331: 181–191.

***

PLENARY LECTURES

Keratinophilic Fungi and their Potential

Verekar SA

Dept. of Microbiology, St. Xavier's College-Autonomous, Mumbai
E.mail: shilfa18@gmail.com

Keratinophilic fungi constitute an important ecological group of microbes, which are able to colonize and degrade structurally very hard and stable animal protein – the keratin. Keratinophilic fungi are distributed in wide range of habitats including road side, cattle farm, poultry farm, river streams, school, parks, forests, pastures, etc. These can be differentiated by several traditional parameters including microscopic and colony morphology, nutritional requirement, growth temperature, pigmentation, hair perforation, and mating reactions etc. The morphology and microscopy-based identification is confirmed by 18S rDNA sequence comparisons or internal transcribed spacer (ITS1 and ITS2) and 5.8S rDNA sequence examination. Several of these fungi have known teleomorphs in order Onygenales of Ascomycetes and majority of them are the representative of families Arthrodermaceae, Gymnoascaceae, Myxotrichaceae, and Onygenaceae.

Keratinophilic fungi are of great importance for three main reasons. Firstly, these fungi play a very important role in ecosystem functioning and degrade a major portion of soil keratin. Secondly these fungi are potential producer of industrially important secondary metabolites. Thirdly, they are very important medically. The need of an extensive survey of this group of fungi from unexplored areas and exploitation of their ability to produce secondary metabolites is expressed. The need of culture collection of this group of fungi will be highlighted.

***

PLENARY LECTURES

Fungal Endophytes: Better players of biodiversity, host protection, antimicrobial production and nanotechnology

R N Kharwar

Mycopathology and Microbial Technology Labaratory, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005
Email: rnkharwar@yahoo.com

Fungi are a group of microbes which have been the important source of bioactive compounds since long back in addition to great diversity. Overall, it has been estimated that only about 5-8% of fungal species are yet known, and in this regard 'fungal endophytes' are being considered as a new and alternative source of diversity and bioactive products. Since endophytes (that grow in healthy plant tissues) live in unique biotope that may lead to produce novel bioactive compounds. Presently, the study on endophytic fungi is confined only to some regions especially to temperate and cold whereas from India, only a few reports are available in this line. Viewing India's rainforests and great biodiversity, the idea to isolate the endophytic fungi from Indian medicinal plants and to screening their abilities to produce bioactive compounds may be of great interest.

Endophytic fungi found promising in producing natural bioactive having new mechanism of action within the cellular metabolism. The endophytic fungus isolated from neem plant was also used for biosynthesis of antimicrobial silver and gold nano-particles. Thus we explored fungal endophytes of *Azadirachta indica* and found new bioactive compounds with interesting results. Some interesting fungal genera isolated from neem were *Alternaria, Aspergillus, Cladosporium, Choridium, Curvularia, Drechslera, Fusarium, Gliomastix, Nigrospora, Periconia, Pestalotiopsis, Phoma, Phomopsis, Stenella, Trichoderma, Verticillium* including Mycelia-Sterilia. Potential fungal strains of *Azadirachta indica* were identified for their activity against pathogenic bacterial /fungal strains and the fungus was kept for fermentation for isolation and purification of compound. Finally, by using standard spectroscopic methods the crystallized bioactive component was identified as "Javanicin". The calculated best-fit empirical formula of this parent compound is $C_{15}H_{14}O_6$ and calculated MW of javanicin $-H^+$= 290.1120. The bacteria that seemed to be most sensitive to the Javanicin (2 µg/mL) were *Pseudomonas aeruginosa* and *P. fluorescens.*

The biosynthesis of metal nanoparticles using fungi is considered as a unique and eco-friendly method as it is free from any solvent or toxic chemical, capping agents and also easily amenable to large-scale production The fungal isolate *Aspergillus clavatus*, was used for biosynthesis of silver and gold nanoprticles using aqueous solution of silver nitrate ($AgNO_3$), and tetra auro chlorate ($HAuCl_4$) respectively. *A. clavatus* induced AgNps were antimicrobial in nature while AuNps were non-toxic and stable with triangular shape. Transmission electron microscopy (TEM), Atomic force microscopy (AFM), UV-Vis spectroscopy, and X-ray diffraction (XRD) were used to decide the sizes and shapes of Nps.

***

PLENARY LECTURES

Why Pharmaceutical Industry Should Work On Fungal Endophytes ?

Deshmukh Sunil Kumar

TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), Darbari Seth Block, IHC complex, Lodhi Road, New Delhi – 110003, India
E mail sunil.deshmukh@teri.res.in

Plants harbors a large number of microorganisms mainly fungi and are known as endophytes. These are asymptomatic microorganisms that colonize internal tissues of plants. These fungi came in limelight after the discovery of taxol followed by compounds like camptothecin, vincristine and cryptocandin.

The focus of our research activity is on the discovery of bioactive metabolites from Indian subcontinent. Indian subcontinent offers a great opportunity to discover novel endophytes with pharmaceutical potential, as it is blessed with two hotspots of biodiversity, out of the total twelve mega biodiversity centers in the world. Moreover, out of 17500 flowering plants available in India, over one-third of these are endemic. These plants enjoy diverse habitats in different geographical regions and climatic zones ranging from tropical to alpine regions (Himalayas) and from coastal regions to the deserts. Some of them withstand the extreme climatic conditions viz. the deserts, cold deserts, hot springs, craters etc. Most of our activities are related to drug discovery using various cell based, target based and enzyme based screening to get anti-cancer, anti-inflammatory and anti-microbial compounds.

During the course of our study, we have screened more than 4770 endophytic fungi isolated from plants of diverse habitats with medicinal properties to get bioactive molecules. The most prolific producer of biologically active compounds were the species of *Mycoleptodiscus*, *Phoma*, *Phomopsis*, *Xylaria, Chaetomium*, *Alternaria*, *Nigrospora*, *Curvularia, Fusarium, Arthrinium* and sterile forms which yielded compounds like PM181110, heptelidic acid, altersolanol A, nectripyrone, ergoflavin, A52688 antibiotic complex, arthrichitin, and herbarin. An overview of such examples will be presented here.

***

PLENARY LECTURES

'Conservation of Indigenous Fungi'

Singh SK

National Fungal Culture Collection of India- A DST National Facility, Biodiversity and Palaeobiology Group
MACS' Agharkar Research Institute, G.G. Agarkar Road, Pune 411004, MS
Email: sksingh@aripune.org

Fungi have received special consideration as the largest group of organisms in number on Earth, next only to insects, and treated different from other microbes. Biodiversity and conservation of these fungi have become topics of global concern attracting attention to explore various habitats and unusual niches for recovering hidden fungi, before they are lost due to various factors like human interference and climate change. This adversely affects prospects of fungi used in basic and applied researches. Though, ex situ and in situ conservation of fungi are known since long, they were never considered as part of main stream of conservation biology. Recent years, however have drawn the attention of researcher for conservation of taxonomically diverse fungi for screening of valuable active compounds which have shown great promise in generating bio-economy.

On-going destruction of natural habitats necessitates development of suitable alternative strategies for safeguarding fungal genetic resources. Short- to- long term preservation and maintenance of fungi in laboratory condition can compliment to conservation strategy and may be achieved by preserving live cultures in germplasm banks and dried/exsiccate material in mycological herbaria. Subsequent to the Convention on Biological Diversity (CBD), many member countries have progressively acted in conformity with the convention. As a part of concerted efforts, preservation of indigenous fungi is being practiced in National Fungal Culture Collection of India (NFCCI), a national facility set up by the Department of Science and Technology (DST), Govt. of India. This is an exclusive repository created for conservation of authenticated germplasm of indigenous fungi, and is the unique centre of repository, research, and knowledge based services. Presently more than 4000 pure cultures belonging to about 265 genera of fungi isolated from different substrates, habitats and from distant geographical locations are maintained by applying various methods. Glimpse of the various classical methods of preservation and long term maintenance of indigenous fungi being practiced at NFCCI shall be discussed in detail for the benefit of increasing awareness of conserving mycological heritage of India for future generations.

***

RESEARCH ARTICLES

RESEARCH ARTICLE

Hymenochaetales from Yeoor Hills, Thane, Maharashtra, India.

Hajirnis Sarita[1] and Mishra Raj[2]

[1]Department of Botany, Satish Pradhan Dnyanasadhana College of Arts, Science and Commerce, Thane (W), India
[2]Principal LSPM College, Chondi, Raigad.
*Corresponding author Email: hsarita64@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Hajirnis Sarita and Mishra Raj (2016) Hymenochaetales from Yeoor Hills, Thane, Maharashtra, India, Int. J.of. Life Sciences, Special Issue, A7:1-6.

Acknowledgements:
I am very much thankful to my guide Dr. R. L. Mishra, Principal LSPM College, Chondi for rendering valuable guidance for the present research work. I am also thankful to Principal Dr. C. D. Marathe of my Parent institution for his administrative and technical support. Lastly thanks to my family and all my well-wishers.

ABSTRACT

The wood rotting Hymenochaetaceous fungi were collected from 'Yeoor Hills' situated in the city of Thane, Maharashtra, India in the monsoon months, June to October of the year 2014 and identified on the basis of macroscopical and microscpoical characters. Three species of Phellinus namely, *P. allardi, P. discipes and P. linteus* and one species each of *Coltricia cinnamomea* and *Inonotus tabacinus* were recorded for which an artificial key for the three genera was prepared and similarly for the three species of Phellinus.

Keywords: Hymenochaetales, Wood-rotting, Yeoor Hills, Artificial key.

INTRODUCTION

The variety and galaxy of fungi and their natural beauty occupy prime place in the biological world. Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists have to unravel the unexplored and hidden wealth.

Fungi are among the few organisms that can effectively break down wood. Wood is composed primarily of cellulose, hemicellulose and lignin. Lignin is a complex polymer that is highly resistant to degradation, and it encrusts the more readily degradable cellulose and hemicellulose. Wood rotting fungi, most of which are also members of Basidiomycota, infect trees through wounds, branch stubs and roots and decay the inner heartwood of living trees. Wood rot is of two main types viz. brown rot and white rot. Brown rot fungi are fungi responsible for causing the Brown rot in plants. They selectively degrade cellulose and hemicellulose in wood, leaving behind the more recalcitrant lignin. The decayed wood is brown in color White rot fungi degrade cellulose, hemicellulose, and lignin at approximately equal rates. The decayed wood is pale in color, light in weight, and has a stringy texture. White rot fungi are the only organisms that can completely degrade lignin. Lignin is one of the most abundant organic polymers, accounting for 30% of the organic carbon on the planet only cellulose is more abundant (Boerjan et al. 2003). Extensive decay weakens the tree, and reduces the quality of wood in trees harvested for timber.

The members of the Family Hymenochaetaceae of Order Hymenochaetales are mostly tropical, saprophytic and more rarely parasitic on living plants, always causing a white rot of dead and living wood. As described by Donk (1948), basidiomes are characteristically perennial, leathery or woody, resupinate to stipitate, clavaroid or coralloid with a smooth, rugose, irpiciform, hydnoid or poroid hymenophore distinctly brown and generative hyphae always with simple septa. Setoid elements are variably present in the hymenium, trama or context or on the pileal surface. **Setae** when present are dark brown. Spores are smooth or ornamented and hyaline or brownish. Members of the family Hymenochaetaceae respond positively towards the Xanthochroic reaction i.e. blackening with KOH (Fiasson & Bernillon 1977, Fiasson 1982).

MATERIALS AND METHODS

Fungal specimens were collected from 'Yeoor hills', Thane, Maharashtra, India. Photographs were taken on-field and necessary characters noted down. For morphotaxonomic studies the monographic works of Largent (1977) and Singer (1986) were referred. For microscopic studies sections from the dehydrated pileus and stipe were rehydrated, stained and observed. All measurements were taken under 100X magnification except for tramal patterns which were under 40X magnification and expressed in microns (µm). The books used for identification of the specimens were Ryvarden and Johansen (1980) and Sharma (1995). The systematic arrangement in the present article follows Hibett et al. (2007). Name corrections, authorities and taxonomic assignments of all taxa reported in this work were checked against the (<Mycobank.org>), In addition, websites of international mycological centers such as (< index fungorum.org>) and (<species fungorum.org>) were also referred.

RESULTS AND DISCUSSION

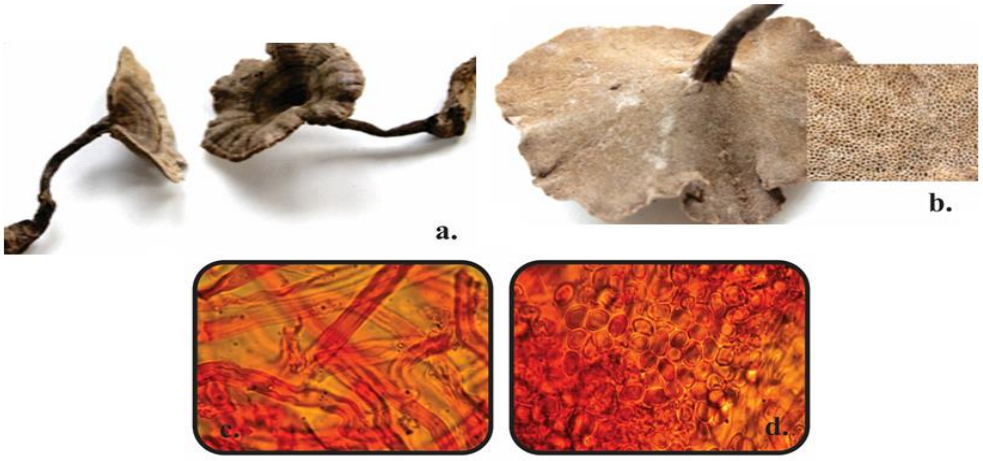
Out of the total specimens collected, 5 of them were identified to belong to Family Hymenochaetaceae of Order Hymenochaetales of Class Agaricomycetes of Phylum Basidiomycota of Sub-kingdom Dikarya of Kingdom Fungi. Out of the 5 species identified, 3 species belonged to genus Phellinus and 1 species each to genus Coltricia and Inonotus.

Artificial Key for Genera

1	Basidiomes pileate, centrally stipitate	***Coltricia***
1	Basidiomes resupinate to pileate, sessile or with a lateral tapering base	2
	Perennial, hyphal system dimitic, basidiomes woody and hard	
2	Annual, hyphal system monomitic, basidiomes thin, flexible when fresh and	***Phellinus***
2	brittle and fragile when dry.	***Inonotus***

Coltricia cinnamomea (Jacq.) Murrill, Bulletin of the Torrey Botanical Club 31 (6): 343 (1904) [MB#119718]

Specimen no.1 **Plate 1**



a. Habit b. Hymenial view & part magnified
c. Hyphal system d. Badiospores

Basidiomes annual, solitary, pileate, laterally stipitate but appearing central as the two edges of the pileus connect behind the stipe, corky when wet, leathery on drying; Pileus 70 × 40 × 4 mm, dimidiate to circular (when projects behind the stipe) plane but shallowly depressed in the centre, surface concentrically zonate, yellowish white towards margin (3B2) and rosewood (9D5) towards the centre, texture velvety with presence of minute hair, margin expanded, wavy, acute; Stipe 53 × 8 mm, violet brown (10F6), eccentric, terete, equal throughout but slightly expanded at the base; Hymenophore porate, pores circular to angular, 1-2 per mm, greyish brown (10D3); Context 2 mm thick, colour rose wood (9D5); Hyphal system monomitic, generative hyphae with hyaline wall, variously thickened, septate and width 6-8 µm; Basidiospores (6.5-)7-8.5(-8.5) × (5-)5.5-5.5(-6.5), (7.63±0.54 × 5.66±0.43) µm, Q = 1.34, broadly ellipsoid to ellipsoid, wall thick, smooth, hyaline with apicular germ pore, dextrinoid. No black reaction with KOH.

Holotype: Terricolous (on soil), gregarious, India, Maharashtra, Thane, Yeoor Hills, Temperature: 28ºC, Humidity: 83 %, GPS Coordinates: 19º20´53´´N 72º91´94´´E, Altitude 110.05 m asl, 21 Sept. 2014, S. Hajirnis

Remarks: The rose wood colour, velvety texture and the concentric zones on the pileus, a thin, long stipe, monomitic hyphal system and ellipsoid basidiospores with an apicular germ pore are some of its distinguishing characters.

The stip is principally the only characteristic that separates the genus from genera like *Inonotus, Coltriciell* and *Phyloporia.*

Inonotus tabacinus (Mont.) G. Cunn., Bulletin of the New Zealand Department of Industrial Research 78: 3 (1948) [MB#121567], **Specimen no. 2**

Basidiomes annual, lignicolous, pileate, sessile, scattered, solid, hard; Pileus 28 × 18 × 4 mm, dimidiate to flabelliform, narrow at the base, reddish brown (9E7), velutinate, hair grey, dry; margin entire and acute. Hymenophore poroid, pores minute, 8-9 pores per mm, angular, 1mm deep, reddish brown; Context 5 mm thick, reddish brown (9D6); Hyphal system monomitic, generative hyphae thin walled, hyaline, septate, with a broad lumen, 3-4 wide µm.Setae 18.69 × 8.97 µm, hymenial setae abundant, ventricose; Basidia 9 × 4 µm, broadly clavate with four sterigmata at their tip; Basidiospores (6.0-)6.5–6.5(-8.0) × (4.0-)4.5–5.0(-6.0), (6.67±0.48 × 4.88±0.43), Q = 1.36, ellipsoid, spore wall thin, hyaline smooth, ventricose, 17.5 × 6.3 µm, non-amyloid and non-dextrinoid; Black reaction with KOH.

Holotype: Terricolous (on soil), Gregarious, India, Maharashtra, Thane, Yeoor Hills, Temperature: 28ºC, Humidity: 83 %, GPS Coordinates: 19º20´53´´N 72º91´94´´E, altitude 110.05 m asl, 21 Sept. 2014, S.Hajirnis

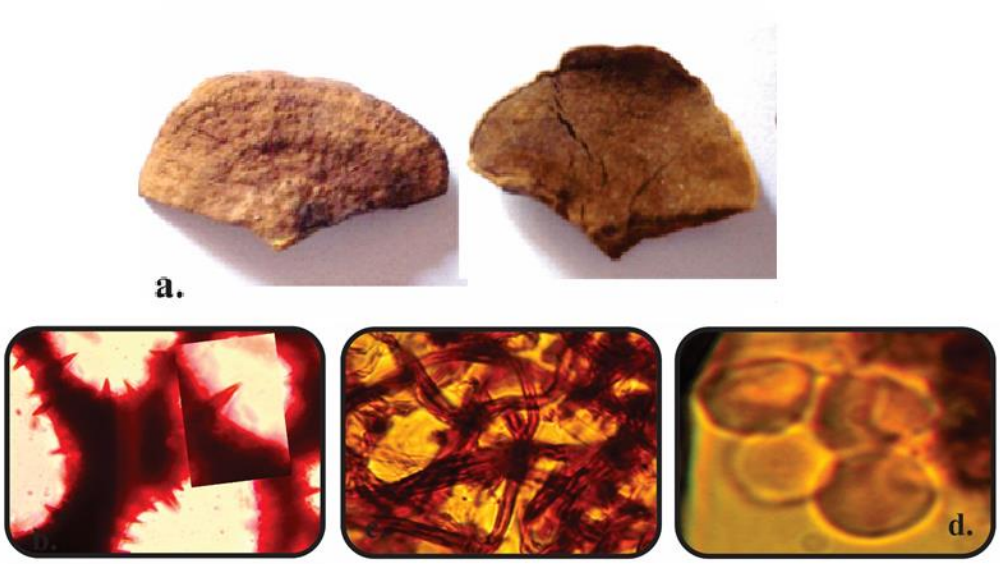


Plate 2 : **a. Habit & Hymenial view b. Pores showing setae (seta magnified) c. Hyphal system d. Badiospores**

Remarks: The present species can be easily identified by its thick and hard pileus, reddish brown colour, dimidiate shape, grey hair on its surface and poroid hymenium. Also monomitic hyphal system, hymenial setae, ellipsoid spores and a black reaction with KOH are some more characters helping in confirming the species. It differs from the type species *Inonotus hispidus* which has the surface of the basidiocarp hairy at maturity, absence of branched setae and larger spore size

***Phellinus* is the largest genus of all polypores.**

Artificial key for species to genus *Phellinus*

Key to species

1 Basidiomes resupinate *P. allardi*

1 Basidiomes pileate…… 2

2 Basidiomes annual, Hymenial setae present……*P. linteus*

2 Basidiomes perennial, Hymenial setae absent……*P. discipes*

Phellinus allardii (Bres.) S. Ahmad, Basidiomycetes of West Pakistan: 57 (1972) [MB#319735]

Specimen No. 3

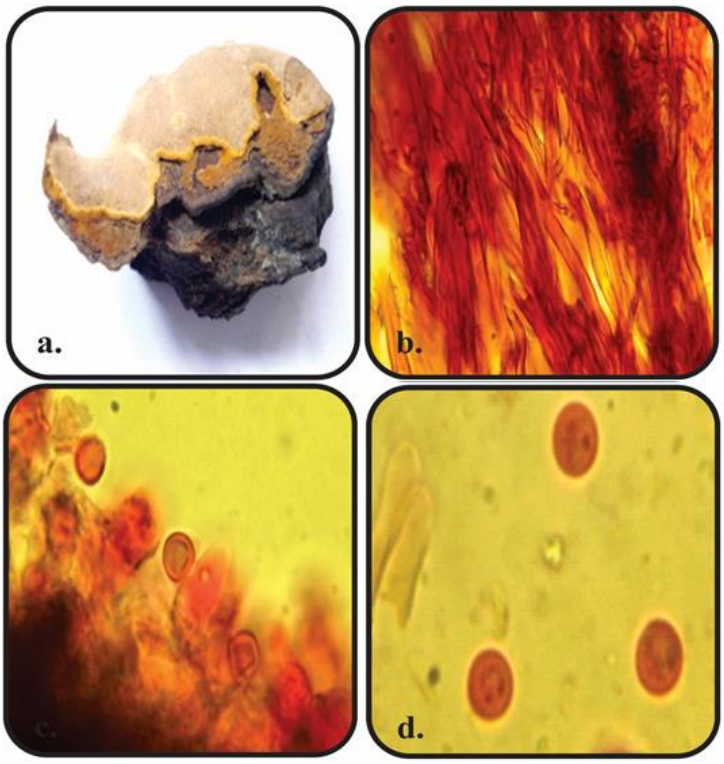


Plate 3. a. Habit b. Hyphal system c. Basidia d. Badiospores

Basidiomes, annual, lignicolous, resupinate, present as an entire large patch on the substratum; Hymenophore exposed, brownish orange (6C3) in the centre and brownish orange (6C6) towards the periphery, porate, pores minute; Context 0.1mm thick, brownish orange (6C6) Hyphal System dimitic, generative hyphae with a thin to thick hyaline wall, broad lumen, septate, 4 µm wide, skeletal hyphae unbranched, thick walled, solid, 3.7-4.5µm wide; Setae absent; Basidia ellipsoidal, 12 × 6 µm; Basidiospores (4.5-)5–5.5(-5.5) × (4-)4.5-5(-5), (5.18±0.27 × 4.70±0.21) µm, Q = 1.1, sub globose, spore wall thin and smooth; dextrinoid; Black Reaction with KOH.

Holotype: Terricolous (on soil), Gregarious, India, Maharashtra, Thane, Yeoor Hills, Temperature: 28ºC, Humidity: 83 %, GPS Coordinates: 19º20´53´´N 72º91´94´´E, altitude 110.05 m asl, 21 Sept. 2014, S. Hajirnis

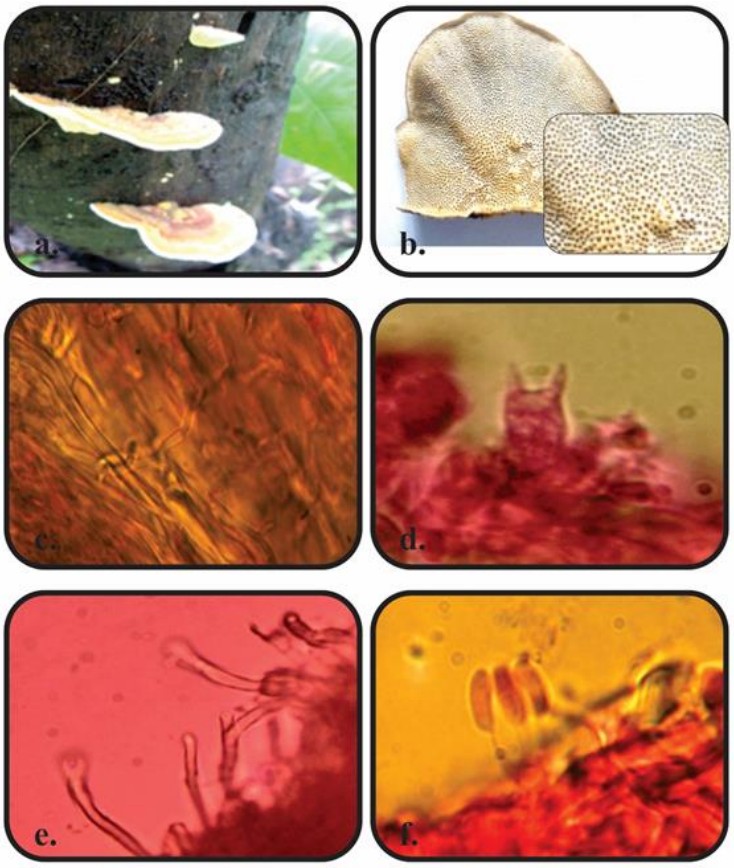


Plate 4 : **a. Habit b. Hymenial view with part magfied c. Hyphal system d. Basidia e. Cystidia f. Badiospores**

Remarks: The resupinate habit of this species with a brownish orange hymenophore lined by a conspicuous margin makes this species easily recognizable from its other closely related species. Also the absence of setae, non-dextrinoid reaction, rusty brown and sub-globose spores are typical of the species.

Phellinus discipes (Berk.) Ryvarden, Kew Bulletin 31 (1): 88 (1976) [MB#319752]
Specimen no. 4 **Plate 4**.

Basidiomes annual, lignicolous, scattered, pileate, sessile, attached by a broad margin, coriaceous when fresh to hard when dry; Pileus 28 × 18 mm, dimidiate, yellowish brown (5D5), concentrically zonate, surface strigose, hair unbranched, margin plane, entire, 1 mm thick; Hymenophore porate, pores circular to angular, 3-4 per mm, pore depth 0.05 mm towards the centre; Context 1-2 mm, greyish yellow (1B3); Hyphal system dimitic, generative hyphae thin to thick walled, hyaline, with a broad lumen, septate and feebly branched with width 2.5-3.5 µm, skeletal hyphae thick walled, solid, unbranched with width 13.5-15.5 µm; Setae absent; Basidia10 × 4.5 µm, broadly clavate with four sterigmata; Basidiospores (4.5-)5.5-6(-6.5)×(2-)2.5-2.5(-3)µm, (5.71±0.40 × 2.41±0.22) µm, Q = 2.36, cylindric, smooth, hyaline, dextrinoid; Black with KOH.

Holotype: Terricolous (on soil), Gregarious, India, Maharashtra, Thane, Yeoor Hills, Temperature: 28ºC, Humidity: 83 %, GPS Coordinates: 19º20´53´´N 72º91´94´´E, altitude 110.05 m asl, 21 Sept. 2014, S. Hajirnis

Remarks: The yellowish brown, dimidiate and hairy pileus and a porate hymenophore make the species easily identifiable in the woods. Besides the dimitic hyphal system, absence of setae, and cylindric basidiospores also contribute to the distinguishing characters of the species. The present species differs from the original species in the size of the spores being smaller.

Phellinus linteus (Berk. & M.A. Curtis) Teng, Zhong Guo De Zhen Jun [Fungi of China]: 762 (1963) [MB#319769] **Specimen no. 5.**

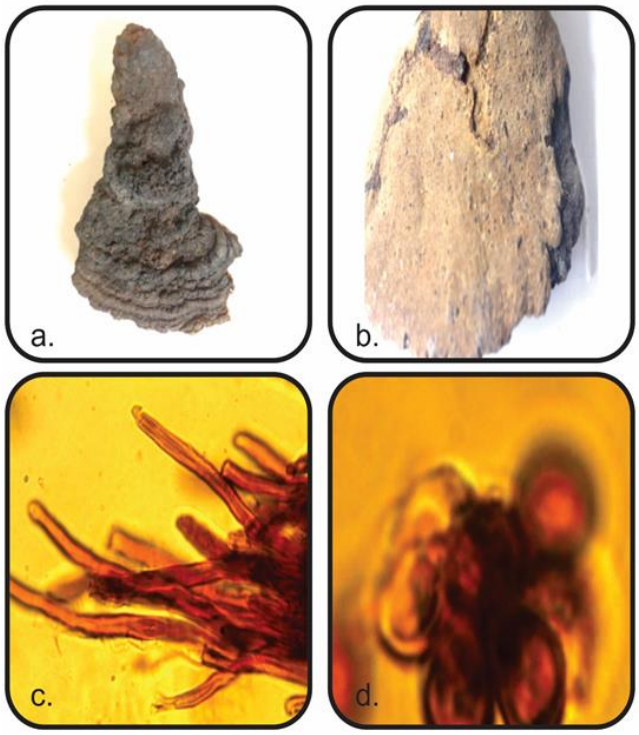


Plate 5 : **a. Habit b. Hymenial view c. Hyphal system d. Badiospores**

Basidiomes perennial, lignicolous, solitary, pileate, sessile, dry, thick, hard and woody; Pileus 140 × 77 × 25 mm, aplanate and flabelliform, brownish red (9E7), concentrically zonate towards the periphery, surface rough, often cracking, margin thick; Hymenophore poroid, pores minute, indistinctly angular, greyish red (8D5), hymenial setae present, acuminate; Context 4 mm thick greyish orange (6D5); Hyphal system dimitic, generative hyphae thin walled, hyaline, with a broad lumen, sparingly branched, width 3.5-4 µm and skeletal hyphae thick walled, solid, unbranched, width 5-6 µm; Basidia 11.71 × 5 µm, cylindrical with four sterigmata at their tips; Basidiospores (4-)4-4.5(-5.5) × (3-)4-4(-5) µm, (4.45±0.45 × 4±0.51) µm , Q = 1.22, sub-globose, rusty brown, slightly thick walled, hyaline, smooth, dextrinoid; Black reaction with KOH.
Holotype: Terricolous (on soil), Gregarious, India, Maharashtra, Thane, Yeoor Hills, Temperature: 28ºC, Humidity: 83 %, GPS Coordinates: 19º20´53´´N 72º91´94´´E, altitude 110.05 m asl, 21 Sept. 2014, S. Hajirnis

Remarks: The perennial habit, dark coloured, hard, woody and aplanate pileus are characters which help to identify the species in the woods. Also the dimitic hyphal system, hymenial setae, sub-globose and rusty brown spores are its diagnostic characteristics

REFERENCES

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol **54**: 519–546

Donk MA (1948) Notes on Malesian fungi 1. Bulletin of the Botanic Gardens Buitenzorg Series III. 17: 473-482.

Fiasson JL (1982) Distribution of Styrylpyrones in the basidiocarps of various Hymenochaetaceae (Aphyllo-phorales, Fungi) - Biochem. Syst. & Ecol. 10: 289-296.

Fiasson JL, Bernillon J (1977) Chemical identification of styryl pyrones in 4 hymenochetaceae fungi aphyllophorales. *Canadian J. of Botany* 55(23): 2984-2986

Hibbett DS (2007) A higher-level phylogenetic classification of the Fungi..*Mycological Research*, 111: 509-547.

Kornerup A, Wanscher JH (1978) Methuen handbook of colour. 3rd ed. Methuen and Co. Ltd., London. 5-252

Ryvarden L, Johansen I (1980) A preliminary polypore flora of East Africa: 1-636

Sharma JR (1995) Hymenochaetaceae of India. Botanical Survey of India. Calcutta: 1-219

Web Bibliography:
http://www.indexfungorum.org/
http://www.mycobank.org/.

RESEARCH ARTICLE

Positive interaction of am fungi with *Capsicum annum* L. (Chilli)

Kelkar Tushar S[1] and Bhalerao Satish A[2]

[1]Environmental Sciences Research Laboratory, Department of Botany,
Wilson College, Mumbai – 400 007, University of Mumbai
[2]S. I. C. E. Society's Degree College of Arts, Science and Commerce,
Jambhul Phata, Chikhloli, Ambernath (W) 421 505, University of Mumbai
*Correspondng author: drsatishbhalerao60@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Kelkar Tushar S and Bhalerao Satish A (2016) Positive interaction of am fungi with *Capsicum annum* L. (Chilli), Int. J.of. Life Sciences, Special Issue, A7:7-12.

ABSTRACT

Arbuscular Mycorrhiza is well known symbionts for majority of angiospermic plants. Mycorrhiza is more common than the exception for higher plants. Symbiotic relationship is developed because of requirement of carbon source by the fungus, which is present and can be produced by higher photosynthetic plants. In return, higher plants get enhanced phosphate uptake along with many other minerals like magnesium, iron etc. This symbiotic association proved to be beneficial for both of the symbionts. The benefits of this symbiotic association can be easily checked in host plants which are often a higher plant like angiosperm.

In present investigation *Capsicum annum* L. plant commonly known as chilli was screened and assessed for such benefits shared by mycorrhiza. In present paper biochemical aspects were studied in controlled (non mycorrhizal) and treated (mycorrhizal) plants of chilli. The estimation of total chlorophyll content, total soluble proteins, phosphate phosphorous, Ca and Mg content of leaf was carried out in mycorrhizal chilli plants and is compared with non mycorrhizal plants of chilli. Results from these estimation showed that there is a significant increase in all the studied biochemical parameters in mycorrhizal chilli plants than the non mycorrhizal chilli plants. These results may contribute to the increment in overall yield of chilli plants.

Key words: Arbuscular mycorrhiza, *Capsicum annum* L., total chlorophyll content, total soluble proteins, phosphate phosphorous, Ca and Mg content.

INTRODUCTION

Arbuscular Mycorrhiza is well known symbionts for majority of angiospermic plants. Mycorrhiza is more common than the exception for higher plants. Symbiotic relationship is developed because of requirement of carbon source by the fungus, which is present and can be

produced by higher photosynthetic plants. In return, higher plants get enhanced phosphate uptake along with many other minerals like magnesium, iron etc. This symbiotic association proved to be beneficial for both of the symbionts. The benefits of this symbiotic association can be easily checked in host plants which are often a higher plant like angiosperm.

Capsicum annuum is a perennial herbaceous plant in the family Solanaceae. Although the species name *annuum* means "annual" (from the Latin *annus* - year), the plant is not an annual. The numerous varieties that have been developed are categorized in five major groups: 1) Cerasiforme (cherry peppers); 2) Conoides (cone peppers); 3) Fasciculatum (red cone peppers); 4) Grossum (bell or sweet peppers); and 5) Longum (chili or cayenne peppers). Chillies are used fresh, cooked, or dried in an enormous variety of dishes characteristic of different regional cuisines. They are high in vitamins A and C. Some varieties have been developed to use as ornamentals, often for indoor pots; these often have small, brightly-coloured, persistent fruits. One of the major biochemical active compounds one can obtain from fruits of chilli (*C. annuum* and other *Capsicum* species) is Capsaicin, is an intense skin and eye irritant, and is the ingredient used in pepper sprays sold for self-defense. However, it also has numerous medical uses, including topical pain relief for muscle soreness, shingles, skin irritations, and rheumatism, and as an anti-inflammatory. Recent medical research has also documented antimicrobial and antifungal activity of capsaicin obtained from several *Capsicum* species, and on-going studies are exploring its use in cancer treatment.

There are many references available on the mycorrhization in chilli plant throwing a light of enhancement in yield of chilli plant by use of mycorrhiza [Bhuvaneswari *et al* (2014), Selvakumar and Thamizhiniyan (2011), Gaur *et al* (1998), Vyas and Vyas (2014)]. Kavitha *et al* (2004) studied the use of mycorrhiza in biocontrol of Damping-Off in Chilli. In present paper effect of mix culture of *Glomus* (*Glomus mosseae* and *Glomus fasciculatum*) on *Capsicum annum* L. plant is studied in terms of some of the biochemical parameters. They are Ca and Mg content of leaf, phosphate phosphorous in leaf, protein content of leaf and total chlorophyll content of leaf. Estimation was done in mycorrhizal (controlled) plants of chilli along with non mycorrhizal (controlled) plants of chilli.

MATERIALS AND METHODS

Eighteen cm diameter, bottom holed plastic pots twelve in number were used for the experiment. Out of twelve pots, six were maintained as control and six were used for the treatment with mycorrhiza culture. From nursery suppliers garden soil was obtained in bulk. Sand was collected from Girgaon Chowpatty sea shore and thoroughly washed with plenty of tap water to remove soluble salts. Mixture of soil and sand (volume / volume) in 3: 1 proportion was made in trays. As mycorrhizae are aerobic microorganisms, aeration in pot is essential and that is why sand was added because to support aeration (Potty, 1988).

The above mixture was sterilized at 121^0 C for 1 hour in autoclave at 15 lbs pressure to kill microorganisms and insect present if any. This sterilized mixture was allowed to cool down to normal room temperature and was used as a growth medium for pot experiment. Initially $3/4^{th}$ capacity of each pot was filled up with sterilized soil mixture. 10 g of AM inoculation was added to six pots as experimental or treated pots. The inoculum was distributed evenly in the pot and was covered with a layer of 4 cm of sterilized soil mixture. Fifteen water soaked seeds of *Capsicum annum* L. (Chilly) were sown in each pot, both control and treated and covered with a layer of soil. The pots were watered with watering can having small pores to avoid the disturbance of the soil surface. After intervals of 15 days after sowing (15 DAS), 30 DAS, 45 DAS and 60 DAS, chilli plants were carefully uprooted and all necessary precautions were taken unless and until plant leaves were processed for making aliquots for estimation the biochemical parameters. Various standard biochemical assays wer followed as mentioned. Estimation of Calcium and Magnesium is done by complexometric titration using ethylene diamine tetra acetic acid (EDTA) which is the most reliable method (Jackson, 1973). Phosphate phosphorus in the oven dry leaf material was extracted by the method of wet digestion (Jackson, 1967). Soluble protein contents in the fresh leaf material were analysed by the method of Lowry *et al.,* (1951). The method of Arnon (1949) was used for estimating the chlorophyll content of the leaves. Unpaired t-test is carried out to check the effect of mycorrhizal inoculation of *Capsicum annum* L. plants. All the statistical analysis are carried out with the help of R i386 3.3.3. Ink

RESULTS AND DISCUSSION

Calcium content (mg / g of leaf material)of *Capsicum annum* L. leaf - Table no. 1 depicts that the calcium content of experimental plant of *Capsicum annum* L is significantly high than the control ones throughout the experimental period.

Magnesium content (mg / g of leaf material) of *Capsicum annum* L. leaf - Table no. 2 reflects that similar to that of calcium content, magnesium content of experimental plant leaves were higher than that of control plant leaves.

Phosphate phosphorus content (mg / g of leaf material) of *Capsicum annum* L. leaf - Table no. 3 depicts phosphate phosphorus content in leaves of experimental and control plants of *Capsicum annum* L. It clearly indicates that the mycorrhizal plants of *Capsicum annum* L shows higher phosphate phosphorous than that of non – mycorrhizal plant leaves.

Protein content (mg / g of leaf material) of *Capsicum annum* L. leaf – Protein content in mycorrhizal plants was significantly higher to that of non – mycorrhizal plants. Table no. 4 reflects the same fact.

Total chlorophyll content (mg / g of leaf material) of *Capsicum annum* L. leaf – Table no. 5 depicts the higher amount of total chlorophyll content in experimental leaf of *Capsicum annum* L. than that of control leaves of *Capsicum annum* L.

Table 1: Calcium content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Calcium content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	1.789	1.892	2.112	2.489
Experimental	1.920	2.325	2.889	3.102
Calculated t	-17.048	-497.14	-566.69	-819.96
Level of significance (p)	0.05	0.05	0.05	0.05

Table 2: Magnesium content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Magnesium content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	1.121	1.285	1.394	1.676
Experimental	1.749	1.862	1.999	2.212
Calculated t	-284.33	-699.72	-179.98	-384.97
Level of significance (p)	0.05	0.05	0.05	0.05

Table 3: Phosphate phosphorous content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Phosphate phosphorous content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	0.122	0.149	0.154	0.160
Experimental	0.189	0.252	0.262	0.282
Calculated t	-29.963	-53.547	-43.374	-12.104
Level of significance (p)	0.05	0.05	0.05	0.05

Table 4: Protein content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

Protein content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	1.302	1.384	1.323	1.485
Experimental	2.229	2.321	2.415	2.502
Calculated t	-603.69	-320.4	-484.32	-477.68
Level of significance (p)	0.05	0.05	0.05	0.05

Table 5: Total chlorophyll content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants

Total chlorophyll content (mg / g of leaf material)	15 DAS	30 DAS	45 DAS	60 DAS
Control	20.100	21.290	22.319	22.345
Experimental	30.212	32.485	33.421	33.481
Calculated t	-138.09	-454.33	-3178.5	-2534.4
Level of significance (p)	0.05	0.05	0.05	0.05

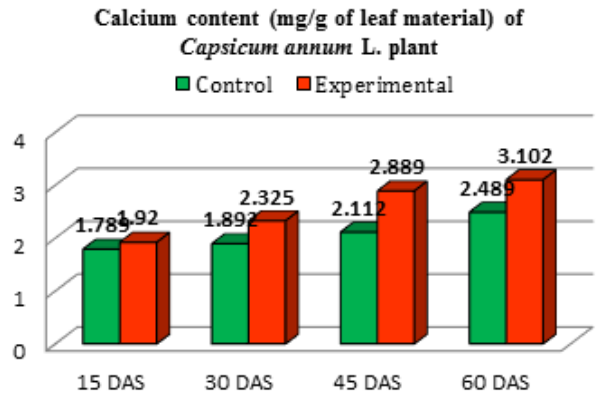


Fig.1: Calcium content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

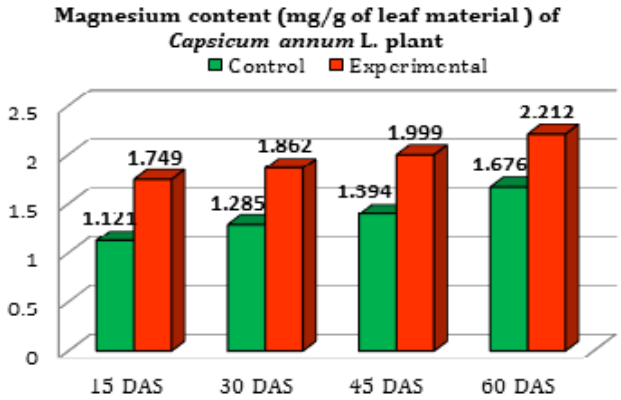


Fig. 2: Magnesium content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

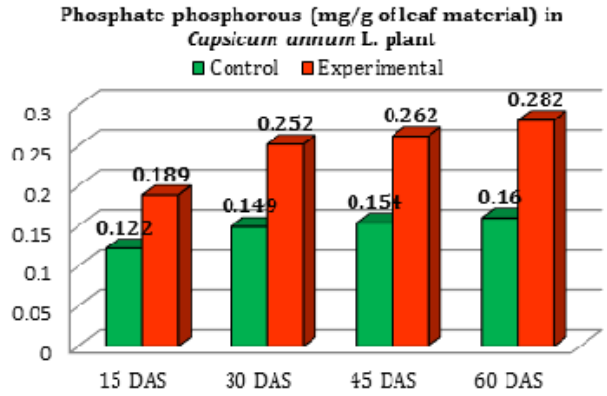


Fig. 3: Phosphate phosphorous content of *Capsicum annum* L leaf in experimental and control(mycorrhizal and non – mycorrhizal respectively) plants

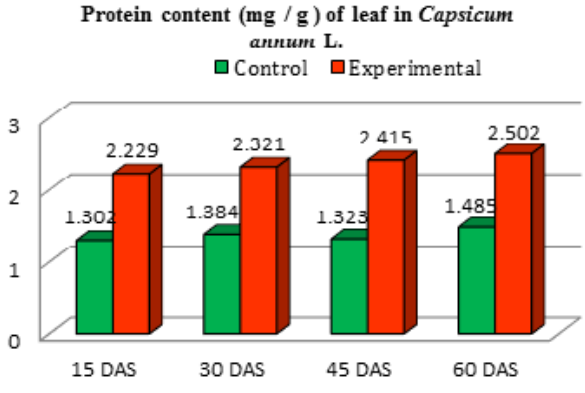


Fig. 4 – Protein content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants

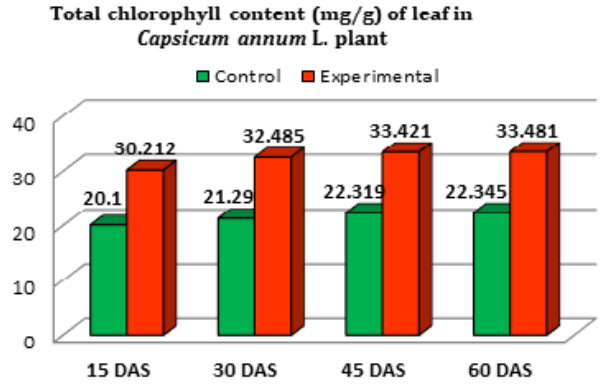


Fig. 5: Total chlorophyll content of *Capsicum annum* L leaf in experimental and control (mycorrhizal and non – mycorrhizal respectively) plants.

It is seen that in the above experiments, enhancement in all the five biochemical parameters studied in mycorrhizal (experimental) plants of *Capsicum annum* L. in comparison with the non mycorrhizal (controlled) plants of *Capsicum annum* L. plants. Similar results have been reported by various researchers in various other plants viz Singh (2004) reported higher concentrations of Ca and Mg in shoots of maize in AM maize plants with comparison to non AM maize. Sitaramamaiah *et al* (1998) observed 55 % increase in Ca and 50 % increment in Mg content in maize plants by *Glomus fasciculatum* inoculation as compared to that of non inoculated maize plants. Mycorrhizal maize leaf shows higher amount of phosphates then the non mycorrhizal ones was shown by McGonigle and Miller (1993). Roy-Bolduc and Hijri (2011) in their review paper throws a light on increase phosphate concentration in mycorrhizal plants with respect to non mycorrhizal ones. Krishna and Lee (1987) reported increased phosphorous uptake and plant growth in pearl millet and sorghum in semi arid tropics. Azcon *et al* (1996) reported increased protein content in Lettuce *Lactuca sativa* plant inoculated with *Glomus fasciculatum* than the non mycorrhizal plant. Fattah and Asrar (2012) reported increased protein content in leaves of mycorrhizal maize plant than the non mycorrhizal plants in saline conditions. Shinde and Thakur (2015) carried out detail experiments on pea plants in water stress condition with mixture of AM fungi species of *Acaulospora denticulata, A. gerdemannii, Glomus macrocarpum, G. maculosum, G. fasciculatum and Scutellospora minuta*. It was observed that protein content of leaf decreases in water stress conditions in non mycorrhizal plants whereas no such decrease was observed by them in mycorrhizal plants of pea in water stress conditions. They concluded that such increased in protein content was because of mycorrhiza, as mycorrhiza is well known stress reducing symbiont. Shinde and Khanna (2014) reported that proteins in mycorrhizal plants are significantly high than that of non mycorrhizal plants of potato. Shinde and khanna (2014), Shinde and Thakur (2015) reported that in mycorrhizal plants of pea and potato, significant increment of chlorophyll content than non mycorrhizal one. Thakur *et al* (2005) reported such increment in total chlorophyll content in leaves of mycorrhizal apple plants in comparison with apple plants which are non mycorrhizal. They predicted that such increase in chlorophyll content is because of increment in Mg content in mycorrhizal plants as Mg is essential for chlorophyll development. Similar kinds of reports are given by Khare *et al* (2008).

REFERENCES

Arnon DI (1949) Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris* Plant Physiology 24 (1): 1-15.

Azcon R, Gomez M and Tobar R (1996) Physiological and nutritional response of *Lactuca sativa* L. ti nitrogen sources and mycorrhizal fungi under drought conditions. Biology and fertility of soils 22(1 – 2): 156 – 161

Fattah-Abdel GM and Asrar AA (2012) Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil Acta Physiol Plant 34: 267

Bhuvaneswari G, Reetha S, Sivaranjani R and Ramakrishnan K (2014) Effect of AM fungi and Trichoderma species as stimulations of growth and morphological character of chilli (*Capsicum annuum* .L) International Journal of Current Microbiology and Applied Science3(3): 447-455

Selvakumar G and Thamizhiniyan P (2011) The Effect of the Arbuscular Mycorrhizal (AM) Fungus *Glomus intraradices* on the Growth and Yield of Chilli (*Capsicum annuum L.*) Under Salinity Stress World Applied Sciences Journal 14 (8): 1209-1214

Gaur Atimanav, Alok Adholeya, Krishna G Mukerji (1998) A comparison of AM fungi inoculants using Capsicum and Polianthes in marginal soil amended with organic matter Mycorrhiza Vol. No. 7 (6): 307-312

Jackson ML (1967) Soil and chemical analysis Prentice Hall of India Pvt. Ltd. New Delhi: 38 – 82

Jackson ML (1973) Soil Chemical Analysis, Prentice Hall India Pvt Ltd, New Delhi, India: 498-516.

Sitaramaiah K, Rahul Khanna and Trimurtulu N (1998) Effect of *Glomus fasciculatum* on growth and chemical composition of maize Journal of mycology and plant pathology Vol. 28 (1): 38 – 40

Kavitha K, Meenakumari KS and Sivaprasad P (2004) Arbuscular Mycorrhizal Fungi for Biocontrol of Damping-Off in Chilli Journal of Mycology and Plant Pathology, Vol. 34, No. 2:349 – 353.

Khare V, Singh SK, Singh S and Kheri HK (2008) Effect of AM fungi in improving the growth performance of *Cyamopsis tetragonoloba* under saline soil conditions. Mycorrhiza news 20(2): 17 – 19.

Krishna KR and Lee KK (1987) Management of vesicular arbuscular mycorrhiza in tropical cereals In Mycorrhiza in the next decade NACOM 1987, proceedings of 7th North American conference on Mycorrhizae Florida, USA: 43 – 45.

Lowry OH, Rosebrought S, Farr Al and Randall RJ (1951) Protein measurement with Folin phenol reagent Journal of Biological Chemistry Vol. 193 (1): 265 – 275

Mc Gonigle TP and Miller MH (1993) Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. Plant and Soil 210: 33 – 42.

Roy-Bolduc A and Hijri M (2011) The Use of Mycorrhizae to Enhance Phosphorus Uptake: A Way Out the Phosphorus Crisis. J Biofertil Biopestici 2:104.

Shinde BP and Jaya Thakur (2015) Influence of Arbuscular mycorrhizal fungi on chlorophyll, proteins, proline and total carbohydrates content of the pea plant under water stress condition International Journal of Current Microbiology and Applied Science (2015) 4(1): 809-821

Shinde BP and Manjusha Khanna (2014) Impact of AM fungi on biochemical changes in potato plants International Journal of Current Microbiology and Applied Science Volume 3 (7): 1018-1027

Singh S (2004) Effect of soil moisture on arbuscular mycorrhizal development in plants (Part II) In pulses, vegetables and misclellaneous crops Mycorrhiza News 16 (1): 2- 11

Thakur JS, Sharma YP and Lakhanpal TN (2005) Mycorrhizal Innoculations and Mineral Status of Apple (*Malus domesticus* Borkh.) Journal of Mycology and Plant Pathology, Vol. 35, No. 1:58 –63

Vyas Mohnish and Anil Vyas (2014) Field Response of Capsicum Annuum Dually Inoculated with AM Fungi and PGPR in Western Rajasthan International Journal of Research Studies in Biosciences Vol. 2(3): 21-26.

RESEARCH ARTICLE

Use of antifungal lactic acid bacteria (LAB) for bread preservation

Khendkar Bhagyashri C and Purandare Krupa A

Department of Microbiology, G.N. Khalsa College, Matunga, Mumbai – 400 019
E-mail: bhagyashree2188@gmail.com, krupash_purandare14@yahoo.co.in

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Khendkar Bhagyashri C and Purandare Krupa A (2016)Use of antifungal lactic acid bacteria (LAB) for bread preservation, Int. J.of. Life Sciences, Special Issue, A7:13-18.

ABSTRACT

Fungal spoilage of food and feed is a common and global phenomenon. The global food industry sector is under constant pressure from both consumers and regulatory bodies to provide high quality fresh food with minimal processing. The consumers prefer safe preservative-free products. Fungal spoilage is the main cause of economic loss in the bakery industry. In present study, the antifungal starter culture was developed using selected antifungal lactic acid bacteria (LAB) and was used for bread preservation. The best biopreservation effect was obtained with 50% antifungal slurry SL-AL2 containing *Lactobacillus ingluviei* against *Penicillium* sp., which was more effective than 0.4% Calcium propionate (Pca), a common preservative used in bakery products. The antifungal starter culture SL-AL2 extended the shelf life of packaged bread to 19 days, more than 4 times longer shelf life than breads prepared with only 0.4% PCa.

Key words: Lactic acid bacteria (LAB), Antifungal activity, Antifungal starter culture, Biopreservation.

INTRODUCTION

Food and feed spoiling moulds and yeasts cause great economic losses worldwide. In addition to economic losses, food spoilage also represents a health hazard for consumers, especially when it is contaminated with mycotoxigenic molds (Legan, 1993).

Bakery products are the important staple foods in most countries. These products are subjected to spoilage problems which include physical, chemical and microbial spoilage. Since the most common factor of bakery products is water activity (a_w = 0.94 - 0.97) and a pH of approximately 6, mould growth is of major concern in bakery products.

The fungal spores are killed during baking and the airborne moulds recontaminate the baked goods during the processing of bread such as cooling, slicing, wrapping and storage operations. The most common spoilage moulds isolated from bakery goods belong to the genera *Rhizopus, Mucor, Penicillium, Aspergillus*, *Monilia, Endomyces, Cladosporium* and *Fusarium*. Several different approaches have been

adopted to control mould growth in bread, including the addition of propionic acid and its salts, benzoates, sorbates, modified atmosphere packaging, irradiation and pasteurization of packaged bread etc. In present work, the efficacy of antifungal LAB isolates as bio-preservative in bread making was evaluated

MATERIALS AND METHODS

Formulation of the antifungal starter culture (slurry): The strains AL2 (*L. ingluviei*) and IB2 (*Weissella confusa*) used in this study were obtained from lassi and idli batter respectively. Before experimental use, the strains were grown twice in de Man Rogosa Sharpe (MRS) broth without acetate at 30^0 C for 24 h. The cultures were used as single cultures and inoculated (15% v/v) in a mixture of 250 g of wheat flour, 5 g sucrose, 12.5 g of skimmed milk, 0.75 liters of tap water. After homogenization, the pH was adjusted to 5.90 $\pm$ 0.15 with Na_2HPO_4, and fermentations were allowed to proceed at 30^0 C for 24 h with continuous stirring at 100 rpm. Each resulting semisolid fermented product was named slurry (SL) and was used in the dough formula.

LAB growth was determined by the plate dilution method using MRS agar, the plates were incubated at 30^0 C for 48 h, and results were expressed as log CFU per milliliter. The rate of slurry acidification was determined by measuring pH and total titratable acidity (TTA) (Potentiometric method with Domic solution [0.1 N NaOH] using Phenolphthalein as an indicator) in 10-ml samples. Results were expressed in milliliters of Domic solution needed to achieve a pH of 8.3 to 8.6.

Dough fermentation and bread manufacture:
The lactic slurries were used for the production of wheat bread. The doughs were prepared as follows: 1,000 g of commercial wheat flour, 10 g of NaCl, 20 g of sucrose, 50 g of skim milk, 20 g of fat, and 0.5 liters of tap water. To incorporate the fermented slurry, tap water was partially replaced (50%) in the dough preparation by equal amounts of the slurry fermented by each of the selected LAB isolates. A commercial strain of *Saccharomyces cerevisiae* (baker's yeast) was used as the leavening agent. Dough containing yeast only (Y-dough) and doughs with yeast plus 0.4 % (wt/wt) Calcium propionate (PCa) (YCP-dough) were used as controls.

The doughs were individually placed in aluminum pans for fermentation (2 h, 30^0 C). The pH was determined using a pH meter (Hanna Instruments). The leavening power, determined by fermenting a 50-g portion of dough in a beaker at 30^0 C for 2 h, was calculated as V (cm^3) = Vf – Vi, where Vf and Vi are the final and initial leaven volumes, respectively. After fermentation, the doughs were baked in oven (160^0 C for 60 min), and the bread loaves were cooled at room temperature for 90 min. The volume of the baked breads (length by width by height) was expressed in cm^3. The bread loaves were inoculated (1 ml per 100-g loaf) with a conidial suspension (10^4 conidia per ml) of fungi; then they were packed into polyethylene bags and stored at room temperature. The bread shelf life was defined as the time (in days) for moulds to become visible on the surface of the packaged loaves. Observations were performed daily.

RESULTS AND DISCUSSION

The chemical and microbiological characteristics of the slurries obtained with each LAB strain (SL-AL2 and SL-IB2) are summarized in Table 1.

After 24 h of fermentation, pH values for both the slurries were found to be approximately same i.e. 3.8 for SL-AL2 and 3.9 for SL-IB2 and cell counts were 9.1 and 9.4 log CFU per ml for slurries SL-AL2 and SL-IB2 respectively. SL-AL2 yielded the higher value for TTA (14.8 ml of 0.1 N NaOH per 10-ml sample) and titratable acidity in terms of lactic acid was 1.33 Gm% for this slurry. TTA value for SL-IB2 was 14.5 and yielded 1.30 Gm% lactic acid.

Each fermented slurry was subsequently used (50% vol/vol) to produce doughs (YLB doughs) that were identified with the different LAB strains. Dough without incorporation of slurry (Y dough) and dough with 0.4% wt/wt Calcium propionate as a chemical preservative (YCP dough) were used as controls. All doughs were prepared with commercial yeast (Baker's yeast). The pH values and leavening power was calculated for each dough type.

Doughs prepared with fermented slurries (YLB doughs) showed significantly higher leavening power and attained lower pH (5 to 5.6) as compared to Y-doughs (pH 6.5). YLB-IB2 dough had the highest leavening power (115.46 cm^3). Results are presented

in Table 2. Differences in shelf life were also seen for breads prepared with only Yeast (Y), SL and PCa. Y-breads were spoiled within 2 days of ambient storage.

Fungal growth was initiated on Day 4 after preparation in bread sample with yeast and 0.4% calcium propionate (PCa) as chemical preservative as well as bread sample with yeast and SL-IB2 slurry as starter culture, which indicates that biopreservation effect obtained with 50% antifungal slurry containing SL-IB2, was as effective as 0.4% calcium propionate (PCa). Promising results were observed with breads prepared from doughs containing SL-AL2 (50% v/v) where no visible fungal growth was observed up to 19 days, which was very much comparable with the preservative activity exhibited by Calcium propionate where shelf life was found to be more than 4 times longer than breads prepared with only 0.4% PCa.

Table 1: Characteristics of the slurries fermented with Lactic Acid Bacteria

Slurries[a]	**Colony Counts**[b] (log CFU per ml)	**pH**	**TTA**[c]	**Titratable acidity in**[d] **terms of Lactic acid (Gm%)**
SL-AL2	9.1	3.8	14.8	1.33
SL-IB2	9.4	3.9	14.5	1.30

[a] The slurries (SLs) were fermented with LAB isolates AL2 and IB2 at 30^0 C for 24h. Semiliquid slurries contained an inoculated mixture: Wheat flour, 250 g; Sucrose, 5 g; Skimmed milk, 12.5 g; and Tap water, 0.75 liters

[b] Colony counts of LAB expressed as log CFU per ml of slurry

[c] TTA – Total titratable acidity, reported as milliliters of 0.1 N NaOH needed to achieve a pH of 8.3 to 8.6 in 10-ml samples

[d] Titratable acidity in terms of Lactic acid, is calculated using the formula:

$$\textbf{Lactic acid Gm \%} = \frac{0.1 \text{ X B.R. X } 9}{\text{Volume of the sample}}$$

Where, 0.1 → Normality of NaOH
B.R. → Burette reading
9 → Equivalent weight of Lactic acid

Table 2: Characteristics of doughs prepared with fermented slurries as a starter culture and without fermented slurries i.e. controls – Y dough and YCP dough

Dough Type[a]	**Initial Volume (V_i) cm^3**	**Final leaven Volume (V_f) cm^3**	**Leavening**[b] **Power (V_f - V_i) cm^3**	**pH**	**Volume of baked doughs cm^3**
Y dough	88.51	138.54	50.03	6.5	324
YCP dough	111.61	165.48	53.87	5.5	323
YLB-AL2 dough	92.36	173.18	80.82	5.2	432
YLB-IB2 dough	115.45	230.91	115.46	5.6	435

[a] All doughs were prepared with commercial yeast (Baker's yeast). When no PCa (Calcium propionate) was used (0% PCa), the resulting control dough was designated as Y dough.

YCP dough: PCa, Calcium propionate, a chemical preservative added to the dough at 0.4% (wt/wt).Slurries SL-AL2 and SL-IB2 were incorporated by partially replacing (50%) tap water in the dough preparation by equal amounts of the slurry fermented by each of the selected LAB strains

[b] The leavening power, determined by fermenting a 50 g portion of dough in a beaker at 30^0 C for 2 h, was calculated as V (cm^3) = V_f - V_i, where V_f and V_i are the final and initial leaven volumes respectively.

Table 3: Effect on preservation of bread inoculated with mould spores

Target mould	Visible mould growth observed on the surface of packaged loaves* (Days) Dough types					
	I] Bread prepared with Slurry SL-AL2 as starter culture			II] Bread prepared with Slurry SL-IB2 as starter culture		
	Y	YLB-AL2	YCP	Y	YLB-IB2	YCP
Aspergillus flavus	Day 2	Day 2	Day 3	Day 2	Day 3	Day 3
Aspergillus fumigatus	Day 2	Day 2	Day 3	Day 2	Day 2	Day 3
Aspergillus niger	Day 2	Day 3	Day 2	Day 2	Day 3	Day 2
Fusarium sporotrichioides	Day 3	Day 7	Day 7	Day 3	Day 3	Day 7
Penicillium sp.	Day 2	Day 7	Day 5	Day 2	Day 2	Day 5

* Day on which mould growth was initiated on the surface of packaged loaves

(A) **(B)**

Fig. 1 : The extent of growth and sporulation of *A. flavus* (A) and *A. niger* (B) was highly reduced in bread with SL-AL2 slurry as compared to Y-Doughs

The overall extent of growth and sporulation of moulds *A. flavus* and *A. niger* was highly reduced in presence of AL2 (Photograph 1) as well as IB2 as the starter culture. The results were promising with both LAB strains against *Aspergillus niger*, as fungal growth was initiated on Day 2 after preparation in bread sample prepared using Calcium propionate as a preservative; whereas the fungal growth was initiated on Day 3 in bread sample with slurries SL-AL2 and SL-IB2 as a starter culture. Use of these slurries lengthened shelf life by one day with respect to controls prepared using only yeast and yeast plus PCa as chemical preservative. A remarkable antifungal activity was observed in bread sample with yeast and SL-AL2 as the starter culture against *Fusarium* and *Penicillium sp.*, wherein mould growth of was delayed up to 7 days. It was noteworthy that, the bread sample prepared with Calcium propionate showed initiation of *Penicillium* growth on Day 5, whereas in presence of AL2, growth was delayed up to 7th day. LAB AL2 inhibited the growth of *Fusarium* and *Penicillium* and lengthened the shelf life one to three fold with respect to bread prepared using only *Saccharomyces cerevisiae*. The extent of fungal growth as well as sporulation was highly limited in the bread sample with LAB IB2, as compared to bread prepared with only yeast as a starter culture, however no significant results were obtained with LAB isolate IB2 against *Fusarium.* Also, no significant antifungal activity was observed with the LAB isolates AL2 and IB2 against *A. fumigatus* (Table 3).

DISCUSSION

LAB play an important role in sourdough starter, in improving the flavor, taste and shelf life of final product. During the last decade, the main technological advancements in the baking industry have been shortened leavening time and the availability of ready-to-use starter cultures. Although baker's yeast (*S. cerevisiae*) reduces the time of bread making, the finished bread is less flavorful than bread prepared using specific strains of LAB (Brandt, 2007). The

antifungal slurries (SL-AL2 and SL-IB2) developed in this study contains the LAB strains *Lactobacillus ingluviei* and *Weissella confusa* respectively along with low-cost ingredients that are compatible with the food matrix (wheat dough). Since the slurry was developed to partially replace tap water in the dough, no major changes in the conventional process of bread making are introduced.

In present study, YLB doughs showed significantly higher leavening power than Y-doughs. Dough with SL-IB-2 had the highest leavening power (115.46 cm^3). Isolate IB2 (*Weisella confusa*), an obligate hetero-fermenter, produces CO_2 as one of the end-products. This indicates that, the ability of *S. cerevisiae* to produce CO_2 was enhanced in the presence of lactic starters. These results verify that the LAB played an important role for improving the leavening ability of yeast. These results are in good accordance with those reported by Martinez- Anaya et al. (1990), Gobbetti (1998) and Häggman and Salovaaraa (2008).

The YLB-doughs attained a significantly lower pH (5 to 5.6) than Y dough (pH 6.5), which may be due to production of organic acids during fermentation by LAB strains. This result is in agreement with Arendt *et al.* (2007) and Iacumin *et al.* (2009).

LAB strain *Lactobacillus ingluviei* tested was found to be active against *A. niger, F. sporotrichioides* and *Penicillium,* however, *Weisella confusa* was found to be active only against *A. flavus* and *A. niger.* The fungal growth was delayed as well as effects on sporulation were observed by addition of slurry to the doughs, an effect that was dependent on the LAB strains involved. The antifungal effectiveness of slurries at 50% were comparable with breads prepared with 0.4 PCa (wt/wt), as a chemical preservative. The slurries (SL-AL2 and SL-IB2) prepared from each lactobacilli strain also delayed mould growth, thus allowing an extended shelf life as compared to Y-breads prepared without preservatives. Also, extent of sporulation of moulds was found to be affected when slurries were used as starter culture. Use of SL-IB2 produced the biopreservative effect (4-days shelf life), which is similar to that obtained using 0.4% PCa. The best biopreservative effect was observed with SL-AL2 slurry, where no visible fugal growth was observed upto 19 days. The antifungal effect of the slurries was mainly related to the production of various organic acids by lactic acid bacteria and presence of PLA and OH-PLA (which was observed in our previous study (data not published), thus favoring the undissociated active fraction of the acids (Piper, Calderon, Hatzixanthis and Mollapour, 2001). After fermentation, the dough attained a low pH (pH 5.0 – 5.6), which favored the undissociated fraction of the organic acids and the antifungal effect of the slurry. The anti-mould activity of LAB against moulds have been recently reviewed by Hassan and Bullerman (2008). Studies have shown that, LAB strains in sourdough breads offer very good protection against a wide range of mould spoilage organisms including *P. corylophillum, P. expansum, E. fibuliger, A. niger and F. graminearum* (Lavermicocca et al., 2000). Recently *Lb. rossiae* LD108 and *Lb. paralimentarius* PB127 were used in the production of bread and panettone, and found to prevent growth of *A. japonicus* with shelf lives ranging from 11-32 days as compared to bread prepared with baker's yeast dough (Garofalo et al., 2012).

In our study, supplementing traditional doughs prepared with commercial yeast, with slurries prepared using lactic acid bacteria significantly delayed the mould spoilage of bread and therefore this property can be exploited to enhance shelf life of bread.

REFERENCES

Arendt E, O'Brien C, Schober T, Gormley T, Gallagher E (2002) Development of gluten-free cereal products. *Farm Food.* 12: 21–27.

Brandt MJ (2007) Sourdough products for convenient use in baking. *Food Microbiol*. 24: 161-164.

Brul S and Coote P (1999) Preservative agents in foods, Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology*. 50: 1-17.

Clarke CL and Arendt EK (2005): A review of the application of sourdough technology to wheat breads. *Adv. Food Nutr. Res*. 49:137-161.

Clarke CL, Schober TJ, Dockery P, O'Sullivan K and Arendt EK (2004) Wheat sourdough fermentation: effects of time and acidification on fundamental rheological properties. *Cereal Chem*. 81: 409-417.

Corsetti A (2013) Technology of sourdough fermentation and sourdough applications, p 183–217 *In* Gobbetti M, Ganzle MG, editors. (ed), *Handbook of sourdough biotechnology*. Springer, Heidelberg, Germany.

Corsetti A, Gobbetti M, Rossi J and Damiani P (1998) Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Applied Microbiology and Biotechnology.* 50: 253-256.

Corsetti A and Settanni L (2007) *Lactobacilli* in sourdough fermentation. *Food Res. Int*. 40: 539-558.

Dal Bello F, Clarke CI, Ryan LAM, Ulmer H, Schober TJ, Ström K, Sjögren J, van Sinderen D, Schnürer J and Arendt EK (2007) Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. *J. Cereal Sci.* 45: 309–318.

Garofalo C, Zannini E, Aquilanti L, Silvestri G, Fierro O, Picariello G and Clement F (2012) Selection of Sourdough *Lactobacilli* with Antifungal Activity for Use as Biopreservatives in Bakery Products*. J. Agric. Food Chem*. 60: 7719–7728.

Gerez CL, Torres MJ, Font de Valdez G, Rollán G (2013) Control of spoilage fungi by lactic acid bacteria. *Biological Control*. 64: 231–237.

Gerez CL, Torino MI, Rollán G and Font de Valdez G (2009) Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties," *Food Control*. 20(2): 144–148.

Gobbetti M, De Angelis M, Corsetti A and Di Cagno R (2005) Biochemistry and physiology of sourdough lactic acid bacteria. *Trends Food Sci. Technol*. 16: 57-69.

Iacumin et al., (2009) Description of the microflora of sourdoughs by culture-dependent and culture-independent methods*. Food Microbiol*, 26: 128-135.

Khendkar B and Purandare K (2013) Screening of lactic acid bacteria for antifungal properties. *Journal of the National Integrated Medical Association.* Vol.55 - No.6, June 2013.

Kulp K (2003) Baker's yeast and sourdough technologies in the production of U.S. bread products, p. 97-143. In K Kulp and K. Lorenz (ed.), *Handbook of dough fermentations*. Marcel Dekker Inc., New York.

Lavermicocca P, Valerio F, Evidente A, Lazzaroni S, Corsetti A and Gobbetti M (2000) Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Applied and Environmental Microbiology.* 66: 4084-4090.

Legan JD (1993) Moulds spoilage of bread: the problem and some solutions. *Int. Biodeterior. Biodegrad.* 32: 33-53.

Legan JD and Voysey PA (1991) Yeast spoilage of bakery products and ingrethents. *Appl. Bacteriol*. 70: 361-371.

Martínez-Anaya MA et al. (1990) Microflora of the sourdoughs of wheat flour bread. X. Interactions between yeasts and lactic acid bacteria in wheat doughs and their effects on bread quality. *Cereal Chem.* 67: 85–91.

Marina Häggman, Hannu Salovaara (2008) Microbial re-inoculation reveals differences in the leavening power of sourdough yeast strains. *LWT- Food Science and Technology*. 41(1):148-154.

Messens W and De Vuyst L (2002) Inhibitory substances produced by *Lactobacilli* isolated from sourdoughs. *Int. J. Food Microbiol*. 72: 31-43.

Piper P, Calderon CO, Hatzixanthis K, Mollapour M (2001) Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. *Microbiology* 147:2635–2642

Rosenquist H and Hansen A (1998) The antimicrobial effect of organic acids, sourdough and nisin against *Bacillus subtilus* and *B. licheniformis* isolated from wheat bread. *Appl. Microbiol*. 85: 621-631.

Ryan LAM, Dal Bello F and Arendt EK (2008) The use of sourdough fermented by antifungal lactic acid bacteria to reduce the amount of calcium propionate in bread. *Food Microbiol*. 125: 274-278.

Salovaara H (2004) Lactic acid bacteria in cereal-based products, 3rd ed, p 431–452 *In* Salminen S, von Wright A, Ouwehand A, editors. (ed), *Lactic acid bacteria*. CRC Press, Boca Raton, FL.

Thiele C, Ganzle MG and Vogel RF (2002) Contribution of sourdough *lactobacilli*, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. *Cereal Chem.* 79: 45-51

Zhang C, Brandt MJ, Schwab C, Gänzle MG (2010) Propionic acid production by cofermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. *Food Microbiol*. 27:390–395.

Zhang X, Zhang S, Shi Y, Shen F and Wang H (2014) A new high phenyl lactic acid-yielding *Lactobacillus plantarum* IMAU10124 and a comparative analysis of lactate dehydrogenase gene. *FEMS Microbiol Lett*. 356: 89–96.

RESEARCH ARTICLE

Production of Fungal Cellulase under Solid State Fermentation Conditions

Nirmalkar Vaishali*, Shaikh Asfiya and Ansari Raana

Department of Botany, K.M.E Society's G.M.Momin Women's College, Bhiwandi, Dist. Thane (MS), India – 421302
*Corresponding author email: vaishu_p2025@yahooo.co.in

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Nirmalkar Vaishali, Shaikh Asfiya and Ansari Raana (2016) Production of Fungal Cellulase under Solid State Fermentation Conditions, Int. J.of. Life Sciences, Special Issue, A7:19-24.

Acknowledgement
The corresponding author is thankful to the Principal Dr. M.J. Kolet and Management of the College for supporting and giving necessary facilities for the research work. Also special thanks to Dr. Lal Saheb Yadav, Assistant Professor, CHM College, Ulhasnagar for helping in the identification process of the fungi.

ABSTRACT

Filamentous fungi are important due to their high enzyme production potential. Many enzymes produced by fungi have been related to biotechnological applications in several industrial sectors. The purpose of this study was to isolate filamentous fungi from different sources and to screen for cellulase production potential. This study was focused on the evaluation of cellulolytic fungi isolated from different soil samples collected from different Textile Sizing sites of Bhiwandi city. Amongst the twenty isolated fungi, two cellulase producing isolates identified as *P. citrinum* and *A. flavus* were subjected for Solid State Fermentation. SSF was carried out using two substrates namely sugarcane bagasse and sweet lime bagasse. Both the fungi showed maximum cellulase activities at 72 hours of incubation. *P. citrinum* was found to be producing cellulase higher than *A. flavus* using both the substrates. Isolation of cellulase producing fungi from Textile Sizing Site will help in the bioremediation of sizing processing wastes' environment.

Key words: Cellulase, Solid State Fermentation, *P.citrinum, A.flavus*

INTRODUCTION

Enzymes are globular proteins and are important biocatalyst for various industrial and biotechnological purposes and produced by microorganisms, animals and plants. Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature (Sri Lakshmi and Narasimha, 2012). Nowadays, the use of enzymes in industrial sector is increasing due to increase in the number of industries especially food, beverages, textile, leather and paper industries (Ogbonna et al., 2014). Enzymes can work in many adverse conditions compared to chemical catalyst. Microorganisms are preferred as a source of enzyme because of their short life span, high productivity rate, cost effective, and also free of harmful chemicals that are found in enzyme from plant and animal source (Khatri et al., 2015). Fifty percent of available enzymes are originated from fungi and yeast; 35 % from bacteria, while the remaining 15 % are either of plant or animal origin (Khatri et al., 2015). Fungi constitute a group of micro-

organisms that are widely distributed in environment especially in soil (P. Lakshmi Narasimha, 2014). Filamentous fungi are important organisms for production of useful enzymes and biological active secondary metabolites due to extracellular release of various enzymes like amylases, cellulases, xylanases, etc. These fungi produce high levels of polysaccharide degrading enzymes and are frequently used for the production of industrial enzymes. Therefore, there is a growing interest in assessing soil biodiversity and its biological functioning (Barrios E, 2007).

Cellulases

Cellulases are enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze hydrolysis of cellulose. Degradation of the cellulosic materials is achieved chemically, enzymatically or by the combination of both chemical and enzymatic methods (P. Lakshmi Narasimha, 2014). Cellulases bring about the hydrolysis of cellulose, a homopolymer of -1,4 linked glucose units that comprises amorphous and crystalline regions, by synergistic action of its constituent enzymes. Cellulases are group of extracellular enzymes commonly employed in many industries for the hydrolysis of cellulolytic material. Bioconversion of cellulosic biomass to fermentable sugars through biocatalyst cellulases derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce the use of fossil fuels and reduce environmental pollution (Lynd et al., 1999). Cellulases have been used for several years in food processing, feed preparation, waste-water treatment, detergent formulation, textile production and other areas. Additional potential applications include the production of wine, beer and fruit juice. Adverse spectrum of extensively studied mesophilic fungi degrades organic material aerobically (Falcon MA et al., 1995). Nearly, all the fungi that have been reported for the production of cellulases are mesophilic fungi and the best known cellulase producers include *Trichoderma sp., Aspergillus sp., Acremonium sp., Penicillium sp., Rhizopus sp., F. solani and Chaetomium sp.,* among other mesophiles (Kuzmanova S et al., 1991; Teeri T and Koivula A, 1995; Bhat M and Bhat S, 1997; Schulein M, 1997 and P. Lakshmi Narasimha et al., 2014). Cellulase enzymes have a wide range of applications, economic importance in agriculture, biotechnology, and bioenergy (Nathan VK et al., 2014; Phitsuwan P et al., 2012).

Solid State Fermentation

Solid-state fermentation is traditionally defined as those processes in which microbial growth and products formation occur on the surfaces of solid substrates in the mere absence of free water. Due to this low amount of water available in solid-state bioprocessing, the class of microorganisms that are most commonly used is fungi (Martin et al., 2004). SSF is suitable for fungi growth because of its low moisture content and permitting of the penetration of fungi mycelium through the solid substrates. This technology usually uses agro-industrial waste as support and carbon source for production of various value added products, such as single cell protein, industrial enzymes, secondary metabolites and fine chemicals (Grover et al., 2013). Several agro-industrial waste and by-products such as orange bagasse, sugar cane bagasse, wheat bran (Martin et al., 2004) and other food processing waste are effective substrates for depolymerizing enzyme production by solid-state fermentation. Solid-state fermentations are simple, low cost, and provide high yields of appropriate enzymes. Solid-state fermentation has gained renewed interest and fresh attention from researchers owing to its importance in recent developments in biomass energy conservation, in solid waste treatment and in its application to produce secondary metabolites (Elliaiah P et al., 2002; Grover et al., 2013).

Taking all the above facts into consideration the present research work was aimed to study various fungal isolates isolated from textile sizing site for the production of cellulase using cheaper substrates under the solid state fermentation conditions.

MATERIALS AND METHODS

Selection of sample sites

Five different textile sizing sites of Bhiwandi city were selected for the sample collection i.e Ram Raheem Sizing, Moksh Sizing, Master Sizing, Kulsum Sizing, Indra Sizing.

Collection of soil samples

The samples were collected from 6 different spots in each site in zip lock polythene bags. Almost 5-6 soil samples were taken from each sizing industries. The soil sample was mixed well and processed next day.

Isolation Of fungi

Fungal colonies were isolated from soil samples by serial dilution method where in SDA (Sabouraud dextrose agar) media was prepared, autoclaved and poured in sterile petriplates. 50µl of soil samples diluted upto 10-5 dilutions were spread on respective solidified SDA plates with the help of sterile spreader. The inoculated petriplates were incubated at 28°C for 48 h. 20 different fungal isolates differentiated on the basis of physical characteristics obtained after incubation were selected for the further processes. The isolates were further inoculated on SDA plates by point inoculation and incubated at 28°C for 48 h in order to obtain pure fungal cultures (Khan and Yadav, 2011).

Primary Screening of Fungal Isolates for Cellulase Production

The isolated fungal cultures were screened for their ability to produce cellulase. Czapek-Dox medium used in this method contained (g/l): sucrose – 30, $NaNO_3$ – 2, K_2HPO_4 - 1, $MgSO_4$ – 0.05, KCl – 0.5, $FeSO_4$ – 0.01, carboxyl- methyl cellulose – 1%, Agar agar – 20 g. pH of the medium was adjusted to 5. After autoclaving at 121°C and 15 lbs pressure the medium was poured into petri plates and allowed to solidify. The plates were incubated at room temperature (28 ± 2 °C) for three days to allow fungal growth, then again incubated for 18 h at room temperature for cellulase activity. After incubation, 10 ml of 1% Congo-red staining solution was added to the plates. The Congo - red staining solution was then discarded and then de-stained with 10 ml of 1 N NaOH solution for 15 minutes. Finally 1 N NaOH was also discarded and the staining of the plates was analyzed by noticing the formation of yellow zones around the fungal growth **(**Sri Lakshmi A. and G. Narasimha., 2012). The diameter of zone of decolorization around each colony was measured. The fungal colony showing largest zone of decolorization was selected for cellulase production.

Identification of isolates

The isolated strains were carefully identified by morphological characteristics include color of the colony and growth pattern studies, as well as their vegetative and reproductive structures observed under the microscope (M. Charitha Devi and M. Sunil Kumar., 2012). Among the characteristics used were colonial characteristics such as surface appearance, texture and colour of the colonies. In addition, microscopy revealed vegetative mycelium including presence or absence of cross-walls, diameter of hyphae. Mycological identification keys and taxonomic descriptions (Ogbonna Cet al., 2014). Appropriate references were made using monograph and morphological characters (Pitt John, 1988; Raper and Thom, 1949).

Production of cellulases by Solid State Fermentation using Cheap Substrates

Cheap substrates used were Sugarcane bagasse and Sweet lime bagasse. Both the substrates were collected from the local market of Bhiwandi and were washed thoroughly with water twice and thrice to remove excess dirt and fungal spores. After that these substrates were sundried for 4 days. Dried substrates were cut in small pieces and finely grinded into powder.

Sugarcane Bagasse Media:(hence forth referred to as **S1**)

Six gram of sugarcane bagasses were transferred into 250 ml cotton plugged conical flasks (13) and moistened with 40ml of defined Media (Murashige and Skoog, 1962), mixed and sterilized for 15 minute in an autoclave and cooled thereafter at room temperature.

Sweet lime Bagasse Media:(hence forth referred to as **S2**)

Six gram of sugarcane bagasses were transferred into 250 ml cotton plugged conical flasks (13) and moistened with 20ml of defined Media (Murashige and Skoog,1962), mixed and sterilized for 15 minute in an autoclave and cooled thereafter at room temperature.

Solid state fermentation

The highly productive isolates in primary screening were selected. Spore suspension was prepared by dipping 4mm disc of fully grown fungal isolates in 10ml of sterile distilled water.1ml of spore suspension of each isolates was added in both the media under the sterile conditions. The cultures were incubated at 28ºC and were harvested at the gap of 24 h for 8 consecutive days.

Extraction of Crude Enzyme

Crude enzyme was extracted from fermented media by adding 50ml of 100mM Tris buffer pH 6.2, agitating the flask in shaker at 180rpm for 1h, the mixture was filtered and centrifuged at 8000 rpm at 4ºC for 10 min. The supernatant was collected and treated as crude enzyme (Khan and Yadav, 2011).

RESULTS AND DISCUSSION

Isolation of Fungi

The soil samples were collected from sizing industry sites, following spread plate technique. Twenty different fungal colonies were obtained and pure culture of these isolates was obtained for the further processes.

Primary Screening of Isolates for Cellulase Production:

All the 20 pure cultures were screened for their Cellulase producing potential. The isolates were subjected to screening procedure on Carboxy Methyl Cellulose (CMC) plates and incubated for 3 days. After completion of incubation period, varied degree of cellulose utilization was seen after flooding with Congo red solution and observed for zone of hydrolysis. The two isolates which gave maximum zone were selected for fermentation (Table. 1).

Identification of the Isolate Showing Maximum Hydrolysis

Based on morphological and microscopical studies, the isolate no. 9 was identified as *Penicillium citrinum* and isolate no. 1 was identified as *Aspergillus flavus.*

Solid State Fermentation Using Cheap Substrates

The cultures showing maximum zone during screening and identified as *A.flavus* and *P. citrinum* were inoculated in SSF flasks containing different substrates.

CELLULASES

The local isolate of *A. flavus* and *P. citrinum* in the present study were able to efficiently utilize sugarcane bagasse and sweet lime bagasse for production of cellulase enzyme by solid state fermentation method.

In *P. citrinum* the amount of extracellular cellulase in sugarcane bagasse (S1) gradually increased since 48 h. Maximum secretion of extracellular cellulose enzyme was found to be 42.89 mg/ml equivalent of glucose at 72 h followed by 144 h and expressed as 36.12 mg/ml equivalent of glucose. Least secretion of extracellular cellulase was recorded at 24 h of incubation (Fig.1). When S2 was used as growth medium, cellulase activity was maximum on the second and third day of incubation period and expressed as 23.93mg/ml equivalent of glucose and 16.84mg/ml equivalent of glucose respectively followed by a rapid decline. Least activity was observed at first day of incubation period and expressed as 0.25mg/ml equivalent of glucose (Fig.2).

For *A. flavus* the cellulase activity in S1 gradually increased at 48h of incubation. The content of extracellular cellulase on sugarcane bagasse was maximum with 37.47mg/ml equivalent of glucose at 72 h followed by 144 h and expressed as 31.83mg/ml equivalent of glucose. Minimum activity was found at 24 h of incubation period (Fig.3). Highest amount of cellulase activity for S2 was recorded with 15.46 mg/ml equivalent of glucose at 120 h of incubation period followed by 72 h of incubation and expressed as 14.21mg/ml equivalent of glucose. Least activity was observed at first day of incubation (Fig.4).

Both the fungi showed maximum cellulase activity at 72 h after which decline was observed in both the substrates. But again rise was observed in the later period of incubation i.e at 144 h. The production was highest done by *P.citrinum* when S1 is used as a substrate at 72 h. *P.citrinum* is found to be producing cellulase higher than *A. flavus* using S1 and S2 both. It is reported earlier that cellulase activity is exhibited maximum at 3rd day of incubation (Lakshmi, 2014). Also in many of the earlier reports *Aspergillus sp*. are found to be the potential cellulase producers. However, it would be concluded that sugarcane bagasse served the best substrate followed by sweet lime bagasse in supporting maximum production. And among these two fungi *P. citrinum* was found to be the best for the cellulase production than that of *A. flavus* for both S1and S2 substrates. Fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular. Even though there are many reports on fungi producing cellulases (Shin et al. 2000), only a few have proved high activities for commercial success (Sri Lakshmi A. and G. Narasimh., 2012). A wide range of *Aspergillus sp.* have been identified to possess all components of cellulases complex (Vries and Visser, 2001).

Large demand of cellulases has increased their prices to a large extent and the major reason is the cost of substrate, and fermentation procedure. It is the need of the time to search for cheaper substrates and reduced fermentation cost so that the production cost can be reduced to a large extent. The present investigation also focused on the same and hence search for substrates with zero cost was attempted.

Table 1: Primary Screening for Cellulase Production

Isolate no.	Result	Isolate no.	Result
1	++++	11	-
2	-	12	++
3	-	13	-
4	++	14	+++
5	-	15	++
6	+++	16	++
7	++	17	-
8	-	18	-
9	++++	19	-
10	++	20	-

*Maximum zone of hydrolysis: ++++, each + sign shows level of rise in hydrolysis

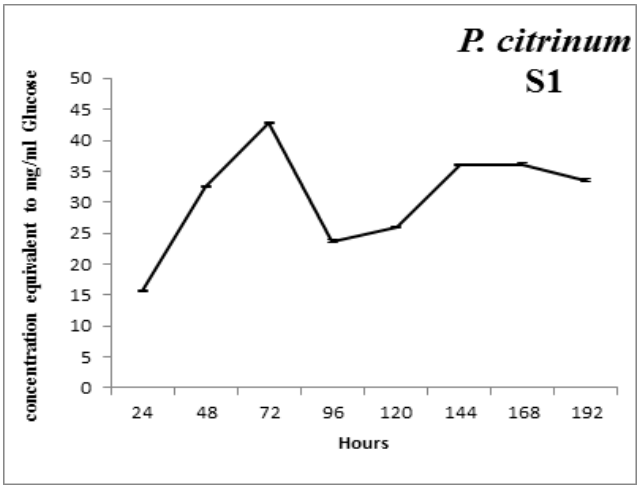


Fig.1: Cellulase activity of *Penicillium citrinum* in Subtrate1

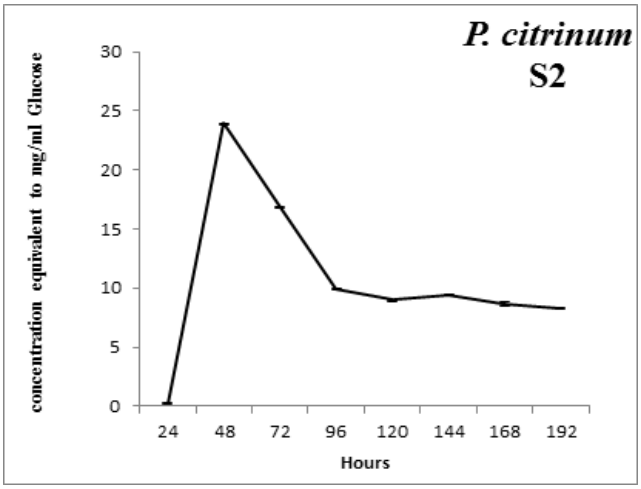


Fig.2: Cellulase activity of *Penicillium citrinum* in Subtrate2

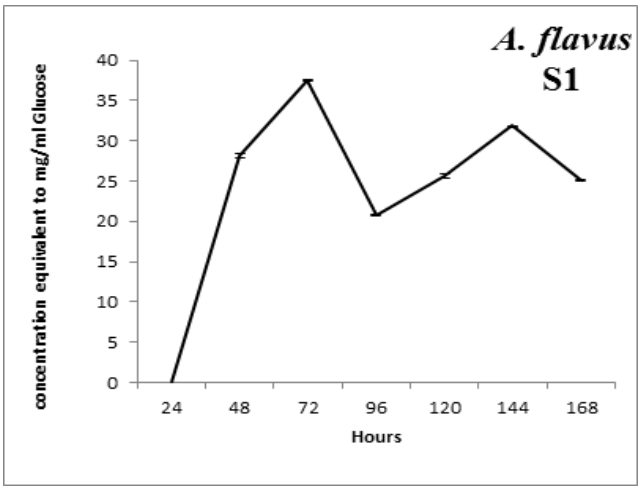


Fig.3: Cellulase activity of *Aspergillus flavus* in Subtrate1

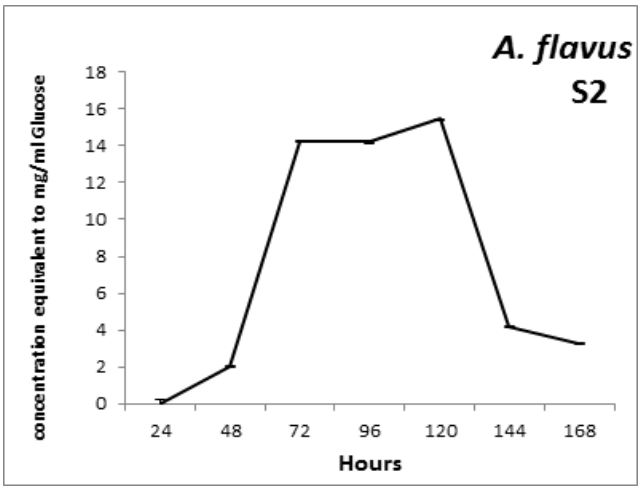


Fig. 4: Cellulase activity of *Aspergillus flavus* in Subtrate2

REFERENCES

Barrios E (2007) Soil biota, ecosystem services and land productivity. Ecological Economics. 64(2): 269 285.

Bhat M and Bhat S (1997) Cellulose degrading enzymes and potential industrial applications. Biotechnology Advances. 15(3-4): 583-620.

Elliaiah P, Adinarayana K, Bhavani Y, Padmaja P and Srinivasula B (2002) Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus species*. Process Biochem 38: 615-20.

Falcon MA, Rodriguez A and Carnicero A (1995) Isolation of microorganism with lignin transformation potential from soil of Tenerife Island. Soil Biology Biochemistry, 27(2): 121-126.

Grover Anshikaa, Arora Maninder and Sarao Loveleen Kaur (2013) Production of fungal amylase and cellulase enzymes via solid state fermentation using *Aspergillus oryzae* and *Trichoderma reesei*. International Journal of Advancements in Research & Technology, Volume 2, Issue 8.

Khan Jahir Alam and Yadav Sachin Kumar (2011) Production of alpha amylases by *Aspergillus niger* using cheaper substances employing solid state fermentation. International J. Plant, Animal Environ. Sci. 1(3):100-108.

Khatri Bhim Prakash, Bhattarai Tribikram, Shrestha Sangita and Maharjan Jyoti (2015) Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolated from Manaslu Conservation Area, Gorkha, Nepal. Springer Plus 4: 488.

Kuzmanova S, Vandeska E and Dimitrovski A (1991) Production of mycelial protein and cellulolytic enzymes from food waste. Journal of Industrial Microbiology. 7(4): 257-261.

Lynd LR, Wyman CE, Gerngross TU, (1999) Biochemical engineering. Biotechnology Progress 15: 777-793.

Charitha Devi M and Sunil Kumar M (2012) Production, Optimization and Partial purification of Cellulase by *Aspergillus niger* fermented with paper and timber sawmill industrial wastes. J. Microbiol. Biotech. Res., 2 (1):120-128.

Martin Natalia, de Souza Simone, da Silva Regina, Roberto and Gomes Eleni (2004) Pectinase Production by Fungal Strains in Solid-State Fermentation Using Agro-Industrial Bioproduct. Brazilian Archives of Biology and Technology, 47(5): 813-819.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures; Physiol Plant; 15: 473-479.

Nathan VK, Rani ME, Rathinasamy G, Dhiraviam KN and Jayavel S (2014) Process optimization and production kinetics for cellulase production by *Trichoderma viride* VKF3. Springer plus. 3: 92.

Ogbonna CN, Okpokwu N, Okafor CU and Onyia CE (2014) Isolation and screening of amylase producing fungi obtained from garri processing site. Int. J. Biotechnol. Food Sci. 2(5): 88-93.

Lakshmi P Narasimha R, Suresh Babu B, Radhaiah A and Sreeramulu A (2014) Screening, Identification and Isolation of Cellulolytic fungi from soils of Chittoor District, India. *Int.J.Curr.Microbiol. App.Sc*i., 3(7): 761-771.

Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu KL and Ratanakhanokchai K (2013) Potential applications of cellulases in Agriculture, *Biotechnology, and Bioenergy Folia Microbiologica*, 58(2):163-176.

Pitt John I (1988) A laboratory guide to common *Penicillium species*. 2nd ed. ISBN 0643048375, North Ryde, N.S.W. CSIRO, Division of Food Processing, 1988. p. 180-183.

Raper KB, Thom C (1949) A manual of the penicillia. The Williams & Wilkins Company, Baltimore.

Schulein M (1997) Enzymatic properties of cellulases from Humicolainsolens. *Journal of Biotechnology*. 57(1-3): 71-81.

Sri Lakshmi A and Narasimha G (2012) Production of cellulases by fungal cultures isolated from forest litter soil. Ann. For. Res. 55(1): 85-92.

Teeri T and Koivula A (1995) Cellulose degradation by native and engineered fungal cellulases. Carbohydr. Eur., 12: 28-33.

Vries RP, Visser J (2001) *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharide. *Microbiology and Molecular Biology Reviews*, 65: 497-522.

RESEARCH ARTICLE

Comparative Account of Antifungal Activity of two weeds

Tutakne Neeraja* and Golatkar Vikas

Assistant Professor, Department of Botany, SIES College, Sion (W) Mumbai 400022, India.
*Corresponding author Email: neerajaba@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Tutakne Neeraja and Golatkar Vikas (2016)Comparative Account of Antifungal Activity of two weeds, Int. J.of. Life Sciences, Special Issue, A7:25-32.

Acknowledgements:
Authors are grateful to Principal, SIES College Sion (W), Mumbai and Principal, D. G. Ruparel College, Matunga, Mumbai, for providing support and all the facilities. Thanks are also due to Librarians, D. G. Ruparel College, Matunga, Mumbai; Haffkin Institute, Mumbai, and UICT, Mumbai for providing library facilities. The authors would also like to acknowledge the faculty members, Department of Microbiology, Bhavans College, Andheri, Mumbai, for their help in providing the authentic bacterial cultures.

ABSTRACT

The weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. were subjected to different solvent extractions to prepare crude extracts. The antifungal activity of these crude extracts was tested against two plant pathogenic fungi, viz., *Alternaria alternata* and *Fusarium oxysporum* and also against three animal pathogenic (dermatophytic) fungi, viz., *Candida albicans*, *Microsporum gypsium* and *Trichophyton mentagrophytus*. All the extracts efficiently inhibited the growth of all the fungi understudy.

Keywords: Antifungal activity, inhibition zone, plant pathogenic fungi, animal pathogenic fungi, dermatophytes

INTRODUCTION

The term 'Weed' refers to any wild harmful unwanted plant, that grows on cultivated land and not only compete with the crop plants for light, space, moisture and soil nutrients but also harbours harmful insect pests and diseases. These weeds interfere with agricultural operations, increase labour, add to the cost of crop cultivation, impair the quality of crops and finally reduce the crop yields. The main problem of the environmentalists and agriculturists is, how to control the rapid expansion of the weeds across the region. The weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. are notorious shrubs with strong odour. They have received the status of world's worst weeds because of their invasiveness, potential for spread and economic and environmental impacts.

As per ancient Indian literature, however, every plant on this earth possesses some useful medicinal properties for human beings, animals and also for other plants (Oudhia, 1999 a, b and c). It is sheer the ignorance of human beings that they have classified some plants as useful and others as weeds.

The use of higher plants and their extracts to treat infections is an age-old practice. Ethnopharmacologists, botanists, microbiologists and natural-product chemists are combing the Earth for phytochemicals which could be developed for the treatment of infectious diseases (Marjorie, 1999).

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Patel *et al.,* 2007). Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attribute. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also been increased. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized (Patel *et al.,* 2007). If this medicinal (antifungal) property resides in a weed then that will be of great advantage. Therefore, in the present investigations, the weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. were screened to explore their antifungal potentials.

The extracts of *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. prepared in petroleum ether, chloroform, methanol and water were screened against two plant pathogenic fungi, viz., *Alternaria alternata* and *Fusarium oxysporum* and also against three animal pathogenic (dermatophytic) fungi, viz., *Candida albicans*, *Microsporum gypsium* and *Trichophyton mentagrophytus*.

MATERIALS AND METHODS

Collection of Plant material:

Fresh wild samples of the weeds, *Lantana camara* Linn. (Yellow-Orange variety) and *Parthenium hysterophorus* Linn. were collected in monsoon by conducting the field excursions to different parts of Mumbai and Thane districts in the months of June to September.

Extraction of weeds:

The cleaned, washed aerial parts (stem, leaves, flowers and fruits) of the weeds were dried at 45^0C in hot air oven. The dried aerial parts of both these weeds were homogenized separately in the mixer to get fine powder of 30 mesh (500µm) particle size. The extractions of both the weed powders in petroleum ether, chloroform and methanol were carried out separately by Soxhlet's extraction method while aqueous extraction was carried out by hot decoction method.

Soxhlet's Extraction:

50g of weed powder was wrapped in the thimble of filter paper and placed in Soxhlet extractor along with the respective solvent for extraction. The powder : solvent proportion was 1:10. The extraction was continued till the solvent became colourless. The extracts so obtained were concentrated; collected in glass vials and dried completely at 40^0C in hot air oven. The glass vials containing extracts were given identification codes and stored in a refrigerator at 4^0 C.

Aqueous Extraction:

10g of weed powder was mixed with distilled water in the proportion of 1:10. The mixture was boiled for two hours. It was then filtered through Whatman's filter paper no. 1 and the filtrate was concentrated to achieve the consistency of thick paste. The extract was collected in glass vial; given identification code and stored in refrigerator at 4^0C. The identification codes given to the extracts, before storing, were as follows:

Solvent used for extraction	**Identification code given to the extracts**	
	Lantana camara	***Parthenium hysterophorus***
Petroleum ether	LP	PP
Chloroform	LC	PC
Methanol	LM	PM
Water	LW	PW

All the extracts except water extract, were insoluble in water hence there was a need to dissolve them in a suitable vehicle to carry out further experiments. Tween 80 (Polysorbate 80) was used as a vehicle (not more than 1%) and six different concentrations viz., 5%, 10%, 20%, 40%, 80% and 100% of each extract were prepared. Different concentrations of water extract were made in similar manner but without using Tween 80.

Screening of weed extracts for antifungal activity:

a. Standardization of Fungal inoculum:

Stock cultures of fungi under study were maintained at 4°C on PDA medium. Each fungal culture was standardized separately.

***For animal pathogenic (dermatophytic) fungi under study*:**

Three dermatophytic fungi (animal pathogens) were standardized by the standard protocols given by Clinical Laboratory Standard Institute (NCCLS, 1998).

The standardized inoculum values for these fungi were as follows:

Fungi under study	Standardized Values of the Inocula
▪ *Candida albicans*	10^{-3}
▪ *Microsporum gypsium*	10^{-4}
▪ *Trichophyton mentagrophytus*	10^{-3}

For plant pathogenic fungi under study:

The plant pathogenic fungi viz; *Alternaria alternata* and *Fusarium oxysporum*, could not form uniform spore suspensions, hence, could not be standardized. Therefore, fungal discs of uniform size, cut out with the help of sterilized cork borer having 4.5mm diameter, from the eight days old pure culture of the fungi, were used for inoculation.

b. Testing Antifungal activity:

Each weed-extract was screened separately at six different concentration levels against five fungi under study.

For animal pathogenic (dermatophytic) fungi under study:

Fresh standard inoculum of each dermatophyte under study was prepared separately and used for testing the antifungal activity as follows:

0.1mL of standardized fungal inocula were added to the separate flasks containing 20mL sterilized PDA having moderate temperature and mixed well. The plates were poured and allowed to solidify. The well was scooped out at the centre of each solidified plate with the help of sterilized cork borer having the diameter of 1cm.

Six different concentrations viz; 5%, 10%, 20%, 40%, 80%, 100% of the weed-extract were prepared separately. 100µl of each concentration was added to the wells of six different petriplates indicating the concentrations. Control plate for the dermatophytic fungus was set in similar way except that the extract was replaced by adding 1per cent Tween 80. The plates were kept for incubation at room temperature for two days for *Candida albicans* and four days for the remaining two dermatophytic fungi. The experiments were carried out in triplicates.

At the end of incubation period, the zone of inhibition, if present, was observed and measured. To observe the persistence of the activity, the plates were incubated further for next two days and the observations were noted. Photographs were taken and the results were tabulated.

For plant pathogenic fungi under study:

For plant pathogenic fungi under study, flasks containing 20mL of sterilized PDA were prepared. The plates were poured and allowed to solidify. The wells were dug out in similar manner as in case of dermatophytic fungi. The plates were inoculated from three sides, equidistant from the well, by using fungal discs of uniform size (4.5mm diameter) cut out from of eight days old pure cultures of the fungus.

Six different concentrations viz; 5%, 10%, 20%, 40%, 80%, 100% of the weed-extracts were prepared separately. 100µl of each concentration was added to the wells of six different petriplates indicating the concentrations. Control plate for the plant pathogenic fungus was set in similar way except that the extract was replaced by adding 1 per cent Tween 80. The plates were kept for incubation at room temperature for eight days. The experiments were carried out in triplicates.

At the end of incubation period, the zone of inhibition, if present, was observed and measured. To observe the persistence of the activity, the plates were incubated further for next two days and the observations were noted and the results were tabulated.

RESULTS AND DISCUSSION

In general, both the weeds under study, viz., Lantana and Parthenium exhibited wide range of antifungal activity and proved to be effective antifungal agents. Similar reports are available in which antimicrobial activities of different medicinal plants such as *Gyanandropsis gynandra*, *Buchholzia coriaceae*, *Sanguisorba officinalis*, *Tussilago farfara*, *Vernonia amygdalina*, *Carum copticam*, *Artemisia afra*, *Backhousia citriodora*, *Callistemone citrinus*, Clove, *Jambolan*, Thyme, Pomgranate, Rosmerry, Guava, Lemongress, Papermint, Bay, Oregano, *Mikania micrantha*, *Ocimum gratissimum*, *Xylopia aethiopica*, *Acalypha* fruticosa, *Peltophorum pterocarpum*, *Cassia auriculata*, *Momordica charantia*, *Caesalpinia pulcherrima, Citrus aurantifolia*, *Parkia biglobosa,*

Parkia bicolor, *Catharanthus roseus*, *Polyalthia longifolia* etc. have been reported against the same microorganisms under study (Ajaiyeoba, 2000; Dagmar *et al.*, 2003; Mintesnot and Mogessie, 1999; Cock *et al.*, 2008; Gislene *et al.*, 2000; Hammer *et al.,* 1999; Facey *et al.,* 1999; Ijeh *et al.*, 2005; Veeramuthu *et al.*, 2006; Martinez *et al.*, 1996; Parekh and Chanda 2007; Onyeagba *et al.*, 2004; Ajaiyeoba, 2002; Nayak *et al.*, 2006 and Saha *et al.*, 2005).

Table 1: Inhibition Zones exhibiting antifungal activity of Lantana and Parthenium extracts

Antifungal activity of Petroleum ether extract of Lantana (LP)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	0.3	0.5	0.5
M. gypsium	**x**	**x**	**x**	1.0	1.3	1.5	1.5
T. mentagrophytus	**x**	**x**	**x**	**x**	1.3	1.5	1.5
A. alternate	**x**	**x**	**x**	**x**	0.5	0.6	0.6
F. oxysporum	**x**	**x**	**x**	**x**	0.7	0.7	0.9
Antifungal activity of Chloroform extract of Lantana (LC)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	0.5	0.6	0.6
M. gypsium	**x**	**x**	**x**	1.2	1.4	1.5	1.5
T. mentagrophytus	**x**	**x**	**x**	1.5	1.5	1.6	1.6
A. alternate	**x**	**x**	**x**	**x**	0.5	0.5	0.6
F. oxysporum	**x**	**x**	**x**	**x**	0.8	0.9	0.9
Antifungal activity of Methanol extract of Lantana (LM)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	0.7	1.0	1.0
M. gypsium	**x**	**x**	2.5	2.6	2.6	2.8	2.9
T. mentagrophytus	**x**	**x**	**x**	2.5	2.6	2.6	2.7
A. alternate	**x**	**x**	0.7	0.7	0.7	0.8	0.9
F. oxysporum	**x**	**x**	**x**	0.6	0.7	0.8	0.8
Antifungal activity of Water extract of Lantana (LW)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	**x**	1.0	1.2
M. gypsium	**x**	**x**	**x**	**x**	1.8	1.8	1.9
T. mentagrophytus	**x**	**x**	**x**	**x**	1.5	1.6	1.6
A. alternate	**x**	**x**	**x**	**x**	0.6	0.8	0.8
F. oxysporum	**x**	**x**	**x**	**x**	0.7	0.9	1.0
Antifungal activity of Petroleum ether extract of Parthenium (PP)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	1.2	1.3	1.3
M. gypsium	**x**	**x**	**x**	**x**	1.0	1.4	1.4
T. mentagrophytus	**x**	**x**	**x**	1.3	1.3	1.5	1.5
A. alternate	**x**	**x**	**x**	**x**	**x**	**x**	**x**
F. oxysporum	**x**	**x**	**x**	**x**	**x**	**x**	**x**

Table 1: Continued…

Antifungal activity of Chloroform extract of Parthenium (PC)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	1.2	1.4	1.5
M. gypsium	**x**	**x**	**x**	1.7	1.8	1.9	1.9
T. mentagrophytus	**x**	**x**	**x**	2.1	2.2	2.4	2.5
A. alternate	**x**	0.5	0.6	0.6	0.8	0.8	0.8
F. oxysporum	**x**	0.7	0.7	0.7	0.8	0.9	0.9
Antifungal activity of Methanol extract of Parthenium (PM)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	1.7	1.7	1.8	1.9
M. gypsium	**x**	2.0	2.3	2.3	2.5	2.5	2.5
T. mentagrophytus	**x**	2.0	2.2	2.3	2.3	2.5	2.6
A. alternate	**x**	**x**	**x**	**X**	0.5	0.6	0.7
F. oxysporum	**x**	**x**	**x**	**X**	0.8	0.8	0.9
Antifungal activity of Water extract of Parthenium (PW)							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
C. albicans	**x**	**x**	**x**	**x**	1.3	1.4	1.4
M. gypsium	**x**	**x**	**x**	1.0	1.4	1.5	1.5
T. mentagrophytus	**x**	1.0	1.0	1.1	1.1	1.3	1.4
A. alternate	**x**	**x**	**x**	**x**	0.5	0.5	0.6
F. oxysporum	**x**	**x**	**x**	0.8	0.9	0.9	1.0
(**x** – No inhibition zone and hence No antifungal activity)							

Table 2: Best antifungal concentrations of Lantana and Parthenium extracts and their persistence

Microorganisms Tested	**Lantana Extracts**											
	LP			LC			LM			LW		
	C	Z	D	C	Z	D	C	Z	D	C	Z	D
C. albicans	40%	0.3	3	40%	0.5	4	40%	0.7	5	80%	1.0	5
M. gypsium	20%	1.3	5	20%	1.2	5	10%	2.5	7	40%	1.8	7
T. mentagrophytus	40%	1.3	5	20%	1.5	5	20%	2.5	7	40%	1.5	7
A. alternate	40%	0.5	7	40%	0.5	7	10%	0.7	8	40%	0.6	8
F. oxysporum	40%	0.7	7	40%	0.8	7	20%	0.6	8	40%	0.7	8
	Parthenium Extracts											
	PP			PC			PM			PW		
	C	Z	D	C	Z	D	C	Z	D	C	Z	D
C. albicans	40%	1.2	3	40%	1.2	4	20%	1.7	5	40%	1.3	5
M. gypsium	40%	1.0	6	20%	1.7	6	5%	2.0	7	20%	1.0	7
T. mentagrophytus	20%	1.3	6	20%	2.1	6	5%	2.0	7	5%	1.0	7
A. alternate	NA			5%	0.5	7	40%	0.5	8	40%	0.5	8
F. oxysporum	NA			5%	0.7	7	40%	0.8	8	20%	0.8	8

LP: Lantana – Petroleum-ether, **LC**: Lantana – Chloroform, **LM**: Lantana – Methanol, **LW**: Lantana – Water, **PP**: Parthenium – Petroleum-ether, **PC**: Parthenium – Chloroform, **PM**: Parthenium – Methanol, **PW**: Parthenium – Water, **C**: Best antifungal concentration, **Z**: inhibition zone produced (cm), **D**: Number of days for which activity persisted, **NA**: No Activity.

Petroleum ether, chloroform, methanol and water extracts of Lantana were effective in controlling the growth of all the dermatophytic fungi under study viz., *Candida albicans*, *Microsporum gypsium* and *Trichophyton mentagrophytus*. This is in accordance with Dabur *et al.* (2007); Prashantkumar *et al.* (2006) and Bhakuni *et al.* (1969). However, some of the reports show that *Lantana camara* did not exhibit antifungal activity against *C. albicans* (Ahmad and Beg, 2001 and Rajakaruna *et al.*, 2002). All the extracts of Lantana possessed antifungal activity against both the plant pathogenic fungi under study viz., *Alternaria alternata* and *Fusarium oxysporum*. These findings are in line with the observations made by Mamatha and Ravishankar Rai (2004), Tiwari *et al.* (2004) and Bhatnagar *et al.* (2004). Besides these, there are also reports available where in antimicrobial activity of Lantana oil is reported (Deena and Thoppil 2000; Tania *et al.*, 2000 and Juliani *et al.*, 2002).

The survey of literature revealed that antifungal activity of Parthenium is well documented as compared to its antibacterial activity. Petroleum ether, chloroform, methanol and water extracts of Parthenium proved to be effective against all the dermatophytic fungi under study. These findings are in accordance with Rai *et al.* (1999) and Dhawan *et al.* (1980).

All the extracts, expect the petroleum ether extract of Parthenium restricted the growth of plant pathogenic fungi viz., *Alternaria alternata* and *Fusarium oxysporum*. Similar kinds of observations were also reported by Rai *et al.* (1999); however, Bajwa *et al.* (2004), obtained surprising results by testing effect of aqueous extract of Parthenium shoot on *Drechslera hawaiiensis*, *Alternaria alternata*, *Fusarium moniliformae*. According to their findings, lower concentrations of the extract (10 to 50 per cent) proved to be antifungal while that of higher concentrations like 60, 70 per cent stimulated the fungal biomass production.

There are other evidences showing antifungal activity of Parthenium against other plant pathogenic fungi which further confirm the antifungal potential of Parthenium (Polyanna *et al.*, 2008; Bajwa *et al.*, 2003; Sinha *et al.*, 2004; Hsieh *et al.*, 2005 and Patel *et al.*, 2007). Besides these, there is also a report on antimycotic activity of Parthenium oil in which the oil is proved to be the best antimycotic agent against *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Microsporum* sp., *Trichophyton* sp. etc. (Sharma and Sharma, 1989).

Over all, all the extracts of Lantana and Parthenium except petroleum ether extracts of Parthenium inhibited the growth of 100 per cent of the fungi tested. Petroleum ether extract of Parthenium found to be least effective amongst all as it inhibited the growth of only 60 per cent of the fungi tested.

The dermatophytic fungi tested found to be more susceptible than the plant pathogenic fungi tested. This variation in susceptibility of the fungi tested may be due to varying permeability of their mycelial wall as well as spore wall.

It was also observed that the diameter of inhibition zone may increase with the increase in concentrations which may be due to increase in the antifungal principles of the extract at higher concentrations. However, after certain limit, the diameter of inhibition zone remains constant. With very few exceptions, almost in all cases the inhibition zone produced at 80 per cent is similar to that of 100 per cent. This may be because of the reduced fluidity of the extracts and thereby that of the active antifungal principle of the extract at higher concentrations. Hence, even being more potent, the highest concentrations of the extracts can not produce larger inhibition zones but gives the inhibition zones similar to that of the immediate lower concentrations.

It was also noted that though the same concentration of the extract is used against different fungi, the diameter of the inhibition zone varies with respect to the fungi tested. This suggests that different antifungal constituents of the same concentration of the extract may be responsible to arrest the growth of different fungi. The difference in the qualitative and quantitative levels of the antifungal constituents of the extracts may be responsible to produce difference in the inhibitory effect.

The persistence of the antifungal activity is checked in terms of the number of days for which the activity persisted. It was observed that the persistence of the antifungal activity of the extracts was varying depending upon the fungi tested. The minimum antifungal concentration of the extract which produced large inhibition zone and remained active

for maximum number of days was considered to be the best antifungal concentration. (Table no. 2).

For both the weeds, the methanol extract gave best antifungal results by producing large inhibition zones at low concentrations and remained active for comparatively more number of days, against maximum number of the fungi tested. Next to methanol, water and chloroform extracts proved to be the best for Lantana and Parthenium respectively.

Though, methanol extract of Lantana and Parthenium proved to be the best extracts, it should not be overlooked that the water extract of both the weeds also exhibited overwhelming antifungal results by producing more or less similar persistent inhibition zones but at higher concentrations. (Table no. 1)

Scanning of literature revealed that variety of research work has been carried out on isolation of chemical constituents of the plants to check its antimicrobial activity. However, in the present investigations, the author has reported the significant antifungal activity of the whole extract of the weeds. Our traditional Ayurvedic medicinal system also supports the concept of using whole plant extract instead of using isolated individual phyto-constituent, for treating various diseases. It is a bitter fact which one should not overlook that individual phyto-constituents many times show side effects, however, whole plant preparations like Ayurvedic preparations are always reported to be more effective and safe. Besides this, the isolation and identification of phyto-constituents involve complicated methods which are time consuming and expensive.

It was very interesting to note that the water extracts of both the weeds obtained, by famous Ayurvedic hot decoction method, exhibited significant antifungal activity. Besides that, its persistence was also praiseworthy. These results are encouraging, because for a common man, it is more feasible to use safest and cheapest water extract than using isolated phyto-constituents by tedious and costly extraction methods. Similarly, these findings have further confirmed and also justified the folkloric uses of these weeds by traditional healers in the treatment of skin disorders or infections.

CONCLUSION:

Conclusively, most of the extracts of Lantana and Parthenium displayed wide range of antifungal activity. There is need for the development of new antibiotics due to acquired resistance, more importantly from natural sources as this delays resistance (Ajaiyeoba, 2000). The weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. screened in present investigations provide very good opportunities for drug development in this area.

REFERENCES

Ahmad, Iqbal and Arina Z Beg (2001) *Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens.* Journal of Ethnopharmacology 74:113–123.

Ajaiyeoba EO (2000) *Phytochemical and antimicrobial studies of Gynandropsis gynandra and Buchholzia coriaceae extracts.* African Journal of Biomedical Research 3(3):161-165.

Ajaiyeoba EO (2002) *Phytochemical and antibacterial properties of Parkia biglobosa and Parkia bicolor leaf extracts.* African Journal of Biomedical Research 5(3): 125-129.

Bajwa Rukhsana, Afia Khalid and Tabinda Shahid Cheema. 2003. *Antifungal activity of allelopathic plant extracts III: Growth response of some pathogenic fungi to aqueous extract of Parthenium hysterophorus.* Pakistan Journal of Plant Pathology 2(3): 145-156.

Bajwa, Rukhsana, Sobiya Shafique, Tehmina Anjum and Shazia Shafique. 2004. *Antifungal activity of allelopathic plant extracts IV: Growth response of Drechslera hawaiiensis, Alternaria alternata and Fusarium monilifrome to aqueous extract of Parthenium hysterophorus.* International Journal of Agriculture and Biology 6(3): 511-516.

Bhakuni DS, Dhar ML, Dhawan BN and Mehrotra (1969) *Screening of Indian plants for biological activity Part III*. Indian J Experimental Biol. 7: 250-262.

Bhatnagar Kalpana, Bhim Sen Sharma and H. S. Cheema. 2004. *Efficacy of plant extract against Fusarium oxysporum f. sp. cumini Wilt in Cumin.* Journal of Mycology and Plant Pathology 34(2): 360-361.

Cock IE (2008) *Antibacterial activity of selected Australian native plant extracts.* The Internet Journal of Microbiology 4(2).

Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM and Lavekar GS (2007) *Antimicrobial activity of some Indian medicinal plants.* African Journal of Traditional, Complementary and Alternative Medicines 4(3): 313-318.

Dagmar Janovska, Katerina Kubikova and Ladislav Kokoska (2003) *Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine.* Czech J. Food Sci. 21(3): 107–110.

Deena MJ and Thoppil JE (2000) *Antimicrobial activity of the essential oil of Lantana camara.* Fitoterapia 71(4): 453-455.

Dhawan BN, Dubey MP, Mehrotra BN, Rastogi RP and Tondan JS (1980) *Screening of Indian Plants for Biological activity:Part IX.* Indian Journal Experimental Biology 18: 594-606.

Facey PC, Pascoe KO, Porter RB and Jones AD (1999) *Investigation of plants used in Jamaican folk medicine for antibacterial activity.* Journal of Pharmacy and Pharmacology 51(12): 1455-1460.

Gislene GF, Nascimento, Juliana Locatelli Paulo C, Freitas, Giuliana L Silva (2000) *Antibacterial activity of plant extracts and Pphytochemicals on antibiotic-resistant bacteria.* Brazilian Journal of Microbiology 31(4).

Hammer KA, Carson CF and Riley TV (1999) *Antimicrobial activity of essential oils and other plant extracts.* Journal of Applied Microbiology 86: 985–990.

Hsieh TF, Huang JH, Hsieh LJ, Hu MF and Ko WH (2005) *Antifungal effect of plant extracts on phytopathogenic fungi.* Plant Pathology Bulletin 14(1): 59-66.

Ijeh II, Omodamiro OD and Nwanna IJ (2005) *Antimicrobial effects of aqueous and ethanolic fractions of two spices, Ocimum gratissimum and Xylopia aethiopica.* African Journal of Biotechnology 4(9): 953-956.

Juliani Hector R(Jr.), Fernando Biurrun, Adolfina R Koroch, Oliva MM, Mirta S Demo, Victorio S Trippi and Julio A Zygadlo (2002) *Chemical constituents and antimicrobial activity of the essential oil of* Lantana xenica. Planta Medica 68(8): 762-764.

Mamatha T and Ravishankar Rai V (2004) *Evalution of fungicides and plant extracts against Fusarium solani Leaf blight in Terminalia catappa.* Journal of Mycology and Plant Pathology 34(2): 306-307.

Marjorie Murphy Cowan. (1999) *Plant products as antimicrobial agents.* Clinical Microbiology Reviews 12(4): 564-582.

Martinez MJ, Betancourt J, Alonso-Gonzfilez N and Jauregui A (1996) *Screening of some Cuban medicinal plants for antimicrobial activity*. Journal of Ethnopharmacology 52: 171-174.

Mintesnot Ashebir and Mogessie Ashenafi (1999) *Assessment of the antibacterial activity of some traditional medicinal plants on some food-borne pathogens.* Ethiop. J. Health Dev. 13(3): 211-16.

National Committee for Clinical Laboratory Standards (NCCLS) (1998) *Performance standards for antimicrobial susceptibility testing; Eighth informational supplement.* M100-S8. Vol. 17, No. 1. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania.

Nayak BS and Lexley M Pinto Pereira (2006) *Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats.* BMC Complementary and Alternative Medicine 6: 41.

Onyeagba RA, Ugbogu OC, Okeke CU and Iroakasi O (2004) *Studies on the antimicrobial effects of garlic (Allium sativum Linn), ginger (Zingiber officinale Roscoe) and lime (Citrus aurantifolia Linn)* African Journal of Biotechnology 3(10): 552-554.

Oudhia. (1999) a, b and c. Cited at: http://www.iprng.org/IPRNG-parthenium_a&w12.htm

Parekh, Jigna and Sumitra Chanda. 2007. *In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants*. Turk J Biol 31: 53-58.

Patel, Seema, Nitin Venugopalan and S. Pradeep. 2007. *Screening for antimicrobial activity of weeds.* The Internet Journal of Microbiology 4(1).

Polyanna Alves Silva, Denilson Ferreira Oliveira, Ney Robson Taironi do Prado, Douglas Antônio de Carvalho and Gilvane Aparecida de Carvalho. 2008. *Evaluation of the antifungal activity by plant extracts against Colletotrichum gloeosporioides Penz. Cienc. agrotec.* Lavras 32(2): 420-428.

Prashanthkumar V, Chauhan Neelam S, Padh Harish and Rajani M (2006) *Search for antibacterial and antifungal agents from selected Indian medicinal plants.* Journal of Ethnopharmacology 107(2):182-188

Rai MK and Acharya D (1999) *Screening of some Asteraceous plants for antimycotic activity.* Compositae Newsletter 34: 37-43.

Rajakaruna, Nishanta, Cory S Harris and Towers GHN (2002) *Antimicrobial activity of plants collected from Serpentine Outcrops in Sri Lanka.* Pharmaceutical Biology (Formerly International Journal of Pharmacognosy) 40(3): 235 – 244.

Saha D, Dasgupta S and Saha A (2005) *Control of Foliar Tea Diseases by leaf extracts of Polyalthia longifolia.* Journal of Mycology and Plant Pathology 35(1): 132-136.

Sharma and Sharma. 1989. **Cited at:** http://www.issg.org/database/species/ecology.asp?fr=1&si=56

Sinha K, Verma KP, Agarwal KC, Toorray NK and Thakur MP (2004) *Antifungal activities of different plant extracts against Colletotrichum capsici.* Advances in Plant Sciences 17(1): 337-33.

Tania Maria de Almeida Alves, Andreia Fonseca Silva, Mitzi Brandao, Telma Sueli Mesquita Grandi, Elza de Fatima A Smania, Artur Smania Junior and Carlos Leomar Zani. 2000. *Biological Screening of Brazilian Medicinal Plants.* Rio de Janeiro 95(3): 367-373.

Tiwari BK and Srivastava KJ (2004) *Studies on bio-efficacy of some plant extracts against pathogens of onion.* News Letter - National Horticultural Research and Development Foundation 24(1): 6-10.

Veeramuthu Duraipandiyan, Muniappan Ayyanar and Savarimuthu Ignacimuthu. 2006. *Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India.* BMC Complementary and Alternative Medicine 6: 35.

RESEARCH ARTICLE

Effect of AM Fungi on *Mentha spicata* L.

Birje Rachana R* and Golatkar VV

Department of Botany, D. G. Ruparel College, Mahim, Mumbai 400 016.
*Corresponding author Email: rachanabirje@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Birje Rachana R and Golatkar VV (2016) Effect of AM Fungi on *Mentha spicata* L. Int. J.of. Life Sciences, Special Issue, A7: 33-40.

Acknowledgement
Authors are grateful to Dr. Desai, Principal, D. G. Ruparel College, Mahim, Mumbai, for providing the laboratory and internet facilities and to Dr. Karnik, Librarians, D. G. Ruparel College, Mahim, Mumbai for providing library facilities.

ABSTRACT

In order to study the effect of various doses of AM fungi on growth of spearmint (*Mentha spicata* L.) plants, a series of experiments were conducted in pot cultures in randomised block design (RBD). A commercial AM fungal consortium 'Rhizagold' was used as the Arbuscular mycorrhizal fungal (AMF) culture. The various growth parameters such as dry weight, chlorophyll content, relative water content, Nitrogen content, Phosphate Phosphorous content and potassium content of spearmint were tested after 60, 90 and 120 DAS. Per cent root colonisation and spore count were also tested. The results were encouraging and demonstrated that there was a significantly positive effect of increasing doses of AMF culture on various growth parameters of spearmint plants throughout the experiment. Amongst the various treatments, the treatment T_2 and T_3 were proved to be better for harvest at 90 DAS than the remaining treatments.

Key words: AM fungi, spearmint (*Mentha spicata* L.), Rhizagold, pot cultures, growth, harvest.

INTRODUCTION

The Arbuscular Mycorrhizal fungi (AMF), which were formerly known as Vesicular Arbuscular Mycorrhiza (VAM) are unique group of ubiquitous soil microorganisms known to form symbiotic association with roots of economically important crop plants (Pragatheswari *et al.*, 2004). The arbuscular mycorrhizal fungi are the associations between higher plants and fungi where the plants normally provide carbon to fungus and fungus provides nutrients and water to plant. The AM fungi infect the root system and invade several layers of the outer root cortex. AM fungal hyphae penetrate the individual cells and form unique structures called arbuscules and vesicles. Arbuscules are the much-branched structures within the cells while vesicles (may be intracellular or intercellular) are balloon-like structures and probably have a function of storage (Harley and Smith, 1983). The fungal hyphae do not penetrate the protoplast, but invaginate the cell membrane. The structure of the arbuscules greatly increases the contact surface area between the hypha and the cell cytoplasm to facilitate the transfer of nutrients between them.

Spearmint (*Mentha spicata* L.), is an annual herb of the family Lamiaceae (Labiatae). Spearmint is chiefly employed in cooking especially as a condiment to flavour the curries, soups, chutneys, sauces, etc. Spearmint is a useful source of essential oil and has been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry. As the spearmint oil is stimulant, antiseptic, restorative, carminative and antispasmodic, it is used in various medicines. Due to its low menthol (0.5 per cent compared to 40 per cent in peppermint) and menthone content, these medicines can be safely given to children and old people. Spearmint has a great export potential (Kumar *et al*., 1997). Thus, there is a need to focus on the cultivation of spearmint plant to enhance its production.

Due to public awareness programs and various government policies, the farmers have realized the hazardous effect of chemical fertilizers and hence, they are increasingly shifting to sustainable agricultural practices which include use of natural resources and organic inputs to get maximum yield without harming the ecosystem. In the past few decades, Arbuscular mycorrhizal (AM) fungi have emerged as potential biofertilizers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava *et al*., 1996).

Therefore, in the present experiment, the symbiotic efficiency of AM fungi on growth performance, root colonization and spore density in *Mentha spicata* L. was evaluated. Present study also aimed to select the optimum dose of AM fungal culture in order to harness the maximum benefit from the AMF inoculum at the time of harvest.

MATERIALS AND METHODS

The soil used for the experiment, was procured from Pathare Nursery, Kalyan, India having pH 6.75, electric conductivity (EC) 0.816 mS, water holding capacity 112%, organic carbon 1.031%, nitrogen 0.761 mg/gm soil, phosphate phosphorous 1.763 mg/gm soil and potassium 0.336 mg/gm soil. A commercial AM fungal consortium 'Rhizagold' (200 viable spores per 10 gram) manufactured by 'Biotrack Tech. Pvt. Ltd.' purchased from Tamil Nadu, India was used as the Arbuscular mycorrhizal fungal (AMF) culture. Four doses of AMF culture viz., 1 gm (T_1), 2 gm (T_2), 3 gm (T_3) and 4 gm (T_4) were tested by mixing each dose with 3 kg of sterilized soil separately as each plastic pot could accommodate this much of soil. Four replications of control pots and four replications of each treatment pot were maintained. 10 pieces of spearmint suckers having 1 – 2 inches length were sown in each pot. Pots were watered on alternate days.

The effect of various concentrations of AM culture on the vegetative growth of spearmint plants was studied separately after 60, 90 and 120 days of sowing (DAS) with respect to various parameters viz., Dry weight, Chlorophyll content (Arnon, 1949), Relative Water Content (Noggle and Fritz, 1983), Nitrogen by micro-kjeldahl (Sadasivam and Manickam, 2008), Phosphate phosphorous by colorimetry (Bhargava and Raghupathi, 1993) and Potassium by flame photometry (Bhargava and Raghupathi, 1993). Per cent root colonization (Phillips and Hayman, 1970; Koske and Gamma, 1989 and Grace and Stribley, 1991) and spore count (Gerdemann and Nicolson, 1963) were also studied after 60, 90 and 120 days of sowing.

Statistical analysis

The experiment was laid in randomized block design (RBD) with four replicates of control pots and four replicates of each treatment pot. Data were expressed as mean value of these four replicates. The mean values were subjected to statistical analysis and the one way Analysis of Variance (ANOVA) was constructed. The test was carried out by referring the 'F' value obtained to the standard 'F' value at 5% level of significance. The standard error and critical differences were also calculated. All the calculations were made by using data analysis tool pack for Microsoft Excel 2007 and Windows 7.

RESULTS AND DISCUSSION

Dry weight of aerial shoot

The treatments T_2 and T_4 produced significant effect on dry weight of shoots throughout the study (Table 1.A). At 120 DAS, all the treatments produced significant effect on mean dry weight of arial shoot of spearmint plants. The probable reason for the significant increase in dry weight, is the increase in supply of nutrients (Furlan *et al*., 1983; Marschner and Dell, 1994; Habte and Soedrajo, 1996) and enhanced nutrient absorption especially phosphorous and greater rates of photosynthesis in inoculated plants

(Cooper, 1984; Sankaran, 2004). Increase in shoot biomass of spearmint plants by mycorrhiza was also reported by Kumar (2012). Similar significant increase in dry weight of shoot was also reported by Khaliq *et al*. (2001) in peppermint, Zolfaghari *et al*. (2013) in *Ocimum basilicum*, by Padmavathi (2009) in *Ocimum sanctum*, Rasouli-Sadaghiani *et al.* (2010) in Basil and by Rashmi and Roy (2003) in *Eleusine coracana* (Finger millet).

Chlorophyll content

The present study showed that the AMF treatments had significant impact on chlorophyll content of spearmint leaves (Table 1.A). The treatments T_3 and T_4 were proved themselves to be more promising by exhibiting significant impact on total chlorophyll content throughout the experiment. The treatment T_2 produced significant positive effect at later stage of development i.e. at 90 and 120 DAS. This may be due to relatively lower root colonization in treatment T_1 and T_2 at 60 and 90 DAS. The increase in chlorophyll content under the influence of AMF may be due to the increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Levi and Krikun, 1980; Hayman, 1983). Rate of photosynthesis is susceptible to the inorganic phosphate concentration in the chloroplast (Beever and Burns, 1980). The AM fungi not only help the plants in better absorption of phosphorous but also help indirectly in increased uptake of other macro and micro nutrients (Safir *et al*., 1972) which are essential for chlorophyll synthesis. Thus, improved phosphorous nutrition through mycorrhizal application may have contributed for the enhanced chlorophyll content in the present investigations.

Significantly higher amount of total chlorophyll content in the leaves of AMF inoculated *Vigna unquiculata* plants than that in control plants was observed by Rajasekaran and Nagarajan (2005) while that in Mulberry plants was reported by Baqual *et al*. (2005). Jadhav (2011) had also got parallel results while working on Patchouli and Ashwagandha plants. Haripriya *et al*. (2010) reported ameliorative effect of AM inoculation on chlorophyll content of Ashwagandha.

Table 1: Effect of various levels of AMF culture on growth of spearmint plants

A. Dry weight, chlorophyll content and relative water content (RWC) under various levels of AMF culture									
	Dry weight (gm)			**Total Chlorophyll content** (mg per gm fresh leaves)			**RWC of leaves** (per cent)		
Treatments	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**
C	0.053	0.041	0.058	1.325	1.578	1.561	85.284	89.310	90.452
T_1	0.065	0.044	0.062	1.560	1.830	1.696	88.852	93.552	93.552
T_2	0.075	0.070	**0.147**	1.617	1.841	2.151	88.402	**94.316**	92.036
T_3	0.066	**0.083**	0.131	1.821	2.055	2.187	**90.597**	94.087	**95.788**
T_4	**0.086**	0.063	0.096	**1.898**	**2.093**	**2.297**	89.512	93.534	93.742
F Test	Significant	Significant	Significant	Significant	Significant	Significant	Non-Significant	Significant	Significant
S.E.	±0.009	±0.010	±0.032	±0.182	±0.102	±0.252	–	±1.601	±1.410
C.D.	0.020	0.022	0.067	0.389	0.216	0.537	–	3.410	3.004
B. NPK content of spearmint plants under various levels of AMF culture									
	Nitrogen content (mg per gm dry plant material)			**Phosphatephosphorous content** (mg per gm dry plant material)			**Potassium content** (mg per gm dry plant material)		
Treatments	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**	**60 DAS**	**90 DAS**	**120DAS**
C	22.584	20.442	20.466	0.105	0.216	0.255	2.476	2.700	2.291
T_1	22.811	27.579	32.848	0.156	0.353	0.406	2.532	4.815	**4.154**
T_2	28.434	34.561	34.596	0.169	0.482	0.475	4.454	4.562	3.879
T_3	**32.406**	35.187	**35.382**	**0.255**	**0.537**	**0.555**	**5.174**	**5.647**	3.482
T_4	29.926	**35.747**	30.633	0.236	0.345	0.419	4.376	4.423	3.963
F Test	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
S.E.	±3.427	±5.027	±4.595	±0.067	±0.078	±0.059	±0.450	±0.406	±0.474
C.D.	7.299	10.708	9.787	0.142	0.166	0.126	0.959	0.866	1.011

Relative water content

Though the AMF treatments were non-significant for relative water content at 60 DAS, all of them gave significant results at 90 DAS while at 120 DAS, the treatments T_1, T_3 and T_4 were significantly effective (Table 1.A). Poor response with respect to relative water content at 60 DAS may be the result of lower root colonization at 60 DAS. Maiti *et al*. (2009) found out that higher AM fungi colonization in rice roots maintained elevated leaf RWC over that of treatments with lower colonization. Higher RWC due to AMF inoculation was also reported by Shivaputra *et al*. (2004[b]) in papaya plant and by Devachandra *et al*. (2009) in *Syzygium cuminii* Skeel. (Jamun). According to Singh (2004), AM inoculated plants showed improved resistance to wilting and recover their leaf turgor faster than that in non-mycorrhizal plants.

Nitrogen content

The effect of AMF culture on nitrogen content was found to be commendable throughout the experiment (Table 1.B). At 60 DAS only treatments T_3 and T_4 of AMF exhibited significant results while at 90 DAS, the T_2 also started producing significantly higher nitrogen content whereas at 120 DAS, all the treatments were significantly praiseworthy. The higher plant nitrogen content in AMF inoculated plants could be attributed to hyphal uptake. Extra radical hyphae permit the transfer of nutrients such as nitrogen (Marschner and Dell, 1994). Besides, inorganic form of ammonium nitrogen can be absorbed by AM fungi (Ames *et al*., 1983). The increasing response of spearmint plants over a period may be attributed to increasing root colonization and activity of AMF. Increase in nitrogen content in AMF inoculated plants was also reported by many workers (Rasouli-Sadaghiani *et al*., 2010; Shivaputra *et al*., 2004[a]; Kessel *et al*., 1985).

Phosphate-phosphorous content

Mycorrhizal fungi are known to have better access to the pools of phosphorous which otherwise not readily available to the plants (Sharma *et al*., 2014). In the present study also, the effect of AMF culture on phosphate phosphorous was noteworthy (Table 1.B). Each treatment exhibited significant results at least at one stage of the development. The AMF treatment T_3 was successful enough for producing significant increase in phosphate phosphorous content throughout. Increase in phosphate uptake by mycorrhiza leads to increase in plant phosphate concentration (Filter, 1991). Previous work has shown that arbuscular mycorrhizal fungi increase plant phosphate content (Rasouli-Sadaghiani *et al*., 2010; Bolan, 1991). McGonigle and Miller (1993) have reported higher amount of phosphorous in the leaves of AM associated maize plants than that in control maize plants.

A number of factors may contribute to the increased rate of phosphorous uptake measured in mycorrhizal plants (Smith and Read, 1997). Mycorrhizal fungi increase the availability of phosphorous either by organic acids production or by enhancing the phosphatase activity in the rhizosphere (Sharma *et al*., 2014). An extensive network of hyphae, extending from roots enables the plants to explore a greater volume of soil, thereby overcoming limitations imposed by the slow diffusion of phosphorous in the soil. The mycorrhizal fungi may also be able to scavenge phosphorous from the soil solution more effectively than other soil fungi. The plant fungus association could, therefore, enables the plant to compete more effectively with soil microorganisms for the limited amount of available soil phosphorous. Mycorrhizal fungi may also be able to acquire phosphorous from organic sources that are not available directly to the plants (Jayachandran and Shetty, 2003).

Potassium content

The potassium content of spearmint plants was significantly affected by AMF treatments. Almost all the treatments showed significantly higher potassium content of spearmint plants throughout the experiment (Table 1.B). This higher nutrient uptake in mycorrhizal plants might be attributed to the contribution of fungal external mycelia which explore a large volume of soil and thus absorb more nutrients (Gupta and Janardhanan, 1991). Khaliq *et al*. (2001) reported significant increase in potassium uptake by peppermint due to application of VAM fungi at 90 DAS. Besides, Balasubramaniun and Nambisan (1989) marigold seedlings, Gupta *et al*. (1990) in palmrosa, Kennedy and Rangarajan (2001) in papaya proved the efficiency of AMF inoculum in potassium uptake.

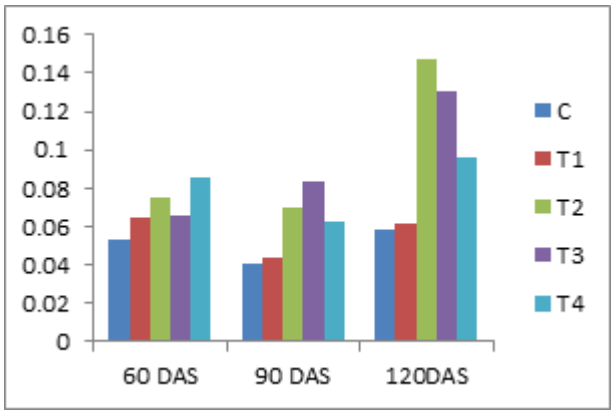
Spore count and Root colonization in AMF inoculated plants

The data showed that throughout the study, the increase in dose of AMF culture successfully resulted in increase in spore count of mycorrhizal fungi in soil (Table 2). The treatment T_1 which received lowest

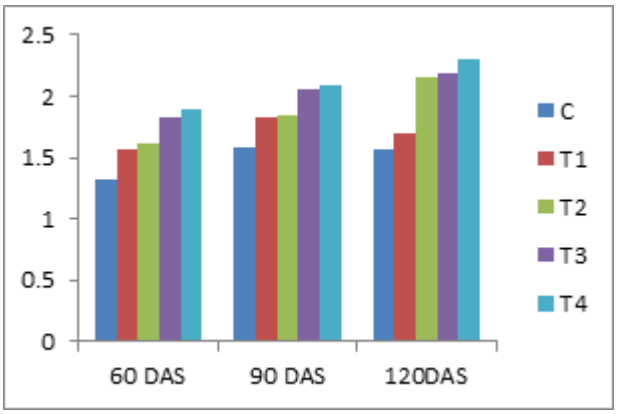
dose of AMF culture showed lowest mean spore count whereas treatment T_3 and T_4 showed almost two folds higher spore count than that of treatment T_1. At 120 DAS, the highest spore count was recorded in the soil from treatment T_4. The per cent root colonization followed a similar trend. Throughout the experiment, the treatment T_1 showed lowest root colonization. Treatments T_3 and T_4 showed almost same extent of root colonization. At 120 DAS, all the treatments possessed higher root colonization.

Table 2: Spore count and per cent root colonization in AMF treated plants

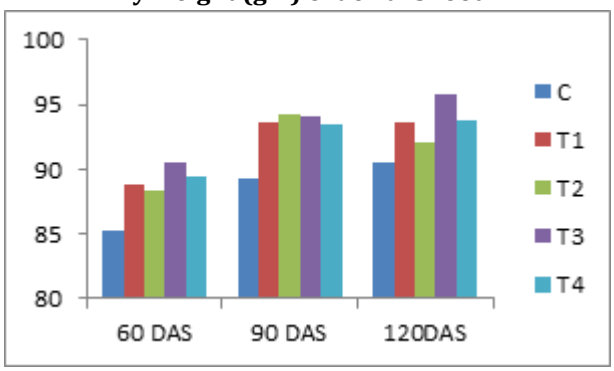
	Spore count			% Root colonization		
Treatments	**60 DAS**	**90 DAS**	**120 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
T_1	15.75	28.00	62.75	27.33	33.35	55.67
T_2	25.75	43.25	70.75	36.00	58.33	73.33
T_3	31.25	64.50	105.25	43.67	68.67	88.05
T_4	33.75	60.75	118.75	45.00	76.01	90.89



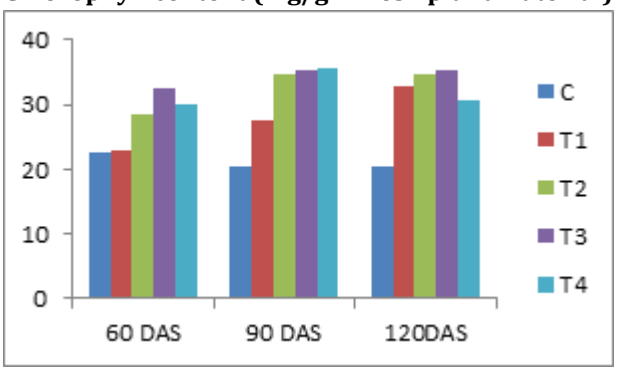
Dry weight (gm) of aerial shoot



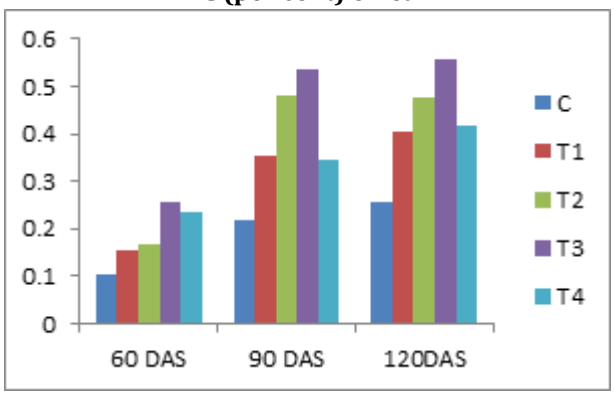
Chlorophyll content (mg/gm fresh plant material)



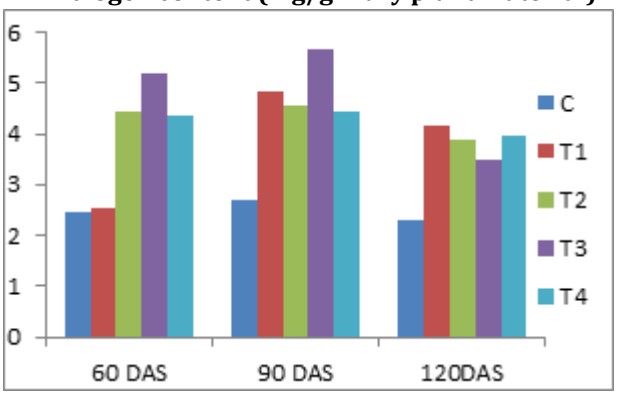
RWC (per cent) of leaf



Nitrogen content (mg/gm dry plant material)



Phosphate P content (mg/gm dry plant material)



Potassium content (mg/gm dry plant material)

The spore count and root colonization studies indicate the extent of association between host plants and AM fungi. The present investigations showed that the higher dosage of mycorrhiza gave better establishment of mycorrhiza in terms of spore count and root colonization in spearmint plants. At 60 DAS, the spore count and root colonization was low which increased considerably at 90 and at 120 DAS. Draft and Nicolson (1972) demonstrated that the higher root colonization allows more mycorrhiza – host contact and more exchange of nutrients, hence, the better plant growth. The low level of infection at 60 DAS might be due to the time taken by AM fungi for initial colonization of the roots as reported by Ramirez *et al*. (1975) and Kennedy and Rangarajan (2001).

Determination of optimum dose of AMF culture

From the present study, it is apparent that the AMF culture had a stimulating effect on growth, productivity and quality of spearmint plants throughout the course of study. 90 DAS stage is considered as very vital from harvest point of view (Sud and Kumar, 2004), hence, to determine the optimum dose of AMF culture, the results obtained at 90 DAS were analyzed, compared and significant results were counted (Table 3).

Table 3: Determination of optimum dose of AMF culture

Parameters Tested	AMF			
	T_1	T_2	T_3	T_4
1. Dry Weight	-	S	S	-
2. Total Chlorophyll	S	S	S	S
3. RWC	S	S	S	S
4. Nitrogen	-	S	S	S
5. Phosphorous	-	S	S	-
6. Potassium	S	S	S	S
Total	3	6	6	4
Best Treatments	**T_2 and T_3**			

'S' : Significant, '–' : Non-significant

Treatment T_2 and T_3 showed a remarkable effect on spearmint plants as all growth parameters were significantly influenced by the same at 90 DAS. It was very surprising that in spite of highest dose, treatment T_4 was not as effective as T_3. Kapooria (2006), in his study, also noticed the same behavior and reported that beyond a certain inoculum level, the mycorrhizal fungus failed to produce desired stimulatory effect.

CONCLUSION

It is evident from the data that application of AM Fungi played a vital role in improving plant biomass, photosynthetic activity in terms of chlorophyll content, water uptake and enhanced uptake and accumulation of nutrient elements in spearmint plants. This must have improved the host nutrition by increasing the delivery of phosphorous and other minerals to roots of the plant. It was also observed that to obtain better growth and yield of spearmint plants, high inputs of biological fertilizers were not needed. In fact, spearmint plants flourished well even in lower doses of AMF culture. AMF treatment T_2 and T_3 (2 gm and 3gm AMF culture per pot respectively) were proved to be the better than the remaining treatments from harvest point of view. Thus, the application of AMF for enhancing the growth of spearmint plants has promising future. This will not only reduce the dependence of spearmint plants on hazardous chemical fertilizers but will also ensure the maintenance of soil health, owing to eco-friendly nature of AM fungi.

REFERENCES

Ames RN, Reid CPP, Porter LK and Cambaedella C (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae,* a vesicular-arbuscular mycorrhizal gungus. *New phytologist*., 95: 351-396.

Arnon DI (1949) **Cited in:** Sadasivam S. and A. Manickam. 2008. Biochemical Methods. New Age International Publishers, Third edition. pp 201-202.

Balsubramanan J and Nambisan KMP (1989) Studies on combined effect of *Azospirillum*, VA-mycorrhizae and inorganic fertilizers on growth and performance of French marigold (*Tagetes patula* L.). South Indian Hort.: 311-312.

Baqual MF, Das PK and Katiyar RS (2005) Effect of arbuscular mycorrhizal fungi and other microbial inoculants on chlorophyll content of mulberry (*Morus* spp.). Mycorrhiza News 17 (3): 12-14.

Beever RE and Burns DJ (1980) Phosphate uptake, storage and utilization by fungi. Advances in Botanical Research 8. Ed. H. W. Woolhouse. Academic Press Inc. (London) Ltd. pp 127-129.

Bhargava BS and Raghupathi HB (1993) **Cited in:** Methods of Analysis of soils, plants, water and fertilizers. Ed. HLS Tandon. Fertilizer Development and Consultation Organization (New Delhi). pp 49-82.

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants. Plant and Soil 134: 189-207.

Cooper KM (1984) Physiology of VA mycorrhizal associations**. Cited in:** Padmavathi, T. 2009. Effect of Arbuscular Mycorrhizal fungal inoculation on growth performance of *Oscimum sanctum.* Kavaka 37 and 38: 25-30.

Devachandra N, Patil CP, Patil PB, Swamy GSK and Durgannavar MP (2009) Mycorrhizal inoculation in combating low moisture stress in Jamun (*Syzygium cuminii* Skeel.). Mycorrhiza News 20(4): 7-10.

Draft MJ and Nicolson TH (1972) Effect of endogone mycorrhiza on plant growth: Quantitative relationship between the growth of the host and development of endophyte in tomato and maize. New Phytologist 71: 287-295.

Filter AH (1991) Costs and benefits of mycorrhizas: Implementation for functioning under natural conditions. Experientia. pp 47.

Furlan V, Fortin JA and Plenehette C (1983) Effects of different vesicular arbuscular mycorrhizal fungi on growth of *Fraxinus americana.* Canadian Journal of Botany 4: 589-593.

Gerdemann JW and Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting method. Transaction of British Mycological Society 46: 235-244.

Grace C and Stribley DP (1991) A safer procedure for routine staining of Vescicular Arbuscular Mycorrhizal fungi. Mycol. Res. 95(10): 1160-1162.

Gupta ML and Janardhanan KK (1991) Mycorrhizal association of *Glomus aggregatum* with palmarosa enhances growth and biomass. Plant Soil 131: 261-263.

Gupta ML, Janardhanan KK, Chattopadhyay A and Akhar Husain (1990) Association of *Glomus* with palmrosa and its influence on growth and biomass production. Mycological Research 94(4): 561-563.

Habte M and Soedarjo M (1996) Response of Acacia mangium to vesicular arbuscular mycorrhizal inoculation, soil pH and soil P concentration in an oxisol. Canadian journal of Botany 74: 155-161.

Haripriya M, Vijaya T and Mouli KC (2010) Effect of water stress on growth, metabolic activities of *Withania somnifera* – an important phytoceutical plant: Ameliorative effects of VAM. Journal of Global Pharma Technology 2(3): 112-116.

Harley JL and Smith SE (1983) Mycorrhizal symbiosis. Academic press. pp 408.

Hayman DS (1983) The physiology of Vesicular arbuscular endomycorrhizal symbiosis. Canadian Journal of Botany 61: 944-963.

Jadhav RS (2011) Studies on effect of AM (Arbuscular Mycorrhiza) Fungi as biofertilizer on growth and yield of medicinal and aromatic plants. Ph.D. Thesis, University of Mumbai, pp 290.

Jayachandran K and Shetty G (2003) Growth response and phosphorus uptake by arbuscular mycorrhizae of wet prairie saw grass. Aquatic Botany 76: 281-290.

Kapooria RG (2006) Mycorrhizal diversity of Zambian ecosystems and the effect of vesicular-arbuscular mycorrhizal fungi on tomato. **Cited in:** Mycorrhiza. Ed. A. Prakash and V. S. Mehrotra. Scientific publishers (India). pp 1-4.

Kennedy ZJ and Rangarajan M (2001) Biomass production, root colonization and phosphate activity by six VA-mycorrhizal fungi in papaya. Indian Phytopathology 54(1): 72-77.

Kessel C, Paul WS and Heinz JH (1985) Enhance N-transfer from a soybean to maize by vesicular-arbuscular mycorrhizal fungi. Plant Physiology 79: 562-563.

Khaliq A, Gupta ML and Kumar S (2001) The effect of vesicular-arbuscular mycorrhizal fungi on growth of peppermint. Indian Phytopathology 54(1): 82-84.

Koske RE and Gamma JN (1989) Modified Procedure for staining the roots to detect VA Mycorrhiza. Mycol. Res. 92(4): 486-505.

Kumar A (2012) The influence of Bioinoculants on growth and mycorrhizal occurrence in the rhizosphere of *Mentha spicata* linn. Bulletin of Environment, Pharmacology and Life Sciences 1(6): 60-65.

Kumar N, Md. Abdul Khader JBM, Rangaswami P and Irulappan I (1997) Introduction to Spices, Plantation crops, Medicinal and Aromatic plants. Oxford and IBH Publishing Co. Pvt. Ltd. pp 22.23.

Levi Y and Krikun J (1980)Effect of Vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. New Phytologist 85: 25-31.

Maiti D, Barnwal MK and Mandal NP (2009) Exploring possibilities of partial drought mitigation in upland rice (*Oryza sativa* L.) through enhancing native arbuscular mycorrhizal association. Mycorrhiza News 21(3): 29-30.

Marschner H and Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159: 89-102.

McGonigle TP and Miller MH (1993) Mycorrhizal development and phosphorous absorption in maize under conventional and reduced tillage. Plant and Soil. 210: 33-42.

Noggle GR and Fritz GJ (1983) Introductory plant physiology, Prentice Hall of India, pp 372-373.

Padmavathi T (2009) Effect of Arbuscular Mycorrhizal fungal inoculation on growth performance of *Oscimum sanctum*. Kavaka 37 and 38: 25-30.

Phillips JN and Hayman DS (1970) Improved procedures for clearing root and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. 55: 158–160

Pragatheswari D, Manjunath A and Madhaiyan M (2004) Effect of inoculation with vesicular arbuscular mycorrhizal (VAM) fungus (*Glomus mosseae*) on selected tropical grain legumes. **Cited in:** Biofertilizers Technology. Ed. S. Kannaiyan, K. Kumar and K. Govindarajan. Scientific publishers (Jodhpur). pp 342-346.

Rajasekaran S and Nagarajan SM (2005) Effect of dual inoculation (AM fungi and *Rhizobium*) on chlorophyll content of *Vigna unguiculata* (L.) Walp. var. Pusa 151. Mycorrhiza News 17 (1): 10-11.

Ramirez BN, Mitchell DJ and Schenck NC (1975) Establishment and growth effects of three vesicular-arbuscular mycorrhizal fungi on papaya. Mycologia 67: 1039-1041.

Rashmi and Roy AK (2003) Effect of VAM fungi and *Azospirillum brasilense* on growth performance of Finger Millet. Journal of Mycology and plant pathology 33(3): 403-405.

Rasouli-Sadaghiani M, Hassani A, Barin M, Danesh YR and Sefidkon F (2010) Effects of arbuscular mycorrhizal (AM) fungi on growth, essential oil production and nutrients uptake in basil. Journal of Medicinal Plants Research 4(21): 2222-2228

Sadasivam S and Manickam A (2008) Biochemical Methods. New Age International Publishers, Third edition. pp 32-35.

Safir GR, Boyer JS and Gerdemann JW (1972) Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant physiology 49: 700-703.

Sankaran KV (2004) Status of arbuscular mycorrhizal associations in *Acacia auriculiformis* plantations in Kerala state, India. Kavaka 32: 113-121.

Sharma YP, Watpade S and Thakur JS (2014) Role of mycorrhizae: A component of Integrated Disease Management strategies. Journal of Mycology and Plant pathology 44(1): 12-20.

Shivaputra SS, Patil CP, Swamy GSK and Patil PB. (2004a) Cumulative effect of VAM fungi and vermicompost on nitrogen, phosphorous, potassium and chlorophyll content of papaya leaf. Mycorrhiza News 16(2): 15-16.

Shivaputra SS, Patil CP, Swamy GSK and Patil PB (2004b). Effect of vesicular-arbuscular mycorrhiza fungi and vermicompost on drought tolerance in papaya. Mycorrhiza News 16(3): 12-13.

Singh S (2004) Effect of soil moisture on arbuscular mycorrhizal development in plants. Part II. In pulses, vegetables and miscellaneous crops. Mycorrhiza News 16(1): 2-8.

Smith SE and Read DJ (1997) Mycorrhizal symbiosis. Second Edition, Academic Press, London. pp 605.

Srivastava D, Kapoor R, Srivastava SK and Mukerji KG (1996) Vesicular arbuscular mycorrhiza – an overview.

Sud RK and Kumar S (2004) Herbs: Culinary, Medicinal, Aromatic (Secretes and Human Happiness). Scientific Publishers (India). pp 107-115.Sud and Kumar, 2004

Zolfaghari M, Nazeri V, Sefidkon F and Rejali F (2013) Effect of arbuscular mycorrhizal fungi on plant growth and essential oil content and composition of *Ocimum basilicum* L. Iranian Journal of Plant Physiology 3(2): 643-650.

RESEARCH ARTICLE

Studies on copper induction for laccase enzyme production by *Trichoderma erinaceum*

Ingle MR[1] and Mishra RL[2]

[1]Department of Botany, S. P. Dnyanasadhana College of Arts, Commerce and Science, Thane- 421604
[2]Professor and Principal, LSPM College, Chondhi – Kihim, Alibag, Raigad- 402 201
*Corresponding author: rajmandakini@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Ingle MR and Mishra RL (2016) Studies on copper induction for laccase enzyme production by *Trichoderma erinaceum,* Int. J.of. Life Sciences, Special Issue, A7:41-46

Acknowledgements
We sincerely thank Dr. S.K. Singh, Scientist and Dr. P. N. Singh, Scientist and Curator, National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India for molecular identification studies. Also we are grateful to National Facility for Bio-Pharmaceuticals, G.N. Khalsa College, Matunga, Mumbai for carrying out Protein purification and gel electrophoresis studies

ABSTRACT

Trichoderma erinaceum was isolated from decomposing coconut coir and was screened for its ability to produce laccase enzymes. Production of extracellular laccase enzyme from *Trichoderma erinaceum* was carried out by submerged fermentation. The laccase enzyme was partially purified by acetone precipitation from the culture filtrates of *Trichoderma erinaceum*. SDS-PAGE analysis showed the purified laccase to be a monomeric protein of 38 kDa. Laccase assay was carried out using ABTS as substrate. *Trichoderma erinaceum* exhibited laccase activity both under constitutive and copper induced conditions. Copper sulphate with 300 µm concentration added to the media highly induced production of laccase.

Keywords: *induced, laccase, SDS-PAGE, submerged fermentation, Trichoderma erinaceum*

INTRODUCTION

Laccase (E.C.1.10.3.2, p-benzenediol:oxygen oxidoreductase) is a multi-copper enzyme belonging to the group of blue oxidase that catalyzes the one electron oxidation of a broad range of organic substrates including phenols, polyphenols, anilines, benzene thiols and even certain inorganic compounds with a concomitant four electron reduction of oxygen to water (Sandhu and Kalra, 1982). Laccases catalyze the oxidation of a variety of phenolic compounds, diamines and aromatic amines (Solomon *et al*., 1996).They are widely distributed in nature in higher plants, bacteria and fungi (Mayer and Staples;2002).Laccases find enormous industrial, biotechnological and environmental applications. They are used in food industry, pulp and paper industries, textile industry, cosmetics, bioremediation and biodegradation of environmental pollutants (Rao et *al*., 1993).Due to its versatile importance, there is a crucial need to induce both its expression and production through up-regulation of the enzyme coding genes. Although genetic manipulation is an effective tool, it is highly complex and expensive technique. Therefore increasing the enzyme yield by adding inducer is perceived as simple and

cost- effective (Skorobogat'ko *et al*., 1996; Levin *et al*., 2010). There are many different inducers for laccase production such as aniline (Bollag and Leonowicz, 1984), methoxy phenolic acids (Rogalski and Leonowicz, 1992,), lignin preparations (Rogalski *et al*., 1991) but the most common is copper. Copper is considered to be an efficient putative inducer for laccase production (Palmieri *et al*., 2000).

Among microorganisms, fungi are the efficient producers of lignocellulolytic enzymes. It is well known that over 60 fungal strains belonging to Ascomycetes, Basidiomycetes and Deuteromycetes show laccase activity (Gianfreda *et al*., 1999). Among Basidiomycetes, white rot fungi produce laccase enzyme more efficiently (Ruiz-Dueñas, and Martínez, 2009). *Trametes versicolor*, *Chaetomium thermophilum* and *Pleurotus eryngii* produce laccase and it has been reported that *Trichoderma* species also has the ability to produce polyphenol oxidase (Gochev and Krastanov, 2007). *Trichoderma* species actively participates in delignification and biodegradation of lignocellulosic compounds in nature. Only a few publications are concerned with laccase producing *Trichoderma* species (Holker *et al*., 2002).*Trichoderma atroviride*, *T*. *harzianumT*. *longibrachiatum* and *T*. *erinaceum* have been reported to produce laccase (Blum *et al*., 1987; Gianfreda *et al*.,1999; Niku-Paavola *et al*.,1988; Ingle and Mishra, 2016).

The present study focuses on isolation and screening of laccase producing *Trichoderma erinaceum* and also the effect of copper on the production of laccase by submerged fermentation.

MATERIALS AND METHODS

Isolation of fungi:
Trichoderma erinaceum was isolated from decomposing coconut coir using a tenfold serial dilution-plating technique on potato dextrose agar (PDA) plates. The plates were incubated at 28°C. The pure culture was then transferred to PDA slants and maintained at 4°C and sub-cultured every month.

Primary screening for Laccase production by qualitative methods:
ABTS – Plate screen test: Plates containing Lignin-agar basal medium supplemented with 0.1% ABTS and 0.01% of 20% w/v aqueous glucose solution were inoculated with test fungus and incubated at 28º C (Pointing,1999). The formation of green halo around the fungal colony indicates the production of laccase enzyme.

Guaiacol assay:
Guaiacol assay was performed by the method given by Kiiskinen *et al*., (2004) with slight modifications. The test fungus was inoculated on to the plates containing PDA medium supplemented with 0.01% guaiacol and then incubated at 28ºC for about 6 days. Appearance of reddish brown halos around the colony suggested laccase positive strain.

Inoculums Preparation for submerged fermentation:
Four mycelial plugs (8mm diameter) from a 7 day old culture PDA plate were cut with the help of a potato borer. The mycelial mats on the plugs were carefully scraped so as to remove agar and aseptically added to the sterilized 250ml Erlenmeyer flasks containing 10ml of Sabouraud's broth. The inoculated flasks were incubated at 28º $\pm$ 2ºC on an orbital shaker at 150 rpm for 48 hrs to obtain large quantity of active mycelia.

Cultivation Media for Laccase enzyme Production:
Laccase enzyme production was carried out by using two media:

Media A: The culture broth as given by Tien and Kirk (1988) was prepared with slight modifications. The media was supplemented with 300 µm $CuSO_4$.

Media B: Tien and Kirk's medium was prepared.
The pH of the media was adjusted to 4.5 .The volume was brought up to 1000ml using distilled water.

Enzyme Production by Submerged Fermentation:
25 ml of the medium was dispensed into 250 ml of Erlenmeyer flask and autoclaved at 121°C for 15 minutes. The flasks were inoculated with 5ml of spore suspension (inoculum) and then incubated for 6 days at 28°C on rotary shaker at 150 rpm.

Enzyme extraction:
After six days of cultivation the contents of the flasks were filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 5,000 rpm for 15 minutes. The supernatant was used as the crude enzyme extract for further analysis.

Partial purification and SDS-PAGE:

Partial purification of laccase was carried out with slight modifications (Sun *et al*., 2013) for both the samples i.e. culture filtrate from media A and media B. Cold acetone precipitation was carried out and the precipitate was then re-suspended in 20mM Tris pH 8.0. To determine the purity of the protein and its molecular weight, SDS-PAGE was performed (Laemmli, 1970) with 10% polyacrylamide gel and the protein was visualized by staining the gel with silver staining (Blum *et al*., 1987) using standard molecular weight markers.

Protein determination

Protein concentration was determined by Bradford method using BSA as standard (Bradford, 1976).

Laccase assay:

Laccase assay was carried out for the crude enzyme extract and precipitated enzyme from both the media. Laccase activity was determined by monitoring the oxidation of ABTS (ε = 29,300 M^{-1} cm^{-1}) (2, 2'- azinobis -3-ethyl-benzothiozoline-6-sulfonic acid) (Niku-Paavola *et al*., 1988). The reaction mixture contained 0.5 ml of 0.2 mM ABTS in 50 mM sodium acetate buffer pH 4.5 and 0.5 ml enzyme extract. The oxidation of ABTS was measured spectrophotome-trically at 405 nm as an increase in absorbance at 1 min interval. One unit of enzyme activity (U) is defined as the amount of enzyme that released 1µmole of oxidized product per minute, expressed as µmole/ min /L.

Statistical analysis:

All experiments were performed in replicates of five and the average values were given with standard deviation.

RESULTS AND DISCUSSION

Primary screening for laccase production:

ABTS Plate screen test and Guaiacol assay for Laccases:

The formation of a green halo in the ABTS supplemented plates and reddish brown halo in guaiacol supplemented plates indicated laccase production by *Trichoderma erinaceum* (Fig.1: a and b). *Trichoderma* strains have been reported to produce polyphenol oxidases (Blum *et al.,* 1987). Recently *Trichoderma atroviride* and *T. harzianum* have showed positive results for laccase (Gianfreda *et al*., 1999; Gochev and Krastanov 2007).

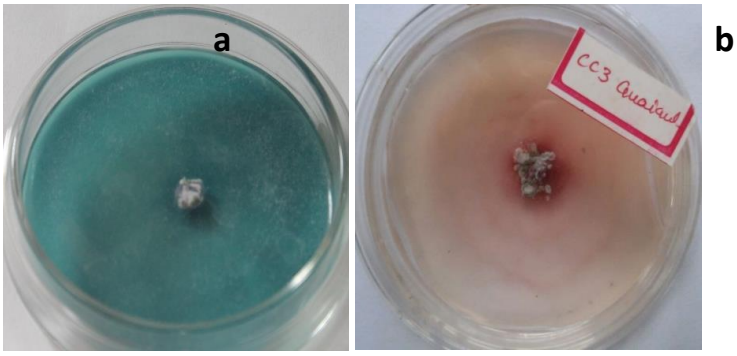
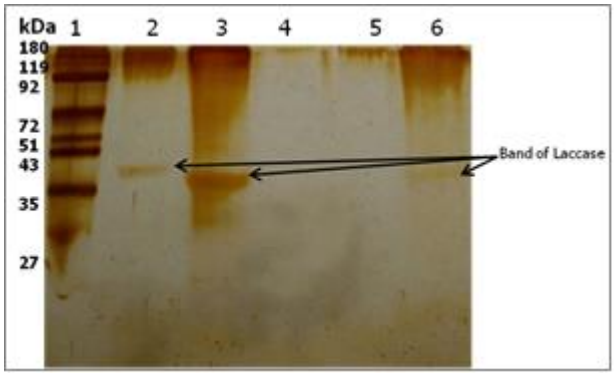


Figure 1: Qualitative screening (a) ABTS plate assay; (b) Guaiacol plate assay.



Lane No.	Sample ID
Lane 1	Protein Ladder
Lane 2	Crude A
Lane 3	Precipitated A
Lane 4	Blank
Lane 5	Crude B
Lane 6	Precipitated B

Fig 2: 10 % PAGE, silver stained, under reducing condition showing Acetone precipitation of Laccase.

Table 1 : Laccase activity profile before precipitation and after precipitation from Media A and Media B

Sample		Specific Activity (U/mg)	Laccase activity (U/L)
Media A: (with inducer)	Crude	48.344	1.429
	Precipitated	77.350	2.286
Media B: (without inducer)	Crude	6.397	0.429
	Precipitated	27.594	1.857

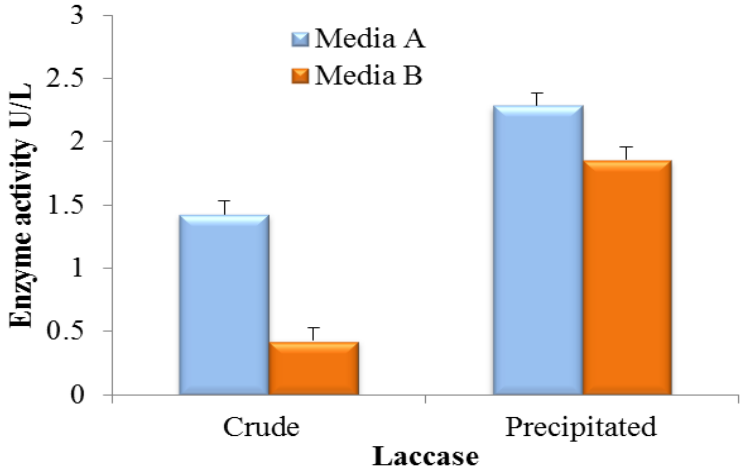


Fig. 1: Laccase production media

ABTS and Guaiacol are considered as best laccase substrates in the absence of hydrogen peroxidase, therefore confirming that the enzyme is true laccase (Sivakumar *et al*., 2010; Sandhu and Kalra, 1982). The chromogen ABTS is a very sensitive substrate that allows rapid screening of laccase producing fungal strains by means of a color reaction (Laemmli, 1970]. Guaiacol is used as a marker for extracellular oxidative enzymes, supports the above result (De Jong *et al*., 1994).

Molecular mass

SDS-PAGE analysis was carried out for both the crude and purified enzyme extracts obtained from both the media. The purified laccase showed a single band on SDS-PAGE with a mobility corresponding to the relative molecular mass of 38 kDa as visualized by Silver nitrate staining (Fig.3); this is very close to the molecular weight of laccase from *Pleurotus* sp. having molecular weight of 40 kDa as previously reported (More *et al*., 2011]. Assavanig *et al*., (1992) has reported molecular weight of 71 kDa for *Trichoderma* sp. According to Kunamneni *et al*. (2007) fungal laccases usually have molecular weights ranging from 50 to 100 kDa and are covalently linked to carbohydrate-moiety, which may contribute to high stability of the enzyme (Duŕan *et al*., 2002). From Fig.3; it is observed that laccase produced in the media A showed a darker band than media B. This indicates that the media supplemented with 300 µm CuSO4 has an inducing effect on laccase production. Lane 5 did not show any band of laccase, which might be due to negligible production of laccase, however Lane 6 (precipitated protein) showed a light band of laccase.

Laccase enzyme activities:

The laccase enzyme activities of *Trichoderma erinaceum* for the crude extract and the purified precipitate obtained from media A and media B are given in the Table 1. The laccase enzyme production in the Media A and Media B showed marked variations (Graph 1). Media A supplemented with 300 µm CuSO4 had an inducing effect on laccase production. It is worth noting that the addition of Cu+2 in the cultivation media stimulated laccase production increasing it almost three times higher than the control .This may be due to the filling of Type-2 copper binding sites with Copper ions (Nagai, *et al*., 2002).

Copper has been reported to be a strong inducer in several species such as *T. versicolor* (Collins and Dobson, 1997) and *P. chrysosporium* (Dittmer *et al*., 1997).

Similar observations have been recorded by Sivakumar *et al*. (2010) while working with *Ganoderma* sp. Niladevi and Prema (2007) have reported maximum laccase activity when Copper sulphate was used at a concentration of 1mM. However, Sadhasivam *et al*., (2008) reported that Copper at concentration of 10mM had a great inducing effect on laccase production by *Trichoderma harzianum* WL1 strain.

Also it was observed that the purified enzyme (media A) showed almost double enzyme activity (2.286 U/L) compared to the crude form (1.429 U/L).

CONCLUSION

The purified laccase from *Trichoderma erinaceum* showed molecular mass of 38kDa.The production of laccase by *Trichoderma erinaceum* occurs constitutively as well as in presence of inducer. The metal ion copper effectively increases the production of laccase by submerged fermentation. Further studies on standardization of concentration of copper for laccase production are needed

REFERENCES

Assavanig A, Amornkitticharoen B, Ekpaisal N, Meevootisom V and Flegel TW (1992) Isolation characterization and function of laccase from *Trichoderma*. *Applied Microbiology and Biotechnology*, 38: 198-202.

Blum H, Beier H and Gross HJ (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis*, 8:93-99.

Bollag J and Leonowicz A (1984) Comparative studies of extracellular fungal laccases. *Applied and Environmental Microbiology*, 48:849-854.

Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principles of protein dye binding. *Analytical Biochemistry*, 72:248-254.

Collins PJ and Dobson ADW (1997) Regulation of laccase gene transcription in *Trametes versicolor*. *Applied Environmental Biology*, 63:3444-3450.

De Jong E, Field JM and De Bont JAM (1994) Aryl alcohols in the physiology of lignolytic fungi. *FEMS Microbiology Reviews*. 13:153-188.

Dittmer JK, Patel NJ, Dhawale SW and Dhawale SS (1997) Production of multiple laccase isoforms by Phanerochaete chrysosporium grown under nutrient sufficiency. *FEMS Microbiology Letters*, 149:65-70.

Duŕan N, Rosa MA, Annibale D and Gianfreda L (2002) Applications of laccases and tyrosinases (phenol oxidases) immobilized on different supports: a review. *Enzyme and Microbial Technology*, 31 (7): 907-931.

Gianfreda L, Xu F and Bollag JM (1999) Laccases: A useful group of oxido-reductive enzymes. *Bioremediation Journal*, 3: 1-26.

Gochev VK and Krastanov (2007) Isolation of laccase producing *Trichoderma* Spp. *Bulgarian Journal of Agricultural Science*, 13: 171-176.

Hölker U, Dohse J and Höfer M (2002) Extracellular laccase in ascomycetes *Trichoderma atroviride* and *Trichoderma harzianum*. *Folia Microbiologica*, 47(4):423-427.

Ingle MR and Mishra RL (2016) Production of laccase by *Trichoderma erinaceum*. *Indian Journal of Applied Research*, 6 (10): 141-144.

Kiiskinen LL, Ratto M and Kruus K (2004) Screening for novel laccase producing microbes. *Journal of Applied Microbiology*, 97: 640-646. DOI: 10.1111/j.1365-2672.02348.x.

Kunamneni A, Ballesteros A, Plou FJ and Alcalde M (2007) Fungal laccase – a versatile enzyme for biotechnological applications. In: A. Méndez-Vilas Ed. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, Spain: Formatex, Badajoz, 1:233-245.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the bacteriophage T4. *Nature*, 227 (5259):680-685.

Levin L, Melignani E, Ramos AM (2010) Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white - rot fungi: Dye decolorization by selected culture filtrates. *Bioresource Technology*, 101:4554-4563.

Mayer AM and Staples RC (2002) Laccase: new functions for an old enzyme. *Phytochemistry,* 60:551-565.

More SS, Renuka PS, Pruthvi K, Swetha M, Malini S and Veena SM (2011) Isolation, Purification and Characterization of Fungal Laccase from *Pleurotus* sp. *Enzyme Research*, 2011:1-7. DOI:10.4061/2011/248735

Nagai M, Sato T, Watanabe H, Saito K, Kawata M, Enei H (2002) Purification and characterization of an extracellular laccase from the edible mushroom Lentinula edodes and decolorization of chemically different dyes. *Applied Microbiological Biotechnology*. 60 (3): 327-35.

Niku- Paavola ML, Karhunen E, Salola P and Raunio V (1988) Ligninolytic enzymes of the white-rot fungus *Phlebia radiata. Biochemical Journal*, 254:877-884.

Niladevi KN and Prema P (2007) Effect of inducers and process parameters on laccase production by *Streptomyces psammoticus* and its application in dye decolorization. *Bioresource Technology*. 99:4583-4589.

Palmieri G, Giardina P, Bianco C, Fontanella B and Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. *Applied and Environmental Microbiology*, 66(3): 920-924.

Pointing SB (1999) Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Diversity*, 2: 17-33.

Rao DS, Panda T and Subba-Rao D (1993) Comparative analysis of calcium gluconate and sodium gluconate techniques for the production of gluconic acid *Aspergillus niger*. *Bioprocess Engineering*, 8:203-207.

Rogalski J, Lundell T, Leonowicz A and Hatakka A (1991) Influence of aromatic compounds and lignin on production of ligninolytic enzymes by *Phlebia radiata*. *Phytochemistry*, 30: 2869-2872.

Rogalski J and Leonowicz A (1992) *Phlebia radiata* laccase forms induced by veratric acid and xylidine in relation to lignin peroxidase and manganese- dependent peroxidase. *Acta Biotechnology*,12: 213- 221.

Ruiz-Dueñas FJ and Martínez ÁT (2009) Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. *Microbial Biotechnology*, 2 (2):164-177.

Sadhasivam S, Savitha S, Swaminathan K and Lin F (2008) Production, purification and characterization of mid-redox potential laccase from a newly isolated *Trichoderma harzianum* WL1. *Process Biochemistry*, 43:736-742.

Sandhu DK and Kalra MK (1982) Production of cellulose, xylanase and pectinase by *Trichoderma longibrachiatum* on different substrates. *Transactions of the British Mycological Society*, 79(3): 409-413.

Sivakumar R, Rajendran R, Balakumar C and Tamilvedan M (2010) Isolation, screening and optimization of production medium for thermostable laccase production from *Ganoderma* sp. *International Journal of Engineering Science and Technology*. 2(12):7133-7141.

Sokorobogat'ko OV, Stepanova EV, Gavrilova VP and Yaraplov AI (1996) Effects of inducers on the synthesis of extracellular laccase by *Coriolus hirsutus*, a basidial fungus. *Applied Biochemistry and Microbiology*, 32:524-528.

Solomon EI, Sundaram UM and Machonkin TE (1996) Multicopper oxidases and oxygenase. *Chemical Reviews*, 96 (7):2563-2606.

Sun S, Zhang Y, Que Y, Liu B, Hu K and Xu L (2013) Purification and characterization of Fungal Laccase from *Mycena purpureofusca*. *Chiang Mai Journal of Science*, 40(2):151-160.

Thurston CF (1994) The structure and function of fungal laccases. *Microbiology*, 140:19-26.

Tien M and Kirk TK (1988) Lignin peroxidase of *Phanerochaete chrysosporium*. In: Wood, Willis A.; Kellogg, Scott T., eds. *Methods in enzymology*, San Diego, CA: Academic Press, Inc.:161:238-249..

RESEARCH ARTICLE

Diversity of AM Fungi in *Sesamum indicum* L. from Sanjay Gandhi National park, Borivali, Mumbai, Maharashtra, India

Belose Shweta*and Chahar Sunita

NES Ratnam College of Arts, Science and Commerce, Bhandup (West), Mumbai, India *Corresponding author
Email: beloseshweta@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Belose Shweta and Chahar Sunita (2016) Diversity of AM Fungi in *Sesamum indicum* L. from Sanjay Gandhi National park, Borivalli, Mumbai, Maharashtra, India, Int. J.of. Life Sciences, Special Issue, A7:47-51

Acknowledgement: The authors are thankful to the Principal, NES Ratnam College of Arts, Science and Commerce for providing lab and shade house facilities for carrying out the work

ABSTRACT

The present study was carried out to evaluate the status of Arbuscular Mycorrhizal Fungi (AMF) colonizing the rhizosphere of *Sesamum indicum* L. by setting up soil trap culture and to compare the species diversity in trap cultures with natural conditions. *Sesamum indicum* L. is a common wild medicinal herb, belonging to family Pedaliaceae. The study revealed that spore number was amazingly high in natural conditions. It was found to be 135spores/10g soil. The trap cultures after 3 months showed only 90 spores as against expected. The type of spores found in the natural conditions belonged to ten different types of species .The spores isolated from trap cultures belonged to only seven species. The spores isolated from the natural rhizospheric soil belonged to *Glomus geosporum, Glomus callosum, Glomus clarum, Glomus tortuosum , Acaulospora myriocarpa, Ambispora appendicula, Scutellospora sp. Gigaspora margarita, Glomus clavisporum, Kuklospora sp* . The spores isolated from trap cultures belonged to *Glomus halonatum, G. constrictum , G.aureum, Gigaspora decipiens , Ambisporum, Pacispora boliviana and Acaulospora sp*. The study of trap culture demonstrated that all AM Fungi do not sporulate in three months period , but had good amount of root colonization percentage. The sections of roots from trap cultures showed plenty of fungal hyphae, vesicles and arbuscules. Other endophytes with swollen hyphae and bulbous structures were observed in natural and trapped cultures.

Key words: AM Fungi, *Sesamum indicum* L., *Kuklospora, Ambispora, Glomus clavisporum*

INTRODUCTION

Arbuscular mycorrhizal Fungi are widely distributed in terrestrial ecosystems and can be found in both natural and agricultural areas. Arbuscular Mycorrhizal Fungi (AMF) belonging to the phylum Glomeromycota are important soil organisms that form mutualistic associations with plants, and which are involved in the uptake and transport of mineral nutrients to plant roots (Barea *et al*. 2002). Up to 90 % of analysed plant species are able to form this symbiosis (Smith & Read

1997). AM Fungal ubiquitous presence and their taxonomic, genetic and functional diversity are directly related to plant and soil processes and therefore there is an increasing interest in the assessment of the biodiversity and functions of AM Fungal communities. Although biodiversity has been a major research topic in terrestrial ecology, it has been largely ignored in terms of soil biota mainly in the tropical regions (Patrícia Lopes Leal *et al*.,2009).

The documentation of new patterns of species distribution is necessary for the accurate estimation of the diversity and distribution of this important group of symbiotic fungi. Diversity of AM Fungal species is measured mainly by extracting, counting and identifying their field collected asexual spores, the fungal propagule that possess morphological characters to define species in this group of organisms although molecular techniques have been revealed as useful tool for characterization and identification of AM Fungi.

Field-collected spores, however, are found in some circumstances in low numbers, parasitized, lacking informative taxonomic characteristics impairing a more accurate identification as components of spore walls are susceptible to alteration and deterioration by a wide sort of agents in the soil. Establishment of trap cultures using bulk soil or by mixing rhizosphere soil and root pieces with sterilized diluents and growing with suitable hosts, represents a strategy to yield a large number of healthy spores which can be readily identifiable and supplement the assessment of local species diversity in different ecosystems. This methodology not necessarily allows the identification of all species, because sporulation of the fungal community may be affected by the plant host chosen for trapping whereas in some cases it can promote the sporulation of cryptic AM Fungal species that were not sporulating at the sampling time or natural field conditions. Despite this, trap cultures have been widely used to access AMFungal diversity and isolate indigenous AMFungi (Patrícia Lopes Leal *et al*., 2009).The purpose of this work was to record the number and type of AM Fungal species that colonized *Sesamum* plant in Sanjay Gandhi National Park.

MATERIALS AND METHODS

Soil sampling : Root samples and rhizosphere soil of *Sesamum* was collected from Sanjay Gandhi National Park, Borivali, Mumbai and preserved in sterile polythene bags and stored in refrigerator at 4^0 C until use. Soil sample up to 20 cm depth was collected . Root samples were cut into 1cm bits and preserved in FAA until use.

Trap Culture was done by Rodrigues & Muthukumar method, 2009. It is frequently observed that isolation of spores directly from field soil has drawbacks viz. they appear intact but may not be viable as they may persist in the soil as spore cases for years, they change appearance in their structural characters in response to root pigments, soil chemistry, temperature, moisture and microbial activity. Therefore trap cultures have to be prepared to recover intact , fresh and healthy spores.

Spore extraction was done by Gerdeman and Nicolson method ,1963 and t he spore number was counted by Gaur and Adholeya method ,1994, Taxonomic identification of spores up to species level was made using the identification manual of Schenck and Perez and description provided by the International collection of VAM (INVAM).

Root Colonization of AM Fungi was done by Philips and Hayman method, 1970 and percentage of root colonization was calculated by Read *et al*.,1976.

RESULTS AND DISCUSSION

Ten species of AM Fungi were identified from natural field soil (fig 1) and seven species sporulated in 3 months period in trap cultures (fig 2). The species identified are listed in the table. Spore density was also less in trap culture compared to natural conditions. Percent root colonization was less in trap cultured roots of *Coleus* compared to the *Sesamum* from field conditions. Cuenca et al. (2003) reported that AM Fungal propagation in trap cultures exhibits difficulties because exact natural conditions cannot be reproduced; this causes a bias towards proliferation of species that are able to tolerate greenhouse conditions, and that associate better with the plant host under the specific trap culture conditions.

Table 1: AM Fungal status of *Sesamum indicum* L.

Sr. No.	Habitat	AMF Structure in Roots				% of Root Colonization	Spore Density	No. of Spore Sps.	Type of Spore Species
		H	A	V	Endophytes				
1	Natural Rhizosperic Soil	+++	--	++	+	100%	135±14.5	10	*Glomus geoporum, G.callosum,G. clarum, G. tortuosum, Acaulospora sp, Ambispora appendicula, Scutellospora sp. Gigaspora margarita, Glomus clavisporum, Kuklospora sp.*
2	Trap Culture	+++	+++	++	+	90%	90±5.57	7	*Glomus halonatum, G. constrictum, G. aureum, Gigaspora decipiens,, G.ambisporum Pacispora boliviana, Acaulospora sp.*

+Poor, ++Moderate, +++Good, ++++ Excellent, - Absent

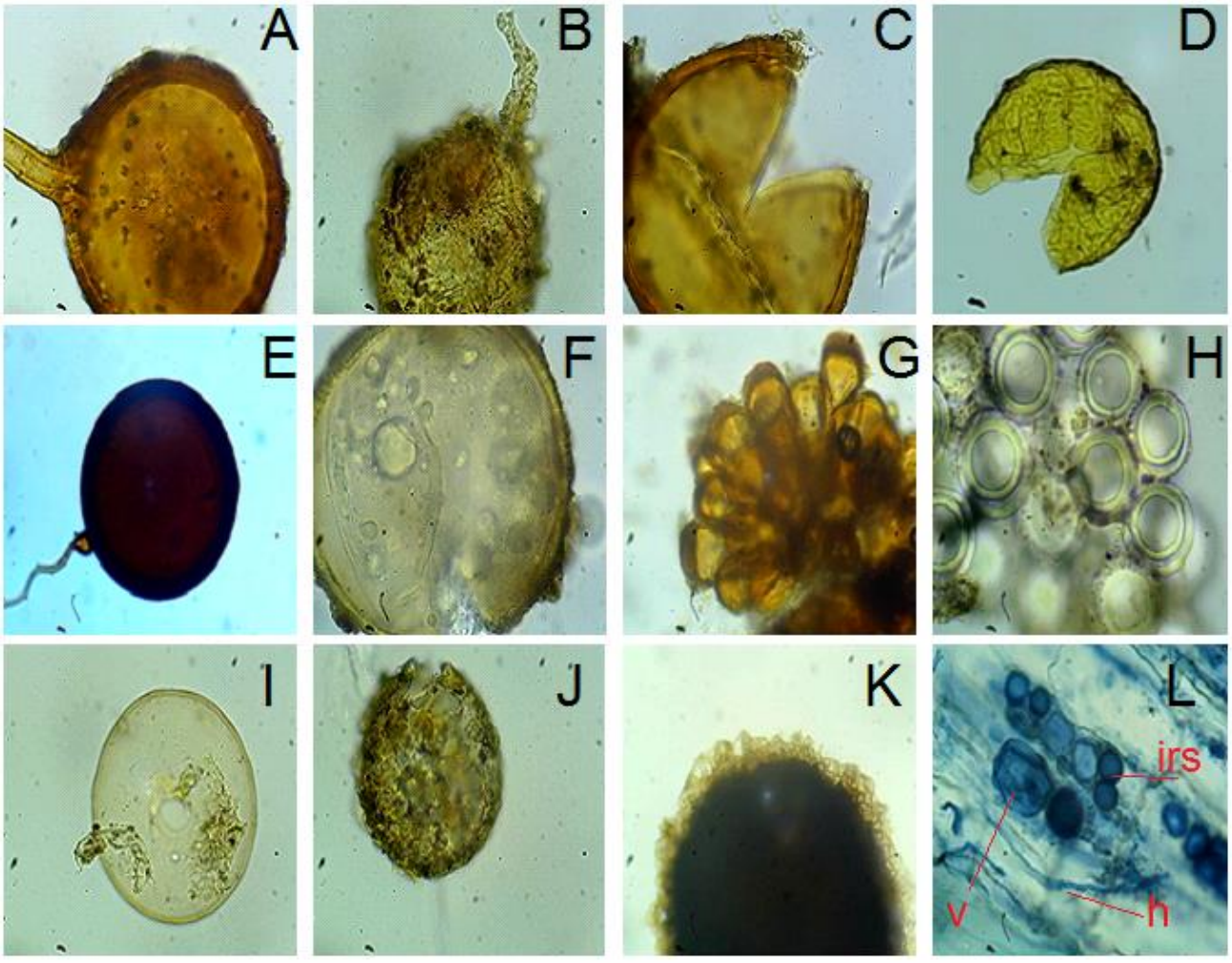


Fig. 1. AM fungal status of *Sesamum indicum* L. in Natural habitat. **A.** *Glomus geosporum* **B.** *Glomus callosum* **C.** *Glomus clarum* **D.** *Ambispora appendicula* **E.** *Gigaspora margarita* **F.** *Acaulospora sp* **G.** *Glomus clavisporum* **H.** *Kuklospora sp* I. *Scutellospora sp* **J.** *Acaulospora sp* **K.** *Glomus* tortuosum **L.** AMF-colonized roots showing different internal structures indicated by arrows: hyphae **(h)**, and vesicles **(v)** ,intraradical spores **(irs).**

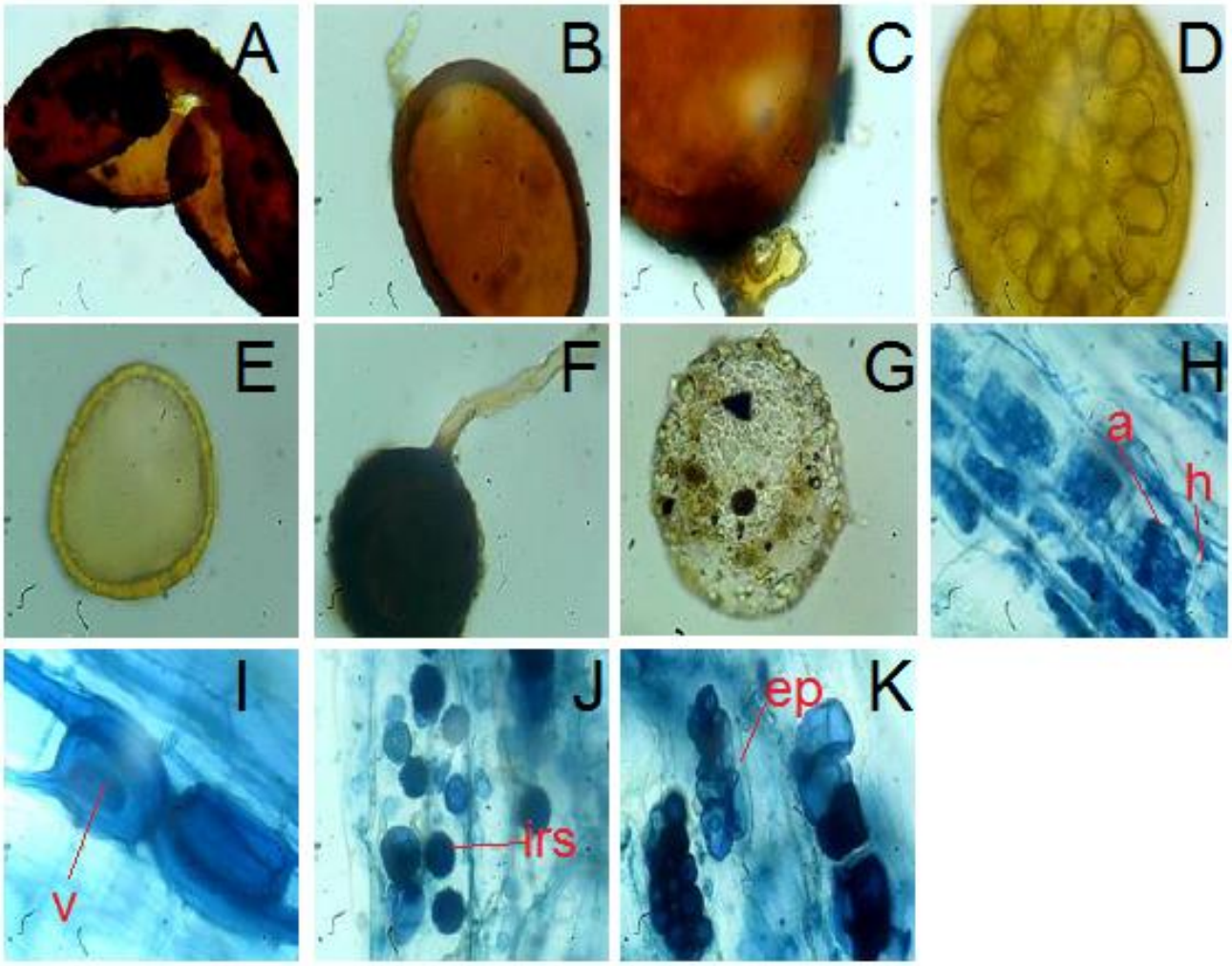


Fig. 2. AM fungal status of *Sesamum indicum* L. in Trap Culture. **A.** *Gigaspora decipiens* **B.** *Glomus halonatum* **C.** *Glomus constrictum* **D.** Sporocarp of Glomus aureum **E.** *Pacispora boliviana* **F.** *Glomus ambisporum* **G.** *Acaulospora sp* AMF-colonized roots showing different internal structures **H.** hyphae **(h)**, arbuscule **(a)** **I**. vesicles **(v)** J. intraradical spores **(irs)** **K.** endophytes **(ep).**

In the present study, the mycorrhizal structures observed were arbuscules, vesicles and hyphae in trap cultures. The arbuscule percentage was very poor whereas vesicle percentage was excellent. The vesicles were elliptical type with oil droplets very clearly seen. The arbuscules were of coiling (Paris) type. Some endophytes were also observed in roots. Very few studies describe the selective pressure on AM Fungal species when they are taken from their natural environments to trap cultures (Antunes,2012, Oliveira 2010). *Acaulospora and Glomus* genera were common in both Natural habitat and Trap culture. In trap culture *Glomus halonatum, G. constrictum, G. aureum, Gigaspora decipiens, Pacispora boliviana* were found to be sporulating which were not observed in natural habitat. According to Adholeya 1994, the non-sporulating species can often be coaxed to sporulate in 'trap cultures'. The genus *Glomus* is the most dominant AMFungal genera isolated and identified in *Sesamum* followed by *Acaulospora.*

CONCLUSION

Sesamum indicum L. is a medicinal herb. The soil trap culture using *Coleus* as the host plant showed presence of mycorrhizal structures - coiling (Paris) type of arbuscules, hyphae and elliptical intra and

intercellular vesicles along with Intraradical spores. Arbuscules were not observed in the roots from field.

The spores isolated from trap cultures belonged to *Glomus halonatum, G. constrictum , G.aureum, Gigaspora decipiens , Ambisporum, Pacispora boliviana and Acaulospora sp*.

The spores isolated from the natural rhizospheric soil belonged to *Glomus geosporum, Glomus callosum, Glomus clarum, Glomus tortuosum , Acaulospora myriocarpa , Ambispora appendicula, Scutellospora sp. Gigaspora margarita, Glomus clavisporum, Kuklospora sp*

REFERENCES

Barea JM, Azcón R, Azcón-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antone van Leeuwenhock81: 343–351.

Rodrigues BF and Muthukumar T (2009) A manual of identification protocols, Goa University.

Cuenca G, de Andrade Z, Lovera M, Fajardo L, Meneses E, Márquez M, Machuca R (2003) Pre-selección de plantasnativas y producción de inóculos de hongosmicorrízicos arbusculares (HMA) de relevanciaen la rehabilitación de áreasdegradadas de La Gran Sabana, estado Bolívar, Venezuela. *Ecotrópicos*16:27–40.

Gaur A and Adholeya A (1994) Estimation of VAM spores in the soil - A modified method. Mycorrhiza News 6(1): 10-11.

Gerdemann JW and Nicolson TH (1963) Spores of mycorrhizal *Endogone species* extracted from soil by wet sieving and decanting. *Trans. Br. MycoL Soc.* 46:235-244.

Hemavani C and Thippeswamy B (2013)International Journal of Plant, Animal and Environmental Sciences, 3(2),2013,pp 105-110

Oliveira RS, Boyer LR, Carvalho MF, Jeffries P, Vosatka M, Castro PML, Dodd JC (2010)Genetic, phenotypic and functional variation within a *Glomus geosporum*isolate cultivated with or without the stress of a highly alkaline anthropogenic sediment. *Applied Soil Ecology* 45: 39–48.

Patrícia Lopes Leal, Sidney Luiz Stürmer, José Oswaldo Siqueira (2009) Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the amazon, Brazil Brazilian Journal of Microbiology,40:111-121.

Phillips JM and Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. MycoL Soc. 55:* 159-161.

Smith SE and Read DJ (1997) Mycorrhizal Symbiosis. Second edition. Academic Press. London, 605 pp.

RESEARCH ARTICLE

Fusarium oxysporum as a potential fungus for bioremediation

Usmani Asra and Sashirekha S*

Department of Botany, Mithibai College, Vileparle [West], Mumbai 400056
*Corresponding author Email: sashirekhas52@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Usmani Asra and Sashirekha S (2016) *Fusarium oxysporum* as a potential fungus for bioremediation, *Int. J.of. Life Sciences, Special Issue,* A7:52-56

ABSTRACT

Fungi occupy many different ecological niches. Fungi are well known to tolerate and detoxify metals by several mechanisms including extra- and intracellular precipitation, and active uptake. In the present paper soil fungi is isolated from forest soil samples from different locations of SGNP. *Fusarium oxysporum* was examined for its pH tolerance, Metal tolerance and biosorption capacity to Zn and Pb metals. The effect of the metal pollutants on the fungi and response of the fungi was analysed using ICP AES. The changes in the mycelial characteristics were observed. *Fusarium oxysporum* proves to be a potential candidate for bioremediation

Key words: *Fusarium oxysporum*, biosorption

INTRODUCTION

Metals may be present in soils as free metal ions, complexes with organic matter or they may be chemically precipitated into insoluble compound such as oxalates, carbonates and hydroxides. The degree of toxicity of the metal to the organisms depends on its relative availability (solubility) within the soil solution. This availability is dependent upon edaphic factors such as pH, organic matter and clay content. Soil microfungi are able to tolerate concentrations of various metals and less sensitive at higher pH levels. Fungi are able to restrict the entry of toxic metal species into the cells by extracellular metal sequestration, binding to the cell wall, reducing its uptake by intercellular chelation (Berthlin *et al* 1995; Gadd 2007).

Fungi which are a major and often dominant component of the microbiota of soil are important as decomposer organism, animal and plant symbionts and pathogens and they play an important role in biogeochemical cycle of elements. Certain fungal processes solubilise metal from minerals and bound locations thereby increasing metal bioavailability, whereas other fungal processes immobilize and reduce their bioavailability (Fomina *et al* 2005). Naturally fungi have a variety of extracellular proteins, organic acids and other metabolites. Fungi can adapt in any ecosystem and any environmental conditions.

Rapid urbanization and industrialization has led to increased occurrence of heavy metals in the environment. The resultant degradation and contamination of ecosystem become a major threat to all living organism globally open water and aquatic ecosystems are contaminated with several heavy metals to human activities. Bioremediation is considered alternative processing methods for removing the heavy metal ions from polluted areas(Hardman et al 1993).

The present paper focuses on the role of *Fusarium oxysporium* as potential fungi for bioremediation. The fungi was investigated for the metal tolerance capacity, biosorption capacity and the changes in mycelia characteristics.

MATERIALS AND METHODS

Soils samples from different locations in the forest of Sanjay Gandhi National Park were collected.

Soil sampling

Soil samples were collected by taking composite samples up to a depth of 10cm, after scraping off 5cm of surface soil with a sterile trowel. Soil samples were collected in fresh polyethene bags. The soils were brought to the laboratory for further study. Soil was dried and then used for isolating soil fungi.

Isolation of fungi

Soil fungi were cultured on PDA, soil agar and Czapek dox .The Petri plates were incubated at 28^0 C for 7 days. The fungi were isolated and maintained as pure cultures. They were examined under light microscope and were identified using standard manuals. Identification of soil fungi was done on the basis of microscopic and colony characteristics and the basic identification keys were used. Digital Photographs of fungi were taken using MIPS.

Isolated soil fungi were *Absidia, Acremonium sp, Alternaria sp, Aspergillus sp, Cladosporium sp, Cylindrocarpon, Fusarium oxysporum, Fusarium solani, Geotrichum sp, Gliocladium sp, Mucor, Paecilomyces, Penicillium sp, Rhizopus, Trichoderma ,Verticillium.* It was observed that *Fusarium oxysporum* was found in most of the collected soil samples. Of the isolated fungi *Fusarium oxysporum* was selected for further experiments.

pH tolerance

F.oxysporum was cultured on PDA with varying pH to check its tolerance .pH was adjusted in the range of 5 to 14 using Na_2CO_3.Cultures were grown for 15 days. Growth of the fungus in pH 5 to pH 14 was expressed in terms of biomass weight. It was observed that *F.oxysporum* is growing in pH 5 -11. (Table 1)

Metal tolerance and biosorption capacity

Fusarium oxysporum cultures were grown on Czapek dox broth (CZB) amended with metal solutions (Zn and Pb) of varying concentrations (200, 400, 600, and 800 ppm). Control flask containing Czapek dox broth inoculated with culture was kept as reference. The flasks were kept at 28+-2 c for 15 days on shaker. The biomass was harvested and filtered through Whatman no. 42 filter paper after 15 days of growth. The weight of biomass in each concentration was determined.

The harvested mat was oven dried until a constant weight was reached. After the dry weight was measured 1 gm of dried mat was digested in 10 ml of concentrated nitric acid. After appropriate dilutions with double distilled water, metal ions contents of the solutions and culture filtrates were analyzed for the presence of Zn and Pb using Inductive coupled plasma (ICP AES) at SAIF IIT Mumbai.

Determination of metal tolerance index

The metal tolerance index (MTI) was determined to assess the ability of the fungi to grow in the presence of a given concentration of Pb and Zn according to the following equation:

$$\text{MTI (\%)} = \frac{\text{DW of treated culture}}{\text{DW of control culture}} \times 100$$

Where DW is the dry weight of the culture

Determination of biosorption capacity

The biosorption capacity of Zn and Pb was calculated using the formula

$$Q = \frac{(ci - cf)}{m} V$$

Where Q = milligram of metal ions uptake per gram biomass (mg/g)

C_i = initial concentration of metallic ions (mg/L)

C_f = final concentration of metallic ions (mg/L)

m = dried mass of biosorbent in reaction mixture (gm)

V = volume of reaction mixture (ml)

Change in mycelial characteristics of *Fusarium oxysporum*

The mycelia from the different flasks of varying concentrations were harvested after 15 days growth. Changes in the morphological characteristics of the mycelium were noted using light microscope.

RESULTS AND DISCUSSION

25 Soil samples were collected from different locations of Sanjay Gandhi National Park forest. Around 35 fungi were isolated in culture. Of all the isolated fungi, *Fusarium oxysporum* (**Fig. a**) was observed in all the soil samples. Tolerance of this fungus ranged from pH 5 to pH 11. Growth in the different pH was measured as biomass weight (**Table 1**). This shows the versatility of *Fusarium oxysporum* to adapt to acidic and basic pH conditions.

The culture was subjected to varying concentrations of Zn and Pb to check the metal tolerance index. It was observed that metal tolerance index for Zn in 200 ppm was 51% , 400ppm 28%,600 ppm 7% and in 800 ppm 3.7 % showing decrease in metal tolerance index with increase in concentration.(**Table 2**)

In Pb amended media it was observed 200 ppm was 53% , 400ppm 25%,600 ppm 11% and in 800 ppm 1% showing decrease in metal tolerance index with increase in concentration.(**Table 3**).

Biosorption capacity of the Zn and Pb in the different concentrations 200, 400, 600 and 800 was 8.8, 7.4, 3.2 and 7.8 for Zn. Similarly for Pb it was 3.0, 3.8, 6.0 and 11.6 respectively.

Certain fungal processes solubilise metals from minerals and bound locations, thereby increasing metal bioavailability, whereas other fungal processes immobilize metals and reduce their bioavailability. Flexible mycelial growth strategies and the ability to produce and exude organic acids, protons, and other metabolites make fungi important biological weathering agents of natural rock, minerals, and building materials.

Table 1: pH tolerance of *F.oxysporum*

pH	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Biomass (mg)	8.5	9.2	8.8	6.2	4.1	2.8	1.2	00	00	00

Table 2: Metal tolerance index of *F.oxysporum* Zn amended media

Concentration(ppm)	**Wet weight(mg)**	**Dry weight(mg)**	**MTI (%)**
200	5.9	3.8	51
400	4.61	2.1	28
600	1.734	0.583	7
800	0.938	0.278	3.7
Control	15.26	7.45	--

Table 3: Metal tolerance index of *F.oxysporum* Pb amended media

Concentration(ppm)	**Wet weight(mg)**	**Dry weight(mg)**	**MTI (%)**
200	6.24	3.98	53
400	4.12	1.92	25
600	1.24	0.893	11
800	0.544	0.123	1
Control	15.26	7.45	--

Table 4: Biosorption capacity of *F.oxysporum* in metal amended media

Concentration	**200 ppm**	**400 ppm**	**600 ppm**	**800 ppm**
Zn amended media (mg/g)	8.8	7.4	3.2	7.8
Pb amended media (mg/g)	3.0	3.8	6.0	11.6

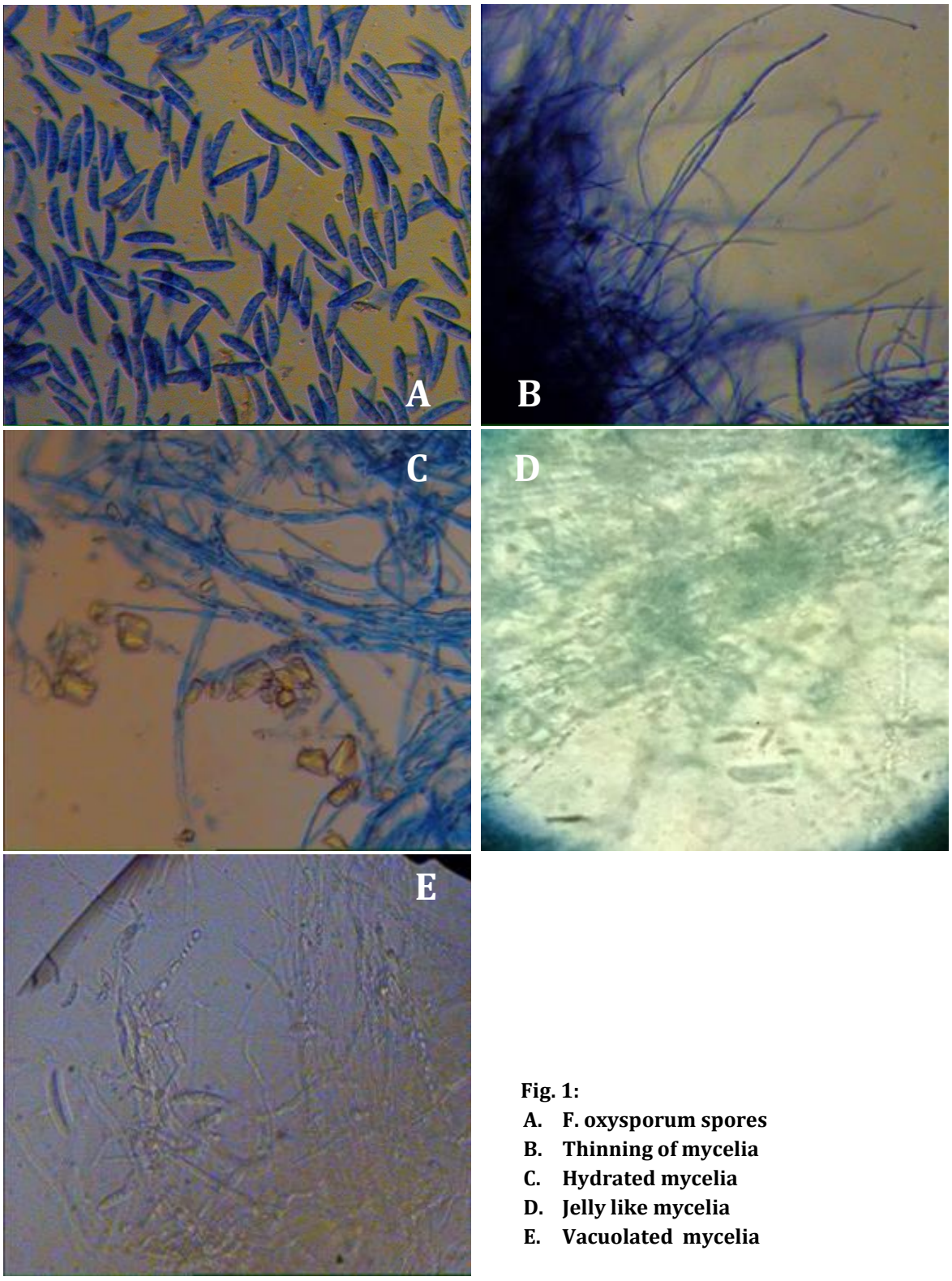


Fig. 1:

A. F. oxysporum spores
B. Thinning of mycelia
C. Hydrated mycelia
D. Jelly like mycelia
E. Vacuolated mycelia

In *F.oxysporum* the following changes in the mycelia were observed. Mycelia Straight branched length 12µ m and 4.5 µm in control culture, whereas the growth with metal media (200 and 400ppm) it has shown the changes in mycelia thin dense soft vacuolated, mucilaginous 12µm length and 4.5µm width spore size 54µm length and 4.5µm width. Formation of mycelia covered by a thick hydrated mucilaginous sheath leading to formation of jelly like mass which provide a micro environment for chemical reactions, crystal depositions and growth.(**Figs b, c, d, e**,) Thereby leading to reduced mobilization of metals.

CONCLUSION

As mineral component contain considerable quantities of metals as well as other elements which are biologically unavailable, the influence of such processes on metal mobility are of economic and environmental significance and may be important in the treatment of contaminated soil. From the above observations *F.oxysporum* proves to be a potential candidate for bioremediation.

REFERENCES

Amna Javaid *et al,* (2010), Biosorption of heavy metals using A dead macro fungus *Schizophyllum commune fries: evaluation* of equilibrium and kinetic models, Pak. J. Bot., 42(3): 2105-2118.

Berthelin J Munier-Lamy C.Leyval C (1995), Effects of microorganism on mobility of heavy metal in soils.In :Huang PM,Berthelin J, Bollag JM, McGill WB (eds) Metals , other metal in soils. Inorganics, and microbial activities : Environmental impact of soil component interactions.Lewis, Boca Raton, pp 3-17.

Dilna Damodaran, Raj Mohan Balakrishnan, and Vidya K. Shetty, (2013), The Uptake Mechanism of Cd(II), Cr(VI), Cu(II), Pb(II), and Zn(II) by Mycelia and Fruiting Bodies of *Galerina vittiformis,* BioMed Research International Volume, Article ID 149120, 11 pages

Fomina M, Burford EP and Gadd GM. Toxic metals and fungal communities in J. Dighton , J.White and P. Oudemans (Ed.) , The fungal community: its organization and role in ecosystem, in press. CRC Press, Boca Raton, Fla.

Gadd GM (2015) Fungi in Bioremediation, Fungal remediation of soils contaminated with persistent organic pollutants pp. 79-96 Cambridge University Press,

Iram Shazia, Uzma *et al,* ((2013), Bioremediation of Heavy Metals Using Isolates of Filamentous Fungus *Aspergillus fumigatus Collected from Polluted Soil of Kasur, Pakistan, International Research Journal of Biological Sciences ISSN 2278-3202* Vol. **2**(12), 66-73.

Fomina M, Hillier S, Charnock JM, Melville K, Alexander IJ and Gadd GM (2005) Role of oxalic acid overexcretion in transformation of toxic metal minerals by *Beauveria caledonica,* vol **71** (1), 371-381

Shazia Iram, Kousar Perveen *et al,* (2013) Tolerance potential of different Species of *Aspergillus* as bioremediation tool - Comparative analysis, E3 Journal of Microbiology Research Vol. **1**(1). pp. 001-008..

RESEARCH ARTICLE

Antifungal effect of phyto-fabricated Silver Nanoparticles

Maurya C and Pius J

Department of Botany, Ramnarain Ruia College, Matunga, Mumbai-19, India
Mauryachanda10@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Maurya C and Pius J (2016) Antifungal effect of phyto-fabricated Silver Nanoparticles, Int. J.of. Life Sciences, Special Issue, A7: 57-60

Acknowledgements
The authors are sincerely grateful to the Principal and Staff of Department of Botany, R. Ruia College for their whole hearted support and cooperation. We, also express our gratitude to IIT- Bombay,CIRCOT, BARC for their help in various analysis

ABSTRACT

Silver ions have long been known to possess broad spectrum antimicrobial activities. In the present work Silver nanoparticles were fabricated using four different organic wastes. Synthesized particles were characterized by UV-Vis spectroscopy and TEM analysis. Antifungal effects of the synthesized silver nanoparticles were carried out by MIC and Radial growth assay method. The obtained result showed that the MIC values against *F. oxysporum* was 40ppm and against *A.niger* at 50 ppm. *F. oxysporum* was found to be more sensitive to all synthesized AgNPs compared to *A.niger.* Percentage inhibitory activity to radial growth was 100% for both the fungi at 40 and 50ppm of AgNPs.

Keywords: Antifungal, Antimicrobial**,** Silver nanoparticles

INTRODUCTION

Silver ions and silver salts are well-known antimicrobial agents (Silver and Phung 1996) in various fields due to their growth inhibitory abilities against microorganisms. The antimicrobial nature of silver nanoparticles is the most exploited in the medical field. Many studies have reported that AgNPs can damage cell membrane of microorganisms leading to structural changes, which render them more permeable (Hashimoto *et al.,* 2012 and Lazer, 2011). This effect is highly influenced by the nanoparticles' size, shape and concentration (Lu *et al.,* 2010). In the present work silver nanoparticles (AgNPs) were fabricated from different organic wastes (a green approach). These fabricated particles were tested for their antifungal activity against selected fungi.

MATERIALS AND METHOD

Synthesis and Characterization of silver nanoparticles:

Silver nanoparticles (AgNPs) were fabricated using extracts of Banana stem (BS), cauliflower leaf (CL), pigeon pea seed coat (PP-SC) and saw dust (SD) with 1mM $AgNO_3$ solution. The change in colour from colourless to yellow indicated the formation of AgNPs. The nanoparticles were characterized using UV-Visible Spectroscopy (Shimadzu UV 1800) and TEM (Transmission electron microscope) analysis. Particles with an

average of less than 30nm size were used for the study. 10-100 ppm of AgNPs synthesized was used for the study.

Fungal cultures:
Aspergillus niger (NFCCI 161) and *Fusarium oxysporum* (NFCCI 245) cultures were obtained from Agarkar Institute, Pune and was maintained on Potato Dextrose Agar (PDA) medium.

Minimum Inhibitory Concentration (MIC) was carried out by broth dilution method (Astiti and Suprapta, 2012 - modified**).** After 8 days, the concentration at which the fungal growth was inhibited was considered as the minimum inhibitory concentration (MIC) which was determined by visual observation.

Radial growth rate assay (Miyashira *et al.,* 2010):
Sterile Petri dishes were prepared with 15 ml of culture medium and 30, 40, 50 and 70 ppm of AgNPs. After the medium solidified, a mycelial plug (5mm in diameter) of *A.niger* and *F. oxysporum* was placed in the centre of the plate. The cultures were incubated for 7 days in the dark at room temperature. After one week the diameter of fungal colony was measured. The inhibitory activity by the radial growth (IR) was determined according to the formula.

$$IR\% = \frac{dc-dt \times 100}{dt}$$

Where, IR= inhibitory activity to the radial growth
dc = average increase in mycelia growth in control plates.
dt= average increase in mycelia growth in treated plates.
Simultaneously a positive control was run with antifungal bavistin and a negative control plates was run without the AgNPs and bavistin.

Statistical analysis: The data were subjected to Analysis of Variance (ANOVA). Statistical analysis was performed using IRRISTAT software (IRRI,2003). Treatment means were compared using Least Significance Difference (LSD) values at $p \leq 0.05$. Differences among treatments were tested by Ducan's New Multiple Range Test (DMRT). In the tables given in results, mean values followed by same alphabets in superscript (a,b,c,d...) within a column are not significantly different at ≤ 0.05 level.

RESULTS AND DISCUSSION

Synthesized nanoparticles were characterized using UV-Visible Spectroscopy and TEM analysis. The particles showed absorption maxima at 415-445 nm and the average size was found to be in the range of 5 to 30 nm. It was evident that irrespective of source of synthesis of AgNPs, all synthesized AgNPs were very active against the selected fungi. Antifungal activity of the synthesized AgNPs in terms of MIC ranged between 40-60 µg/ml (Table 1). The obtained result showed that MIC of all AgNPs synthesized irrespective of their source, against *F. oxysporum* was 40µg/ml and against *A. niger* MIC ranged between 50-60 µg/ml. Synthesized AgNPs were more active against *F. oxysporum* than *A. niger*.

Radial growth rate assay
The results of determination of Radial growth rate assay of AgNPs fabricated from the various organic wastes against *A.niger* and *F. oxysporum* have been tabulated in table 2& Fig.1. *F. oxysporum* was found to be more sensitive to all synthesized AgNPs compared to *A.niger.* Both fungi showed 100% inhibition in growth with selected concentration of AgNPs.

Table 1: Minimum inhibitory concentration of fabricated AgNPs from organic waste extracts against *A. niger* and *F. oxysporum.*

Source of fabrication of AgNPs	MIC of AgNPs (µg/ml)	
	A.niger	***F. oxysporum.***
BS-AgNPs	60[a]	40[a]
CL-AgNPs	50[b]	40[a]
PP(SC)-AgNPs	60[a]	40[a]
SD-AgNPs	50[b]	40[a]
LSD p=0.05	0.987	1.31

Table 2 Radial growth inhibition percentage of AgNPs synthesized from different organic wastes against *A. niger* and *F. oxysporum.* (Values in the column in parenthesis are arcsine transformed values and were used for comparing treatments by ANOVA).

	Radial inhibition rate of AgNPs (%)	
AgNPs	***A.niger***	***F. oxysporum***
BS-AgNPs	100[a] (96.98)	100[a] (96.98)
CL-AgNPs	100[a] (96.98)	100[a] (96.98)
PP(SC)-AgNPs	100[a] (96.98)	100[a] (96.98)
SD-AgNPs	100[a] (96.98)	100[a] (96.98)
Control	0.00	0.00
LSD	0.00	0.00

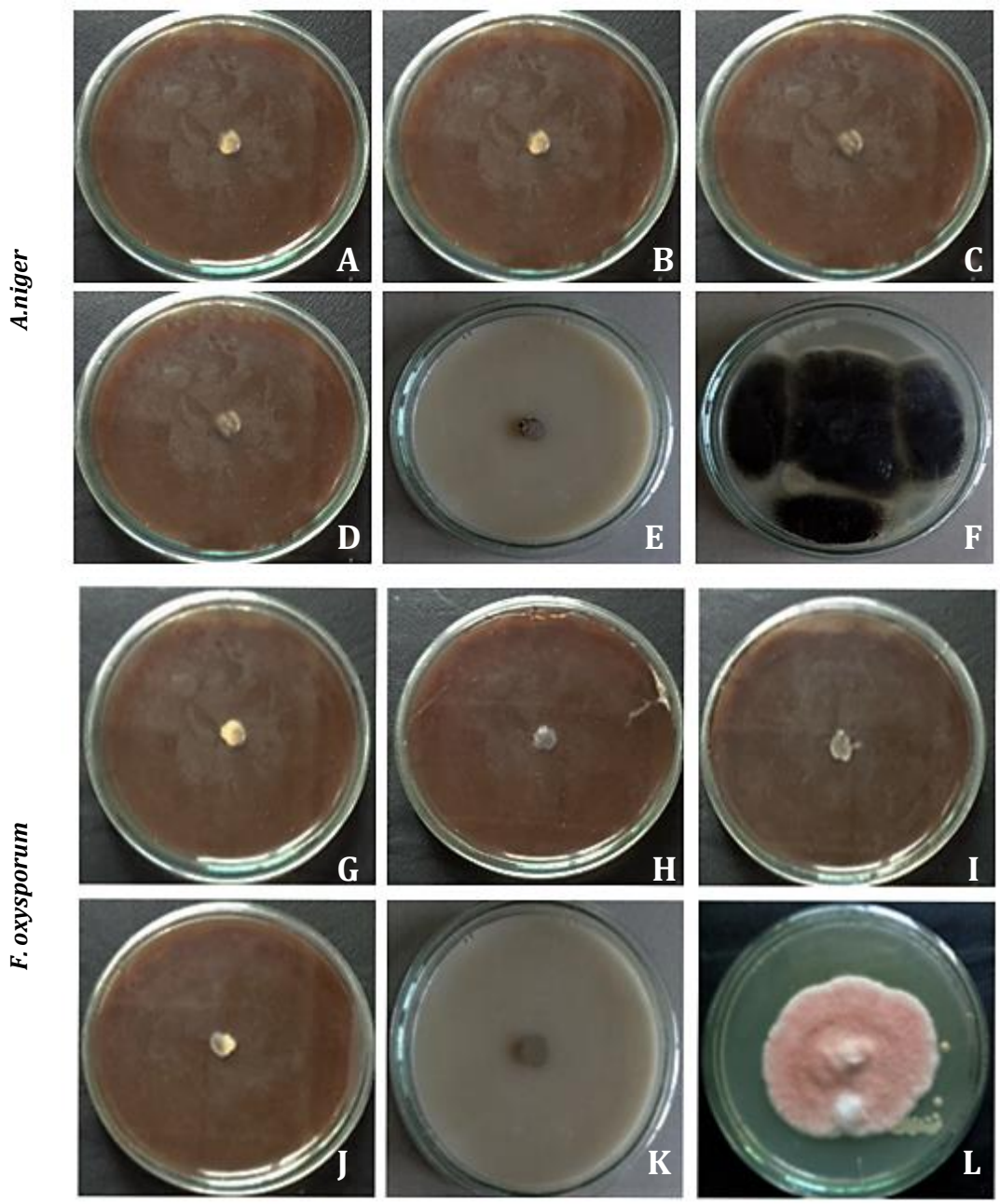


Fig. 1 Radial growth rate assay of AgNPs synthesized from different organic wastes. A&G-BS-AgNPs, & H-CL-AgNPs, C & I-PP (SC)-AgNPs D& J –SD-AgNPs E& K-Positive control, F & L- Negative control against *A.niger* and *F. oxysporum* respectively.

In the present study the size of the synthesized AgNPs ranged from 5-25nm. Strong antimicrobial potency of AgNPs in the range of 10–15 nm has been reported (Siddhartha *et al.,* 2007). Several studies have shown that activity of AgNPs is strongly dependent on the size (Tamayo *et al.,* 2014 and Wu *et al.,* 2014). Smaller nanoparticles seem to have a superior ability to penetrate into the microorganism. Also the interactions with the membranes and any resulting damage, which may lead to cell death, are certainly more evident in the case of nanoparticles with smaller diameter and a positive zeta potential (Gianluigi *et al.,* 2015; Rai *et al.,* 2014; Roe *et al.,* 2008).

Potential antimicrobial activity of Synthesized AgNPs from *Strychnos potatorum* seed extract and banana peel extract have been reported (Suganya *et al.,* 2014; Haytham and Ibrahim,2015). Kruthika and Somanathan (2014) reported lethal effects of fruit waste mediated AgNPs against *Aspergillus and Candida.* AgNPs are effective and fast-acting fungicide against a broad spectrum of common fungi including genera such as *Aspergillus*, *Candida* and *Saccharomyces* (Vikas *et al.,* 2014). Our results indicated the potential application of phytofabricated AgNPs against selected fungi.

REFERENCES

Astiti NPA and Suprapta DN (2012) Antifungal activity of teak (*Tectona grandis* L.f) leaf extract against *Arthrinium phaeospermum* (Corda) M.B. Ellis, the cause of wood decay on Albizia falcataria (L.) Fosberg. *J. ISSAAS*.18(1):62-69.

Gianluigi F, Annarita F, Stefania G, Luciana P, Mahendra R, Giancarlo M and Massimiliano G (2015) Silver Nanoparticles as Potential Antibacterial Agents. *Molecules.* 20:8856-8874.

Hashimoto MC, Prates RA, Kato IT, Nunez SC, Courrol LC and Ribeiro MS (2012) Antimicrobial photodynamic therapy on drug-resistant *Pseudomonas aeruginosa*-induced infection. An *in vivo* study. *Photochem. Photobiol*. 88: 590–595.

Hyatham and Ibrahim (2015) Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J Radiation Res & Applied Sci.* 8(3): 265–275.

Kruthika N and Somanathan T (2014) Eco-synthesis of silver nanoparticles and its use to antibacterial and antifungal activity. *J Chem & Pharmaceutical Scie.*4:74-76.

Lazar V (2011) Quorum sensing in biofilms—How to destroy the bacterial citadels or their cohesion/power? *Anaerobe.*17:280–285.

Lu Z, Dai T, Huang L, Kurup DB, Tegos GP, Jahnke A, Wharton T and Hamblin MR (2010) Photodynamic therapy with a cationic functionalized fullerene rescues mice from fatal wound infections. *Nanomedicine*.5:1525–1533.

Miyashira CH, Tanigushi DG, Gugliotta AM and Santos DYA (2010) Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa*forel in two culture media. *Braz J. Microbiol.*41:2.

Rai M, Deshmukh SD, Ingle AP, Gupta IR, Galdiero M and Galdiero S (2014) Metal nanoparticles: The protective nanoshield against virus infection. *Crit. Rev. Microbiol*.1:11.

Roe D, Karandikar B, Bonn-Savage N, Gibbins B and Roullet JB (2008) Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J Antimicrob Chemother,* 61:869-876.

Siddhartha S, Tanma B, Arnab R, Gajendra S, Ramachandrarao P and Debabrata D (2007) Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnol* . 18:22.

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. *Annu Rev Microbiol* 50: 753-789.

Suganya C, Guru V and Packialakshmi N (2014) Antibacterial activity and green synthesis of silver nanoparticles using strychnos potatorum seed and bark extract packialakshmi .*A J Phytomed & Clinical Res*. 2:127–138.

Tamago LA, Zapata PA, Vejar ND, Azocar MI, Gulppi MA, Zhou X, Thompson GE, Rabagliati FM and Paez MA (2014) Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into Listeria monocytogenes. *Mater. Sci. Eng. C Mater. Biol. Appl*. 40:24–31.

Vikas S, Krishan S and Manjit KS (2014) Nanosilver potent antimicrobial agent and its biosynthesis. *A. F. J. Biotechnol*. 13 (4):546-554.

Wu D, Fan W, Kishen A, Gutmann JL and Fan B (2014) Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. *J. Endod.* 40:285–290.

RESEARCH ARTICLE

Antifungal activity of *Mirabilis jalapa*. L against selected fungi

Kumthekar Shrutika[1] and Pius Jessy[2],

[1]DDU, Kaushal Kendra, Greenhouse Management,Ramnarain Ruia College
[2]Department of Botany, Ramnarain Ruia College, Matunga, Mumbai-19.
shrutika2186@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Kumthekar Shrutika and Pius Jessy (2016) Antifungal activity of *Mirabilis jalapa*. L against selected fungi, Int. J.of. Life Sciences, Special Issue, A7:61-65

ABSTRACT

The Four-o'clock plant, *Mirabilis jalapa* L.of family Nyctaginaceae is a popular ornamental plant grown worldwide for the beauty of its flowers and sweet fragrance. The plant is rich in many active compounds and has been used in traditional medicine due to the presence of these biomolecules of pharmacological importance. Evaluatation of antifungal activity of leaf, flower, root and seed of four cultivars (white, pink, yellow and multicolour flower) of *M. jalapa* on selected fungi –*Aspergillus niger* and *Fusarium oxysporum* was carried out by Minimum Inhibitory Concentration (MIC) and effect of extracts on spouraltion. The results of this study showed that extracts from all parts of four cultivars of *M. jalapa* were very active against selected fungi. MIC of the extracts was found to be in range of 11- 15 mg/ml. The extracts also significantly inhibited the sporulation in selected fungi. Among the cultivars Pink flower Cultivar and Multicolour flower cultivar and White flower cultivar showed potent antifungal activity against *F.oxysporum* compared to that of *A.niger*.

Key words: Antifungal activity, Minimum Inhibitory Concentration, *Mirabilis jalapa*,

INTRODUCTION

Plants are very important commercial source of chemical compounds. Secondary metabolities such as flavonoids, alkaloids and terpenoids produced by plants act as chemical defense against pests and diseases. Medicinal plants represent a rich source of antimicrobial agents. Uniyal *et al*. (2006) stated that a wide range of extracts of medicinal plant parts are used as raw drugs and possess varied medicinal properties. Some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use and many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, a vast majority have not been adequately evaluated (Balandrin *et al*., 1985).

Available literature on the phytochemical constituents of *M. jalapa* showed that the plant is rich in many active compounds including proteins, alkaloids, terpenes, flavonoids and steroids. It is a perennial herb and has been used in traditional medicine in many parts of the world for the treatment of various diseases (Nair *et a*l., 2004).

In the present study attempts were made to evaluate antifungal potential of leaf, flower, root and seed of four cultivars [white(WFC), pink(PFC), yellow(YFC) and multicolour flower(MFC)] of *M. jalapa* on two selected fungi.

MATERIALS AND METHODS

Fungal cultures:
Aspergillus niger (NFCCI 161) and *Fusarium oxysporum* (NFCCI 245) cultures were obtained from Agarkar Institute, Pune and was maintained on Potato Dextrose Agar (PDA) medium. Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method (Astiti and Suprapta, 2012 - modified).

Spore Culture:
Spores were harvested in sterile d/w from the cultures of *A.niger* and *F. oxysporum* culutured on PD broth. This spore culture was used for carrying out MIC and effect of extracts on sporulation and fungal biomass. MIC was determined using dilution broth method (Astiti and Suprapta, 2012, modified). 1% - 1.5 % concentration of various plant extracts of *M.jalapa* were taken and diluted by the PD broth to make the total volume to 5 ml. 500 µl of spore culture was added in all test tubes. For each extract, positive and negative control with fungal spores and without fungal spores were also prepared. All the test tubes were incubated in dark at room temperature for 8 days. After 8 days, the concentration at which the fungal growth was inhibited was considered as the minimum inhibitory concentration (MIC) of the extract which was determined by visual observation and also by taking the fungal biomass.

Effect of extracts on Sporulation
Since the fungal growth was totally inhibited when 1.5% extract was used (from the earlier MIC experiment) effect of various extracts on sporulation was carried out only for 1, 1.1, 1.2, 1.3, 1.4 and 1.5% of extract prepared from different parts of four cultivars of *M.jalapa*. Total volume was made up to 5 ml with PD broth. 500 µl of spore culture was added in these test tubes. For each extract positive and negative control with fungal spores and without fungal spores were also prepared. All the test tubes were incubated in dark at room temperature for 8 days. After 8 days the effect of extracts on sporulation at various concentrations were carried out by counting the number of spores using haemocytometer under a light microscope.

Statistical analysis:
The data were subjected to Analysis of Variance (ANOVA). Statistical analysis was performed using IRRISTAT software (IRRI,2003). Treatment means were compared using Least Significance Difference (LSD) values at $p \leq 0.05$. Differences among treatments were tested by Ducan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Minium Inhibitory Concentration (MIC)
The results of determination of MIC of the extracts from various parts of *M. jalapa* against *A.niger* and *F. oxysporum* have been tabulated in table 1. It is evident that irrespective of cultivars, in general extracts from all parts were very active against selected fungi. There was no much difference in MIC. The extracts from various parts of *M.jalapa* exhibited MIC ranging from 11 to 15mg/ml against *A.niger* and *F. oxysporum*.

Among the cultivars, pink flower cultivar (PFC) and multicoloured flower cultivar(MFC) was found to be more effective against *A.niger* and White flower coloured cultivar(WFC) and MFC against *F. oxysporum*. Among the fungi the inhibitory effect was found to be moreagainst *F. oxysporum* compared to *A.niger* (Table 1).

Effect Of Extracts On Sporulation
Among the various concentrations of extracts, irrespective of cultivars and plant parts significant reduction in spore density was observed at 13 or 14mg/ml of the extract. Complete inhibition (100% inhibition) of sporulation in *A.niger* was observed when 14mg/ml of extract was used where as in *F.oxysporum* it was at 13 mg/ml. A decrease in spore density was observed with increasing concentrations of the extracts (Table 2).

Table 1: Minimum inhibitory concentration of extracts of flower, leaf, root, and seed of white, pink, yellow and multicolour flower cultivars of *M.jalapa.* against *A. niger* and *F. oxysporum.*

***M.jalapa* Plant part** / **Cultivars**	**MIC of Plant extracts (mg/ml)** ± SD	
	A.niger	***F. oxysporum.***
Flower		
WFC	14 ± 0.04	**11± 0**
PFC	15 ± 0.02	15 ± 0.02
YFC	15 ± 0	14 ± 0.04
MFC	15 ± 0	12 ± 0
Leaf		
WFC	14 ± 0.02	13 ± 0.04
PFC	**13 ± 0.04**	13 ± 0
YFC	14 ± 0.04	13 ± 0.02
MFC	15 ± 0	**11 ± 0**
Root		
WFC	15 ± 0	**11 ± 0**
PFC	**13 ± 0**	14 ± 0
YFC	14 ± 0	15 ± 0
MFC	14 ± 0.04	15 ± 0
Seed		
WFC	15 ± 0	14 ± 0.02
PFC	14 ± 0	14 ± 0
YFC	**13 ± 0**	15 ± 0
MFC	15± 0	**13 ± 0**

Table 2: Inhibitory activity of various concentrations of flower, leaf, root and seed extract of *M.jalapa* on sporulation of *A. niger* and *F. oxysporum*.

	Spore density ml^{-1} of *A.niger* (x 10^5 spores)				**Spore density ml^{-1} of *F.oxysporum* (x 10^5 spores)**			
	EXTRACTS							
Concentration (mg/ml)	**Flower**	**Leaf**	**Root**	**Seed**	**Flower**	**Leaf**	**Root**	**Seed**
0 (control)	65.1^a	65.1^a	65.1^a	65.1^a	143.1^a	143.1^a	143.1^a	143.1^a
10	34.7^b	23.2^b	17.2^b	37.8^b	15.2^b	13.2^b	16.0^b	16.0^b
11	27.1^c	17.3^c	11.0^c	26.7^c	7.5^c	9.5^c	5.8^c	8.3^c
12	17.8^d	13.0^d	6.6^d	24.6^c	4.7^d	6.6^d	2.3^d	5.3^d
13	9.7^e	9.5^e	1.8^e	8.1^d	3.0^e	**0^e**	**0^e**	1.5^e
14	0^f	0^f	0^f	0^e	0^f	0^e	0^e	0^f
15	0^f	0^g	0^g	0^f	0^g	0^e	0^e	0^g
LSD	1.0	1.3	1.5	2.2	1.4	1.5	1.4	1.6

Among the **cultivars** irrespective of various concentrations significant reduction in sporulation in *A. niger* and *F.oxysporum* was observed when extracts from **various parts of WFC** was used (Table 3). It was evident that irrespective of cultivars, and plant parts all extracts were significantly active against sporulation of selected fungi. Extracts were found to be more active against *F. oxysporum* than *A.niger.* In positive control, spore density was found to be 64 x 10^5 spores for *A.niger* and 145 x 10^5 spores for *F. oxysporum*.

Table 3: Inhibitory activity of flower, leaf, root and seed extract of four cultivars of *M.jalapa* on sporulation of *A.niger* and *F.oxysporum*.

Cultivars	EXTRACTS							
	Spore density ml^{-1} of *A.niger* (x 10^5 spores)				Spore density ml^{-1} of *F.oxysporum* (x 10^5 spores)			
	Flower	Leaf	Root	Seed	Flower	Leaf	Root	Seed
WFC	**17.7^{d}**	**10.5^{c}**	**12.4^{c}**	**15.0^{d}**	**22.0^{c}**	**21.5^{c}**	**20.7^{c}**	**20.8^{c}**
PFC	22.2^{b}	17.8^{b}	15.6^{a}	18.2^{c}	29.4^{a}	29.2^{a}	26.2^{a}	25.5^{b}
YFC	19.8^{c}	27.3^{a}	14.1^{b}	29.0^{b}	23.7^{b}	23.7^{b}	26.2^{a}	27.7^{a}
MFC	28.5^{a}	18.4^{b}	16.0^{a}	30.9^{a}	24.3^{b}	24.0^{b}	22.3^{b}	25.6^{b}
LSD	0.7	1.0	1.2	1.6	1.1	1.1	1.1	1.2

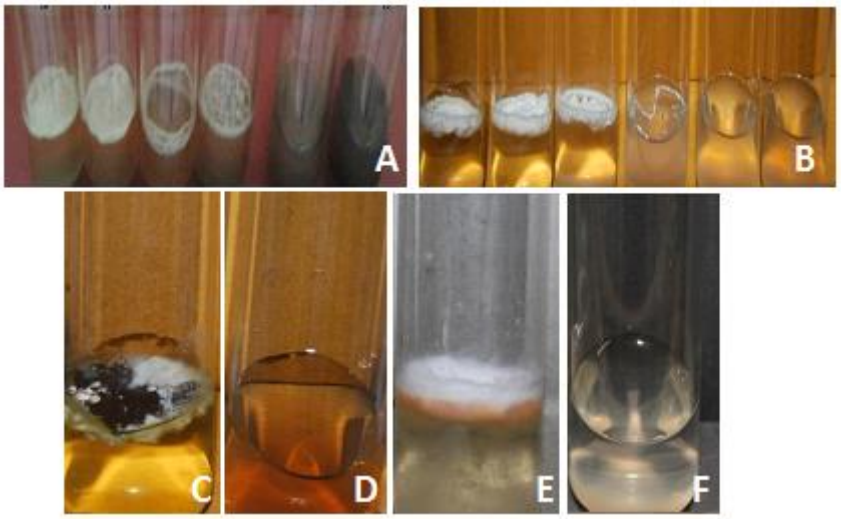


Fig.1 Effect of flower extracts of white flower cultivar of *M.jalapa* against sporulation in
A: *A.niger* **B:** *F.oxysporum* **C & D:** Positive and negative control -*A.niger*
E & F : Positive and negative control -*F.oxysporum*

In this study it was observed that extracts from all parts of four cultivars of *M. jalapa* were very active against selected fungi -*A.niger* and *F.oxysporum*. In general, significant reduction in sporulation and biomass in *A.niger* and *F. oxysporum* was observed when treated with flower and root extracts. Antifungal potential of the extracts were found to be significantly high against *F. oxysporum* compared to *A.niger*.

Though there are many reports on antibacterial activity of *M. jalapa* (De Bolle *et al.,* 1996; Kusamba *et al.,* 1991; Dimayuga,1998; Oladunmoye *et al*., 2007; Oskay and Sari, 2007; Sharma *et al*., 2010 and Ullah *et al.,*2011; Zachariah *et al.,* 2012), only few reports are available on its antifungal activity (Hajji *et al.,* 2010; Kumar *et al.,* 2010 and Muthumani *et al.,* 2008). To our knowledge there are no reports, where a comparative study of antifungal activity was carried out with various parts of four cultivars of *M.jalapa.* Aqueous tuber extract (Hajji *et al.,* 2010), leaf extract (Muthumani *et al.,* 2008) and Biocidal protein – Mj – AMP (Ikeda *et al.,* 1987) from *M.jalapa* was reported to show a wide range of antifungal activity. *Mucuna pruriens* seeds showed antifungal activity against *A. carneus, A.flavus* and *Candida albicans* (Marimuthu *et al.,*2013).

Inhibitory activities exhibited by *M.jalapa* may be due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils (Selvakumar *et al.,*2012; Akintobi *et al.,* 2011; Erasto *et al*.,2004). Similar observations were also made in the present study, where the highest antimicrobial activity exhibited by flower and root extracts also showed high amount of phenolics, flavonoids, tannins and alkaloids.

REFERENCES

Akintobi OA, Aggunbiade SO, Okonko IO and Ojo OV (2011) Antimicrobial evaluation and phytochemical analysis of leaf extracts of *Mirabilis jalapa* against some human pathogenic bacteria. *Nature and Science* 9(10): 45–53.

Astiti AN and Suprapta DN (2012) Antifungal activity of teak (*Tectona grandis* L.F) leaf extract against *Arthrinium phaeospermum* (Corda) M.B. Ellis, the cause of wood decay on *Albizia falcataria* (L.) Fosberg. *J. Int. Soc. Southeast Asian Agri. Sci.* 18(1): 62–9.

Balandrin MF, Klocke JA, Wurtele ES and Bollinger WH (1985) Natural plant chemicals. *Sources of Industrial and Medicinal materials Science* 228: 1154–60.

De Bolle MF, Osborn RN, Goderis IJ, Noe L, Acland D, Hart CA, Torrekens S, Van Leuven F and Broekaert WF (1996) Antimicrobial peptides from *M. jalapa* and *Amaranthus caudatus*: expression, processing, localization and biological activity in transgenic tobacco. *Plant Mol. Biol*. 31: 993–1008.

Dimayuga RE (1998) Antimicrobial activity of medicinal plants from Baja California Sur/Mexico. *Pharm. Biol*. 36: 33–43.

Erasto P, Bojase-Moleta G, Majinda RRT (2004) Antimicrobial and antioxidant flavonoids from the roots wood of *Bolusanthus spesiosus*. *Phytochem*. 65: 875–80.

Hajji M, Jarraya R, Lassoued I, Masmoudi O, Damak M and Nasri M (2010) GC/MS and LC/MS analysis and antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis jalapa* tubers. *Process Biochem.* 45: 1486–93.

Ikeda T, Takanami Y, Imaizumi S, Matsumoto T, Mikami Y and Kubo S (1987) Formation of anti-plant viral protein by *Mirabilis jalapa L*. cells in suspension culture. *Plant Cell Rep*.6: 216–8.

Kusamba C, Byaman K and Mbuyi WM (1991) Antibacterial activity of *Mirabilis jalapa* seed powder. *J. Ethnopharmacol*. 35(2): 197–9.

Marimuthu M, Gurumoorthi P and Krishnamoorthi K (2013) Comparative antifungal activity of two different germplasm of *Mucuna*. *J. Microbiol. Biotech. Res*. 3(3): 143–6.

Muthumani P, Devi P, Meeri R, Kamashwari B and Eswarapriya B (2008) *Invitro* antimicrobial activity of various extracts of *Mirabilis jalapa* leaves. *Int. J. Microbiol*. 7(2).

Nair R, Kalariya T and Sumitra C (2004) Activity of some selected Indian medicinal flora. *Turkey J. Biol*. 29: 41–7.

Oladunmoye MK (2007) Comparative evaluation of antimicrobial activities of leaf extract of *Mirabilis jalapa. Trends Med. Res*. 2(2): 108–12.

Oskay M and Sari D (2007) Antimicrobial screening of some Turkish medicinal plants. *Pharma. Biol*. 45: 176–81.

Sharma M, Mohan V and Abraham M (2010) Antimicrobial potential of the phytoextratcs of some Nyctaginaceae members. *African J. Biotech*. 9(46): 7942–7.

Selvakumar P, Kaniakumari D and Loganathan V (2012) Phytochemical screening and antioxidant activity of red flowered *Mirabilis jalapa* leaf in different solvents. *Int. J. Pharm. Bio. Sci*. 3(4): 440–6.

Ullah N, Khan M, Ali H, Shakoor N, Ahmed G and Minhaj D (2011) Importance of white flowered *Mirabilis jalapa* with respect to its phytochemical and antimicrobial screening. *African J. Pharm. Pharmacol.* 5(24): 2694–7.

Uniyal SK, Singh KN, Jamwal P and Lal B (2006) Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. *J. Ethnobiol. Ethnomed*. 2: 1–14.

Zachariah S, Aleykutty N, Jaykar B, Viswanad V and Halima A (2012) Evaluation of antioxidant and total flavonoid content of *Mirabilis jalapa*. Linn. using *in vitro* models. *Int. Res. J. Pharm.* 3(3): 187–91.

RESEARCH ARTICLE

Studies in discomycetes: genus *Leotia persoon*

Patil Anjali[1], Dangat BT[2] and Patil MS[3]

[1]Department of Botany, Rajaram College, Kolhapur 416 004 (M.S.), India

[2]Department of Botany, Shivaji University, Kolhapur 416 004 (M.S), India

[3]Retd. Prof. and Head, Department of Botany, Shivaji University, Kolhapur 416 004 (M.S), India

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Patil Anjali Dangat BT and Patil MS (2016) Studies in discomycetes: genus *Leotia persoon* Int. J.of. Life Sciences, Special Issue, A7:66-68.

ABSTRACT

Present paper describes three species of the genus *Leotia* Pers. viz. *L. albiceps* (Peck) Mains, *L. viscose* Fr. and *L. sahyadriensis* sp. nov., new record to the fungi of India.

Key words: Mycotaxonomy, Discomycetes, *Leotia*

INTRODUCTION

The genus *Leotia* was established by Persoon (1797) with *L. lubrica* Fr. as the type species (Mains,1956). *Leotia* has multiguttulate and hyaline ascospores, similar to those found in *Microglossum* (Geoglossaceae). But the colour of ascocarps and structure differ from all other genera of family Geoglossaceae. Hence, its systematic position and status has been changed numerous times (Corda,1842; Korf, 1973; Eriksson and Winka,1997, Lumbsch *et al.,*2007; Carpenter,1988). Molecular studies based on ITS and RPB_2 sequences failed to establish the phylogenetic relations (Zhihong, *et.al.*, 2004). Erikson and Hawksworth (1987) accepted 108 genera under family Leotiaceae while Lumbusch, *et.al.* conservatively accepts only 06 genera. Mains (1956) has given elaborate key to the families, genera and species. Hence limitations within genera, families and orders are not well defined. Number of *Leotia* Pers. Species are not definite, though four species are accepted and the type species is widely distributed.

MATERIAL AND METHODS

Specimens were collected during field visits during rainy season, detail observations of habitat and habit were recorded, morphological characters were studied under research microscope by preparing hand cut sections in lacto phenol-cotton blue. Microphotography and measurements were taken using C-MOS digital camera. Herbarium specimens have been deposited. Up-to-date literature has been sighted.

RESULTS AND DISCUSSION

Key to the species studied

1 Ascospores 5.5 x 13(-15) µm; ascocarp hymenium watery-white to ochraceous brown …… …… *L. albiceps*

1' Ascospores larger; colour of ascocarps variable …….. 2

2 Hymenium of the ascocarp dark green and ascospores 11-13 x 18-25 µm …… *L. viscosa*

2' Hymenium and stalk of ascocarps amber brown coloured; ascospores 5 x 60 (-65) µm ……. *L. sahyadriensis* sp.nov.

1. *Leotia albiceps* (Peck) Mains, *Mycologia* 48:700, 1956.

= *Ombrophila albiceps* Peck, *Ann. Rep. N.Y. State Mus.* 42: 34, 1889.

Habit - on decaying wood, Radhanagari, Dist. Kolhapur (M.S.), India, 3/9/1985, leg. D. N. Ghadge, deposited under Fungi of Western India, Department of Botany, Shivaji University, Kolhapur (M.S.) as W.I.F. no. 77.

Remarks: Ascocarps are gelatinous, watery white to ochraceous brown coloured,with broad, flattened stipe. Ascospores are slightly longer than *L.albiceps* (Peck) Mains. However, most of the characteristics such as gelatinous ascocarps; watery-white to ochraceous- brown hymenium; hyaline, non-septate, guttulate ascospores are similar thus, referred to it. Mains (1956) has reported the species from Michigan (USA) on decaying wood. It makes a new record to the fungi of India.

2. *L. viscosa* Fr., *Syst. Mycol.* 2: 30, 1822.
= *L. stipitata* Schroeter, *Engl. Prantl. Nat. Pfl.* 1: 166, 1894.

Habit: on damp soil, Kulu Valley (H.P.),18/7/1979, collected by Shri. Gaikwad, identified by M. S. Patil, W.I.F. No. 76.

Remarks: Ascocarps fleshy, with green pileus, yellow-orange, terete, cylindrical stipe. Asci 12(-13) x 165 (-198) µm. It has been reported from N.America (Mains,1956) as *L. viscose.* The same species has been reported from N. E. Himachal Pradesh as *L. stipitata* Schroeter (Bilgrami, K.S., *et.al.*,1991), described by Sharma, M. P.(1983) as *L. viscosa* Fries. The present collection matches well with *L. viscosa* Fries. hence referred to it.

3. *L. sahyadriensis* sp. nov.

Habit: on damp soil, Amba, Anjali Patil, August 2015, 2016.

Ascocarps cespitose gregarious,scattered, 2-4 cm long, gelatinous-viscid, throughout brown- amber coloured, pilei convex, somewhat furrowed or lobed above smooth, 1.2-1.4 cm thick or in diameter when globose, stipe stout, thick, round, viscid, enlarging above to hymenium as a continuation 5-10 mm thick and 1.2-3 cm long and made of three layers, central gelatinous layer, middle non gelatinous and outer is of gelatinous hyphal layers (in V.S. & T.S. of the stipe), asci clavate, unitunicate, 8- spored, J negative (pore not blue by iodine solution – Melzers reagent), inoperculate, 12.5 X 150-175 um, ascospores arranged in one group i.e. fasciculate, or biseriate, ends overlapping, clavate-cylindrical, multiguttulate, hyaline, smooth, ends rounded, straight or curved, 5 X 62.5 um, paraphyes numerous, filiform, somewhat enlarged at the apices and agglutinated with gelatinous amorphous matter.

Table 1: Comparison of present material with type specimen

Species type	Ascocarps	Asci	Ascospores	Remarks
L. lubrica Fr.	0.5-1 X 2-7 cm, buff ochraceous; Cinnamon/ olivaceous, stalk squamulose	7-10 X 115-150 um	4-6 X 16-23 um, fusoid, ellipsoid, guttulate	Pilei distinct from stipe, wide geographical distribution
Present Collection	2-4 cm long stipe: 1-3 cm long and 0.5 – 01 cm thick, amber brown throughout	12.5 X 150-175um	5 X 62.5 um	No such distinction stipe enlarged as clavate, globose lobed hymenium, limited to a locality Amba, ditrict Kolhapur, Maharashtra, India

Remarks:
Overall morphology (size, shape, colour, texture) of the ascocarps along with larger asci and ascospores, which are very distinct keeps the present material or collection as a new and thus a new species viz. *Leotia sahyadriensis* sp. nov. has been proposed based on the Sahyadri Ghats from where the material has been collected.

REFERENCES

Bilgrami KS, Jamaluddin and Rizwi MA (1991) *Fungi of India list & references*. Today and Tomorrow Printer and Publishers, New Delhi, 798p

Corda ACJ (1842) Icones fungorum hucusque cognitorum. 5:1-92.

Eriksson OE and Winka K (1997) Supraordinal taxa of Ascomycota. *Myconet* **1** (1): 1-16.

Eriksson OE, Hawksworth DL (1987) An alphabetical list of the generic names of ascomycetes – 1987. Systema Ascomycetum 6 (1): 1-109.

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Lumbsch HT, Lutzoni F, Matheny PB, Mc Laughlin DJ, Powell MJ, Redhead S, Schoch CL et al. (2007) A higher-level phylogenetic classification of the Fungi. *Mycological Research* 111: 509-547.

Korf RP (1973) Discomycetes and Tuberales. In Ainsworth GC, Sparrow FK, Sussmann AS (eds) The fungi - An advanced treatise, 4A, pp 249-319. Academic press New York and London

Mains EB (1956) North American species of the Geoglossaceae.Tribe Cudonieae. – Mycologia 48: 694-710.

Persoon CH (1797) Commentatio de Fungis Clavaeformibus.1-124

Zhong Z, Pfister DH (2004) Phylogenetic relationships among species of *Leotia* (Leotiales) based on ITS and RPB2 sequences, *Mycological Progress,* 3 (3): 237–246.

RESEARCH ARTICLE

Effect of Arbuscular Mycorrhizal interactions on growth, productivity, and nutrient content of *Vigna catgang* Walp.

Ayare Komal S

V.K. Krishna Menon college, Bhandup (East)

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Ayare Komal S (2016) Effect of Arbuscular Mycorrhizal interactions on growth, productivity and nutrient content of *Vigna catgang* Walp., Int. J.of. Life Sciences, Special Issue, A7:69-73

ABSTRACT

The mutually beneficial relation between feeder roots of plants and fungi is called 'Mycorrhiza'. The term 'Mycorrhiza' was coined by Frank in 1885 to describe symbiotic association of plant roots and fungi. The word 'Mycorrhiza' originate from two greek words 'Mycos' meaning fungus and rhiza meaning root. Arbuscular mycorrhizae (AM) are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that association with AM fungi facilitates better nutrient uptake enhancing growth in leguminous plants. Hence to exploit these biological tools, pot experiments were carried out and response on growth, productivity and nutritional aspects of *Vigna catgang* Walp**.** were studied. AM inoculums brought from Tamil Nadu Agricultural University containing the mixture of Glomus species was directly used as an inoculum to study the effect of AM on *Vigna catgang* Walp**.** Pot experiments were conducted in mixture of sterilized garden soil and sterilized sand in the ratio 3:1. The experiment was conducted with AM (treated) and non AM [control] plants of *Vigna catgang* Walp**..** Phosphate phosphorus content, chlorophyll content, Alpha amino acid content, nitrate content and nitrate reductase activity, soluble protein content, calcium and magnesium content in the leaves and fruits of treated and control plants of *Vigna catgang* Walp**..** were estimated at an interval of 15 days after sowing the seeds (DAS), 30DAS, 45DAS and at 60DAS and the percent of productivity was checked. The association of AM fungi enhances the growth in all the treated plants. The significantly high growth rate and yield was observed in treated plants than control plants. Significantly higher amount of above mentioned aspects was observed in the leaves of treated plants of *Vigna catgang* Walp**.** than that in control ones.

Keywords: Arbuscular Mycorrhizal, productivity, *Vigna catgang* Walp

INTRODUCTION

Arbuscular mycorrhizae (AM) are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that

association with AM fungi facilitates better nutrient uptake enhancing plant growth. The association of AM fungi enhances the ability of leguminous plants to withstand the various stresses to some extent. When the nutrient uptake levels and growth rate were estimated in AM and control leguminous plants in drought and saline stresses, the AM associated leguminous plants showed more growth rate and nutrient levels than the ones without AM association. It was found that percentage variation in growth rate (i.e. root and shoot length and root and shoot dry weight) and nutrient uptake in leguminous plants under drought and different levels of salinity stress condition were directly proportional to the percentage of mycorrhization (Kumar and Muraleedhara, *2003).*

MATERIALS AND METHODS

AM inoculum – AM inoculums was brought from Tamil Nadu Agricultural university which contained the mixture of *Glomus* species was directly used as an inoculum to study the effect of AM on *Vigna catgang* Walp*.*

Preparation of control and treated pots – Twelve large sized plastic pots with holes at the bottom having an internal diameter of 18 cm were used for the experiment of which six were maintained as control and six were used for treatment with 'Mycorrhiza'. Garden soil was obtained in bulk from nursery suppliers. Similarly sand was procured from sea shore and was washed thoroughly in running water for several hours to remove soluble salts. Both garden soil and sand were mixed in proportion of 3:1 by volume in large trays. Sand help in improving aeration in pot and thereby help AM fungi to grow as mycorrhizae are aerobic microorganisms. This soil sand mixture was sterilized at a temperature of 200^0c for 2 hours in hot air oven, to kill soil microorganisms and insects. This sterilized mixture was used as a growth medium for pot experiments. Out of 12 pots six were maintained as treated and remaining six as control. Initially ¾th part of each pot was filled up with sterilized soil mixture. 10 g of AM inoculums was added to each treated pot the inoculums was distributed evenly in the pot and was covered with a layer of 4 cm. of sterilized soil mixture. Twelve water soaked seeds were sown in each pot and covered with a layer of soil. The pots were watered with watering can having small pores to avoid the disturbance of the soil surface.

Following physiological parameters from the leaves and fruits of the plants of *Vigna catgang* Walp*.* both control and treated were studied.

1) Soluble protein content,
2) Alpha amino nitrogen content,
3) Nitrate content,
4) Nitrate reductase activity,
5) Calcium and Magnesium content

Soluble protein contents in the fresh leaf material and fruits were analysed by the method of Lowry *et al.,*(1951).

Alpha amino nitrogen was estimated in the fresh leaf material and fruits by the method of Moore and Stein (1948). Nitrate content of fresh leaves and fruits was estimated by the method of Johnson and Ulrich, (1950). The *in vivo* assay of nitrate reductase (NR) activity in the leaves was carried out according to the method of Kleeper *et al*. (1971). Calcium and Magnesium content of fresh leaves was estimated by the method of Jackson, (1973).

All the parameters from leaves were studied on 15th, 30th, 45th and 60th day after sowing the seeds. The roots of *Vigna catgang* Walp*.* were screened to obtain percentage of AM colonization at 15, 30, 45 and 60 DAS. Isolation and quantification of spores from rhizosphere soil of *Vigna catgang* Walp*.* was also carried out before sowing the seeds and at 60 DAS. Screening of the roots was carried out to study the per cent of root association by AM fungi in treated pots by the method described by Grace and Stribley (1991). The percent of root infection was calculated by using Nicolson's formula (1955).

RESULTS AND DISCUSSION

Soluble protein content, alpha amino nitrogen content, nitrate reductase activity and Calcium and Magnesium content in the leaves of treated plants of *Vigna catgang* Walp*.*. was significantly higher than that of control ones. Nitrate content in the leaves of control plants of *Vigna catgang* Walp*.* was higher than the treated ones at 15 DAS, 45 DAS, and at 60 DAS.

Before sowing the seeds 10 g of AM inoculums was found to contain 57 AM spores while at 60 DAS it showed presence of around 78 spores. The results are tabulated in following tables.

Table 1: Soluble protein content in the leaves of treated and control plants of *Vigna catgang* Walp.. (mg per 100mg fresh leaf)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	1.686	2.603	3.886	2.163
Control	0.953	1.503	2.53	1.246
Calculated 't'	4.014	4.246	3.395	3.223
Level of significance	++	++	++	++
Standard error (S.E.)	± 0.181	± 0.258	± 0.398	± 0.283

Table 2 -Alpha amino nitrogen content in the leaves of treated and control plants of *Vigna catgang* Walp. (mg / 100 mg fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	0.096	0.183	0.143	0.133
Control	0.070	0.12	0.103	0.086
Calculated 't'	2.064	3.450	3.464	2.567
Level of significance	0	++	++	+
Standard error (S.E.)	± 0.0128	± 0.0181	± 0.0114	± 0.0180

Table 3:Nitrate content in the leaves of treated and control plants of *Vigna catgang* Walp. (mg per 100 mg fresh leaf)

	15 DAS	30 DAS	45 DAS	60 DAS
Treated	0.088	0.096	0.052	0.036
Control	0.096	0.032	0.076	0.056
Calculated 't'	0.278	3.957	1.243	1.546
Level of significance	0	++	0	0
Standard error (S.E.)	± 0.028	± 0.0099	± 0.0571	± 0.0129

Table 4 –Nitrate reductase activity in the leaves of treated and control plants of *Vigna catgang* Walp. (NR activity is expressed as micromole nitrate g^{-1} fresh leaf hour $^{-1}$.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	3.625	1.4	6.575	3.825
Control	3.125	3.225	4.375	2.275
Calculated 't'	1.342	3.160	4.498	6.242
Level of significance	0	+	++	+++
Standard error (S.E.)	± 0.372	± 0.295	± 0.488	± 0.248

Table 5 – Calcium content in the leaves of treated and control plants of *Vigna catgang* Walp. (mg per g dry leaf)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	32.5	34	33.5	33
Control	27.5	29	30	30
Calculated 't'	5.274	5	4.342	2.373
Level of significance	+++	+++	++	+
Standard error (S.E.)	± 0.978	± 0.1	± 0.806	± 1.264

Table 6 – Magnesium content in the leaves of treated and control plants of *Vigna catgang* Walp (mg per g dry leaf)

	15 D A S	**30 D A S**	**45 D A S**	**60 D A S**
Treated	20.5	21	22.5	21.5
Control	17.5	18.5	18	18
Calculated 't'	3.164	3.101	4.025	4.342
Level of significance	+	+	++	++
Standard error (S.E.)	± 0.948	± 0.806	± 1.118	± 0.806

Table 7 – Analysis of fresh seeds of treated and control plants of *Vigna catgang* Walp

	Protein content mg/100 gm	**Alpha amino** nitrogen mg / 100 g	**Nitrate content** Mg / 100 g	**Nitrate reductase** g / fresh leaf hr.
Treated	4.07	0.330	0.112	2.275
Control	2.676	0.216	0.072	1.575
Calculated 't'	6.520	5.477	3.024	4.506
Level of significance	+++	+++	+	++
Standard error (S.E.)	± 0.213	± 0.0207	± 0.0132	± 0.155

Table 8 – Analysis of dry seeds of treated and control plants of *Vigna catgang* Walp

	Protein content mg/100 gm	**Alpha amino** nitrogen mg / 100 g	**Nitrate content** Mg / 100 g	**Nitrate reductase** g / fresh leaf hr.
Treated	4.656	0.280	0.096	1.875
Control	3.446	0.163	0.052	1.275
Calculated 't'	3.883	4.679	3.867	4.224
Level of significance	++	+++	++	++
Standard error (S.E.)	± 0.311	± 0.0249	± 0.0113	± 0.141

Level of significance 'o' = Difference of mean not significant, '+' = Difference of mean significant (P=0.05), '++' = Difference of mean significant (P=0.01), '+++' = Difference of mean significant (P=0.001), DAS = Days after sowing. Each value is a mean of six replicates

Table 9 – Results of the screening of roots of *Vigna catgang* Walp to obtain percentage of AM colonization

Days after sowing	**% colonization of AM roots**	**AM structures observed in the root cortex**
15	16	Mycelium
30	45	Mycelium + vesicles
45	72	Mycelium + vesicles
60	87	Mycelium + vesicles

As percentage of AM colonization increases in root system of AM plants, the soluble protein content, Alpha amino nitrogen content and Calcium and Magnesium content of leaves also increases. At 60 DAS both soluble protein content and Alpha amino nitrogen content of the leaves shows reduction in both AM and non AM plants. This may be due to the utilization of these nutrients by plants for flowering and fruiting. At 15 DAS. The nitrate content of leaves of AM associated *Vigna catgang* Walp. plant was less than that in the leaves of non AM plants but, no significant difference was observed in two values. Nitrate reductase activity was significantly high in AM plants than non AM plants at 15 DAS. At 30 DAS, nitrate content in leaves of AM plants was significantly higher than that in the leaves of non AM plants. NR activity at this stage was

significantly higher in non AM plants than that in AM plants. As NR activity is high, utilization of nitrate is also higher. Again at 45 DAS and at 60 DAS nitrate content of leaves of AM plants was less than that of leaves of non AM plants. This can be correlated with high NR activity in AM plants than that in non AM plants at this stage. More nitrate might have been utilized for flowering and fruiting in AM plants.

Lactuca sativa (lettuce) when inoculated with *Glomus fasciculatum* showed increased growth, nitrate reductase activity and protein content compared to that of non AM plants. (Azcon*et al.,*1996).

Cliquet and Stewart (1993) have reported higher amino acid concentration in AM inoculated Maize roots as compared to that of non AM inoculated maize roots. Major component of the free amino acid pool were glycine, glutamic acid, alanine, serine, aspargine and 4-amino-n-butyric acid.

AM inoculated *Ziziphus mauritiana* showed increase in soluble protein concentration in both roots and shoots. Different AM species varied in their efficacy to increase soluble protein concentration in both the organs. *Glomusfasciculatum*can increase soluble protein concentration most efficiently in both the organs. (Mathur and Vyas, 1995).

The effect of VAM fungus *Glomus fasciculatum*on growth and nitrogen assimilation was measured on *Allium cepa* grown under drought conditions. Under water limitations, the effectiveness of *Glomus fasciculatum*to increase nitrate reductase activity in plant was enhanced. The proportion of nitrate assimilation into root was increased in VAM plants. AM plants reached a high specific and total Glutamine synthetase activity in shoots and roots. AM plants can utilize nitrate form more efficiently than ammonium form. Lettuce, when inoculated with AM fungi, *Glomus fasciculatum or Glomus mosseae*showed higher nitrate reductase activity than the plants not inoculated with AM fungi, particularly under water stress conditions. Control plants had 57 per cent less nitrate reductase activity than that in *Glomus deserticola* colonized plants under well watered conditions, with a reduction in nitrate reductase activity by 79 per cent when the plants were subjected to drought stress. It was suggested that either the AM fungi increased the nitrate reductase activity in the host plant or that AM fungi have enzymatic activity per se. Drought stress decreased the nitrate reductase activity but the decrease was less in AM inoculated plants (Singh, 2007).

AM inoculated *Vigna sinensis* L. when grown in saline soil, showed significant increase in nitrogen content. In this case also the nitrogen uptake was directly propotional to the percentage of mycorrhization (Kumar and Muraleedhara, 2003). Increased nitrate reductase activity has also been reported in mycorhiza inoculated plants (Manoharachary*et al*., 2009).

REFERENCES

Azcon R, Gomez M and Tobar R (1996) Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. *Biology and fertility of soils* 22 (1-2):156-161.

Cliquet JB and Stewart GR (1993) Ammonia association in *zea mays* L infected with vesicular arbuscular fungus *Glomusfasciculatum* Plant physiol.1993 101: 865-871

Grace C and Stribley DP (1991) A safer procedure for routine staining of vesicular arbuscular Mycorrhizal fungi. Mycol. Res. 95(10) : 1160 - 1162.

Johnson CM and Ulrich A (1950) Determination of nitrate in plant material. Analytical Chem. 22:1526.

Jackson ML (1973) Soil and Chemical analysis. Prentice Hall Inc. Newjersey, U.S.A.

Kleeper L, Flesher D and Hageman RH (1971) Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. *Plant Physiol*. 48:580.

KumarAnil KK and Muraleedhara Kurup GM (2003) Effect of Vesicular ArbuscularMycorrhizal fungi Inoculation on growth and nutrient uptake of leguminous plants under stress conditions. *Journal of mycology and*

Lowry OH, Rosebrought NS, Farr AL and Randall RJ (1951) Protein measurement with Folin Phenol reagent. *Biol.Chem*.199:265.

Mathur N and Vyas A (1995) Changes in nitrate reductase and glutamine synthetase activities in *Ziziphusmauritians*by different VAM fungi. Current science 68(11): 1144-1146.

Moore S and Stein WH (1948) In "Methods in Enzymology" Eds. Colowick, S. P. and N. D. Kaplan Academic Press new York, 3:468

Nicolson TH (1955) The mycotrophic habit in grass. As cited by Hasan A. 2000 In: Arbuscularmycorrhizal association and Incidence of root - knot nematode on some cucurbits in Sstern Uttar Pradesh a Prelininary survey report. *Mycorrhiza news* 12(2): 15-18.

Singh S (2007) Nitrogen assimilation in Mycorrhizal fungi onroots. *Mycorrhiza news* 18 (4) : 2-11.

RESEARCH ARTICLE

Fungal Diseases of Vegetables grown in Greenhouse

Chaudhuri Sanchita[1], Pius Jessy[2*], Naik Vishwesh[1] and Gaikwad Sneha[1]

[1]DDU Kaushal Kendra, Greenhouse Management, RamnarainRuia College,
[2]Department of Botany, RamnarainRuia College, Matunga, Mumbai-19
*Corresponding author email: jessypius@yahoo.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Chaudhuri Sanchita, Pius Jessy, Naik Vishwesh and Gaikwad Sneha (2016) Fungal Diseases of Vegetables grown in Greenhouse, Int. J.of. Life Sciences, Special Issue, A7: 74-76.

ABSTRACT

Warm, humid greenhouse environment favours a number of plant pathogens that can infect various plant parts. The disease can be soil/ peat born, air born or water born. The aim of this paper is to give an over view of fungal diseases commonly observed on the vegetables grown in Greenhouse of RamnarainRuia College. In protected cultivation optimal conditions for growing vegetables in soil and soilless method need to be maintained. However improper maintenance stimulates the activity of pathogens. The diseases observed include Powdery mildew, Downy mildew, Damping-off, Tikka disease and fruit rot. This paper also gives organic control measures for the same.

Key words: Fungal diseases, Greenhouse, vegetables

INTRODUCTION

With the exponential growth in population, global warming resulting erratic changes in climatic conditions and ever increasing need for food (Anirudh Garg and Rekha Balodi ,2014) as well as demand for seasonal crops throughout the year has led to cropping up of greenhouses. A greenhouse has the capability to meet the demand for increased production of food, avail seasonal crops throughout the year under protected and controlled environment. Thus growing crops in greenhouses is the need of the hour.

Greenhouses are designed to protect crops from many adverse conditions, however it is impossible to exclude several pathogens and pests (Kharwar, 2014; Colucci and Holmes, 2010, Lim *et al*.,2004; Stallknecht and Schulz-Schaeffer,1993;Subrahmanyam *et al.,*1985). Air borne and soil-borne pathogens enter through the doorway, ventilator and also adhere to footwear and machinery, aquatic fungi through irrigation water, insects that enter the greenhouse can transmit viruses and can carry and spread bacteria and fungi as well. Once inside a greenhouse, pathogens are difficult to eradicate. Moreover high humidity level in a greenhouse is an open invitation to fungal infections. This calls for constant monitoring and efficient pest and disease management.

MATERIALS AND METHODS

Studies were carried out to identify and control the fungi associated with the vegetables grown in greenhouse. The leaves of the infected plants were collected from the greenhouse and were thoroughly inspected for disease symptoms to identify the pathogen. The general health of the infected plant was also monitored.

Organic fungicides like Neem oil(1%) and / Tobacco decoction was sprayed on the plants to check the infection. A fungicide was prepared by mixing clove oil, black pepper, *Aloe vera* gel and basil to successfully fight downy mildew.

RESULTS AND DISCUSSION

Here we record the observations made pertaining to the various fungal diseases encountered while growing vegetables in greenhouse and shade net house in the College premise under a skill based B.Voc course-Green house Management recognised by UGC and University of Mumbai. All the infected materials are preserved in the department.

Fungal diseases like Powdery mildew on *Arachis hypogaea*(*Oidium*sp.), Downy mildew of *Trichosanthus cucumerina*(*Pseudoperenospora* sp.),Tikka disease/ leaf spot disease of *Arachis hypogaea*(*Cercospora arachidicola, C. personata*), late blight of *Lycopersicon esculentum* fruit (*Phytophthora sp.*) and damping-off of *Amarathus* seedlings (*Pythium* sp.)(Fig.1)were observed.

Powdery Mildew of *Arachis hypogaea*:
Causative agent -*Oidium arachidis*. The infected leavesdeveloped pale white spots on the under surface of the leaf, at the on set of the disease. Later the spots were visible on the upper surface of the leaf. The spots later became larger and whiter. These spots covered the upper surfaces of leaflets and superficial sporulating fungal growth gave them a powdery white appearance (Fig.1). The center of the spots later became brown and necrotic.

Powdery mildew on Groundnut leaf *Oidium*sp.

Downy mildew in Snake gourd leaf *Pseudoperenospora*sp

Damping -off of Amaranthus seedling *Pythium* sp.

Early leaf spot Disease of Groundnut leaf *Cercospora arachidicola*

Late leaf spot Disease of Groundnut leaf *Cercospora personata*

Fruit rot of Tomato *Phytophthora sp.*

Fig. 1 : Fungal Diseases observed in the greenhouse grown vegetables

Downy mildew of *Trichosanthus cucumerina*: Causative organism-*Pseudoperenospora sp.* Typical symptoms consist of chlorotic lesions on upper leaf surfaces and premature defoliation.Sporulation was observed on the lower leaf surface**.** The infected leaves in severe cases appear burnt and skeletanised.

Tikka disease/ leaf spot disease of *Arachis hypogaea*:
Causative organism-*Cercospora arachidicola*. Early leaf spot showed brown lesions (spots) that were surrounded by a yellow halo.Early leaf spot was found as early as 30 days after planting. Tufts of silvery, hair-like spores on the top of the leaf could be seen with the help of a good magnifying glass.

Late leaf spot of *Arachis hypogaea:*
Causative organism- *Cercospora personata.*Late leaf spot disease showed circular and darker spots than early spot disease with or without yellow halo.

Late blight of *Lycopersicon esculentum* fruit:
Causative organism- *Phytophthora sp.* The fruits were brown blighted also turned greasy and oilivaceous brown.

Damping-off of *Amarathus* seedlings :
Causative organism- *Pythium sp.* Seedlings are attacked by the pathogen.Affected roots showed a glassy rot and light brown colour at the level of the collar

Organic control measures:
In the present study 1% Neem oil when sprayed, spread of the fungal disease was controlled. This could be probably due to the protective coating on the leaf surface that blocked the germination of the spore.This was effective against powdery mildew. Tobacco decoction was not as effective as neem oil against fungal pathogen, but was very effective against insect pests. However, it was effective in controlling fruit rot of tomato. A fungicide that was prepared by mixing clove oil, black pepper, *Aloe vera* gel and basil leaf extract was used successfully to fight downy mildew.

In order to control the fungal infection the following precautions were taken and this resulted in reduction of the infection. **i)** The infected plants were sprayed with organic fungicide ii) Highly infected plants were uprooted and destroyed iii) The hinges, the doors and the tools used in the greenhouse were throughly cleaned and disinfected **iv)** The humidity of the greenhouse was controlled by increasing the time interval between the two consecutive sprays of the foggers v) Number of people entering the greenhouse on regular basis were restricted.

CONCLUSION

Greenhouse could be susceptible to fungal attack if proper hygiene is not maintained. Many a times the doors, the tools and implements used or unregulated visitors in the greenhouse attribute to the fungal attack.Use of organic fungicides showed that to some extent they can control the pathogen which could be due to its antifungal potential which inhibits fungal mycilial growth or spore germination.

REFERENCES

Colucci SJ and Holmes GJ (2010) Downy Mildew of Cucurbits. The Plant Health Instructor. DOI: 10.1094/PHI-I-2010-0825-01

Garg Anirudh and Balodi Rekha (2014) Recent Trends in Agriculture: Vertical Farming and Organic Farming Volume 1 Issue 4

Kharwar RN and Upadhyay R, Dubey Nawal (2014) Microbial Diversity and Biotechnology in Food Security Technology & Engineering

Lim CHO and White PJ (2004) .A Cellular Hypothesis for the Induction of Blossom-End Rot in Tomato Fruit. Annals of Botany Volume 95, Issue 4Pp. 571-581.

Stallknecht GF and Schulz-Schaeffer JR (1993) Amaranth rediscovered. p. 211-218. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York.

Subrahmanyam P, Moss JP, McDonald D, Subba Rao PV and Rao VR (1985) Resistance to leaf spot caused by *Cercosporidiumpersonatum* in wild *Arachis species*. Disease 69:951-954.

William RB and Allen PJ (1962) Induction of Disease Symptoms in Barley by Powdery Mildew .Plant Physiol Jan; 37(1): 50–5.

RESEARCH ARTICLE

Dectection of seed-borne Mycoflora of Rice Cultivar Priyanka (*Oryza sativa* L.) seeds

Pawar NB[1], Rathod LR[1*], and Suryawanshi NS[2]

[1]Department of Botany, Mahatma Phule A. S. C. College, Panvel | [2]Department of Botany, K. V. Pendharkar College, Dombivali | *Correponding author email: lrathod78@yahoo.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Pawar NB, Rathod LR, and Suryawanshi NS (2016) Dectection of seed-borne Mycoflora of Rice Cultivar Priyanka (*Oryza sativa* L.) seeds, Int. J. of. Life Sciences, Special Issue, A7: 77-80.

ABSTRACT

Fungi associated with seeds of Priyanka cultivars of rice was isolated by Agar plate, blotter paper methods and seed washates. A total of 40 rice seed samples were obtained from different region of Konkan. Totally eleven genera of fungi viz., *Pyricularia oryzae*, *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Bipolaris oryzae, Alternraria alternata, Curvularia lunata*, *Cercospora janseana, Curvularia lunata*, *Ephelis oryzae, Rhizoctonia solani*, *Scrocladium oryzae, Sclerotium rolfsii.* Comprising 13 species were found to be associated with the rice seed sample. Among them the most dominant *Pyricularia oryzae*, *Aspergillus flavus* and *Aspergillus flavus* which are associated with higest percent incidence followed by *Fusarium oxysporum.* A least percent incidence were observed with *Scrocladium oryzae* and *Sclerotium rolfsii* and *Ephelis oryzae.*

Key words - Rice (*Oryza sativa* L.) Cv. Priyanka, Screening methods.

INTRODUCTION

India is one of the world's largest producers of white rice, accounting for 20% of all world rice production. Rice is India's pre-eminent crop, and is the staple food of the people of the eastern and southern parts of the country. Rice (*Oryzae sativa* L.) belongs to the family Poaceae and is cultivated throughout the tropical and subtropical region of the world (Ezuka and Kaku, 2000). Rice seed is affected by fungal pathogens. So far more than 13 different fungi have been detected on rice seeds (Neergard, 1977). Rice is infested by number of diseases causing fungi and some of them are perpetuated through seed thereby affecting seed germination causing poor quality grains and low yields. Many micro-organisms including fungi have been recorded as seed borne in paddy. 13 fungal isolates on Priyanka cultivars were reported from Panvel of which Pyricularia oryzae, Aspergillus flavis and Aspergillus niger were most common. The present investigation was carried out to establish whether seed-borne fungi are responsible for seed shriveled, seed broken and seedling abnormalities. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al*., 2002).

MATERIALS AND METHODS

Collection of seed samples (Cultivars)

The seed sample of Rice were collected from various region of Konkan, Local farmar, market places, Kharland Research Station Panvel and Agriculture Research Station Karjat and Palghar. During the course of studies, seed samples were separately collected and stored in plastic containers without any treatment of fungicide/insecticide at laboratory conditions.

Detection of Seed Mycoflora on Paddy

These samples assessed for the detection of seed borne fungi through Agar plate, blotter paper and Seed washates (ISTA, 1966). These seed lots surface sterilized with the 0.5 % HgCl2. In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. A pair of sterile white blotter papers of 8.5 cm diameter were soaked in sterile distilled water and were placed in pre-sterilized petriplates of 90 mm diameter. The plates were incubated at $28^0 \pm 2^0$C under diurnal conditions. On seventh day of incubation, seeds were first examined under stereoscopic microscope for determining the various fungal growth. The plated plates incubated for 7 days at 25 °C. After incubation fungi developed on each seed were examined under different magnifications of a stereomicroscope and were identified by colony, color, speculation.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 reveal that this cultivar yielded thirteen fungi such as *Aspergillus flavus, Aspergillus niger, Pyricularia oryzae, Pyricularia grisea, Alternaria alternata, Fusarium oxysporum, Sclerotium rolfsii, Bipolaris orizae, Cercospora janseana, Rhizoctonia solani, Ephelis oryzae, Sarocladium oryzae and Curvularia lunata.* In case of agar plate, *Aspergillus flavus* (48.6%) gave highest percent incidence followed by *Aspergillus niger* (46.6%), *Pyricularia oryzae* (44.6%) and *Fusarium oxysporum* (41.6%). *Bipolaris orizae* (38%), *Alternaria alternata* (34.3%), *Curvularia lunata* (31%), *Cercospora janseana* (29%), *Pyricularia grisea* (25.6%), *Rhizoctonia solani* (22.6%), were found to be intermediate within range of 22.6 – 38 %. *Sclerotium rolfsi*, *Ephelis oryzae* and *Sarocladium oryzae* were found to be least.

In case of standard blotter paper, the percent incidence of *Aspergillus flavus* (42.3%) gave highest percent incidence followed by *Aspergillus niger* (39%) and *Pyricularia oryzae* (36%). *Fusarium oxysporum, Bipolaris orizae, Alternaria alternata, Cercospora janseana, Curvularia lunata* and *Pyricularia grisea* were found to be intermediates within the range of 20-33.6% . *Sclerotium rolfsii, Rhizoctonia solani, Ephelis oryzae* and *Sarocladium oryzae* were found to be least. In case of seed washates, the percent incidence of *Aspergillus flavus* (40%) gave highest percent incidence followed by *Aspergillus niger* (34%) and *Pyricularia oryzae* (31%).

Table1: Fungi associated with seeds of Rice (*Oryza sativa* L.) Cv. Priyanka

Sr. No.	Name of Fungi	Percent (%) incidence of Mycoflora		
		Agar plate	Standard blotter paper	Seed washates
1	***Aspergillus flavus***	48.6	42.3	40.3
2	***Aspergillus niger***	46.6	39	34
3	***Pyricularia oryzae***	44.6	36	31
4	***Fusarium oxysporum***	41.6	33.6	28.6
5	***Bipolaris orizae***	38	31.6	26
6	***Alternaria alternate***	34.3	28.3	21.3
7	***Curvularia lunata***	31	24.6	19
8	***Cercospora janseana***	29	22.3	17.3
9	***Pyricularia grisea***	25.6	20	16
10	***Rhizoctonia solani***	22.6	18.3	14.6
11	***Sclerotium rolfsii.***	19.6	16	12.3
12	***Ephelis oryzae***	18	13.6	11.3
13	***Sarocladium oryzae***	16.6	11.3	10.3
	SE ±	7.01	6.3	5.9
	CD at 0.05 %	19.83	17.9	16.8

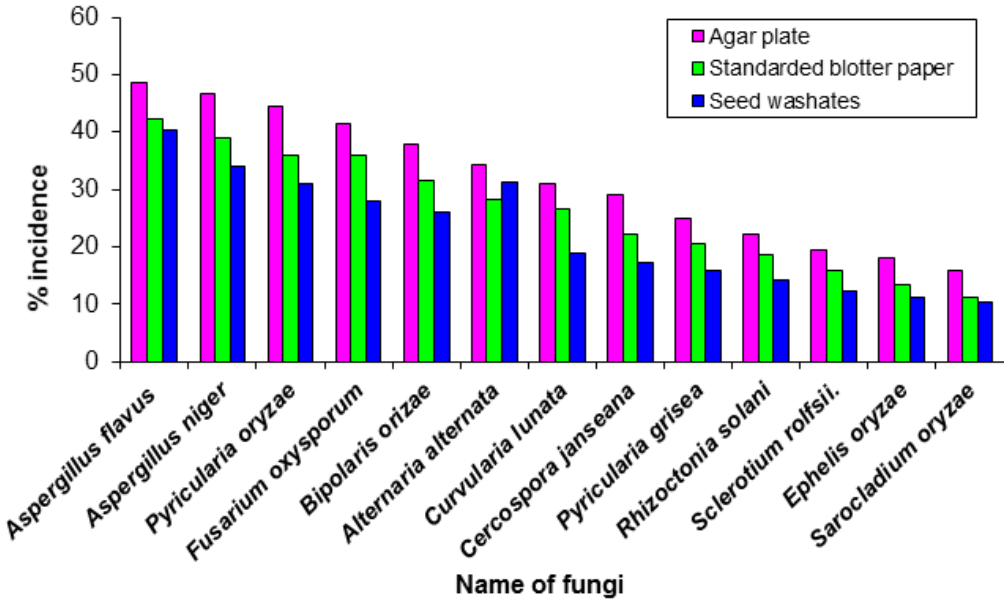


Fig.1. Fungi associated with seeds of Rice (Oryza sativa L.) Cv. Priyanka

Fusarium oxysporum, Bipolaris orizae, Alternaria alternata, Cercospora janseana, Curvularia lunata and *Pyricularia grisea* were found to be intermediates within the range of 16-28.6% . *Sclerotium rolfsii, Rhizoctonia solani, Ephelis oryzae* and *Sarocladium oryzae* were found to be least.

The present study was conducted to verify the presence of different mycoflora both in local and imported paddy seed lots. All fungal pathogens found in this study are known to be seed borne in nature. Rice crop is affected by a large variety of fungal pathogen and most of them are seed borne, which have been identified from rice seeds in *invitro* condition. Javaid (2002) studied seed mycoflora in rice. Mandhare (2008) studied Seed Health Evaluation in Paddy varieties (*Oryza sativa* L.). Gopalakrishnan et al. (2010) observed survey of Seed-Borne fungi associated with Rice. Butt et al. (2011) studied the seed Borne Mycoflora of Stored Rice Grains and its Chemical Control. Rathod et al. (2012) evaluated Seed borne Mycoflora from legume seeds. Ora et al.(2011) observed of Seed Borne Pathogens from Some Cultivated Hybrid Rice.

REFERENCES

Agrawal RL (1995) Seed technology. 2nd Edition. Oxford and Ibh Publishinh Co. Pvt. Ltd, New Dehli.

Butt AR, Yaseen SI and Javaid A (2011) Seed Borne Mycoflora of Stored Rice Grains and its Chemical Control. *J. Animal Plant Sci.,* 21(2): 193-196.

Ezuka A and H Kaku (2000) A historical review of bacterial blight of rice. Department of Genetic Resource II and I. Bull. Natl. Inst. Agrobialresour. 15: 1-207.

Fakir GA, Hossain I, Ahmed MU, Doullah MAU and Alam, M (2002) Quality of farmers Boro and T. Aman rice seeds collected before sowing from Bogra, Rajshahi and Rangpur districts of Bangladesh. A paper presented in the review and planning meeting of the Rice Seed Health Improvement (SHIP), PETRRA project held on 17- 18 April at BRRI, Gazipur, Bangladesh.

Franco DF, Ribeiro AS, Nunes CD and Ferreira F (2001) Fungi associated with seed of irrigated rice in Rio Grande do Sul. Revistabrsileira- de-Agrociencia. 7(3):235-236.

Gopalakrishnan C, Kamalakannan A and Valluvaparidasan V (2010) Survey of Seed-Borne Fungi Associated with Rice Seeds in Tamil Nadu, Production Unit, 24(1): 37-41. *India. Libyan Agric. Res. Cen. J. Intl*. 1(5): 307-309.

IRRI (2006) International Rice Research Institute. www. knowledge bank. irri. org/ ppfm/ storage/ 6.B. fungi. htm.

ISTA (International Seed Testing Association) (1993) International rules for seed testing. Rules amendments. *Seed Sci. Technol*. 29: 1-127.

ISTA (1996) International Rules for Seed Testing. e-Bangla Agricultural University for providing financial. *Seed Sci. Technol*., 24: 39-42.

Javaid MS, Wahid A, Idrees M, Gill MA and Saleem A (2002) Seed mycoflora studies in rice. *Pak. J. Phytopathol*. 14: 132-134.

Mandhare VK, Gawade SB and Game BC (2008) Seed Health Evaluation in Paddy varieties (*Oryza sativa* L.). *Agric. Sci. Digest.* 28 (4) : 289 – 291.

Mathur SB, Kongdal E (2003) Common laboratory seed health testing method for detection fungi. First edition. The International Seed Testing Assosiation (ISTA).

Neergard P (1977) Seed Pathology. The Macmillan Press Ltd., London, pp. 1187.

Neergard P (1979) Seed Pathology. The Macmillan press ltd., London and Basingstoke, pp.1191.

Ora N, Faruq AN, Islam MT, Akhtar N and Rahman MM (2011) Detection and Identification of Seed Borne Pathogens from Some Cultivated Hybrid Rice Varieties in Bangladesh. *Middle-East Journal of Scientific Research.* 10 (4): 482-488.

Ou SH (1985) Rice Diseases. CAB International Mycological Institute, Kew, Surrey, U.K. pp. 395.

Porta Puglia A (2004) Major seed borne diseases of rice. Informatore-Fitopatologico. 54(3): 18-21.

Rathod LR Jadhav MD Mane SK Muley SM and Deshmukh PS (2012) Seed borne Mycoflora of Legume seeds. *International Journal of Advanced Biotechnology and Research.* Vol 3, Issue 1, pp -530-532.

Salar Monajjem, Ebrahim Zainali, Farshid Ghaderi-Far, Elias Soltani, Maryam Hosseini Chaleshtari and Maryam Khoshkdaman (2014) Evaluation Seed-born Fungi of Rice (*Oryza sativa* L.) and that Effect on Seed Quality. *Plant Pathology & Microbiology*. 5-4 pp. 1-7.

Uma V and Wesely EG (2013) Seed borne fungi of rice from South Tamil Nadu. *J. Acad. Indus. Res.* Vol. 1(10).

RESEARCH ARTICLE

A screening tool for Quality of Mycorrhizal bio fertilizer- Mycorrhiza Inoculum potential

Shweta Jain* and Chahar Sunita

NES Ratnam college of Arts, Science and Commerce, Bhandup west, Mumbai-400071, India
*Corresponding author Email: jsweet.jain@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Shweta Jain and Chahar Sunita (2016) A screening tool for Quality of Mycorrhizal bio fertilizer- Mycorrhiza Inoculum potential, Int. J.of. Life Sciences, Special Issue, A7:81-84.

Acknowledgement: The authors are thankful to the Principal, NES Ratnam College of Arts, Science & Commerce for providing lab and Shade house facilities for carrying out the work.

ABSTRACT

Mycorrhiza is a known bio-fertilizer since long and has been found to be mobilizing various nutrients, including phosphorus, iron and zinc in adequate quantities. Mycorrhizal Inoculum Potential or Infectivity Potential is an indicator of mycorrhizal activity in the plant and soil. It allows the quality/infectivity of inoculum to be evaluated and is used as a biological indicator. Mycorrhizal Inoculum Potential is the number of infectious fungal propagules in a sample which can be tested by bioassay using plant seedlings as host and measurement of mycorrhizal colonization after a defined period. The method used in this study is Mean Infection Percentage, an effective method for assessing Mycorrhiza Inoculum Potential. Five mycorrhizal bio-fertilizer samples were used to assess the quality and an in house developed mycorrhizal bio-fertilizer was used to compare the results. The quality of samples was decided based on its spore count, viability of Infective Propagules, Mycorrhizal Infectivity Potential and presence of other endophytes after 15 days. Samples: "A,B,C,D,E" were fertilizer samples from the market and sample "F" was in house developed bio-fertilizer. Spore count in sample A,B,C,D and E was found to be 10,000 /gm, 101/gm, 6 /gm, 2/gm and 50/gm respectively . Spores of Glomus genera were the main Mycorrhizal constituent of all the samples and their Mycorrhizal infectivity potential after 15 days was found to be 0%, 30%, 0%, 10%, and 0% respectively. As compared to the market samples, F sample which is an inhouse developed Mycorrhizal bio fertilizer had 3spores/gm and showed 50% Mycorrhizal Inoculum Potential. Other endophytes were observed in all the roots of host plant (*Zea mays* plant) inoculated with fertilizer samples. In conclusion, the in house, in vivo developed product was of good quality than other in-vitro developed bio-fertilizer samples.

Keywords: Mycorrhiza Inoculum Potential, Mycorrhiza bio-fertilizer, *Glomus*, Endophytes

INTRODUCTION

Field trials with Arbuscular mycorrhizas are still beset with the issues of huge scale production of AM Fungi, its storage and application to the crop

An alternate approach is to control Indigenous Arbuscular mycorrhizal (AM) fungi by cultural practices or by the utilization of soil amendments that increase the effectiveness of the natural inocula. (Daft, 1992). Several bioassays are developed to indirectly count the quantity (or relative amount) of infectious fungal propagules during a sample use. (www.INVAM). Inoculum of Vesicular-Arbuscular Mycorrhizal fungi consists of various kinds of infective propagules: spores, vesicles (members of Glomineae only), hyphal fragments, and hyphae from mycorrhizal root fragments. whereas some analysis has indicated that hyphae and roots are most infective, this conclusion can't be generalized to all or any isolates of a species or perhaps to all or any species. (Abbott and Robson, 1981) The advantages and edges of adopting mycorrhizae in agriculture, permits us to raise, visualize the scope of this development at the crop level and , intern, the impact of its long- term adoption on the standard of life in improvement in nutrition, tolerance to water stress, proof against low temperature, transformation of root design, diversity of microbes in soil development, resistance against pathogens, enhanced synthesis of primary or secondary metabolites and improvement within the quality and amount of agricultural product. (Abbott and Robson, 1991b). Development and production of AM fungal inocula and bio-fertilizer is a laborious and cost consuming process because of obligate and bio tropic nature of AM fungi, hence to standardize the production process of AM fungi inocula and bio-fertilizer is of very much importance. Numerous plant species may be utilized in infectivity assays, since most have some dependence on the mycorrhizal association and can become inhabited. The foremost dependent plant species of grasses are most popular to optimize.

The main aim of this study was to find out and compare the performance of market fertilizer samples with inhouse developed bio- fertilizer sample

MATERIALS AND METHODS

1. Host plant used for checking mycorrhiza infection is *Zea mays*.
2. The red laterite soil and pot used for MIP testing was sterilized.
3. Sterilization technique used for soil is autoclaving soil at 1.5Kg/cm^2 at 128^{0}C for 30 minutes.
4. Pot was sterilized using alcohol swab.
5. Experiment was set up in the pot as shown in figure 1
6. Amount of mycorrhizal bio-fertilizer used for Mycorrhizal inoculums potential is 5 gm of each sample in each pot.
7. The plant was uprooted 15 days after germination and roots were checked for mycorrhizal infection by Modified Philips and Hayman,1970

Spore count and Spore isolation

by Gerdeman and Nicolson's method, 1963 (wet sieving and decanting)

Soil based inoculum

This is the most commonly used inoculant technique. Soil inocula are produced using traditional pot culture technique by multiplying AM inoculant in the soil mixture. The success of good soil inoculum production depends on selection of good host plant, efficient AM strain and a suitable substrate in which AM Fungus can be mass multiplied. (Bagyaraj et al., 2002) (Singh et al., 2016).

Fig. 1: method for developing in vivo mycorrhiza or setting up MIP

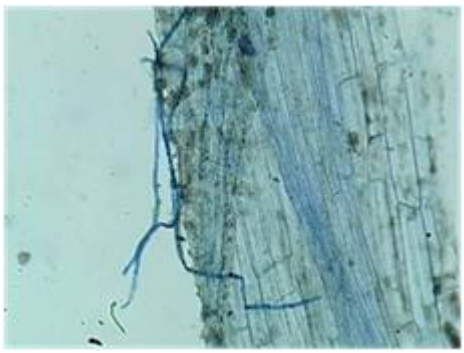


Fig.2: Mycorrhizal hyphae point

Preparation of in vivo mycorrhizal bio-fertilizer

3 sets of Mycorrhizal pots were taken each containing 5kg of substrate soil: sand in the ratio of 3:1 and 50gm of coco peat was added in each pot. 5 gms of *Funneliformis mosseae* containing 50 spores were inoculated at the time of germination of seeds. The plants were watered every alternate day. The plants were uprooted 90 days after sowing and analyzed for Spore density. (Shweta et.al,2016) 50 spores in 5kg of soil was added. After 90days spore density was found to be 60 spores per 20gm of soil, which is much higher compared to the results obtained by (Mala et al., 2010) i.e 769/50 g at the 6thmonth after inoculation. Here the time period was also less and spore density was high within 3 months only.

RESULTS AND DISCUSSION

The Bio fertilizer samples used A,B,C,D and E in the proposed study were containing Mycorrhizal propagules which were in vitro produced. These samples were subjected to MIP studies to assess the quality. Sample F was in vivo developed in house product with soil as carrier material. In vivo mycorrhizal inoculation treatments had high infectivity potentials, whereas the equivalent in vitro treatments were less effective at colonizing the plant's root system. Although all types of propagules of AM fungi (spores, root fragments, and hyphae) are able to initiate AM symbiosis. (Mosse, 1988) the production system of AM fungi itself can have implications in experimental results, thus requiring thorough investigation of mechanisms involved as well as monitoring of responses in cropping systems. (Calvet 2013) . Spore count in sample A,B,C,D and E was found to be 10000/gm, 101/gm, 6 /gm, 10 spores/5gm and 50/gm respectively. 3spores /gm was present in in vivo developed inhouse product sample F. As per (FCO 1985), requirement, a fertilizer sample should have 60 spores/g. Per that rule only sample A and B are passing the requirement but are not found to be effective in the field. However we suspect more number of spores in the bio-fertilizer samples as the spores must have changed their morphology when other ingredients are added in fertilizer sample. Changes in morphology of spores were not studied. This could be one of the reason for less spore count.

As per the procedure given in FCO, 1985, after 15 days of growth plant is uprooted and checked for Myorrhizal Inoculum Potential i.e. whether mycorrhiza is showing any entry point in plant root or not which is termed as infectivity potential. Mycorrhizal infectivity potential was 0%, 30%, 0%, 10%, and 0% respectively. As compared to the market samples, F sample which is a inhouse developed Mycorrhizal biofertilizer shows 50% Mycorrhizal Inoculum Potential. Root colonization achieved by in vivo root fragments when used as inoculums was much higher despite the uncertain density of propagules within the plant roots. A previous study done by (Plenchette et al., 1996) says Loss of infectivity along generations of inocula produced in vitro, both for spores and mycorrhizal root pieces, might reduce the infectivity and the effectiveness of the subcultures. This could be one of the reason for 0 % or no infection seen in bio-fertilizers sample A,B,C,D and E inoculated maize plants.

Table 1: Results after 15 days

Samples	A	B	C	D	E	F
Total spore count	10000/gm	101/gm	6 /gm	10 spores/5gm	50/gm	3/gm
Type of spore present	*Glomus intraradices*	*Accaulospora and Glomus*	*Glomusintraradices*	*Glomus etunicatum*	*Glomus intraradices/ Glomus Fasciculatum*	*Glomus mosseae*
Infectivity potential	0%	30%	0%	10%	0%	50%
Presence of other endophytes	present	present	Present	present	Present	Absent

Dominant myorrhizal type found in all types of bio-fertilizer samples was *Glomus*. A research conducted by Krishnamoorthy, 2015 says that T-RFLP and DGGE analysis confirmed the dominance of *Funneliformis mosseae* and *Rhizophagus intraradices* spores in major type of fertilizers and natural environments which was conducted to find out abundant mycorrhizal species.

Other endophytes were not identified during spore isolation from sample but when roots were checked for Myorrhizal Infection Potential, endophytes were observed in the roots. They grow much faster than mycorrhiza. The size of the mature chlamydospores varies between (14) 16 in length and (9) 10and 17(20) mm in width (Kost et al,2013)

CONCLUSION

In conclusion, Infection unit originates from entry points and represent the initial phase of fungal growth within the root cortex which may lead to general colonization of roots. (Cox G et.al 1974), Mycorrhiza Inoculum Potential is a useful tool for deciding quality of mycorrhizal bio-fertilizer. The in house in- vivo developed bio -fertilizer was found to be much effective compared to the in –vitro developed biofertilizers which had more spore count .Thus the Mycorrhiza Inoculum Potential test is a useful tool to find out quality and efficiency of AM fungal bio-fertilizers.

The above study and our results suggests that infective propagules need not be only spores, they may be infected root pieces with vesicles and arbuscules.

REFERENCES

Abbot LK and Robson AD (1991) Factors influencing the occurrence of arbuscular mycorrhizae, Agri. Ecosyst. Environ,35:121- 150

Cinta Calvet et al. (2013) Plant Growth Stimulation and Root Colonization Potential of In Vivo versus In Vitro Arbuscular Mycorrhizal Inocula, HORTSCIENCE 48(7):897–90

Cox G and Sanders P (1974) Ultrastructure of the host fungus interface in a Vesicular arbuscular mycorrhiza. New Phytol 73: 901-912

Daft MJ (1992) Use of mycorrhizas in agriculture: Problems and prospects. In: Read, D.J.; Lewis, D.H.; Fitter, A.H.; Alexander, I.J. (Eds.). Mycorrhizas in ecossystems. Wallingford: CAB International, 1992. P.198-201

Dhananjaya Pratap Singh, Harikesh Bahadur Singh, Ratna Prabha (2016) Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications, ISBN-978-81-322-2642-0 DOI 10.1007/978-81-322-2644-4

FCO, Fertilizer Control Order 1985, p206-2011

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizalendogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46:235- 244.

Gerhard Kost et al,2013, Morphology and Ultrastructure of Piriformosporaindica, sebainales and their biotechnological applications, p397, ISBN-978-3- 642-33801-4

Abbott L and Robson A (1981) Aust. J. Agric. Res. 32:631-639.

Mala WJ, Kumari IS, Sumanasena HA, Nanayakkara CM (2010) Effective Spore Density of Glomusmosseae, Arbuscular Mycorrhiza (AM), for Inoculation of Rooted Cuttings of Black Pepper (Piper nigrumLinn.) Trop. Agri. Res., 21(2): 189–197.

Mosse B (1988)Some studies relating to 'independent' growth of vesicular–arbuscularendophytes. Can. J. Bot. 66:2533–2540

O'halloran PI, Miller MH, Arnold G (1986) Absorption of P by corn (Zea mays L.) as influenced by soil disturbance. Canadian Journal of Soil Science, Ottawa, 66:287-302

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of colonization. Trans Br MycolSoc 55:158-160

Plenchette C, Declerck S, Diop TA and Strullu DG (1996) Infectivity of monoxenic subcultures of the arbuscular mycorrhizal fungus Glomusversiforme associated with Ri-T-DNA transformed carrot root. Appl. Microbiol. Biotechnol. 46:545–548.

Ramasamy Krishnamoorthy et al. (2015) Arbuscular Mycorrhizal Fungi Community Structure, Abundance and Species Richness Changes in Soil by Different Levels of Heavy Metal and Metalloid Concentration, PLoS ONE 10(6): e0128784. doi: 10.1371/journal.pone.0128784

Jain Shweta B and Sunita Chahar (2016) Effect of AM Fungi Funneliform ismosseaeon the Growth of Ocimum sanctum. *Int.J.Curr.Microbiol.App.Sci.* 5(4): 64-67. doi:http://dx.doi.org/10.20546/ijcmas.2016.504.010

Smith SE and NA walker (1981) A quantitative study of mycorrhizal infection in Trifolium: Separate determination of the rates of infection and of mycelia growth, New. Phytol 89; 225-240

invam.wvu.edu

RESEARCH ARTICLE

Study of effect of magnetized water treated with varying temperature on growth of *Penicillium chrysogenum*

Vaidya Vinit [1], Yadav Udaybhan [1], Gupta Deepak [1], Bhat Lalita [2] and Papaiya Shruti[3],

[1]Department of Botany, Thakur College of Science and Commerce, Kandivali (East), Mumbai 400101
[2]Department of Physics, Thakur Junior College, Kandivali (East), Mumbai 400101
[3]Department of EVS, Thakur Junior College, Kandivali (East), Mumbai 400101

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Vaidya Vinit, Yadav Udaybhan, Gupta Deepak, Bhat Lalita and Papaiya Shruti (2016) Study of effect of magnetized water treated with varying temperature on growth of *Penicillium chrysogenum* , Int. J.of. Life Sciences, Special Issue, A7:85-88.

Acknowledgement:
Authors are indeed grateful to the Management and Dr. (Mrs.) C. T. Chakraborty, Principal of the College to provide infrastructural facilities and also the moral support throughout the tenure of the project. We are also very much thankful to Dr. C. P. Shukla, the Head; Mr. Bholanath Yadav, Laboratory Assistant and Mr. Pravin Singh (Sonu), the Peon from the Department of Botany for their support and cooperation during the project

ABSTRACT

It has been proved that the magnetic field has positive impact on biological activities to accelerate rates of most of the reactions such as photosynthesis, respiration, blood circulation etc. When water is magnetized, the ions of the water are oriented as per the magnetic field and they acquire magnetic properties. This water can be used in various processes to study the effect of magnetic field on the biological activities. In the present studies, a bar magnet with known power of 70 Gauss was used to magnetize the distilled water, which was used with the parameter of varying temperature for preparation of Potato Dextrose Broth (PDB) for inoculation of *Penicillium chrysogenum*. The effect of magnetized water treated at different temperature on growth of *Penicillium chrysogenum* and production of mycotoxins by the fungal organism was studied.

Key words: Magnetized Water, Varying Temperature, Biological Activities

INTRODUCTION

Liquid water is required for life to start, continue and sustain. Recently, various theories have been proposed but without a consensus except for the key involvement of liquid water. Water possesses particular properties which cannot be found in other materials and that are required for life. These properties are brought about by the hydrogen-bonded environment particularly evident in liquid water. The hydrogen bond in liquid water is highly affected by electrical and magnetic fields. It is found that some physical and chemical properties change when water pass through magnetic field (Hirota *et. al.,* 1999). Therefore the so called "magnetized water" has different chemical and physical properties and action than ordinary water (Madsen, 2004).

The physical and chemical properties of magnetized water have a series of changes which lead to special functions (Dandan and Shi, 2013). Magnetic water improved the plant growth characteristics and nutrients uptake in tomato and soybean (Carbonell, 2011; Radhakrishnan and Kumari, 2012), root function (Aladjadjiyan, 2010), influenced the

chemical composition of plants, activate plant enzymes (Alikamanoglu and Sen, 2011; Shabrangi, 2011), in wheat (Hozayn and Abdul Qados, 2010), Maize (Zepeda, 2011).

Till 1980, a little was known about how the magnetic field can stimulate plant growth or even prevent (Mahmoud and Amira, 2010). Recent, there has been a rapid increase in the use of technologies employing magnetic water. The magnetized water is made by ordinary water which can get through the magnetic field of certain intensity with a certain flow rate, along with a direction perpendicular to the magnetic field lines.

Hay (1873) received first patent for introduction of a device for magnetization of water. Since, then various experiments were devised to study the effect of magnetized water on the biological activities in the organisms. Water molecule composed of positive hydrogen ions and negative hydroxyl group along with the dissolved minerals are diamagnetic. (Busch *et. al*., 1985). Kovacs et. al. (1997) proved that magnetic field has positive impact on water, which can be utilized to enhance biological activities in the organisms. It has also been proved that varying temperature also has effect on the magnetic power of the water (Yadav, 2016). Iverson (2004) and Hoadley (2006) also proved that varying temperature has positive impact on the magnetic power.

Hence, in the present investigation authors have tried to study the impact of magnetic water on the growth of *Penicillium chrysogenum* and even effect of varying temperature on the magnetic power of the water.

MATERIALS AND METHODS

Part I: Preparation of Potato Dextrose Broth (PDB)

Potato – 200 gms
Dextrose – 20 gms
Distilled Water – 1000 ml

200 gms of peeled and sliced potatoes were boiled in 500 ml distilled water to make a slurry and filtered to separate the slurry from the potato pieces. 20 gms of dextrose was dissolved in 500 ml of distilled water separately. Both the solutions were mixed together and volume was adjusted to 1000 ml with distilled water. The pH was adjusted to 5.6. The solution was autoclaved at 120°C temperature and pressure of 15 lb/inch^2 for 20 minutes. The solution after cooling to the room temperature was used as the PDB for inoculation of the fungus.

Obtaining the fungal organism

20 ml of the autoclaved PDB was poured into pre-autoclaved Erlenmeyer's flasks (150 ml) and a loopful pure culture of *Penicillium chrysogenum* was inoculated in the flasks in aseptic conditions. The flasks were incubated at 28 ($\pm$ 2) °C for 5 days to get luxuriant growth of the fungal organism. This was treated further for the experiment.

Part II: Quantitative Estimation of Biomass:

Four types of PDBs were used in the experiment. They were as follows:

1. PDB prepared in regular distilled water and autoclaved (120°C)
2. PDB prepared in magnetized distilled water without autoclaving or chilling (Room Temp.)
3. PDB prepared in magnetized distilled water and autoclaved (120°C)
4. PDB prepared in magnetized distilled water and chilled (0°C)

A loopful of pure culture was inoculated in the four flasks containing above mentioned PDBs and incubated at 28 ($\pm$ 2) °C for 5 days. After 5 days, all the cultures were filtered separately by using pre-weighed Whatman No. 1 filter papers and weight of biomass in each flask was calculated. The experiment was repeated 3 times and average weight of fungal biomass was noted down.

Part III: Colorimetric Estimation of Mycotoxins:

When fungal biomass was separated from each flask, the filtrate was collected separately and concentrated on the water bath (60°C) to obtain the Mycotoxins in concentrated form. These Mycotoxins were subjected to quantitative estimation as follows:

1 ml of each concentrated filtrate was taken in a test tube in which 1 ml solvent containing (Chloroform and Acetone - 9:1) was added. It was allowed to stand for 10 minutes. 1 ml of 1 N HCl followed by 1 ml of 1 N NaOH was added in each test tube to provide ionization gradient. The test tubes were incubated for 10 minutes at room temperature to separate the two layers of immiscible liquids as

aqueous and organic solvents. 1 ml of Ninhydrin solution was added to all the test tubes, whereby blue colour was obtained for aqueous solvent. This aqueous solution from each test tube was separated from the organic solvent by using separating funnel and subjected to colorimetric estimation of Mycotoxins. The Optical Density (O. D.) was calculated for each test tube by using red filter at 600 nm..

RESULTS AND DISCUSSION

As per Table No. 1, the amount of fungal biomass obtained in autoclaved distilled water, magnetized distilled water without autoclaving or chilling, magnetized and autoclaved distilled water and magnetized, chilled distilled water was 3.91, 4.98, 5.18 and 7.07 gms respectively.

This clearly indicates that there is a positive impact of magnetized water on growth of the fungal organism, which enhanced with lowering of the temperature. When temperature was increased, the impact of magnetic water was reduced.

As per Table No. 2, the highest amount of toxins were detected to be present in magnetized and chilled water (0.73) followed by magnetized and autoclaved distilled water (0.61), magnetized distilled water without autoclaving or chilling (0.34) while least amount of Mycotoxins were obtained in autoclaved distilled water (0.10) in terms of Δ O. D.

This shows that magnetized water has positive effect not only on growth of the fungal organism but even on the production of the mycotoxins by the organism.

Table No. 1: Quantitative Estimation of Biomass

Sr. No.	Particulars	Weight of Biomass (gms)
1	Autoclaved Distilled Water	3.91
2	Magnetized Distilled Water without Autoclaving or Chilling	4.98
3	Magnetized and Autoclaved Distilled Water	5.18
4	Magnetized and Chilled Distilled Water	7.07

Table No. 2: Colorimetric Estimation of Mycotoxins

Sr. No.	Particulars	O. D.	Δ O. D.
1	Blank	0.95	---
2	Autoclaved Distilled Water	1.05	0.10
3	Magnetized Distilled Water without Autoclaving or Chilling	1.29	0.34
4	Magnetized and Autoclaved Distilled Water	1.56	0.61
5	Magnetized and Chilled Distilled Water	1.68	0.73

1. *Penicillium chrysogenum* 2. Growth of Cultures in Treated PDBs 3. Extraction of Mycotoxins

Fig. 1: Growth of cultures in treated PDBs Extraction of Mycotoxins *Penicillium chrysogenum*

CONCLUSION

Above mentioned observations and results clearly indicate the positive effect of magnetized water on growth of *Penicillium chrysogenum*. It is also been seen that magnetized water helps the organism to release more amount of Mycotoxins. If we use magnetized water for preparation of PDB and autoclave it then it has more impact for production of biomass as well as secretion of Mycotoxins. It is revealed further that preparation of PDB in magnetized water followed by chilling treatment enhances the impact to produce more amount of biomass of *Penicillium chrysogenum* along with release of more amount of Mycotoxins. These observations are in tune with the fact that magnetic properties of water can be enhanced by chilling treatment at 0°C

REFERENCES

Kovacs PE, Valentine RL and Alvarez PJ (1997) "The effect of static magnetic fields on biological systems: Implications for enhanced biodegradation". *Crit. Rev. Environ. Sci. Technol*., 27**,** 1997, pp. 319-382.

Yadav A (2016) "Effect of Temperature on Electric Current, Magnets and Electromagnet". *International Journal of Advancements in Technology [ISSN 0976-4860] 7:167*.

Hay AT, US Patent No. 140, 196, June 24, 1873.

Hirota N, Nakagawa J and Kitazawa K (1999) "Effects of a magnetic field on the germination of plants " *Jou. Appli. Phy. , vol.85, no.8.*

Madsen HEL (2004) "Crystallization of calcium carbonate in magnetic field in ordinary and heavy water". *J. Crystal Growth 267 251-255.*

Dandan L and Shi Y (2013) "Effects of Magnetized Saline on Growth and Development of Winter Wheat Seedlings". *Adv. J. Food Sci. Technol., 5: 1596-1599.*

Carbonell MV, Florez M, Martinez E, Maqueda R and Amaya JM. (2011). "Study of stationary magnetic fields on initial growth of pea (*Pisum sativum* L.) seeds". *Seed Science and Technology, 39: 673-679.*

Radhakrishnan R and Kumari BDR (2012) "Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean". *Plant Physiology and Biochemistry, 51: 139-144.*

Aladjadjiyan A (2010) Influence of stationary magnetic field on lentil seeds". *Int. Agrophys., 24: 321-324.*

Alikamanoglu S and Sen A (2011) "Stimulation of growth and some biochemical parameters by magnetic field in wheat (Triticumaestivum L.) tissue cultures". *African Journal of Biotechnology, 10: 10957-10963.*

Shabrangi A, Majd A and Sheidai M (2011) Effects of extremely low frequency electromagnetic fields on growth, cytogenetic, protein content and antioxidant system of *Zea mays* L". *African Journal of Biotechnology, 10: 9362-9369.*

Zepeda RB, Hernandez CA, Suazo FL, Dominguez AP, Cruz AO, Martinez EO and Hernandez LMS (2011) "Physical characteristics of maize grain and tortilla exposed to electromagnetic field". Int. *Agrophys., 25: 389-393.*

Hozayn M and Abdul Qados AMS (2010) "Magnetic water application for improving wheat (Triticumaestivum L.) crop production". *Agric. Biol. J. N. Am., 1: 677-682.*

Busch KW and Mariana A Busch (1997) Laboratory Studies on Magnetic Water Treatment and Their Relationship to a Possible Mechanism for scale reduction". *Desalination 109 (1997) 131 - 148. Elsevier Publications.*

Iverson AA (2004) Does the Temperature of a Magnet Affects its Strength?' *California State Science Fair Abstract.*

Hoadley R (2006) "What affects the Strength of Magnet?". *Magnet Basics.*

Mahmoud H and Amira MS (2010) "Magnetic water application for improving wheat (Triticum aestivum L.) crop production". *Agric. Biol. J. N. Am., 1(4): 677–682*.

RESEARCH ARTICLE

Terrestrial Orchid Mycorrhiza and Non- Mycorrhizal Endophytes from Kolhapur District (M.S.) - III

Patil Anjali, Kambale Madhuri and Patil Sunita*

Department of Botany, Rajaram College, Kolhapur 416 004, Maharashtra State, India
*Corresponding author email: patilsunita790@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Terrestrial Orchid Mycorrhiza and Non- Mycorrhizal Endophytes From Kolhapur District (M.S.) - III J.of. Life Sciences, Special Issue, A7: 89-97

ABSTRACT

Orchid Mycorrhiza (OM) is a specialized group of endophytes, mutually associated with roots of almost all terrestrial orchids. They play an important role, not only in germination of seeds, but also for nutrient uptake throughout the entire adult life of many orchids, so much so that they are identified as mycoheterotrophs. As many as 45 numbers of terrestrial orchids belonging to the genera *Habenaria*, *Nervelia*, *Malaxis*, *Peristylus*, *Pectilis*, *Geodorum*, *Zeuxine*, *Cheirostylus* and *Eulophia* are recorded from Kolhapur District of Maharashtra state. Till date there are no reports of studies on OM of terrestrial orchids in the study area. Fungi isolated from orchids until now belong to the genus *Rhizoctonia*, Agaricales and other Basidiomycetes. In the present study OM from roots of *Habenaria brachyphylla* (Lindl.) Aitch., *Habenaria commelinifolia* (Roxb.) Wall. Ex Lindl., *Habenaria diphylla* (Nimmo) Dalzell, *Habenaria digitata* Lindl., *Habenaria foliosa* A. Rich.,*Habenaria foetida* Blatt. and McCann, *Habenaria furcifera* Lindl., *Habenaria gibsonii* Hook.f., *Habenaria heyneana* Lindl., *Habenaria longicorniculata* J. Graham, *Habenaria longicornu* Lindl., *Habenaria marginata* Colebr.,*Habenaria plantaginea* Lindl.,*Habenaria rariflora* A.Rich., *Habenaria roxburghii* Nicolson, *Malaxis versicolor* (Lindl.) Abeyw., *Nervelia infundibulifolia* Blatt. and McCann, *Nervelia crociformis* (Zoll. and Moritzi) Seidenf, *Pecteilis gigantea* (Sm.) Raf., *Peristylus densus* (Lindl.) Sant. and Kapad., *Peristylus lawii* Wight., *Peristylus plantagineus* (Lindl.) Lindl. are isolated and identified for the first time from Maharashtra state. During the present study non-mycorrhizal endophytes have also been recorded.

Keywords: Orchid Mycorrhiza, *Habenaria*, *Nervelia, Peristylus, Pecteilis, Malaxis.*

INTRODUCTION

Orchidaceae is one of the largest family in the world consisting about approximately 600-800 genera and over 25000-35000 species, has a worldwide distribution and largest number of the species are in the tropics. As many as 45 number of terrestrial orchid species belonging to the genera *Habenaria, Nervelia, Malaxis, Peristylus, Pecteilis, Geodorum,*

Zeuxine, Cheiristylus and Eulophia are recorded from Kolhapur District of Maharashtra state. The seeds of orchids are minute and contain very less reserve food. Hence, colonization by compatible fungus is essential for germination and early seedling development.

Several Orchid species remain achlorophyllus during their entire life cycle , depending on fungi for carbon compounds derived either from the breakdown of organic matter in the soil or from mycorrhizal linkage with autotrophic plants. There are 3 groups of Mycorrhizal fungi,

1) *Rhizoctonia*
2) Mycelium radicis atrovirens (Non –Sporulating Hyphomycetes)
3) Hyphomycetes

Orchid Mycorrhiza are unique in that they occur only within the Family Orchidaceae which is one of the largest family of flowering plants. The diagnostic feature of OM is the formation of hyphal coils (pelotons) within host root cells. OM can, therefore, be considered within the broad category of endomycorrhizas. The present investigation of terrestrial orchids has focused on both mycorrhizal and non- mycorrhizal fungi. Orchid mycorrhiza is the well known symbiotic relationship between orchid roots and a variety of fungi belonging to Fungi Imperfecti like *Rhizoctonia* and Basidiomycetes like *Tulasnella, Sebacina, Ceratobasidium* and Thanetophorus, etc. Orchid seeds are very minute, dust like and need symbiotic association of the suitable fungi for their germination, further growth and development into protocorm like bodies as the seeds lack sufficient endosperm.

Endophytes belong to a wide range of organisms - Bacteria and Fungi, inhibiting the healthy plant tissues without causing visible pathological symptoms. Endophytic fungi are polyphyletic, functionally diverse and serve as; latent pathogens, mutualists (e.g. Mycorrhiza) saprophytes involved in decomposition, nutrient turnover anti-herbivory, symbiotic increase of host plant fitness to abiotic stresses and improve adaptability to various environmental conditions.

MATERIALS AND METHODS

33 species of 09 genera of terrestrial orchids were collected from Kolhapur district. Field photography was done with a Nikon Digital camera. Healthy Roots of orchids were collected and preserved in 4% Formalin solution.

Isolation of Mycorrhizal fungi:
Mycorrhizal fungi were isolated using a modification of Masuhara and Katsuya method. Root & root hair were used as inoculants. PDA, CDA, NDY, NA and OA was used for the growth of fungal cultures in petriplates and test tubes, incubated at room temp. (25º C). Pure cultures were maintained on. PDA,CDA,NDY,NA and OA medium.

Anatomical Studies:
Thin**,** free hand sections of the roots were taken and stained with trypan blue (0.1% Lactophenol) and observed under the research microscope for presence of fungal hyphae in cortical cells in the root.

OBSERVTATIONS AND RESULT

Table 1: Localities of collection

Sr. No.	Genera	Field visits					
		2012-13	Locality	2013-14	Locality	2014-15	Locality
1.	*Cheirostylis parvifolia* Lindl.	-	-	14/09/13	Tillari, Chandgad	03/08/14	Amba
2.	*Habenaria brachyphylla* (Lindl.) Aitch.	20/08/12	Kas, Thosegar, Bamnoli, Satara	08/10/13	Kas	19/08/14	Kas, Satara Bamnoli, Thoseghar
3.	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	08/09/12-09/09/12	Malshej Ghat, Bhimashankar, Vichitragad	16/08/13 - 17/08/13	Malshej, Vichitragad	15/08/14-18/08/14	Uran, Malshej

4.	*Habenaria diphylla* (Nimmo) Dalzell	08/09/12-09/09/12	Malshej Ghat, Bhimashankar, Vichitragad	08/10/13	Satara, Kas, Bamnoli, Thoseghar	14/09/14	Gaganbawada
		Sept 2012	Matheran, Uran, Lonavala	15/09/13	Gaganbawada		
		07/08/12	Kas, Thosegar, Bamnoli, Satara Thosegar	16/08/13 - 17/08/13	Malshej, Vichitragad		
5.	*Habenaria digitata* Lindl.	07/08/12	Kas, Thosegar, Bamnoli, Satara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	19/08/14	Kas, Satara Bamnoli, Thoseghar
6.	*Habenaria foliosa* A. Rich.	05/08/12	Ugwai Devrai, Radhanagari	30/08/13	Amba		
		05/09/12	Gavase Devrai, Ajara	22/09/13	Patgaon		
		Sept 2012	Matheran, Uran, Lonavala	15/07/13	Radhanagari		
				02/10/13	Gavase Devrai Ajara		
7.	*Habenaria foetida* Blatt. and McCann	22/07/12	Ajara	30/08/13	Amba	10/08/14	Patgaon, Pal, Bhatwadi
		05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	30/11/14	Radhanagari
		29/07/12	Patgaon	02/10/13	Gavase Devrai Ajara	19/08/14	Kas, Satara Bamnoli, Thoseghar
8.	*Habenaria furcifera* Lindl.	Sept.2012	Tillari	02/10/13	Ajara	29/07/14	Ajara, Tillari
		Oct.2012	Barki, Anuskura Ghat			12/10/14	Kasar Kandgaon
		12/09/12	Amba	30/08/13	Amba ghat	15/08/14-18/08/14	Uran, Malshej
		08/09/12-09/09/12	Malshej ghat, Bhimashankar,Vichitragad	13/10/13	Karanj, Mumbai	07/09/14	Patgaon, Pal
		Aug.- Oct.	Tillari	16/08/13 - 17/08/13	Malshej, Vichitragad	03/08/14	Amba
		Sept 2012	Matheran, Uran, Lonavala	22/09/13	Patgaon	29/07/14	Ajara, Tillari
		July2012	Katyayni Devrai, Kolhapur	28/07/13	Masai Pathar, Panhala	29/07/14	Kasar Kandgaon
		Oct.2012	Kas, Thosegar, Bamnoli, Satara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	29/07/14	Ajara
9.	*Habenaria gibsonii* Hook.f.	Aug.- Oct.	Ratnagiri	15/07/13	Radhanagari	29/07/14	Kasar Kandgaon
				02/10/13	Gavase Devrai Ajara		
10.	*Habenaria heyneana* Lindl.	Oct.2012	Kas	15/07/13	Radhanagari	19/08/14	Kas, Satara Bamnoli, Thoseghar
		05/08/12	Ugwai Devrai, Radhanagari	Sept.2013	Amboli Choukul	21/09/14	Amboli Choukul
11.	*Habenaria longicorniculata* J. Graham	Aug.-Sept.	Vaibhavwadi	30/08/13	Amba	17/07/14	Radhanagari
		12/09/12	Amba	14/09/13	Tillari, Chandgad	19/08/14	Kas, Satara Bamnoli, Thoseghar
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	15/07/13	Radhanagari	07/09/14	Patgaon, Pal

		Aug.-Sept.	Tillari	16/08/13 - 17/08/13	Malshej, Vichitragad	15/08/14-18/08/14	Uran, Malshej
		05/09/12	Gawase Devrai, Ajara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		Sept 2012	Matheran, Uran, Lonavala	Sept.2013	Amboli Choukul	03/08/14	Amba
		Aug.- Oct.	Karanj, Mumbai	08/11/13	Bugate-Alur	29/07/14	Ajara, Tillari
		Aug.- Oct.	Bugate-Alur	16/08/13 - 17/08/13	Bhimashankar	29/07/14	Kasar Kandgaon
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad			29/07/14	Ajara, Tillari
12.	*Habenaria longicornu* Lindl.	22/07/12	Ajara	16/08/13	Uran-Raigad	10/08/14	Patgaon, Pal, Bhatwadi
		Sept.2012	Kolhapur				
13.	*Habenaria marginata* Colebr.	Oct.2012	Kolhapur				
		Sept 2012	Matheran, Uran, Lonavala	20/08/13	Kolhapur	Jun-Oct.2014	Kolhapur
		05/08/12	Ugwai Devrai, Radhanagari	10/08/13	Patgaon,	21/09/14	Amboli Choukul
		Aug.-Sept.	Gaganbawada	08/10/13	Satara, Kas, Bamnoli, Thoseghar	03/08/14	Amba
		Aug.-Sept.	Amboli			30/11/14	Radhanagari
14.	*Habenaria multicaudata* Sedgw.	08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	02/10/13	Ajara	10/08/14	Patgaon, Pal, Bhatwadi
15.	*Habenaria ovalifolia* Wight					17/07/14	Radhanagari
		Aug.-Sept.	Tillari	14/09/13	Tillari, Chandgad		
16.	*Habenaria plantaginea* Lindl.	Sept 2012	Matheran, Uran, Lonavala	14/09/13	Tillari, Chandgad		
		Sept.- Oct.	Mahabaleshwar				
17.	*Habenaria rariflora* A.Rich.					03/08/14	Amba
						29/07/14	Ajara, Tillari
18.	*Habenaria roxburghii* Nicolson	Sept.2012	Sutagatii	09/09/13	Sutgatii Ghat	14/09/14	Sutakatti, Dist. Belgavi
19.	*Habenaria stenopetala* Lindl.	Oct.2012	Koyna, Nawaza	Oct.2013	Koyna, Nawaza	Oct.2014	Koyna, Nawaza
20.	*Liparis nervosa* (Thunb.) Lindl.	05/08/12	Ugwai Devrai, Radhanagari	15/09/13	Radhanagari, Patgaon, Ajara	29/07/14	Kasar Kandgaon
		Aug.-Sept.	Tillari				
21.	*Malaxis versicolor* (Lindl.) Abeyw.	Aug.-Oct.	Thosegar	30/08/13	Amba,	17/07/14	Radhanagari
		12/09/12	Amba	14/09/13	Tillari, Chandgad	19/08/14	Kas, Satara Bamnoli, Thoseghar
		Aug.-Oct.	Tillari	15/09/13	Radhanagari, Patgaon, Ajara	07/09/14	Patgaon Pal Devrai
		Oct. 2012	Barki, Anuskura Ghat			15/08/14-18/08/14	Uran, Malshej
		Sept 2012	Matheran, Uran, Lonavala			10/08/14	Patgaon, Pal, Bhatwadi

			Mahabaleshwar			03/08/14	Amba
						29/07/14	Ajara, Tillari
						29/07/14	Kasar Kandgaon
						29/07/14	Ajara, Tillari
22.	*Nervelia concolor* (Blume)Schltr. (= N. aragoana)	Aug.-Oct.	Thosegar	15/07/13	Radhanagari	17/07/14	Radhanagari
		20/08/12	Kas, Thosegar, Bamnoli, Satara	10/08/14	Patgaon, Ajara	19/08/14	Kas, Satara Bamnoli, Thoseghar
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad			29/07/14	Ajara, Tillari
		Aug.- Oct.	Tillari				
		Oct.2012	Koyana nagar, Nawaza				
		Aug.-Oct.	Mahabaleshwar				
23.	*Nervelia infundibulifolia* Blatt. and McCann (=N. infundibliformis)	05/09/12	Gawase Devrai, Ajara	30/08/13	Amba,	12/10/14	Kasar Kandgaon
		05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	21/09/14	Amboli Choukul
		12/09/12	Amba	02/10/13	Ajara	10/08/14	Patgaon, Pal, Bhatwadi
24.	*Nervelia crociformis* (Zoll. and Moritzi) Seidenf *(N.prainanana)*	Aug.-Oct.	Patagaon			17/07/14	Radhanagari
		05/09/12	Gawase Devrai, Ajara			07/09/14	Patgaon, Pal
		22/07/12	Chaloba Devrai, Ajara			19/08/14	Kas, Satara Bamnoli, Thoseghar
		Oct.2012	Kas			15/08/14-18/08/14	Uran, Malshej
		08/09/12-09/09/12 Aug.-Oct.	Malshej ghat, Bhimashankar, Vichitragad Karanj			10/08/14	Patgaon, Pal, Bhatwadi
						03/08/14	Amba
		Sept 2012	Matheran, Uran, Lonavala			29/07/14	Ajara, Tillari
						29/07/14	Kasar Kandgaon
						29/07/14	Ajara, Tillari
25.	*Pecteilis gigantea* (Sm.) Raf.	Aug.Sept.	Tillari	29/07/14	Tillari, Chaloba Devrai,	17/07/14	Radhanagari
		05/09/12	Gawase Devrai Ajara	15/07/13	Radhanagari	29/07/14	Ajara, Tillari
		05/08/12	Ugwai Devrai, Radhanagari	22/09/13	Patgaon, Ajara	29/07/14	Ajara, Tillari
		Aug.- Oct.	Amboli	02/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		July 2012	Amba			19/08/14	Kas, Satara Bamnoli, Thoseghar
		20/08/12	Kas, Thosegar, Bamnoli,Satara			29/07/14	Ajara, Tillari
26.	*Peristylus densus* (Lindl.) Sant. and	05/08/12	Ugwai Devrai, Radhanagari	14/09/13	Tillari, Chandgad	29/07/14	Ajara, Tillari

	Kapad.	20/08/12	Kas, Thosegar, Bamnoli,Satara	22/09/13	Patgaon, Ajara	29/07/14	Ajara, Tillari
		05/07/12	Gawase Devrai Ajara	02/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		Sept 2012	Matheran, Uran, Lonavala			17/07/14	Radhanagari
27.	*Peristylus goodyeroides* (D.Don) Lindl.	05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	17/07/14	Radhanagari
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	02/10/13	Ajara	29/07/14	Ajara, Tillari
		Sept 2012	Matheran, Uran, Lonavala			03/08/14	Amba
						29/07/14	Ajara, Tillari
28.	*Peristylus lawii* Wight.	Sept 2012	Matheran, Uran, Lonavala			10/08/14	Patgaon, Pal, Bhatwadi
		Sept.-Oct.	Pratapgad				
29.	*Peristylus plantagineus* (Lindl.) Lindl.	29/07/12	Patgaon	15/07/13	Radhanagari	17/07/14	Radhanagari
				02/10/13	Gawase Devrai Ajara	29/07/14	Ajara, Tillari
30.	*Peristylus aristatus* Lindl.	05/08/12	Ugwai Devrai, Radhanagari				
		Aug.-Oct.	Tillari				
31.	*Zeuxine longilabris* (Lindl.) Trimen					29/07/14	Kasar Kandgaon

Table 2: : Fungi isolated

Sr No	Host	Media	Fungal Isolates.
1.	*Habenaria digitata* Lindl.	PDA	Vegitative mycelium
2.	*Habenaria foliosa* A. Rich.	PDA	*Aspergillus sp.*
3.	*Habenaria foetida* Blatt. & McCann	PDA	*Aspergillus sp.*
4.	*Habenaria heyneana* Lindl.	CDA	Vegitative mycelium
5.	*Habenaria longicorniculata* J.Graham	CDA	*Rhizoctonia solani* J. G. Kuhn
6.	*Habenaria marginata* Colebr.	NDA	*Rhizoctonia solani* J. G. Kuhn
7.	*Habenaria multicaudata* Sedgw.	PDA	*Rhizoctonia solani* J. G. Kuhn
8.	*Habenaria ovalifolia* Wight	PDA	*Rhizoctonia solani* J. G. Kuhn
9.	*Habenaria rariflora* A.Rich.	PDA	*Rhizoctonia solani* J. G. Kuhn
10.	*Habenaria roxburghii* Nicolson	PDA	*Rhizoctonia solani* J. G. Kuhn
11.	*Liparis nervosa* (Thunb.) Lindl.	PDA	*Aspergillus sp.*
12.	*Malaxis versicolor* (Lindl.) Abeyw.	PDA	*Aspergillus sp.*
13.	*Nervelia concolor.*(Blume)Schltr.	PDA	*Fusarium sp.*
14.	*Nervelia infundibulifolia* Blatt. &McCann	PDA	*Rhizoctonia solani* J. G. Kuhn
15.	*Nervelia crociformis* (Zoll.& Moritzi) Seidenf	PDA	Vegitative mycelium
16.	*Pecteilis gigantea* (Sm.) Raf.	PDA	*Rhizoctonia solani* J. G. Kuhn
17.	*Peristylus lawii* Wight.	PDA	*Rhizoctonia solani* J. G. Kuhn
18.	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	CDA	*Rhizoctonia solani* J. G. Kuhn
19.	*Habenaria brachyphylla* (Lindl.) Aitch.	CDA	Vegitative mycelium
20.	*Habenaria rariflora* A.Rich.	PDA	*Rhizoctonia solani* J. G. Kuhn
21.	*Peristylus goodyeroides* (D.Don) Lindl.	PDA	*Trichoderma , Gonytrichum& Glioladium*
22.	*Peristylus densus* (Lindl.) Sant. and Kapad.	PDA	*Rhizoctonia solani* J. G. Kuhn
23.	*Habenaria diphylla* (Nimmo) Dalzell	PDA	Vegitative mycelium

C. ***Habenaria crinifera*** **Lindl D.** ***Nervelia concolor*** **(Blume)Schltr E.** ***Pecteilis gigantea*** **(Sm.) Raf F.** ***Habenaria ovalifolia*** **Wight**
G. ***Habenaria commelinifolia*** **(Roxb.) H.** ***Malaxis versicolor*** **(Lindl.) Abeyw I.** ***Habenaria brachyphylla*** **(Lindl.) Aitch**

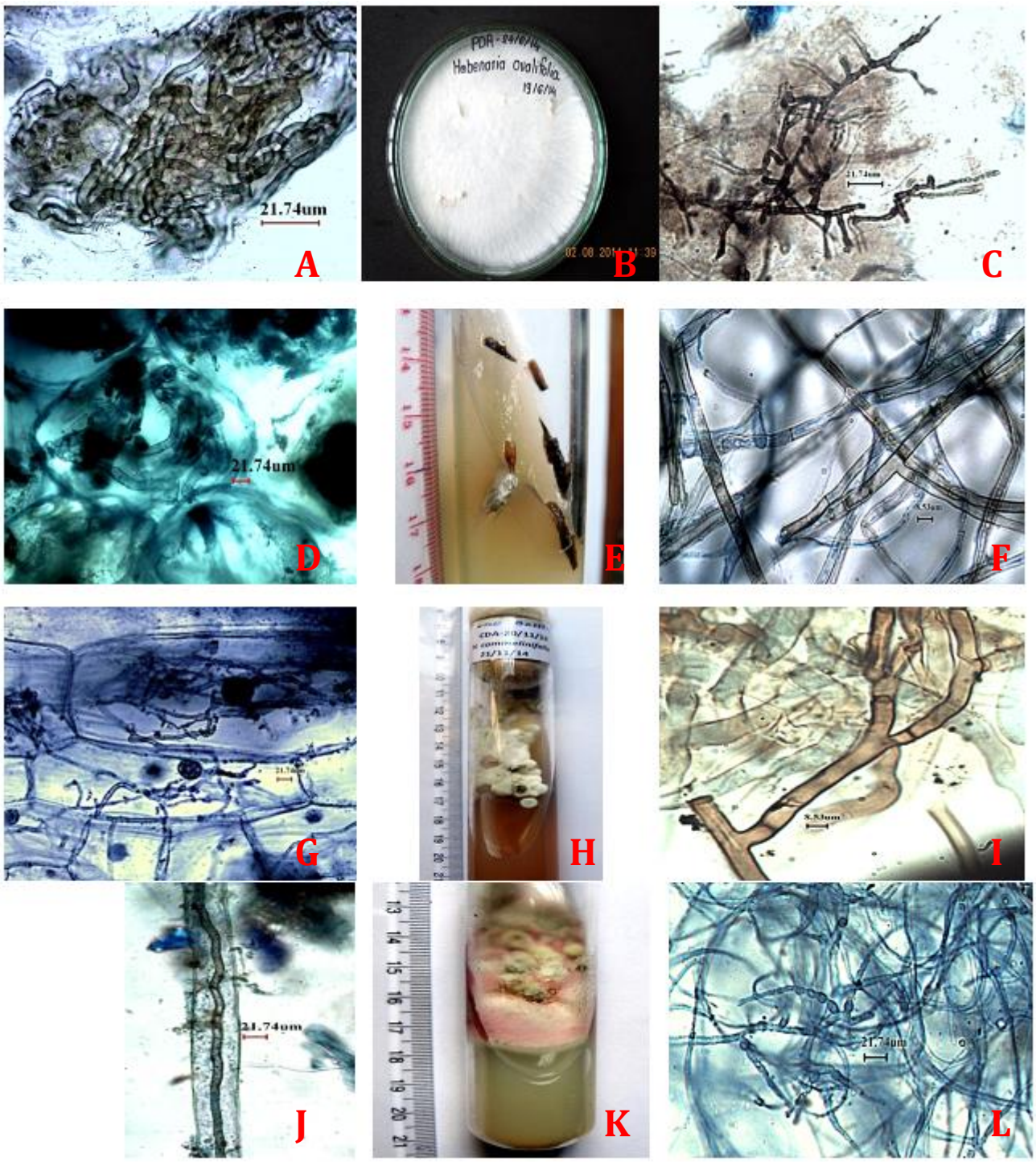


Fig. 2: A. *Rhizoctonia* peloton in *Habenaria roxburghii* Nicolson root cells; **B.** Pure isolate of *Rhizoctonia* sp.from *Habenaria ovalifolia* Wight roots; **C.** *Rhizoctonia* sp.(100X) from *Habenaria ovalifolia* Wight; **D.** *Rhizoctonia* peloton in *Habenaria marginata* Colebr root cells; **E.** Pure isolate of *Rhizoctonia* sp. from *Habenaria multicaudata* Sedgw. roots; **F.** Vegetative mycelium (100X) from *Malaxis versicolor* (Lindl.) Abeyw ;**G.** *Rhizoctonia* sp. peloton in *Nervelia plicata* (Andrews) Schltr. root cells; **H.** Pure isolate of *Rhizoctonia* sp. from *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl. root cell; **I.** *Rhizoctonia* sp.(100X) from *Habenaria marginata* Colebr; **J.** *Rhizoctonia* sp. peloton in *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl. root cell; **K.**Pure isolate of *Rhizoctonia* sp. from *Habenaria foetida* Blatt. and McCann root; **L.** Vegetative mycelium (40X) from *Habenaria multicaudata* Sedgw.

REFERENCES

Agerer R (1995) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 685–734

Ashford AE, Allaway WG(1982)A sheathing mycorrhiza in Pisonia grandis R.Br. (Nyctaginaceae) with development of transfer cells rather than a hartig net. *New Phytol* 90:511–519.

Alexander C, Alexander IJ, Hadley G (1984) Phosphate uptake by *Goodyera repens* in relation to mycorrhizal infection. *New Phytol* 97:401-411.

Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ (2004) Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proc Royal Soc Lond B* 271:1799-1806.

Bidartondo MI (2005) The evolution of myco-heterotrophy. *New Phytol* 167:335-352.

Bonnardeaux Y, Brundrett M, Batty A, Dixon K, Koch J, Sivasithamparam K (2007) Diversity of mycorrhizal fungi in terrestrial orchids: compata-bility webs, brief encounters, lastingrelationships and alien invasions. *Mycol Res* 111:51-61.

Brundrett MC, Scade A, Batty AL, Dixon KW, Sivasithamparam K (2003) Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. *Mycol Res* 107:1210-1220.

Cameron DD, Leake JR, Read DJ (2006) Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol 171:405-416.

Cameron DD, Johnson I, Leake JR, Read DJ (2006) Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. *Ann Bot* 99:831-834.

Dickson S (2004) The Arum-Paris continuum of mycorrhizal symbioses. *New Phytol* 163:187–200.

Dickson S, Schweiger P, Smith FA, Söderström B, Smith S (2003) Paired arbuscules in the Arum-type arbuscular mycorrhizal symbiosis with Linum usitatissimum. *Can J Bot* 81:457–463.

Duddridge JA, Read DJ (1982) An ultrastructural analysis of the development of mycorrhizas in Monotropa hypopitys L. *New Phytol* 92:203–214.

Ellis MB (1971 & 1976) More Dematiaceous Hypomycetes. 42,65,148 pp.

Gebauer G, Meyer M (2003) 15N and 13C natural abundance of autotrophic and mycoheterotro-phic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytol* 160:209-223.

Girlanda M, Selosse MA, Cafasso D, Brilli F, Delfine S, Fabbian R, Ghignone S, Pinelli P, Segreto R, Loreto F, Cozzolino S, Perotto S (2006) Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. *Mol Ecol* 15:491-504.

Gams (2000) The Mycota. 323pp.

Hadley G (1982) Orchid Mycorrhiza. In: Arditti J, ed. Orchid biology: reviews and perspectives, II. Ithaca, NY, USA: Cornell University Press, 83–118.

Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA (2005) Mixotrophy in orchids: insights from a comparative study of green individuals and non photosynthetic individuals of *Cephalanthera damasonium*. *New Phytol* 166:639-653.

Leake JR (1994) The biology of myco-heterotrophic ('saprophytic') plants. *New Phytol* 127:171-216.

Leake JR (2005) Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the "saprophytic" plant myth. *Mycologist* 19:113-122

Masuhara G, Katsuya K (1994) *In situ* and *in vitro* specificity between *Rhizoctonia* spp. And *Spiranthes sinensis* (Persoon) Ames. var. amoena (M. Biebertsien) Hara (Orchidaceae). *New Phytol* 127:711-718.

McCormick MK, Whigham DF, O'Neill J (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. *New Phytol* 163:425-438.

McCormick MK, Whigham DF, Sloan D, O'Malley K, Hodkinson B (2006) Orchid-fungus fidelity: A marriage meant to last? *Ecology* 87:903-911.

McKendrick SL, Leake JR, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. *New Phytol* 145:539-548.

Naga MS, Kandilkere RS(2012)Non mycorhizal fungal endophytes in two orchids of Kaiga forest (Western Ghat), *India J. of Forestry Research* 23(3) : 453-460.

Robertson DC, Robertson JA (1982) Ultrastructure of Pterospora andromedea Nuttal and Sarcodes sanguinea Torrey mycorrhizas. *New Phytol* 92:539–551.

Robertson DC, Robertson JA (1985) Ultrastructural aspects of Pyrola mycorrhizae. *Can J Bot* 63:1089–1098.

Rasmussen HN (2002) Recent developments in the study of orchid mycorrhiza. *Plant Soil* 244:149-163.

Smithm SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, San Diego.

Selosse MA, Weiß M, Jany JL, Tillier A (2002a) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) 53. L.C.M. Rich. and neighbouring tree ectomycorrhizae. *Mol Ecol* 11:1831-1844.

Selosse MA, Richard F, He X, Simard SW (2006) Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecol Evol* 21:621-628.

Shefferson RP, Weiß M, Kull T, Taylor DL (2005) High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. *Mol Ecol* 14:613-626.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, Cambridge.

Taylor DL, Bruns TD (1997) Independent, specialized invasions of ectomycorrhizal

mutualism by two nonphotosynthetic orchids. *Proc Natl Acad Sci* USA 94:4510-4515.

Taylor DL, Bruns TD, Szaro TM, Hodges SA (2003) Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. *Am J Bot* 90:1168-1179.

Xiaoya M, Kang J, Nontachaiyapoom S, Wen T and Hyde KD (2015) Non-Mycorrhizal endophytic fungi from Orchids. *Current Science* 109 (1): 72-87.

RESEARCH ARTICLE

Mobile phones of roadside food vendors: An exogenous source of infections

Patil Vaishali G, Sonawane Adhiraj A, Gaikwad Shraddha N and Saha Moitreyee

Department of Botany, VPM's B.N. Bandodkar College of Science, Thane
e-mail: sahamoitreyee@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Patil Vaishali G, Sonawane Adhiraj A, Gaikwad Shraddha N and Saha Moitreyee (2016) Mobile phones of roadside food vendors: An exogenous source of infections, Int. J. of. Life Sciences, Special Issue, A7: 98-101.

Acknowledgments: Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities under Research Scholar Programme. We are also thankful to DBT-Star College Scheme for providing the instruments required for the research work.

ABSTRACT

Mobile phones have become an indispensable tool across all age groups in professional and social life. The potential role of mobile phones as an exogenous source of infection is a major concern. The objective of this study was to explore the prevalence of fungal contamination on mobile phones of various food vendors on road side food shops in vicinity of VPM's B. N. Bandodkar College of Science, Thane. These roadside food stalls are popular and are frequented by the students, staff members of the college and the public. Twenty swab samples were randomly collected from the roadside food vendors and were cultured to observe the presence of fungal microflora. The predominant fungi isolated were *Aspergillus flavus*, *Aspergillus niger, Mucor indicus, Rhizopus stolonifer, Penicillium* sp., *Candida albicans* and *Cladosporum* sp.

Key words: *fungal isolates, Mobile phones, roadside food vendors*

INTRODUCTION

Street foods are foods and beverages sold by roadside vendors and hawkers. These food items are usually sold near public places and customers from various economic strata benefit from these low-cost meals. Customers however overlook aspects of hygiene or sanitation in these roadside stalls as availability and accessibility determine street food consumption. Mobile phone used by the food vendors constitutes a major health hazard as their phones are seldom cleaned. Their mobile phones may harbour various potential pathogens, thus becoming an exogenous source of infections and possible vector for transmission of pathogens. Colonization of potentially pathogenic organisms on various objects such as pagers, personal digital assistants, hands, and mobile phones has been reported (Singh *et al.* 2002). Level of contamination depends on the clinical and geographical setting (Heyba *et al.*, 2015). Microbiologists say that the combination of constant handling and the heat generated by the phones creates a prime breeding ground for all sorts of microorganisms that are normally found on our skin (Brady *et al.,* 2006). The aim of this study was to investigate the fungal contamination of mobile phones belonging to food vendors with their stalls in close

vicinity of VPM's B. N. Bandodkar college of Science, Thane. These roadside food stalls are very popular with students and staff members of the college. The public also visit the food stalls as the Thane railway station and CIDCO bus stop are nearby. The food served in these stalls are tasty, of good value, available to everyone almost around the clock, best bet for grabbing a quick bite and are affordable.

MATERIALS AND METHODS

Study area: The study was conducted to investigate fungal contamination of mobile phones belonging to roadside food vendors in the vicinity of VPM's B. N. Bandodkar College of Science, Thane, Maharashtra.

Sample collection: In this cross-sectional study total twenty swab samples were randomly collected from the roadside food vendors. Sterile swab sticks were immersed in sterile normal saline and then used to swab the phones at the earpiece, mouthpiece, keypad, and the sides to ensure that microorganisms on the phone adhere to the swab sticks appropriately (Ekrakene and Igeleke, 2007) and then placed in sterile sealable plastic bags (one swab in one bag) and immediately transported to laboratory. For control mobile phone of a food vendor was thoroughly wiped and sterilized with 70% isopropyl alcohol and swab sample was collected.

Inoculation and Isolation: The specimens were inoculated onto potato dextrose agar (PDA) and incubated at room temperature for 72 hours (Chinedu *et al.,* 2007).

Identification and characterization of the isolates: Preliminary identification and characterization of the isolate fungi was done on the basis of culture appearance, colonial morphology, mycelia, spores and colour according to cotton blue test. Fungal isolates were characterized as described by Barnett and Hunter (1972) (Barnett and Hunter, 1972). Identification of fungal culture was also done by Biosource Biotech laboratories, Pune.

RESULTS AND DISCUSSION

Total 20 mobile phones belonging to roadside food vendors in the vicinity of VPM's B. N. Bandodkar College of Science, Thane, Maharashtra were analyzed. Fungal contamination was observed in all 20 samples when compared with control (Table 1). *Aspergillus flavus*, *Aspergillus niger, Mucor indicus, Rhizopus stolonifer, Penicillium* sp., *Candida albicans* and *Cladosporum* sp. were isolated and identified from these samples. The morphological characteristics of the fungal isolates were studied (Table 2).

Fungal contaminants like *Aspergillus* spp cause different forms of hypersensitivity diseases such as allergic asthma and aspergillosis. Chronic granulomatous disorder (CGD) with pulmonary infection is caused by *Penicillium* spp. *Mucor* spp is the reason behind gastrointestinal disorders. Root cause of mucocutaneous, pulmonary and urological infections is *Rhizopus* spp. *Candida albicans* is the causative agent for vaginal and urinary tract infection. *Cladosporium* species have been reported to cause infections of the skin. The airborne spores of *Cladosporium* species are significant allergens and in large amounts they can severely affect asthmatics and people with respiratory diseases (Deshmukh and Rai, 2005).

Table 1: Identification of fungal isolates from different food vendor's mobile phone

	C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
a	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	-	-
b	-	+	-	-	-	+	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-
c	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
d	-	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
e	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+	+
f	-	+	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-
g	-	+	-	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-

a : *Aspergillus flavus*, b: *Aspergillus niger*, c: *Mucor indicus*, d : *Rhizopus stolonifer*, e: *Penicillium sp.*, f: *Candida albicans,* g: *Cladosporum sp.*

Table 2: Morphological characteristics of fungi isolates

Sr. no.	*Colony description*	*Morphological characteristics*	*Organism*
1.	Pale brown colonies	Septate branched, mycelium, green-brown conidia	*Aspergillus flavus*
2.	Black colonies	Septate branched, mycelium, Blackish conidia	*Aspergillus niger*
3.	Greyish brown	Broad hyphae, non-septate sporangiophores	*Mucor indicus*
4.	Brownish colonies	Cottony surface, non-septate mycelium	*Rhizopus stolonifer*
5.	Blue green colonies	Septate branched, mycelium, with conidiophores	*Penicillium* spp.
6.	Yellow-white colonies	Pseudohyphae, septate	*Candida albicans*
7.	Green to dull green, then olive-grey	cylindrical, aseptate, pigmented, smooth, long chain of conidia	*Cladosporum* sp.

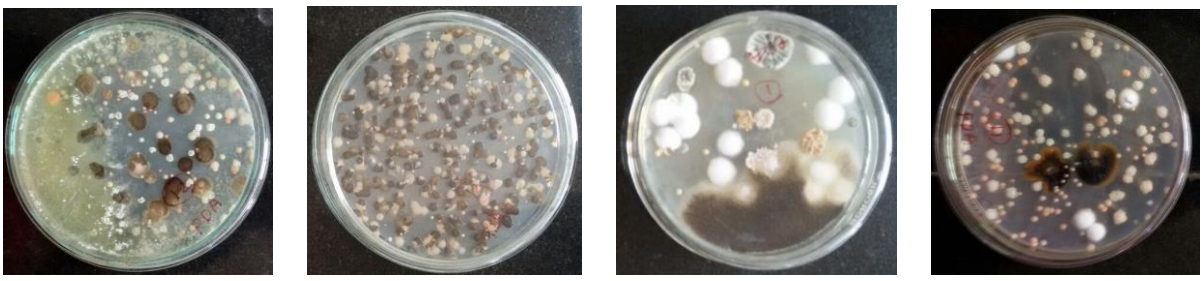


Plate 1: Fungal isolates from different food vendor's mobile phone

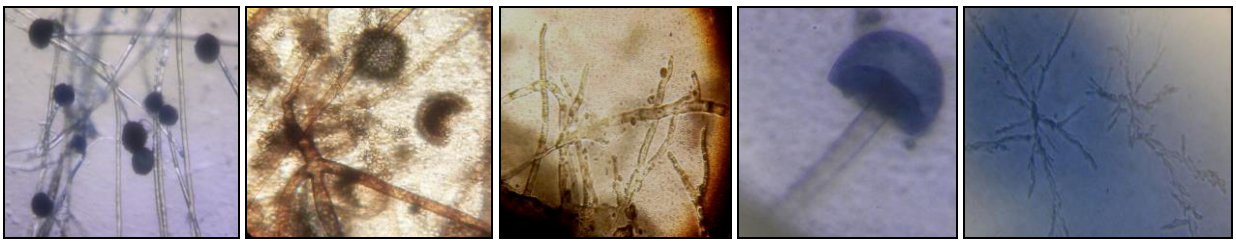


Plate 2: Microscopic morphology of fungal isolates from different food vendor's mobile phone

Poor personal hygiene and sanitation, unhygienic environment, unsafe storing and handling of food by the roadside food vendors pose a major health hazard. The potential role of mobile phones of these road side food vendors as an exogenous source of infection may be confirmed from this investigation as a variety of microbes were found on their mobile phones. The high prevalence of fungal agents isolated from their mobile phones was attributed to the poor hygienic and sanitary practices associated with the low level of education among marketers and food vendors.

The presence of the isolated fungi showed that the food vendor's phones and environment had been contaminated by fungal spores (Ekrakene and Igeleke, 2007). Most of the isolated fungi are also natural inhabitants of the soil and air. These pathogens may cause food borne infections, lowering of semen, brain disorder, cancer, headache, nosocomial infections, cell damages, etc. (Flavia *et al.,* 2001).

Since the restriction of the use of mobile phones is not effective for the prevention of the spread of infections, it is necessary to develop effective preventive strategies that will include creating awareness in relation to environmental decontamination, hand hygiene, surveillance and contact isolation for the prevention of infections (Farr *et al.,* 2001). Simple cleaning of mobile phones with 70% isopropyl alcohol may decrease the microbial load (Neely and Sittig, 2002). This study showed that fungal contamination of food vendors' mobile phones in different food shops in vicinity of VPM's B.N.Bandodkar college of Science, Thane was high. These mobile phones could be marked as an exogenous source of infections however simple cleaning of mobile phones with 70% isopropyl

alcohol, good hygiene practice and safe food handling may decrease the fungal load.

CONCLUSION

Mobile phones are frequently used as they are an effective means of communication. The objectives of this cross-sectional study were to determine the level and type of fungal contamination of the mobile phones used by roadside food vendors. The predominant fungi isolated were *Aspergillus flavus*, *Aspergillus niger, Mucor indicus, Rhizopus stolonifer, Penicillium* sp., *Candida albicans* and *Cladosporum* sp. which can cause infection in humans. Students, staff and general public frequenting these food stalls are at a greater risk of acquiring and spreading infections, thus roadside food vendors require the stringent implementation of infection control guidelines.

REFERENCES

Barnett HL and Hunter BB (1972) Illustrated Genera of Imperfect Fungi. Burgress Pub. Co. Minneapolis, pp: 218.

Brady R, Wasson R, Stirling A, McAllister I and Damani NN (2006) Is Your Phone Bugged? The Incidence of Bacteria Known to Cause Nosocomial Infection in Healthcare Workers Mobile Phones. *Journal of Hospital Infection.* 62: 123-125.

Chinedu M, Stephen O and Uchenna U (2014) Microorganisms Associated with The Use of Commercial Mobile Phones in Umudike, Ikwuano Local Government Area of Abia State, Nigeria. *N.Y Sci J*, 7(7): 74-77.

Deshmukh SK and Rai MK (2005) *Biodiversity of fungi : their role in human life*. Enfield, NH: Science Publishers. p. 460.

Ekrakene T and Igeleke CL (2007) Microorganism Associated with Public Mobile Phones along Benin. Sapele Expressway. *Journal of Applied Sciences Research*, 3: 2009-2012.

Farr BM, Salgado CD, Karchmer TB and Sherertz RJ (2001) Can antibiotic-resistant nosocomial infections be controlled? *Lancet Infect Dis.*, 1: 38–45.

Flavia PC, Marilene da SC and Francisco CN (2001) Isolation of Filamentous Fungi From Public Telephones of the Metropolitan Region of the City of Recite, Pe, Brazil.

Heyba M, Ismaiel M, Alotaibi A, Mahmoud M, Baqer H, Safar A and Al-Taiar A (2015) Microbiological contamination of mobile phones of clinicians in intensive care units and neonatal care units in public hospitals in Kuwait. *BMC Infectious Diseases*, 15: 434.

Neely AN and Sittig DF (2002) Basic microbiologic and infection control information to reduce the potential transmission of pathogens to patients via computer hardware. *J Am Med Inform Assoc.*; 9:500–8.

Singh D, Kaur H, Gardner WG and Treen LB (2002) Bacterial contamination of hospital pagers. *Infect Control Hosp Epidemiol* ; 23:274–6.

REVIEW ARTICLE

Insight into Endophytic fungi in medicinal plants – A Review

Raut Asmita and Sashirekha S

Mithibai College, Vile Parle West, Mumbai 400056
*Corresponding author Email: sashirekhas52@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Raut Asmita and Sashirekha S (2016) Insight into Endophytic fungi in medicinal plants – A Review, Int. J.of. Life Sciences, Special Issue, A7:102-106.

ABSTRACT

Fungi are group of organisms having a great biodiversity. They are the second largest group after insects and key component of tropical ecosystems throughout the world. Endophytes are group of microorganisms that resides asymptomatically inside the living plant tissues. Endophytic fungi are unexplored group of organism that has enormous potentials for new pharmaceutical substances. Medicinal plants and their endophytes are important resources for discovery of natural products. Plant endophytic fungi have been recognized as an important and novel resource of natural bioactive products with potential application in agriculture, medicine and food industry.

Key words: Endophytes, Medicinal plants, bioactive compounds

INTRODUCTION

Endophytes are microorganisms that inhabit plant hosts for all or part of their life cycle. They protect their host from infectious agents and adverse conditions. Thus endophytes play an important role in physiological and ecological role in their host life. The distribution of endophytic mycoflora differs with the host. It is found out that Endophytes can be transmitted from one generation to the next through the tissue of the host seed or vegetative propagules. (Carroll, 1988). Endophytic fungi were isolated from leaves of the weed Parthenium hysterophorus in order to establish whether the endophytes were the same fungi as had previously been recorded on scenescent or diseased leaf tissues. Seven surface sterilization methods were used. *Alternaria zinniae, A. helianthi, Cylindrocarpon* sp., *Curvularia brachyspora, Fusarium* sp., *Nigrospora oryzae, Penicillium funiculosum and Periconia* sp. were isolated The methods used to isolate endophytes may represent a tool for the identification of biological control agents of weeds (Romero 2001). Grass endophytes occur as non-sporulating , systemic infectious and are transmitted vertically from maternal plant to offspring through seeds (Clay ,1990). Bacon and White (2000) give an inclusive and widely accepted definition of endophytes—"microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects".

The mutual relationship benefits the endophytic fungi through provision supply of energy, nutrients, shelter as well as protection from environmental stress. On the other hand fungal endophytes indirectly benefit plant growth by producing special substances mainly secondary metabolites and enzymes, which are responsible for the adaptation of plants to abiotic stresses such as light, drought, and biotic stresses such as herbivore, insect and nematode attack or invading pathogens. Some endophytic fungi have developed bioactive compounds as those originated from the host plants. Endophytes may produce a plethora of bioactive metabolites that may be involved in the host-endophyte relationship and may serve as potential sources of novel natural products for exploitation in medicine, agriculture and industry (Bacon and White 2000).

Fungal endophytes act as potential producers of novel and biologically active compounds. Many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered from the endophytic fungi. These bioactive compounds could be classified as alkaloids, terpenoids, steroids, quinines, lignans, phenols and lactones (Xu L, Zhou L, 2008).

Plant selection

It is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making novel bioactive products. A specific rationale for the collection of each plant for endophyte isolation and natural-product discovery is used. Strobel and Daisy (2003) discussed the following methods of selection:

(i) Plants from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival are seriously considered for study.

(ii) Plants that have an ethnobotanical history (use by indigenous peoples) that are related to the specific uses or applications of interest are selected for study. These plants are chosen either by direct contact with local peoples or via local literature. Ultimately, it may be learned that the healing powers of the botanical source, in fact, may have nothing to do with the natural products of the plant, but of the endophyte (inhabiting the plant).

(iii) Plants that are endemic, that have an unusual longevity, or that have occupied a certain ancient land mass, such as Gonwanaland, are also more likely to lodge endophytes with active natural products than other plants.

(iv) Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity. Just as plants from a distinct environmental setting are a promising source of novel endophytes and their compounds, so too are plants with an unconventional biology.

Endophytic fungi from medicinal plants

In recent years, it is seen that the isolation of new compounds from medicinal plants has become a fascinating area of research. Plants with ethno-pharmaceutical importance are being exploited because of their healing properties. However, a large scale harvesting of medicinal plants has already become a major threat to biodiversity. As an alternative, microbes which live inside such plants (endophytes) may offer tremendous potential source of novel medicinal compounds. (Endophytes infected plants often grow faster than non-infected plants) which could be utilized for potential applications. It has been estimated that there may be 1.5 million fungal species, while only about 100,000 species are presently known (Hawksworth 2004).

Western Ghats of India is thus a rich source of biodiversity of micro-organisms, which plays a vital role during the growth of fungi. Western Ghats of India are known to be an active hotspot region with enormous biodiversity wealth.

The studies of endophytic fungi and medicinal plants with a emphasis on the factors that possibly influence the population structure and distribution of endophytic fungi. It also provides new insights into drug discovery and clinical utility which can be further improved by investigating endophytes further as these have the potential of playing a key front line role in the treatment of various diseases.

Isolation of Endophytes

Endophytes can be isolated from various plant parts such as seeds, leaves and stems. The collected plants for studying endophytic communities should look apparently healthy and disease free plant, i.e. they do not display any visual symptoms of diseases, in order

to minimize the presence of plant pathogenic and saprobic species, and to prevent the isolation of localized pathogenic endophytic microorganisms.

The most important step for the isolation of endophytic fungi that reside in plant tissues is surface sterilization and the plant parts under investigation should be cut into small pieces to facilitate sterilization and isolation processes. (Strobel 2003)

Petrini (1991) isolated Endophytic fungi from medicinal plants more likely exhibit pharmaceutical potentials. Plant endophytic fungi have been found in each plant species examined and it is estimated that there are over one million fungal endophytes existed in the nature. The secondary metabolites produced by endophytes associated with medicinal plants can be exploited for curing diseases.

Rajagopal and Suryanaryanan(2000) isolated five endophyes from the leaves of Neem. Of these, four were sterile forms and one was *Fusarium avenaceum*. The result showed that colonization frequency percentage of endophytes was significantly higher in the monsoon season (49.6%) than during the dry seasons (24.3%).

Suryanarayanan and Rajagopal (2000) isolated 963 isolates belonging to 36 fungal species from the bark tissues of ten tropical forest trees. Of these, four were Ascomycetes, one belonged to Coelomycetes and eleven were Hyphomycetes rests were sterile mycelia forms.

Raviraja (2005) reported on the fungal endophytes in five medicinal plant species from Kudremukh Range Western Ghats of India. He isolated 18 species of endophytic fungi from bark, stem and leaf segments of five medicinal plant species growing within Kudremukh rang. pe in the Western Ghats of India. The dominant species were *Curvularia clavata , C. lunata*, *Callescens and F. oxysporum*. The higest species richness as well as frequency of colonization of endophytic fungi was found in the leaf segments rather than the stem and bark segments of the host plant species. The greatest number of endophytic fungal species were found within Callicarpa tomentosa (11 species), whereas *Lobelia nicotinifolia* harbored the lowest number of fungal endophytes (5 species). The study provides evidence the fungal endophytes are host and tissue specific.

Tejesvi et.al. (2006) isolated fungal endophytes from inner bark segments of , ethnopharmaceutically important medicinal tree species namely *Terminalia arjuna Crataeva magna, Azadirachta indica , Holarrhena antidysentrica, Terminalia chebula, Butea monosperma* growing in different regions of Southern India and species of *Fusarium, Pestalotiopsis*, *Myrothecium, Trichoswema, Verticillium and Chaetomium* were isolated.

Gangadevi and Muthumary (2007) studied on endophytic fungal diversity in young, old and senescent leaves of *Ocimum basilicum L*. a medicinal plant. Not much is known on the temporal and spatial variation of fungal endophytes inhabiting the foliage of medicinally important plants. This study provides the first report on diversity of endophytic fungi of medicinal plants from Chennai city Southern India. Added to it one of the isolates *Phyllosticta sp.* was found to produce taxol in artificial culture media . the endophytic fungus is thus expected to artificial culture media. The endophytic fungus is thus expected to be a potential source of natural bioactive agent.

Mohanta et al., (2007) studied the antimicrobial potentials as endophytic fungi inhabiting three Ethno-medicinal plants of Similipal Biosphere Reserve India. Nearly 60 fungal endophytes belonging to genera were isolated out of which 31 endophytes (51.66%) were obtained as filamentous forms and 29 of them (48.33%) as yeast colonies. Species of *Curvularia, Fusarium*, *Alternaria* and *Penicillium* were isolated as dominant and host specific endophytes. Among the potent strains of about 13 isolates, 19.3% displayed both antibacterial activity against all the test pathogens. The study reinforced the assumption that endophytes of ethnomedicinal plants could be a promising source of anti- microbial substances.

Devi (2014) studied the antioxidant activity of the endophytic fungus *Penicillium* sp. isolated from medicinal plant *Centella asiatica* was evaluated by its ability to scavenge DPPH (1,1- diphenyl -2- picryl-hydrazyl) free radicals. Bioactive metabolites present in the ethyl acetate extract from the endophytic fungus *Penicillium sp*. were analysed by using GC-MS. The metabolites were investigated for cytotoxic activity.

Karunai Selvi (2014) isolated fifty one fungal endophytes belonging to twenty one genera were isolated from medicinal plants in Virudhunagar

District. The crude metabolite of endophytic fungus *Cladosporium sp*. displayed a significant antimicrobial activity against all test pathogens. Phytochemical analysis of the ethyl acetate solvent extract revealed the presence of saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins in *Alternaria alternate and Cladosporium* sp. The extract of *Acyranthus aspera* was effective against all test organisms except *Bacillus cereus, B. subtilis and Proteus sp.* Endophytes can reduce the growth of the harmful bacteria in plants by different mode of action.

Shiva (2015), reported Endophytic fungi isolated from four different populations of *Urginea indica*. The plants were collected from 4 different localities which includes Seethampundi (Tamil Nadu), Yediyur (Karnataka), Allepy (Kerala) and Udupi (Karnataka).It is an important medicinal plant found distributed in India, Africa, andMediterrean regions and widely used for the treatment of Edema, Dropsy, Gout, Rheumatic pain , in trating cancer and as cardiac stimulant. Plant portions viz., pseudostem, outersheath, inner leaf sheath and root parts were inoculated on potato dextrose agar medium amended with chloromphenico The presence of 12 endophytic fungi belonging to Hyphomycetes and Coelomycetes were identified from *U. indica* by means of morphological and cultural features . Sterile forms were common to the host. A total of 6 fungal species viz., *Acremonium, Aspergillus niger, Cladosporium*, *Curvularia lunata, C. brachyspora, Penicillium* species belonging to the class Coelomycetes and some sterile forms were recorded. The present study provided a clue that the existence of endophytic fungi in *U. indica* and it can be of great importance if further studies conducted on to elucidate their role in the host plant and for bio-prospecting.

The most valuable application is to utilize the advantages of endophytic fungi that can promote the accumulation of secondary metabolites originally produced by plants. Through such an application, we can enhance the synthesis and accumulation of bioactive compounds by adding particular endophytic fungi to the plants. This application may open a complete new dimension to produce natural medicines in an extremely effective manner, given that the relationship between endophytic fungi and their host medicinal plants is completely understood.

CONCLUSION

This review represents data on microbial diversity of Western Ghats of India, with an emphasis on exploiting them for potential applications for socio-economic development of India. A variety of relationships can coexist between endophytes and their host plants, ranging from mutualism or symbiosis to antagonism or slightly pathogenic (Schulz and Boyle 2005, Arnold 2007). The host-endophyte relationships can be described in terms of host-specificity, hostrecurrence, host-selectivity, or host preference (Zhou and Hyde 2001, Cohen 2006). As the endophytic fungi are a good source for bioactive compounds and a great demand arises for new drugs, there arises a need to exploit the endophytic fungi associated with medicinal plants. Innovative and effective approaches should be made to strengthen fledgling participation of various research groups and civil societies to catalyze exploitation and conservation of Western Ghats biodiversity.

REFERENCES

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biology Reviews 21, 51–66.

Bacon CW and White JF (2000) Microbial endophytes. Marcel Dekker Inc., New York, N.Y.

Carrol G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69: 2-9.

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69: 10-16.

Cohen SD (2006) Host selectivity and genetic variation of *Discula umbrinella* isolates from two oak species: analyses of intergenic spacer region sequences of ribosomal DNA. Microbial Ecology 52, 463–469

Devi NN (2014) Bioactive metabolies from an endophytic fungus *Penicillium* sp isolated from *Centella asiatica*. Current Research in Environment and Applied Mycology 4(1), pp 34-43

Gangadevi and Muthumary (2007) Endophytic fungal diversity from young, mature and senescent leaves of senescent leaves of *Ocimum basilicum* L. with special reference to Taxol special reference to Taxol production production production, Indian Journal of Science and Technology,1(1) http://www.indjst.org

Gangadevi V and Muhumary J (2008) Taxol an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi , isolated from a medicinal plant, *Aegle marmelos* Correa ex Roxb., World Journal of Microbiology and Biotechnology 24(5), 717-724.

Hawksworth DL (2004) Fungal diversity and its implications for genetic resource collections. Studies in Mycology 50, 9– 18.

Karunai Selvi B and Balagengatharathilagam P (2014) "Isolation and Screening of Endophyte fungi from Medicinal plants of Virudhunagar district fir antimicrobial activity ", International Journal of Science and Nature, vol .5(1),. pp:147-155

Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi HN (2007) Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L.f. Sci. Res. Essay 2(11): 486-490.

Petrini O (1991) Fungal endophytes in the tree leaves , In: Andrew JH, Hirano SSed. Microbial Ecology of leaves,Spriger, New York, 179-197

Rajagopal K and Suryanarayanan TS (2000) Isolation of endophytic fungi from leaves of neem (*Azadirachta indica* A. Juss.) Current Science 78: 1375-1378.

Raviraja NS (2005) Fungal endophytes in five medicinal plant species from Kudremukh Range, Western Ghats of India. J. Basic. Microbiol., 45: 230-235.

Romero A, Carrion G and Rico-Gray V (2001) Fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* (Asteraceae). Fungal Diversity 7: 81-87

Schulz B, Boyle C (2005)The endophytic continuum. Mycological Research 109, 661–686.

Shiva Kameshwari MN, Mohan B and Thara Saraswathi KJ (2015) Isolation and Identification of Endophytic fungi from *Urginea indica*, a medicinal plant from diverse regions of South India ", International Journal of Latest Research in Science and Technology ,vol 4(1), pp:75-80.

Strobel G (2003) Endophytes as sources of bioactive products. Microbes and Infection 5, 535–544

Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V and Shetty HS (2006) Fungal endophyte assemblages from ethnopharmaceutically important medicinal trees. Canadian Journal of Microbiology 52: 427-435.

Xu L, Zhou L, Zhao J, Li J, Li X, Wang J (2008) Fungal endophytes from *Dioscorea zingiberensis* rhizomes and their antibacterial activity. Lett Appl Microbiol.; 46(1) :68–72. Doi: 10.1111/j.1472-765

Zhou D, Hyde KD (2001)Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycological Research 105, 1449–1457.

REVIEW ARTICLE

A Review on Bioluminescent fungi: A Torch of Curiosity

Kushwaha Vinodkumar and Hajirnis Sarita

Department of Botany, Satish Pradhan Dnyanasadhana College of Arts, Science and Commerce, Thane (W), India
Email: kushwahavinod30@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Kushwaha Vinodkumar and Hajirnis Sarita (2016) A Review on Bioluminescent fungi: A Torch of Curiosity , Int. J.of. Life Sciences, Special Issue, A7:107-110.

Acknowledgements
I would like to thank one of the dynamic personality Mrs. Sarita Hajirnis, Vice-Principal and HOD Dept. of Botany for outstanding assistance, grateful discussion and critical reading of manuscript. Also thanks to the Principal Dr.C.D. Marathe, Satish Pradhan Dnyanasadhana College, Thane for his support and encouragement

ABSTRACT

Fungi are one of the world's least studied life forms and there are presumably hundreds of species remaining to be found in the form of bioluminescence. Bioluminescence is a natural light emitting phenomenon that is emitted by several living organism, verified in 71 of 100,000 described species in the kingdom fungi. Bioluminescence results when energy from a chemical reaction is released as light, this occurs when an enzyme, such as luciferase catalyzes the oxidation of an organic molecule luciferin. These bioluminescent fungi are applied for biotechnological applications such as Luciferase systems in genetic engineering as reporter genes, environment monitoring, heavy metal extraction and many more. The aim of this review is to address the current studies on bioluminescent fungi and their future applications.

Keywords: Fungi, Bioluminescence, luciferin, luciferase

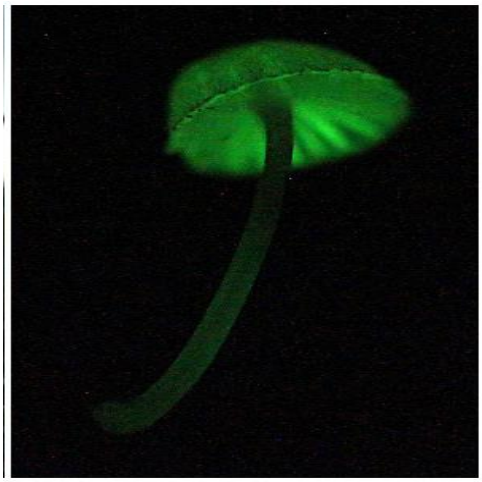
INTRODUCTION

Bioluminescent organisms have attracted the attention of mankind since ancient world. Aristotle(382BC) and the Roman scholar Pliny the Elder observed the effect of fungal bioluminescence when they described the glowing light of the cold "fire" of damp wood, this probably later became known as "foxfire" cause in old French "*fois"* means "false". The next mention of luminous wood in the literature occurred in 1667 by Robert Boyle who noticed glowing earth and noted that heat was absent from light. As quoted Johnsons and Yata, 1966 and Newton 1952, many early scientists such as Conrad Gesner, Francis Bacon and Thomas Bartolin observed and made notation of luminous earth. The first mention that the light of luminous wood was due to fungi occurred from a study of luminous timbers used as supports in mines by Bishoff in 1823. Fabre established the basic parameters of bioluminescence fungi, that is, (1) Light without heat. (2) Light ceased in a vacuum, in hydrogen, and carbon dioxide (3) The light was independent of humidity, temperature, and did not burn any brighter in pure oxygen. A Dutch consul in 1700 reported that Indonesian people used fungal fruits to illuminate forest pathways. The phenomenon of bioluminescence is most common in marine environments and a number of theories have been put forward to

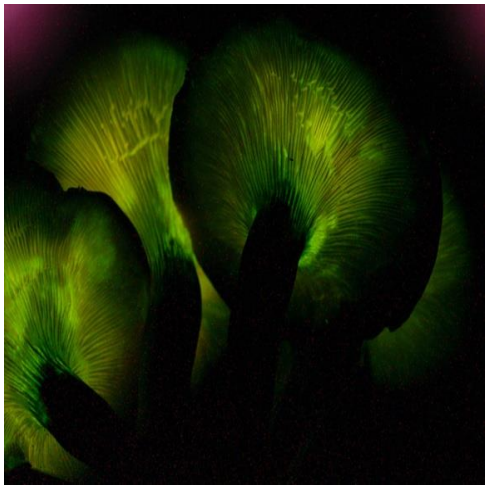
account for its selective advantage in the dark of Deep Ocean. From the 1850's to the early part of the 20th century the identification of the majority of fungal species exhibiting bioluminescent traits was completed. The research of bioluminescent fungi stagnated from the 1920's till 1950's (Newton, 1952 and Herring, 1978). After which extensive research began involving the mechanisms of bioluminescence and is being still carried out. Amongst fungi the majority of bioluminescence occurs in the Basidiomycetes and only one observation has been made involving the Ascomycetes; specifically in the Ascomycete genus *Xylaria* (Harvey 1952). At present there are 42 confirmed bioluminescent Basidiomycetes that occur worldwide and share no resemblance to each other visually, other than the ability to be bioluminescent. Of these 42 species that have been confirmed 24 of these have been identified just in the past 20 years and as such many more species may exhibit this trait but are yet to be found. Luminescence may not confer a significant selective advantages as there are both luminescent and non-luminescent strains of the same species and species that only have luminescent mycelium. (Herring,1994). The two main genera that display bioluminescence are *Pleurotus* which has at present 12 species which occur in continents of Europe and Asia and genus *Mycena* which has 19 species identified to date with a worldwide distribution range. In North America only 5 species of bioluminescent basiodiomycetes have been reported. These include the Honey mushroom *Armillaria mellea*, the common Mycena -*Mycena galericulata* , the Jack O'Latern - *Omphalotus olearius, Panellus stipticus* and *Clitocybe illudens*.

Panellus stipticus

Mycena chlorophos



Mycena singeri



Omphalotus olearius

PHYSIOLOGY OF BIOLUMINESCENCE

Bioluminescence in fungi is an oxygen-dependent reaction involving substrates generically termed luciferans, which are catalyzed by one or more of an assortment of unrelated enzymes referred to as luciferases. In fungi, both the luciferans and luciferases involved remain largely unidentified. During the luciferans- luciferase reaction, unstable chemical intermediates are produced which when decompose excess energy is released as light emission, causing the tissues in which this reaction occurs to glow or luminesce. Although the older literature reports some fungal species as producing white or blue light, all recent studies and observations indicate that bioluminescent fungi emit a greenish light with a maximum of 520-530nm.

Fungal Bioluminescence (Airth & Foerster, 1962)

$$L + NAD(P) + H^{+} \xrightarrow{\text{reductase}} LH_2 + NAD(P)^{+}$$

$$LH_2 + O_2 \xrightarrow{\text{Luciferease}} LO + H_2O + hv$$

L-Luciferin, **LH_2**-reduced luciferin, **LO**-oxyluciferin

ROLE OF LUMINESCENCE IN FUNGI

Bioluminesce fungi offer many advantages like,
1) Attracting insects for dispersal of fungal spores. This hypothesis is supported by the presence of luminescence more strongly in the gills (*P. stipticus*) or in the spores region (*Mycena rorida var. lamprospora*). (Bermudes et al., 1992).
2) Functions as predators of fungivores,
3) repulsion of negative phototropic fungivores and
4) as a warning signal to nocturnal fungivores. (Sivinski 1981). Another hypothesis suggests that bioluminescence is a by-product of a biochemical reaction and has no ecological value. For example, a relationship of biolumenescenc to lignin degradation has been suggested where it may act to detoxify peroxides that are formed during lignolysis. (Bermudes et al., 1992; Lingle, 1993).

CURRENT RESEARCH APPLICATIONS

Luminescence is the only biochemical process that has a visible indicator than can be measured. Luminometer is a device used to measure luminescence which can detect small amounts of light given off in the bioluminescent reaction. It is used in scientific research involving biological process applications. E.g.

Biosensors

The property of bioluminescence can be used as biosensors in Bioremediation for detection of heavy metal ions like mercury and aluminium. This can be achieved by using bacteria with light genes fused to their ion resistant regulons.
For example, if a bacteria that is resistant to Hg is in the presence of Hg, the genes coding for its Hg resistance will be activated which in turn will activate the luciferase gene fused to it, so the bacteria will produce luciferase whenever Hg is present. Adding luciferin and testing for light production with a luminometer reveals the presence of the metal ion in the solution. This technique is especially useful in testing for pollutants in the water supply when concentrations are too low to detect by conventional means (Herring 1978, and Patel 1997).

Biosensors also has application in *Tuberculosis Test***.** Testing for tuberculosis has long been a problem because of the long time it takes for the species (mycobacterium) to grow to a size that is detectable by modern medicine. By the use of bioluminescence in the TB test has found to sharply reduced the diagnosis time to as two days. The technique involves inserting the gene through a viral vectors that codes for luciferase into the genome of the TB bacterial culture taken from the patient. The bacteria now start producing luciferase. When luciferins are added the amount of light produced needed to code for enough luciferase to produce a detectable amount of light, is reduced to only 2-3 days. By reducing the time needed to prescribe the correct drugs for treatment, this application of bioluminescence will someday be ready to save some of the 3 million killed each year by tuberculosis (Patel 1997).

Other applications

Fungal luciferin chemically differs from other known luciferins because it exhibits a different mechanism of light emission. This attribute of it is used in photochemistry, biochemistry and evolution. Bioluminescence is also used in scientific research including evolution, ecology, histology, physiology, biochemistry, biomedical applications, cytology and taxonomy.

FUTURE SCOPE

1. Creation of an autonomously luminescent plant

Scientists may now be able to explain not only why certain fungi glow in the dark, but how. Unlike the other luciferins, fungal luciferin is compatible with plant biochemistry, hopefully this will eventually allow Plant to biosynthesize luciferins by itself. So, they are nearer to creating glowing trees as a novel form of street lighting, replace electricity-draining conventional streetlights, lit- up road signs and interior lighting. The trees would come "on" at night and go "off" during the day. The trees would need only air, water, and soil nutrients to maintain their urban lighting duties.

2. Agricultural Signs.

When crops need water or nutrients, they will be able to tell farmers. Plants could even go to red, yellow or green "alert" to give farmers early warning about disease and invasions by harvest- destroying pests. Bioluminescence will provide a new dimension of Lighting, Healthcare and Food industry. Adoption of these technologies will lead to a massive growth of Bioluminescence.

CONCLUSION

When technology matures and becomes economically feasible, it will definitely offers a superior value proposition.

REFERENCES

Airth RL and Foerster GE (1962) The isolation of catalytic components required for cell-free fungal bioluminescence, Archives of Biochemistry and Biophysics 97: 567-573.

Bermudes D, Peterson RH and Neason KN (1992) Low level bioluminescence detected in Mycena haematopus basidiocarps,Mycologia 84: 799-802.

Burr GJ (1985) Chemiluminescence and Bioluminescence. Marcel Dekker, Inc. New York, U.S.A.

Campbell AK (1988) Chemiluminescence: Principles and Applications in Biology and Medicine. VCH, Chichester

Desjardin DE, Capelari M and Stevani C (2005) A new bioluminescent agaric from São Paulo, Brazil. Fungal Diversity 18:9-14.

Harvey EN (1952) Bioluminescence. Academic Press, New York, NY.

Herring PJ (1978) Bioluminescence in Action. Academic Press. New York. U.S

Johnson FH and Yata H (1966) Bioluminescence in progress. Princton, New Jersey, Princeton University Press

Lincoff GH (1981) The Audubon Society field guide to North American Mushrooms. Knopf Inc. New York. U.S.A

Lingle WL (1993) Bioluminescence and ligninolysis during secondary metabolism in the fungus panellus. Journal of Bioluminescence and chemiluminescence 8:100

Newton HE (1952) Bioluminescence. Academic Press. New York. U.S.A.

Oliveira AG, Desjardin DE, Perry BA and Stevani CV (2012) Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. Photochem. Photobiol. Sci. 11, 848–852.

Tarr CL, *Patel* JS, Puhr ND, Sowers EG, Bopp CA, Strockbine NA (1997)*;83:727–736*

Sivinski J (1981) Arthopods attracted to luminous fungi Psyche 88:383-390

Weitz HJ (2004) Naturally bioluminescent fungi. Mycologist 18(1):4-5.

REVIEW ARTICLE

Applications of DNA bar coding in molecular systematics of fungi: A review

Gosavi Mahavir C

Department of Botany, SIES College of Arts, Science & Commerce, Sion (W), Mumbai 400022.
mahavirg@sies.edu.in

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Gosavi Mahavir C (2016)
Applications of DNA bar coding in molecular systematics of fungi: A review, Int. J.of. Life Sciences, Special Issue, A7:111-115.

ABSTRACT

Fungi are a large, diverse and economically important group of organisms. Estimates of the actual number of fungal species vary widely from 1.5 million to 13.5 million, with fewer than 100,000 now known. Some fungi have relative complex and conspicuous morphologies, but others have very simple morphologies. Identification of fungi isolated from samples in laboratories is mainly based on observing morphology under the microscope and use of various cultural techniques. These conventional methods are very time-consuming and laborious. It usually requires several weeks for the fungi to grow sufficiently on culture media, and the identification processes on fungal phenotypic structure. DNA barcoding techniques have provided standardized, reliable and cost-effective methods for fungal species identification. Gene sequencing and phylogenetic analysis targeting the internal transcribed spacer (*ITS*) region in the fungal genomes are the most commonly used molecular methods for fungal identification. The *ITS* region has been heavily used in both molecular methods and ecological studies of fungi, due to its high degree of interspecific variability, conserved primer sites and multiple copy nature in the genome. It has been recently accepted as a suitable marker for barcoding fungi. This approach has immensely being used currently for both phylogenetic reconstruction and species recognition.

Key Words: DNA bar coding, Molecular systematics of fungi, rDNA, *ITS.*

INTRODUCTION

Fungal taxonomy is an essential part of biological research especially in the context of its ecological and economic implications (Shenoy *et al*., 2007). Traditional methods for fungal species identification require diagnostic morphological characters and are often limited by the availability of fresh fruiting bodies and local identification resources. About 100,000 fungal species have been identified yet, but it has been estimated that there may be from 1.5 to 13.1 million species. According to Hibbett *et al.*, 2011, over the last decade, about 1200 new species of fungi have been described in each year. At that rate, it may take up to 4000 years to describe all species of fungi using current specimen-based approaches. The use of morphology is extremely useful in several cases to

assign organisms to well-defined categories but reliable diagnostic procedure can be time-consuming and require the expertise of different taxonomists (Pereira *et al*., 2008).

DNA barcoding techniques have provided standardized, reliable and cost-effective methods for fungal species identification. Gene sequencing and phylogenetic analysis targeting the internal transcribed spacer (*ITS*) region in the fungal genomes are the most commonly used molecular methods for fungal identification (Das & Deb, 2015). The *ITS* region is now perhaps the most widely sequenced DNA region in fungi. It has typically been most useful for molecular systematics at the species level, and even within species. Because of its higher degree of variation than other genic regions of rDNA that allow selective amplification of fungal sequences (Bruns & Gardes, 1993).

DNA Barcoding:

Taxonomy is the science of classifying living organisms according to their morphology. Today identification of organisms has become highly critical as effects of global warming and accelerating habitat destruction. Classical taxonomy falls short in this race to catalog biological diversity before it disappears http://www.dnabarcoding101.org/introduction.html.

DNA barcoding is a method for identifying living organisms to species. It makes use of a short, highly variably regions of the genome that evolves fast enough to differ between closely related species. DNA barcodes function as molecular identifiers for each species, in the same way as the machine-readable black-and-white barcodes are used in the retail industry to identify commercial products. Using Polymerase Chain Reaction (PCR) to amplify a targeted genetic marker and DNA sequencing to determine the content of that sequence, DNA barcoding allows for the comparison of an informative sequence from an unknown fungal sample against a database of identified sequences (Harrington *et al*., 2014).

In effect, DNA barcoding in its modern form was popularized in a paper by Hebert *et al.* (2003), who proposed to use the mitochondrial gene *COI* (cytochrome c oxidase subunit) as the standard barcode for all animals.

A region of the chloroplast gene *rbc*L – RuBisCo large subunit – is used for barcoding plants (Chase *et al.,* 2005). The most abundant protein on earth, RuBisCo (Ribulose-1,5-bisphosphate carboxylase oxygenase) catalyzes the first step of carbon fixation. A region of the mitochondrial gene *COI* is used for barcoding animals. Cytochrome c oxidase is involved in the electron transport phase of respiration. *COI* in fungi is difficult to amplify, insufficiently variable, and some fungal groups lack mitochondria. Instead, the nuclear internal transcribed spacer (*ITS*), a variable region that surrounds the 5.8s ribosomal RNA gene, is targeted. Like organelle genes, there are many copies of *ITS* per genome, and the variability in fungi allows for their identification. During the last 15 years the *ITS* of nuclear DNA has been used as a target for analyzing fungal diversity in environmental samples, and has recently been selected as the standard marker for fungal DNA barcoding (Bellemain *et al*., 2010).

Internal transcribed spacer (ITS) in DNA barcoding of Fungi:

The nuclear ribosomal coding cistron (rDNA) has been widely utilized for detecting variation among isolates of a fungus. The rDNA is composed of tandemely repeated units, each unit being composed of 5S, 25S and 18S rDNA. Two noncoding regions exits in each repeat: The internal transcribed spacer (*ITS* 1 and *ITS* 2) and and intergenic non-transcribed spacer (*IGS*). It has typically been most useful for molecular systematics at the species level, and even within species. Because of its higher degree of variation than other genic regions of rDNA (for small- and large-subunit rRNA), variation among individual rDNA repeats can sometimes be observed within both the *ITS* and *IGS* regions. There are two *ITS's* in eukaryotes; *ITS1* is located between 18S and 5.8S rRNA genes, while *ITS2* is between 5.8S and 25S rRNA genes (*Lafontaine & Tollervey, 2001*). *ITS* has proven especially useful for elucidating relationships among species and closely related genera in clinically important yeast species (Chen et al., 2011). The *ITS* region is the most widely sequenced DNA region in molecular ecology of fungi (*Peay et al., 2008). Schoch et al. (2012), proposed that* among the regions of the ribosomal cistron, *ITS* region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecific variation. The *ITS* region has been proposed as the prime fungal barcode species identification (http://www.allfungi.com/its-barcode.php). The *ITS* region of fungi varies in length from 450bp to 750bp. Approximately 172,000 full-

length fungal *ITS* sequences are available in Genbank. *ITS* marker exists in multiple copies in most fungal cells and is retrievable by relatively strong primers with an established record of reliability. From some latest review papers on fungal barcoding, it is observed that in ascomycetes, *ITS* had the most resolving power for species discrimination (Das & Deb, 2015).

DNA barcoding procedure

The process from field samples to species abundance data involves a long series of steps, from sampling via laboratory handling to bioinformatics treatment. At each step, there is a risk of losing and distorting information.

Lindahl *et al*., (2013) has reported a standard procedure for DNA Barcoding using *ITS* region in fungal identification. It involves following steps.

1. Collection of Samples:

Collection of fungal samples vary according to season, mycelia, growth pattern and fruiting bodies For fungi, use of fruiting bodies is always best since it is easier to obtain DNA from fruiting bodies than mycelia (http://www.dnabarcoding101.org). Multiple samples from same should be collected that appears similar, avoiding the contamination by other fungi. Fresh samples work well for DNA isolation (Kelly *et al*., 2011).

2. Handling of samples:

Freeze-drying at -20° C enables long-term storage at room temperature, and may also aid later sample homogenization. It also restricts sporulation and rapid growth of opportunists (Lindahl *et al*., 2013).

3. Homogenization and sub sampling:

Fungal tissue ~10–20 mg should be obtained from the sample. If working with more than one sample, care should be taken not to cross contaminate specimens (Lindahl *et al*., 2013 & http://www.dnabarcoding101.org). The samples are normally homogenized using appropriate lysis mixtures followed by storing at -20° C.

4. Extraction and purification of DNA:

DNA is extracted from the fungi using standard protocols (http://www.dnabarcoding101.org). Extraction should yield high and uniform amounts of DNA. To achieve this, same extraction protocol should ideally be used for all samples (Tedersoo *et al*., 2010). The DNA precipitation may have to be further purified by binding of DNA to a silica matrix (Lindahl *et al*., 2013).

5. Markers and primers: The ideal marker for fungal community studies should have primer sites that are shared by all fungi, be of appropriate length for efficient amplification and sequencing, have high interspecific variation but low intraspecific variation, and be possible to align across all fungi. This is the main reason why the *ITS* region has been particularly attractive for mycologists (Gazis *et al*., 2011).

6. Multiplying DNA by PCR: DNA is extracted from the fungi, and the barcode portion of the ITS gene is amplified by PCR. After thermal cycling, the amplified DNA is stored on ice or at -20 °C. Before sequencing, PCR products from different samples are mixed in equimolar proportion, so that the DNA sequence output is evenly distributed across all samples. The PCR products have to be purified, to remove primers and short DNA fragments (http://www.dnabarcoding101.org).

7. Analysing PCR Products by Gel Electrophoresis & Sequence alignment: The amplified sequence (amplicon) is submitted for sequencing in one or both directions (Das & Deb, 2015). The PCR samples are subjected to gel electrophoresis and the bands in each lane of the gel are interpreted (http://www.dnabarcoding101.org). *ITS* primers amplify differently sized products that migrate to different positions on the gel. DNA sequencing of *ITS* amplicon is required to determine the nucleotide sequence that constitutes the DNA barcode. A single, good-quality barcode from the forward strand is sufficient to identify an organism.

8. Bioinformatics analysis: The sequencing results are then used to search a DNA database. A close match quickly identifies a species that is already represented in the database (Das & Deb, 2015). Novel DNA barcodes can be submitted to GenBank (www.ncbi.nlm.nih.gov). For the fungi primers, the hits should all be to the nuclear internal transcribed spacer of the 5.8s ribosomal RNA gene.

9. Data interpretation: Computer based analysis are applicable for DNA barcoding (Das & Deb, 2015). Barcode Sequences were deposited in GenBank and

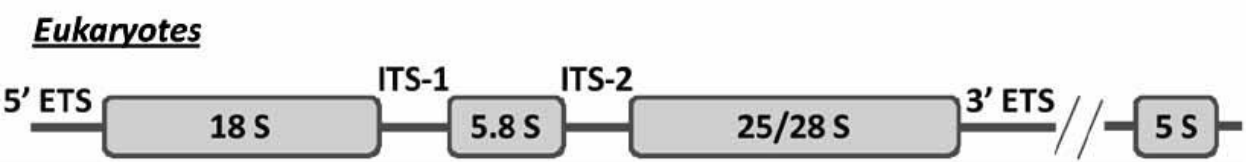


Fig: Ribosomal RNA genes

The genomic organization of ribosomal RNA (rRNA) genes - responsible for the synthesis of RNA species (the core of ribosomes). Almost all eukaryotes have several copies of each rDNA cluster organized in tandem repeats. In this case, each cluster contains the 18S, 5.8S and 25/28S rRNAs, while the 5S gene is present in separate repeat arrays in the majority of eukaryotes. Where ITS=internal transcribed spacers, ETS=external transcribed spacers.

Cited from: Pereira *et al*,. 2008. "Identification of Species with DNA-Based Technology: Current Progress and Challenges" *Recent Patents on DNA & Gene Sequences* 2008, Vol. 2, No. 3, P. 187-200.

compared against those sequences already found in the databases using the Basic BLAST search option of BLAST 2.0 (http://www.ncbi.nlm.nih.gov/BLAST & Photita *et al*., 2005). *ITS1* and *ITS2* including 5.8S sequences were aligned using the multiple sequence alignment program CLUSTAL W (http://www.dnabarcoding101.org).

Reports of identification of fungal species using DNA barcoding:

Robideau *et al*., (2011) had reported use of DNA barcoding in species identification of oomycetes especially in case of genera like *Phytopthora* and *Pythium*, they tried DNA barcoding for 1205 isolates representing 23 genera.

Seena, *et al*., (2010) examined the suitability of *ITS1*-5.8S-*ITS2* rRNA gene region to identify aquatic hyphomycetes, by sequencing and comparing these regions in 94 fungal isolates belonging to 19 species collected in Portuguese streams with different environmental conditions.

Khaund & Joshi (2014) had characterized 10 species of wild mushrooms of Meghalaya, viz. *Gomphus floccosus*, *Lactarius deliciosus*, *Lactarius volemus*, *Cantharellus cibarius*, *Tricholoma viridiolivaceum*, *Inocybe* aff. *sphaerospora*, *Laccaria vinaceoavellanea*, *Albatrellus ellisii*, *Ramaria maculatipes* and *Clavulina cristata*. They also reported that the final species identity generated by the *ITS* marker matched more accurately with the morphological characteristics/appearance of the specimens indicating the *ITS* region as a reliable barcode for identifying wild edible mushrooms. Irinyi *et al*. (2016) depicted standardization of *ITS* sequence based identification of fungi causing infections in humans and animals.

Khodadadi *et al*. (2014), in their studies tried to identify the clinically rare yeast isolates. 49 out of 855 (5.7%) yeast isolates which formerly remained unidentified by PCR-RFLP method were subjected to sequence analysis of *ITS* regions. These species include: *Hanseniaspora uvarum, Saccharomyces cerevisiae, Sporidiobolus salmonicolor, Pichia fabianii, Pichia fermentans, Candida famata, Candida inconspicua, Candida maqnoliae, Candida guilliermondii, Candida kefyr, Candida rugosa, Candida lusitaniae, Candida orthopsilsis*, and *Candida viswanathii*.

Geiser *et al*. (2007) published complete genome sequences of eight *Aspergillus* species (*Em. nidulans*, *A. oryzae, A. fumigatus, N. fischeri*, *A. terreus, A. clavatus, A. niger, A. flavus*) based on DNA barcoding.

Wang *et al*. (2016), evaluated six different loci in their study as potential barcodes in *Chaetomium* indicated that the 28S large subunit (LSU) nrDNA and the internal transcribed spacer regions and intervening 5.8S nrRNA (*ITS*) gene regions were unreliable to resolve species, whereas β-tubulin (*tub2*) and RNA polymerase II second largest subunit (*rpb2*) showed the greatest promise as DNA barcodes for differentiating *Chaetomium* species.

REFERENCES

Bellemain Eva, Tor Carlsen, Christian Brochmann, Eric Coissac, Pierre Taberlet and Håvard Kauserud (2010) "ITS as an environmental DNA barcode for fungi: an in silicoapproach reveals potential PCR biases". BMC Microbiology 2010, Vol.10, Issue.189 P.1-10 cited from.

Bruns TD and Gardes M (1993) Molecular tools for the identification of ectomycorrhizal fungi taxon-specific oligonucleotide probes for suilloid fungi Mol. Ecol., 2(4):233–242.

Chase MW, Salamin N, Wilkinson M, Dunwell JM, Kesanakurthi RP, Haidar N and Savolainen V (2005) "Land plants and DNA barcodes: short-term and long-term goals". Philosophical Transactions of the Royal Society, 360, P.1889-1895.

Chen YC, Eisner JD, Kattar MM, Rassoulian-Barrett SL, Lafe K, Limaye AP, and Cookson BT (2001) "Polymorphic Internal Transcribed Spacer Region 1 DNA Sequences Identify Medically Important Yeasts, J. Clin. Microbiol., 39(11,):4042–4051.

Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts". *Mol. Ecol.*2(4): 113-118.

Gazis R, Rehner S, Chaverri P (2011) "Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences". Mol. Ecol. Vol 20, P. 3001–3013.

Geiser DM, Klich MA, Frisvad JC, Peterson SW, Varga J and Samson RA (2007) "The current status of species recognition and identification in Aspergillus". Studies in Mycology, 59, P. 1–10.

Harrington AH, Bigott AF, Anderson BW et al. (2014) "Sampling Local Fungal Diversity in an Undergraduate Laboratory Using DNA Barcoding". *Journal of the Arkansas Academy of Science*, 68, P.65-72.

Hebert PDN, Cywinska A, Ball SL and0 deWaard JR (2003) "Biological identifications through DNA barcodes". *Proceedings of the Royal Society B* 270: 313-322.

Hibbett David S, Anders Ohman, Dylan Glotzer, Mitchell Nuhn, Paul Kirk, R. Henrik Nilsson (2011) "Progress in 0molecular and morphological taxon discovery in Fungi and options forformal classification of environmental sequences". *Fungal Biology Reviews*, 25(1) 38-47.

Irinyi Laszlo, Michaela Lackner, G. Sybren de Hoog, Wieland Meyer. 2016. "DNA barcoding of fungi causing infections in humans and animals". *Fungal Biology*, 120(2):25-136.

Kelly Laura J, Peter M Hollingsworth, Brian J Coppins Christopher J Ellis, Paul Harrold, James Tosh and Rebecca Yahr (2011) "DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context". New Phytologist, Vol. 191. P. 288–300.

Khaund Polashree, Joshi SR (2014) "DNA barcoding of wild edible mushrooms consumed by the ethnic tribes of India." Gene, 550(1) 123–130.

Khodadadi Hossein, Ladan Karimi, Hossein Mirhendi. 2014. Identification of uncommon species of the clinical isolates of the yeasts by ITS-sequencing. In Proc. Of 58th Annual Meeting of JSMM, 2014. P. 21.

Korabecna M (2007) The Variability in the Fungal Ribosomal DNA (ITS1, ITS2, and5.8 S rRNA Gene): Its Biological Meaning and Application in Medical Mycology. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. A. Méndez-Vilas (Ed.). P.783-787.

Lafontaine DLJ, Tollervey D (2001) "The function and synthesis of ribosomes". Nature Reviews Molecular Cell Biology. Vol.**2,** Issue7, P. 514.

Lindahl Bjorn D, Henrik Nilsson R, Leho Tedersoo, Kessy Abarenkov, Tor Carlsen, Rasmus Kjoller, Urmas Koljalg, Taina Pennanen, Soren Rosendahl, Jan Stenlid and Havard Kauserud. 2013. "Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide". New Phytologist, Vol.199, P. 288–299.

Peay KG, Kennedy PG, Bruns TD (2008) "Fungal community ecology: a hybrid beast with a molecular master". BioScience. Vol. 58, P. 799–810.

Pereira Filipe, João Carneiro and António Amorim. 2008. "Identification of Species with DNA-Based Technology: Current Progress and Challenges" Recent Patents on DNA & Gene Sequences 2008, Vol. 2, No. 3, P. 187-200.

Photita, Wipornpan, et al. (2005) Morphological and molecular characterization of Colletotrichum species from herbaceous plants in Thailand." *Fungal Diversity*, 18, P**.** 117-133.

Robideau GP, Arthur WAM, De Cock, Michael D Coffey et al., (2011) DNAbarcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molcular Ecology Resources Vol. 11, P.1002–1011.

Sanghita Das and Bibhas Deb (2015) "DNA barcoding of fungi using Ribosomal ITS Marker for genetic diversity analysis: A Review". ISSN: 2320 – 7051**,** Int. J. Pure App. Biosci. Vol.3 Issue, 3. P. 160-167.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge, JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW (2012) "Nuclear Ribosomal Internal Transcribed Spacer (ITS) Region as a Universal DNA Barcode Marker for Fungi". PNAS. Vol.109, Issue 16, P. 6241–6246.

Seena S, Pascoal C, Marvanová L and Cássio F (2010) DNA barcoding of fungi: a case study using ITS sequences for identifying aquatic hyphomycete species". Fungal Diversity, October 2010, 44(1): 77–87.

Shenoy, BD, Jeewon R and Hyde KD (2007) "Impact of DNA sequence-data on the taxonomy of anamorphic fungi". Fungal Diversity, 26, P. 1-54.

Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Bahram M, Bechem E, Chuyong G, Koljalg U. 2010. "454 pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases". *New Phytologist* 188(1)291–301.http://www.newphytologist.com

White TJ, Bruns T, Lee S and Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics". P. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds

Web References:

http://www.dnabarcoding101.org/introduction.html

http://www.ncbi.nlm.nih.gov

http://www.ncbi.nlm.nih.gov/BLAST.

REVIEW ARTICLE

Immobilisation of fungal Beta-galactosidase – Review

Kiran Maurya[1*] and Unnati Padalia[2]

[1]Kiran Maurya (M.Sc.-II, Microbiology, Department of Microbiology, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai- 400077, Maharashtra, India).
[2]Unnati Padalia (Associate Professor, Department of Microbiology, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai- 400077, Maharashtra, India).
*Corresponding author Email id – maurya.kiran666@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Maurya Kiran and Padalia Unnati (2016) Immobilisation of fungal Beta-galactosidase – Review , Int. J.of. Life Sciences, Special Issue, A7:116-118.

ABSTRACT

Beta-galactosidase enzyme catalyses conversion of lactose to its monosaccharides. The importance and use of fungal Beta-gaalctosidase is mentioned in this review article. Immobilisation of Beta-galactosidase using convanalin A, chitosan, calcium alginate, starch alginate, barium alginate and cobalt alginate entrapment beads were discussed here. For all these immobilisation techniques enzyme activity and stability of the immobilised enzyme complexes were reported by the researches. Along with this, therapeutic effect of the Concanavalin A, calcium-starch alginate enzyme complex is studied and reported by researchers. The hydrolysis of milk and whey using immobilised Beta-galactosidase are also reported and the maximum concentration of glucose after enzyme treatment was around 1404 mg/decilitre after 15 minutes of enzyme treatment.

Keywords: Beta-galactosidase, Immobilisation, Concanavalin A, Hydrolysis

INTRODUCTION

Beta-galactosidase is a member of hydrolase family that brings about the cleavage of glycosidic bond present between carbohydrates. Enzyme Beta-galactosidase converts lactose, a disaccharide to its monosaccharides, galactose and glucose by breaking of the beta-galactosidic bond present within them and provides various health benefits like providing a healthy alternative for lactose intolerance individuals (Parmjit *et al*., 2016). Beta-galactosidase is naturally present in animal organs e.g. Placenta and brain and also present in plants like peaches, apicoats and almonds (Soares *et al*., 2001). Along with plants and animals, microbes are also considered as important & naturally occurring sources of Beta-galactosidase. Microbes can give high yield of enzyme and reduces the cost production by growing on agro-waste (Holsinger *et al*., 1991). Synthesis of potential prebiotic with different nutritional health benefits is a result of transgalactosylation reaction catalyst by Beta-galactosidase enzymes which adds on to the industrial application of Beta-

galactosidase (Pernosil *et al*., 1987). The prebiotics synthesised are lactulose and galactooligosaccharides (GOS). Various microorganisms have been screened for high amount production of Beta-galactosidase. Effect of temperature and pH for optimal activity of the enzyme will differ from source to source. Amongst all the microbes, fungi are the most preferred source of Beta-galactosidase as it synthesises thermostable and extracellular enzyme. The optimum pH of fungal Beta-galactosidase is reported to be around 2.5-4.5 and optimum pH for bacterial Beta-galactosidase is around 6-7. The variation in optimum pH makes the enzyme suitable for their specific applications such as whey as hydrolysis of sweet whey and milk. The fungal Beta-galactosidase can be used for acidic whey hydrolysis. It was found that lactic acid bacteria isolated from various dairy products are the major source of this enzyme. Use of soluble enzyme in industries has many disadvantages like high sensitivity to several denaturing agents, non-reusability and presence of some inhibitory molecules in reaction mixture or samples. These obstacles can be overcome by using immobilised form of enzymes. Various immobilisation techniques are used to immobilise enzymes. These are Entrapment, Covalent Bonding, Membrane confinement and Adsorption.

MATERIALS AND METHODS

Immobilisation of beta-galactosidase using concanavalin A was done by using jack bean extract was done and further this complex was crosslinked with gluteraldehyde (Toshiba *et al*., 2007). Calcium alginate entrapped enzyme was used in packed bed reactor and stirred batch process for the hydrolysis of lactose in milk / whey (Toshiba *et al*., 2009). A novel technique was used as a therapeutic agent. In this technique, a concanvaline layered calcium alginate – starch beads were developed (Toshiba *et al*., 2008). Another novel method of Beta-galactosidase immobilisation was carried out by using starch alginate beads preparations (Ates *et al*., 1997). Stability of gluteraldehyde - activated chitosan in hydrolysis of lactose and in galactooligosaccharrides synthesis was performed (Manuela *et al*., 2013). The enzyme was also immobilised on chitosan a naturally occurring polysaccharides (Carlos *et al*., 1994). For all these enzyme preparations optimum enzyme activity was studied.

RESULTS AND DISCUSSION

The enzyme activity of Concanavalin A –Beta galactosidase complex was 92% and the same complex when crosslinked with gluteraldehyde showed decrease in its enzyme activity which was around 88%. The Vmax value of the calcium alginate entrapped enzyme used in whey hydrolysis was around 4.2 X $10^{-4.}$ Beads prepared were spherical in shape. The area and volume of the calcium –starch alginate beads were calculated and was found to be 341.94 X 10^{-3} and 18.80 X 10^{-3} respectively. Immobilised enzyme shows enzyme activity in wide range of pH, which is not seen in case of soluble enzyme preparations. Immobilized β- galactosidase maintain around 84% and 95% enzyme activity at pH 3.0 and 5.0, respectively. In case of soluble enzyme, activity retained is around 52% and 78% of the initial enzyme activity under similar conditions of pH 3.0 and 5.0 respectively. The results obtained indicates no of effect of prolonged inucation on the enzyme activity of the immobilised enzyme. (Toshiba *et al*., 2008; 2009). In this method, entrapment of Beta-galactosidase in cobalt alginate beads were performed. The relative enzyme activity of cobalt alginate beads was found to be 83%. The study to get the effect of pH and temperature on immobilised and free enzyme was performed. The pH range used for the study was 4 - 9.2 and the maximum activity was observed in range of 4 - 4.9 for both forms of enzyme. The maximum productivity obtained was 212 mg/decilitre/min at a residence time of r = 1.4 min. The highest concentration of glucose obtained was 1404 mg/decilitre when the treatment of immobilised enzyme was given for 25 minutes (Ates *et al*., 1997). The GOS productivity of chitosan immobilised enzyme in the PBR related to the operational flow rate indicates that the maximum of 484.5 g L^{-1} h^{-1} at 15 mL min^{-1} of GOS production can be achieved. Immobilisation technique of β-galactosidase using chitosan Allows researchers to fix the enzyme with the possibility of holding more than 75 % of the initial activity during 183 days at a half-life of 108.9 h (Carlos *et al*., 1994). There were number of studies conducted on stability and enzyme activity of immobilised enzyme by researchers.

REFERENCES

Carlos Carran and Amelia Rubiolo (1994) *Immobilisation of β-galactosidase on Chitosin.* Biotechnological Progress, Page No. 220-224.

Soares I, Tavora Z, Barcelos RP and Baroni S (2001) "*Microorganism produced enzymes in food industry." Journal of Agriculture & Biological Science.* Volume – 488, Page No. 83-94.

Manuela P Kleina, Lucas P Fallavena, Jéssie da N. Schöffer, Marco AZ Ayub, Rafael C Rodrigues, Jorge L Ninow, Plinho F Hertz (2013) *High stability of immobilized Beta- galactosidase for lactose hydrolysis and galactooligosaccharides synthesis.* Carbohydrate Polymers, Page No. 465– 470.

Parmjit S Panesar, Shweta Kumari, and Reeba Panesar (2010) *Potential Applications of Immobilized β-Galactosidase in Food Processing Industries.* SAGE-Hindawi Access to Research Enzyme Research, Volume 2010, page 16.

Prenosil JE, Stuker E and Bourne JR (1987) *Formation Of oligosaccharides during enzymatic lactose hydrolysis. Part I: State of art.* Biotechnol. Bioeng. 30:1019–1025.

Ates S and Mehmetoglu U (1997) *A new method for immobilisation of β-galactosidase and its utilization in plug flow reactor*. Process Biochemistry, Volume 32, Issue No. 5, Page No. 433-436.

Toshiba Haider, Qayyum Husain (2008) *Concanavalin A layered calcium alginate–starch beads immobilized galactosidase as a therapeutic agent for lactose intolerant patients.* International Journal of Pharmaceutics, Page No.1-6.

Toshiba Haider, Qayyum Husain (2007) *Calcium alginate entrapped preparations of Aspergillus oryzae galactosidase: Its stability and applications in the hydrolysis of lactose.* International Journal of Biological Macromolecules, Page No.72-80.

Toshiba Haider, Qayyum Husain (2009) *Hydrolysis of milk/whey lactose by β-galactosidase : A comparative study of stirred batch process and packed bed reactor prepared with calcium alginate entrapped enzyme.* Chemical Engineering and Processing: Process Intensification, Page No. 576-580.

Holsinger VH, Kligerman AE (1991)*Application of lactase in dairy foods and other foods containing lactose".* Food and Technology, Volume -167, Page No. 1938-1953.

RESEARCH REPORT

Monitoring of aeromycoflora of herbal garden of SIES College of ASC Sion (west) - A case study.

Deshpande Ashwini* and Nadar Evangelin

Dept of Botany, SIES College of ASC, Sion (W)
*Corresponding author email: ashwini_d1@ yahoo.com, evangelinsoloman5@gmail.com

Manuscript details:

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Deshpande Ashwini and Nadar Evangelin (2016) Monitoring of aeromycoflora of herbal garden of SIES College of ASC Sion (west) - A case study, *Int. J.of. Life Sciences*, Special Issue, A7:119-120.

ABSTRACT

Aeromicrobiology is the study of living microbes which are suspended in the air. These microbes are refered to as bioaerosols and also includes various dormant fungal spores. Though there are significantly less atmospheric microorganisms than there are in oceans and in the soil, there is still a large enough number that they can affect the atmosphere. Once suspended in the air column , the fungal spores get an opportunity to travel long distance with the help of wind and precipitation, increasing the occurrence of widespread fungal diseases. There is also an ecological significance as they can be associated with diseases in humans, animals and plants as they settle on surfaces in calm environment. Specially nurtured college herbal garden plants are also subjected to fungal infections. Thus there is a potential threat to medicinal, ornamental, rare and exotic garden species.Spores suspended in air can settle on an agar nutrient medium as it provides readymade nutrients for germination with optimum conditions required for growth, as a result of which fungal colonies are produced which can be further identified well in advance as a part of garden maintenance. A comprehensive aeromycological study was conducted in herbal garden of SIES College of ASC, Sion (west). Present studies revealed presence of *Aspergillus*, *Alternaria*, *Fusarium, Pythium*, *Rhizopus* etc and subsequently necrosis in the form wilt, blight, damping off, stem and foot rot were found to be occurred on the leaves of *Ficus elastica*, *Exora,* Papaya etc. Thus, continuous monitoring of aeromycoflora is a crucial step in garden maintenance.

Key words: Aeromycoflora, bioaerosols, fungal pathogens, *Alternaria*, *Aspergillus, Fusarium*, *Pythium*, *Rhizopus*, necrosis, blight, stem and foot rot, wilting, damping off

INTRODUCTION

All samples of air contain some water in the form of vapour or mist along with gaseous mixture and suspended matter consisting of dust, bacteria, yeasts, moulds, pollengrains etc. Air is not a natural environment for the growth and reproduction of microorganisms. It doesnot contain the necessary amount of moisture and kinds of nutrients that can be used by

fungal spores. The fungal spores when land on a plant can germinate and cause infection. This can cause loss of valuable garden plants and infection also spreads rapidly. In this case, the study of aeromycoflora is considered as the basis of public health, yield and thereby the economy of nation. Being saprophyte, when fungal spores land on exposed agar plates, they germinate as in culture medium, potato provides nitrogen, carbon and vitamins. Dextrose is the source of carbon; Agar is used as solidifying agent. There are very few reports regarding the study of indoor aeromycoflora of occupational environment. Herbal garden of SIES college has been awarded first Prize in 'Best Garden competition' in 2014.It has spread over front and backyard of the college where medicinal, ornamental rare plants are significant and most delicate sections. It is important to study qualitative prevalence of aeromycoflora. Thus, continous monitoring of aeromycoflora and subsequent preventive measures or control measures becomes crucial aspect of garden maintenance.

MATERIALS AND METHODS

Nutrient agar plates were prepared in asceptic condition. Different localities from college herbal garden which are far apart were selected for exposing the plates. Thus, sedimentation method of studying the air mycoflora was preferred. The plates were exposed for half an hour. Then covered with lids and incubated at 37degree centigrade for 48 hours. Wet staining in cottonblue stain was performed. Mycelia were mounted in lactophenol and their colony characteristic, hyphal and spore structures were analysed as basis for identification of pathogens. Garden plants also were thoroughly scrutinized for disease symptoms.

RESULTS AND DISCUSSION

With respect to observations of colony characters, hyphal structures, reproductive structures, the potential threat of fungal species such as *Aspergillus, Alternaria, Fusarium, Pythium, Rhizopus were* identified and subsequent occurrence of necrosis in the form of blight, wilting, stem and foot rot, damping off were observed.

Continuous monitoring of aeromycoflora by sedimentation plate exposed technique is fairly a good precautionary step to avoid opportunistic fungal pathogen attack on fragile garden plants. It will alert local farmers about prevaing pathogenic spores and diseases to be spread in future. The necessary precautionary measures can be undertaken to control spread of diseases in that locality. It is fairly an inexpensive less equipped, simple microbiology technique even can be applied in non-sophisticated labs providing sterile conditions for inoculation of fungal spores.

REFERENCES

Aneja KR (2006) Experiments in Microbiology, Plant pathology and Biotechnology, Fourth Revised Edition, New Age International (P) Ltd. Publishers, New Delhi, India, p465-466

Leslie JF and Summerell BA Illustrated by Bullock, S(2006) The Fusarium Laboratory Manual. Blackwell Publishing Iowa, UAS, p16-18.

Shah Mukhtar Hussain and Bashir Uzma (2008) Air-flora,Mycoflora of Rohtas Fort, Pakistan, Mycopath, 6(1&2)p71-73.

Shukla P and Misra JK (1986) Fungi as potent biopollutant in the indoor air of Ginneries in Lucknow. Bio.Mem 12:101.

Subramanyam M (1987) Seasonal variation and vertical stratification of the atmospheric fungal flora in Bombay. Atm. Bio-pollution, Editor N. Chandra. Environmental Publication, Karad:43.

Thom C and Raper KB (1945) A manual of *Aspergilli*. The Williams and Wilkins Company, U.S.A.

Lodge DJ (1997) Factors related to diversity of decomposer fungi in tropical forests, Biodiversity. Conservation 6,681-688.

Atlas RM (1984) Diversity of Microbial communities. Advances in Microbial ecology 7:147.

Whittaker RH (1972) Evolution and measurement of species diversity Taxon 21:213-251.

D'Silva AM and Freitas YM (1981) The role of the aerial mycoflora of Bombay in respiratory allergies proc. Nat. Conf .Env. Bio 63.

Shah NR and Arya A (2000) Studies on fungal biodeteriogens. ISBN 81-86050-19-1. Bhartiya Kala Prakashan , Delhi.p-5-143.

Pera LM, Romero CM, Baigari MD and Casiro GR (2006) Catalytic properties of lipase extracts from *Aspergillus niger.* 44(2)247-252.

ABSTRACTS

Isolation of seed-borne fungi from Pigeon pea (*Cajanus cajan* L.) seeds

Gaikwad PB, Jitekar RC, Rathod LR and Mote MR
Department of Botany, Mahatma Phule A. S. C. College, Panvel

Pigeon pea (*Cajanus cajan* L.) Cv. BSMR-853 was collected from pulses research center Badnapur, field place and market places. Mycoflora associated with the Pigeon pea was isolated using three different techniques. A total number of nine moulds: *Aspergillus flavus, Aspergillus niger, Cladosporium herbarum, Alternarias tenuis, Alternraria alternata, Penicillium citrinum, Curvularia lunata, Rhizopus nigricans* and *Fusarium oxysporum f. sp. udum.* were isolated from both healthy and infected Pigeon pea seeds. Findings from this study have revealed that while members of the *Aspergillus flavus Aspergillus niger and Alternaria tenuis* are the dominant fungi which are causing seed rot and seedling blight of Pigeon pea.

Key words - Pigeon pea (*Cajanus cajan* L.) Cv. BSMR-853, *Standard blotter paper methods, Agar plate, Seed washates method*

***

Identification of some Leather Degrading Fungi & Preliminary Investigation of their Growth Inhibition using Extracts of *Trichoderma viride*

Mayur Kale, Rohit Inkar, Mukta Salunkhe, Neha Sawant, and Sakshi
Department of Botany, D.G. Ruparel College, Matunga (W), Mumbai-400016, India

Different types of finished leathers are prone to various types of fungal attacks. These leathers are stored in warehouses, where the moisture & humidity makes them susceptible to such attacks. Consequently, this has become a problem to the leather industries. Hence, a need to identify and curb the growth of these fungi on different leathers, arises. For this project three types of leather were used, viz., cow leather, goat leather, and sheep leather. The fungi growing on these leathers were identified using standard manuals. Among the fungi identified *Aspergillus niger*, and *A. flavus*, were selected, and investigated further for their growth inhibition by extracts of *Trichoderma viride*. The anti-fungal activity of two different extracts (obtained by using the solvents, n-butanol and ethyl acetate) of *T. viride* was tested against the two selected fungi using agar well diffusion method and broth culture method. Different concentrations of both the extracts were taken. It was observed that both the extracts were more effective against the growth of *A. flavus*, than *A. niger*. The extracts obtained from the more economical solvent among the two, i.e., ethyl acetate, can be further investigated.

Keywords: *Aspergillus niger*, *A. flavus*, *Trichoderma viride*, leather degrading fungi, and antifungal activity.

***

Determination of Crude Oil Degradation Potential of some Filamentous Fungi

Vishal Darode, Ashwini Jadhav, Shubham Burande, Nikhil Pawar, Vivek Pawar, Sushant Bornak and Sakshi
Department of Botany, D.G. Ruparel College, Matunga (W), Mumbai-400016, India

Mycoremediation offers a promising means to reclaim soil contaminated with oil spills. Treatment of the contaminated site with an appropriate inoculum of microorganisms shows potential to enhance the biodegradation of hydrocarbons. Fungi show remarkable range and adaptability in consumption of different organic molecules as a carbon source. Environments polluted with oil spills can be remediated by introducing these organisms at the problem areas. The project aimed at identifying the potential of certain fungi isolated from sites contaminated with crude oil. Soil samples, contaminated with petroleum hydrocarbons, were collected from the car garages, and the fungi growing in such environments were isolated and identified using standard manuals. Three fungi viz., *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., were considered. The biodegradability of these fungi was verified and the linear growth was examined, after growing them on media having crude oil as the sole carbon source. Fungi were also grown in broth cultures, and their per cent biodegradation was examined at 10th, 20th and

30th day after incubation. *Aspergillus* sp., showed the most potential while the other two selected fungi showed a considerably good degradation potential. Hence, there is a prospective of introducing the consortia of such organisms in the environment to remediate it.

Keywords: *Aspergillus sp.*, *Fusarium sp.*, *Trichoderma sp.*, mycoremediation, biodegradation of hydrocarbons, crude oil.

***

Investigation of Extracts of Different Spices on the Growth and Aflatoxin Production of *Aspergillus flavus*

Rahate Kshitija, Sherekar Prachi, Deshmukh Pradnesh, Parab Pranil and Sakshi
Department of Botany, D.G. Ruparel College, Matunga (W), Mumbai-400016, India

Aflatoxins are one of the most common and widely known mycotoxin contaminants. The aflatoxin producing fungi are broadly distributed in nature and can grow over a wide range of environmental conditions. Many Indian spices are capable of producing anti-fungal compounds, which can be used against these fungi. Hence, the extracts of these spices hold promise to check the growth, and inhibit the production of aflatoxins by such fungi. For this project, three different spices were taken, viz., Cinnamon, Star anise, and Fennel. For each spice two different types of extracts (extracts obtained by using water and ethyl acetate as solvents) were prepared. Different concentrations of these extracts were made. The activity of these extracts was checked against *Aspergillus flavus*, a major contaminant, and an aflatoxin producing fungi. Using the broth culture method, the efficacy of the extracts was tested by assessing the biomass and aflatoxin production by *A. flavus*. The qualitative estimation of the aflatoxins was done by thin layer chromatography. The extracts of Cinnamon, Star anise and Fennel all turned out to be very effective. At higher concentrations, the production of aflatoxins ceased, even though the mycelium was being produced. The aqueous extracts were moderately effective, as opposed to ethyl acetate extracts. Since, aqueous extracts are easy to prepare and economical, they can be used against such fungi, as an alternative.

Keywords: *Aspergillus flavus*, aflatoxins, mycotoxins, spices, and antifungal activity.

***

Systematic Study in Family Xylariaceae from Kolhapur District

Patil Anjali and Patil Ketaki
Department of Botany, Rajaram College, Kolhapur 416 004, Maharashtra State, India

The Family Xylariaceae belongs to class Ascomycetes. Ascomycota is the largest group of fungi representing the rank of Phylum or Division in the Kingdom fungi. Worldwide genera is 47 there are 13 genera of Xylariaceae representing from India and 6 from Maharashtra respectively. Kolhapur District collected genera is *Daldania* sp.10, *Poronia* sp.2, *Hypoxylon* sp. 11, *Nemania* sp1, *Podosordaria* sp.1, *Poronia* sp.2, *Rophalostroma* sp.3, *Roselina* sp.1, and *Xyleriaceae* sp.25.etc.

Keywords: Ascomycota, Mycotaxonomy, Xylariaceae.

***

Diversity of Terrestrial Orchid Mycorrhiza from Kolhapur District, Maharashtra - I

Patil Anjali, Kambale Madhuri and Patil Sunita
Department of Botany, Rajaram College, Kolhapur 416 004, Maharashtra State, India

Orchids, an unique group of plants, comprising the largest family of flowering plants- 'Orchidaceae' is represented by 25,000 to 35,000 species belonging to 600 to 800 genera (Garay, 1960; Schultes and Pease, 1963; and Arditti, 1979).Majority of orchids are photosynthetic at maturity. However, more than 100 species of myco-heterotrophic (MH) orchids are completely achlorophyllous and are nutritionally dependent on their fungal partners throughout their lifetime (Leake,1994, 2005; Bidartondo, 2005). During germination, seeds of orchids get supply of sugar, vitamins, etc. through fungal hyphae. Mycorrhizal fungi breakdown starch into simple sugars which facilitate germination of orchid seeds (Knudson, 1922, 1924 and 1925). The germination of orchid seeds in

nature is very low due to their requirement of specific mycorrhizal association for germination. In the present paper (Part I) 45 species of terrestrial orchids collected during the present work, belonging to the genera Habenaria, Nervelia, Malaxis, Peristylus, Pectilis, Geodorum, Zeuxine, Cheirostylus and Eulophia are recorded from Kolhapur District of Maharashtra state.

Keywords: Terrestrial Orchids, Habenaria, Eulophia, Geodorum, Nervelia, Peristylus, Pecteilis, Malaxis.

***

Diversity of Terrestrial Orchid Mycorrhiza from Kolhapur District, Maharashtra - II

Patil Anjali, Kambale, Madhuri and Patil Sunita
Department of Botany,Rajaram College, Kolhapur 416 004, Maharashtra State,India

Orchid mycorrhiza (Mark,1991) are basically endophytic. The fungi form intra-cellular coils (pelotons) in root cortical cells or less regular hyphal aggregates within host tissue. Nearly all known orchid mycorrhiza are formed with fungi of the Basidiomycotina (Rasmussen, 1995; Taylor, et al., 2002). The identification of orchid mycorrhizal fungi is a critical first step in exploring the biology of this symbiosis, on which all orchids so far studied depend to complete their life cycles in nature (Arditti *et al.*, 1990). Orchid seeds are minute with few stored reserved food (endosperm), colonization by a specific endophytic mycorrhiza (fungus) is essential for germination and/or early protocorm development (Smith and Read, 1997). In the present investigation 33 species belonging to 09 genera of orchids were collected and their mycorrhizal associations were studied. Anatomical studies of roots show fungal coils in the cells of cortical region, i.e. presence of mycorrhizal association. Pure culture of the fungus associated with the root cortical region of the plant was obtained and identified as *Rhizoctinia solani*, along with some non-mycorrhizal endophytes.

Keywords: Orchid Mycorrhiza, Pelotons, *Rhizoctonia solani*, non-mycorrhizal endophytes.

***

Isolation of fungi as pollutant From Indoor Air

Rambal Kavita
Department of Botany, Maharishi Dayanand College of Arts, Science & Commerce. Parel, Mumbai

Indoor air refers to air in the indoor environment within a home, building or commercial facility. The quality of indoor air is very essential for the people living indoors. Air being essential for survival should be free from any type of pollution, but can become polluted by pollutants of chemical and biological origin. Indoor air can get affected by microbial pollutants or contaminants which include fungi and moulds. Since people spend more time indoors than outdoors, exposure to these pollutants can be hazardous. Present study was undertaken in one of the rooms of Maharshi Dayanand College being used by vice Principals Arts & Commerce. They complained of irritation in eyes and throat, dizziness, fatigue and other respiratory problems. Present study aimed at isolating and identifying the major fungal organisms there. A total of six genera and seven species were recorded. The most dominant fungi were *Rhizopus stolonifer and Aspergillus niger.*

Key words: Fungi, humidity, Allergy, *Rhizopus*, *Aspergillus niger,* ventilation.

***

Diversity study of cellulolytic filamentous fungi of Matheran forest

Shete Pooja, Chandralekha and Yadav Lal Sahab
Department of Botany, Smt. C.H.M. College, Ulhasnagar

Fungal diversity and decomposition of leaves were studied in Matheran forest. This study focused on fungal community involve in degradation of leaf litters and diversity of fungi on forest floor. Diversity of filamentous fungi monitored during two consecutive rainy seasons. Total 28 species of microfungi belongs to eight genera were encountered very frequently. The dominant genera among them were *Aspergillus, Penicillium, Fusarium* and Mucoracious fungi, few rare genus are also encountered during isolation on three different media. The numbers of Mucoracious fungi

were higher in leaf litter samples than in soil and forest floor sample like fallen fruits and wood logs. Screening of isolated fungi from different samples viz. leaf litter, soil and fallen fruits for their cellulose degrading potential was done on solid media CMC selected agar using congored as indicator. The enzyme cellulase assay also done for selected isolates. Five isolates were discovered to be highly cellulolytic compare to the rest.

Key words: Diversity, Cellulolytic filamentous fungi, enzyme cellulase

***

Arbuscular mycorrhizal associa-tion with pteridophytes in areas around Mumbai.

Omkar Khache, Pranil Parab, Maanasi Marathe and Shyam Palkar
Department of Botany, D.G. Ruparel College, Mumbai 400016

Pteridophytes are considered the first true land plants, that evolved after algae and bryophytes. Mycorrhizae are soil borne fungi that form symbiotic association with the roots of the plant. They are found in a variety of ecosystems, and are naturally documented as much in pteridophyte as in gymnosperms and angiosperms. The Ferns were collected from 3 sites : SGNP, Tungareshwar and Matheran. Species of ferns collected were *Asplenium*, *Adiantum*, *Chilanthus* and *Aspidium*. These ferns were analysed by technique of Isolation of AM fungi from rhizosperic soil performed by Sieve decanting method of (Gerdemman and Nicolson, 1983). Root colonization by AM, staining technique of (Philips and Hayman, 1970). Majority of ferns collected showed mycorrhizal association. Asplenium showed the highest amount of root colonization. The species of spore isolated was *Glomus*.

***

The Assessment of Fungal Bioaerosol Contamination around the Municipal Landfill Site in Mumbai, India

Patil Neeraj S, Saitawadekar Aadity S and Kakde Umesh B*
The Institute of Science, Madam Cama Road, Fort, Mumbai, Maharashtra, India 400032

In India, general method of municipal solid waste disposal is the dumping of waste on open land outside the cities. This procedure leads to air pollution with harmful gases as well as bioaerosols. The present study was carried out on occurrence of culturable aeromycoflora for three seasons- summer, winter and Monsoon in the year 2015-16 in the vicinity of Mulund dumping site in Mumbai, India. The highest concentration of fungal bioaerosol was found in monsoon season which is 1370 cfu/m^3 and the lowest result recorded in winter season which 220 cfu/m^3. The *Aspergillus* species were most abundant in the air followed by *Penicillium*, *Alternaria*, etc. The occurrence of bioaerosol is positively correlated with the Relative Humidity and Temperature.

Keywords: aeromycoflora, Bioaerosol, India, Mumbai, Municipal landfill site,

***

Antibacterial activity of metabolites of alkaliphilic fungi against Methi-cillin resistant *Staphylococcus aureus* (MRSA)

Bari Kishor P and Padalia Unnati
Department of Microbiology, K.J. Somaiya College of Science and Commerce, Vidyanagar, Vidyavihar, Mumbai-400077

The study was undertaken to screen antagonistic activity of metabolites of alkaliphilic fungal isolates against clinical antibiotic resistant Methicillin resistant *Staphylococcus aureus* (MRSA) isolates. Cell free extracts were prepared by filtration and extracted with methanol, ethanol, ethyl acetate and diethyl ether. Study of antibacterial activity was performed with these extracts. Cell free extract of alkaliphilic

fungus exhibited significant activity against MRSA. Ethyl acetate extracts of alkaliphilic fungus showed more antibacterial activity as compared with ethanol extracts and diethyl extracts.

Keywords: Methicillin resistant *S. aureus* (MRSA), Antibacterial activity, Cell free extract.

***

Role of fungal symbionts in the promotion of *Capsicum annuum*

Menon Shailaja S and Padalia Unnati
Department of Microbiology, K.J.Somaiya College of Science & Commerce, Vidyavihar,Mumbai-77.

This is an era of reduction in the use of chemical fertilizers as it is hazardous to the living being. We are enriched with the natural resources., which not only promote the well being of the ecosystem but prevent and reduce the rate of environmental pollution. Agriculture is the main stay of this nation. This research projects address the issues of peak production and pollution free environment. The study highlights the use of Mycorrhizal Fungi in the production of *Capsicum annuum* or Chillies, member of Solanaceae in an environment friendly way. This natural biofertilizer is easily available. Arbuscular Mycorrhiza(AM) is a microbial source of accepted bio-fertilizers. It can be introduced in any desired field. Mycorrhiza , a collection of precise symbionts, a fungi and the roots of higher plants. These fertilizers are present both in the rhizosphere soil and in the root cells. The present study was carried out across ten locations of greater Mumbai.. There were abundance of genera like *Acaulospora* and *Glomus* and in a few, there were *Gigaspora* too. Inoculum of Mycorrhiza was prepared and introduced in the cultivation of *Capsicum annuum*. The study focused on the changes in Vegetative and reproductive growth in these plants. The results were encouraging as there wasa decline in the dormancy period, resulting in early germination of treated seeds. It was also observed that the rate of germination was high and the physiological parameters were significant. The treated plants were showing early maturity and mass production of quality fruits. The plants remained wild but the quality had improved which preserves the original germplasm. This plant is well known as it adds spiciness to the culinary activities and in the production of Capsaicin, a well known ingredient of pain relieving preparations of various diseases. The availability and the easy introduction of Arbuscular Mycorrhiza to crop plants for better results provide a comfort zone to the ailing Agriculture quarter.

Key words: *AM Fungi, Rhizosphere, Dormancy, symbionts Capsaicin, germplasm.*

***

Antifungal activity of Silver nanoparticles

Riddhi Gada and Unnati Padalia
Department of Microbiology, K. J. Somaiya College of Science And Commerce, Vidhyavihar, Mumbai-400077, India.

Antifungal activity of silver nanoparticles against various fungal pathogens have been reported. Silver nanoparticles have been synthesized by the researchers from algal extract, plant extract and raspberry extract. Synthesis of these nanoparticles were confirmed by visual observation and by using UV-Visible Spectrophotometer. These synthesized nanoparticles were used to determine antifungal activity against *Candida albicans, Candida tropicalis, Candida kefyr* and *Aspergillus niger*. Development of eco-friendly, safe and nontoxic methods for preparation of Silver nanoparticles by various researchers have been reported.

Keywords: *Silver nanoparticles, Antifungal activity, Eco friendly.*

***

Study of mycotoxins and its impact on humans

Saitawadekar Aadity S and Kakde Umesh B
The Institute of Science, Madam Cama Road, Fort, Mumbai, Maharashtra, India 400032

Molds are microscopic fungi that live on organic matter. In nature they play a huge role in the breakdown of plant and animal matter and return it to the soil to be used again. Around ten thousands to perhaps 300,000 or more species of fungi has been discovered. Most of the fungi are filamentous

(threadlike) organisms. A number of fungal genera such as *Aspergillus, Penicillium, Alternaria, Fusarium* etc. are known to produce mycotoxins under certain conditions. Mycotoxins are secondary metabolites of molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses. The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. Often more than one mycotoxin is found on a contaminated substrate. Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of molds, they can also be found in temperate zones. The diseases caused by exposure to mycotoxins are known as mycotoxicoses.

Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and these substances vary greatly in their toxicity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. India is a prime example of a country in which the economy is affected heavily by mycotoxins. In a study in the Bihar region from 1985 to 1987, nearly 51% of the 387 samples tested were contaminated with molds. Of the 139 samples containing AF, 133 had levels above 20 µg/kg. Study in cosmopolitan city like Mumbai, study is not yet carried out. Mycotoxins have various acute and chronic effects on humans. Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth. The economic impact of mycotoxins include loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem. Although efforts have continued internationally to set guidelines to control mycotoxins, practical measures have not been adequately implemented.

Keywords: Mycotoxins, Fungal secondary metabolites, types of mycotoxins, effect on human health.

***

AUTHOR INDEX (Research Articles)

AUTHOR INDEX (Abstracts)

ROYAL MICRO
IRRIGATION SYSTEMS

Nero Drainage Cell
30mm

WE ARE AUTHORISED DISTRIBUTOR
FOR RAINSMART SOLUTIONS IN INDIA

RAINSMART
SOLUTIONS
www.rainsmartsolutions.com

Nero Pave
50mm

Cell: +91 98211 35027 / 93209 26511 / 7045452110 / 9322226521

Email :info@royalmicroirrigation.com • royalmicroirrigation@gmail.com

www.royalmicroirrigation.com

Wherever I go, my bank goes with me!

बैंक ऑफ महाराष्ट्र
Bank of Maharashtra
भारत सरकार का उद्यम
एक परिवार एक बैंक

Banking
- Balance Enquiry
- Mini statement

Card Services
- Apply for debit/ credit card
- Reset ATM PIN
- Block debit card

Fund Transfer
- Transfer to own account
- Transfer to other accounts
- Transfer to other bank a/c with NEFT
- View/add/modify/ delete beneficiaries
- Transfer to other bank a/c with IMPS

Service Request
- Search past transactions
- A/c statement request
- Cheque Book Request
- Cheque Status Enquiry
- Stop Cheque
- Demand Draft Request
- Open new a/c

Bill Pay
- Add Billers/Instant Pay
- Delete Billers

VAS
- Log Complaint
- Mobile Passbook
- Change MPIN & MTPIN

Send an SMS MAHAMOBILE TO 9223181818 to get the download link or find us on the app stores.

Available on the App Store

GET IT ON Google play

Terms & Conditions apply

Toll Free No: 1800 - 233 - 4526 Website: www.bankofmaharashtra.in Net Banking: www.mahaconnect.in

PULSE
LIFE SCIENCE

MICROSCOPES

IMAGING SOLUTION FOR MICROSCOPES

IMAGE ANALYSIS SOFTWARES

UV/VIS SPECTROPHOTOMETERS

AUTOMATIC COLONY COUNTER

GEL DOCUMENTATION SYSTEM,

DATA LOGGERS & GSM BASED

TEMPERATURE MONITORING SYSTEMS

BOD INCUBATORS, STABILITY CHAMBERS,

DEEP FREEZERS, WATER BATHS,

LAMINAR AIR FLOW, BIOSAFETY CABINETS,

CENTERIFUGES, MUFFLE FURNACE,

AUTOCLAVES, INCUBATORS,

VACCUM OVENS & HOT AIR OVENS.

B/ 107 Vrindavan B. Morwala C.H.S, Majaswadi, Nr. Gajanan Temple, Jogeshwari(E), Mumbai 400 060.
www.pulselifescience.com , www.pulselifescience.co.in Mob. : 9022592910 / 9324479648

सेन्ट्रल बैंक ऑफ़ इंडिया Central Bank of India

1911 से आपके लिए "केंद्रित" "CENTRAL" TO YOU SINCE 1911

FESTIVE SEASON'S Big Bonanza

Complete Waiver of Processing Fees on:

- Cent Home Double Plus ++
- Cent Vehicle — Loan for Vehicle
- Cent mortgage — Loan against mortgage of property
- CENT RENTALS — Loan against rent
- CENT PERSONAL GOLD LOAN — Loan/Overdraft against Gold
- CENT TRADE SCHEME — Overdraft Facility for Traders
- XYZ STORES — Cent Shop SCHEME
- CENT PERSONAL LOAN

Like us on: https://www.facebook.com/CentralBankofIndiaMumbai

Follow us on: https://twitter.com/centralbank_in

Festive Offer Available for a limited period

To know more about our customer oriented products & services, please contact our nearest Branch or log on to **www.centralbankofindia.co.in**

गृह आणि वाहन
कर्ज महोत्सव

महा सुपर हौसिंग लोन

आकर्षक व्याजदरासोबत

- जास्तीत जास्त रक्कम कर्ज म्हणुन उपलब्ध
- कमीत कमी व्याजदर
- कोणत्याही प्रकारचे छुपे खर्च नाहीत
- रु. १० लाखापर्यंत कंझ्युमर लोन

महा सुपर कार लोन

आकर्षक व्याजदरासोबत

- जास्तीत जास्त रक्कम कर्ज म्हणुन उपलब्ध
- कमीत कमी हप्ता
- कोणत्याही प्रकारचे छुपे खर्च नाहीत

अधिक माहितीसाठी जवळच्या शाखेशी संपर्क साधावा.

बँक ऑफ महाराष्ट्र
Bank of Maharashtra
एक परिवार - एक बँक

नियम व शर्ती लागू.

*For Limited Period Only.

ठाणे आंचल कार्यालय, महाबँक भवन, बी. ३७, वागळे इंडस्ट्रीयल इस्टेट, ठाणे - ४०० ६०४.

Toll-Free No.: 1800-233-4526 website: www.bankofmaharashtra.in Net Banking: https://www.mahaconnect.in

INSTRUCTION FOR PREPARING THE MANUSCRIPT:

International Journal of Life Sciences publish research papers, case studies and short communications containing original research work of good standard and review papers of contemporary relevance from all over the world. Following types of contributions are considered for publication:
RESEARCH PAPERS (Full Length - Maximum of 12 pages-A/4 size).
CASE STUDIES
SHORT COMMUNICATIONS
REVIEW PAPERS including Mini Reviews

Author can send manuscript as an attachment on email to editorilsci@gmail.com OR editor@ijlsci.in.
Manuscript format should be in MS word only. Charts, Tables and Diagrams should be in MS Excel or MS Word and images in JPG format using maximum possible 150 dpi resolution. All the matter should be typed in Times New Roman Font size - 12 point with single spacing.

AUTHORISATION AND DECLARATION

Authors must accept full responsibility for the content of their articles. The Members of the Editorial Board and the Publisher of the journal are not responsible for the statements and opinions expressed by the authors in their articles/write-up published in the journal. To use the copyrighted material (for example table, figure etc.), the author/s must seek the permission from the Author/s and Publisher of the material and send the copy of the permission letters via e-mail to the Administrative Editor for records. In case, if it is not possible than do not use that material.
While submitting the paper the author (s) must give a declaration that "the article has not been published or sent for publication elsewhere".

INSTRUCTIONS FOR MANUSCRIPT-FORMAT

a) Page Layout: A4 (21cm × 28 cm) leaving 2 cm margin on all sides of the text. All the text Should be single spaced and the pages should be numbered consecutively.
b) Use MS word (2003-2007) for text and TIFF, JPEG or Paint for figures.
c) The first page should contain:
Title-bold, 16 point size
Name/s of author/s-bold, 10 point size (Surname or Family name first in bold) Affiliation/s-address of all the author/s followed by email of corresponding author in 10 point size,
Abstract: up to 200 words in 10 point size
Keywords: at least 5 keywords in 10 point size
d)Main Text- The paper should be divided into the following sections:
Introduction, Material and Methods, Results and Discussion, Conclusion, Acknowledgement and References.
Tables and Figures of good resolution (150 dpi) should be numbered consecutively and given alphabetically by surname or family name in the order of their appearance in the text.
e) Reference style
Text: All citations in the text should refer to:
1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by "et al." and the year of publication.
Citations may be made directly (or parenthetically).
Groups of references should be listed first alphabetically, then chronologically.
Examples: "as demonstrated (Baig, 2004a, 2004b, 2010; Chavhan and Pawar, 2011). Jadhao et al., (2000) have recently shown…

Reference to a journal publication:
Chavhan AB, Pawar SS, Jadhao RG and Patil KG (2013) Distribution of CC-chemokine receptor-5-Δ32 allele among the tribal and caste population of Vidarbha region of Maharashtra state, *Indian J Hum Genet.*, 19 (1) 65-70.

A Book:
Durbin R, Eddy SR, Krogh A, Mitchison G (1999) Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. Cambridge University Press.

A Chapter in a Book:
Leach J (1993) Impacts of the zebra mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra mussels: biology, impacts and control, Eds., Nalepa, T. and D. Schloesser. Ann Arbor, MI: Lewis Publishers. pp: 381-397.

Report:
Makarewicz JC, Lewis T, Bertram (1995) Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference Proceedings:
Chavhan AB (2012) Biomedical Waste Management: Awareness and Practices among Healthcare Providers in Amravati". Proceeding of national conference on Recent Trends and Innovative Development in the Frontiers of Life Science. pp- 148-153. (ISBN-978-93-81733-04-2).

A Thesis:
Chavhan AB (2012) Genetic polymorphism of Chemokine receptors in some tribes of Vidarbha, Maharashtra. Ph. D. Thesis, RTM University, Nagpur,India;.

Web references:
As a minimum, the full URL should be given and the date when the reference was last accessed.

PROOFS

Proofs will be sent to the corresponding author via e-mail as an Acrobat PDF (Portable Document Format) which should be returned to the Editorial office within 2 working days.

PROCESSING FEES

Please send the Membership subscription or processing fees. Processing fees (Rs. 2000/- for India & US$ 120/- for Overseas) has to be paid every time when one submits the manuscript. Life members need not pay processing fees. If one is Annual member, then one can submit two manuscripts during the annual membership. If one submits more than three manuscripts in a year, one has to pay processing fees from fourth manuscript onwards.

ISSN: 2320-7817 (Print) ISSN: 2320-964X (Online)
Journal home page : www.ijlsci.in

Contact : +91-997 055 9438
+91-942 077 5527

INTERNATIONAL JOURNAL OF LIFE SCIENCES

An International Peer Review Open Access Journal

G-3 Dattamandir, Vinayak nagar, Nagpur Road,
Amravati- 444603, India

MEMBERSHIP FORM

The Editor,

I wish to be a member of **International Journal of Life Sciences (IJLSCI)** and agree to abide by the rules setup by you. The filled in proforma is:

(1) Full Name (in block letters) :

(2) Gender :

(3) Nationality :

(4) Working status and Department :

(5) Field of Research :

(6) Mailing Address :

(7) City : State :

Country : Pin :

(8) Phone (O):..... (R): ..... (M): .....

(9) E-mail :

(10) Nature of Subscription: Annual Membership / Life Membership / Fellow Membership

MEMBERSHIP	ANNUAL		LIFE		FELLOW	
	Indian	Overseas	Indian	Overseas	Indian	Overseas
Individual :	INR. 2,500/-	US $ 150	INR. 10,000/-	US $ 550	INR. 15,000/-	US $ 850
Institutional :	INR. 3,500/-	US $ 250	INR. 15,000/-	US $ 850	INR. 25,000/-	US $ 1700

Mode of Payment

INRs./US$/Euro can be remitted by online payment/E-Money Transfer/Wester Union Money Transfer/Bank Demand Draft/Cheques (at par only)/Cash mentioning Receipt No. and date.
Detail of transfer process is as follows:

A/c No.	:	31033988127
MICR Code.	:	444002976
Branch Code	:	003866
IFS Code	:	SBIN0003866
Branch Address	:	State Bank of India, Old Biyani College Road Tapowan Road Distt Amravati Maharashtra 444602
Full Name of Receiver	:	**ARVIND BHIMRAO CHAVHAN**
Full Address (Including City, Street & Postal Code)	:	G-3 Dattamandir, Vinayak nagar, Nagpur Road, Amravati- 444603, India.

Date .....

Signature.....

Name .....

Email this Membership form along with Scan copy of Fees deposit receipt to editorijlsci@gmail.com / editor@ijlsci.in