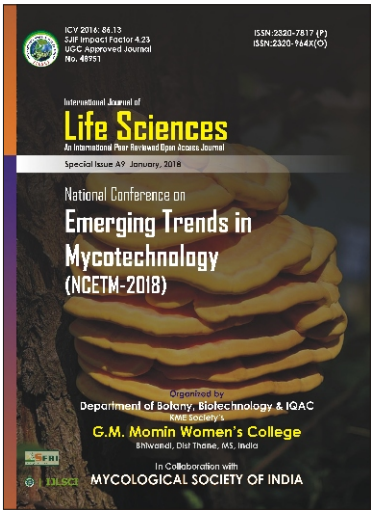
ICV 2016: 86.55
UGC Approved Journal No. 48951
Cosmos Impact Factor 4.369
SJIF Impact Factor 4.23

ISSN:2320-7817 | eISSN:2320-964X
Special Issue, A 9, January 2018

INTERNATIONAL JOURNAL OF Life Sciences

AN INTERNATIONAL PEER REVIEWED OPEN ACCESS JOURNAL



National Conference on
EMERGING TRENDS IN MYCOTECHNOLOGY
(NCETM-2018)

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Organized by
Department of Botan, Biotechnology & IQAC
KME Society's
G.M. Momin Women's College
Bhiwandi, Dist Thane, MS, India

In Collaboration with
MYCOLOGICAL SOCIETY OF INDIA

| IJLSCI

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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.

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Submission of Paper: Submission of paper is encouraged by email to editorijlsci@gmail.com
Subscription Plan: Rs. 3500 /- or 250 US$ (Individual Annual); Rs. 4500/- or 350 US$ (Institutional)
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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, Google Scholar, OCLC WorldCat, INNOSPACE (SJIF), CiteFactor, ROAD, OAJI, AcademicKeys, J-index.net, ScholarSteer, ResearchBible, DRIJ, DIIF, SIS, SIS, ASI, Directory of Science

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MESSAGE

THE KONKAN MUSLIM EDUCATION SOCIETY OF THANE DISTRICT

H. No. 158, Rais High School Campus, Thana Road, Bhiwandi - 421 302, Dist. Thane. Phone : 02522-225441
(Registration No. 427 Dt. 10-10-1927) & (F-19 (T)
Email : kmesociety1927@gmail.com / kmesociety@yahoo.com Website : www.kmesociety.org

Ref. No.

Date 27-12-17

MESSAGE

I am delighted to pen greetings to the National Conference devoted to fungal research. It aims to cover various aspects related to Fungi in the fields of medicine, agriculture and industry.

I am sure that the conference would be highly beneficial for the amateur researchers as well as the entire teaching and scientific fraternity.

I wish the entire organizing committee, delegates and participants, all success in the deliberations and interaction and may the outcome benefit all the constituent stakeholders and society at large.

Mr. Aslam Fakih
President
K.M.E.Society

MESSAGE

THE KONKAN MUSLIM EDUCATION SOCIETY OF THANE DISTRICT

H. No. 158, Rais High School Campus, Thana Road, Bhiwandi - 421 302, Dist. Thane. Phone : 02522-225441

(Registration No. 427 Dt. 10-10-1927) & (F-19 (T)

Email : kmesociety1927@gmail.com / kmesociety@yahoo.com Website : www.kmesociety.org

Date 27/12/2017

MESSAGE

It is indeed a matter of privilege and pride that our K. M. E. Society's G. M. Momin Women's College is organizing Two Days National Conference on "Emerging Trends in Mycotechnology".

Fungi are ubiquitous in nature & influence mankind in diverse manners. They not only play an important role in conservation of ecosystem but also have relevance in modern technology. The Konkan Muslim Education Society is offering heart rendering services, for more than 89 years in the field of education by establishing Primary and Secondary schools, Jr. Colleges, Women's College and College of Education. The G. M. Momin Women's College has created its own niche in academics as well as in extracurricular activities. It has motivated a lot of under privileged women to pursue their higher education.

I am sure that the discussions in the conference will motivate the teachers, research scholars and students to generate novel ideas. The participants coming from all over India will be enriched from the deliberations at the conference.

I wish the conference a grand success.

Dr. Musaddiq Patel

Hon.General Secretary
K.M.E Society

MESSAGE

MYCOLOGICAL SOCIETY OF INDIA

CENTRE FOR ADVANCED STUDIES IN BOTANY
LIFE SCIENCES BUILDING
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Prof. N. RAAMAN, Ph.D., FNABS, FMSI, FSAB
SECRETARY

Email : msi73secretary@gmail.com
E-mail : raaman55@gmail.com

It gives me great pleasure in felicitating the KME Society's G.M.Momin Women's College, Bhiwandi, Dist. Thane, Maharashtra and MSI Mumbai Unit for organizing the National Conference on Emerging Trends in Mycotechnology on 5 and 6 January 2018. On behalf of the Mycological Society of India (MSI), I wish the KME Society's G.M.Momin Women's College and MSI Mumbai Unit a meaningful and resourceful conference.

The MSI and its officers, both past and present, and members have contributed so much in the development and improvement of Mycology nationwide since its inception at Chennai in 1973. A good number have also been active in the international scene, presenting papers, creating collaborations, and even actively participating in the affairs of several international associations. One of the objectives of the MSI is to establish, maintain and strengthen partnerships and linkages with institutions and organizations within India and abroad for the welfare and benefit of mycologists of India and abroad.

I thank the MSI Mumbai Unit for organizing the National Conference. I am sure that such initiatives will propagate the role of budding scientists in the field of Mycology in that region and will promote newer ideas in them.

I congratulate the MSI Mumbai Unit, Convener, Organizing Secretary and all the members of Organizing Committee for conducting such a grand conference in Bhiwandi. I wish this National conference a grand success.

With Best Wishes,

Raaman

PROF. N. RAAMAN
Secretary, Mycological Society of India

MESSAGE

Javed Gulam Mohd. Dalvi
Mayor
Bhiwandi Nizampur City Muncipal Corporation
Office Tel (02522) 250056
Resident 79 Bunder Road Bhiwandi Thane Maharashtra Mob 9823053912

To,
Hon'ble. Principal,
K.M.E. Socitey's G.M. Momin Women's College,
Bhiwandi.

Date:

Respected Sir,

It gives me immense pleasure to know that K.M.E. Society's G.M. Momin Women's College & Mycological Society of India organizes National Conference on "Emerging Trends in Mycotechnology" on 5th & 6th January, 2018.

The theme of conference is related to fungi. I hope that the conference will have meaningful conversations & thought provoking speeches from resource persons. It is therefore essential that our researchers keep themselves updated with emerging trends in fungi.

I am sure the students & young scholars in particular would be enriched from the discussions at the conference.

I wish the conference a great success.

Your's,

Javed Gulam Mohd. Dalvi
Mayor,
Bhiwandi Nizampur City Municipal Corporation

MESSAGE

FROM THE PRINCIPAL & CONVENER

Principal
Dr. Moses J. Kolet

Wish you all a very Happy New Year 2018.

It gives me much happiness and pleasure to conduct the National Conference on 'Emerging Trends in Mycotechnology' in our KME Society's G. M. Momin Women's College in Bhiwandi. I thank the Mycological Society of India, the Mumbai Unit of Mycological Society of India and the University of Mumbai for extending their support and collaboration in this venture. It also gives me great pleasure to present the special issue volume of UGC approved 'International Journal of Life Sciences' comprising papers presented during the conference.

In spite of scientific advancements, our knowledge of mycological diversity and the roles played in nature by these apparently insignificant entities could be equated as only the 'tip of the iceberg', with many types yet to be recorded or worse still, getting extinct before their being discovered; leave alone being gainfully utilized: mainly owing to human interference and interventions. Utilization of fungi for the benefit of mankind comprises Mycotechnology; emerging trends of which comprise the theme of the conference. A sound mycological base therefore is inevitable and a prerequisite for any Mycotechnology to be effectively applied for benefit of humanity.

I take this opportunity to thank our parent body, the Konkan Muslim Education Society of Thane District, our patrons Hon'ble Mr. Aslam Fakih, Dr. Musaddiq Patel, Dr. P.K.S.M. Rahman and our former Vice Chancellor Dr. Sanjay Deshmukh, members of the national advisory and local organizing committees, the conference organizing Departments of Botany, Biotechnology and Internal Quality Assurance Cell; support staff, student council, student volunteers and all our ever enthusiastic students; all support services, especially the administrative office, Department of Information Technology, Department of Library and Information Science, printing and catering services and above all my Teaching and Non-Teaching staff. I also thank all donors and well wishers. I extend sincere thanks to all who have contributed towards success of this national event. I extend a warm welcome to all our guests, invitees, speakers, delegates, contributors, participants and student delegates who have come from far and near for this conference.

We have made a sincere attempt to give a platform to researchers working in mycology and bring the scope of this branch in front of the students and motivate them to march ahead with research in this field. I am sure that the researchers from all over the country assembled under the umbrella of this national conference will contribute their best to unravel the Emerging Trends in Mycotechnology, thereby successfully igniting the minds of young researchers and post-graduate students, to prepare them for bright careers in Mycology and Mycotechnology. Let us have many more young mycologists joining this vibrant group. My best wishes to the Organizing Secretary and Organizing Committee.

I wish the conference a great success!

MESSAGE

Dr. Sashirekha Suresh Kumar
President, Mycological Society of India

Natural ecosystems are abound with myriad of fungi. About 1.5 million of fungi are present globally involved in variety of functions. Fungi include hundreds of species which are of tremendous economic importance to man. Bioactive molecules from them has led to a breakthrough in curing diseases which were once thought to be difficult. Taxol an anticancerous compound was reported from *Taxomycessp, Pestalotiopsismicrospora, P. menezisiana*, and many more. They are also useful in biomineralization, biocontrol and bioremediation. Industrialisation and clearing of the natural vegetations have led to loss of natural remedies and resources.**Ecological studies emphasize patterns of the mycobiota, of host genera and families, or of specific habitat types (Petrini and Carroll 1981; Petrini et al. 1982; Petrini 1985).**The efforts of eminent mycologists have unearthed the treasure world of fungi for us to further explore the miraculous abilities of the fungi to correct the damage caused due to the ever-increasing polluting activities of man. Young research scholars must take this up seriously and work had to identify potential fungi which could be an asset to solve these problems. It is important that we start with the younger students.The importance of basic sciences needs to be taught to them.

With this objective Mycological Society of India Mumbai unit has taken the responsibility of spreading the mycological research among the students through activities like National seminars and workshops in collaboration with different colleges of University of Mumbai. Every year MSI Mumbai has held seminars on specific themes of Fungi. This year we are doing the National conference on **"Emerging Trends in Mycotechnology" with G. M. Momin Women's College. I am sure the** participants and students will interact with the renowned scientists during the deliberations and be enlightened. My sincere best wishes to the organizing committee for a grand success.

Thank you.

Dr. Sashirekha Suresh Kumar
President,
MSI Mumbai Unit

MESSAGE

from Organizing Secretary

Message

It is a great pleasure and an honour to extend a warm invitation to you for the National Conference on 'Emerging Trends in Mycotechnology' organized in our College. This conference is jointly organized by the Departments of Botany, Biotechnology and IQAC of G.M.Momin Women's College with Mycological Society of India (Mumbai Unit). Fungi have immense economic importance hence a centre of attraction to a wide spectrum of researchers belonging to diverse areas of science. The research work in fungi at college level is required to a great extent where students must learn to safely deal with fungi and understand these very important entities present in nature. I am sure that the young researchers especially students would definitely inculcate interest in working with fungi and thought process would be propelled up. The Conference will provide a wonderful forum to exchange the knowledge base and discover the innovations in Mycology. This platform will provide a grand opportunity to meet and interact with the leading scientists, researchers and participants coming from all over the Nation.

I thank the Management and Principal of our College for their motivation, guidance, and inspiration and also for giving me the opportunity to organize this National Conference in our College. I am especially grateful to all my colleagues, teaching, non teaching, administrative staff and all my dear students for extending their helping hands in this venture.

Sincere thanks once again and Wishing you a happy New Year 2018.

Dr. Vaishali Nirmalkar

K.M.E Society's

G. M. Momin Women's College

Bhiwandi 421302 Dist. Thane

&

Mycological Society of India (Mumbai Unit)

Organizes

National Conference

on

"Emerging Trends in Mycotechnology"

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National Conference
on
"Emerging Trends in Mycotechnology"
5 & 6 January, 2018

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K.M.E.Society's G.M.Momin Women's College, Bhiwandi

Joint Secretary
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K.M.E.Society's G.M.Momin Women's College, Bhiwandi

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IJLSCI

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

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

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Special Issue A9 January; 2018

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ADDRESSES

INVITED TALK

REVIEW ARTICLES

ORIGINAL ARTICLES

Inaugural Address

Diversity of algae, fungi, lichens and non-flowering and flowering plants of India: an overview

Lakshminarasimhan P[1] and Arisdason W[2]

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E-mail: lakshminarasimhanp@yahoo.co.in
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India being one of the tropical countries in the world with diverse climate and topographic features supports a rich floral and faunal diversity. It has 12 biogeographical provinces, five biomes and three bioregion domains with diverse array of habitats or ecosystems from sea level to the highest mountainous ranges in the world. The flora of the country is generally considered as the confluence of floristic elements from three major global biogeographic realms namely Indomalesian, Eurasian and Afro-tropical. However, the Indian flora exhibits its own floristic significance with about 28% of vascular plants being endemic to its present political region. The country also has four (out of 35) global biodiversity hotspots, where the rich and unique floral and faunal diversity of the country are concentrated. The species diversity in flowering and non-flowering plant groups, algae, fungi and lichens, endemism, endemic centres, wild relatives of edible plants, medicinal plants, threats to the existing biodiversity, status and conservation strategies are discussed in detail here.

***

Presidential Address

Highlights on the Macrofungi of South West Coast of Karnataka, India

Sridhar KR

Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka
E-mail: kandikere@gmail.com

This study addresses macrofungal composition of coastal habitats (scrub jungles, coastal sand dunes and mangroves) of south west coast of Karnataka. Up to 124 species of macrofungi have been recorded with a highest of 95 species in scrub jungles followed by coastal sand dunes (36 spp.) and mangroves (31 spp.). Ten species were common to all habitats and wood inhabiting *Dacaryopinax spathularia* was frequent. Edible macrofungi were highest (34 spp.) followed by ectomycorrhizal (26 spp.) and medicinal (21 spp.) macrofungi. Soil inhabiting *Lycoperdon utriforme* was edible, ectomycorrhizal and medicinal fungus. Many macrofungi were eaten based on traditional knowledge of local people. Habitat degradation is most threatening to macrofungi, which results in soil erosion, substrate depletion and elimination of host tree species. The current status and strategies of habitat conservation in favour of macrofungi have been discussed.

Keynote Address

Emerging trends in Mycotechnology

Rodrigues BF

Department of Botany, Goa University, Goa 403 206.
Email: felinov@gmail.com

Fungi are lower eukaryotes classified into a separate kingdom, 'The Fifth Kingdom', based on their absorptive mode of nutrition. They secrete a wide range of powerful enzymes into the environment and then absorb these 'pre-digested' foodstuffs back into their cells. They are diverse in morphology, physiology and ecology, and many of them have a negative impact on human welfare as agents of plant disease such as smuts, blights, wilts, rusts, bio-deterioration e.g. rots and mildews, and or as animal pathogens producing toxins and causing mycoses.

They have been important in both ancient and modern biotechnological processes. They range from microscopic molds and yeasts to macro-scopic mushrooms and truffles. Several species of macro-fungi are considered delicacies and are either gathered or cultivated for human food.

Beer, wine, bread, and other foods and beverages fermented by yeasts have been part of the human diet for centuries their origins lost in antiquity. Enzymes, alcohols, organic acids, and pharmaceuticals from filamentous fungi are central to the development of modern biotechnology.

Soil fungi play important role in major processes such as soil formation and nutrient cycling. They form an essential link between soil nutrient availability and plant productivity as they are directly involved in the cycling of nutrients through the transformation of organic and inorganic forms of nutrients. Arbuscular Mycorrhizal (AM) fungi belonging to Phylum Glomeromycotina are ubiquitous soil fungi which form symbiotic association with roots of vascular plants. This mutualistic association provides the fungus with carbohydrates and in return, the plant benefits through the high absorptive capacity of the fungal mycelium for acquisition of water, nutrients (mostly immobile P) and provide other benefits to their host plants thereby constituting a vital component of terrestrial ecosystems. Recognizing the potential of AM spore production by *in vitro* culture technique, efforts are being made to produce and multiply carrier based AM fungal bioinocula. This will bridge the gap of developing a suitable carrier formulation to facilitate the transfer and increase the efficacy of *in vitro* produced AM fungal propagules in the rhizosphere, and encourage the use of carrier based *in vitro* produced AM fungal bio-inocula in agriculture and forestry.

Fungal endophytes have the ability to colonize internal plant tissues of healthy leaves, petioles, stems, twigs, bark, root, fruit, flower, and seeds without causing any apparent harm or pathogenic infection to their host plants. It has been estimated that more than one million different endophytic fungal strains inhabit about 300,000 various plant species. Fungal endophytic metabolites are useful resources for natural products which effectively have a wide range of application in medicine, agriculture, and industry. Besides, they also promote plant growth through the production of ammonia and phytohormones.

Marine fungi too have great potential in biotechnology. The unique marine environment has possibly conferred marine fungi with special physiological adaptations that could be exploited in biotechnology. It is likely that these fungi may be the actual producers of many bioactive compounds. Fungi occurring in decomposing plant organic material or

detritus in the sea have been shown to be the source of several wood-degrading enzymes of importance in paper and pulp industries and bioremediation. One of the major applications of the thraustochytrids occurring in marine detritus and sediments is the production of docosahexaenoic acid (DHA), an omega-3 fatty acid used as nutraceutical. An understanding of the adaptations of extremotolerant fungi in such habitats is likely to provide us with a greater insight into the adaptations of eukaryotes and an avenue from which to discover novel genes.

From historical record, it is evident that people indirectly knew about microorganisms such as molds and yeasts by their activities. Scientific study of these activities through the use of microscope has led to disciplines now called microbiology and biochemistry. For instance, Louis Pasteur termed the living organisms (mostly yeasts) seen under a microscope during sugar fermentations as 'organized ferments' and the changes that occurred in solutions without any detectable microorganisms were called 'unorganized ferments'. Later, when it became apparent that the 'unorganized ferments' (later termed as enzymes) were generally the metabolic products of 'organized ferments'. One of the early and best studied enzymes diastase (amylase) was originally isolated from germinating barley and used for the malting step in the production of beer. Jokichi Takamine (1894) was probably the first to isolate this enzyme from the Japanese koji mold, *Aspergillus oryzae, who* reported its use as substitute for malting enzyme and as a digestive aid for the treatment of dyspepsia. By the year 1983, approximately 30 different classes of enzyme were identified for common commercial use, approximately 50% of which were of fungal origin. The number of commercial enzymes is increasing at a rapid rate.

Fungal enzymes form an important cornerstone of modern biotechnology. For instance, the Laccases can degrade both phenolic and non-phenolic compounds. They also have the ability to detoxify a range of environmental pollutants. Due to their property to detoxify a range of pollutants, they have been used for several purposes in many industries including paper,

pulp, textile and petrochemical industries. Other applications of laccase are included in the food processing industry, and in medical and health care. Further more recently laccase has found application in the design of biosensors and nanotechnology.

The period from 1940 to 1950 is rightly called as the 'Golden Age of Antibiotics'. It may be noted that the discovery of penicillin and its subsequent development into a 'wonder drug' was a turning point in the development of modern industrial microbiology. During the past 50 years, several major advancements in medicine have come from lower organisms such as molds, yeasts, *etc*. Fungi are extremely useful in making high value products like mycoproteins and act as plant growth promoters and disease suppressors. Fungal secondary metabolites are important to our health and nutrition and have tremendous economic impact. Microscopic species belonging to the genera *viz., Aspergillus, Penicillium* and *Saccharomyces*, are best known for their positive impact on humans. They have been harnessed either as producers of degradative enzymes or synthesizers of useful metabolites. This in turn triggered searches for other secondary metabolites with antibacterial activity, as well as stimulated research on fungal physiology, fermentation technology, and industrial strain development. This also resulted in a systematic search for drugs with activities other than anti-infective. Laboratories in Japan were particularly innovative in establishing new screens to look for anti-tumor, anti-hypertensive, immunostimulant, anti-diarrheal, anti-mutagenic and other similar biological activities. In this context the immunosuppressant cyclosporins from *Tolypocladium inflatum* and the anti-hypertensive mevalonins from *Aspergillus terreus* are the most important pharmaceuticals to be discovered from filamentous fungi. Another example of

filamentous fungal product in the development of modern biotechnology is citric acid. First isolated from citrus fruits, it was known by the end of the 19th century that filamentous fungi also produced citric acid and is used in food and beverage industry. It is also used in tablets, cosmetics, detergents, antifoaming, textile treatment, and as a preservative for stored blood. Many aspects of modern fermentation technology were developed with an objective of improving citric acid yields by manipulating culture conditions, by developing submerged processes, and improving product recovery.

In addition to this, fungi are extremely useful in carrying out biotransformation processes. Recombinant DNA technology, which includes yeasts and other fungi as hosts, has markedly increased the market for microbial enzymes. The advent of recombinant DNA technology has in fact revolutionnized biology. Large scale genomics analysis has placed yeasts and filamentous fungi in the forefront of contemporary commercial applications. Most definitions of biotechnology are comprehensive and encompass fermentation processes from wine to penicillin, as well as a broad spectrum of contemporary methodologies that grow out of recombinant DNA technology. Thus, the term 'mycotechnology' is more appropriately used to describe the enormous impact of fungi on biotechnology or more clearly as 'mycology making money'.

Today, fungi are major constituents of the global industry due to its mind blowing potential such as their role in designing of vectors, as food of high protein and low calorific value, as rich source of SCP, sources of secondary metabolites of pharmaceutical importance, as biofertilizes, as bioherbicides, as biodegraders and used in hazardous waste remediation. Besides, entamopathogenic fungi have entemocidal properties.

Fungi, the industrial workhorses of traditional fermentations, are in the forefront of molecular biotechnology. These lower eukaryotes remain important models for basic biology and commercial manufacture. The yeast genome is the platform for discoveries in functional genomics and DNA microhip technology. In the coming years, biotechnologists will continue to rely on yeasts and filamentous fungi to develop new paradigms for research and development**.**

Valedictory Address

Translating Nanotechnology Research to Agriculture Applications

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Nanotechnology is a promising field of interdisciplinary research. It opens up a wide array of opportunities in various fields like electronics, pharmaceuticals, and agriculture. The potential uses and benefits of nanotechnology are enormous. These include management of crops through the formulations of nanomaterials-based formulation to be used as Nano-fertilizer, Nano-pesticides, Nano-biosensors. These technologies are better than traditional methods used in agriculture. For example application of chemical fertilizer/pesticides have adverse effects on animals and human beings apart from the decline in soil fertility. Therefore, nanotechnology would provide green and efficient alternatives for the managing of crops in agriculture without harming the nature. The presentation will cover the translation of nanotechnology research in the agriculture application along with the facility and trends of research at TERI Deakin Nanobiotechnology centre.

Lead Lectures

Diversity of Epigeous Ectomycorrhizal Fungi from Swami Ramanand Teerth Marathwads University Campus, Nanded, Maharashtra

Mulani R.M.

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Swami Ramanand Teerth Marathwada University Campus is located in Nanded district of Maharashtra (19"06' 00.3" N 77"17'15.6'E) occupies approximately 525 acres area and consisting of *Acacias sp*., *Prosopis sp*., *Dichrostachys sp*. and large number of plants cultivated along the road side in the campus. Number of plants species belonging to both monocots and dicots have shown ectomycorrhizal association.
Ectomycorrhizal fungi belong to group of macrofungi having mutually associated with woody, vascular plants. Ectomycorrhizae is the commonest type of mycorrhia comes under the Division Basidiomycetes (mushroom and puff ball groups) and some Ascomycetes (without Gills fungi) which are mutually associated with many monocots and dicots plants that may be either angiosperms or gymnosperms especially it is dominantly noticed with woody vascular temperate forest trees. It plays vital role enhancing plant growth and also useful in increasing soil fertility and soil remediation. It plays crucial role in channelize the macro and micro nutrients from soil to host improving the uptake of water and nutrients from soil to roots of host plants. Enhance the growth and productivity of plants mainly in forest ecosystem. Act as physical barrier and provide protection to host roots from harmful root pathogens. Most of the mushrooms are good source of protein and vitamins. Some epigeous and hypogeous species act as important food source for human being, animals and invertebrates. Few ectomycorrhizal fungal species have medicinal properties.

The present investigation gives in detail about diversity of epigeous ectomycorrhizal fungi from Swami Ramanand Teerth Marathwada University Campus, Nanded. The total eleven ectomycorrhizal fungal species were collected and identified from above mentioned research area which were associated with plants especially *Azadirachta indica*, *Jatropha sp*., *Acacia sp*., *Peltoforum, ferruginium* and some weed such as *Cynodon dactylon*, *Euphorbia sp*. and some unknown species of weeds, etc.

The 11 ectomycorrhizal fungal species such as *Termitomyces heimii, Termitomyces clypeatus, Coprinus plicatilis*, *Galerina sp., Agaricus arvensis*, *Panaeolus sp*., *Macrolepiota procera*, *Volvoriella bombycina*, *Volvoriella bombycina var. flaviceps*, *Agaricus placomyces* (basidiomycetes) and *Cordiceps sp*. (ascomycetes) were collected from the three different sites of the study area such as Botanical Garden, Lake area and Pangari area in which *Termitomyces heimii* and *Termitomyces clypeatus* having the most dominant species as compared to other species.

In my present study three ectomycorrhizal fungal species namely *Termitomyces heimii, Termitomyces clypeatus* and *Volvoriella bombycina var. flaviceps* have been cultured.

Lead Lectures

Impact of New Concepts in *Fusarium* Identification and Systematics

Sanjay K. Singh

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Members of the genus *Fusarium* are common inhabitant of soil. They survive in most soil; arctic, tropical, desert, cultivated and non-cultivated field. Due to their potent pathogenic behaviour they make their worldwide presence and cause a range of diseases leading huge losses. Although, virtually most economically important crops are infected each year, it is the cereals that are mostly damaged due to the infection by Fusaria, concerning yield reduction and contamination of the grain with mycotoxins. The taxonomic assignment of *Fusarium* genus has been based on the analyses of the morphological traits, such as conidial shape, arrangement and mycelium characteristics for many years. However, the difficulty with classical taxonomic markers in *Fusarium* has been well illustrated by researchers from time to time and emphasized to resolve the taxonomic ambiguities. Development of a phylogenetic species concept and availability of better sequencing techniques resulted in the accumulation of sequence information stored in GenBank databases. Though, ITS region has been widely used for species identification in many fungal lineages, and functions as a de facto barcode, it has been found to have limited usefulness in case of *Fusarium* taxonomy and phylogenetic analysis. Low interspecific variability, especially in several groups within the filamentous ascomycetes make other markers, such as elongation factor a more popular choice. Another potential problem of ITS reported is the presence of multiple divergent copies of ITS in some species of *Fusarium*. Besides, TEF-1-alpha gene that has gained wider interest.

Review of literature reveals to more that 300 records of different species and *forma specialis* belonging to more than 40 different species of *Fusarium* reported from various hosts/substrates in India. They are mainly identified and characterized based morphotaxonomic and cultural characteristics only, However, recent changes in new concept/rules governing nomenclature of fungi (Melbourne codes, 2011) have resulted in taxonomic confusion need thorough understanding and awareness at large. About 150 isolates isolated from diverse substrates and geographic locations in India were studied at NFCCI. During identification it has been realized that colony characters and morphological traits present in many isolates did not match exactly with the set of characters available in standard keys to different sections, and species given in manuals which led to great confusion in making a clear judgment of species identification, while others were well identified following conventional approach. In order to resolve this taxonomic issues, combined approach of morpho-taxonomy, multigene sequencing and phylogeny were used. Compliance of one fungus one names helped in retaining generic name '*Fusarium*' that includes virtually all species of importance in plant pathology, mycotoxicology, medicine and basic research. The phylogenetically-guided circumscription of *Fusarium* species helped in delimiting other generic entity including teleomorphs, of species grouped within the same clade. As such a glimpse of the new generic/species concept and highlights of the status of research and development of *Fusarium* in India shall be presented during conference.

Lead Lectures

Status of mycorrhizal fungi in different forest ecosystems in south india and their importance to Humankind

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Scientist – G

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Microbes are ubiquitous in nature. They can live in extreme conditions such as volcanic vents (350◦C) to frigid waters of Antarctic lakes, a top high mountains, polar snow, deserts and forest ecosystems. The microbes are both beneficial and harmful to human mankind. Without microbes, the earth would have been converted into a monstrously large garbage can. Microbes play several key roles in the environment. Microbial inoculants/ Bio-fertilizers are "living" fertilizer composed of microbial inoculants or groups of useful micro organisms or preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms. These are used for application to the seed, soil or composting areas with the objective of increasing their numbers and accelerate certain microbial processes to augment the extent of availability of nutrients in a form which can be easily assimilated by plants. Bio-fertilizers constitute beneficial microorganisms both symbiotic and free living forms in the soil that provide nutrients to the plants in available form through natural processes. Symbiotic organisms like *Rhizobium* contribute a good deal to the amount of nitrogen fixed into the biosphere and produce root nodule which is not merely a store house or protein but has a casual relation with the assimilation of free nitrogen. Some of the free living organisms like Blue Green Algae also fix the atmospheric nitrogen. Besides fixing atmospheric nitrogen, Blue Green Algae synthesizes and excrete several vitamins and growth substances with contribute towards better growth of plants. The other important symbiotic microbes are the "Mycorrhizal Fungi" literally means "Fungus Root", is the association between specialized root-inhabiting fungi and the roots of living plants. In this mutually beneficial association or symbiosis, each partner or symbiont receives essential nutrients and other benefits and also contributing to the other partner's survival. The mycorrhizal roots lack root hairs. The fungal sheath (mantle) together with the hyphae extending to the soil, absorb nutrients and other essential elements and supply to the host plants. They afford better intake of nutrients such as nitrogen, phosphorous and potassium from the surrounding soil. Metabolites produced by the mycorrhizal fungi influence the structure and morphology of root system. This symbiotic association is especially critical to forest trees and in disturbed areas or areas that have been progressively degraded over time since the rhizosphere organisms can be affected by shifts in land management practices. Much of this biological diversity is hidden from view beneath the soil surface. The biological soil resource is one of the most important factors governing soil fertility. Through close mutual interaction between the trees and soil organisms, conditions are created that govern the forest ecosystems and productivity. Maintaining mycorrhizal diversity helps to minimize site degradation by assuring plant adaptability to unpredictable or varying environments. This has

special significance in forest ecosystems that now face unprecedented changes due to human activity. Even though a reasonable amount of research has been done on various aspects of mycorrhizal fungi, the diversity of mycorrhizal fungi and their interaction and benefits derived in the field of forestry have not been fully studied. This paper highlights the diversity status of different mycorrhizal fungi in varied forest ecosystems in South India. The ectomycorrhizal (ECM) fungi *Pisolithus albus* and *Thelephora ramarioides* were reported in association with different species of Acacias, Casuarinas and Eucalypts. Some of the ECM fungi *viz., Alnicola* sp., *Amanita* sp., *A. muscaria*, *Cortinarius* sp., *Geastrum* sp., *Hebeloma* sp., *Inocybe* sp., *Laccaria fraterna, L. laccata*, *Leucophleps* sp., *Lycoperdon perlatum, Rhizopogon luteolus, Russula parazurea, Russula delica., Scleroderma citrinum, Scleroderma bovista*., *Suillus brevipes, S. subluteus* and *Thelephora terrestris* were recorded in association with *Acacia mearnsii, A. melanoxylon, Cupressus macrocarpum, Eucalyptus globulus, E. grandis, Hopea parviflora* and *Pinus patula*. Another well known mycorrhizal fungi on tree species is Arbuscular Mycorrhizal (AM) fungi, which are widespread and about 80-90% of the plant species can form AM fungal association in their roots. It was recorded that 32 different AM fungi belonging to four genera such as *Acaulospora, Gigaspora, Glomus* and *Scutellospora* were recorded in different forest ecosystems. Among them, the genus *Glomus* was found to be the dominant with 22 species. It was observed that the climatic and edaphic factors have profound influence on the distribution of these fungi in different ecosystems. Experiments have shown that the mycorrhizal colonization and growth parameters have positive correlation in mycorrhzial inoculated plants in the nursery. Significance of these findings with reference to the ecosystem functioning, exploitation and conservation of the valuable natural resources of mycorrhizal fungi is discussed.

Key words: Arbuscular Mycorrhizal fungi, Ectomycorrhizal fungi, *Amanita muscaria, Laccaria laccata, Laccaria fraterna, Acaulospora, Glomus, Gigaspora, Scutellospora*

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Lead Lectures

"The old order changeth yielding place to the new": the changing face of mycology in the new millennium

Srinivasan MC

Former Head, Biochemical Sciences Division, National Chemical Laboratory, Pune

Developmental morphology, taxonomy and classification of fungi have been placed on a strong foundation during the nineteenth and twentieth centuries by contributions from botanists although fungi are not phylogenetically related to plants. Significant contributions to basic biology from fungi include the origin of Biochemical Genetics and the discovery of truly anaerobic rumen chytrids. Recent developments in Mycology have led to recognition of "Mycota" as a distinct class, polyphyletic origin of fungi and conceptual changes in the taxonomy and classification of fungi.Molecular taxonomy based on DNA Bar Coding and Amplified Fragment Length Polymorphism (AFLP) has added a new dimension to the classical phenotype-based taxonomy.

Mycotechnology or the application of fungal systems for the manufacture of bioactive metabolites and industrial enzymes is a rapidly advancing frontier of biology. This involves greater focus on isolation and identification of naturally occurring fungal biodiversity. high productivity mutants and recombinant strains to produce even mammalian proteins like Insulin and Chymosin by fermentation. Increased application of molecular and other newer techniques to mycology and mycotechnology necessitates mycologists to closely align themselves in team work along with chemists and process technologists and contribute to significant progress in Mycotechnology

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Lead Lectures

Translating Research on Endophytic fungi towards Pharma and Food Application

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Endophytic fungi are defined as fungi that live asymptomatically within the tissue of higher plants. These fungi came in limelight after the discovery of taxol and taxane from an endophytic fungi *Taxomycesandreanae*, of Pacific yew. Later on a large number of bioactive metabolites from endophytic fungi have been isolated from endophytic fungi from tropical countries. Still there is a great opportunity to discover unexplored fungi with industrial potentials. There is a need to discover these fungi from less explored ecosystems viz. cold desert, hot desert, Antartica, mangroves along with other sources like lichens, Bryophytes, orchids. Some of the strategies of cultivation of these fungi to stimulate the production of secondary metabolites under laboratory conditions are needed to explore the diversity of bioactive compounds. These strategies include variations in media composition, pH, temperature, aeration, or shape of culturing flask; biotic elicitation by co-culture of different strains; abiotic elicitation by physical or chemical stresses; and epigenetic modulation by chemical epigenetic modifiers. These fungi are also known to produce antioxidants, food colors and enzymes. Some of the work done in Pharmaceutical and food Industries and its aspect of translation of endophytic fungi research into industrial applications will be discussed.

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Lead Lectures

Need of Mycotaxomist Conservation in India

Dr. Kiran Randive

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Now a day we come across many research institutions working in excellent manner on molecular aspects but at the same time very less records resending the exact number of species reported from India till date in every group.

Some where our students are also thinking that the applied sciences are the only recent and useful sciences as compared to the taxonomic study. One can see the decreasing amount of taxomists in India also because of the same. If this will continue for few more years than it is quite possible that we many not yet any single authentic taxonomist who could tell us the exact identification of the fungus or lichen.

As we all know that forest degradation is taking place and because of the same very few species are there in actual condition, as the awareness is lacking. If we have to solve this problem in near future then we have to plan it well and do the segregation of students as per their interest in to many facets of myco-study like basic taxonomy, molecular taxonomy, biochemical study, etc.

In India we get least collaboration in the researches of various disciplines & because of the same also our work is not getting multidimensional. If we start some of the programs under the Biodiversity Departments of the states then I think that we can bring once again all those branches of Mycology at a very nice level where our students will get more benefit & the future taxomists will be conserved in a proper way.

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ISSN: 2322-0015

INTERNATIONAL RESEARCH JOURNAL OF
SCIENCE & ENGINEERING
An International Peer Reviewed Open Access Bi-Monthly Online Journal
http://www.irjse.in

UGC Approved Journal No. 63628)
SJIF Impact Factor-4.115
Cosmos Impact Factor-3.662

Published in: February, April, June, August, October & December

EDITOR-IN-CHIEF:
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Cell : (+91) 9970559438 | 9420775527

International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Review Article Open Access

Recent Advances in Myco-remediation of Xenobiotics : Review

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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:
Desai Sanjay M (2018) VAM association in some Ferns of Bhiwandi Maharashtra, *Int. J. of. Life Sciences*, Special Issue, A9: 1-5

ABSTRACT

A xenobiotic is a class of chemical substances which usually are not produced within an organism naturally or expected to be present within. It can also cover substances that are present in much higher concentrations than are usual.

The term xenobiotics, however, is very often used as synonym of pollutants such as dioxins and polychlorinated biphenyls, because xenobiotics are considered as substances which are not found in entire biological system, i.e. synthetic substances, which did not exist in nature or purely manmade. Degradation of such compounds by physical and/or chemical processes is costly and often produces undesirable products which are toxic. Biological methods, being eco-friendly and cost cheap techniques, were proposed for xenobiotic degradation purposes in order to overcome these problems. Compared to bacteria, most of the fungi are robust organisms and generally more tolerant to high concentrations of pollutants. It explains why they have been extensively investigated since the mid-1980s for their bioremediation capacities.

A wide number of fungal species have shown incredible abilities to degrade a growing list of persistent and toxic industrial waste products and chemical contaminants to less toxic form or non-toxic form. Fungi posses' very peculiar mode of metabolizing their substrate by using array of enzyme systems. Extracellular nature of these enzymes proven superiority over any other enzyme system in nature.

The potential of fungal enzymes can be harnessed for remediation of the environment. In depth knowledge and understanding about these enzymes will surely revolutionize the waste treatment in near future.

Key words: Xenobiotics, Myco-remediation, Fungi, Biodegradation

INTRODUCTION

A xenobiotic is a class of chemical substances which usually are not produced within an organism naturally or expected to be present within. It can also cover substances that are present in much higher concentrations than are usual.

The term xenobiotics, however, is very often used as synonym of pollutants such as dioxins and polychlorinated biphenyls, because xenobiotics are considered as substances which are not found in entire biological system, i.e. synthetic substances, which did not exist in nature or purely man made.

Degradation of such compounds by physical and/or chemical processes is costly and often produces undesirable products and intermediates which are toxic. Biological methods, being eco-friendly and cost cheap techniques, were proposed for xenobiotic degradation purposes in order to overcome these problems. Compared to bacteria, most of the fungi are robust organisms and generally more tolerant to high concentrations of pollutants. It explains why they have been extensively investigated since the mid-1980s for their bioremediation capacities.

A wide number of fungal species have shown incredible abilities to degrade a growing list of persistent and toxic industrial waste products and chemical contaminants to less toxic form or non-toxic form.

Fungi possess very special mode of metabolizing their substrate by using array of enzyme systems. Extracellular nature of these enzymes has proven superiority over any other enzyme system in nature. The potential of fungal enzymes can be harnessed for remediation of the environment. In depth knowledge and understanding about these enzymes will surely revolutionize the waste treatment in near future.

MATERIALS AND METHODS

White Rot Fungi in mycoremediation

A wide number of fungal species have shown incredible abilities to degrade a toxic industrial waste products and chemical contaminants to less toxic form or non-toxic form. Mycelium reduces toxins by different enzymatic actions to restore the natural pollutant free environment. White rot fungi have successfully been utilized in degradation of environmental pollutant like polyaromatic compounds, pesticides etc. Prakash (2017). Use of White rot fungi for degradation was reported as early as in1991 Higson FK (1991), White rot fungi were among the organisms tried for degrading xenobiotics. This group of fungi includes many saprophytic and few parasitic fungi like honey fungi.

White rot fungi such as *P. chrysosporium* was tried for degrading the non-repeating, non-stereo selective, insoluble polymer lignin under conditions of nutrient limitation Higson FK (1991). The attack on lignin principally involved extracellular enzymes like peroxidases (ligninases) and hydrogen peroxide. The *P. chrysosporium* system reported to be active against diverse substrates as DDT, lindane, PCBs, TNT and crystal violet. Some like biphenyl and triphenylmethane dyes are structurally related to lignin substructures. Normally less toxic intermediates were generated. Methods of optimizing ligninase activity in fungal reactors have been described, such as the addition of surfactants and veratryl alcohol to the medium. This work initiated the search for bio remediating activity of white rot fungi Higson FK (1991).

Among biological processes for degradation of xenobiotics, fungal degradation, being eco-friendly and cost effective, have been investigated extensively because most of basidiomycetes are more tolerant to high concentrations of pollutants Ellouze and Sami (2016). Fungal bioremediation is a promising technology using their metabolic potential to remove or reduce xenobiotics. Basidiomycetes are the unique microorganisms that show high capacities of degrading a wide range of toxic xenobiotics. They act via the extracellular ligninolytic enzymes, including laccase, manganese peroxidase, and lignin peroxidase. Their capacities to remove xenobiotic substances and produce polymeric products make them a useful tool for bioremediation purposes. During fungal remediation, they utilize hazardous compounds, even the insoluble ones, as the nutrient source and convert them to simple fragmented forms.

Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to the less hazardous or non-hazardous forms with less input of chemicals, energy and time. White rot fungi are unique organisms that show the capacities of degrading and mineralizing lignin as well as organic, highly toxic and recalcitrant compounds. The key enzymes of their metabolism are extracellular lignolytic enzymes that enable fungi to tolerate a relatively high concentration of toxic substrates. Tišma *et al*. (2010) gave a brief review of many aspects concerning the application of white-rot fungi with the purpose of the industrial contaminants removal.

A wide number of fungal species have shown incredible abilities to degrade a growing list of persistent and toxic industrial waste products and chemical contaminants to less toxic form or non-toxic form Prakash (2017). Mycelium reduces toxins by different enzymatic mechanism to restore the natural flora and fauna. White rot fungi have successfully been utilized in degradation of environmental pollutant like polyaromatic compounds, pesticides etc. The present review gives a insights on degradation aspects of heavy metals, PAH especially using different fungal species. White rot fungi has potential to degrade contaminants using wide range of enzymes. Mycoremediation is promising alternative to replace or supplement present treatment processes.

Bhattacharya *et al*. (2012) reported mycoremediation of Benzol [a] pyrene by *Pleurotus ostreatus*. Benzo[a]pyrene (BaP) is a ubiquitous environmentally significant compound which is considered as persistent bioaccumulative toxin. The present study was carried on to determine the degradation potentials of three natural isolates of the white rot fungi *Pleurotus ostreatus* towards BaP.

Use of Mushrooms in Degrading Xenobiotics

Mushrooms posses mycoremediation potential Kulshreshtha *et al*. (2014). Mushroom uses different methods to decontaminate polluted spots and stimulate the environment. These methods include - Biodegradation, Biosorption and Bioconversion. Mushrooms are known to produce extracellular enzymes such as peroxidases, ligninase (lignin peroxidase, manganese peroxidase and laccase), cellulases, pectinases, xylanases and oxidases. These enzymes are able to oxidize many of the pollutants. Activities of these enzymes are typically induced by their substrates.

In recent times as there is an advancement in the knowledge about fungal interactions with pollutants. Khan *et al*. (2017) shown that some of the white rot fungi like *T. versicolor* and *P. ostreatus* have been recognized to be the major decomposers of biopolymers via laccase-mediated transformation. Moreover, the ligninolytic fungal strains carrying enzyme Mn-peroxidase activity demonstrated the maximum degradation of naphthalene (69 %). They have shown that biotransformation of the hazardous pollutants to less toxic substances or their complete mineralization represents an economical substitute to clean up soil and water. Fungi possess an array of extracellular enzymes which is capable of biodegrading any naturally existing biopolymers and some of the synthetic polymers as well. Degradation of polymers is largely dependent on the fungal extracellular enzymes, namely, oxidoreductases and hydrolases. Many non-ligninolytic species degrade polycyclic aromatic hydrocarbons (PAHs). Remediation of nitro-aromatics has been described by utilizing fungal species such as *Phanerochaete chrysosporium* or *Pseudomonas* sp. The need for discovering the new beneficial fungal strains and isolation, engineering, and sequencing of new useful enzymes is highlighted. This may speed up the remediation of contaminated soil.

Tiberius and Cătălin (2013) reported that ligninolytic fungi are seen with the ability to degrade the synthetic dyes, a class of xenobiotics, resistant to biological degradation. The degradation strategies can be planned according to the category and nutrients present. *Bjerkandera adusta* and *Trametes hirsuta*, were tested on different structural classes of dyes: azo, thiazine and arylmethane.

The use of white-rot fungi was also reported way back in 1995 by Paszczynski and Crawford (1995). Since this group produces an unusual enzyme system, characterized by a specialized group of peroxidases, that catalyzes the degradation of the complex plant

polymer lignin. This ligninolytic system shows a high degree of non-specificity and oxidizes a very large variety of compounds in addition to lignin. Among these compounds are numerous environmental pollutants. The white-rot fungi show considerable activity as bioremediation agents for use in the remediation of xenobiotic molecules. One such white-rot fungus, *Phanerochaete chrysosporium*, has been studied and reported for their ligninolytic enzymes system and the degradation of xenobiotics. It has been widely promoted as a bioremediation agent. This article examines literature concerning the degradation of xenobiotic compounds by *Phanerochaete chrysosporium* and attempts to critically assess this organism's real potential as a bioremediation tool.

Rabinovich *et al* (2004) had reported in their detailed review about transformation of natural and synthetic aromatic compounds by fungi (causative agents of white rot, brown rot, and soft rot, as well as soil filamentous fungi). Major enzyme types, their role in the transformation of lignin and aromatic xenobiotics. The article refers aspects like activity regulation under the conditions of secondary metabolism and oxidative stress. Coupling of systems degrading polysaccharides and lignin and non-phenolic lignin structures is analyzed, together with nonenzymatic mechanisms. Metabolic pathways resulting in the formation of aromatic and haloaromatic compounds in fungi are described. Consideration is given to the mechanisms of fungal adaptation to aromatic xenobiotics.

CONCLUSION

The ability of fungi to use various substrates as carbon and energy sources can be exploited for elaboration of cost effective strategies for the mycoremediation of xenobiotics using cheap materials such as agricultural residues. Mycoremediation is promising alternative to replace or supplement present treatment processes.

White rot fungi have potential to degrade contaminants using wide range of enzymes. These enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, and cosmetic industrial sectors. Their capacities to remove xenobiotic substances and to produce others, which are less or non-toxic, make them a useful tool for bioremediation purposes. The white-rot fungi show considerable promise as bioremediation agents for use in the restoration of environments contaminated by xenobiotic molecules.

Mushroom cultivation used in mycoremediation may help in subsiding the the world's major problem i.e. waste accumulation. There is a need for further research towards exploring potential of mushroom as bioremediation tool. The safety issues involved in consuming mushrooms as product need to be addressed.

The potential of white-rot fungi can be harnessed thanks to emerging knowledge of the physiology and morphology of these microorganisms. This knowledge could be transformed into reliable and robust waste treatment processes. The importance of high extracellular levels of these enzymes to enable the efficient degradation of xenobiotic compounds.

Importance of white-rot ligninolytic fungal strains such as *T. versicolor* and *P. ostreatus* have been recognized to be the major decomposers of biopolymers via laccase-mediated transformation. Utilization of fungal species such as *Phanerochaete chrysosporium* may be of great use. This underlines importance of white rot fungi in developing new techniques in future.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: 2018.
UGC Approved Journal No 48951

Review Article Open Access

Preservatives used for control of fungal spoilage of bread

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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:
Thakre Jayashree, Nirmalkar Vaishali and Kolet Moses (2018) Preservatives used for control of fungal spoilage of brea, *Int. J. of. Life Sciences*, Special Issue, A9: 6-10.

ABSTRACT

Bread has been a staple human diet since historical times. Generally, loaves of bread are baked fresh daily in local bakeries, but commercial brands of processed and packaged sliced bread, which comprise a sizable chunk of the urban sales of bread, do come with shelf life of few days. Bread, having a short shelf life like all bakery products, loses its desired texture and taste characteristics upon storage and is also subject to spoilage by fungi. To avoid this spoilage and increase shelf life of the product, natural and chemical preservatives are added. This communication deals with natural and chemical preservatives added to dough to delay spoilage of bread by fungi.

Keywords: Antifungal activity, Bread, Preservatives, Calcium propionate, Butylated Hydroxytoluene (BHT), ascorbic acid.

INTRODUCTION

Bread has been a staple diet of man since historic times a fact that is well documented in the history of mankind and is believed to have originated even before the advent of agriculture (Kim, 2013). Traditionally, loaves of bread were prepared using flour, sugar, yeast; and baked daily in baking pans. Quality of bread depends on several factors encompassing method of preparation of dough, baking conditions, packaging and sanitary conditions during the processes. Freshness also depends on various features comprising flavour, appearance, crispness of bread-crust, volume of bread and hardness of crumb, of which taste is of paramount importance as criterion of acceptability to consumers (Plessas *et al*., 2011). Breads, as all bakery products have a short shelf life (Arendt *et al*., 2007) and lose freshness during storage, indicated by loss of desirable characteristic flavour, texture, taste and acceptable appearance to consumers, accompanied by increase in hardness of crumb; all commonly referred to as staling of the bread (Heenan *et al*., 2008) and are also subject to spoilage by fungi

namely molds, yeasts as well as bacteria (Mentes *et al*., 2007). Modern manufacturing technology has freed us of the task of laborious baking of our bread as was done in earlier times and has yielded a processed pre-packaged product with a prolonged shelf life. This extended shelf life is mainly due to the use of chemicals that inhibit microbial growth. Both natural and artificial preservatives used in bread to prevent early spoilage by molds. Preservatives and packaging accompanied by storage of the product in ideal recommended conditions make loaves last for three to four days. Various preservatives are used to inhibit growth of molds and keep off spoilage in almost all processed food products and bread as a pre-packaged processed food commodity is no exception.

Fungal spoilage of bread:

A large share of spoilage of bread is generally attributed to activity of molds, followed by yeasts. The culprit organisms actually responsible for spoilageof bread are limited to a small group comprising species of *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, and in some cases, *Fusarium, Chrysonilia, Hyphopichia, Saccharomyces* and other yeasts which are all more or less common in display of such spoilage activity all over the world (Unachukwu and Nwakanma, 2015; Ravimannan *et al*., 2016). These fungi prefer starchy substrates, and bread along with other bakery products are ideal for their amylolytic properties. Banwart (2004), describing the sequence of arrival of the fungi responsible for the spoilage reported *Mucor* and *Rhizopus* as initial colonizers on loaves, followed by *Aspergilli* and *Penicillium*. In addition to their visible growth indicating spoilage, fungi are responsible for the development of characteristic flavour and producing carcinogenic mycotoxins and allergenic compounds in bread, which were traced back to exist from the cereal grain stage (Versilovskis and Bartkevics, 2012; Gimenez *et al*., 2014), however, in spite of contamination, bakery products were reported to cause food poisoning in relatively rare cases (Cook and Johnson, 2010).

Preservatives used for inhibition of fungal spoilage:

To avoid spoilage and ensure an extended shelf life, preservatives are incorporated during processing of bread to inhibit the growth of molds. Application of furnace heat during baking of the bread kills and inactivates the molds that may be present in dough and other ingredients (Ponte and Tsen, 1978). Secondary contamination during cooling, slicing, packaging and storage till consumption exposes the bread to airborne molds which contaminate the bread and this is where the role of preservatives begins. Chemical Preservatives have no nutritional value and can actually be harmful to health. Chemical preservatives currently used in packaged sliced bread available in Indian market are listed along with their reported side effects in Table 1. Calcium propionate is the most common class II preservative used in bread (Vazhacharikal *et al*., 2015). It has been known to cause side effects such as stomach ulcers, behavioral irritability in children and headaches. Butylated Hydroxytoluene (BHT) is a synthetic chemical that is added to bread as a preservative. It slows down the autoxidation rate of ingredients that causes deterioration in the taste or color. It is known to produce problems upon ingestion such as abdominal pain, confusion, dizziness, nausea and vomiting. It has long term toxic effects on lungs, liver and kidneys. Sulfur Dioxide is regularly used in bread for its properties as a preservative and antioxidant. It is mixed with the flour and serves as its bleaching agent. Though it increases the shelf life, it is harmful to individuals suffering from asthma and sulfite sensitivity (food for life, 2017).

Dalmasso (1985) partly connected longer shelf life of bread to chemicals of the likes of potassium sorbate, sodium sorbate and sodium benzoate that inhibit mold growth. Wang *et al.,* (2004) studied the effects of commercial fungicides on wheat, analysing various quality parameters and flour processing properties, inclusive of baking quality. Shahnawaz *et al*., (2012) carried out a comparative study of calcium propionate and calcium lactate as preservatives for bread. Inhibition of yeasts by some preservatives also prevents bread dough from rising properly thereby affecting quality of the product. The list of permitted chemical preservatives for bread apparently differs in different parts of the world (Govt. of Canada, 2017) with calcium or sodium propionate, sorbic acid or its sodium, potassium or calcium salts, acid calcium phosphate, sodium diacetate, ammonium bicarbonate and acid sodium pyrophosphate permitted singly or in combination in India (fssai, 2011)

Alternatives to chemical preservatives:

Nielsen and Rios (2000) investigated the effect of various essential oils of plant origin on the fungi that commonly spoil bread; reporting mustard, cinnamon, clove and garlic oils to be most potent in inhibiting the growth of spoilage microorganisms. Lotfinia *et al.* (2013) used starch foam containing vegetable oil to prevent mold growth and improve shelf life of packaged bread where cinnamon oil, absorbed to foam starch micro particles, acts as antimicrobial agent. The study showed fungal growth on bread to reduce with increasing concentration of cinnamon oil. Starch foam powders containing1000 and 1500 ppm of cinnamon essential oil in bulky bread packages inhibited the growth of microorganisms for six days.

Habeebulla (2013) compared ginger and honey as natural preservatives in bread and reported honey to best suit the purpose and suppress molds. Among natural compounds, there are essential oils and herbal extracts that are gaining interest as preservatives in recent years.

Incorporation of a small percentage of milk solids increases moisture retention after baking, thus retarding formation of moisture film between the bread crust and the wrapper. Milk solids-free bread releases its moisture more rapidly from crust to the air space within the wrapper. Humidity was shown to be an important factor in the rate of growth of molds (Fustier *et al*., 1998). Bakery sanitation was found to be more important in reducing mold related troubles than the process of baking and cooling. Packaging system can be considered as an operative part in food production lines, because of its ability of improving food safety and prolonging food's shelf life. Even addition of Biofilms in the packaging bags adds natural preservatives to the packing material instead of chemicals to the fresh bread. The compounds used in active packaging come from plants such as clove, which have natural antimicrobial properties (Caio *et al*., 2014). The edible films are placed inside the plastic bags used to store bread, and the researchers have found that bread remains mould-free for 15 days at room temperature. Such techniques could be used on a larger scale where natural preservatives can be used in either the bread loaf itself or inside the packaging bags.

Gamma radiation of flour was reported to reduce the microbial spore load without affecting baking quality (Agundez-Arvizu *et al*., 2006) while microwave treatment was reported to significantly increase the shelf life of bread for long periods of storage (Lakins et al., 2008). Berni and Scaramuzza (2013) recommended ethanol to avoid or slow down fungal spoilage of bakery products, while Lafarga *et al*. (2013) successfully experimented with chitosan as a bioactive preservative. Safe bio-preservatives were tested to partly replace and reduce the amount of chemical preservatives in bread (Ryan *et al*., 2008). Application of such bio-preservatives, eliminating side effects of chemical preservatives, is an upcoming field of research aimed at preserving freshness and extending shelf life of packaged sliced bread to few weeks (Barman *et al*., 2017). Denkova *et al*. (2014) reported success in production of bread with longer shelf life, without using preservatives, by slightly increasing the proportion of sourdough starter. Giannone *et al.* (2016) reported an innovative sanitizing treatment comprising hydrogen peroxide and silver solution to reduce spoilage of bread by yeasts. Axel *et al.* (2017) reviewed and suggested consumer friendly and ecologically sustainable preservation techniques as alternatives to chemical preservatives in bread.

CONCLUSION

Having come a full circle, today, consumers are again demanding preservatives derived from natural sources due to their ability to deliver the required shelf life and allay harmful effects of chemical and synthetic preservatives. Since1980's, bread industry all over the world has put in great efforts to reduce the number of additives and synthetic preservatives and produce natural fresh bread, however class II preservatives such as calcium propionate continue to be used in Indian bread. Breads containing chemical preservatives are a matter of concern and should be controlled to achieve healthy life style. Active principles from the natural sources giving comparable preservative effect need to be explored and incorporated in baking technology for avoiding harmful side effects of chemical preservatives and achieving long term health benefits. These natural preservatives may even be studied for their

enhancement of freshness characteristics of the bread and prove to be the better choice in the market.The findings of this investigation indicated that novel natural preservatives and bioactive preservatives hold the future in replacement of chemical preservatives in the bread and bakery industry.

Acknowledgment

The authors are grateful to the Management and Principal of G. M. Momin Women's college for their support and providing essential facilities required.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Review Article Open Access

Indoor Air Pollution – A Cause of Concern

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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:
Rambal Kavita (2018) Indoor Air Pollution – A Cause of Concern, *Int. J. of. Life Sciences*, Special Issue, A9: 11-14.

ABSTRACT

Indoor air pollution which is of chemical and biological origin is a cause for worry. Various studies related to indoor air pollution have revealed that it can cause many complications like irritation in the eyes and throat, dizziness, fatigue, allergy, rhinitis, and other respiratory problems besides sick building syndrome and poor visibility. Indoor air pollution affects all especially children and immunocompromised people or people suffering from other ailments. Studies have revealed that the outdoor environment also affects it. Especially fungal spores or mould spores which are normally present in the outdoor air intrude into the indoor environment. In the present paper a review of literature related to indoor air fungi as pollutant has been undertaken and to find ways to improve the indoor air quality.

Keywords: Pollution, Indoor, Outdoor, Particulate matter, Agricultural wastes, Indoor plants.

INTRODUCTION

Outdoor air pollution has been a cause of worry from a lot of time and lot of studies pertaining to it have been carried out. It being one of the reasons for indoor air pollution has become a cause of concern, as people spend more time indoors as compared to outdoors. It is a burning problem, for instance Delhi the air pollution level has crossed the permissible levels and is causing havoc. It has become a health hazard. One can imagine what must be the levels indoors. Pollution be it outdoors or indoors has chemical and biological components. Increasingly attention is being paid to microbial components present in the indoor air, as exposure to microbial components especially fungi. Fungi are ubiquitous and can grow on almost all natural and synthetic materials and can cause a spectrum of diseases. In the present study an attempt has been made to find possible ways to overcome this problem.

Indoor Air Pollution

Indoor air pollution like outdoor pollution is of chemical and biological origin. The main source of indoor air pollution is outdoor pollution and it becomes more harmful as it gets concentrated indoors. Since people spend more time indoors, it directly affects the health and wellbeing of individuals. Indoor air pollution refers to physical, chemical, and biological characteristics of air in the indoor environment or in other words it refers to chemical, biological, and physical contamination of indoor air. Both outdoor and indoor sources determine the constituents of indoor air. Since people spend 80 – 95% of their time indoors the indoor air quality directly affects their health and wellbeing (Dacarro, et al., 2003).

Biological contaminants or pollutants which affect indoor air qualityinclude allergens such as pollen from plants, hair from pets, fungi, and some bacteria. Fungi infested pollution of indoor environments is considered and rather is a serious threat to public health. (Samet and Spengler, 2003),(Khan, et al., 2009). Lot of work related to isolation of fungi from the indoor environments has been carried out world over. (Agarwal, et al., 1969, Agarwal and Shivpuri, 1974), (Burge, 1990,2001), Davies, 1969, Dubey, et al., 2011, (Gravesen, 1972, 1978), Jain, 1994, Lumpkin's, et al., 1973, Santra and Surnimal, 1989, Singh, 2005, Portnoy, 2003, Usha, et al., 1989, Verma, 1987, Rambal,2012 (Bhuvaneshwari and Vittal, 2005), (Tilak, 1989, 2009), (WHO, 1990, 2009), (Gravesen, et al., 1999). Indoor air fungi are recognised as second only to cause respiratory allergy and other related diseases in humans (Agarwal, et al., 1969,74, Agarwal and Shivpuri, 1974).

Indoor air mould fungi and their metabolites are gaining importance as they contribute to a spectrum of clinical diseases and sick building syndrome, (Bhuvaneshwari, 2005), WHO, 1990.These also release chemicals which include allergens, glucans, Mycotoxins, Trichothecenes and microbial volatile compounds (MVOC'S) which can cause many diseases like toxin induced inflammation, allergy, or infection. These fungi are also responsible for musty odour. (Curtis, et al., 2004, Gordon, et al., 1993, Yoshida et al., 1989.

Allergenic nature of hyphal fragments and spores of Rhizopus, Alternaria, Aspergillus and Curvularia etc have been proved by clinical investigations, WHO 1990, 2009, Yoshida and Araki, 1989. Studies carried out in India, indicate the following allergenic spore types and hyphal fragments of following fungi Rhizopus, Chaetomium, Basidiospores, Alternaria, Aspergillus, Penicillium, Cladosporium, Curvularia. (Singh 2005, Tobin, et al., 1987).

Presence of fungi is associated with presence of moisture and humidity. Paper and glue used in indoor surfaces have been reported as good growth substrates for most of the fungi besides, fiber glass insulation, ceiling &tiles etc. The fungi frequently isolated include Aspergillus, Cladosporium and Penicillium species (Yazicioglu, et al., 2004). Fungi even colonize inorganic materials as these absorb dust and moisture serve as good substrates. Painted surfaces and acrylic painted surfaces have been reported to be colonized by fungi like Alternaria, Cladosporium and Aspergillus (Shirakawa, et al., 2011)., even air filters and ventilation ducts have also been reported to be colonised by fungi (Noris, et al., 2011).

Fungi indoors have been associated with allergy, Neuro psychiatric problems and immune diseases. Volatile fungal metabolites released by fungi have been reported to cause respiratory irritation and allergy. Volatile organic compounds, released have been associated with headache, nasal irritation, dizziness, fatigue, and nausea. (Weinhold, 2007, Burton, et al., 2008).

There are many factors responsible for the growth and colonization of fungi indoors which include moisture, humidity, organic matter (dust and dirt), etc. poor ventilation and leaking air conditioners and coolers are the cause for their colonisation.

Ways to improve the indoor air quality

From the literature available and studies so far carried out the measures include.

Primarily, the indoor Environment should completely be moisture free and humidity needs to be controlled by using dehumidifiers. Indoor area be it building, rooms, hall etc should be cleaned daily and crawl

spaces should be cleaned regularly. No dust dirt should be allowed to settle on the shelves and other spaces. Leaking pipes and coolers should be fixed. Proper ventilation needs to be carried out. Ventilation with proper management of humidity and temperature needs to be taken care of. It should be distributed effectively throughout spaces and stagnant air zones need to be avoided (WHO 1990). Besides all these control measures indoor plants need to be planted. NASA has even suggested some indoor plants like Dracaena, Spathiphyllum, Chrysanthemum, Anthurium, Pothos etc. These plants have been found to purify the air and keep the indoors safe in a natural way. Volatile phytochemicals released by leaves of these plants have been reported to play an important role in controlling airborne microbes and mould spores. (Kobayashi, et al., 2007). Installation of artificial air filters should be avoided under all circumstances, as air outside cannot be filtered. Burning and non-administered decomposition of agricultural wastes in the open should be avoided as it indirectly adds to the indoor air pollution.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

IAD: The first online initiative to the documentation of Aphyllophorales Fungi from India

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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:
Ranadive Kiran R, Jagtap Neeta, Khare Harshavardhan, Ranade Vijay, Jite PK and Vaidya JG (2018) IAD: The first online initiative to the documentation of Aphyllophorales Fungi from India, *Int. J. of Life Sciences*, Special Issue, A9: 15-24.

ABSTRACT

This is the first on-line database i.e. IAD- Indian Aphyllo-Fungal Database has been launched on the website www.fungifromindia.com. In all there are 1646 records of Indian Aphyllophorales has been put from 52 families, 190 genera and 1217 species of a much neglected group of Basidiomycetes fungi. Every species has been given a unique identity number that can be cited in the publication where the nomenclatural novelty is introduced. Every record of this database has been linked with the world reputed myco-database (www.mycobank.org). This Indian Aphyllofungal database contributes to the first initiative for the international biodiversity documentation from India, where they will further be linked to other databases from world.

Key words: Aphyllophorales, IAD, Myco-Bank, mycology, online database.

INTRODUCTION

Fungi are among the most diverse and important organisms. If the estimate is correct, then sites share enough species to make broad-scale inventory work possible yet harbors sufficient number of unique species to make valuable contributions to our understanding of fungi biodiversity and the ecological, evolutionary and genetic processes of these fungi and their associated organisms (Mueller Bills and Foster, 2004).

Aphyllophorales:
Aphyllophorales order was proposed by Rea, after Patouillard, for Basidiomycetes having macroscopic basidiocarps in which the hymenophore is flattened (Thelephoraceae), club-like (Clavariaceae), tooth-like (Hydnaceae) or has the hymenium lining tubes (Polyporaceae) or sometimes on lamellae, the poroid or lamellate hymenophores being tough and not fleshy as in the Agaricales. Traditionally the order has had a core of four families based on hymenophore shape, as described above, but recent detailed microscopic studies of basidiocarp structure has shown these groupings to be unnatural and the taxonomy of the order is at present in a state of flux. Donk (1964), who recognized 22 families are now followed, (Hawksworth *et al.*1991). Keys to 550 spp. in culture are recognized by Stalper (1978).

The ultimate aim of the present study was to compare occurrence and distribution of wood rotting Aphyllophorales from the Pune district and to give easy access to researchers and the students for the Indian Aphyllophorales Fungal information. It has tremendous mycological significance and we are sure that it will update the knowledge of wood rotting Aphyllophorales of Pune and India. This is the first Indian database on a much neglected group of Basidiomycetes. The database will inspire the students as well as researchers to study the Aphyllophorales from India. Such type of database gives the information in very short period which will help researchers to save their energy and time for further research.

MATERIALS AND METHODS

Digitization work- Database preparation (Indian Aphyllo-fungal Database i.e. IAD)

Card Preparation:
More than 1700 reference cards of size 17.5 x 12.5 cms were prepared from extensively surveyed Indian literature including reference books, research papers, explainatory notes etc. The latest nomenclatural change has been added on the card by different coloured ink. The basic reference is quoted at the right hand corner of the ruled card. The important references of the same species are added on the back side of the reference card. The information on the card is kept in similar format for all the cards as follows.

Digitization:
Now a days digitization is a need in every field of information generation, processing, preservation and access. Many institutions and agencies run the activities at National and international level. In India the digization programmes are on the first step and becoming more focused activities now a days. The technologies are a complex process of experimentation with gains and losses, triumphs and failures (Dasgupta, 2005; Nagarkar 2000)

Because of digital technology expectations of people's hopes are increased for facing the challenges bridging the gap between information rich and poor countries and also upgrading the level of development in all its different facets. Now after all this, the responsibilities rests on may persons like decision makers, technological experts etc. and also the local institutions for roles in bringing digital information to the diverse mob of the country

For getting multi-lingual and multi-sectoral information more sophisticated technology is needed based on available technical infrastructure. As the rate of development is seen after few years every corner of India will be having digital technology. Many Indian decision makers have now realized the value of information is power and Government of India with other agencies are also taking necessary efforts to enrich IT based Information for substantial improvement in the quality of life of every Indian personnel. (Dasgupta, 2005).

Card:

Short form of the reference at right hand side top corner
Recent name of the species
Old name of the species
Family
Host
Locality
Distribution
Reference sited

Data Feeding in Excel:

The data on the above said cards is feed in different columns sequence wise as that of the card information so that the uniform system is developed in the database. MS Office 2007 has been used for creating the MS Excel sheets. The advantage of the excel sheet data was that we could import the same data in any usable format, which was not possible in other forms so easily. More than 1700 entries of the records have been done from all over India very keenly.

Database Building:

In this database the software's which are used as follows:

DBMS	:**MySQL Server 5.0**
Serverside script	: **PHP 5.2.9**
Server	: **Apache 2.2**
Javascript library	: **Scriptaculosus**

Being reference data it is not complex in terms of relationships between the files. But the complexity is present in terms of repeating phrases/words and different words with same/similar meaning. The primary key for the record table is defined by the collection of three columns viz. genus, species and original reference. The original reference field had to be added in the primary key since there can be repeating genus-species combination obtained from different sources of data. Uid is the unique identification number for each record. The columns in the database are as follows:

Software:

Between the physical database itself (i.e. the data as actually stored) and the users of the system is a layer of software, usually called the database management system or DBMS. DBMS provides a view of the databases that is elevated somewhat above the hardware level and supports user operations that are expressed in terms of that higher level view.

Description of All Pages of Database:

The website contains the pages as follows Home page, General search (Simple search), Advanced Search, Output of the search, Browse, Card viewer, Card, References page, Contact page, Site Map, Help Page, Publications and Data entry page.

Figures 1: Database Home page

Home page:
It contains introductory information and links to other pages. Data statistics given on the front page will change automatically as per the updates in the data records. **(Figure No. 1).**

General search (Simple Search) Page:(Figure No. 2)
It contains a text input field where user can type keywords to be searched. There is also a dropdown box for selecting the operate (AND/OR). A button is added to the page which is used to initiate the querying process. When this search button is pressed on 'onclick' event is fired and subroutine is called. This subroutine (written in java script) takes key words and operator from corresponding fields of the pages and sends it to the serverside script that accesses the database (here it is simple search.php). This script works in backend to search for the data.

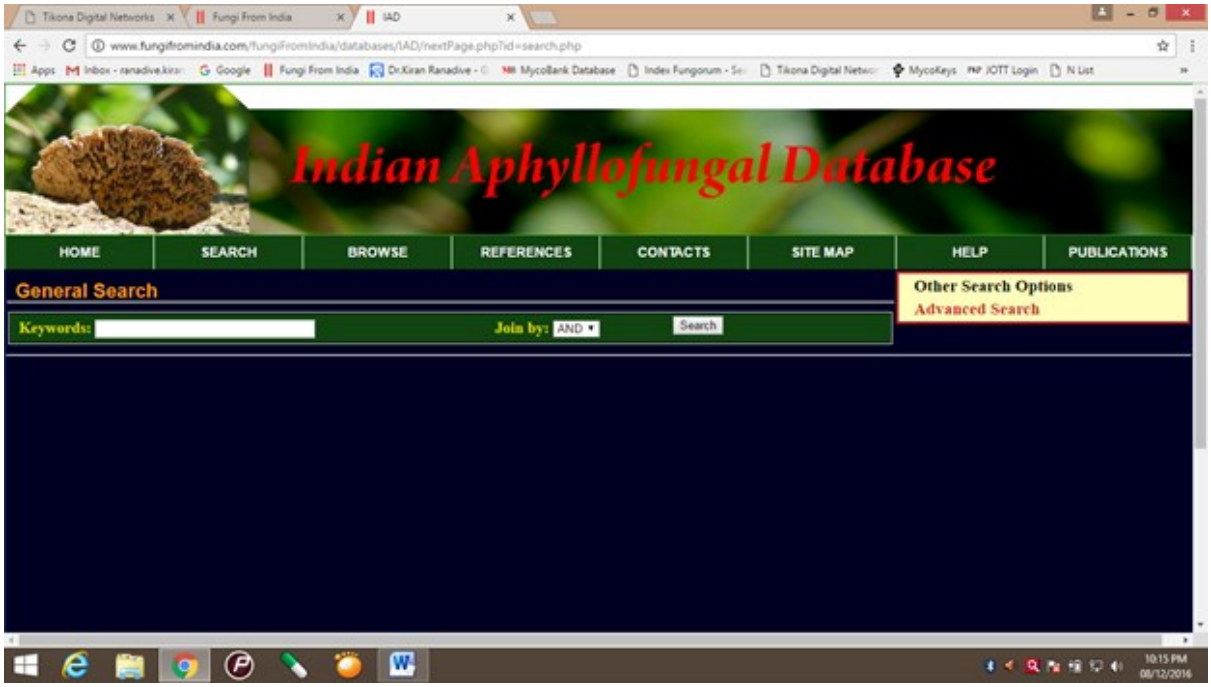

Figure 2 : Database Simple Search and Advanced Search page:

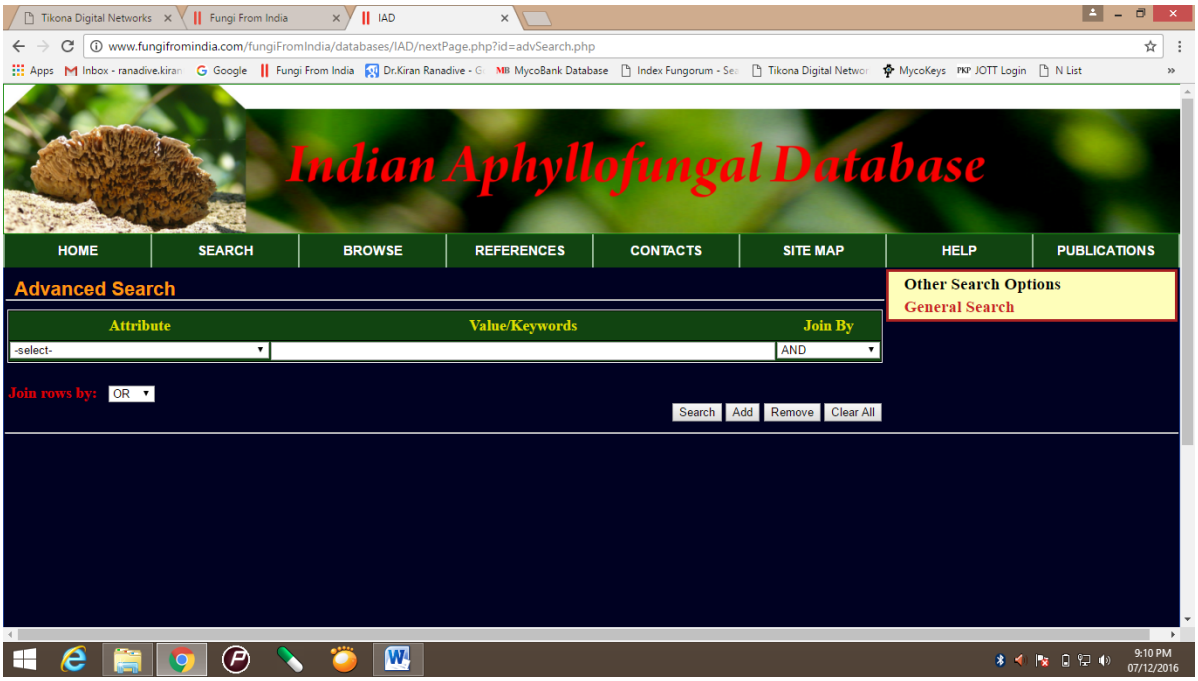

Figure 3 : Database Advanced Search page:

If the data matching to the query is found, the script generates a tabular output in html format and returns it to the client. Being in the "html format", the data can then be viewed in browser as the html page that contains the search results.

Advanced Search Page: (Figure No. 3)
This page contains three input fields as follows
a. Dropdown box- For selecting database field to be searched.

b. Text input field - For Keywords, dropdown box for logical Operate.

c. Row join operator-For joining multiple searches.
The set of these 3 basic input fields mentioned above form a single search. More than one searches can be performed in one go by adding such sets of three field s and joining them by 'row join operator'. For this add and remove buttons are given.

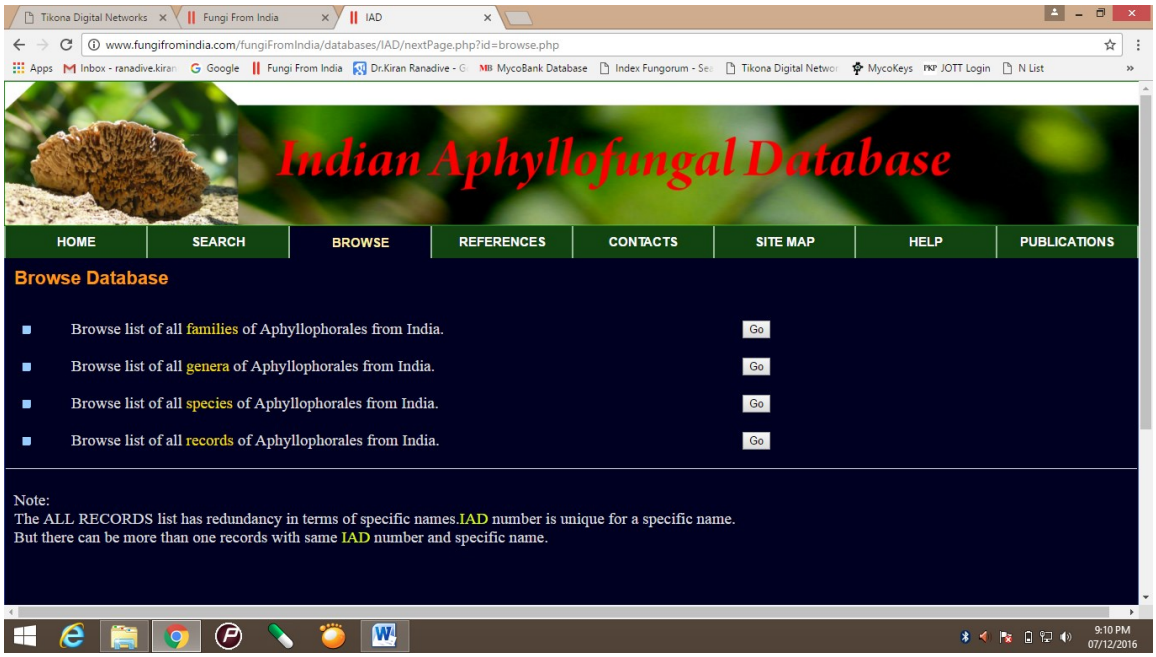

Figure 4-a: Database Browse page

Figure 4-b : Database Search page result:

Output of the Search:
Both simpleSearch.php and advSearch.php generate output in same format. The searched records are presented in a tabular format with serial number, IAD-ID number genus name, species name and family name as the columns. The output generated by this script is in a card format and is not presented to the user as different page but is dynamically embedded in the existing search results page. This achieves the purpose of both user-friendliness and the minimum amount of data transfer. The embedded page is formatted in such a way that the user feels as if looking the hardcopy of the card. The card can be closed using the provided 'close' button and other cards can be reviewed in the same page without querying the database again and again.

Browse page:(Figure No. 4a and 4b)
Browse: Family
Browse: species specific query
Output of browse queries is same as that for search queries.

Card Viewer:(Figure No. 5)
This page will display the card as well as the background showing glimpses of the result of the query put by the user/visitor. This function will help the user/visitor to get back to the species in which lies client's second interest. Every record is connected with the world reputed Mycobank website (http://www.mycobank.org/mycotaxo.aspx)[8]

Figure 5 : Database Card viewer:

Card:(Figure No. 6) The card is the source of all basic information like

IAD Number (The Unique Indian Aphyllofungal Database Number**)**
Name of the species
UID (Unique ID number given to every Record from The Database)
Family Name
Rot Type Recorded
Host
Locality
Geographical Distribution
Original Reference (From where the data of the is taken)
Research Work Reference (Any other research reference to be added)

Card:

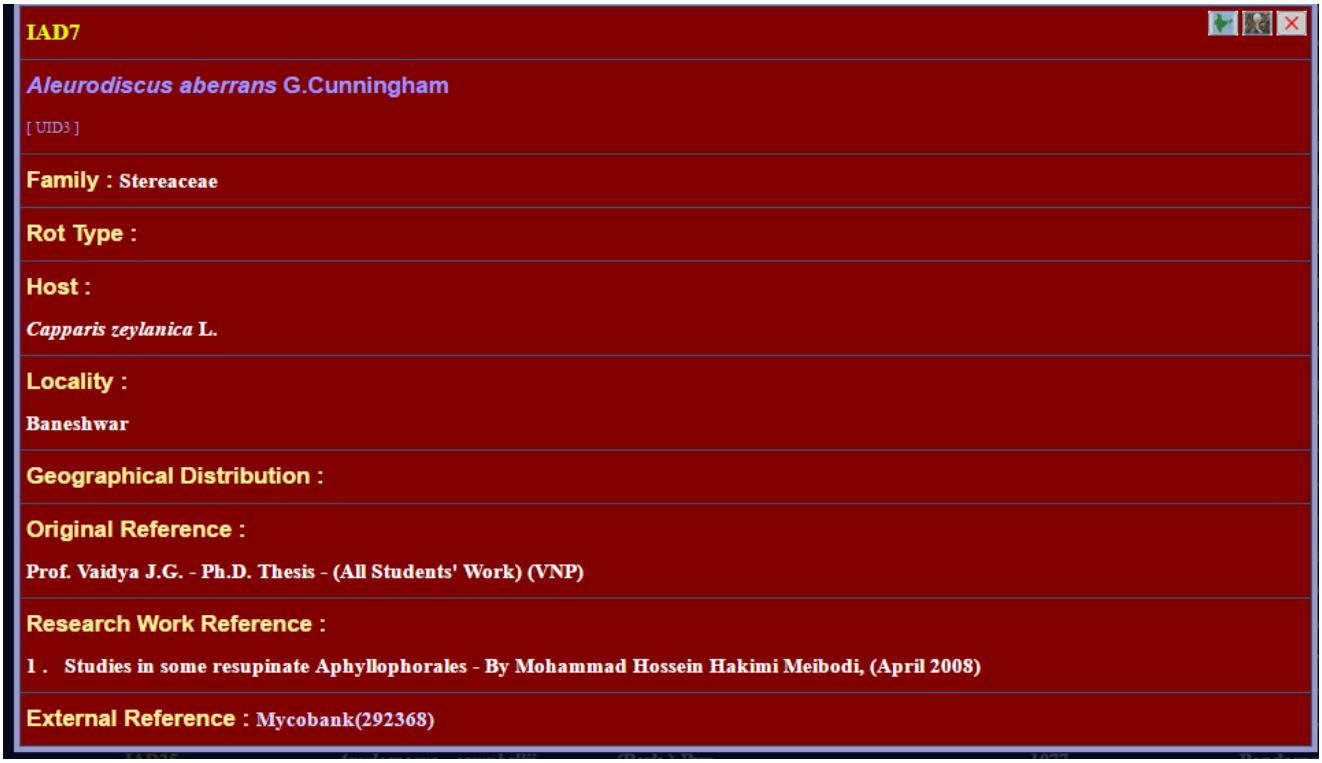

Figure 6 : Database Card :

References Page:(Figure No. 7)

This page includes more than 19 major references used for this Database. Reference books, Ph.D. Thesis of the related subjects are given sequentially.

Figure 7 : Database Reference page:

Contacts Page:(Figure No. 8)

This page shows the photographs and biodatas of the authors Database for any further queries about the Database.

Figure 8: Database contact details page:

Site Map:(Figure No. 9)

In this page the short cut links are given for every major part of topics of the Database which occurs on the task bar of the Database. It is very much easier for every visitor of this Database.

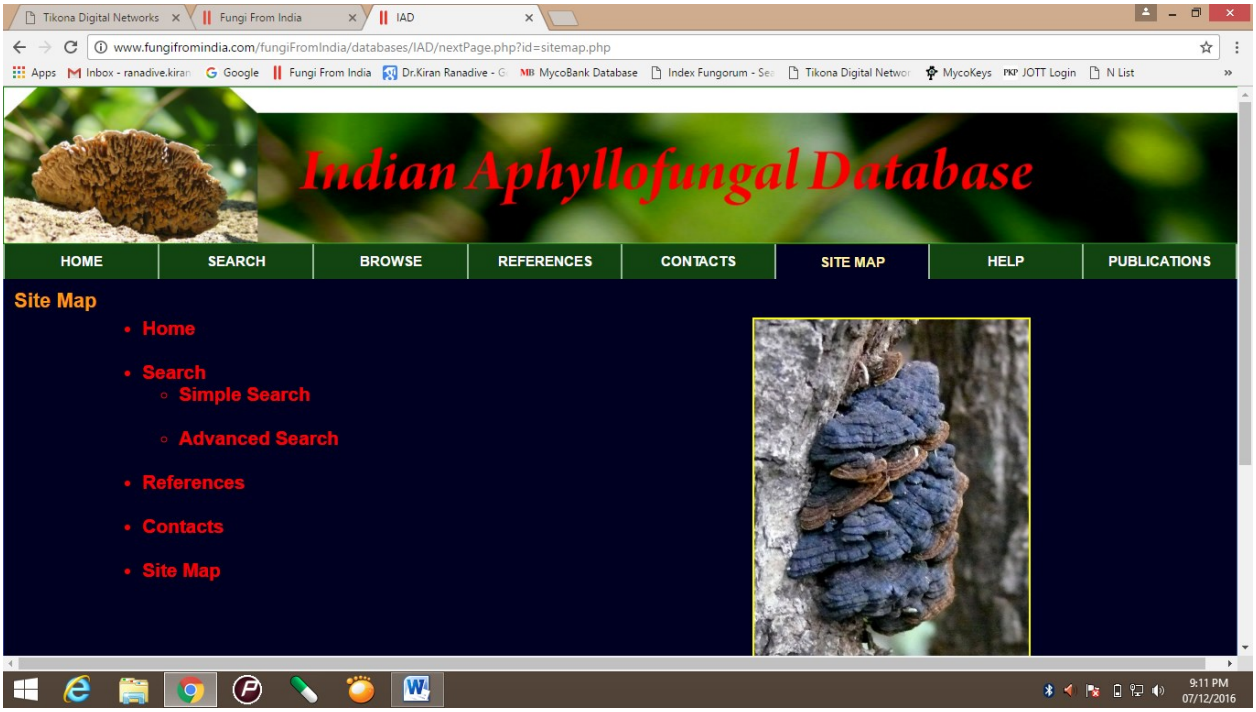

Figure 9: Database Site map page:

Help Page:(Figure No. 10)

This page is really the encouraging page for every visitor of the Database because it gives every help regarding (How to use the database?) its use. The page is having the screen shots of all the pages which will help the student as well as researchers to solve their difficulties regarding the use of the Database.

Figure 10: Database Help page:

Publications:

This is the section containing the publications done by author of this database. **(Figure No. 10)**

Figure 10 : Database Publication page:

RESULT

The present work has contributed for the first time in India about the Aphyllophorales Reference database. The in this study more than 1700 reference cards were prepared in a standard way. The database shows total 1646 records in which total 1217 species were recorded from 52 families and 190 genera of Aphyllophorales from all over India. This IAD Database has been launched online on www.fungifromindia.com. Every species of this database has been linked to the www.mycobank.org.

DISCUSSION

The literature on fungi is scattered in journals, not easily accessible to the Indian students. The unavailability of the related literature may develop the disliking of the subject, so in such case our IAD-Reference Database (The database giving all Aphyllophorales references from India on a single click, i.e. on IAD- Indian Aphyllofungal Database) will minimize the efforts and time for the survey of literature. This is the first Indian effort to do such contribution in the history of Indian Mycology and just made available free of cost for all researchers from world. This database will help to the new comers in the field and it is available free of cost online. This database work has been completely funded by the author himself only. This is expected to serve as an initial step towards better understanding of the Aphyllophorales from any locality of India.

Acknowledgement:
It gives me great pleasure to avail this opportunity in acknowledging all those who were of immense help to me during the course of my research work. First and foremost I express my deep sense of gratitude to Late Prof. J. G. Vaidya for suggesting the problem and providing constant guidance, cooperation and freedom during the entire period of my research work. I am grateful to our Principal Dr. Nitin Ghorpade, Waghire College, Saswad, Tal- Purandar and all higher authorities of Poona District Education Association for encouragement. I can't forget the help and guidance extended by well known Indian Mycologists Prof. Anjali Roy, Dr. AshitBaren De and Prof. P. Ganesh, regarding basic Aphyllophorales taxonomy. I am highly indebted to my mother (Vimal) and father (Ramchandra) who steered the way through towards accomplishment of my goals, giving me a constant encouragement and by being besides me in every thick and thin, showing unfailing patience throughout the course of my work. I can't express my feelings in words about the help extended by Prof. Leif Ryvarden, Dr. David Minter, Dr. Paul Kirkand Dr. Vincent Robert of www.mycobank.org for giving me free of cost linking to every species of this database and taxonomic literature help. I am especially grateful to Harshavardhan Khare for his extreme help in database development, without whom the database would have had no existence. My sincere thanks are due to Mr. Rahul Kale (ARI Pune, Library) who helped me a lot during the survey of literature. I am unable to conclude the acknowledgment without expressing my special thanks to Ashish Deodhar and all family members for their constant support and encouragement.

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

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http://www.mycobank.org/mycotaxo.aspx

International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Macrofungi from KME Society's educational campus in Bhiwandi, dist. Thane Maharashtra and their associated Myco-technologies

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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:
Moses Kolet, Asba Ansari, Ayesha Divkar and Ayesha Momin (2018) Macrofungi from KME Society's educational campus in Bhiwandi, dist. Thane Maharashtra and their associated Myco-technologies, *Int. J. of. Life Sciences*, Special Issue, A9: 25—30.

ABSTRACT

Macrofungi, especially mushrooms; their wide variations in morphological characteristics have fascinated all; mycologists and laymen alike. Mushrooms, owing to their secretive life, have been associated with several animals, fictitious creatures, mysterious entities, super natural beings and happenings through the ages which are also reflected in their vernacular names. These small, apparently insignificant components of biodiversity play an important role as food, dietary supplements, therapeutics, sources of drugs, pharmaceuticals and novel compounds; in modern scientific research, biodegradation and bioremediation. In the present study, macrofungi from KME Society's G.M. Momin Women's College campus in Bhiwandi were documented by the survey method. The study recorded 17 types of macrofungi inclusive of several mushrooms; prominent among them being the genera *Auricularia*, *Daldinea*, *Trametes*, *Mycena*, and *Schizophyllum* among several others, details of which, and the current and emerging mycotechnologies associated with the types recorded, are mentioned in the paper.

Key Words: biodiversity, mushrooms, macrofungi, Bhiwandi.

INTRODUCTION

The word fungus was long ago used to denote mushrooms, following which, its colloquial usage expanded to include other related groups such as molds, polypores, puff balls and many others. The wide variations in colours, shades, sizes, shapes and other morphological characteristics exhibited by macrofungi, particularly mushrooms, accompanied with the mysterious and extremely secretive status

attached to their way of life; the mystifying appearance and disappearance of fruiting bodies, have always fascinated all; laymen and experts alike. Through the ages, in all civilizations, mushrooms have been associated with animals, superstitions and related entities, supernatural and mysterious entities, fairy tales, mythology and folk legends. Their vernacular names bear testimony to such mysterious associations, as well as their morphology, habitat and several other associative characteristics.

The dietary value of mushrooms is well documented (Bahl, 1998, Feeney *et al*., 2014). Cultivation of edible mushrooms has always attracted research attention and the trends are also visible in remote areas, as an answer to dietary deficiencies (Sharma and Thakur, 2010; Valverde *et al*., 2015). Amongst the several other uses of mushrooms and macro fungi are their utilization as sources of therapeutic and anti-cancer agents (Patel and Goyal, 2012; Sharma *et al*., 2017), neutraceuticals (Rathore *et al*., 2017), novel bioactive compounds (Chatterjee and Patel, 2017); in biotransformation and bioremediation (Raj *et al*., 2011), as dyes (Bessette and Bessette, 2001), in dye sensitized solar cells (Zalas *et al*., 2015) and several aspects of modern scientific research. In spite of their utilitarian aspects, literature on macrofungi from the mega city of Mumbai is scarce and scattered and there are practically no reports of this component of biodiversity from Bhiwandi region, adjoining Mumbai. Hence the current investigation was undertaken to study and document mushrooms and macrofungi from KME Society's campus and G.M. Momin Women's College campus in Bhiwandi city.

The area of study viz. G.M.Momin Women's College, popular amongst locals as G.M. college or Rais High School campus is a 6.5 acre campus situated in the Kaneri area of Bhiwandi city, Dist. Thane near Mumbai, the commercial capital of India. Apart from housing some of the best schools and colleges in Bhiwandi, the campus, developed since 1927 on agricultural plots which were originally paddy fields, also sports a reasonably rich biodiversity of vegetation. Various mushrooms and macrofungi appear on the educational campus, especially during the monsoon season, which prompted the present study. The study was also undertaken to spread awareness on the presence of these small but nevertheless important entities in the area of study and the current and emerging mycotechnologies associated with them.

MATERIALS AND METHODS

The study was carried out by the survey method, for collection and documentation of data during the monsoon and post-monsoon season from June to November 2017; wherein a survey of macrofungi was carried out in the area of study. The five locations of study in the campus were the gardens adjoining main building of the G.M. Momin Women's College (Location no. 1), area adjoining extension building of the college (location no. 2), area adjoining staff quarters (location no. 3), lumber and timber storage area adjoining KME Society's College of Education building (location no. 4) and area adjoining KME Society office building (location no. 5). The specimens were identified in the field and in the department of botany, G. M. Momin Women's College, using standard literature (Bakshi, 1971; Lawrence and Harniess, 1991; Keizer, 1997; Polese, 2000) and techniques suggested by Buczacki (1992) and Kaul (1999).

RESULTS AND DISCUSSION

A total of 17 types of macrofungi, comprising 15 genera, were recorded during the investigation. Amongst the fungi recorded, 2 forms comprising 2 genera belonged to Ascomycetes, while 15 forms comprising 13 genera were attributed to Basidiomycetes. The findings are presented in Table 1. Forms such as *Auricularia auricula*, *Daldinea concentrica*, *Trametes*, *Mycena*, *Schizophyllum commune* and *Schizophyllum sp.* were prominently represented, albeit at their respective site locations. The forms encountered were found to be in most of the cases, limited to their respective location and generally did not overlap with other locations of study, most probably owing to the typical and characteristic circumstantial conditions prevalent at every location. While location no. 1 showed conditions characteristic of gardens, with soil rich in organic matter, the others

were dry in comparison. Location no. 2 and 3 were characterized by few uprooted dead trees, tree stumps and logs of wood; location no. 4 was characterized by conditions typical of a dry timber depot, while location no. 5 revealed dry garden-like conditions with some dead tree stumps. The findings on biodiversity of macrofungi are in agreement with those of Todawat and Papdiwal (2012) and Kumar *et al.* (2015). Mushrooms such as *Coenocybe tenera*, *Psilocybe* sp. and *Mycena* sps. were common during rains on the garden soil freshly amended with cowdung manure; the latter, most probably being their source of origin. Many of the macrofungal forms recorded in the current investigation had remarkable abilities related to wood rotting and biodegradation of agro-industrial wastes, dietary value, production of bioactive compounds of medicinal importance (Zhang *et al*, 2016; Kinge *et al*, 2017), and are cultivated in different parts of the world for their valued products (Zervakis and Koutrotsios, 2017).

From among the macrofungal forms reported herein, *Daldinia concentrica* is well documented as a wood decay fungus (Hiscox and Boddy, 2017); has applications in traditional medicines and ethnomycology (Akpaja *et al*., 2005), yields secondary metabolites with anti-HIV (Qin *et al*., 2006), nematicidal (Anke *et al*., 1995), phytotoxic (Lee *et al*., 2006), antimicrobial (Shen *et al*., 2017) activities and is known for its health benefits (Karun *et al*., 2017). *Hypoxylon* has been successfully exploited for metabolites exhibiting anti-bacterial and anti-fungal activity (Yuyama *et al*., 2017). Widely believed to be the earliest cultivated fungus for food (Royse, 2014), *Auricularia auricula* has been in the lime light for its antioxidant and antimicrobial activities (Yu and Oh, 2016), hypoglycemic (Yuan *et al*, 1998) and therapeutic properties (Lu *et al*., 2018), dietary aspects (Misaki and Kakuta, 1995; Vallee *et al*, 2017), novel cultivation practices (Onyango *et al*., 2011), molecular aspects (Du *et al*., 2016), commercial food value (Zou, *et al*., 2017) and mycoremediation potential (Song *et al*., 2017).

Table 1: Macrofungi recorded on KME Society's Educational Campus, Bhiwandi

S. No.	Botanical Name	Common Name	Location site				
			1	2	3	4	5
Ascomycetes							
1	*Daldinia concentrica* (Bolton) Cesati & de Notaris	King Alfred's cakes, carbon balls		*	*		
2	*Hypoxylon sp.*	hypoxylon		*	*		
Basidiomycetes							
3	*Auricularia auricula* (Bull.) J. Schrot	Jelly ear, Jew's ear, Tree ear			*		
4	*Auricularia polytricha* (Mont.) Sacc.	Cloud ear fungus			*		
5	*Schizophyllum commune* Fries	Split-gill, Common Schizophyllum		*	*	*	
6	*Pleurotus ostreatus* (Jacq ex Fr.) P. Kumm.	Oyster mushroom	*				
7	*Polyporus sp.*	Bracket fungus				*	
8	*Poria sp.*	Poria				*	
9	*Trametes sp.*	Many zoned polypore					*
10	*Daedalea sp.*	Maze gill				*	
11	*Coenocybe tenera* (Schaeff.) Fayod	Cone cap mushroom, brown dunce cap	*				
12	*Laccaria laccata* (Scop.) Cooke	Deceiver	*				
13	*Marasmius sp.*		*				
14	*Psilocybe sp.*		*				
15	*Mycena sp.*(1)	Common mycena	*				
16	*Mycena sp.*(2)	Common mycena	*				
17	*Psathyrella sp.*	Cone capped agaric	*				

A. polytricha is known for its antimicrobial (Gbolagade and Fasidi, 2005) and antidiabetic (Wu *et al*., 2014) properties as well as culinary and medicinal importance (Afiukwa *et al*., 2013). *Schizophyllum commune* was reported as a respiratory allergen (Singh *et al*., 2013) but also produces commercially valuable biopolymers (Mohammadi *et al*., 2017) and has been attributed with anti-inflammatory property (Du *et al*., 2017).

The popular edible oyster mushroom, *Pleurotus ostreatus*, is reported to have nutritive, medicinal and antimicrobial attributes, neutraceutical potential (Kunjadia *et al*., 2014) along with biodegradation capabilities, mycoremediation potential (Purnomo *et al*., 2017), apart from being source of novel compounds and enzymes (Piscitelli *et al*., 2017). *Polyporus*, *Poria* and *Trametes,* well known for their wood rotting activity, also have great therapeutic value (Stamets 2012) and applications in alternative medicine. Genus *Trametes* is reported as an excellent source of enzyme laccase (Bucic-Kojic *et al*., 2017) and for its mycoremediation potential (Wolfand *et al*., 2016). Apart from production of laccase and potent role in biodegradation, *Daedalea* and *Marasmius* have also been attributed with biotransformation abilities (Rizqi and Purnomo, 2017; Vantamuri and Kaliwal, 2017). The small agaric *Mycena* was reported as source of several novel volatile compounds (Palazzolo *et al*., 2017) and as symbiont enhancing germination and growth in rare orchid species (Lee *et al*., 2017). Similarly, *Psathyrella* sps. are reported endowed with compounds of nutritional and therapeutic value (Atchibri *et al*., 2017). Various species of *Psilocybe* are known for their psychedelic effects and applications in medicine (Nichols *et al*., 2016). The current study revealed a moderately rich biodiversity of macrofungi on KME Society's Educational Campus, which is however significant considering the poor macro mycoflora of Bhiwandi and its surrounding areas.

A survey of mushrooms and macrofungi was conducted during monsoon and immediate post monsoon months in the current year 2017 in Bhiwandi, Dist. Thane, Maharashtra, India. A total of 17 types of mushrooms and macrofungi, belonging to 15 genera, were recorded from the area of study. Amongst the fungi recorded, 2 forms belonged to Ascomycetes, while 15 forms comprising 13 genera were members of Basidiomycetes. All the macrofungi found growing in the area of study were economically and environmentally important. Most of the forms documented, were reported to exhibit exceptional wood rotting capabilities.

Acknowledgements:
The author gratefully acknowledges the co-operation, encouragement and inspiration received from the Konkan Muslim Education Society of Thane District and Dr. S. K. Deshmukh for completion of this project.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Lichens of Cotigao Wildlife Sanctuary, Goa

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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:
Randive Pallavi P, Nayaka Sanjeeva and Janarthanam MK (2018) Lichens of Cotigao Wildlife Sanctuary, Goa, *Int. J. of. Life Sciences*, Special Issue, A9: 31- 36.

ABSTRACT

The detailed lichen study at Cotigao Wildlife Sanctuary, Goa is initiated recently. In the present study earlier report of lichens from the sanctuary and fresh collections were included. The study revealed the occurrence of 99 species belonging to 43 genera and 25 families of which 21 species are new records for the sanctuary and 15 species as new to Goa. Also, *Lepraria jackii* Tønsberg, a leprose lichen is reported as new to India. The sanctuary is dominated by bark inhabiting lichens, crustose forms with 83 species, and members of Graphidaceae family. The luxuriant growth of lichens at sanctuary indicates the availability the shady, moist conditions of the forest and less anthrapogenic disturbances.

Key words: Biodiversity, Lichenized fungi, Protected area, new records, Western Ghats

INTRODUCTION

Lichens are organisms that are products of symbiotic association between algae and fungi. In India a total of 2511 lichen species are recorded so far (Singh and Dash 2017) and Goa is represented by 128 species (Randive *et al*. 2017a, b). Detailed study on lichens of Cotigao Wildlife Sanctuary is recently initiated owing to its rich biodiversity. Cotigao is located towards the southeastern border of the state within the Western Ghats ecosystems and was notified as Wildlife Sanctuary in 1958 (Naithani *et al*. 1997) (Fig. 1). The topography of the sanctuary is largely flat, becoming undulated as it meets the Western Ghats. The sanctuary is surrounded by some of the highest hills in this region on the west, the Anshi National Park (Karnataka) to the southeast and the Netravali Wildlife Sanctuary to the Northwest. The sanctuary covers an area of 85.65 km^2 while large portions of the sanctuary show a forest crown density > 40%. The sanctuary is noted for its lofty tree cover, some trees attaining heights up to 20 m. The undergrowth is mainly

composed of the now familiar scourge, *Eupatorium*. The weed growth is particularly dense in the Eucalyptus and teak plantations (Alvaris, 2002). Regarding the lichens earlier Phatak *et al*. (2004) reported 43 species, while some collections of one the author (PR) is included in the publication of Ranadive *et al*. (2017a, b). The present communication further updates the list of lichens from Cotigao Wildlife Sanctuary with several additions.

MATERIALS AND METHODS

The checklist of lichens included species reported earlier (Ranadive *et al*. 2017a, b) as well as fresh collections. About 350 samples of lichens were collected from Cotigao Wildlife Sanctuary during the year 2015 to 2017. These lichens were mostly growing over tree bark and collected following standard procedure, air dried and preserved at herbarium of Goa University with details. The lichens were identified by studying their morphology, anatomy and chemistry (Nayaka 2014). Orange *et al*. (2001) was followed for chemical analysis of the samples. Morphological details were examined using a stereo zoom Leica S8APO microscope. Anatomical details were studied using a light DM2500 microscopes attached with camera and image analysis software. Hand-cut sections of thalli and ascomata mounted in distilled water, KOH solution (K), lactophenol cotton blue (LPCB) were studied. The amyloid reactions were tested in Lugol's iodine solution without (I) or with pre-treatment with KOH (KI). All measurements were made on material mounted in distilled water. Awasthi (1991, 2007) and other recent literature were consulted for identification of various lichen taxa.

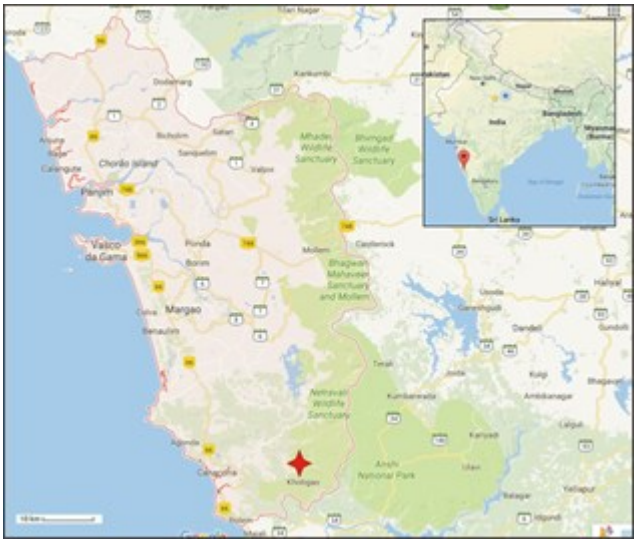

Figure 1. Map of Goa showing location of Cotigao Wildlife Sanctuary (star marked)

RESULTS AND DISCUSSION

The study resulted in 99 species belonging to 43 genera and 25 families in Cotigao Wildlife sanctuary of which 21 species are new records for the sanctuary. Of these 15 species are new addition to Goa lichen biota (Table 1).

Figure 2. (a) *Lepraria jackii* Tønsberg, a new record for India **(b)** *Lepraria jackii* Tønsberg, a new record for India

Table 1: List of lichens from Cotigao Wildlife Sanctuary, Goa [Note: CR = Crustose, FL = Foliose, SQ = Squamulose, LP = Leprose, Corti. = Corticolous, Folii. = Foliicolous,# = New record for Cotigao Wildlife Sanctuary, * = New record for Goa]

Sr. No.	Family	Taxa Name	Growth Form	Substratum
1	**Arthoniaceae**	*Arthonia cinnabarina* (DC.) Wallr.	CR	Corti.
2		*A. confertum* (A.L. Smith) Makh. &Patw.	CR	Corti.
3		*A. dispersula* Nyl.	CR	Corti.
4		*Cryptothecia faveomaculata* Makh. &Patw.	CR	Corti.
5		*C. subnidulans* Stirton	CR	Corti.
6		*C. subtecta* Stirton	CR	Corti.
7		*Herpothallon cinerenum* G. Thor	CR	Corti.
8		*#*Tylophoron nidulans* Stirton	CR	Corti.
9	**Arthopyreniaceae**	*Arthopyrenia alboatra* (Kremplh.) Müll. Arg.	CR	Corti.
10		*A. finkii* Zahlbr.	CR	Corti.
12		*A. grisea* (Schierch.) Körb.	CR	Corti.
13		*A. indusiata* Müll. Arg.	CR	Corti.
14	**Byssolomataceae**	**#Byssoloma permutans* (Nyl.) Lücking	CR	Corti.
15	**Caliciaceae**	*Dirinaria aegialita* (Afz. in Ach.) Moore	FL	Corti.
16		*D. confluens* (Fr.) Awasthi	FL	Corti.,Folii
17		*Pyxine cocoes* (Sw.) Nyl.	FL	Corti.
18		#*Pyxine cylindrical* kashiw.	FL	Corti.
19	**Coenogoniaceae**	*Coenogonium dilucidium* (Kremp.) Kalb & Lücking	CR	Folii
20		**#C. lutescens* (Vezda & Malcome) Malcome	CR	Corti.
21	**Collemataceae**	#*Leptogium austroamericanum* (Malme) C.W. Dodge	FL	Corti.
22		#*L. chloromelum* (Ach.)Nyl.	FL	Corti.
23		*L. denticulatum* Nyl.	FL	Corti.
24		*L. chloromelum* (Sw.) Nyl.	FL	Corti.
25	**Fissurinaceae**	**#Fissurina elaiocarpa* (A.W. Archer) A.W. Archer	CR	Corti.
26		**#F. immerse* B.O. Sharma, Khadilker & Makhija	CR	Corti.
27	**Gomphillaceae**	*Echinoplaca* sp.	CR	Corti.
28	**Graphidaceae**	*Diorygma confluens* (Fée) Kalb, Staiger&Elix	CR	Corti.
29		*Glyphis cicatricosa* Ach.	CR	Corti.
30		*Graphis nigroglauca* Leight.	CR	Corti.
31		*G. adscribens* Nyl.	CR	Corti.
32		*G. cleistoblephara* Nyl.	CR	Corti.
33		*G. pyrrhocheiloides* Zahlbr	CR	Folii
34		*G. cincta* (Pers.) Aptroot	CR	Corti.
35		*Hemithecium echinatum* Aptroot, Lücking& Will-Wolf	CR	Corti.
36		*H. nakanishianum* (Patw. & C.R. Kulk.) Makh.&Dube	CR	Corti.
37		*H. peplophora* (M. Wirth & Hale) V. Tewari &Upreti	CR	Corti.

Table 1: Continued…

Sr. No.	Family	Taxa Name	Growth Form	Substratum
38		*Leucodecton anamalaiense* (Patw. & C.R. Kulk.) Rivas Platas & Lücking	CR	Corti.
39		*Myriotrema subconforme* (Nyl.) Hale	CR	Corti.
40		*Ocellularia groenhartii* Hale	CR	Corti.
41		*Pallidogramme chrysenterodes* (Nyl.) K. Singh & Swarnalatha	CR	Corti.
42		*Phaeographis brasiliensis* (A. Massal.) Kalb & Matthes-Leicht	CR	Corti.
43		*P. platycarpa* Müll. Arg.	CR	Corti.
44		*P. extrusula* (Stirton) Zahlbr.	CR	Corti.
45		*Platygramme wattiana* (Müll. Arg.) V. Tewari &Upreti	CR	Corti.
46	**Lecanoraceae**	*Lecanora andina* Räsänen	CR	Corti.
47		*L. chlorotera* Nyl.	CR	Corti.
48		*L. helva* Stizenb.	CR	Corti.
49		*L. leproplaca* Zahlbr.	LP	Corti.
50		*L. tropica* Zahlbr.	CR	Corti.
51		**#Lecidella sp.*	CR	Corti.
52	**Malmidaceae**	*Malmidea granifera (Ach.) Kalb, Rivas Platas & Lumbsch*	CR	Corti.
53	**Monoblastiaceae**	*Anisomeridium angulosum (Müll. Arg.) R.C. Harris*	CR	Corti.
54		*A. complanatum (Makh. & Patw.) R.C. Harris*	CR	Corti.
55		*A. subnexum (Nyl.) R.C. Harris*	CR	Corti.
56		**#A. tarmuqliense (Makhija & Patw.) R.C. Harris*	CR	Corti.
57		*#Monoblastia pellucida Aptroot*	CR	Corti.
58	**Naetrocymbaceae**	*Naetrocymbe fraxini (A. Massal.) R.C. Harris*	CR	Corti.
59	**Parmeliaceae**	*Parmotrema latissimum (Fée) Hale*	FL	Corti.
60	**Pertusariaceae**	*Pertusaria concinna Erichson*	CR	Corti.
61		*P. punctata Nyl.*	CR	Corti.
62	**Physciaceae**	*Heterodermia obscurata (Nyl.) Trevisan*	CR	Corti.
63		*Physcia tribacia (Ach.) Nyl.*	FL	Corti.
64	**Pilocarpaceae**	*Felhanera bouteillei (Desm.) Vezda.*	CR	Corti.
65	**Porinaceae**	*Porina internigrans (Nyl.) Müll. Arg.*	CR	Corti.
66		**#P. interstes (Nyl.) Harm.*	CR	Corti.
67		*P. kameruensis F. Schill*	CR	Folii
68		*P. nitidula Müll. Arg.*	CR	Folii
69		*P. karnatakensis Makhija, Adawadkar & Patw.*	CR	Folii
70		**#P. rufula (Kremp.) Vain.*	CR	Folii
71		*P. subcutanea Ach.*	CR	Corti.
72		*P. subhibernica Upreti*	CR	Corti.
73		*P. tetracerae (Afz.) Müll. Arg.*	CR	Corti.
74		**#Trichothelium alboatrum Vain.*	CR	Corti.

Table 1: Continued…

Sr. No.	Family	*Taxa Name*	Growth Form	Substratum
75	**Pyrenulaceae**	*Pyrenula approximans (Krempelh.) Müll. Arg.*	CR	Corti.
76		*P. breutelii (Müll. Arg.) Aptroot*	CR	Corti.
77		*P. brunnea Fée*	CR	Corti.
78		**#P. leucotrypa (Nyl.) Uptreti*	CR	Corti.
79		**#P. macularis (Zahlbr.) R. C. Harris*	CR	Corti.
80		*P. mamillana (Ach.) Trevisan*	CR	Corti.
81		*P. nitidula (Bres.) R.C. Harris.*	CR	Corti.
82		**#P. nodulata (Stirton) Zahlbr.*	CR	Corti.
83	**Ramalinaceae**	*Bacidia connexula (Nyl.) Zahlbr.*	CR	Corti.
84		*B. rosella (Pers.) De Not.*	CR	Corti.
85		*Phyllopsora manipurensis (Müll. Arg.) G. Schneider*	SQ	Corti.
86		*P. nemoralis Timdal & Krog*	SQ	Corti.
87		*P. parvifolia (Pers.) Müll. Arg.*	SQ	Corti.
88	**Roccellaceae**	*Enterographa pallidella (Nyl.) Redinger*	CR	Corti.
89	**Stereocaulaceae**	**#Lepraria jackii Tønsberg*	CR	Corti.
90	**Strigulaceae**	*Srtigula nitidula Mont*	CR	Folii
91		**#S. orbicularis Fr.*	CR	Corti.
92		*S. phyllogena (Müll. Arg.) R.C. Harris*	CR	Folii
93		*S. smaragdula Fr.*	CR	Folii
94	**Teloschistaceae**	*Blastenia ferruginea (Huds.) A. Massal.*	CR	Corti.
95	**Trypetheliaceae**	*Laurera meristospora (Mont. & Bosch) Zahlbr.*	CR	Corti.
96		*#Trypethelium eluteriae Spreng.*	CR	Corti.
97		*T. endosulphureum Makh. & Patw.*	FL	Corti.
98		**#T. plicatorimosum Makhija*	CR	Corti.
99		*T. tropicum (Ach.) Müll. Arg.*	FL	Corti.

Graphidaceae family is dominant with17 species followed by Porinaceae (10 spp.), Pyrenulaceae (8 spp.), Arthoniaceae (8 spp.) and Lecanoraceae (6 spp.). The lichens were growing over tree bark while a total of 10 species were also recorded from leaves. The lichen biota of the sanctuary is mostly dominated by crustose forms represented by 83 species, while foliose and squamulose forms is have only 11 and three species respectively. In present study *Lepraria jackii* Tønsberg, a leprose-crustose lichens is reported as new record for India (Fig. 2). The species is found growing on the tree trunk in the sanctuary. It is characterized by leprose, powdery thallus with diffuse margin, lacking lobes and true medulla, sparse to continuous hypothallus, fine to coarse soredia with projecting hyphae, containing atranorin and zeorin secondary metabolites. Earlier, *L. jackii* was known from Europe, North America, Asia, Australia and Central Europe (Saag *et al*. 2009).

CONCLUSION

The present study clearly indicates lichen richness of Cotigao Wildlife Sanctuary. The luxuriant growth of foliicolous lichens at sanctuary indicates the availability the shady, moist conditions of the forest and less anthrapogenic disturbances. This information

would be helpful for biomonitoring studies in the area in future. Also, these lichens can be utilized for bioprospecting for novel biomolecules.

Acknowledgements:
We are thankful to Goa University for financial assistance under Ph.D. programme and to Forest Department of Goa for permission to survey Cotigao Wildlife Sanctuary. Authors PR and MKJ are thankful to Director, CSIR-NBRI, Lucknow for his permission to consult herbarium LWG and library. Authors are also thankful to Dr. D.K. Upreti for his encouragement, to Dr. Siljo Joseph and other members of the laboratory for their cooperation during the study.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Highlights on the Macrofungi of South West Coast of Karnataka, India

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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:
Sridhar KR (2018) Highlights on the Macrofungi of South West Coast of Karnataka, India, *Int. J. of Life Sciences*, Special Issue, A9: 37-42.

ABSTRACT

This study addresses macrofungal composition of coastal habitats (scrub jungles, coastal sand dunes and mangroves) of south west coast of Karnataka. Up to 124 species of macrofungi have been recorded with a highest of 95 species in scrub jungles followed by coastal sand dunes (36 spp.) and mangroves (31 spp.). Ten species were common to all habitats and wood inhabiting *Dacaryopinax spathularia* was frequent. Edible macrofungi were highest (34 spp.) followed by ectomycorrhizal (26 spp.) and medicinal (21 spp.) macrofungi. Soil inhabiting *Lycoperdon utriforme* was edible, ectomycorrhizal and medicinal fungus. Many macrofungi were eaten based on traditional knowledge of local people. Habitat degradation is most threatening to macrofungi, which results in soil erosion, substrate depletion and elimination of host tree species. The current status and strategies of habitat conservation in favour of macrofungi have been discussed.

Keywords: Coastal sand dunes, conservation, ectomycorrhizae, mangroves, scrub jungles, traditional knowledge

INTRODUCTION

Nearly 30% of human population in the world has taken shelter in coastal regions (Hoagland and Jin, 2006). The Peninsular India encompasses coastline over 7,000 km and its magnitude further increases by considering the coastlines of islands (Andaman, Nicobar and Lakshadweep). Area of coast influenced natural habitats (west coast, east coast and islands) constitutes roughly 22,000 km^2 (Rodgers and Panwar, 1988; Mehta, 2000). Bioresources of such habitats drastically differ compared to other aquatic and terrestrial regions. The coastal region of southwest India is represented by a variety of ecological niches with unique flora, fauna and microbiota. Coastal ecosystems of the southwest India could be broadly classified into three major habitats: i) freshwater habitats; ii) marine habitats; iii) terrestrial habitats (Fig. 1). Sand dune forests, mangrove forests and river valley forests of coastal

region play a key role in supporting unique life forms (Sridhar, 2017). Some of the major human interferences of the coastal region include resource depletion, soil erosion and urbanization. Thus, coastal regions need restoration, rehabilitation and revegetation for protection from storms, water purification and to derive sustainable agricultural products.

Macrofungi constitute important natural resource owing to their major role in decomposition, nutrient cycling, mutualistic association and other advantages (nutrition, medicine and metabolites). Mueller *et al*. (2007) predicted the global extent of macrofungi between 53,000 and 110,000. Due to geographically distinct climatic conditions, the Indian Peninsula encompasses up to 850 macrofungi (Manoharachary *et al*, 2006), the list is growing further by checklists and addition of new macrofungi especially from the Western Ghats and Himalayas (Bhosle *et al*., 2010; Mohanan, 2011; Farook *et al*., 2013; Prashar and Lalita, 2013; Senthilarasu, 2014; Gogoi and Prakash, 2015; Vishwakarma *et al*., 2017). Despite studies on various life forms of the coastal habitats, insights on macrofungi are fairly recent. The aim of this contribution is to compare macrofungi of lateritic scrub jungles, coastal sand dunes and mangroves of coastal regions of south west Karnataka to forecast future lines of research.

Assemblage and diversity

Among the three distinct habitats in coastal region, scrub jungles represent up to 77% of macrofungi (124 spp.) (Fig. 2) (Karun and Sridhar, 2016: Greeshma *et al*., 2016; Pavithra *et al*., 2016). In spite of saline conditions and disturbances, coastal sand dunes consist of 29% of macrofungi (Ghate *et al*., 2014; Ghate and Sridhar, 2016a). Although the wet and saline conditions in the mangroves hamper macrofungal growth, substrates like leaf litter and woody litter on the bunds above tide line during monsoon season support up to 25% of macrofungi (Ghate and Sridhar, 2016b). Common macrofungi between habitats ranges from 10.8% (coastal sand dunes and mangroves) to 14.5% (scrub jungles and coastal sand dunes). Ten macrofungi (8.1%) were common to all habitats, among them wood inhabiting edible macrofungi *Dacaryopinax spathularia* and *Lentinus squarrosulus* were dominant in all habitats (Table 1).

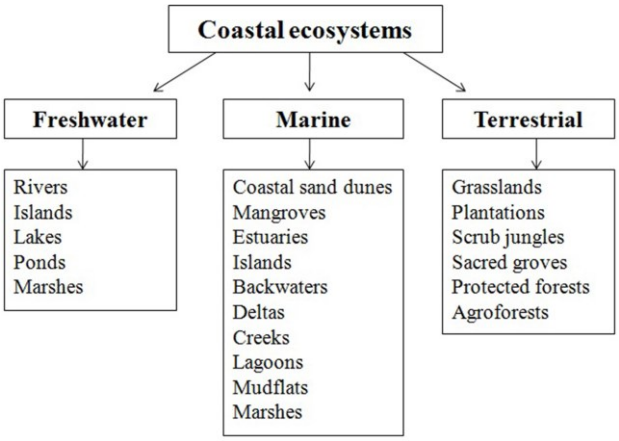

Fig. 1. Ecosystems of coastal region.

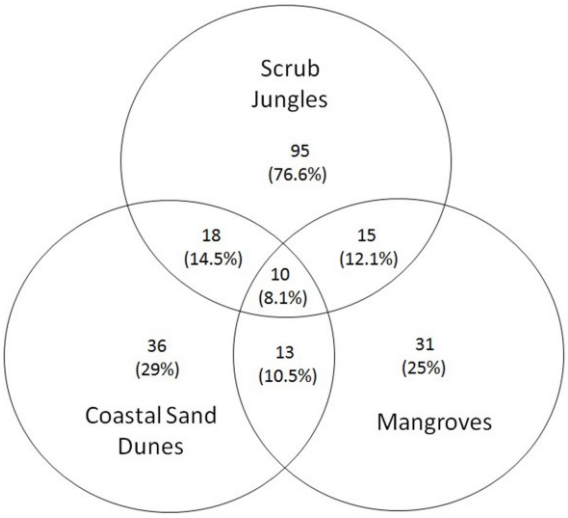

Fig. 2. Comparison of macrofungi occurring in lateritic scrub jungles, coastal sand dunes and mangroves of coastal region.

Table 2 presents edible, ectomycorrhizal and medicinal macrofungi in coastal region. The edible fungi were highest (34 spp.) followed by ectomycorrhizal fungi (26 spp.) and medicinal fungi (21 spp.) (Fig. 3). Fungi common to three coastal habitats ranges from 8.2% (edible and medicinal; ectomycorrhizal and medicinal) to 16.4% (edible and ectomycorrhizal). Thirty four edible macrofungi were recognized in coastal region. The soil inhabiting, edible, medicinal and ectomycorrhizal fungus *Lycoperdon utriforme* although rare it was common to all habitats (Table 2). About 15% macrofungi were edible, 31% medicinal and 54% were ectomycorrhizal in scrub jungles (Greeshma *et al*., 2016). In scrub jungles up to 47% macrofungi preferred soil, 38% preferred woody litter and 15% preferred leaf litter,

Table 1. Macrofungi occurring in three habitats of coastal region of southwest Karnataka

	Scrub jungles	Coastal sand dunes	Mangroves
Coprinus plicatilis	+	+	+
Crepidotus uber	++	+	+
Dacryopinax spathularia	+++	+++	+++
Ganoderma lucidum	+	+	+
Hexagonia tenuis	+	+	++
Lentinus squarrosulus	+++	++	++
Lycoperdon utriforme	+	+	+
Marasmius kisangensis	+	++	++
Microporus xanthopus	++	+	+
Thelephora palmata	++	+	+++

(+, rare; ++, common; +++, frequent).

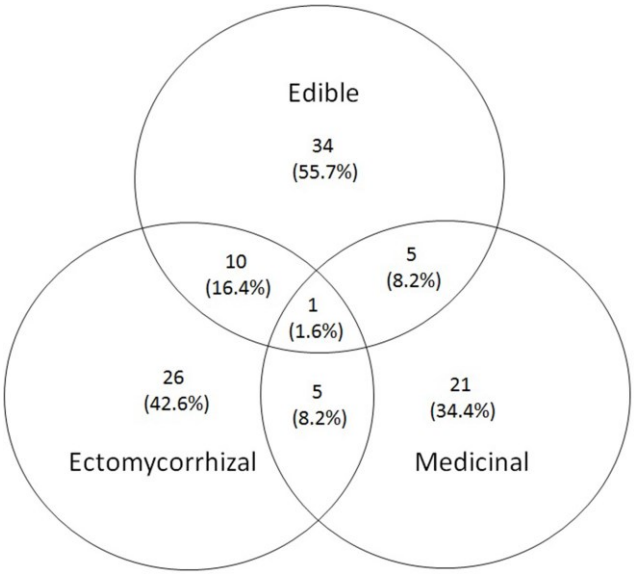

Fig. 3. Comparison of edible, medicinal and ectomycorrhizal macrofungi in coastal region.

Substrate and traditional knowledge

Substrate and substrate quality play important role in supporting macrofungi. Soil (lateritic soil, humus, compost, sandy loam, termite mound, ant-infested soil and decomposing leaf / woody litter mixed soil), fragile substrates (leaf litter, grass shreds, bark, twig, fine roots, grass and ferns) and stable (branch, trunk, stubs and standing dead trees) support a wide range of macrofungi. Besides dead substrates, live roots of many tree species mutualistically associated with ectomycorrhizal fungi. Thirty four edible macrofungi (20 genera) in coastal region grew on soil, woody litter and some were ectomycorrhizal. Twenty six ectomycorrhizal fungi (17 genera) were dependent many tree species especially *Anacardium occidentale, Artocarpus hirsutus, Carya arborea, Holigarna arnottiana, Hopea parviflora, H. ponga, Macaranga peltata, Phyllanthus emblica, Sapium insigne, Syzygium cmmini* and *Terminalia paniculata*. Twenty one medicinal fungi (15 genera) were recorded in coastal region and many of them grew on woody material. A few endemic trees of *Vateria indica* (Dipterocarpaceae) in scrub jungles served as host for two edible and ectomycorrhizal fungi (*Russula adusta* and *R. atropurpurea*) (Pavithra *et al*., 2017).

Several macrofungi serve as delicacy for the local people based on their traditional knowledge (e.g. *Astraeus* spp. *Pleurotus* spp. and *Termitomyces* spp.) (Pavithra *et al*., 2015; Karun and Sridhar, 2016). Termitomycetes are most preferred owing to their high delicacy followed by *Pleurotus* spp. and *Astraeus* spp. *Pleurotus* spp. grow profusely on the fronds of coconut (*Cocos*) and areca (*Areca*). *Astraeus* spp. occupy the lateritic pebble rich soil and tender fruit bodies develop below 1-2 cm (Pavithra *et al*., 2015). Being ectomycorrhizal they grow more profusely underneath several native tree species (e.g. *Artocarpus hirsutus, Holigarna arnottiana, Hopea parviflora, H. ponga, Phyllanthus emblica* and *Syzygium cumini*). Many of them are collected and eaten in tender stage based on traditional knowledge by the local people. Similar to *Astraeus* spp., *Amanita* sp. also occupies the pebble rich lateritic soils and it is ectomycorrhizal on several host tree species (e.g. *Acacia auriculiformis*, *A. mangium*, *Anacardium occidentale, Hopea ponga* and *Terminalia paniculata*) (Karun and Sridhar, 2014). This mushroom will also be eaten when it is in young stage especially prior to opening of volva or partially opened stage (Karun and Sridhar, 2016).

Table 2. Edible, mycorrhizal and medicinal macrofungi documented in coastal region of southwest Karnataka (*, edible, ectomycorrhizal and medicinal).

Edible	Ectomycorrhizal	Medicinal
Agaricus sylvaticus	*Amanita angustilamellata*	*Agaricus sylvaticus*
Amylosporus campbellii	*Amanita aureofloccosa*	*Amauroderma conjunctum*
Astraeus hygrometricus	*Amauroderma conjunctum*	*Amylosporus campbellii*
Astraeus odoratus	*Astraeus hygrometricus*	*Daldinia concentrica*
Auricularia auricula	*Astraeus odoratus*	*Ganoderma applanatum*
Auricularia auricula-judae	*Boletus edulis*	*Ganoderma colossus*
Boletus edulis	*Boletus hongoi*	*Ganoderma lucidum*
Boletus hongoi	*Boletus reticulatus*	*Lentinus betulina*
Boletus reticulatus	*Clavulinopsis dichotoma*	*Lentinus polychrous*
Collyba aurea	*Entoloma brihadum*	*Lentinus squarrosulus*
Coprinus plicatilis	*Entoloma vanajum*	*Lycoperdon lividum*
Dacaryopinax spathularia	*Hygrocybe astatogala*	**Lycoperdon utriforme*
Lentinus patulus	*Hygrocybe aurantioalba*	*Microcarpus xanthopus*
Lentinus squarrosulus	*Inocybe petchii*	*Phallus indusiatus*
Lenzites elegans	*Laccaria laccata*	*Pycnoporus sanguineus*
Lepista hyalodes	*Leucoagaricus rubrotinctus*	*Scleroderma citrinum*
Lycoperdon decipiens	**Lycoperdon utriforme*	*Scleroderma verrucosum*
**Lycoperdon utriforme*	*Macrolepiota dolichaula*	*Termitomyces microcarpus*
Macrolepiota dolichaula	*Macrolepiota rhacodes*	*Trametes versicolor*
Macrolepiota rhacodes	*Pisolithus albus*	*Xylaria hypoxylon*
Oudemansiella canarii	*Russula adusta*	*Xylaria nigripes*
Panus conchatus	*Russula atropurpurea*	
Phallus indusiatus	*Scleroderma citrinum*	
Phallus merulinus	*Scleroderma verrucosum*	
Pleurotus djamar	*Xylaria nigripes*	
Pleurotus flabellatus	*Thelephora palmata*	
Russula adusta		
Russula atropurpurea		
Termitomyces clypeatus		
Termitomyces microcarpus		
Termitomyces shimperi		
Termitomyces striatus		
Termitomyces umkowaan		
Tremella reticulata		

Disturbance and conservation

Human interference is most threatening to macrofungi of the coastal region. Macrofungi are habitat dependent, thus habitat destruction has major impact on their growth and perpetuation. Eliminating the host tree species by clear cutting leads to eradicate tree dependent mycorrhizal fungi. Ecosystem degradation is happening in alarming rate mainly due to clear cutting and transformation of forests (sand dune forests, mangrove forests and river valley forests) into plantations (e.g. areca, coconut and rubber). The habitats of native tree species are occupied by *Acacia spp*. Due to present government policies farmers are afraid of losing buffer zones adjacent to their agricultural lands (which supply organic matter, green manure and other products in support of

agriculture and their livelihood) and started practicing clear cutting of *Acacia* trees. Along with *Acacia* trees native trees are also eliminated and thus scrub jungles succumb for severe soil erosion. Due to such devastating activities, there is severe threat for coastal agroforestry as well as agriculture.

In addition to soil erosion, extraction of fire wood results in depletion of macrofungal substrates. There will be a drastic shift in substrate preference (leaf litter, soil and woody litter) as well as type of macrofungi (edible, medicinal and mycorrhizal fungi) owing to the impact of fire episodes in scrub jungles during summer. Due to fire, ectomycorrhizal fungi reduced from 54% to 15% in scrub jungles (Greeshma *et al*., 2016). However, those fungi colonized the roots underneath the soil escape from the impact of fire and perpetuate on the onset of wet season. Similar to scrub jungles, coastal sand dunes are also threatened by fire due to human impact (clearing vegetation and accumulated debris for recreation purposes) and its impact on macrofungi is yet to be systematically investigated (Ghate *et al*., 2014; Ghate and Sridhar, 2016a).

Many soil and termite dependent macrofungi in plantations suffer due to application of pesticides and weedicides. There is a need to change the cultivation practices in plantations towards agroforestry / silvi-culture in favour of macrofungi. Conservation or cultivation of native tree species has major impact on ectomycorrhizal as well as other fungi. In addition, prevention of soil erosion, retention of termite mounds and allowing minimum woody litter on the ground support growth and perpetuation of several macrofungi. Many of the lateritic scrub jungles have been converted into quarries for extraction of laterite stones and they will be abandoned without proper revegetation. Similarly, coastal sand dunes, mangroves and river mouths are severely affected by sand mining, which is threatening the existing forest cover.

Outlook

Macrofungi being alternate source of nutrition / medicine / metabolites and ectomycorrhizal with valuable tree species needs more attention for their utilization. The coastal ecosystem is endowed with a variety of habitats like lateritic scrub jungles, mountain slopes and valleys, rocky escarpments, sacred groves, mangroves, coastal sand dunes, plantations and grass lands. In spite of human interference, many interesting and stress tolerant macrofungi survive in the coastal region. Some of them were not identified up to species level in coastal region indicates further scope for macrofungal research. Under the existing climatic conditions coastal region supports a variety of edible, medicinal and ectomycorrhizal fungi. Impact of human interferences on macrofungi needs further emphasis to understand their assemblage, diversity and distribution. Coastal habitats posses a variety of feed stock to grow macrofungi. For example, utilization of grasses (e.g. *Pennisetum),* weeds (e.g. *Eupatorium* and *Lantana)* and leaf litter (of several deciduous trees) is worth to cultivate desired macrofungi. Educating the public towards practice of environment friendly agroforestry /silviculture and change in the policies of government towards ecosystem protection is utmost important to preserve the ecosystem. The local educational institutes shoulder major responsibilities in educating people towards sustainable development by showcasing the biodiversity exists in their surroundings.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Structural diversity of AM Fungi in the roots of *Lantana camara* and *Stachytarpheta indica*

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Sunita Chahar (2018) Structural diversity of AM Fungi in the roots of *Lantana camara* and *Stachytarpheta indica*, *Int. J. of. Life Sciences*, Special Issue, A9: 43-48

ABSTRACT

The structural diversity of AM Fungi in the roots of *Lantana camara* and *Stachytarpheta indica* was studied. Soil samples and the roots were collected from approximately20 cm below the soil surface of the two plants present in the NES Ratnam College campus, Bhandup (West), Mumbai, Maharashtra, India . The plants were sown approximately a year ago in the college garden. Both the plants are perennial shrubs and nectar plants for several species of butterflies belonging to family verbenaceae. The AM Fungal spore density was checked in the soil samples and the spore types were identified. The spore density in *Stachytarpheta indica* was 91.34 ± 6.5 and in *Lantana camara* it was 122.67 ± 9.45. *Lantana camara* showed dominance of *Glomus* species spores where as *Stachytarpheta indica* showed dominance of *Acaulospora* species spores in the rhizosphere soil. Hence a thorough study of the AM structures found in the roots was done. The anatomical study of roots revealed that *Lantana camara* showed 95 % root colonization with thin darkly stained hyphae and oval vesicles where as *Stachytarpheta indica* showed 90% root colonization, thick, lightly stained, coiled hyphae with arbuscules and vesicles with oil globules.

Key words: AM Fungi, *Lantana camara, Stachytarpheta indica, Glomus, Acaulospora.*

INTRODUCTION

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). AMF 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 AMF communities.

Diversity of AMF 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 AMF. Hyphae within root also show different structures. Gallaud (1905) observed that VAM associations in different species formed two distinctive morphology types, which he named the *Arum* and *Paris*s eries after host plants. **Linear** (*Arum*) series associations hyphae proliferate in the cortex by growing longitudinally between host cells. This occurs because hyphae grow through longitudinal intercellular air spaces that are present. **Coiling** (*Paris*) series hyphae spread by forming coils within cells because there are no continuous longitudinal air spaces. **Arbuscules** (tree shaped structures) Arbuscules are intricately branched haustoria that are formed within a root cortex cell. Arbuscules are considered the major site of exchange between the fungus and host. Arbuscules are short-lived and begin to collapse after a few days, but hyphae and vesicles can remain in roots for months or years. **Vesicles** develop to accumulate storage products in many VAM associations. Vesicles are initiated soon after the first arbuscules, but continue to develop when the arbuscules senesce. Vesicles are hyphal swellings in the root cortex that contain lipids and cytoplasm. These may be inter- or intracellular in active mycorrhizae (Bago *et al*., 1998). *Gigaspora, and scutellospora* do not form vesicles within the roots .

Mycorrhizas produced by *Glomus* show relatively straight hyphae that ramify along the root cortex (if root anatomy permits), often producing "H" branches which result in simultaneous growth in 2 directions. Staining of these hyphae is usually relatively dark, arbuscules can be dense and compact. Oval vesicles, which usually form between root cortex cells, are present in many cases. These vesicles persist in roots and often develop thickened and/or multi-layered walls. Intraradical spores in Glomaceae are usually globose, subglobose to elliptical.

Mycorrhizas produced by *Acaulospora* show hyphae that are more irregularly branched, looped or coiled than for *Glomus*. Internal hyphae are thin walled, often stain weakly and thus may be very hard to see, but may be visible due to rows of lipid droplets. Intracellular oil-filled vesicles, that are initially rectangular, but often become irregularly lobed due to expansion into adjacent cells, are a characteristic feature. Intraradical spores in *Acaulospora* are pleomorphic, knobby and stain lightly in trypan blue.(https://mycorrhizas.info/vam.html).

MATERIAL AND METHODS

Soil sampling : Root samples and rhizosphere soil of *Lantana camara* and *Stachytarpheta indica* was collected from NES Ratnam College campus, Bhandup (West), Mumbai, Maharashtra, India. Soil sample upto20 cm depth was collected.

Spore extraction (Gerdeman and Nicolson,1963)
The soil samples were subjected to wet-sieving and decanting technique for the isolation of spores. The isolated spores were picked up with the needle under a dissecting microscope and were mounted in polyvinyl lactoglycerol and observed under compound microscope. The spore number was counted by Gaur and Adholeya method ,1994.

Taxonomic identification of spores was done by descriptions provided by the www.invam.caf.wvu.edu. andwww.zor.zut.edu.

Root Colonization of AM Fungi (Philips and Hayman,1970**)**
Root samples were subjected to root clearing and staining technique in which the root samples were cut into 1cm bits and then cleared with 10% KOH for one hour, rinsed with distilled water and cleared with 5N HCl for 3min, and stained with 0.05% trypan blue in

Lactophenol and percentage of root colonization was calculated by Read et.al,1976.

RESULTS AND DISCUSSION

Root Colonization in *Lantana camara* & *Stachytarpheta indica*

The mycorrhizal root colonization was 95% in *Lantana camara*. Themycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were seen. Vesicles were of elliptical shape. The hyphae were of arum type (Linear). Other endophytes were not observed. The photographs of the roots are shown in Figure 1. The mycorrhizal root colonization was 90% in *Stachytarpheta indica*. Themycorrhizal structures observed were coiled, lightly stained mycelium, oval vesicles with prominent oil globules and highly coiled arbuscules. The photographs of the roots are shown in Figure 2.

Species Composition in *Lantana camara* & *Stachytarpheta indica*

The AM spore density was 122.67± 9.45/10g soil in *Lantana camara* and 91.34 ± 6.5/10g soil in *Stachytarpheta indica*. Two genera were identified, *Glomus* and *Acaulospora*. The spores were identified on the basis of their morphological characteristics. The dominant species in *Lantana*was *Glomus* (6 species) and few spores of *Acaulospora* (3 species) were also identified(Figure1).The dominant species in *Stachytarpheta* was *Acaulopspora* (8 species) and *Glomus* were less in number (3 species) (Figure 2).

AM Fungi have been described as keystone mutualists in ecosystems due to their unique position at the root-soil interface (Aditya Kumar et.al 2010).The present study was carried out to study the structural diversity of AM Fungi associated with the roots of *Lantana camara* and *Stachytarpheta indica*. The above results of *Lantana* showing darkly stained, thin walled hyphae which show H-shaped connections are produced by *Glomus* species is confirmed from the website Mycorrhizal associations (Brundrett *et al*. 1985, Brundrett & Kendrick 1988, Brundrett *et al*. 1996, https://mycorrhizas.info/vam.html). The structures seen in the roots of *Stachytarpheta* are lightly stained mycelium, coiled arbuscules and oval vesicles with oil globules are produced by *Acaulospora* species is also confirmed from the website of Mycorrhizal associations. They have also described the similar structures produced by *Acaulospora* species. From the above study differences in the structures of mycelium between *Glomus* and *Acaulospora* was confirmed. Since *Lantana* and *Stachytarpheta* are ecologically important plants, the knowledge of mycorrhizal status will be of immense importance to the researchers.

Table 1: AM Fungal status of *Lantana camara and Stachytarpheta indica*

Plant name	Mean Spore Density	% Root Colonization	Mycorrhizal Spores isolated	AM Structures Observed		
				Arbuscules	Vesicles	Hyphae
Lantana camara	122.67 ± 9.45	95%	*Glomus* (6 Species) *Acaulospora* (3Species)	+ Arum type	+++ Oval, Spherical	+++
Stachytarpheta indica	91.34 ± 6.5	90%	*Glomus* (3 Species) *Acaulospora* (8 Species)	+++ Coiled type	++ Oval with oil globules	+++

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

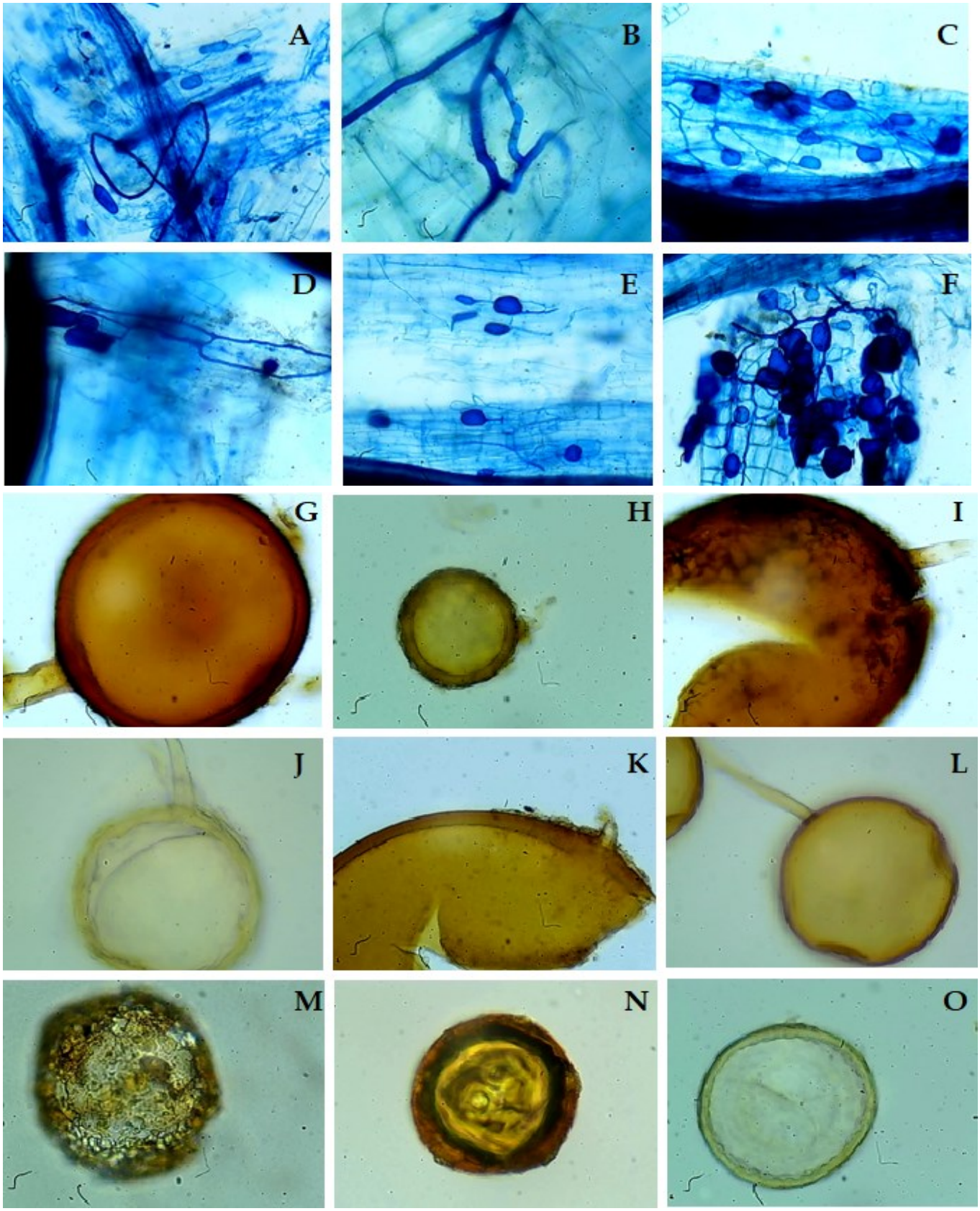

Fig. 1. A-F :*Lantana camara* root showing linear type hyphae with H shaped hyphal connections, oval & spherical vesicles,Spore types in rhizosphere soil **G - L** : *Glomus* species, **M - N** : *Acaulospora* species **O** : *Glomus* species

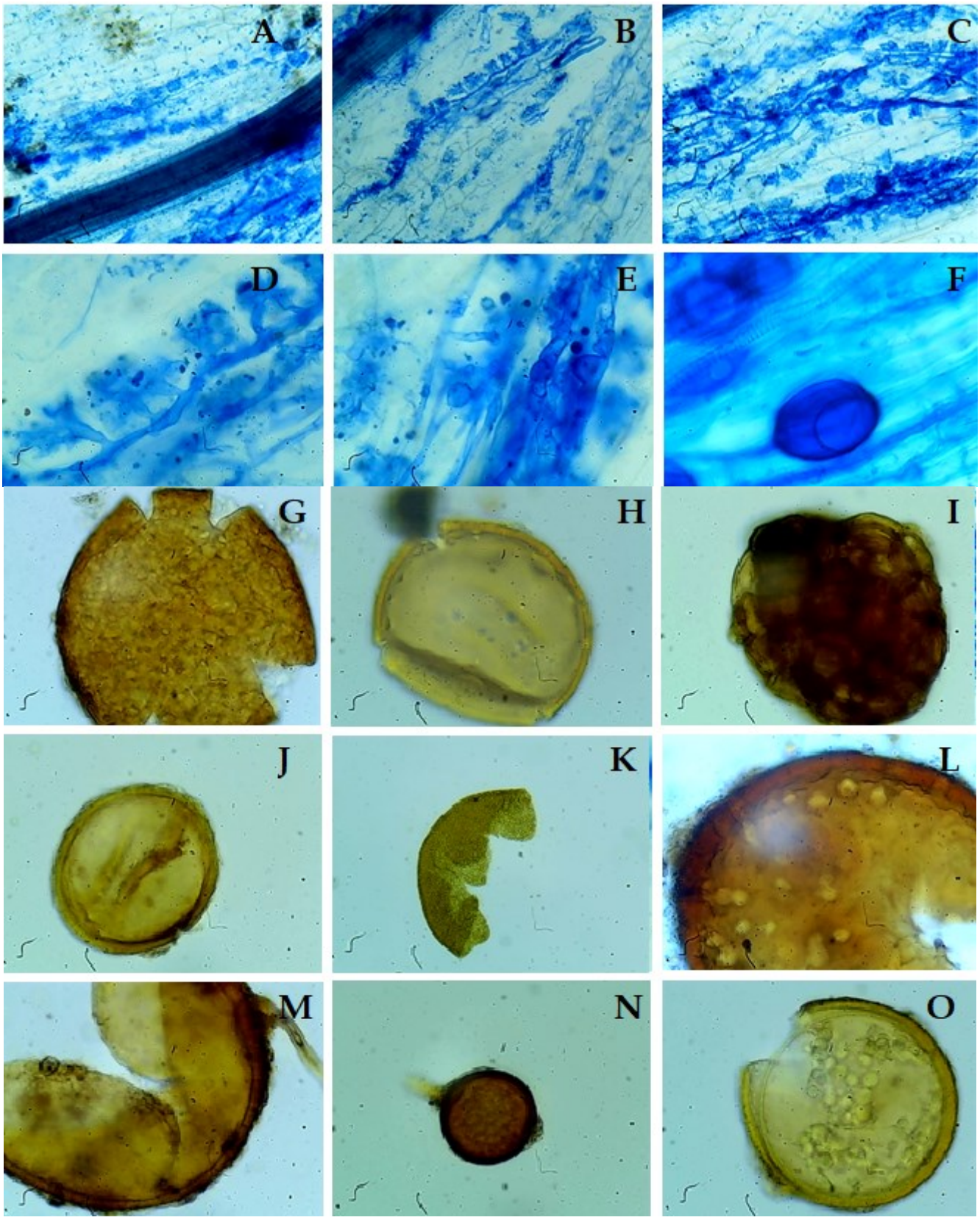

Fig 2. A-F : *Stachytarpheta indica* root showing hyphae with prominent arbuscules, hyphae lightly stained, oval vesicles with oil globules, Spore types in rhizosphere soil G - L : *Acaulospora* species M - O : *Glomus*species,

Acknowledgements

I thank University Grants Commission, Western Region, Pune, India for providing the financial support. I also thank the College for providing the laboratory facilities.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article | Open Access

In vitro efficacy of *Trichoderma harzianum* against major fungal pathogens of Teak and Mahogony seedlings

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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:
Nair Bharath* and Mallikarjunaswamy GE (2018) *In vitro* efficacy of *Trichoderma harzianum* against major fungal pathogens of Teak and Mahogony seedlings, *Int. J. of. Life Sciences*, Special Issue, A9: 49-54.

ABSTRACT

Trichoderma an asexually reproducing filamentous fungi commonly found in varied soil types in all climatic zones. Their ability to grow and multiply rapidly in various substrates makes it a good biocontrol agent. Six isolates of *Trichoderma harzianum* from rhizoplane regions of grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) were tested for their antagonistic activity against major fungal pathogens - *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off and *Colletotrichum gloeosporiodes, Curvularia lunata* and *Pestalotiopsis macqulens* causing foliar diseases of Teak and Mahogany seedlings from Central Nurseries of Kerala by dual culture method. All the isolates showed antagonistic activity against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the *T. harzianum* isolates tested PC-03 was found to exhibit minimal antagonistic activity. However, *Trichoderma* – Pathogen interaction showed variations indicating the activity of *Trichoderma* isolates varied to a greater or lesser extent depending upon the pathogenic species.

Keywords: Fungal diseases, *Trichoderma harzianum*, Rhizoplane and Antagonism

INTRODUCTION

Teak (*Tectona grandis* L.) and Mahogony (*Swietenia macrophylla* King) are mainly known for their timber and a number of nurseries have been established for producing healthy plant stocks. A number of factors affect their successful out plantings. Among seedling diseases are the major ones of which fungi being the primary pathogenic agent (Bakshi, 1976; Bloomberg, 1985). Rots, Wilts, damping off and various foliar

diseases are the major diseases found in seedlings. Chemical agents have been practised for their assured results but indiscriminate use has resulted in environmental pollution. Alternative strategy has been in search there by minimise pollution and other hazards caused by chemicals.

Biological control is being applied as an alternative and a number of microbes have been found to show potentiality as bio-controlling agents. Microbes can be found on the leaves, roots, soil adjacent to roots and even inside plant tissues as endophytes and their interactions provide a wide array of opportunities to explore the complexities in association as well as their interaction in the growth and development. Rhizosphere and Rhizoplane inhabiting micro-organisms competitiveness for water, nutrients and space plays an important role in the growth and ecological fitness of their host (Hartmann *et al*. 2009).

Trichoderma a filamentous fungi have been extensively studied for its potentiality as an antagonistic agent (Henis and Chet, 1975; Hadar *et al.,* 1979 and Elad *et al.,* 1980). Their ability to successively thrive in diverse environment and easy to isolate the species makes it an important biocontrol agent. Besides antagonising, their role in plant growth promotion and inducing defence mechanism have also been reported (Harman *et al.,* 2004 and Vinale *et al.,* 2009).

Grasses form an important component of ecosystem which keeps rejuvenating with each growing season. They produce fibrous roots which homes abundance of diverse microbes. A great diversity of rhizosphere and rhizoplane microorganisms have been described and also in many cases been used as bio-control agents. The present work has been carried out to study the antagonistic activity of rhizoplane fungi *Trichoderma* against Fungal pathogens of Teak and Mahogany seedling diseases from Central Nurseries of Kerala *in vitro*

MATERIALS AND METHODS

Isolation of pathogenic fungi

Disease survey have been carried out in Central nurseries of Kerala located at Cheruvanchery, Valluvassery, Chettikulam and Kulathupuzha. Infected samples were collected and were taken to the laboratory. The samples were washed thoroughly, blotted and were inoculated on antibiotic amended PDA medium. The pathogen was isolated and identified by referring to standard manuals (Arx, 1981; Barnett and Hunter, 1972; Domesch and Gams, 1972; Ellis and Ellis, 2001 and Gilman, 1994).

Isolation of *Trichoderma* sp. from rhizoplane region

Trichoderma sp. were isolated from roots of grass species *Cynodon dactylon* and *Paspalum conjugatum.* Root samples were collected from Northern Kerala parts of Western Ghats, washed in slow running tap water, blotted and were fragmented into 1 cm long segments. Root segments were inoculated on antibiotic amended PDA medium. Fungal colonies were isolated and identified by referring to standard manuals as described earlier.

***In vitro* antagonism by dual culture technique**

Pathogenic fungi isolated from Teak and Mahogany seedlings and test rhizoplane fungi from grasses *Cynodon dactylon* and *Paspalum conjugatum* were cultured on their respective medium under 12/12 hr light and dark cycle at 23±2^{o}C for five days. Five mm diameter disc of selected fungi from grass and test pathogen were taken from the growing edge of a five-day-old pure culture using a cork borer. The control plates were inoculated with the pathogen and antagonists separately. Petri-dishes were incubated at 23±2^{o}C and daily growth measurements of fungal colonies were recorded for seven days. The percentage inhibition of radial growth of the pathogen was calculated using a formula by Vincent (1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control

R_2 – Test organism in Dual culture

Statistical analysis

Antagonistic ability of *Trichoderma* isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

Evaluation of Antagonism

The evaluation of antagonism between the *Trichoderma* and the test pathogen was scored 1-5 (Bell *et al*., 1982). The cultures were observed after seven days of incubation. The given isolate of *Trichoderma* was considered to be antagonist if the score was ≤ 2 and not highly antagonist if the score was ≥ 3.

Colony Interaction	Type of Antagonism
Complete overgrowth of the antagonist over the pathogen	1
75% overgrowth of the antagonist over the pathogen	2
Both the antagonist and the pathogen grow 50% and neither organism dominate	3
75% overgrowth of the pathogen and withstand antagonism	4
Complete overgrowth of the pathogen	5

RESULTS AND DISCUSSION

Disease survey conducted in the Central Nurseries and incubation of samples for the associated pathogens resulted in the isolation of *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off, *Colletotrichum gloeosporiodes* causing leaf spots and blights, *Curvularia lunata* causing leaf spots and *Pestalotiopsis macqulens* causing leaf spots to be major symptoms associated with Teak and Mahogany seedlings. *Trichoderma harzianum* isolated from grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) (Table-1) (Fig-1) were tested for their antagonistic activity against Teak and Mahogany fungal pathogens (Table 2) (Fig-2). The isolates showed inhibition against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the isolates PC-03 was found to exhibit minimal inhibitory activity.

Table 1. Morphological characteristics of *Trichoderma harzianum* isolates isolated from rhizoplane regions of grasses

SL. no.	Grass species and *T. harzianum* Isolate No.	Culture characteristic
1	*Cynodon dactylon* CD-01	Colony initially white with 11mm growth per day later turning into yellow and finally to green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
2	*Cynodon dactylon* CD-02	Colony initially white with 10mm growth per day later turning into green. Reverse light coloured. Phialides (8-11 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
3	*Cynodon dactylon* CD-03	Colony initially white with 14mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-8 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
4	*Paspalum conjugatum* PC-01	Colony initially white with 10mm growth per day later turning into yellow and finally to light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
5	*Paspalum conjugatum* PC-02	Colony initially white with 11mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
6	*Paspalum conjugatum* PC-03	Colony initially white with 11mm growth per day later turning into light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)

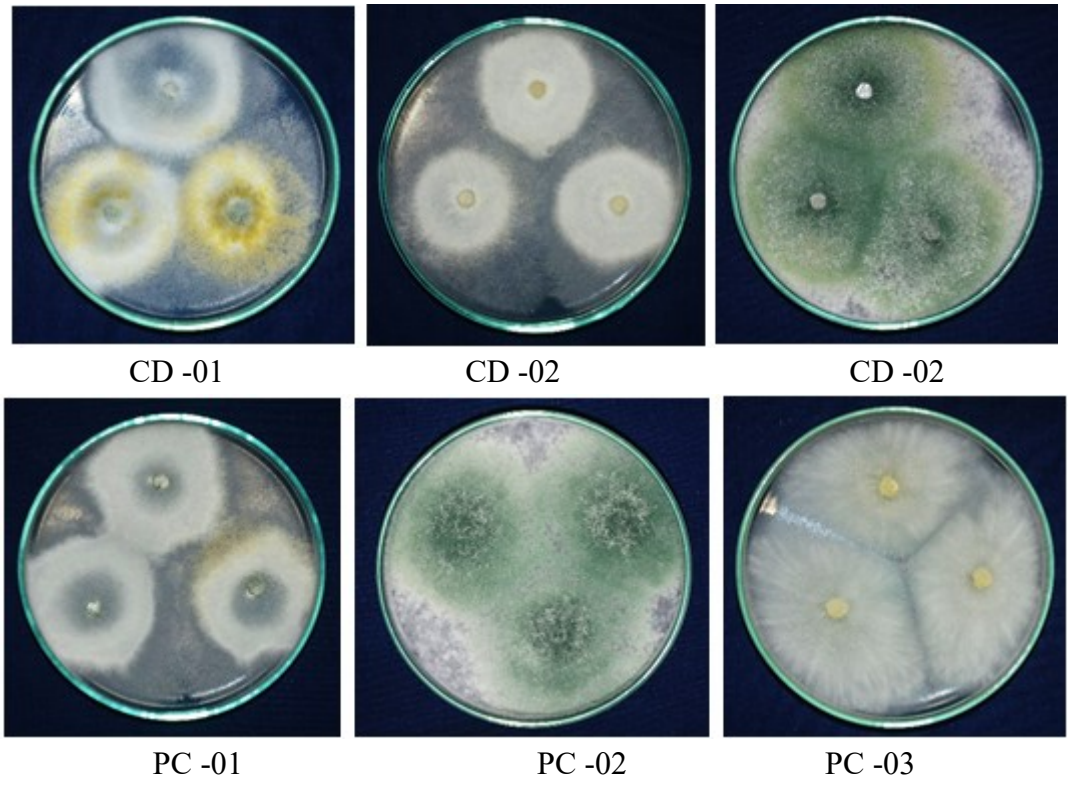

Fig 1. ***Trichoderma harzianum*** **isolates at Five days of incubation**

Table 2. Antagonistic activity of ***Trichoderma*** **isolates and reaction types against fungal pathogens**

Trichoderma harzianum isolates	Percent inhibition and colony interaction types against fungal pathogens											
	F O		*F S*		*S R*		*C G*		*C L*		*P M*	
	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT
CD-01	69.23 ± 0.48[1] e[2]	-	68.57 ± 0.78[1] b[2]	1	60.00 ± 0.23[1] c[2]	3	75.00 ± 0.54[1] c[2]	1	58.82 ± 0.78[1] b[2]	1	61.90 ± 0.36[1] a[2]	1
CD-02	57.69 ± 0.56[1] b[2]	-	60.00 ± 0.86[1] a[2]	1	60.00 ± 0.36[1] c[2]	3	75.00 ± 0.16[1] c[2]	2	64.70 ± 0.54[1] d[2]	1	66.60 ± 0.42[1] a b[2]	1
CD-03	65.38 ± 0.96[1] d[2]	-	68.57 ± 0.18[1] b[2]	1	62.20 ± 0.45[1] d[2]	3	75.00 ± 0.77[1] c[2]	1	70.50 ± 0.49[1] e[2]	1	64.28 ± 0.13[1] a b[2]	1
PC-01	61.53 ± 0.98[1] c[2]	-	71.43 ± 0.73[1] c[2]	1	62.20 ± 0.95[1] d[2]	3	72.50 ± 1.10[1] b[2]	1	64.70 ± 0.76[1] d[2]	1	64.28 ± 0.47[1] a b[2]	1
PC-02	57.69 ± 0.53[1] b[2]	-	71.43 ± 0.73[1] c[2]	1	51.10 ± 0.57[1] b[2]	3	72.50 ± 0.87[1] b[2]	1	61.76 ± 0.83[1] c[2]	1	64.28 ± 0.49[1] a b[2]	2
PC-03	53.84 ± 0.69[1] a[2]	-	60.00 ± 1.13[1] a[2]	2	-	5	52.50 ± 0.69[1] a[2]	2	47.05 ± 0.62[1] a[2]	3	64.28 ± 0.77[1] b[2]	3

Data is an average of three replicates

*RT - Reaction Type [1] Standard deviation

[2] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

F O- Fusraium oxysporum, F S- Fusarium solani, S R- Sclerotium rolfsii, C G- Colletotrichum gloeosporioides, C L- Curvularia lunata, P M- Pestalotiopsis macqulens

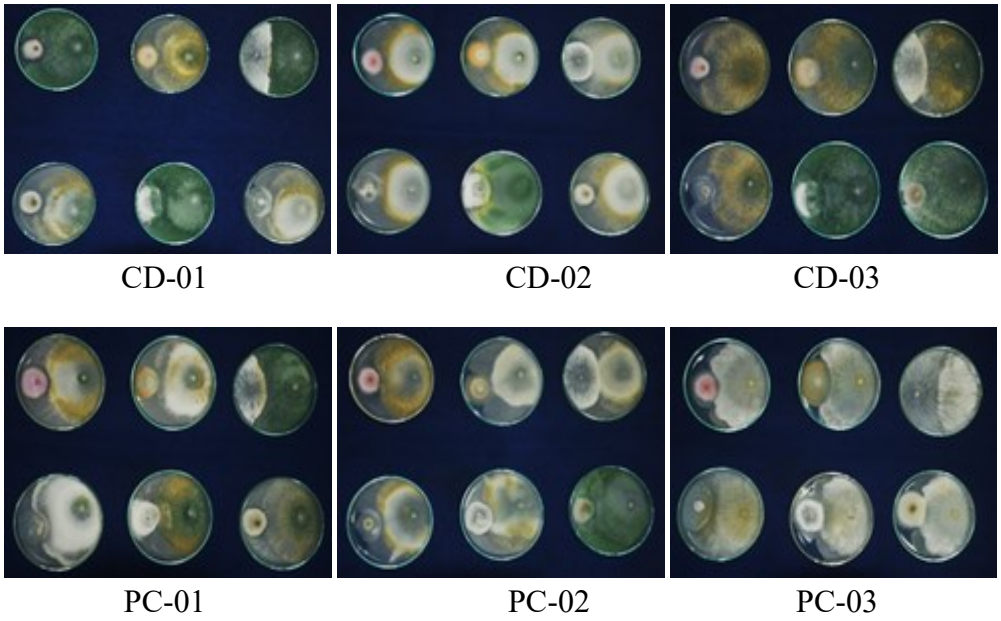

Fig 2. Antagonistic activity of *Trichoderma harzianum* isolates against fungal pathogens *Fusraium oxysporum, Fusarium solani, Sclerotium rolfsii, Colletotrichum gloeosporioides, Pestalotiopsis macqulens and Curvularia lunata*

Various species of *Trichoderma* namely *T. koningii*, *T. harzianum* and *T. viride*, respectively have been studied for their antagonistic activity *in vitro* (Mathew and Gupta, 1998; Prasad *et a*l., 1999; Bunker and Mathur, 2001; Pandey *et al*., 2005; Grosch *et al*., 2007). *Trichoderma* isolates also varied in their reaction types, this was evident in the case of *Sclerotium rolfsii*. Among the root pathogens *F. solani* was more susceptible to the antagonist and this was also evident with the interaction type as the antagonists were able to completely overgrow the pathogen. *Sclerotium rolfsii* exhibited an interaction type where both the pathogen and the antagonist grew 50% and neither dominated on each other except for PC-03 where the pathogen was able to over grow the antagonist. In case of *F. oxysporum* zone of inhibition was observed. In case of foliar pathogens all the species were susceptible to the antagonist. This was also evident with the reaction type as all the antagonists were able to completely overgrow the pathogens. A vast variety of microbes have the ability to be potentially used as biocontrol agent but the selection of an appropriate isolate forms an important aspect for its success in field application. The present work showed the potentiality of rhizoplane mycoflora and its efficacy against various forest plant pathogens and can be further analysed for its use as an alternative to chemical fungicides.

CONCLUSION

Trichoderma harzianum exerted good antagonistic activity against all the pathogens studied and makes this species as a biocontrol agent which can be used as an alternative to chemicals. The variations among the isolates stressed on the selection of effective isolate and needs a series of steps in their appropriate application for their infield success (Ravensberg, 2011). Rhizoplane regions of grasses homes diverse fungal organisms and can be used as biological weapons against various plant pathogens.

Acknowledgement
The authors are grateful to Director, Kerala Forest Research Institute for encouragement and all the staffs of Central Nurseries of Kerala for their help during the survey.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Impact of pond deposit soils for improving Vigour index and AMF status of Soybean in renovated land

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Bhale UN and Bansode SA (2018) Impact of pond deposit soils for improving Vigour index and AMF status of Soybean in renovated land, *Int. J. of. Life Sciences*, Special Issue, A9: 54-61.

ABSTRACT

Glycine max (L.) Merr soybean is one of the important pulse and oilseed crops of India. It grows well during the *kharif* or monsoon season (July-October) in the dry land areas of peninsular India. Pond deposit soil had a rich source of humus, minerals and micronutrients. When it comes in contact with agricultural field then productivity would be increased. This study attributed consecutive two generation data from renovated agricultural land. In M_1 generation, when pond deposit soil amended with newly renovated land decreased the all studied factors than crop land soil+ pond deposit soil, while in M_2 generation it was significantly increased. In M_2 generation significant growth rate was observed in case of nodules, leaves, pods; shoot length, fresh and dry wt of shoot, leaf area and productivity. Length of shoot (87cm) & root (28cm) was increased in M2 generation as compared to cropland + pond deposit soil. In M_2 generation total leaf area are extensively increased due to prosperity of organic matter. Therefore, productivity of soybean was increased. Percent root Arbuscular mycorrhizal fungi (AMF) colonization and spore density was showed potential in both soils in both generations. AMF association was found but suppressed in M_2 generation in some extent. In both soils & generations, *Acaulospora, Gigaspora, Glomus, Entrophospora, Sclerocystis* and *Scutellospora* was found frequently but *Acaulospora, Sclerocystis* and *Glomus* genera was found dominant. Vesicular, arbuscular and hyphal root colonization was found in both soils of soybean fields.

Key words: *Glycine max*, Generations, renovated land, pond deposit soils, productivity, AMF association.

INTRODUCTION

Soybean is one of the important pulse and oilseed crops of India. It grows well during the *kharif* or monsoon, season (July-October) in the dry land areas of peninsular India. The region is rocky and dry with low and uncertain rainfall. The fertility index with respect to Nitrogen

and Potash varies in all the districts of Marathwada. Major crops in this region are Sorghum, Cotton, Pigeonpea, Sunflower, Groundnut, Beans and Sugarcane. Region also contributes to fruit crops like Banana, Orange, Grape, Mango, Papaya, Guava, Ber, Lime and vegetable crops like Tomato, Brinjal, Chilli, Cucurbits, Cauliflower, Cabbage, Onion, Garlic, Leafy Vegetables like Spinach, Fenugreek etc. Agriculture is a major source of income for about 70 % population of rural part. It is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. Fat free (defatted) soybean meal is a significant and cheap source of protein for animal feeds and many prepackaged meals; soy vegetable oil is another product of processing the soybean crop. Arbuscular mycorrhizae (AM) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection, and soil quality. The effectiveness of a mycorrhiza in improving plant growth appears to be governed by the interplay between edaphic factors, the host plant, edaphic factors, and the fungal isolate **(**Bethlenfalvay *et al*.,1985). Government of Maharashtra noticed farmers living on bank of ponds and river to lift the slit soil deposited in pond and river for their agricultural purpose. Soil is a natural resource with crucial ecological, economic and social function. Soil is the essential component of the terrestrial environment and forms the interface between geosphere, atmosphere, hydrosphere and biosphere **(**Doran and Parkin, 1994) Soil provides the medium for the production of plant biomass for use as food, feed and fiber. The capacity of soil to supply sufficient quantities and proportions of essential chemical elements (nutrients) and water required for optimal growth of specified plants as governed by the soil's chemical, physical and biological attributes. Soil is the foundation of an agricultural field and mediates processes essential to the functioning of the system, including: biogeochemical cycling of elements such as carbon and other mineral nutrients; provision of habitat for soil organisms; movement, storage, and decontamination of water; and promotion of plant growth (Brady and Weil, 2002). Soil organic Matter (SOM) encompasses living microorganisms as well as plant and animal tissues in various stages of decomposition (Craswell and Lefroy, 2001).

MATERIALS AND METHODS

Study Site Description

The study was carried out two generation on soybean **(***Glycine max)* crop plant during *Kharif* or monsoon, season (July-October-2013 & 2014) from agricultural land of Naldurg (17.82^0N 76.30^0 E) Osmanabad districts of Marathwada region of Maharashtra. Naldurg is located at an altitude of 566 m and receives an average annual rainfall of 760 mm.

Physico-chemical parameters

Available Nitrogen was assessed by alkaline permanganate method by using Kjeldhal tube (Subbiah and Asija, 1956). Available Phosphorus in soil was determined by Olsens method by using spectrophotometer (Olsen *et al*, 1954) and Bray & Kurtz (1945). Water soluble and exchangeable Potassium was calculated by Ammonium acetate method of Hanway and Heidel (1952) using Flame photometer. Analysis of Ferrous, Copper and Zinc were done by acid digestion of soil (Jackson, 1967).

Biomass Production

Three plants were harvested 8 weeks after planting for soybean. At harvest, the soils from the roots were washed off carefully and the nodule number was counted visually. Fresh weight of root and shoot samples were recorded. Shoots (including fruit & flowers) and roots were separated and oven dried at 60°C for 48 h for the determination of dry mass after recording their lengths **(**Muthukumar and Udaiyan, 2000). Leaf area was measured at harvest by disc method by Vivekanandan *et al*.(1972).

Soil and Root Sampling

Soil samples and roots were collected from the rhizospheric region of soybean plant from pond (PS) soil amended with renovated land (LS) soil. The samples consisting of feeder roots + soil were collected with the help of a soil auger (0-25cm) so as to represent the complex root zone. Root systems of common plant species were excavated taking care to ensure that fine root predominates in the sample and to exclude entangled roots of other species. Sufficient samples were taken to determine, if there is any variation in the constituency and degree of mycorrhizal colonization roots between or within the

sampling sites. Roots were gently washed and immediately fixed in Formalin Acetic Acid Alcohol (FAA) in the field (Kormanik *et al*.,1980). Rhizospheric soil was collected in polythene bags and after drying stored at 4^0C.

Mycorrhizal study

Numerous techniques were available to recover AMF spores from soil. The most basis of this is wet sieving and decanting, which remove the clay, sand and organic matter fractions while retaining spores and other similar sized soil particles on sieves of various with stainless steel mesh (35, 63, 125,150 212 and 355µm). For the isolation, 100g of soil was weighed and is added to1000 ml of water taken in a conical flask. Then the flask was shaken well in a vortex mixture and allowed to sediment for few seconds and was immediately transferred to a series of sieves. The jar was washed twice with water and added in to sieves series. This sieving was collected in respected jars by washing with water. Then transferred the sieving on to a gridded petriplate and observed it under the binocular microscope 400X (Lawrence & Mayo LM-52-3521). The number of spores were counted and expressed as number of spores/100g of soil sample. These isolated spores were picked up using micropipette and were mounted in Poly Vinyl Lacto Glycerol (PVLG) to make permanent slides.

Root Colonization and Spore Density

Collected fresh roots were washed in tap water and cut into 1 cm pieces in length and cleared with 10% KOH and acidified in 5NHCL and stained with Trypan Blue (Phillips and Hayman, 1970). Root colonization percentage was measured according to the by following formula (Giovannetti and Mosse,1980) Hundred grams of rhizosphere soil from each sample was analyzed for spore isolation by wet sieving and decanting method (Gerdemann and Nicolson,1963). Identification of AM fungal species was done by using the manual (Schenck and Perez, 1990).

Statistical Analyses

The arcsine transformed values is used for biomass production meaning that if the difference between three values are different or above, then that difference is significant (McDonald, 2008).

RESULTS AND DISCUSSION

The study was carried out in two generation (Year 2013 &2014) on soybean *(Glycine max)* crop plant during *Kharif* or monsoon season and data represented following.

I. Physico-chemical parameters

Plant health is linked with soil health. Proper management of the soil by conserving and enhancing the soil biota improve crop yields and quality. During investigation, soil studied from two different sites i.e blackish & blackish red & it was found alkaline in nature with pH 7.21 & 7.80. EC responsible for movement of cataions and anions from soil to root was sufficient and ranging from 0.28 to 0.10 dS/m. Nitrogen, Phosphorus & potassium which is important factor for AMF development was deficient in renovated field. Zinc, Ferrous & copper was least increased in renovated field soil (Table 1).

Table 1. Physico-chemical Parameters of soil.

Sr. No	Parameters of soil	Naldurg sites	Renovated field
1	Soil type	Blackish	Blackish red
2	pH	7.31	7.80
3	EC (dS/m)	0.28	0.10
4	Nirogen (kg/ha)	205.84	232.06
5	Phosphrous (kg/ha)	51.32	15.20
6	Potassium (kg/ha)	616	470.86
7	Zinc (ppm)	1.78	1.110
8	Ferrous (ppm)	0.98	1.011
9	Copper (ppm)	0.84	0.98

Table 2. Biomass production of *Glycine max* renovated and crop land field in two generation.

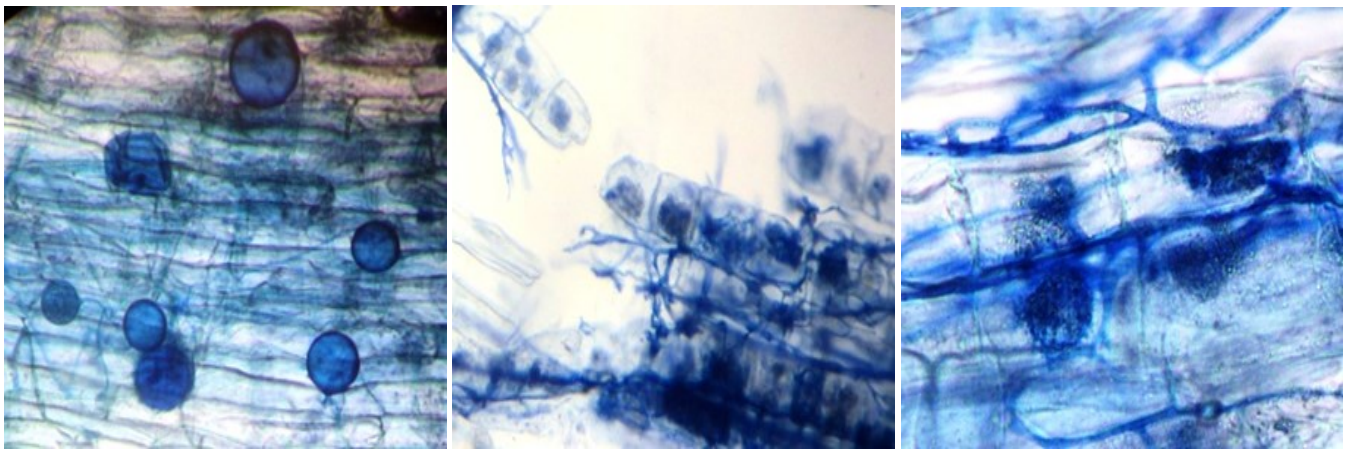
Sr. No.	Parameters	M_1 Generation (2013)		M_2 Generation (2014)	
		R L + PS	CS + PS	R L + PS	CS + PS
1	No of nodules	09 (12.33)	42 (47.51)	67 (70.12)	72 (75.11)
2	No of leaves	10.33 (12.33)	12.66 (14.23)	71 (76.33)	107 (114.54)
3	No of auxiliary branch	07.66 (8.11)	08 (10)	42 (47.11)	71 (76.33)
4	No of pods	61.66 (67.33)	154 (177.22)	103 (110.11)	240 (253.63)
5	Length of Shoot (cm)	52.83 (60.76)	63 (65.11)	87 (93.11)	65 (72.23)
6	Length of Root (cm)	26.66(28.54)	19.26 (23.34)	28 (32.22)	22 (27.27)
7	Fresh Wt of Shoot (g)	29.43 (32,22)	32.62 (34.11)	62.71 (71.11)	120.76 (134.34)
8	Fresh Wt of Root(g)	3.30 (7.11)	3.77 (4.23)	10.69 912.11)	19.38 (26.11)
9	Dry Wt of Shoot (g)	8.69 (10.11)	9.98 (12.33)	38.85 (43.11)	70.39 (74.22)
10	Dry Wt of Root (g)	1.51 (3.51)	1.61 (3.87)	4.07 (7.22)	9.02 (11.11)
11	Area of Total Leaves(cm^2)	2128.49	2449.03	3445.93	4867.16
12	Yield (Quintal/ha)	10 (14)	19 (24)	15 (18)	22 (27)

Legands :Values in parentheses are arcsine transformed values, RL=Renovated Land, PS=Pond Sedimentary Soil, CS= Cropland soil

Table 3. Myorrhizal association of *Glycine max* renovated and crop land field in two generations

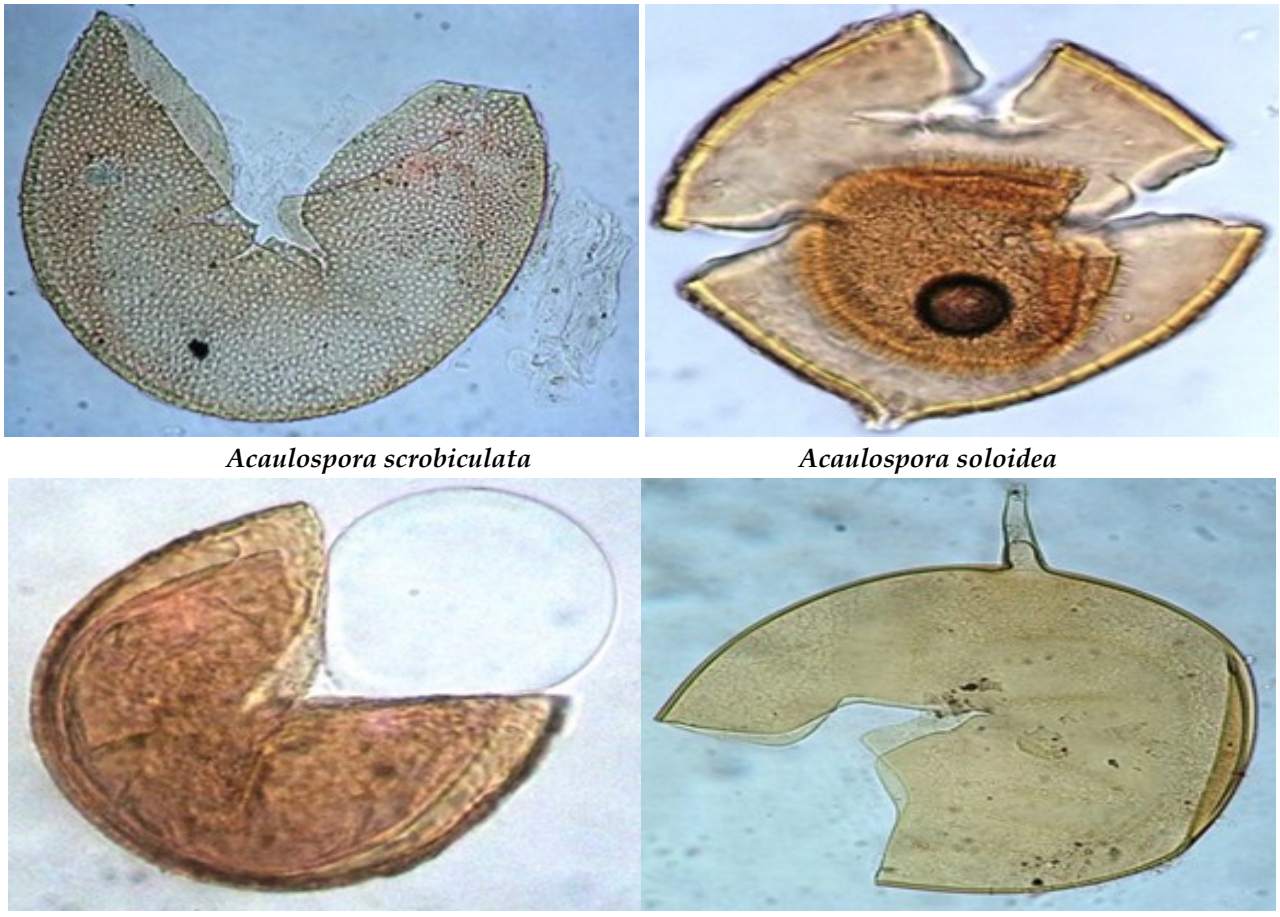
Sr. No.	Parameters	M_1 Generation (2013)		M_2 Generation (2014)	
		R L + PS	CS + PS	R L + PS	CS + PS
1	AMF Root colonization (%)	82.5 ± 2.11	80 ± 0.2525	58.21±10.1	63.75 ±3.0
2	AMF spore Density (per 100g)	730 ± 0.33	735 ±1.1	215 ±21.11	239 ±23.13
3	Type of colonization	Vesicles, Arbuscules & Hyphal	Vesicles, Arbuscules & Hyphal	Vesicles, Arbuscules & Hyphal	Vesicles, Arbuscules & Hyphal
4	Types of spores	*Acaulospora scrobiculata, Acaulospora soloidea, Acaulospora undulata and Glomus mosseae*			

Legands: RL=Renovated Land, PS=Pond Sedimentary Soil, CS= Cropland soil



Vesicles **Arbuscles** **Hyphal**

Fig.1. Types of AMF root colonization (400X magnification).



Acaulospora scrobiculata *Acaulospora soloidea*

Acaulospora morrowiae *Glomus mosseae*

Fig.2. Isolated and identified Arbuscular mycorrhizal fungal spores (400X magnification).

II. Biomass Productivity

This study accredited promising two generation consecutive data from newly renovated agricultural land. In M_1 generation, when pond sedimentary soil (PS) amended with newly renovated land decreased the all studied parameters than cropland soil + Pond soil (CS+PS), while in M_2 generation it was significantly increased. In M_2 generation significant growth rate was observed i.e. no of nodules, leaves, pods, shoot length, fresh wt & dry wt of shoot, leaf area and productivity. Length of shoot (87cm) & root (28cm) was increased in M_2 generation as compared to cropland + pond soil (CS+PS). Consecutively root systems are increased in both generations. In M_2 generation total leaf area are considerably increased due to richness of organic matter progressively. Number of pods (103 & 240) are considerably increased therefore productivity was increased in M_2 generation in both soils (Table 2).

III. Mycorrhizal Status

AMF colonization and spore density were found promising in both soils in both generations. AMF association was found but suppressed in M2 generation. Percent root mycorrhizal colonization (82.5 & 80%) was found in both soils but in M2 generation it was reduced (58.21 & 63.75%). Mycorrhizal spore density was also decreased in M2 generation. In tested soil more vesicles (V) and Paris type arbuscles (A) was developed Vesicular, arbuscular and hyphal mycorrhizal colonization was present in both soils and generations. Arbuscular type of colonization was dominant in treated soybean roots in both generations (Table 3; fig. 1 &2). *Acaulospora, Gigaspora, Glomus, Entrophospora* and *Scutellospora* were found frequently but *Acaulospora* and *Glomus* genera were found dominant i.e. *Acaulospora scrobiculata, Acaulospora soloidea, Acaulospora undulata and Glomus mosseae.*

Results observed during investigation was supported by Vyas and Vyas (2012) where spore density of AMF had a strong positive correlation with soil pH and organic carbon content and a negative correlation with Olsen's P content of the soil. Sreevani and Reddy (2004) studied relation between soil characters and occurrence of AMF where greater number of AM fungal propagules were found in neutral to slightly alkaline (pH 7 to 8) soil whereas alkaline soils (pH higher than 8.0) have not favored mycorrhizal fungi. Nutritionally deficient soils (zinc, copper, nitrogen, phosphorus and potassium) had greater number of AM fungal propagules whereas high levels of these nutrients inhibited the population of AM fungi.

The study was in accordance with earlier workers, Doran and Zeiss (2000) defined soil health as: "the continued capacity of soil to function as a vital living system, by recognizing that it contains the biological elements that are the key to ecosystem function within land use boundaries". The results showed that the VAM fungi helped to stabilize wind-borne soil that settled under dense canopies, enhanced the establishment of colonizer plants in bare soils of disturbed areas and influenced plant associations through differences in the mycotrophic status of the associates **(**Carrillo-Garcia *et al*., 1999). Large populations of *Glomus aggregatum* were associated with dense weed populations in a com-soybean sequence **(**Johnson *et al*.,1991). HarnerNora *et al*. (2011) studied abundance of AMF propagules (colonized roots, spores, and hyphae) within sediments of Tagliamento River in northeastern Italy; Root inoculums in fresh deposits were absent however; some viable spores and hyphae were available. Positive effects of AMF on host-plant growth and development were already observed in low soil fertility conditions and also in drought environments **(**Sylvia and Williams, 1991: Picone.2003 ;). Reclamation of River deposit soil when amended with renovated land decreased biomass productivity in soybean crop plant than crop land soil but cropland soil showed significant growth rate whereas infection of AMF root colonization & spore density found in both the soils (Bhale and Bansode,2013). Pond soil when amended with renovated land soil decreased biomass productivity than crop land soil in soybean crop plant but Cropland soil showed significant growth rate whereas infection of AMF root colonization & spore density found in both the soils (Bansode and Bhale,2014).

CONCLUSION

The results concluded that when sedimentary soil was amended in agricultural land, it was enhanced the growth parameter of Soybean and AMF infection. Sedimentary soil had rich source of humus, minerals and micronutrients and when contact with agricultural field then biomass would be increased even in M_2 generation.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Study of phylloplane fungi isolated from *Lagerstroemia speciosa* and their biochemical screening for alkaloid production

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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:
Ansari Rahat Ubaid and Kumar Sashirekha Suresh (2018) Study of phylloplane fungi isolated from *Lagerstroemia speciosa* and their biochemical screening for alkaloid production, *Int. J. of. Life Sciences*, Special Issue, A9: 62—68.

ABSTRACT

Phylloplane fungi are microbes that reside on the leaf surface without showing any disease symptoms. Plants produce several compounds to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses. Presence of phylloplane mycoflora and their associations with the leaf helps as potential defence and in enhancing the plant growth and productivity. The present paper focuses on the isolation and documentation of phylloplane fungi from the leaf surface of Lagerstroemia speciosa. The presence of fungi was confirmed by SEM analysis. *Chaetomium globosum, Curvularia pallescens* and *Myrothecium verrucaria*, the dominant isolates were selected to study the biochemical analysis. Leaf extract and the three fungal extracts in Methanol, DCM and Ethyl acetate were screened for secondary metabolites like alkaloids, flavonoids, phenols and terpenoids using phytochemical qualitative techniques. The presence of the compounds was confirmed with UV-Vis and FTIR. The biochemical results of the fungal extracts were compared with that of the leaf.

Keywords: Phylloplane fungi, FTIR and alkaloid.

INTRODUCTION

Fungi are achlorophyllous organism found everywhere. Biodiversity of these organism in a particular ecosystem differs.The phylloplane represents an important terrestrial habitat that harbors a wide range of microbial populations. Phylloplane microflora plays an important role affecting the plant - microbe interactions on leaf surface and thereby contribute significantly for beneficial plant growth and disease suppression (Amarjyoti Tanti et. al.). Plant leaf surface serves as a

suitable environment inhabitating a larger proportion of microbial resources.

Lot of investigations have been carried out on the phylloplane flora of leaf surfaces of several plants growing in garden or cultivated in many parts of the world by several researchers (Abdel-Fattah, et al., 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez, et al., 1995; Eicker, 1976; Khallil, and Abdel- Sater., 1993; Mazen, et al., 1985; Nagaraja, 1991; Sharma, 1974). EI-Said (2001). Many physical, chemical and biological factors bring about causative changes in composition of aeromycoflora of an area and different fungal species are restricted to that particular areas with specific environmental conditions (Bajwa et al., 1997; Verma, 1990).

Fungi provide a plentiful and diverse source of unique and often bioactive metabolites, and they have produced a number of medicinally important compounds, including penicillin, mevinolin (Lovastatin) (Gloer, 2007), fingolimod (Strader et al., 2011) and caspofungin (Keating and Figgitt, 2003).

In the present investigation, ***Lagerstroemia speciosa*** leaves were examined for phylloplane fungal flora. Phylloplane fungi may be residing on the leaf surface without any disease symptoms or may be casually present. They were further investigated for the alkaloid production.

MATERIALS AND METHODS

Collection of Plant material :
Fresh leaf of plant specimen was collected from Western Ghats of SGNP, Borivali, India. The plant specimen was identified as ***Lagerstroemia speciosa*** (L.) Pers. (**Fig 2**,) by Blatter Herbarium, St. Xaviers College, Mumbai, Fort.
Leaves were examined for fungi on the surface by the following methods.

Direct method
Leaf section: The leaf was cut with sterile blade. The sections were mounted in lactophenol blue. The blue stained hyphae or spores shows the presence of phylloplane fungi on the epidermal cell wall.

Nail paint impession (Masurovsky and Jordan,1960), technique was performed in which transparent nail polish was applied on the surface of the leaf. The coating of the nail paint was gently peeled off after drying. This peeling was mounted in lactophenol and observed under compound microscope for fungal presence on the leaf surface.

Cellotape impression method (Edward and Hartman, 1952)**,** was carried out in which strips of cellotape was pressed gently against the surface of the leaf. After impression, the strips were stained in cotton blue and was observed in microscope for fungal presence.

Leaf impression :
The leaves of the plant were washed in distill water and allowed to dry to remove the surface contaminants and soil particles. The leaves were surface sterilized and pressed on PDA media in a petri-plate for about five minutes. The plates were kept in at room temperature for 7 days and observed. The different colo nies formed were sub-cultured. The pure isolates were stored as master slants at 4°C. The dominant phylloplane cultures were authenticated and deposited at Agarkar Research Institute, Pune. Among these, three cultures *Chaetomium globosum, Curvularia pallescens* and *Myrothecium verrucaria*, were selected to study the biochemical analysis.

Scanning electron microscopy :
The dried small leaf segments (2 x 10 mm) were mounted ventral side up on aluminum stub mounts using 12-mm carbon adhesive tabs coated with carbon-conducting glue and sputter coated with 6 nm of platinum using a Hummer 6.2 sputtering system (SAIF-IIT, Bombay, Powai). Images were obtained in high-vacuum mode with accelerating voltages at or around 2.0 kV.

Biochemical analysis :
The leaves were washed with water, shade dried and ground to powder using an electronic blender, sieved and the fine powder was stored in air tight container for further study.

Preparation of leaf extract :
100 gram of powder was subjected to methanolic extraction by hot percolation method through Soxhlet

apparatus. The extract was filtered through Whatmann filter paper no. 1. This leaf extract was concentrated using rotary evaporator at 40°C and dried.

Preparation of fungal extract :
The three fungal isolates were cultured on PDA broth and incubated for 21 days at room temperature.

Extraction of fungal cultures :
The culture filtrate was extracted in ethyl acetate and DCM. Culture filtrate was then was evaporated to dryness under vacuum in rotary evaporator. The dried organic extract was reconstituted with 10ml of the same solvents. The culture mats were weighed before and after drying. These mats were then extracted in methanol and dried in rotary evaporator. The dried extracts of leaf and the fungal cultures was analyzed for alkaloid production by phytochemical study, UV & FTIR.

Phytochemical analysis :
Preliminary phytochemical screening for bioactive compound of the above extracts was carried out using standard qualitative methods ([Kotate et al, 2010; Harborne 1998; Egwaikhide and Gimba, 2007; Savithramma et al, 2011).

Detection of Alkaloids (Evans, 1997)
Mayer's test (Evans, 1997) To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Wagner's test (Wagner, 1993) –
To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Detection of Flavonoids
Few drops of dilute NaOH solution was added to the extract, an intense yellow or pink colour was observed. Further on addition of dilute acid it becomes colourless. Thus, indicating presence of flavonoids. To a small quantity of extract dilute H_2SO_4 was added. Appearance of orange colour indicated the presence of flavonoids.

Detection of Phenolic compounds
Ferric chloride test (Mace, 1963) - The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution is added. A dark green or deep blue colour indicates the presence of phenolic compounds.

Lead acetate test
The extract dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagent test
An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Detection of Terpenoids

Salkowski test - The extract was mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (1ml) was carefully added to form a layer. A reddish violet coloration in the interface indicates positive result for the presence of terpenoids.

Libermann's reaction –
The extract was mixed with equal amount of acetic acid. It was heated & colled. Then few drops of concentrated H_2SO_4 was added. Formation of bluish green rings indicates the presence of terpenoids.

UV - Vis analysis
To detect the UV-Vis spectrum profile, the leaf and fungal extracts were scanned in the wavelength ranging from 200-1100 nm on Lambda 25 UV-Vis spectrophotometer - 01 Perkin Elmer model at CIL LAB SPPTM, MUMBAI. The characteristic peaks were detected which confirms the presence of alkaloid in all the extracts. The peak values of the UV-Vis were recorded.

FTIR analysis
The leaf extract, the crude fungal extracts of methanolic, DCM and ethyl acetate were studied under FTIR. A drop of the liquid leaf and fungal crude extract was placed between the two NaCl cells circular and transparent in nature. These liquid samples were scanned from range 400 to 4000cm^{-1} with a resolution

of 4cm^{-1} in FTIR spectroscopy (Spectrum v.5.3.1 Perkin Elmer) at CIL LAB SPPTM, MUMBAI.. The results of FTIR peak values and functional groups are represented in **Table 3** . The FTIR analysis suggest the presence of alkaloid in all extracts.

RESULTS AND DISCUSSION

Plant description :

Lagerstroemia speciosa, (**Fig 1 and 2**) belonging to family Lythraceae is commonly called as the Queens crape myrtle "PRIDE OF INDIA" in English and Arjuna in Hindi. (Flora of Maharashtra).

Direct observations:

The direct observation of the leaf of *Lagerstoemia speciosa,* was seen under stereoscope (**Fig 3 & 4**) and light microscope.

Nail paint and cello tape impression methods

The phylloplane fungal diversity was studied by the presence of mycelial forms impressed on nail paint strips (**Fig 5**) and cello tape strips (**Fig 6**).

Fig 1: Lagerstroemia speciosa(L.)

Fig 2: Specimen identified.

Fig 3: Leaf surface

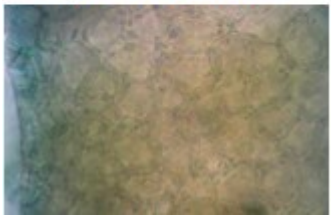


Fig 4: Leaf stained in cotton blue

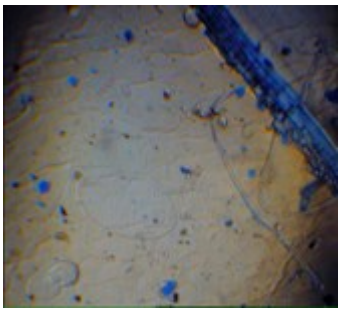


Fig 5: Nail paint impression

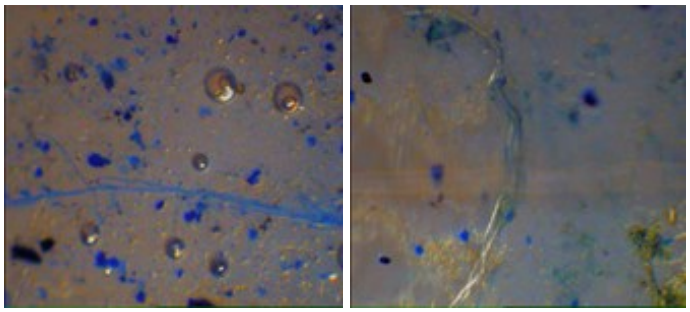


Fig 6: Cello tape impresssion

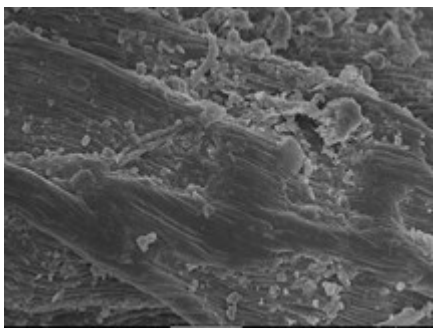


Fig 7: SEM Image of *Lagerstoemia speciosa (magnification X 2,000, bar indicates 1µm)*

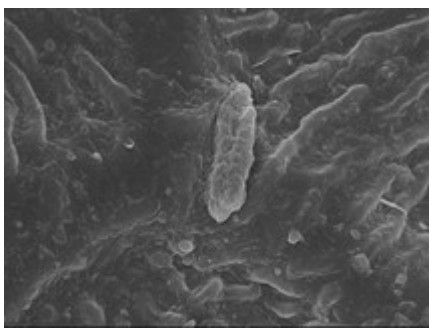


Fig 8: SEM Image of *Lagerstoemia speciosa (magnification X 5,000, bar indicates 1µm)*

The presence of phylloplane fungi was further confirmed by Scanning Electron Microscopy where mycelial forms and spore were observed on the surface of the leaf of Lagerstroemia speciosa (**Fig 7 & 8).**

Leaf impression showing fungal colonies

The fungal colonies (**Fig 9 &10**) were seen on 7th day on the PDA medium. These colonies were isolated into pure cultures and stored in slants at 4°C.

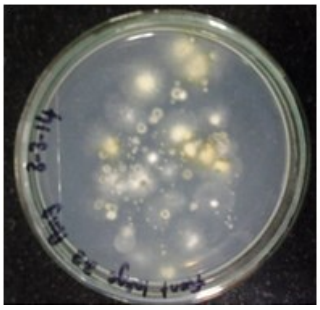


Fig 9: surface view

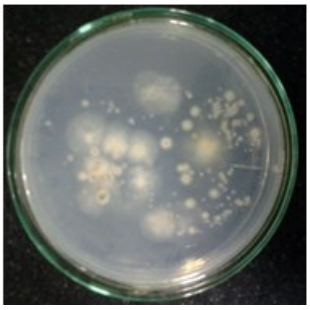


Fig 10: reverse view

Phylloplane fungi

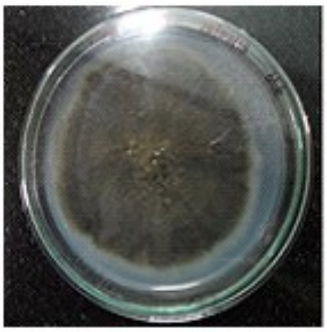
A total number of 14 phylloplane fungi were isolated from surface sterilized leaf impression of *Lagerstoemia speciosa.*

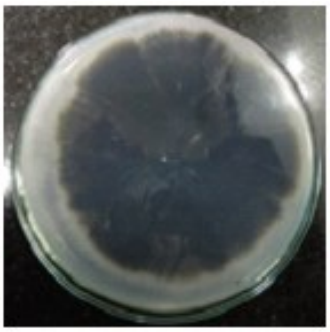
Phylloplane fungi on *Lagerstroemia speciosa*

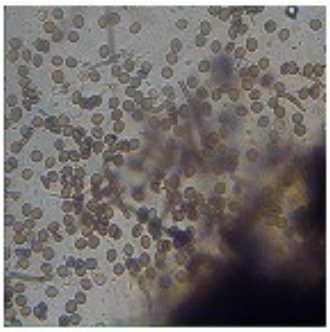
The following were the colonies identified,

Aspergillus sp, Alternaria alternate, Chaetomium globosum, Cuvrularia lunata, Curvularia pallescens, Dematicious sp, Fusarium oxysporum, Gleosporium sp, Myrothecium verrucaria, Mycelia sterilia, Paecilomyces cerevisae, Phoma sp, Rhizopus sp,Torula sp.

Out of these phylloplane fungi, 3 fungal isolates namely *Chaetomium globosum* (**Fig 11**) *Curvularia pallescens* (**Fig 12)** and *Myrothecium verrucaria* (**Fig 13)**, were found to be dominant.







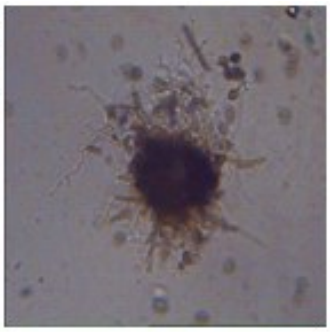


Fig11: Surface Reverse Ascospores Perithecia

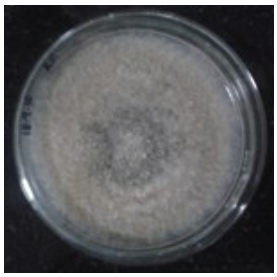
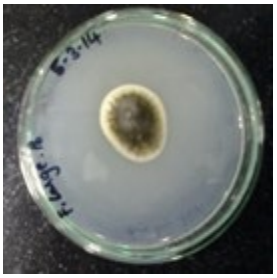
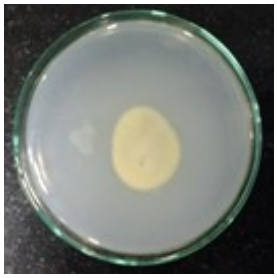


Fig12: Colony Conidia





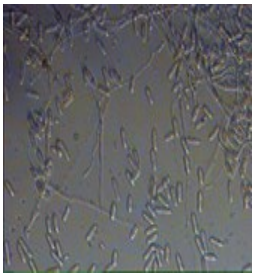


Fig13: Surface Reverse conidia

***Chaetomium globosum* Kunze (NFCCI accession no 4111, family- Chaetomiacea)**

Colony brownish grey and dark black at reverse, growing moderately about 4cm in 5 days. Perithecia ostiolate, sub globose, somewhat elongated with a bluntly pointed base, producing short cirrhi, with dark brown colour rhizoids. Terminal hairs numerous, drooping., slender., septate with spines. Ascospores filled with several large mature dark, brown, ovate & lemon shaped with apiculate 9-13 u in length and 7 u broad.

***Curvularia* aff. *pallescens* Boedijn (NFCCI accession no 1602, family-*Pleosporaceae*)**

Colony velvety creamish, moderate in growth of diameter 7 cm in 4 days, reverse dark brown. Mycelium partly immersed, septate, branched, pale brown 3-4 um wide. Conidiophore mononematous,

nodose, septate, branched, thick walled, dark brown, 40-90um long .Conidia solitary, dry, curved, ellipsoidal, rounded at both ends, thick-walled, smooth, dark brown, 3-septate, end cells paper than central cells.

***Myrothecium verrucaria* @ (Alb & Schwein) (NFCCI accession no 4106, family *Incertae sedis*)**

Slow growing upto 2cm in 12 days. Colony brown in centre and margin creamish in colour. Reverse cream in colour. Sporodochia cushion like, sometimes with marginal hyaline setae; conidiophores subhyaline to coloured, repeatedly branched, bearing conidia terminlly. Philiades cylindrical, densely aggregated, conidia fusiform 1 celled or cylindrical, forming dark, often greenish-black masses.

Biochemical analysis

The three phylloplane isolates were further studied for their biochemical presence of alkaloid by performing phytochemical tests, Uv and FTIR analysis.

The phytochemical tests (Indian Pharmacopia) showed the presence of Alkaloid in all the solvent extracts of leaf and fungi by Wagners tests. The presence of flavonoid was detected in methanol extracts of leaf, *Chaetomium globosum* and *Curvularia pallescens.* Whereas, flavonoid was present in ethyl acetate extract of *Myrothecium verrucaria.* The phenolic compounds were present in methanol extract of leaf and *chaetomium globosum* by ferric chloride and lead acetate test respectively. Terpenoid was only present in ethyl acetate extract of *Chaetomium globosom.* (**Table 1**).

UV-Vis analysis [Harborne, 1998]

The UV spectrum of leaf and all three phylloplane fungi showed sharp peaks by proper baseline correction which confirms the presence of Alkaloids at 234.676nm (Nandha Kumar. et al, 2015) (**Table 2**).

Table 1: Phytochemical analysis

Phytochemical test	Methanolic leaf extract of Lagerstroemia speciosa	Crude fungal extracts of *Chaetomium globosum*			Crude fungal extracts of *Curvularia pallescens*			Crude fungal extracts of *Myrothecium verrucaria*		
		DCM	Ethyl acetate	Methanol	DCM	Ethyl acetate	Methanol	DCM	Ethyl acetate	Methanol
Alkaloid test	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	+	-	-	+	-	+	-
Phenolic compounds	+	-	-	+	-	-	-	-	-	-
Terpenoids	-	-	+	-	-	-	-	-	-	-

Table 2: UV spectrum data of methanol leaf extract and three fungal extracts in DCM,

Solvents	**leaf extracts of *Lagerstroemia speciosa***	**Crude fungal extracts of Chaetomium globosum**	**Crude fungal extracts of *Curvularia pallescens***	**Crude fungal extracts of *Myrothecium verrucaria***
DCM	-	403.21 470.92	236.80	380.88
Ethyl acetate	-	363.38	394.84	323.70 244.47
Methanol	324.83 329.17 470.92	342.18	243.75	381.61

Table 3: FTIR spectrum data of methanol leaf extract and three fungal extracts in DCM, Ethyl acetate and methanol.

Leaf sample and Phylloplane fungi	FTIR peak values for alkaloids presence of crude extract of different solvents		
	DCM	Ethyl acetate	Methanol
Lagerstroemia speciosa	-	-	1651.72
Chaetomium globosum	1729.57	1742.84	1650.73
Curvularia palles	1645.50	1841.82	1654.70
Myrothecium verrucaria	1648.35	1743.37	1657.53

FTIR analysis

From the above observation (**Table 3**) it is evident that alkaloids are present in the leaf and the fungal extracts in the range of 1850 - 1620cm-1 IR radiation region (Robert M Silverstein, 2014) in similar traces, which may be potentially defensive, detection of specific alkaloids needs to be further confirmed by TLC and HPTLC analytical techniques for better understanding their biological activities for future needs.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Bioleaching of toxic elements by *Paecilomyces lilacinus*

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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:
Asra Usmani and Sashirekha S (2018) Bioleaching of toxic elements by *Paecilomyces lilacinus*, *Int. J. of. Life Sciences*, Special Issue, A9: 69—73.

ABSTRACT

Industrialization has led to the production of different kinds of pollutants and toxic elements. Conventional methods for the removal of heavy metals are not economically and environmental friendly. Fly ash was collected from a cement plant from Chandrapur, to investigate the presence of heavy metals. It was found to contain Al, Li and Mn in higher concentrations approaching toxic limits. Physio-chemical techniques for treating fly ash are expensive and time consuming. Naturally fungi have a large variety of extracellular proteins, organic acids and other metabolites. Fungi can adapt and survive in several ecosystems and under different environmental conditions. Most of the soil fungi, especially the filamentous fungi are of great interest in bioremediation. Biological treatment methods are economical and allow the cyclisation of the sediment after treatment. In present investigation *P.lilacinus* was employed as a bioleaching agent, experimentally. The concentrations of metal in its elemental form of Al, Li, Mn and Zn were analyzed by ICP -AES. The formation of oxalates in the culture filtrates bioleached by *P.lilacinus*, after treatment was confirmed using FTIR analytical technique.

Key words: Fly ash, *Paecilomyces lilacinus*, FTIR, Oxalates

INTRODUCTION

The soil and water are frequently contaminated by toxic heavy metals and organic pollutants. As a consequence of human activities become a key concern in environmental and health problems. Several toxic metals (Cd, Cu, Hg, Pb, Mn, As, Ni, Zn etc.) from industrial wastewater and other human activities are directly or indirectly release into the environment. Unlike organic contaminants, these pollutants from heavy metals are non-biodegradable and able to enter the food chain via bioaccumulation.

Rapid developments of industrialization and urbanization have led to direct impact on the environment. Globally, open water and aquatic ecosystem are contaminated with several heavy metals through various human activities that directly and indirectly led to pollutions. High concentrations of heavy metals can change the physical and chemical properties of the water and thereby the profile of water. (Siddiquee *et al*., 2015).

Fly ash is produced worldwide in vast quantities as an incineration waste. Fly ash is hazardous due to the volatile toxic metals that concentrate and accumulate in the ash. Treatment of fly ash may lead to both detoxification and reuse. Fly ash includes substantial amounts of silicon dioxide (amorphous and crystal-line), aluminum oxide (Al_2O_3) and calcium oxide (CaO), are the chief components of the fly ash.

Biological treatment methods are the methods of choice because they are natural, economically viable, and attractive and because they allow the reuse of the sediments after their treatment. Present paper focuses on detoxification of pollutants using filamentous fungi *Paecilomyces lilacinus*.

Fungal leaching of heavy metals is an interesting biological treatment method. It is based on the principle on catalytic conversion of organic acids by various fungi to organic esters such as oxalates, citrates, maleates, tartarates, etc. thereby increasing the solubility of metals in the form of water soluble complexes. Formation of complexes is also associated with substantial decrease in toxicity fungal production of weak organic acids that solubilize metals by forming water soluble complexes with them (Shannon *et al*., 2014).

MATERIALS AND METHODS

Fly ash is collected from Chandrapur cement plant, Chandrapur district is located in Maharashtra near Nagpur. Sampling was done randomly. The fly ash was digested then analyzed for their total heavy metal content by inductively coupled plasma atomic emission spectroscopy (ICP AES). for the digestion 0.5 g of fly ash sediments were digested in 10 ml of concentrated nitric acid for 10 minutes.

Paecilomyces lilacinus was isolated from the soil collected from forest. Six days old culture of *P.lilacinus*was inoculated in Czapek dox broth and examined for its Metal Tolerance Capacity (MTC).Grades of fly ash were prepared by adding 1, 5, 10 and 15 gramsof fly ash in 100 ml of Czapek dox in duplicates. Three control flasks, one with media, media with culture and media with fly ash were incubated for 15 days. pH was checked before and after the treatment. On the 15th day pH was checked, and the culture filtrate was filtered with Whatman's No.42 filter paper and the fresh and dry weight of the biomass was recorded. Culture filtrate and the fungal mats were separately digested and analyzed for the presence of metals. Analysis was done using ICP AES. Culture filtrate was then extracted with Ethyl acetate and vacuum dried. The residue was reconstituted in methanol. Sample was used for the further investigations. The samples of all the flasks were analyzed for presence of organic acid.

Oxalic acid was estimated by titrating culture filtrate with 0.02 N Potassium permanganate solution. The oxalates in the samples were detected and confirmed with the UV spectroscopy and FTIR.

Fungal mats from each concentration were examined to observe the modification of mycelia due to the effect of metals.

RESULTS AND DISCUSSION

Detection of metals by ICP AES

Culture filtrate and the fungal mats of the control and treated flasks were digested with concentrated nitric acid for analysis. It was observed that fly ash showed the presence of Li, Al, Zn and Mn. Fly ash is as such not hazardous but it causes ground water pollution. Filamentous fungi *Paecilomyces lilacinus* was grown in various concentrations of fly ash amended media. Before treatment the pH was 6 and it changed to pH 10 after treatment. Culture mat was weighed and digested with concentrated nitric acid for analysis. (**Table. 1**)

Table. 1. pH and Biomass of the culture

Sample	pH		Weight of biomass in grams	
	before inoculation	after 15 days incubation	(fresh)	(dry)
Control (Media)	6	6	-	-
Control (M+C)	6	9	17.982	4.228
Control (M+FA)	6	6	-	-
1% FA	6	10	16.522	3.821
5% FA	6	10	12.632	2.112
10% FA	6	10	11.222	2.290
15% FA	6	10	11.082	2.082

Table. 2. Concentration of the elements before and after treatment

Sample	Al(ppm)	Li (ppm)	Mn (ppm)	Zn (ppm)
Control (Media)	0	0	0	0
Control (M+C)	0	0	0	0
Control 1%	2.80	0.20	1.28	5.04
1%FA	0.15	ND	0.11	0.285
1%BF	2.23	0.11	1.08	4.36
Control 5%	3.21	0.31	1.98	6.87
5%FA	0.31	ND	ND	0.85
5%BF	2.84	0.23	1.78	5.90
Control 10%	4.01	1.21	2.01	7.59
10%FA	0.28	0.34	ND	2.40
10%BF	3.82	0.85	1.95	5.10
Control 15%	6.83	2.33	2.58	8.58
15% FA	3.60	0.53	0.723	3.28
15% BF	3.12	1.79	1.657	5.25

FA=Culture filtrate, BF=Biomass of culture mat

In control 1% it was observed elemental Al was 2.80 ppm, Li 0.20 ppm, Mn 1.28ppm and Zn 5.04 ppm.In the culture filtrate after treatment Al was 0.15 ppm , Li was not detected , Mn 0.11 ppm and Zn 0.28 ppm. In the culture mat Al was 2.23 ppm, Li 0.11 ppm, Mn 1.08 ppm and Zn 4.36 ppm. (**Table. 2**)

In control 5% it was observed elemental Al was 3.21 ppm, Li 0.31 ppm, Mn 1.98ppm and Zn 6.87 ppm.In the culture filtrate after treatment Al was 0.31 ppm , Li was not detected , Mn not detected and Zn 0.85 ppm. In the culture mat Al was 2.84 ppm, Li 0.23 ppm, Mn 1.78 ppm and Zn 5.90 ppm. (**Table. 2**)

In control 10% it was observed elemental Al was 4.01 ppm, Li 1.21 ppm, Mn 2.01 ppm and Zn 7.59 ppm.In the culture filtrate after treatment Al was 0.28 ppm, Li was 0.34 ppm, Mn not detected and Zn 2.40 ppm. In the culture mat Al was 3.82 ppm, Li 0.85 ppm, Mn 1.95 ppm and Zn 5.10 ppm. (**Table. 2**)

In control 15% it was observed elemental Al was 6.83 ppm, Li 2.33 ppm, Mn 2.58 ppm and Zn 8.58 ppm.In the culture filtrate after treatment Al was 3.60ppm, Li was 0.53 ppm, Mn 0.72 ppm and Zn 3.28 ppm. In the culture mat Al was 3.12 ppm, Li 1.79 ppm, Mn 1.65 ppm and Zn 5.25 ppm. (**Table. 2**)

From the above observation there was a clear reduction of the Al, Li, Mn and Zn in the culture filtrate after treatment. There was an increase in the presence of Al, Li, Mn and Zn in the culture mat. This clearly indicates the biosorption of the elements on the mycelium of *P.lilacinus*.

Organic Acid production

Organic acids qualitative test was done in which oxalic acid was present in control as well treated samples. Estimation of organic acid was done by titration method. Oxalic acid is a well-known chelating agent that has been widely studied because of its ability to dissolve different minerals. Incontrast to other low molecular–weight carboxylic acids with low complexing abilities that erode minerals in acid solution by protolysis. Oxalic acid is able to mobilize metals very efficiently at neutral pH and even in basic solutions. (Fomina *et al*., 2005)

Interpretation of IR spectra

FTIR analysis of the samples under present investigation was performed by the NaCl cell technique. The FTIR was done on liquid extract. Equipment used was Perkin -Elmer Spectrum –GX and samples were scanned in the region of 4000-650 $cm^{-1}$to evaluate the presence of oxalate peaks in the spectra was run using air as a background.

FTIR spectra related to each studied sample are interpreted in terms of wave numbers in $cm^{-1.}$. In present investigation oxalates shows distinct peaks which are in accordance to literature survey. The oxalates in general shows strong peaks in the region of 3500-2880 $cm^{-1.}$

Biosorption and Bioleaching

Metal mobilization by fungi can occur as a result of several mechanisms, including acidolysis (proton promoted), complexolysis (ligand promoted), reductive mobilization and mycelium functioning as sink for soluble metal species. Solublization yield is related to the association of metalsto the acid soluble and reducible fractions. The bioleaching of heavy metals is mainly brought about by organic acids through acido-lysis and complexolysis. Toxic metals may increase oxalate excretion by fungi. The elements are tightly bound to the mat. Al, Li, Mn and Zn were the most solubilized and biosorbed metals in the fungal treatm-ent. In the current study it was

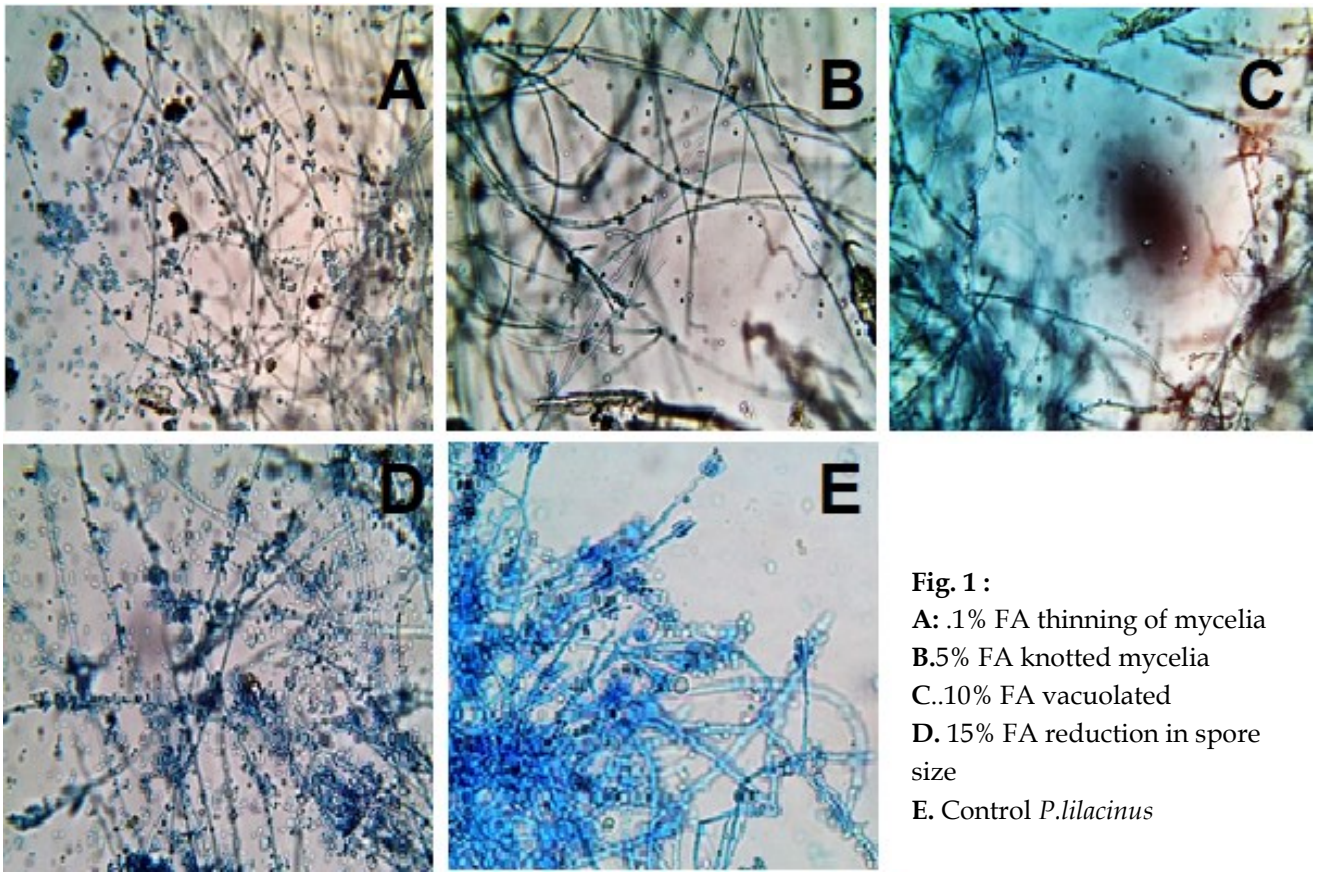

Fig. 1 :
A: .1% FA thinning of mycelia
B.5% FA knotted mycelia
C..10% FA vacuolated
D. 15% FA reduction in spore size
E. Control *P.lilacinus*

observed biosorption of Al, Li, Mn and Zn were efficiently done by *P.lilacinus.*

Microscopic observation

In *P.lilacinus* the changes in the mycelia were observed in the different grades of fly ash treatments. It was observed mycelia was width0.7 µm in control culture, whereas the growth with the fly ash was 0.5µm and 0.3µm in 10% and 15% concentration. It also showed changes in the mycelia as thin (**Fig. 1a**), vacuolated (**Fig.1 c**), knotted (**Fig. 1 b**) and reduction in spore size (**Fig.1 d**). Formation of mycelia covered by a thick hydrated mucilaginous sheath leading to formation of jelly like mass which provide micro environment for chemical reactions. Change in the mycelial growth indicates the aspect of metal tolerance. *P.lilacinus* tolerated the toxic metal stress but maintained a high biomass yield.

Metal toxicity may be reduced if the mobilized toxic metal forms complexes with organic ligands excreted by the fungus and especially if toxic metals are precipitated as highly insoluble oxalates. (Fomina *et al*., 2005). Therefore, overexcretion of oxalic acid probably contributed to the metal tolerance exhibited by the *P.lilacinus*

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

New initiative on developing fungicides: A traditional perspective

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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:
Mudiar Rinky and Varsha Kelkar-Mane (2018) New initiative on developing fungicides: A traditional perspective, *Int. J. of Life Sciences*, Special Issue, A9: 74-78.

ABSTRACT

Global change in *Candida* spectrum has been observed in India along with the world due to uncontrolled use of antifungals. *C.albicans* is the common agent for muco-cutaneous and invasive candidiasis. Existing antifungals seems to have failed fighting this opportunistic pathogen resulting in high rates of morbidity and mortality rates (around 30% mortality), indicated by CDC's surveillance data in 2012. *Candida* has become drug resistant due to expression of efflux pumps which causes low drug accumulation, alteration in target proteins and membrane sterol composition. *Tamarajal, i.e.,* water stored in copper vessels has been proclaimed as health elixir by ancient *Ayurveda*. Bactericidal property of copper vessels used in Indian household has already been established by the western world. In this paper, we report *tamarajal* as cytotoxic agent and pseudo-morphogenic agent for *Candida*. The change in cell structure exposed to *tamarajal* was visualized under Scanning Electron Microscope. Biofilm eradication capacity was also assessed to avoid infection due to medical devices. This will help us find out a possible alternative to combat candidiasis.

Key words: muco-cutaneous, *C.albicans,* fungicides,

INTRODUCTION

Candidiasis is quite prevalent in patients with suppressed immunity, hematologic malignancies, solid organ transplantation, renal failure, autoimmune disorder with prolonged steroid therapy. Topical spreading of *Candida* is promoted by oral thrush, denture related stomatitis, burn wounds, steroid use, diaper dermatitis, vaginal colonization etc. The infection type could be superficial mucosal or more severe systemic one. The differentclasses of drugs available need to be administered at higher doses hence comes with hidden side effects. It ranges from mild headache to severe nephrotoxicity and even heart failure.

To avoid these consequences, rather than killing the pathogen, scientists are now more focused on inhibiting the growth and its virulence of the same. As pathogenicity depends on metabolic pathways, invasion related processes, transcription factors etc., designing an alternative that targets any of these aspects can avoid establishment of infection in the host.

U.S. EPA has registered copper as the only solid surface material to kill bacteria. Dry copper surfaces inactivate cells in a process called contact-mediated killing, by inducing cytoplasmic membrane damage (Quaranta *et al*., 2014). Bactericidal action of copper has been reported on pathogens like Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus*, *Acinetobacterbaumannii, Escherichia coli* etc **(**Kulkarni**,** 2011). Within 2 hours of contact time, copper can kill 99.9% of bacteria (CDA, 2010). Research has strongly claimed that copper based surfaces in hospitals can reduce nosocomial infections (Michels *et al*., 2015). Antibacterial efficacy for copper and brass vessels used in India since ancient times has been referred healthy, recognized and reestablished by Western world (Khamsi, 2005), hence, copper may likely become a metal of choice to combat candidiasis, with lesser side effects.

Microbes do not generally develop resistance mechanism to metals like copper as observed in case of antifungal drugs. In contrast to microbes, human skin is not sensitive to copper. Prolonged use of copper intrauterine devices (IUDs) by women worldwide has proved the metal safe for human (Kimmerle *et al*., 1993). However administering metallic copper based antifungals may result in physical and mental disorders (Jaishankar *et al*., 2014).

Tamarajal is prepared by storing drinking water in copper vessel overnight. Daily practice of drinking this treasured water balances three *Doshas* in our body (Sharma *et al*., 2004). It also boost our immunity and helps perpetuate good health. As per our knowledge, no health issues have been addressed so far due to drinking *tamarajal* even for a very long time. As this does not lead to metal accumulation; it can be a possible alternative to existing antifungals.

In this paper, cytotoxicity of *tamarajal* and its implication on morphogenesis of *Candida*was studied by time kill assay and germ tube inhibition test respectively. Biofilms formed on various medical devices are less susceptible to antifungals are an easy way to invade the host. Here, we have assessed the efficacy of *tamarajal*on biofilm that will help eradication of mature biofilms on such devices.

MATERIAL AND METHODS

1. Preparation of *tamarajal:Tamarajal* was prepared by storing distilled water in a thoroughly cleaned copper vessel for maximum 24h.
2. Fungal isolate: *Candida* species from UTI of a patient was obtained from a general hospital, Mumbai. The isolate was cultured and maintained on Sabouraud's Dextrose agar, for 48h and then stored at 4°C for further use.
3. Media and chemicals: All media were purchased from HiMedia, Mumbai. Commercially available Fluconazole (Flu) tablet (50mg) was used as standard drug. For germ tube inhibition test, serum was collected from a local pathology laboratory.
4. Time kill assay: 10^4 cells/ml were exposed to Flu (32mg/L, MIC for non-resistant *Candida* sp.) &*tamarajal*for 4h. At every hour interval, cell viability was evaluated by spreading 100µl suspension on Sabouraud's media. Cytotoxicity was calculated using CFU count on plates after 48h of incubation at 37°C. Controls were maintained for all experiments with distilled water (Ernst *et al*., 2002).
5. Germ tube inhibition test: Active *Candida*cells at 10^5 cells/ml concentrationwere exposed to the above mentioned concentration of Flu and *tamarajal* for a standardized period of 4h, followed by incubation in serum for germ tube formation. Percentage inhibition of germ tube was calculated for each sample to determine its efficacy. Control as mentioned above was maintained(Acharya, 2015).
6. *Candida* exposed to *tamarajal*was fixed and visualized under SEM. Morphological effects was studied.
7. Biofilm eradication test: 10^7 cells/ml *Candida* cells in RPMI-1640 were allowed to adhere to glass surface at 37°C and 75rpm for 90min. Media was replaced after removing unattached cells (Lal *et al*., 2010). The

biofilm formed after 24h was then exposed to *tamarajal* for another 24h. The biofilm was then washed, dried and stained with crystal violet. The absorbed stain was eluted using 95% ethanol and absorbance was measured at 595nm using Nanodrop (MULTISKAN GO, Thermo scientific). Percentage eradication of *tamarajal* exposed biofilm was calculated.

RESULTS & DISCUSSION

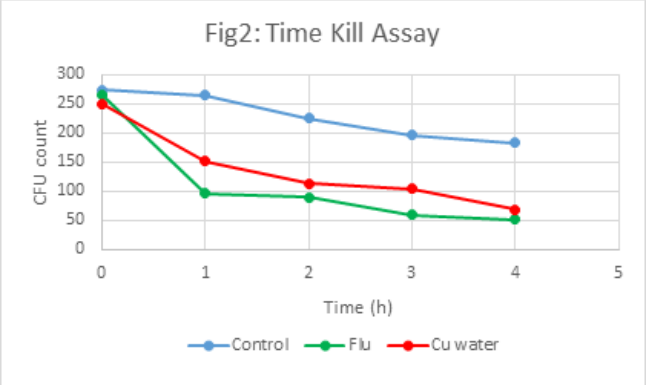
The copper content in *tamarajal* was found to be approximately 1mg/L (ICP-AES, IIT, Bombay), after 24hrs. Within 3h, CFU count indicated 72% cytotoxicity in Flu followed by 59% cytotoxicity with *tamarajal* (Fig. 1). Slow decrease in CFU count in control is attributed to the lysis of cells due to osmotic pressure exerted by distilled water. Correlation between time of exposure (in hours) and cytotoxicity was calculated and the values up to 4h are presented in Table1. Perfectly negative correlation in the control and partially negative correlation were evident in both flu and *tamarajal*.

Table1: Correlation between time of exposure (in hours) and cytotoxicity

Samples	r; correlation
Control	-1;Perfectly negative
Flu	-0.84;Partially negative
Tamarajal	-0.93;Partially negative

Table2: Percentage of Germ tube in *Candida*

Control	Flu	*Tamarajal*
89.67±2.08	19.67±1.16	0±0



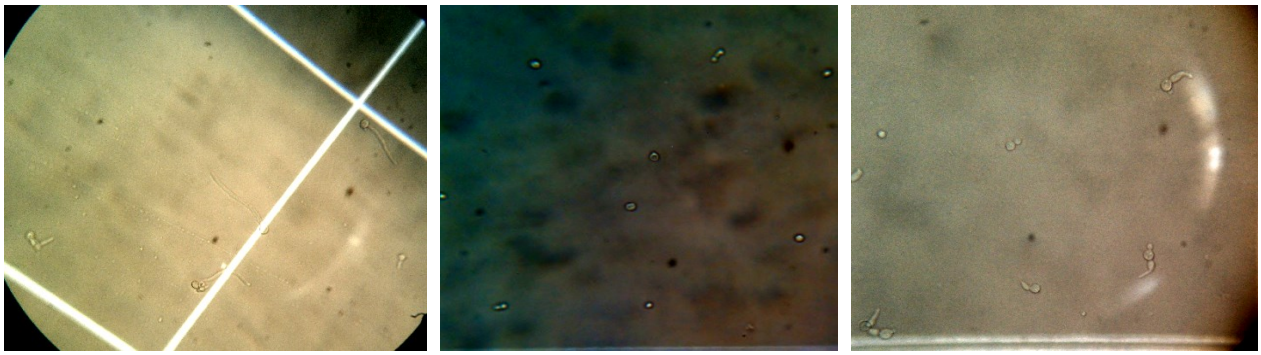


Fig 2: Germ tubes inhibition test: Pre-exposed to A) Control B) Fluconazole C) *tamarajal*

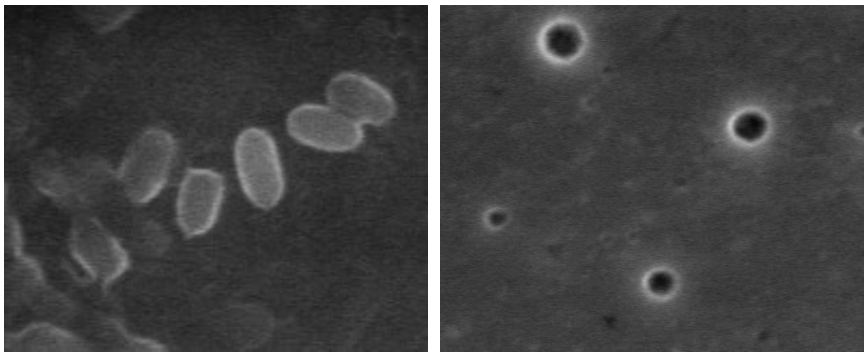


Fig 3: *Candida* under SEM a) control b) *tamarajal*

Inhibition of pathogenicity in *Candida* was effectively and completely observed with *tamarajal Tamarajal* seems to be diverting yeast cells morphogenesis to pseudohyphae rather than true hyphae (Fig 2). The result for germ tube formation are presented in Table2 with percent mean ± SD.

Under SEM, the morphological structure of *Candida* cells were detected to be altered when exposed to *tamarajal*. Brightening surface on the *tamarajal* exposed spherical cells confirmed the deposition of copper ions on the cell membrane.

Biofilm eradication capacity of *tamarajal*is well comparable to the standard drug used. *Tamarajal* effectively destroyed biofilmupto 96% which is comparable to Flu that reduced biofilm upto 98%.

DISCUSSIONS

Drug related health issues and increasing resistance towards commonly used drugs by fungal pathogens like *Candida* urge the development of a highly effective yet safe alternative. Copper surfaces have been reported to kill microbial cells by DNA fragmentation, membrane damage, chelating biomolecules, replacing metal ions of some metallo-proteins etc. (Yamanaka *et al*., 2005). Literature states that copper ion is a non-competitive inhibitor for enzymes, hence permanently damage enzymes. Copper generates hydroxyl radicals which oxidise proteins and helps lipid peroxidation (Hong *et al*., 2005). Copper ions have also been observed to get internalized causing morpho-structural changes in *E. coli*, causing perturbed structure and cytosolic copper accumulation towards the apical ends (Kambli *et al*., 2015). The anti-candidal efficacy of copper may be due to a multifaceted action on the cell. Copper ions can be postulated to bind to the negatively charged cell membrane, making it easier for penetration into the cell. It could be due to ROS generation or cytoplasmic membrane damage.

The present study is probably one of the first reports of the anti-candidal effect of *tamarajal*. A healthy practice of drinking *tamarajal* daily regulates thyroid function, slow down aging, helps relieving from pain and arthritis and stimulate brain. In *tamarajal*, copper gently leaches into the water from the contact surface of the vessel bestowing all its positive properties. As the copper content in *tamarajal* is being well within the normal limits prescribed as 2mg/L by WHO (2003), this proves its safety for application purposes. Detail investigations of *tamarajal* mediated cytotoxicity of *Candida* cells are underway to understand the mode of action.

CONCLUSION

Our results proves that copper in *tamarajal* form, can cause cytotoxicity along with reducing virulence property for pathogenicity. SEM also supports the report by revealing structural alterations of cells, confirming the results. Drug side effects thus could be surpassed using *tamarajal*. It could be used independently or possibly in conjunction with lower doses of existing antifungals to fight pathogens. *In vivo* studies however, must be carried out in future to determine effective dose for the same.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article | Open Access

Isolation and Characterization of dandruff causing fungi & effect of some plant extracts on it

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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 and Randhir Nayan (2018) Isolation and Characterization of dandruff causing fungi & effect of some plant extracts on it, *Int. J. of. Life Sciences*, Special Issue, A9: 79-83.

ABSTRACT

Yeast called *Malassezia* is a causative agent of Seborrheic dermatitis or dandruff. It was isolated from the scalp of human individuals and investigated further. Suitable biochemical tests were performed for the characterization of the organism focusing on its ability to produce Catalase, Urease, utilization of tweens, liquefaction of gelatin and assimilation of different carbohydrates. The organism after its isolation and characterization was further investigated for its susceptibility towards 20 different plant extracts.

Keywords: Seborrheic dermatitis, *Malasezzia*, mDixons agar, antifungal activity, medicinal plants.

INTRODUCTION

Fungi are ancient troublemakers, but the suspicion that they might cause disease is about 100 years old. Seborrheic dermatitis /dandruff is a condition where flaky skin of scalp sheds off. Louis Charles Malassez first saw yeast like substance lurking in the scales of a patient with Seborrheic dermatitis. *Malassezia furfur (Pitryosporum ovale),* is the fungus which plays an important role in Seborrhoeic dermatitis (Faergemann et al., 1996). The genus *Malassezia* comprises lipophilic yeasts found in the normal flora of human skin and other mammals. These yeasts were described as being associated with Pityriasis Versicolor (PV) lesions. The taxonomy and nomenclature of the genus *Malassezia* was controversial for many decades. Nine of the 13 species within the genus, *M. furfur*, *M. sympodialis*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. dermatis*, *M. japonica*, and *M. yamatoensis*, are associated with normal human flora and pathologies. Four species, *M. pachydermatis*, *M. nana*, *M. equina*, and *M. caprae*, are associated with

animals (Cabanes et al., 2007, Khosravi et al., 2008). *Malassezia* species have been associated with diverse dermatological pathologies, including PV, Seborrheic dermatitis (dandruff), Atopic dermatitis, Folliculitis, Psoriasis, Onychomycosis, and Blepharitis.

Despite the difficulty in isolating, maintaining and identifying these yeasts, different characteristics of the genus, such as macroscopic and microscopic morphology and some physiological aspects (e.g., the presence/absence of catalase, urease) gelatin hydrolysis, sugar assimiliation (sorbitol, mannitol, xylose, dextrose, lactose, glucose, sucrose), assimilation of Tween 20 and 80 by *M. furfur*, *M. sympodialis*, allow them to be differentiated as yeasts. *M. furfur (Pitryosporum ovale)*, a lipophilic fungus affects the hair and causes dandruff. Dandruff is a condition which causes small white flakes of skin that separates and fall from the scalp. People who suffer from dandruff have over-active sebaceous glands, which make their scalp oily.

Plant extracts are promising sources for new natural antifungal agents even though they have relatively mild effect against human pathogenic fungi compared with commercial synthetic antifungal drugs (Rukayadi et al., 2006). Plant extracts and essential oils (Pawar and Thaker, 2006, 2008) have been reported to show antifungal activity against a wide range of fungi. The medicinal plants have been used for several purposes including antibacterial as well as antifungal effects. Some Indian medicinal plants have been used widely in treating a variety of skin diseases by the Ayurvedic physicians (Prusti et al., 2008).

In the present research work the characterization of the fungus was performed using different biochemical tests like Catalase, Urease, Gelatin Hydrolysis and Sugar Assimilation. The influence of twenty different plant extracts namely, Shikakai (*Acacia concinna),* curry tree (*Murraya koenigii*), *Tulsi (Ocimum sp).* Black pepper (*Piper nigrum*), Bael (*Aegle marmelos), Hibiscus rosa sinensis*, Betel (*Piper betle*), Cumin seed (*Cuminum cyminum),* Amla (*Emblica officinalis),* Brahmi *(Bacopa monnieri*), Tridax *(Tridax procumbens),* Ajwain (*Trachyspermum ammi*), Reetha *(Sapindus saponaria),* Pudina (M*entha sp.),* Bavanchi (*Psoralea corylifolia*), Horseweed *(Conyza canadensis),* Calendula *(Calendula officinalis),* Kalanchoe *(Kalanchoe luciae),* Aloe *(Aloe vera),* Neem (*Azadirachta indica*), on the growth of dandruff causing organism has also been studied.

MATERIAL AND METHODS

Isolation and culture of the fungus

Total of 12 samples were examined for the occurrence of dandruff causing organism. The sampling was accomplished from 12 human individual's viz., 7 females and 5 males suffering from dandruff. Cotton swab technique was used for the collection from scalp and the samples were cultured on Sabouraud dextrose agar. They were incubated at 36 ± 1°C for 5 days after which the growth of the organisms was observed. The desired colonies were selected and sub cultured on PDA (potato dextrose agar), SDA (Sabouraud dextrose Agar) and mDixon's agar (Kindo et al., 2004). Coconut oil was added to PDA and SDA in the medium and similarly, Olive oil was added to mDixon's agar for the lipid requirement of the organism. The mDixon's agar was modified with olive oil; Tween 80 was used instead of Tween 20 and bile salt was not added into the medium. All the plates were incubated at 32 ± 4°C for 5 days. The organism showing desired cultural characteristics were chosen for direct microscopy with KOH 20% and methylene blue, crystal violet, lacto phenol staining. All slides were examined under 4x, 10x, 40x, 100x magnification.

Biochemical Characterization

Catalase test: It was determined by using method given by Kindo et al., 2004

Urease test: To perform this method Christensen's Urease medium was used (Cox et al., 2000)

Gelatin Hydrolysis Test: The medium contained Meat extract, Peptone, NaCl and Gelatin. All the above ingredients were dissolved in distilled water, adjusted to pH 7.6, and filtered. It was autoclaved for 10 mins at 120 ^{o}C, removed and cooled at 55 ^{o}C, when pre filtered sterilized freshly prepared Ferrous Chloride (10 % -5 ml) solution was added to it. The medium was tubed in narrow tubes and sealed with corks impregnated with paraffin wax. With the help of straight wire, the isolates were swabbed inside the gelatin tube and incubated at 20 ^{o}C for at least 7 days and further observed for results.

Sugar Assimilation Tests: This test was performed to check the utilization of 8 different carbon sources by the isolates. Eight different sugars included: Sucrose, Glucose, Dextrose, Xylose, Lactose, Mannitol, Sorbitol, and Glycerol. The medium was prepared as (Peptone: 3.6g, Sugar: 0.1g, Phenol red: 0.2%) for 100 ml. Culture suspension was added to each sugar and kept for incubation for the assimilation of sugars for 4-7 days.

Preparation of Plant extracts:
Twenty different plants (*A. concinna, M. koenigii, Ocimum sp., P. nigrum, A. marmelos, Hibiscus, P.betle, C.cyminum, E. officinalis, B. monnieri, T. procumbens, T. ammi, S. saponaria,* M*entha sp., P. corylifolia, C. canadensis, C.officinalis, K. luciae, A. vera and A. indica*) were collected from in and around botanical garden of K.T.H.M College, Nashik. The plant parts were washed thoroughly in tap water followed by distilled water and ground by using mortar and pestle. Five grams of plant material was dissolved in 100 ml of distilled water. These samples were refluxed for 2 h at 30-40 ^{o}C and supernatant was collected later. These extracts were oven dried and the dried powder was weighed and used for antifungal activity.

Antifungal assay (disc diffusion method)
The broth culture of the fungal isolates was swabbed over the Dixon's agar by using sterile cotton buds. Sterile 5mm diameter discs prepared from Whatman filter paper no.1 were placed equidistantly (3cm apart) round the margin of the plates. The absorption capacity of paper discs was found to be 5 µl per discs and therefore each sample was calculated as per 5 µl. Three replicates were maintained. The plates were incubated at 30 ± 4 ^{o}C and the zone of inhibition was observed after 2 days. Control was maintained with filter paper discs dipped in distilled water.

RESULTS

Amongst the samples cultured on SDA, PDA and mDixon's agar, 3 isolates were selected which were morphologically similar to dandruff causing organism as isolate 1, isolate 2 and isolate 3. In the present research work it was observed that mDixon's agar with the addition of olive oil as a lipid source proved better for the growth of the organism followed by SDA and PDA with coconut oil. And also it was established that Tween 80 and temperature 32 ± 4°C was suitable for the growth of organism.

Morphological characterization:
The organisms were developed as dirty white colored, smooth and pasty in appearance over the medium. Microscopy revealed that the cells were bottle shaped.

Biochemical Characterization:
Catalase test: The test was performed in duplicates and was found to be positive in all the 3 isolates.
Urease test: The test was performed in duplicates and was found to be positive in all the 3 isolates.
Gelatin hydrolysis test: The test was performed in duplicates and was found to be negative in all the 3 isolates.

Sugar assimilation test: The test was performed in duplicates for the 3 isolates and observed for the assimilation of sugars after 4 days of incubation (Table 1.). Amongst the three isolates viz., isolate 1 did not assimilate sugar; in isolate 2 only glucose was assimilated and in isolate 3 all the sugars were assimilated. Thus on the basis of sugar assimilation isolate 3 was selected for further investigation.

Table 1: Sugar assimilation test (*yellow colour shows utilization of the sugar)

No.	Sucrose	Glucose	Dextrose	Xylose	Lactose	Mannitol	Sorbitol	Glycerol
Control	Red	Red	red	red	red	red	Red	red
Isolate 1.	Red	Red	red	red	red	red	Red	red
Isolate 2.	Red	Yellow	red	red	red	red	Red	red
Isolate 3.	Yellow	Yellow	yellow	yellow	yellow	yellow	yellow	yellow

Table 2: Antifungal activity exhibited by various plants studied

Sr. No.	Plant	Zone of inhibition (mm)	Standard deviation
1	*A. concinna*	17	0.4
2	*M. koenigii*	-	-
3	*Ocimum sp.*	9	0.2
4	*P. nigrum*	-	-
5	*A. marmelos*	8	0.5
6	*Hibiscus*	11	0.3
7	*P. betle*	10	0.5
8	*C.cyminum*	-	-
9	*E. officinalis*	22	0.8
10	*B. monnieri*	-	-
11	*T. procumbens*	-	-
12	*T. ammi*	-	-
13	*S. saponaria*	10	0.2
14	*Mentha sp.*	-	-
15	*P.coryfolia*	-	-
16	*C. canadensis*	-	-
17	*C. officinalis*	12	0
18	*K.luciae*	-	-
19	*A.vera*	-	-
20	*A.indica*	-	-

Antifungal assay:

Amongst the twenty plant extracts tested, *E. officinalis* and *A. coccinna* were found to be most effective than other species. Similarly *Ocimum sp., A. marmelos, P.betle, Hibiscus, Sapindus sp. and C. officinalis* were also found to be active against the tested fungal isolate (Table 1).

DISCUSSION

The dandruff causing organism is yeast like fungus. The cells are oval and budding form similar in appearance to that of *Malassezia.* The *Malassezia* species are difficult micro-organisms to identify and maintain in culture. The present research work showed that the organism grew well at pH 5 ± 1, temperature 32 ± 4°C. The dandruff causing organism could not be grown without the use of lipids. Commonly, Sabouraud's agar is used is used for culturing of dermatophytes (Khosravi et al., 2009). Sabouraud's agar and Potato dextrose agar with coconut oil showed poor growth while mDixon's agar (Guillot et al., 1998) with olive oil improved growth. Although the morphological characteristics (colony and microscopic examination) for *Malasezzia* yeast is used for primary identification; but they do not provide sufficient information for specific identification of isolates (Khosravi et al., 2009). Thus for the specific identification of organism characterization by urease test, gelatin hydrolysis test, sugar assimilation test and Catalase test respectively was carried out in the present study. The sugar assimilation test was found positive for one of the three isolates which was further selected for antifungal activity.

Antifungal activity of plant extracts was tested *in vitro*. In the present research work among the twenty plant extracts; the extract of *E. officinalis and A. concinna* were found to be most effective against the tested fungus. Similarly *Ocimum sp., A. marmelos, P.betle, Hibiscus, Sapindus sp. and C. officinalis* were also found to reduced the growth of organism. Thus it is suggested that by making use of all combination of these plants, more effective results could be obtained as it is better to use natural anti-fungal agents as chemical anti-fungal agents possess lots of side-effects. The extractions of active principle from these plants and their assay against *M. furfur* have been suggested by Vijaykumar et al., 2006. The etiology of SD is poorly understood. Many studies have indicated that *Malassezia* yeasts play an important role in SD (Baysal et al., 2004). Many of these are treatment studies which describe the effectiveness of antimycotics.

Thus in the present research work, dandruff causing organism was isolated from the affected individuals and cultured. It's morphological, microscopical and biochemical characteristics were studied. Further on confirmation of the organism 20 plants extracts were tested for their antifungal activity. The extractions of active principle from these plants and their assay against the organism have been suggested as future course work..

Acknowledgements

The authors are thankful to the Principal Dr. M.J.Kolet of G.M.Momin Women's College, Bhiwandi and Principal Dr. V.B.Gaikwad of K.T.H.M. College, Nashik for rendering all necessary facilities and support for accomplishment of the research work.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Diversity of Marine mitosporic fungi from Maharashtra Coast (India) - II

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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 SA, Pawar NS and Borse BD (2018) Diversity of Marine mitosporic fungi from Maharashtra Coast (India) - II, *Int. J. of. Life Sciences*, Special Issue, A9: 84-88.

ABSTRACT

Present paper deals with six marine mitosporic fungi viz., *Bactrodesmium linderii, Cirrenalia basiminuta, Clavatospora bulbosa, Halenospora varia*, *Hydea pygmea* and *Matsuporium tropicale* which were isolated form intertidal wood samples from the coast of Thane District of Maharashtra. The data provides information on the distribution of these fungi in India, apart from description and illustrations. This data will be useful in the compilation of aquatic fungal biodiversity of India.

Keywords: Hyphomycetes, Biodiversity, Intertidal wood, Marine fungi.

INTRODUCTION

Marine fungi can be defined based on their morphology, physiology and ecology and their ability to reproduce in the marine environment. Early physiological studies of marine fungi focused on their salinity tolerance (Jennings, 1986a, b), temperature requirement (Jones, 1971), nutrient requirement (Amon, 1986), enzyme production (Schaumann and Molitories 1986), aspects of wood decay (Leightly, 1980) and ability to grow on different polysaccharides (Meyers, 1971). Garzoli, et al. (2015) updated knowledge on marine fungi associated with wood substrates in the Mediterranean Sea and hints about their potential to remediate hydrocarbons.

MATERIAL AND METHODS

The study involved the frequent visits to collection sites located in and around coastal region in Thane district of Maharashtra to collect the intertidal drift wood, woody debris, dead stem, root, leaves, fruits of mangroves. specimens were returned to the laboratory and observed

under research microscope for the presence of sporulating structure like mycelium, conidiophores and conidia of Hyphomycetes.

The permanent slides were prepared as suggested by Volkmann- Kohlmeyer and Kohlmeyer (1996). Identification of marine fungi were confirmed with the help of monographs and illustrated keys provided by Kohlmeyer and Kohlmeyer (1979). Hyde et al (2000), and Jones et al (2009). Reports of fungi from India and Maharashtra were confirmed with the help of Kamat et al. (1971), Bhide et al. (1987), Bilgrami et al. (1979, 1981, 1991), Jamaluddhin et al. 2004 and Borse et al. (2012, 2013) and other relevant literature.

Taxonomic account-

1) *Bactrodesmium linderii* (J.L. Crane & Shearer) Palm & Stewart
Mycotaxon, **15-** 319-325 (1982). (Photo.1; Fig.1)
= *Trichocladium linderii* J.L. Crane & Shearer, *Mycologia,* **70-** 866 (1978).
Mycelium is composed of branched, septate, hyaline to brown hyphae, *Conidiophores* are macronematous, mononematous, smooth, thin-walled and hyaline or thick-walled and brown. *Conidiogenous cells* are holoblastic, integrated, terminal or intercalary, smooth, cylindrical, determinate. *Conidia* are solitary, subglobose to obpyriform, 1-2 septate, without constriction, 18-27 x 8-18 µm, becoming 3-6 µm wide at base, apical cell larger, dark brown to black, 11-16 µm high, basal and sub-basal cells smaller, light brown, wall unequal in hight, hence the base of the conidia become curved.

Material examined-
On driftwood, Bordi- Dahanu; S. A. Gosavi 1118 (PGDB), 3 April 2013.

Distribution in India- *East coast-* Andhra Pradesh. ***West coast-*** Maharashtra, Goa, West Bengal and Kerala (Source- Borse et al. 2012, 2013).

Remarks- The measurements of conidia are agreed with that of *B. linderii* (J.L. Crane and Shearer) Palm and Stewart (Crane and Shearer, 1978). Therefore, it is assigned to that species. It is being reported for the first time from Thane district.

2) *Cirrenalia basiminuta* Raghukumar & Zainal (Photo 2; Fig. 2)
In- Raghukumar et al. *Mycotaxon,* **31-** 163 (1988).

Hyphae are 2.5–4.5 µm in diam., septate, pale brown. *Conidiophores* are terminal, integrated, monoblastic, determinate, 8-27 x 1 µm, conidia borne laterally and directly on conidiophore, solitary, helicoid, 28-38 µm x 20-32 µm. *Condia* are 3-4 septate, constricted at the septa, cells increasing in size from base to apex, apical cell 10-14 x 10-13 µm, subglobose, basal cell cylindrical and tapering, 7-14 x 2-6 µm, pigmentation of cells increasing from base to apex, the apical cell light brown with a reddish tinge.

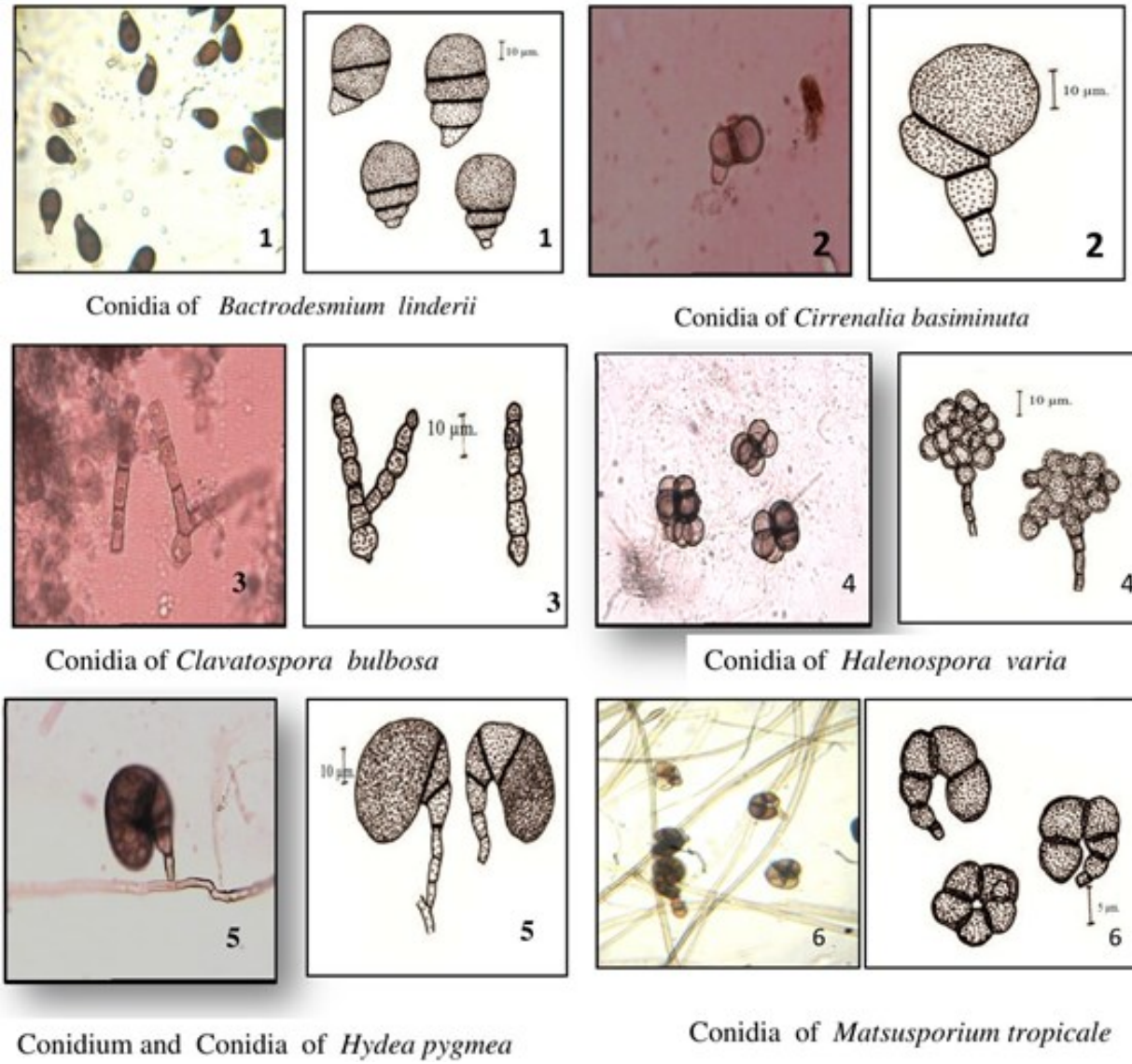
Material examined- On intertidal wood of *Rhizophora mucronata,* Bordi; S. A. Gosavi 1119 (PGDB), 15 Feb. 2012.

Distribution in India- *East coast-* Orissa ***West coast-*** Goa, Kerala, Gujarat and Maharashtra (Source- Borse et al. 2012, 2013).

Remarks- The descriptions of conidia are agreed with that of *C. basiminuta* Raghukumar and Zainal (In- Raghukumar et al. 1988). Therefore, it is assigned to that species. It is an addition to the fungi of Thane district.

3) *Clavatospora bulbosa* (Anast.) Nakagiri & Tubaki. (Photo 3; Fig. 3).
Bot. Mar., **28-** 489 (1985).
= *Clavariopsis bulbosa* Anastasiou, *Mycologia,* **53-** 11, 1961.
Hyphae are 2.5-4 µm in diameter, septate, ramose, and fuscous; *Conidiophores* are 18-78 x 2-4.5 µm, cylindrical, septate, simple or branched, hyaline. *Conidia* are tetra radiate, septate, slightly constricted at the septa, hyaline to light brown, developing by transformation of the inflated apex of the conidiophore, basal arm one–septate, proximal cell 8-16 x 4-9 µm ellipsoidal or ovoid, truncate at the base, light brown; distal cell 7-12 x 6-14µm, cylindrical or shortly three branched, fuscous, three divergent arms arising simultaneously from the inflated distal cell of basal arm, 20-60 x 4-6 µm, cylindrical, one-to-five septate, light brown.

Photo and Figure plates of :



Material examined- On intertidal wood of *Rhizophora apiculata,* Mahim; S. A. Gosavi 1120 (PGDB), 20 April 2014.

Distribution in India- ***East coast-*** Tamil Nadu, Orissa and West Bengal. ***West coast-*** Maharashtra, Kerala, Goa, Gujarat and Pondicherry-Mahe (source- Borse et al 2012, 2013)

Remarks- The descriptions and measurements of conidia are completely agreed with that of C*avatospora bulbosa* (Anast.) Nakagiri & Tubaki (Anastasiou, 1961). Hence, it is assigned to that species. It is being recorded for the first time from Thane district.

4) ***Halenospora varia*** (Anastasiou) E.B.G. Jones (Photo.4; Fig. 4).
In- Jones et al. *Fungal Diversity*, **35-** 154 (2009).
= *Zalerion varium* Anastasiou, *Can. J. Bot*., **41-** 1136 (1963).
Hyphae are septate, branched, immersed, and hyaline, *Conidiophores* are upto 30 µm long, 2-3 µm in diameter, micronematous, simple, cylindrical, septate, sometimes absent, superficial, hyaline to light olive coloured. *Conidia* are 14-62 x 13-44 µm, solitary, irregularly helicoid or coiled in three planes, forming a knot or ball of about 10 to 28 cells; Conidial filament lateral, rarely branched or subtending an additional

conidium; thick–walled, smooth, brown to dark brown, appearing black in mass; cells 6-13x 4-11µm.

Material examined- On intertidal stem of *Avicennia marina,* Bordi; S. A. Gosavi 1122 (PGDB), 15 Octo. 2013.

Distribution in India- ***East coast-*** Tamil Nadu, Orissa and West Bengal ***West coast-*** Maharashtra, Goa, Daman, Gujarat and Kerala (source Borse et al 2012, 2013)

Remarks- The descriptions and measurements of conidia are agreed with that of *H. varia* (Anastasiou) E.B.G. Jones (Jones et al. 2009). Therefore, it is assigned to that species. It is being reported for the first time from Thane district.

5) ***Hydea pygmea*** **(**Kohlm.) K.L. Pang & Jones (Photo.5; Fig. 5)
In- Abdel-Wahab et al. *Mycol. Progress*, **9-** 549 (2010).
= *Cirrenalia pygmea Kohlm., Ber. Drsch. Bot. Ges.,* **79**- 35 (1966).
Hyphae are 2.2-4.5 µm in diam, septate, ramose and fuscous. *Conidiophores* are obsolete. *Conidia* are acrogenous, solitary, igantean, contorted ½ or 1 time contorted, 3-4-septate, not or slightly constricted at the septa, hooked appearance, black or fuscous, fulgent (upper three cells dark, lower two or three cells light-coloured); cells increasing in diameter from base to apex, distinctly dissimilar; spirals 25.5- 31 x 28.5-34 µm; terminal cell 16-23 µm in diam, subglobose to reniform, basely flattened; basal cells 3.5-5.5µm in diam; central cells irregularly conical or almost wedge-shaped. *Note*- It is a common species on mangrove wood, especially *Rhizophora* species, growing on the bark, with slow growth in culture but sporulates readily. It differs from all othe *Cirrenalia* like species by the dark-brown to black hooked nature of the conidia.

Material examined- On decaying driftwood in the intertidal zone, Dahanu; S. A. Gosavi 1123 (PGDB), 5 Mar. 2014.

Distribution in India- ***West Coast-*** Gujarat, Maharashtra, Goa, Pondicherry Mahe and Kerala. ***East Coast-*** Tamil Nadu, Pondicherry, Andhra Pradesh, Orissa and West Bengal (Source - Borse et al .2012, 2013)

Remarks- The descriptions and measurements of conidia are agreed with that of *H. pygmea* (Kohlm.) K.L. Pang and E.B.G. Jones (Kohlm. and Kohlm., 1979). Therefore, it is assigned to that species. It is an addition to the fungi of Thane district**.**

6) ***Matsusporium tropicale*** (Kohlm.) Jones & K.L. Pang
Mycol. Progress, **9-**550 (2010). (Photo -6; fig. - 6).
= *Cirrenalia tropicalis* Kohlm.*Mycologia*, **60**- 267 (1968).

Hyphae are 2-5 µm in diam., septate, superficial or immersed, brown. *Conidiophores* are 24-40 x 2.5-4.5 µm, cylindrical, 0-4-septate, simple, acrongenous or lateral, often remaining connected with detached conidia, sometimes obsolete, straight or curved, light brown. *Conidiogenous cells* are monoblastic, integrated, terminal, and determinate. *Conidia* are acrogenous, solitary, regularly or irregularly helicoid, mostly 1 to 1 ½ times contorted, rarely semicontorted, six - twelve septate, not or slightly constricted at the septa, umber to reddish brown; cells increasing in diameter from base to apex, distinctly dissimilar; spirals 22-35µm in diameter; terminal cell 8.5-14.5 x 11-20 µm, subglobose to ellipsoidal, basally flattened; basal cells 5-10 x 4-5.5 µm; cylindrical; central cells subglobose, obtusely conical or dolliform.

Material examined-On intertidal wood of *Avicennia marina,* Mahim; S. A. Gosavi 1124 (PGDB), 15 Oct. 2013.

Distribution in India-*East coast-* Orissa, West Bengal ***West coast-*** Goa, Kerala- Maharashtra (Source - Borse et al.2012, 2013)

Remarks-The descriptions of conidia are agreed with that of *Matsusporium tropicale* (Kohlm.) Jones and Pang (Kohlmeyer,1968). Hence, it is assigned to that species. It is an addition to the fungi of Thane district.

Acknowledgement -
Authors are thankfull to Incharge Principal of Sathaye college Vile Parle (East) Mumbai. Dr. M.N.Joshi and Miss J.S. Gaikwad (HOD, Botany), The management of N.S. Sanstha Dhule's U. P. College, Dahiwel, Tal.-Sakri, Dist.-

Dhule, Maharashtra for providing necessary laboratory facilities.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Differences in enzyme cellulase production by *Chaetomium globosum* as a effect of UV induce mutagenesis

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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 (2018) Differences in enzyme cellulase production by *Chaetomium globosum* as a effect of UV induce mutagenesis, *Int. J. of. Life Sciences*, Special Issue, A9: 89—93.

ABSTRACT

The current research paperdeals with studies designed to assess the effect of UV induce mutagenesis on enzyme cellulase production by *Chaetomium globosum*and time of UV exposure at which maximum cellulase production take place. Culture of *Cheatomium globosum* expose to UV for different time interval i.e. 10min; 20min; 30min; 40min; 50min; 60min and 0min(control). The cellulase production increases from 10min. to 20min. and decline from 30min. to 60min. The maximum cellulase production was seen at 20min.

Key words: Cellulase, UV induced, *Chaetomium*

INTRODUCTION

Cellulases are a group of hydrolytic enzymes capable of hydrolysing cellulose to smaller sugar components like glucose units. Cellulolytic enzymes play an important role in nature's biodegradation processes where plant lignocellulosic material is efficiently degraded by cellulolytic fungi and bacteria. In industry, these celluloytic enzymes have found novel applications in the production and processing of chemicals, foods and manufactured goods such as paper, rayon and cellophane and the preparation of plant protoplasts in genetic research (Kader *et al* 1999).

There are different kinds of stresses influence the individual's physiological and chemical mechanism such stresses like heat, cold, flood, drought, salinity, UV radiation, chemicals, Abscisic acid, heavy metal and water deficit. Organism strives to survive stress condition by adjusting their gene expression pattern or metabolic activities (Sakpal, 2008). In one of the studies on enzyme cellulase production *in Aspergillus fumigatus, Penicillium chrysogenum and Verticillium terrestre,* isolated from sugarcane field soil, revealed the extracellular production

of amylase, cellulase and lipase was achieved by developing mutants after exposure to UV light (Prabakaran*et al;* 2009). Induced mutations are thought to arise as a result of enzymatic processes utilizing DNA damage as a substrate. In addition, such strains are UV sensitive, X-ray sensitive, and recombination deficient in varying degrees (Jeffrey F; Lemontt, 1970).

UV-induced mutation in fungi, UV-sensitive strains has been selected on the assumption that UV mutagenesis might be related to dark repair of lethal damage. In this study, an attempt was taken to produce mutants of *Chaetomium globosum* by exposing to UV radiation for evaluation of cellulase activities compared to the control and also to evaluate whether it has any considerable effect for the production of such enzymes.

MATERIAL AND METHODS

The effect of cellulosic substrates on the production of extra-cellular cellulases and their cellulolytic activity in *Chaetomium globosum* has been studied in shake flask cultures.

1. Collection of culture :
Culture of *Chaetomium globosum* procured from laboratory of SIES College of arts, commerce, and science.

2. Preparation and Pouring of Culture media:
The different culture media used for proper isolation and growth of *Chaetomium globosum* were follows; Czapek'sDox Agar with Cellulose (Bagool, 1982), Czapek'sDox Agar with Filter paper strips (Subba Rao, 1977), Reese Medium (Mandels& Weber, 1969) . Here cellulose was used as a Carbone source.

3. Inoculation of *Chaetomiumglobosum*:
Pure culture of *chaetomiun globosum* was inoculated into the test tube with media and incubated at room temperature for 9 days. After 9 days, grown culture in test tubes was scrapped lightly by sterile nichrome loop and added 15 ml autoclaved distilled water in it. Water with culture was transferred into new sterilized test tubes which was further well shaken using vortex mixer with addition of tween 80 to obtained uniform spore suspension. This uniform spore suspension was used as inoculum. Seven sterile petri plates with media were taken for inoculation. 1 ml of the suspension was inoculated into each petri plate. These inoculated plates were incubated at room temperature for 9 days.

4. UV treatment:
The UV treatment was given after about 9 days of inoculation into petri plates, when proper growth of culture (*Chaetomiun globosum)* was seen. The UV treatment was given to six out of seven petri plates with culture, where one was kept as control. UV treatment was given to the culture by using technique proposed by *David B. Fankhauser* (2001). Initially UV chamber was cleaned up and sterile by alcohol before using it. One hour before the experiment UV lamp was switched on to sterile the chamber. The petri plates with culture were exposed to UV light without lead one by one at different time interval i.e. 10min; 20min; 30min; 40min; 50min; 60min; which named as C*10*, C*20*, C*30*, C*40*, C*50*, C*60* respectively and one Petri plate was not expose to UV light kept as control which named as C*0.* After UV treatment all treated and one control Petri plates kept at room temperature for one day.

5. Extraction of Enzyme:
Next day after UV treatment and control cultures were used for enzyme extraction. The experiment was based on the methods described by Mandels*et.al.* (1976). Culture from Petri plates i.e. C*0,* C*10*, C*20*, C*30*, C*40*, C*50*, and C60 were scrap lightly by sterile nichrome loop. In each Petri plate added 15 ml sterile distilled water were added. Water with culture was transferred separately into 7 sterile test tubes which were further well shaken using a vortex mixer to obtained uniform spore suspension. Inoculums were further used for enzyme assay. Reese liquid media with cellulose was used as broth. One ml from seven inoculums were further inoculated separately inseven 250 ml conical flasks with 100 ml Reese liquid media with cellulose. These inoculated conical flasks were incubated at room temperature on a rotary shaker at 180 rpm. for 8 days. After 8 days broths were filter through glass wool. These filtrates were stored in freezer which used as enzyme source for determining

cellulase activity. Seven enzymes were extracted from 6 UV treated i.e. 10min; 20min; 30min; 40min; 50min and 60 min. and one control culture of *Chaetomiun globosum* named as E*10*, E*20*, E*30*, E*40*, E*50*, E*60* and E*0* respectively.

7. Cx and C1 enzyme activity:
The seven filtrates (enzymes) were extracted from6 UV treated and one control culture used to determined Cx and C1 enzyme activity. The Cx and C1 enzyme activities of the filtrates were determined by estimating the reducing sugars formed, using DNSA reagent determined by Mandels*et.al* ,1976cited (Gosavi., 2008).

8. Protein estimation:
It was done for enzyme extracted from 6 UV treated and one control culture based on method proposed by Lowry *et al;* (1951).

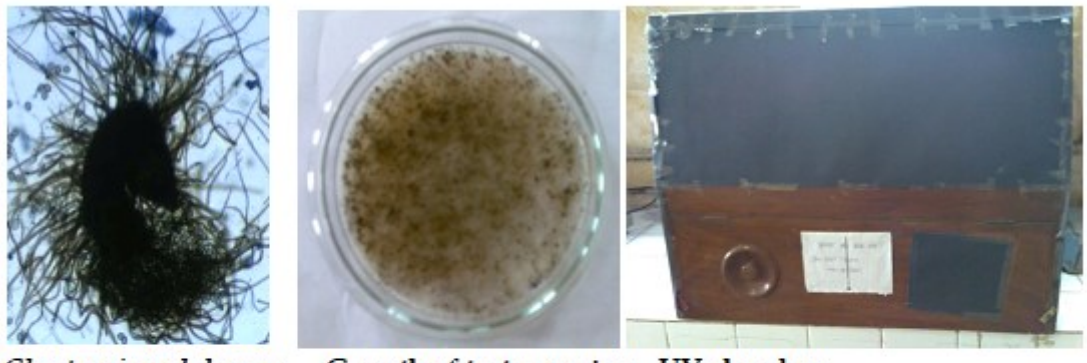
9. Reducing sugar estimation:
Enzymes extracted from UV treated culture and control was further proceeding for estimation of reducing sugar by DNSA method. Estimation of reducing sugar was done by DNSA method determined by Miller (1972).

RESULTS & DISCUSSION

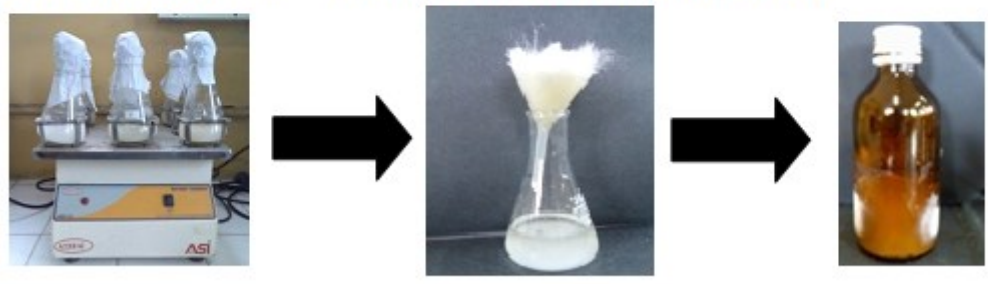
1. The Cx and C1 enzyme activity
Enzyme activity increases from enzyme E*10* to E*20* and again decline from E*30* to E*60.* The enzyme E20 show maximum enzyme activity than other enzyme extracted from treated culture and control culture *Chaetomiunglobosum*. Similar results were seen in *Aspergillusfumigatus* after treatment of UV (Prabakaran*et al;* 2009).

2. Protein estimation:
The mg of protein content per ml of enzyme increased from enzyme E*10* to E*20* and again decline from enzyme E*30 to* E*60.* The maximum protein content was observed in *enzyme E20 than other enzyme extracted from UV treated culture and control culture Chaetomiun globosum*. Similar results of protein estimation were obtained to that Aspergillus *fumigatus, Penicillium chrysogenum and Verticillium terrestre* after treatment of UV(Prabakaran *et al;* 2009).

3. Reducing sugar estimation:
The mg of reducing sugar content per mg of protein in enzyme increased from enzyme E*10* to E*20* and again decline from enzyme E*30 to* E*60.* The maximum mg of



Chaetomiumglobosum Growth of test organism UV chamber



Extraction of Enzyme

TABLE NO.1: Cx enzyme activity

Enzyme	mg of sugar/mg of protein
E0 *(control)*	35
E*10*	20
E*20*	36.36
E*30*	30
E*40*	26.67
E*50*	20
E*60*	20

TABLE NO.2: C_1 enzyme activity

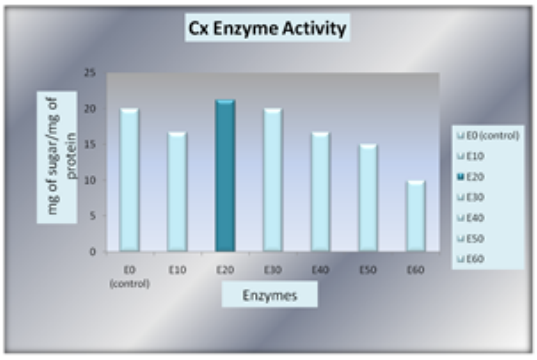
Enzyme	mg of sugar/mg of protein
E0 *(control)*	35
E*10*	20
E*20*	36.36
E*30*	30
E*40*	26.67
E*50*	20
E*60*	20

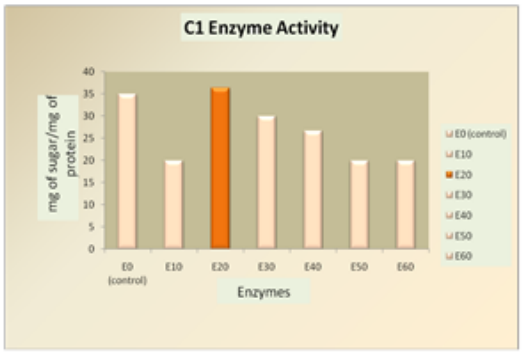
TABLE NO.3: Estimation of Proteins

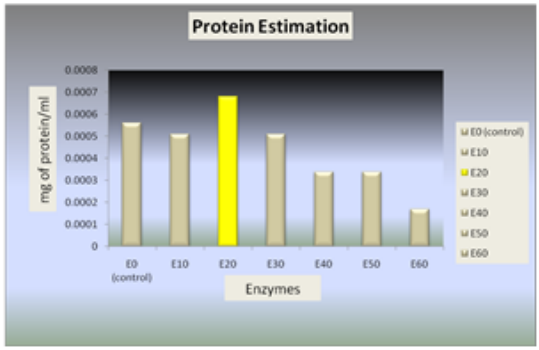
E0 *(control)*	0.000561
E*10*	0.00051
E*20*	0.00068
E*30*	0.00051
E*40*	0.00034
E*50*	0.00034
E*60*	0.00017

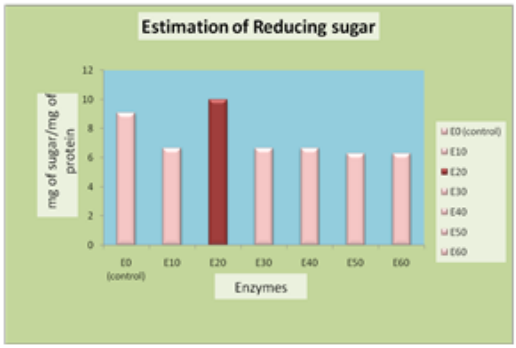
TABLE NO.4: Estimation of Reducing sugars

E0 *(control)*	9.09
E*10*	6.66
E*20*	10
E*30*	6.66
E*40*	6.66
E*50*	6.29
E*60*	6.29









reducing sugar content per mg of protein in enzyme seen in enzyme E*20* than other UV treated and control culture of *Chaetomiun globosum.* This experiment is supported by isolation and partial purification of extracellular enzyme (1,3)-3-D Glucanase from *Trichoderma ressei* (Saravanan*et al*;2007).

In many cases, mutations by UV are harmful, but occasionally it may lead to a better adapted organism to its environment with improved biocatalytic performance. The potential of a microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool. The

challenge is to isolate those strains which are true mutants that carry beneficial mutations *(Prabakaranet al., 2009).*

The above data support the view that cellulase production seen in culture of *Chaetomiun globosum* which exposed to UV light for 20 min. than other culture which exposed to UV light for 10min; 30min; 40min; 50min and 60 min. and control culture of *Chaetomiun globosum.*

Therefore we concluded that Chaetomiun*globosum*get mutated after exposed to UV light, the result of that enzyme cellulase production of *Chaetomiun globosum* after expose of UV light increased till certain time i.e. 20 min. and more expose to UV light enzyme cellulase production of *Chaetomiun globosum* started to decline.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Comparative studies on laccase enzyme production by two different species of *Trichoderma*

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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 (2018) Comparative studies on laccase enzyme production by two different species of *Trichoderma*, *Int. J. of. Life Sciences*, Special Issue, A9: 94-98.

ABSTRACT

Two different species of *Trichoderma viz., T. atroviride and T. erinaceum* were isolated from decomposing coconut coir and were screened for their laccase producing abilities. Production of extracellular laccases by *Trichoderma atroviride and Trichoderma erinaceum* was carried out under submerged fermentation at varying pH of the culture medium and different lignocellulosic agro industrial residues. The optimum pH of the culture medium for laccase production was reported to be 5. Sawdust was found to be the most efficient substrate for laccase enzyme production, followed by sugar cane bagasse. Maximum production of laccase enzyme has been noticed with regard to natural carbon sources than synthetic one.

Keywords: *agro industrial, laccase, lignocellulolytic, Trichoderma atroviride, Trichoderma erinaceum*

INTRODUCTION

The lignocellulosic biomass consists of cellulose, hemicellulose and lignin. Enzymatic hydrolysis of cellulose and other related oligo-saccharides is catalyzed by cellulase enzyme system that includes exoglucanases, endoglucanases and β-glucosidases acting in a synergistic manner (Acharya *et al.*, 2008). Hemicellulose being heterogeneous in nature is hydrolyzed by hemicellulases. Lignin depolymerization is necessary to get access to cellulose and hemicellulose fibers. However, the recalcitrance nature of lignin makes its degradation difficult. The degradation of lignin is brought about by the lignin degrading enzymes.

The ligninolytic enzymes include lignin peroxidase, manganese peroxidase and laccase (Ruiz-Duenas and Martinez, 2009).

Laccase (E.C.1.10.3.2, p-benzenediol: oxygen oxido-reductase) 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 (Thurston, 1994).

Fungi have been recognized as efficient producers of lignocellulolytic enzymes among microorganisms. 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 (Shraddha *et al*., 2011). *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 (Kiiskinen *et al*., 2004). *Trichoderma atroviride*, *T. harzianum, T. longibrachiatum* and *T. erinaceum* have also been reported to produce laccase (Assavanig *et al*., 1992; Hölker *et al*., 2002, Sandhu and Kalra, 1982).

The present study deals with the effect of pH of cultivation medium and different lignocellulosic agro wastes on the production of laccase by *Trichoderma atroviride* and *T. erinaceum*.

MATERIALS AND METHODS

I. **Isolation of fungi:**

Trichoderma atroviride and. Trichoderma erinaceum were isolated from decomposing coconut coir using a ten-fold serial dilution-plating technique on potato dextrose agar (PDA) plates. The plates were incubated at 28°C. The pure cultures were then transferred to PDA slants and maintained at 4°C and sub-cultured every month.

II. Morphological Identification:

The macroscopic characters such as colour, appearance, and diameter of colonies and microscopic (microstructures) characteristics were studied. The fungal isolates were identified according to Barnett and Hunter (1972) and the results were confirmed from Agharkar Research Institute, Pune, Maharashtra, India.

III. Primary Screening for Laccase production:

ABTS – Plate screen test:

Plates containing Lignin-agar basal medium (Pointing, 1999) supplemented with 0.1% ABTS and 0.01% of 20% w/v aqueous glucose solution were inoculated with test fungi and incubated at 28° C. The formation of green halo around the fungal colonies indicates the production of laccase enzyme (Niku- Paavola *et al*., 1988).

IV. Preparation of Inoculum 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 28° $\pm$ 2°C on an orbital shaker at 150 rpm for 48 hrs. to obtain large quantity of active mycelia.

V. Cultivation Media for Laccase enzyme Production:

Tien and Kirk medium (1988) with slight modifications was prepared. The pH of the culture media was adjusted to 4.5 using Citrate phosphate buffer.

VI. Enzyme Production by Submerged Fermentation:

25 ml of the media was dispensed into 250 ml of Erlenmeyer flask and autoclaved at 121°C for 15 mins. The flasks were inoculated with 5 ml of spore suspension and then incubated for 6 days at 28°C on rotary shaker at 150 rpm.

VII. Enzyme extraction:

The contents of the flasks were filtered through Whatman No. 1 filter paper after six days of cultivation. The filtrates were then centrifuged at 5,000 rpm for 15 mins. The supernatants were used as the crude enzyme extracts for further analysis.

VIII. Laccase assay:

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) (Sandhu and Kalra, 1982). 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 spectrophotometrically 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 oxidi-zed product per minute, expressed as µmole/ min /L.

IX. Effect of pH variation:

To study the effect of pH of the culture medium on enzyme production, the culture media was adjusted to different pH using 0.1N HCl and 0.1N NaOH. For laccase enzyme production the pH range of the culture medium used was 3, 4, 5, 6 and 7.

X. Effect of varying carbon sources:

Various agro industrial residues like sugarcane bagasse, fibrous mesocarp of coconut and saw dust were used as carbon sources.

XI. **Statistical analysis:**

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

RESULTS AND DISCUSSION:

I. Morphological characteristics:

***Trichoderma atroviride*:**

The white mycelial colony appears uniformly dispersed, granular with 1-2 concentric rings showing green conidial production. White pustules produced on the green mat of conidia. Conidiophores branching typically unilateral; Phialides 6.0 -10 x 1.0 - 3.0 µm, straight or sinuous , sometimes hooked, whorls of 2-4 often cylindrical; Conidia 1.0- 1.3 µm long sub globose to ovoidal; Chlamydospores produced within 7 days, globose to sub globose, terminal or intercalary. (Fig.1.a, b)

Trichoderma erinaceum:

The colony is flat filamentous, initially white, turning green, conidiophore branches at right angles or less with respect to the main branch, phialides in whorls of 2 or 3, almost cylindrical to swollen in the middle (6.0 to 8.0 µm long), conidia 1.3- 1.5 (L/W) ellipsoidal to broadly ellipsoidal , smooth (Fig.2.a, b).

II. Qualitative screening for laccase production:

ABTS Plate screen test

ABTS has been considered as best substrates for laccase activity (Thurston, 1994). The formation of a green halo in the ABTS supplemented plates indicated laccase production by *Trichoderma atroviride* and *T. erinaceum* (Fig.3). *Trichoderma* strains have been reported to produce polyphenol oxidases (Assavanig *et al*., 1992). Studies have demonstrated laccase activities by *Trichoderma atroviride* and *T. harzianum* (Hölker *et al*., 2002; Kiiskinen *et al*., 2004).

III. Quantitative estimation of enzyme activities:

a. Effect of pH:

The pH of the culture medium influences laccase production by *Trichoderma atroviride* and *Trichoderma erinaceum* (Fig. 4: a). Both *Trichoderma atroviride* and *Trichoderma erinaceum* showed maximum laccase production at pH 5.0 indicating the acidic condition necessary for enzyme secretion. Decrease in laccase activity was noticed below pH 4. A sharp decline in the enzymatic activity with increasing pH was also noticed in the present study. No laccase activity was detectable at pH 7 under the given assay conditions. Studies have shown initial pH for laccase production by fungi to be between 4.5 and 6.0 (Shraddha *et al*., 2011).

Most reports indicated initial pH levels set between pH 4.5 and pH 6.0 prior to inoculation, but the levels are not controlled during most cultivations (Mtui,2012). Nyanhongo *et al*. (2002) reported that an initial pH of 7.0 was the best for optimal growth and laccase production by a newly isolated strain of *T. modesta*.

b. Effect of lignocellulosic substrates on laccase enzyme production enzyme:

It was observed that amongst sugarcane bagasse, saw dust and coconut coir used as a carbon sources in the culture medium, saw dust appeared to be a better lignocellulosic substrate for the production of laccase enzyme. *Trichoderma atroviride* and *Trichoderma erinaceum* showed 1.79428 (U/L) and 1.6228 (U/L) laccase enzyme activity when saw dust was used in the medium. (Fig.4 b)

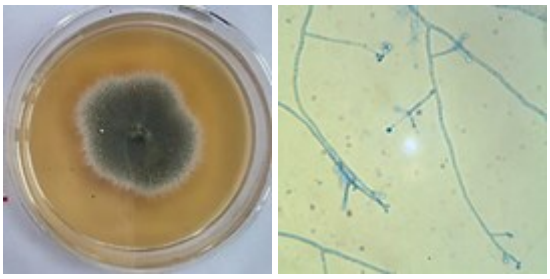

Figure 1: *T. atroviridae* **(a)** Colony morphology (b) mycelia bearing conidiophores and conidiospores after staining and mounting with Lactophenol Cotton Blue

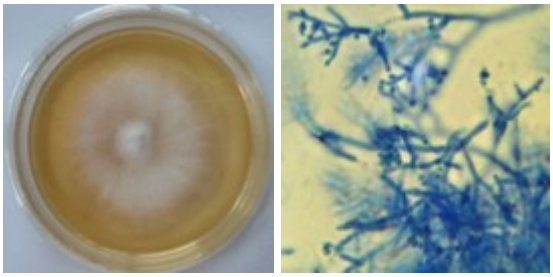

Figure 2: *T. erinaceum* **(a)** Colony morphology (b) mycelia bearing conidiophores and conidiospores after staining and mounting with Lactophenol Cotton Blue

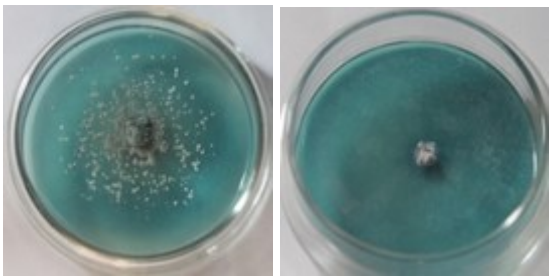

Figure 3: ABTS plate assay : **a.** *T. atroviridae* **b.** *T. erinaceum*

Table 1: Effect of lignocellulosic substrates in the production medium (Laccase)

Sr. No.	Fungal strain	Enzyme activities (U/L)		
		Sugarcane bagasse	Saw dust	Coconut coir
1	*Trichoderma atroviride*	1.44	1.79428	1.31428
2	*Trichoderma erinaceum*	1.16571	1.6228	0.9714

Table 2 : Laccase enzyme activities (U/L) at varying pH of the production medium

Sr.No.	Fungal strains	pH			
		3	4	5	6
1	*Trichoderma atroviride*	0.4457	0.8685	0.6057	0.2514
2	*Trichoderma erinaceum*	0.418	0.7234	0.5257	0.1828

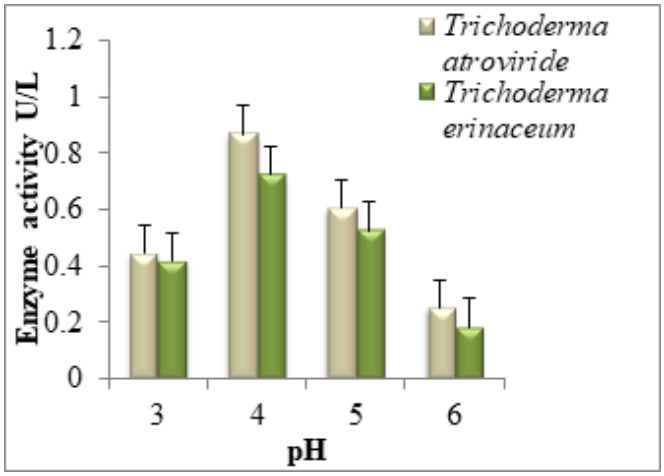

Figure 4. a: Effect of pH on laccase enzyme production

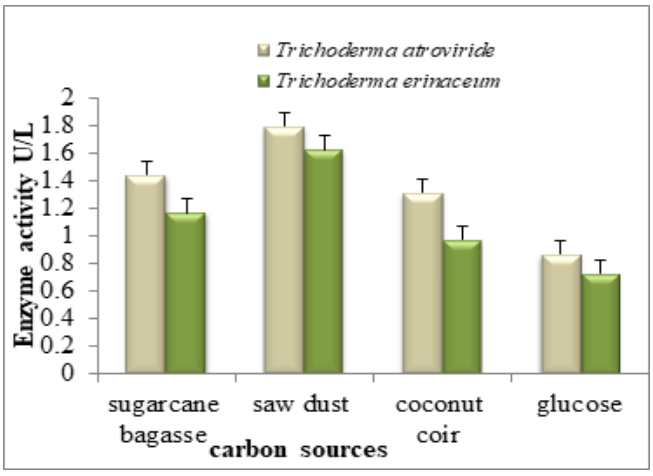

Figure 4 b: Effect of lignocellulosics (carbon) sources on laccase enzyme production

CONCLUSION

The results of the study clearly indicate that the isolates *Trichoderma atroviridae and T. erinaceum* have the ability to produce laccase enzymes, pH optima at 4.0. Sawdust served to be the best substrate for enzyme production.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Study of Intramural Aeromycoflora of the College Premises

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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:
Gupte Archana Ansari, Naziya, Ansari Mariyam, Ansari Saba (2018) Study of Intramural Aeromycoflora of the College Premises, *Int. J. of. Life Sciences*, Special Issue, A9: 99-102.

ABSTRACT

The objective of this study was to know the diversity of fungal flora in the campus environment of the college. In present investigation *Rhizopus sp, Aspergillus sp, Mucor mucedo, Albugo Sp* were found more prevalent in aeromycoflora. Fungal species belongs to Deuteromycetes & Ascomycetes dominated with 37.5% colonies exhibiting highest fungal count followed by Phycomycetes with 25% count. Aeromycoflora was collected by using Petri Plate exposure method on potato dextrose agar. A total of 16 fungi were identified. The present investigation on air borne mycoflora will be helpful in disease forecasting, epidemiology and timely disease management.

Key words: Aeromycoflora, *Rhizopus sp, Aspergillus sp,* Ascomycetes, Deuteromycetes.

INTRODUCTION

Aerobiology is a scientific discipline focused on the transport of microorganism and biologically significant materials. Aerobiological studies are widely used to determine the fungal spectrum in the air (Isabel et.al 2012). It is concerned with the sources of organisms or materials, the release in atmosphere, dispersion, deposition and impact on human and plant system. The distribution of fungal air spora in the environment varies from place to place attributed to variation in climate, season, geographical location, vegetation flora combination (Gali, 2014).

Fungal spores originally created from plant, animal and soil sources get airborne during day time particularly in the afternoon, carried to a long distance, suddenly deposits on epidermal region of plant parts and may cause diseases to diverse group of healthy plants. (Z.I.El Gali 2014) (G.N.Agrios, 2005). They are implicated in damage of food commodities, spoilage of stored grains, fruits, foodstuff, in deterioration of organic material and their high concentration of

mycotoxins may cause health hazards. Usually these fungal spores cause no trouble to most of the human population but they can be harmful by provoking allergic responses or infections and cause disorders such as bronchial asthma (significant global public health issue), allergic rhinitis, migraine, urticaria, eczema, and atopic dermatitis and plant diseases.

The study of fungal spores is of high significance due to its role in the field of human allergy, plant diseases (Ellis1985). Aerobiologists are mainly interested in the entrapment, identification and enumeration of transported biological materials in the atmosphere. Assessment of aeromycoflora is necessary for the control and prevention of fungal diseases so as to protect the human from fungal allergy and to minimize heavy economic losses through spoilage of grains by fungal spores. With the objective to determine the diversity of airborne fungal spores in the environment which will be helpful for further studies on allergy, the aeromycofloral survey was conducted in the campus area of the college.

MATERIAL AND METHODS

Three different locations in a campus area have been selected as sampling site. The samples of different locations were collected on 10 November 2017 on sterile potato dextrose agar (PDA) nutrient medium in Petri plates composed of peeled potato (125 gm/l), dextrose (10 gm/l) and agar (10 gm/l) in distilled water.

Petri dishes containing PDA nutrient medium were exposed in triplicate for 10-15 minutes in the sampling site, in afternoon between 3 to 5p.m. placed at 10 meter height. An exposure time of 10-15 minutes provided to be very suitable, as it gave adequate colony counts. The exposed Petri plates were sealed with cellophane-tape brought them to laboratory and incubated at 25± 2 ^{0}C in incubator for 3 to 4 days depending upon growth of colonies at alternate cycle of 12 hours dark and light. The developed colonies were counted, isolated and identified.

RESULTS & DISCUSSION

Concentration of air borne fungal spores was measured at the campus area of the college. The PDA plates were kept at about 10 m high from ground level at different locations. Fungal spores were classified by appearance and morphological characteristics (color, size, and shape) and identified by comparing with published keys and monographs (Smith 1990) (Tilak 1989). Fungal spores in our college premises has not been estimated or reported earlier. The total of 16 genera of fungal spores were identified from 78 fungal colonies were listed in the photographs.

Among all the fungal spore types the taxonomic group Deuteromycetes & Ascomycetes showed dominance in the total spore contribution with 37.5% followed by Phycomycetes with 25%. Members of Myxomycetes & Basidiomycetes were absent in the result due to the lack of favorable conditions necessary for their growth & development. The results obtained showed a similar pattern with the previous studies (Sharma et al 2011). The *Rhizopus sp* shows maximum dominance with 15.38 % contribution followed by *Drechylera sp* with 10.25% contribution. *Mucor mucedo* and *Bitrimonospora* sp shows equal level of % contribution in the air which was followed by *Aspergillus sp* and *Phytophthora sp*. Rest of the fungi were present in low concentration. Deuteromycetes or the fungi imperfecti represent the species which have thick spore walls that may promote them to remain viable in the air for longer time which may be the reason of getting more fungi from Deuteromycetes type. Similar results were obtain (Kotwal et al 2010) while studying outdoor aeromycoflora at Nasik.

The objective of the present study was to carry out the survey to determine the diversity of airborne fungal spores in a particular period of time and to estimate the variation in aeromycoflora in the college premises atmosphere. Impact of airborne fungal spores including their release, dissemination, deposition and effect is of great significance to identify the health hazards and physiological disorders in living beings.

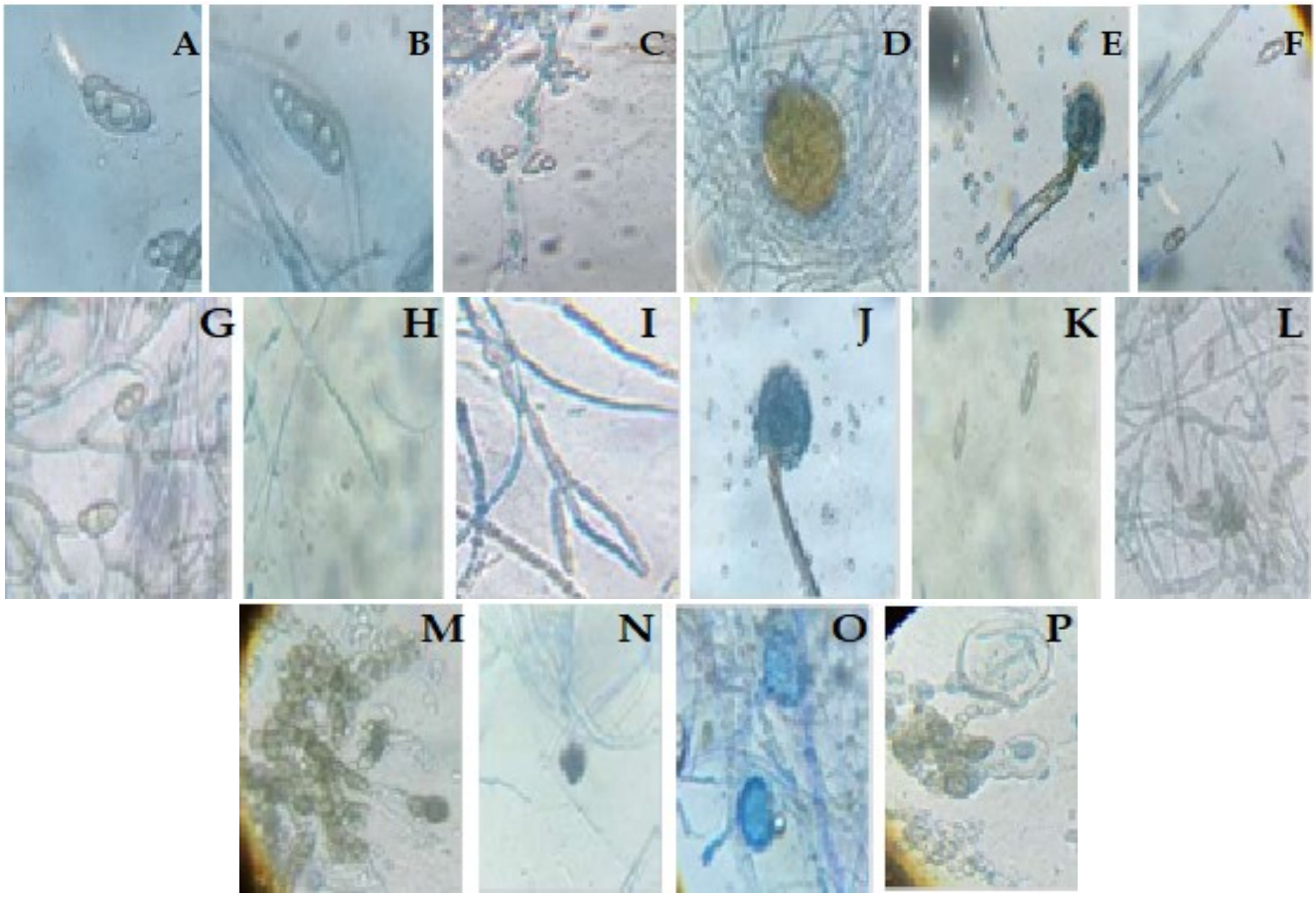

Fig1. Airborne fungi detected in the campus area (A. *Drechylera sp,*
B. *Massarina sp.* **C.** *Aspergillus sp.* **D.** *Phytophthora sp.* **E.** *Mucor mucedo* F. *Apiorhyncostoma* **G.** *Massaria sp* **H.** *Phaetrichoconis sp* **I.** *Tetrapola sp* **J.** *Rhizopus sp* **K.** *Cordona sp* **L.** *Pestalotiopsis sp.* **M.** *Cladosporium sp* **N.** *Harknessia sp* **O.** *Bitrimonosporous sp* **P.** *Albugo sp.)*

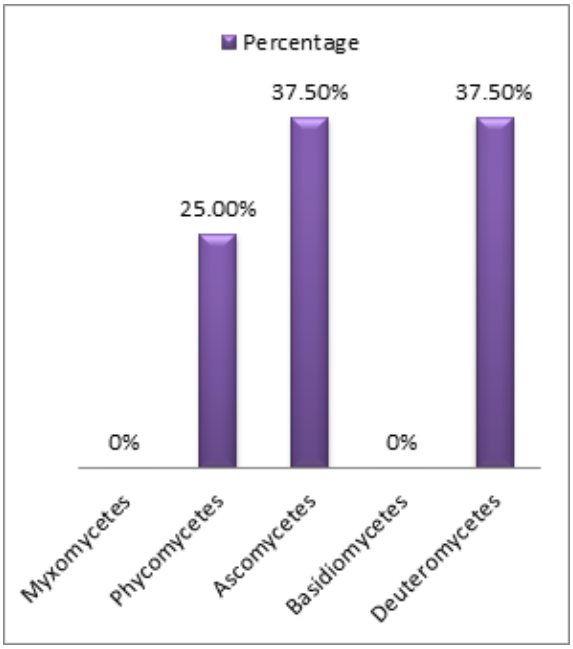

Fig2. Percentage contribution of different class of fungi

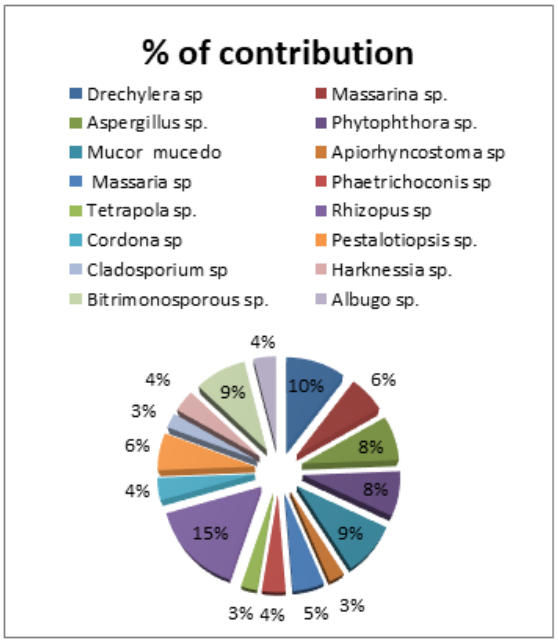

Fig3. % contribution of Aeromycoflora

Study of this aspect is highly interdisciplinary in nature and has tremendous scope to find the significant application in human health and in plants. Exposure to outdoor and indoor airborne inhalant mold allergens develops respiratory symptoms and airway diseases and allergies and at the same time destroys the vegetation which may leads to economic loss. Thus clean environment is of prime importance to reduce the fungal spore load in the air.

In the present study the clear picture of the diversity of fungal spores present in environment of the college during the month of November when the temperature and humidity both were moderate were observed.

CONCLUSION

Environmental micro fungal population is seemed to act as an indicator of the level of environmental biopopulation. In the present investigation aeromycoflora were belonging to Deuteromycetes and Ascomycetes type show maximum, contribution indicating the climate suitable for their growth. Absence of Myxomycetes and Basidiomycetes type indicate absence of crop field in the study area. The airborne fungal spore may provoke variety of respiratory diseases and other health problems. At the same time it may attack plant parts causing diseases in plant system.

Acknowledgement -
We would like to thanks K.M .E Society's G.M.Momin Women's College for providing research facility in the college. We express our sincere thanks to the Principal Dr. M.J.Kolet for his support and guidance. We also gratefully acknowledge the non teaching staff of the Department for their cooperation.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Effect of nitrogen sources on growth and sporulation of five species of *Aplosporella* Speg.

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Kaste PS (2018) Effect of nitrogen sources on growth and sporulation of five species of *Aplosporella Speg*., *Int. J. of Life Sciences*, Special Issue, A9: 103-106.

ABSTRACT

Fungi differ widely in their choice and ability to utilize various sources of nitrogen for their growth and reproduction. In the present study, five different species of *Aplosporella* were investigated for their behavior under the influence of eleven nitrogen sources using liquid basal medium of Asthana and Hawker. Five species of *Aplosporella* so selected for the present investigations were obtained from different plant hosts of diversified host families. These are *Aplosporella labiatae, A. rubiae, A. coniferae, A.citrae and A. brossimumii*. Pure cultures of these five isolates of *Aplosporella* were maintained on Potato Dextrose Agar medium. Inorganic nitrogen sources like Ammonium nitrate, Ammonium chloride, sodium nitrite, sodium nitrate, potassium nitrite,potassium nitrate and organic nitrogen sources like phenyl alanine, aspartic acid, glycine, urea and peptone were taken for present investigation. From the results, it is concluded that the *Aplosporella* grows better in inorganic nitrogen sources than the organic nitrogenous sources.

Key words : *Aplosporella*, nitrogen sources, growth, sporulation

INTRODUCTION

Fungi are specific in their choice of food, but carbon and nitrogen substances are the most important substances required by fungi with regard to their vegetative and reproductive growth. Nitrogen is considered as one of the vital importance as being the chief constituent of proteins and a basic component of protoplasm. Nitrogen, thus, is indispensible for growth of fungi, having both functional and nutritional importance. A particular source of nitrogen is, however, not equally suitable for every fungus because of their differential choice and ability to utilize nitrogen obtained from various sources.

The essentiality of a proper source of nitrogen is an important limiting factor in the nutrition of fungi. Although much work has been done in the past on the nitrogen nutrition of fungi, a generalization of the suitability of different sources of nitrogen for their growth and reproduction is not yet possible. Tandon and Bilgrami (1957) have summarized voluminous literature on this particular aspect.

Generally, nitrates are found to support good growth and sporulation of many fungi, while ammonium salts have shown varying results. Nitrites proved to be toxic to various organisms and seldom promote growth and sporulation of fungi. Organic nitrogen sources, on the other hand, have generally been proved to be more promising and induce better mycelial growth and sporulation. Amino acids are reported to be superior sources of nitrogen than the inorganic sources. Irani (1955), however working with *Phyllosticta papayae* var. *macrospora*, found organic nitrogen sources to be inferior to inorganic ones. Urea and peptone have also been shown to support excellent growth and sporulation in fungi. (Neel *et al*, 1933 ; Lockwood *et al*, 1936, Gotteib, 1946; Leben and Keitt, 1948; Gorden, 1950; Converse, 1953; Aube and Gagnon, 1969; Danielson and Davey, 1973; Sierota, 1977; Jackson *et al*, 1991; Saha & Pan, 1998; Monga, D., 2001)

Nitrogen sources like ammonium nitrate was reported to be a good source for growth of *Diplodia psidii* and *D. viticola* (Shreemali; 1969), *Colletotrichum gloeosporioides* (Tandon & Chandra, 1962) and *Alternaria tenuis* (Mathur, 1978). Amongst nitrogenous inorganic compounds like potassium nitrate and sodium nitrate were observed to be good source for growth and sporulation of majority of the species like *Curvularia, Fusarium, Phoma, Botryodiplodia* etc. (Dandge, 1998).

From the above review it is clear that fungi differ widely in their choice and ability to utilize various sources of nitrogen for their growth and reproduction. In the present study, five different species of *Aplosporella* were investigated for their behavior under the influence of 11 nitrogen sources using liquid basal medium of Asthana and Hawker.

MATERIALS AND METHODS

Five new species of *Aplosporella* obtained from diversified hosts brought into pure culture. These are : *A. labiatae* sp.nov., *A. rubiae* sp. nov., *A. coniferae* sp. nov., A. *citrae* sp.nov. and *A. brossimumii* sp.nov. These five species of *Aplosporella* were designated as isolate L, R, Co, Ci and B respectively. Pure cultures of these isolates were maintained on Potato Dextrose Agar medium. Following nitrogen sources were taken for present investigation.

Inorganic Sources
Ammonium nitrate, Ammonium chloride, sodium nitrite, sodium nitrate, potassium nitrite, potassium nitrate.

Organic Sources
Phenyl alanine, aspartic acid, glycine, urea, peptone.
The original nitrogen source present in the basal medium (viz. potassium nitrate) was replaced individually with different nitrogen sources so as to provide equivalent amount of nitrogen present in the basal medium. 25 ml of the liquid medium was apportioned into 150 ml conical flasks (corning) which were then subjected to fractional sterilization (steaming for 30 minutes for 3 successive days) so as to avoid any decomposition of amino acids etc.

The liquid media containing various nitrogen sources were seeded with the isolates of *Aplosporella* and these were incubated for 15 days under laboratory conditions. At the end, the mats were harvested as usual and average dry weight of the three replicates noted.

RESULTS AND DISCUSSION

Isolate L : In this case, all the nitrogen sources supported good growth and sporulation.

Isolate R : This isolate grew better in aspartic acid, glycine, sodium nitrite, sodium nitrate, potassium nitrite and potassium nitrate while in phenyl alanine, urea, peptone, ammonium nitrate and ammonium chloride, it was poorly grown.

Table 1: Effect of nitrogen sources on growth and sporulation of five isolates of *Aplosporella:*

S.N.	Nitrogen sources	Colony characters	Isolate L	Isolate R	Isolate Co	Isolate Ci	Isolate B
1	Phenyl alanine	Growth Pycnidial development Dry. Wt.in mg	Spreading + 23.87	Spreading + 25.10	Scanty - - - 13.47	Isolated - - - 16.10	Isolated - - - 18.24
2	Aspartic acid	Growth Pycnidial development Dry. Wt.in mg	Spreading + 38.33	Isolated + 47.66	Spreading - - - 31.30	Spreading - - - 27.10	Isolated - - - 20.63
3	Glycine	Growth Pycnidial development Dry. Wt.in mg	Isolated ++ 47.90	Isolated + 53.00	Scanty - - - 23.40	Scanty - - - 31.60	Spreading + 55.33
4	Urea	Growth Pycnidial development Dry. Wt.in mg	Patchy + 34.80	Patchy + 23.37	Isolated - - - 22.67	Spreading - - - 28.67	Spreading + 30.10
5	Peptone	Growth Pycnidial development Dry. Wt.in mg	Patchy + 33.30	Scanty - - - 27.26	Patchy - - - 30.40	Isolated - - - 31.90	Patchy + 32.87
6	Ammonium nitrate	Growth Pycnidial development Dry. Wt.in mg	Congested + 18.67	Congested - - - 22.80	Isolated - - - 28.23	Scanty - - - 12.80	Scanty - - - 23.37
7	Sodium nitrite	Growth Pycnidial development Dry. Wt.in mg	Congested ++ 57.13	Congested + 58.87	Spreading - - - 40.58	Scanty - - - 27.40	Spreading + 61.33
8	Ammonium chloride	Growth Pycnidial development Dry. Wt.in mg	Spreading + 47.27	Isolated - - - 29.10	Isolated - - - 37.67	Patchy + 43.57	Patchy - - - 41.57
9	Sodium nitrate	Growth Pycnidial development Dry. Wt.in mg	Spreading ++ 33.00	Isolated + 31.53	Isolated - - - 27.30	Uniform - - - 25.27	Patchy + 32.90
10	Potassium nitrite	Growth Pycnidial development Dry. Wt.in mg	Spreading ++ 52.87	Spreading + 51.30	Patchy + 47.66	Uniform - - - 33.67	Patchy - - - 41.52
11	Potassium nitrate	Growth Pycnidial development Dry. Wt.in mg	Spreading ++ 32.56	Spreading +++ 44.78	Uniform + 20.12	Isolated + 31.68	Patchy - - - 30.47

Pycnidial development was further classified under four different gradations, viz., - - - Nil; + = Poor ; ++ = moderate ; +++ good.

Isolate Co: Sodium nitrite, potassium nitrite and ammonium chloride has responded well for growth as compared to the rest.

Isolate Ci: For this isolate glycine, peptone, ammonium choloride, potassium nitrite and potassium nitrate supported good growth as

compared to urea, aspartic acid, sodium nitrite, sodium nitrate, phenyl alanine and ammonium nitrate.

Isolate B : For this isolate sources like glycine, urea, peptone, sodium nitrite, sodium nitrate have proved to be good for growth and sporulation while phenyl alanine, aspartic acid, ammonium nitrate, ammonium chloride, potassium nitrite, potassium nitrate were poor for growth and sporulation.

From the table, it is interesting to note that isolate Co, Ci and B failed sporulate in phenyl alanine, aspartic acid and ammonium nitrate whereas isolate L and R showed sparse and poor development of pycnidia. In glycine, all the isolates showed brown colored colony. Isolate Co and Ci did not develop pycnidia while isolate L, R and B developed poor pycnidia. Similar was the response of urea. Ammonium nitrate and peptone, however proved to be a poor source of nitrogen for all the isolates. Sodium nitrite is good for isolate L, R and B and potassium nitrite is good for isolate L, R and Co. Potassium nitrate proved to be a better source for four isolate i.e., L, R, Co, Ci and in isolate B only vegetative growth absorbed. Subhedar (1977) proved that potassium nitrate is a better source for five isolates of *Aplosporella* (out of the seven isolates).

So, it is the concluded that the *Aplosporella* grows better in inorganic nitrogenous sources than the organic nitrogenous sources.

Acknowledgement
The authors are extremely grateful to Principal and Head, Botany Department, Adarsha Mahavidyalaya, Dhamangaon Rly. For providing necessary laboratory facilities and encouragements.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

A study of Rhizosphere fungal populations of two plants from two populations in Ulhasnagar

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Vaidya Sharda (2018) A study of Rhizosphere fungal populations of two plants from two populations in Ulhasnagar, *Int. J. of. Life Sciences*, Special Issue, A9: 107-110.

ABSTRACT

Rhizosphere is a very specific region around the roots where maximum activities of microorganisms take place among themselves and with the plant roots. Smt. C. H. M. College is having 16 acres of lush green area wherein a number cultivated and wild plants grow luxuriously almost throughout the year. Just outside the college, flows the Waldhuni River which is completely polluted. Though the wild populations of plants are same in both, CHM campus and the banks of Waldhuni River, the soil to which they are exposed is completely different. The present project is an attempt to study the differences fungal populations in the rhizosphere of two plants - *Polygonumglabrum* and *Scopariadulcis*. It was observed that in the polluted soils, the number of fungal populations was more.

Keywords: Rhizosphere soil, *Polygonumglabrum, Scopariadulcis, Aspergillus, Penicillium*.

INTRODUCTION

The rhizosphere is a small area of soil around the root. In 1904, Hiltner, the soil bacteriologist and the professor of Agronomy at Technical College of Munich, Germany, for the first time demonstrated that the microbes in the rhizosphere play an important role in the nutrition and growth of the plant. The rhizosphere region is very small covering only a few millimeters. Only in sandy soils itcan be extended up to 1 centimeter. Lynch and Whipps, (1990) and Pinton *et al*., (2001), elaborately studied the peculiarities of this region. According to them, the region is rich in root exudates and hence in soil organic matter. It harbors dense populations of variety of microorganisms. Clark (1949), suggested that actual root surface has still higher concentrations of root exudates and hence it attracts more number of microorganisms. He considered the root surface as the rhizoplane region. It is practically difficult to isolate the two regions and the microorganisms thereof.

Because of this, nowadays a term root soil interface is commonly used for the area that may cover both the regions.

The nature of root exudates varies with the plant species, age of the plant, the vegetative and the reproductive stage of the plant and the region of the root. These exudations are of varied nature from sugars, amino acids, organic acids to secretary and excretory products of the plant. They may include secondary metabolites of the plant. All this not just increases the organic matter in the soil but also decides the type of microorganisms that can grow in the rhizosphere of that plant. The presence of microorganisms also increases the root exudations. (Barber and Martin, 1976). The root exudes these substances from specific regions such as germinating seeds, sloughed off cells, root hairs, root apices, etc. The complex geometry of root soil interface is modified by root hairs and mycorrhizae. This increases the volume of rhizosphere and the heterogeneity of soil porosity. (Hinsinger, *et al*., 2004).

The rhizosphere populations comprise of microflora (bacteria, actinomycetes, fungi, algae and viruses), microfauna (protozoa), mesofauna (nematodes) and macrofauna (insects, mites, termites, millipedes, slugs, snails, earthworms, etc). The different associations, interactions of these organisms with each other and with the plant make a complex food web in this region. There associations include symbiotism, parasitism, nitrogen fixing, phosphate solubilization, etc. (Curl and Truelove, 1986). It is practically impossible to study all these organisms at any one time. Hence the present work includes the study of only fungal populations from such soils.

Smt. C. H. M. College is situated on 16 acres of lush green campus in Ulhasnagar, a suburb near Mumbai on Central Railway. It encompasses 6 colleges, 4 gardens, a playground in the backyards and a foreground. The entire area is covered by more than 200 cultivated and wild plants. Waldhuni River originates in Kakole Lake, Ambernath, flows through Ulhasnagar, Viththalwadi and meets Ulhas River at Mohane Village near Kalyan. Since the river is highly polluted due to release of industrial and domestic sewage, it is often regarded as Waldhuninalla (environmental Status Report, Kalyan). The banks of the river thus become the storage spaces for the pollutants brought by the river water as they gradually settle in the same area. The growth of the plants and the nature of exudates in this region is altered because of these conditions. The fungal populations surviving in these conditions are peculiar and hence this project has been undertaken.

MATERIAL AND METHODS

The plants selected were *Polygonumglabrum* and *Scopariadulcis*.

Polygonumglabrum
Family: Polygonaceae
It is herb with few branches. The stem is reddish below and possesses a reddish ring at the node. The leaves are lanceolate, acuminate, glabrous and with sheathing leaf bases. Flowers are pink in long racemes.

Scopariadulcis
Family: Scrophulariaceae.
It is a small, erect, much branched herb. The leaves are small, opposite or in whorls of three, acute, elliptic and serrate. Flowers are axillary, in whorls of 2-3.

Collection of material-plant and soil-

1. The plants of the selected species were collected from the campus of Smt. C. H. M. College, Ulhasnagar and from the banks of Waldhuni River opposite to College.
2. In the laboratory, the plants were gently shaken to remove the loose soil around the roots. The soil that remained adhered to the root surface was collected with sterile scalpel in sterile petriplates as the soil of root-soil interface (rhizosphere) as suggested by Oritsejafor and Adenjii, 1990).
3. All the soil samples were analyzed for soil pH, texture, moisture, organic matter content and for fungal populations.

Soil analysis

1. The pH of the soil samples was determined with the help of pH meter. (Labindia, PICO).

2. The texture of soil was determined by mechanical analysis method as described by Rai (1998).
3. The organic matter content of soil was determined by rapid titration method (Walkley and Black, 1934).
4. The fungal populations were isolated on Potato dextrose agar (Difco Manual, 1969), Malt extract agar (Difco Manual, 1969), Aspergine Mannitol agar (Thornton, 1922) and Czapeck' Dox agar, (Difco manual, 1969) by serial dilution method (Prammer and Schmidt, 1966).
5. The pure cultures were maintained on respective media at room temperature.
6. The fungi were identified at ARI, Pune.

RESULTS & DISCUSSION

pH:

The pH of the soil samples from the college campus showed slightly higher pH values than those of river banks. It may be due to the diverse types of pollutants having different pH values getting mixed together. The overall range of pH was from 4.0 to 8.5 i. e. from acidic, neutral to basic. Since, *Aspergillus* and *Penicillium* grow well at all these pH values, they grow well in all these soils.

The soil texture of rhizosphere soil of *Polygonumglabrum* from the college campus was clayey with some amount of gravel. That from the

Table 1. pH of the soil samples

Name of the plant	Sample	pH for the sample from the river banks	pH for the sample from the college campus
	I	6.5	5.0
	II	7.0	7.5
	III	7.0	7.5
	I	6.5	4.0
	II	7.0	7.0
	III	7.0	8.5

Table 2: organic matter content

Name of the plant	Sample	Soil sample from the CHM campus	Soil sample from the river banks
Polygonumglabrum	I	1.69	3.25
	II	0.73	1.06
	III	1.57	4.73
Scopariadulcis	I	0.43	0.84
	II	0.32	0.88
	III	0.76	0.82

Table 3: fungal flora isolated on selected media

Name of the plant	Soil samples from river banks				Soil samples from the college campus			
	PDA	AMA	CDA	MA	PDA	AMA	CDA	MA
Polygonumglabrum	*A. candidus*	*A. niger*	*A. ornatus*	*P. decumbens*	*Absidia glauca*	*Fusarium roseum*	*A. glaucus*	*F. solani*
	A. ochraceous	*A. fumigatus*	*A. terreus*	*A. niger*	*P. decumbens*	*Curvularia lunata*	*A. ochraceous*	*P. chrysogenum*
	A. niger	*A. versicolor*	*P. decumbens*		*A. candidus*	*Curvularia pallescens*	*Mucor species*	*Curvularia lunata*
			A. niger		*A. niger*	*Sporotrichum chlorinum*	*Trichoderma viride*	*Sporotrichum chlorinum*
					Nonsporulating mycelium	*A. niger*	*P. chrysogenum*	*A. niger*
Scopariadulcis	*P. steckii*	*A. niger*	*A. niger*	*A. repens*	*A. clavatus*	*A. flavus*	*A. versicolor*	*A. repens*
	A. nidulans	*A. flavus*	*A. flavus*	*P. chrysogenum*	*P. chrysogenum*	*P. frequentans*	*P. chrysogenum*	*Curvularia pallescens*
	A. oryzae	*Rhizopus nigricans*		*A. fumigatus*	*Curvularia lunata*	*P. chrysogenum*	*P. steckii*	*A. clavatus*
	A. niger			*A. niger*	*Sporotrichum chlorinum*	*F. roseum*	*Mucor species*	*A. niger*
					Trichoderma virde	*A. niger*	*A. niger*	

AMA-Aspergine mannitol agar, PDA-Potato Dextrose Agar, MA-Malt extract agar
CZA-Czapeck' Dox agar *A. –Aspergillus* *P. Penicillium* *F.-Fusarium*

river banks was sandy clay. The rhizosphere soil of *Scopariadulcis* from college campus was sandy with lot of gravel. Soil samples from the college campus light coloured with less of decomposing matter. The soil from the river banks was blackish, with lot of decomposing matter and foul smell. The soils from the college campus were dry and favoured the growth of Aspergilli. The soil pH values of both the plants for the two locations are given in the table no. 1.

Organic matter

The soil organic matter from the rhizosphere soil samples ofboth the plants was higher in the soils from the river banks and was slightly less in the soil samples of college campus. The soil organic matter represents the decaying organic matter. The more the organic matter, the more is the growth of saprophytic fungi such as Aspergilli and Penicilli. The Rhizosphere mycoflora of the soil. The organic matter content of the soil samples are given in table no. 2.

The dominant genus in all the rhizosphereic soil samples was *Aspergillus*. The species diversity was found to be maximum for the same genus. *Aspergillus niger* was the dominant species. The number of species isolated was higher in the soil samples collected from the river banks. It may be because of higher amount of organic matter present in the soil.

CONCLUSION

The rhizosphere soils are rich sources of fungal populations. The rhizosphere soils from the river banks (though polluted) are rich in soil organic matter and harbor a vast diversity of fungi.

Acknowledgement:

The author is thankful to the University of Mumbai for sponsoring this project and to the Principal, Smt. C. H. M. College for allowing to carry out this project.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Study in relation to Biodiversity of Aeromycological species in chikhloli, Ambarnath, MS, India

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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:
Patel Alpa K and Patil Vivekkumar V (2018) Study in relation to Biodiversity of Aeromycological species in chikhloli, Ambarnath, MS, India, *Int. J. of. Life Sciences*, Special Issue, A9: 111-113.

ABSTRACT

The present investigation is carried out in Chikhloli area of Ambarnath, which has varied geographical structure. Climate is comparatively less humid and temperature is variable. Extramural aerobiological research includes aero microbial survey at various places of chikhloli region. The Aeromycologocal survey was carried out from March 2016 to February 2017 by using Petri – plate exposure method. Total Twenty two micro fungi trapped from the air. This study is very important with respect to qualitative and quantitative information about the airborne fungi. The prevalence of dominant airspora was found to be correlation with the metrological parameter like Temperature and Humidity. The maximum mycoflora was registered during the monsoon period between July to October, which is gradually decreased during summer from March to June.

Keywords: Aeromycologocal, Metrological parameter, Petriplate exposure method, Micro fungi.

INTRODUCTION

Aerobiology is a scientific and multidisciplinary approach focused on the biodiversity of biological significant materials. It is deal with the science which provides information from various disciplines like ecology, mycology, plant pathology, palynology, bio-chemistry, immunology and clinical medicine. Fungi are the most important aero allergens. Fungal spores constitute a significant fraction of air borne particles. They occur in varying concentration in the atmosphere depending upon the climatic factors, locations.

The present study was carried out to identify prevalence of dominant airspora at the various location of Ambarnath and to study the correlation with the metrological parameter like Temperature and

Humidity. The different locations of chikhloli have been selected, this area has well developed residential sector and hills having good amount of vegetation.

MATERIAL AND METHODS

Petri plates exposure methods were used to know the status of culture airborne fungi at different locations of chikhloli. Petri plates containing potato dextrose agar as culture medium were exposed once in a month for 15 minutes. The petriplates after exposure were incubated at laboratory temperature for 6-7 days till sporulation. The fungal forms were identified and isolated to obtain pure cultures. Identification of fungal colonies up to generic level was done on the basis of relevant literature. (Gilman 1957; Barnett 1991; Ellis 1971 and Subramanian 1971). At the time of petriplate exposure, sterilized medium was poured under aseptic condition in each petriplate later on it covered with lid. Occurrence of culturable fungal colonies was correlated with meteorological factors such as rainfall, relative humidity and temperature.

RESULTS AND DISCUSSION

After the one year of the observation period, culturable molds present in the air from the different sites were collected. In all an average during the period of the present investigation, 22 genera were recorded including fungal fragments and unidentified group of spores (Table 1). Highest colony count (720) was recorded from residential area, while another sites shows count of 544, 321 and 217 number of colony.

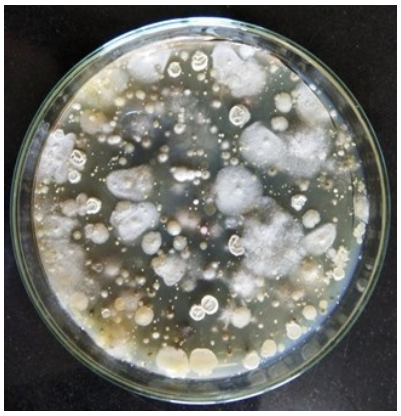

Fig. 1 Fungal spores near chikhloli region

During the period of investigation, spore belonging to group Zygomycotina, Ascomycotina, Basidiomycotina, Deuteromycotina together contributed 92.55% and other types 7.45% to the total air-spora respectively. Among all these types of spores, the group Deuteromycotina contributed highest percentage and lowest percentage contribution was found in group of Zygomycotina.

Out of 22 spore types few of them were belonging to Zygomycotina which includes *Mucor, Cunninghamella,* etc. It is found to be common during August and September due to high humid conditions and considerable rain fall.

The group Ascomycotina was represented by spores belonging to order Sphaeriales, Pleosporales, Hysteriales, Dothidiales etc. Occurrence of many spore types in airspora revealed the presence of many parasitic and saprophytic ascomycetes. Temperature plays significant role in release of ascospores. spores was pronounced prominently in the month of September and October. Less concentration of the spores in July was probably due to less rainfall and humidity.

Group Basidiomycotina was represented by smut spores, Basidiospores, Uredospores, and Ganoderma. Among all the types, Basidiospores showed highest percentage contribution as compare to Smut and Uredospores.

The group Deuteromycotina was represented by maximum different types of spores. The member of Melanconiales and Moniliales showed considerable domination as compared to others. During the period of investigation, spores of *Aspergillus, Alternaria, Cladosporium, Curvularia, Helminthosporium, Penicillium, Periconia* and *Fusarium* found to be contributed in maximum percentage to the total air-spora.

Maximum spore was found in the month of August and September (Fig. 2). Highest percentage of *Aspergillus* and *Penicillium* in the present studies might be due to infected and decaying food grains which were treated as debris present at residential area. The present investigation also includes other types such as fungal fragments and unidentified spore groups

Table 1: List of Fungal taxa identified from the exposed petriplates at different sites

Sr.No.	Fungus Name
1	*Cunnighamella*
2	*Sclerospora*
3	*Alternaria*
4	*Curvularia*
5	*Nigrospora*
6	*Humicola*
7	*Helminthosporium*
8	*Mucor*
9	*Cladosporium*
10	Papularia
11	*Heterosporium*
12	*Penicillium*
13	*Periconia*
14	*Fusarium*
15	*Rhizopus*
16	*Chaetomium*
17	*Aspergillus*
18	*Gleotrichum*
19	*Drechslera*
20	*Pleospora.*
21	*Biospora*
22	*Sporormia*

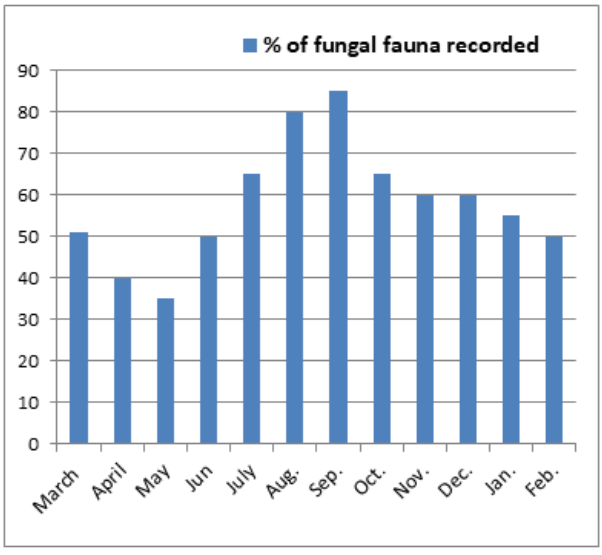

Fig. 2: Month wise variation of fungal spores in percentage

contributed 7.45% to the total air-spora. Unclassified group was found to be changed time to time and change was associated with varied atmospheric conditions. This indicates close relation between spore of this type and meteorological conditions. Maximum percentage of this group was noted in the month of August. The obtained result and conclusion would definitely help to understand the various components of air and their occurrence in the close environment.

CONCULSION

"Aeromycology can be used as a tool for human welfare". Its relation to phytopathology has an aple scope for further investigation. Such studies would bring many useful results like disease forecasting. Biocomponents like fungal spores and pollen grains may initiate allergic responses. Allergic people have an altered capacity to react to potential allergens, causing several types of respiratory and allergic disorders like Asthma and hay fever, eye, skin disorders. Extensive studies on these issues have been carried out in UK and Canada. (Bartzokas 1975). Airborne infections and the resulting diseases threaten the lives and productivity of human beings, animals and plants. Aerobiology thus not simply means the study of microorganism in the atmosphere, but it also take into consideration the allergic properties of various bioparticles like pollen and spores. Last three decade the allergic patients are increasing tremendously in cities as well as villages. The result of the present study will be valuable in solving to cure various diseases and environmental issues.

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

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International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

Effect of fungal elicitation on growth and metabolite production in callus of few medicinal plants

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Meeta Mathur (2018) Effect of fungal elicitation on growth and metabolite production in callus of few medicinal plants, *Int. J. of. Life Sciences*, Special Issue, A9: 114-116.

ABSTRACT

With the developing use of herbal drugs, the need of plants containing secondary metabolites which have medicinal value has increased. Since the intact plant contains low concentrations of active compound, plant callus cultures have employed as an alternative to produce large amounts of these secondary metabolites. Moreover using a bioelicitor the secondary metabolite production can be increased. The objective of this study was to develop a rapid system for the enhanced production of Guggulsterones from *Commiphora wightii*, Zingiberene from *Zingiber officinale* and Falcarinol from *Daucus carrota* *Aspergillus niger* cell extract was used as an elicitor to stimulate the production of secondary metabolite. But inverse results were obtained showing a considerable decrease in Secondary metabolites and growth as well. This is supposed from the results that the mycotoxins may have hindered the metabolite production or probably the biosynthetic pathway was not affected. The same experiment is repeated with cell suspension cultures.

Key words: fungal elicitation *Commiphora wightii, Zingiber officinale, Daucus carrota* *Aspergillus niger.*

INTRODUCTION

The accumulation of secondary metabolites in plants is part of the defense response against pathogenic attack, which is triggered and activated by elicitors, the signal compound of plant defense responses. Therefore, the treatment of plant cells with elicitors has been one of the most useful strategies to enhance secondary metabolites production in plant cell cultures and is recently finding commercial application (Zang et al., 2000). Elicitors are microbe derived molecules which can enhance secondary metabolite production in cultured cells (Dicosmo

and Misawa, 1985). They trigger the increased production of pigments, flavones, phytoalexins and other defense related compounds. Elicitors from fungal origin have been widely employed to increase natural product formation in plant cell cultures and this strategy has been effective in stimulating the production of many chemical classes of secondary metabolites such as terpenoids, coumarin derivatives, alkaloids and flavonoids.

Recently there is a report on increase in production of andrographolide by *Aspergillus niger* and *Penicillium expansum* elicitors in cell suspension culture of *Andrographis paniculata* (Vakil and Mendhulkar, 2013). An oligosaccharin, originating from fungal cell wall is known to induce a number of specific biochemichal changes associated with resistance leading to the synthesis of phytoalexins, lignin and ethylene formation (Ryan, 1988). Since then it has been demonstrated that yeast cell wall preparations, bacterial antibiotics, plant cell wall-derived oligouronides, fungal cell wall derived chitosan, N-acetyl chitohepatose and wounding lead to the rapid synthesis of endogenous jasmonic acid (Muller, 1997).

MATERIALS AND METHODS

Callus cultures of *Commiphora wightii*, *Zingiber officinalis and Daucus carrota* were grown on selected modified Murashige and Skoog (MS) medium. MS modified media [MS-stocks $ABD_{1/2}$ strength (950 mg/l KNO_3, 825 mg/l NH_4NO_3 and 220mg/l $CaCl_2.2H_2O$)] was prepared by mixing salts (in the form of stock solution or weighing fresh every time) and other ingredient in required volume. The media were solidified with 0.8% agar; pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20-25 minutes under 1.05 kg/cm^2 pressures. The callus cultures were initiated on modified Murashige and Skoog (Murashige and Skoog, 1962) medium (950 mg/l KNO_3, 825 mg/l NH_4NO_3 and 220mg/l $CaCl_2.2H_2O$) containing 2, 4-D (0. 5mg/l) and kinetin (0.1 mg/l) (Kumar et al., 2003). Medium was poured in 100 or 250 ml conical flasks contained 20 ml, 35 or 50 ml medium, respectively. Culture were kept vertical to allow cooling and gelling of the medium. Oven dried glassware were used for all experiments. All the cultures were incubated under white fluorescent light 75 µ mol $s^{-1}m^{-2}$ photon flux, 16 hr photoperiod at 26^{0}C temperature and 60% relative humidity. The cultures were monitored daily and the readings were recorded after 4 weeks. The callus cultures were dried and extracted in methanol and HPLC analysis of callus cultures were done and pure or analytical grade chemicals were used throughout the course of study. Preparation of fungal elicitor (Staniszewska *et al.*,2003)

*Aspergillus niger*was grown in 250 ml flasks containing nutrient broth. The flasks were incubated at room temperature under static conditions. At stationary phase, after 21 days, the flasks were autoclaved and the fungal mat separated from the culture medium / filtrate. The culture filtrate was filtered through Whatman No.1 filter paper and made up to a known volume, and autoclaved and stored at 4°C and designed as culture media filtrate.

The fungal mat was washed several times with distilled H2O and an aqueous extract was prepared by homogenizing in a mortar and pestle using acid washed neutralized sand. This extract was filtered through muslin cloth or centrifuged and the clear supernatant was taken. The supernatant is made up to a known volume, autoclaved and stored at 4°C and designated as mat extract. Suspension of *A. niger* cell extract, OD600 = 1.2 (15 ml/l) was added to MS medium. Elicitor was added to MS medium directly before planting cell suspension culture to a new flask.
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RESULTS AND DISCUSSION

Guggulsterones from *Commiphora wightii*, Zingiberene from *Zingiber officinale* (Ernest J.V. Cafino et.al, 2015)and Falcarinol from *Daucus carrota* (Eva M P Wenzig et.al, 2009) were targeted to increase by elicitation with *Aspergillus niger* cell extract. But a considerable decrease in secondary metabolites and growth was observed (Table 1). According to already known fact if the growth retards the secondary metabolite are said to be increased due to the stress created. But here in this case callus from all three medicinal plants didn't showed an increase in growth

Table 1: Effect of elicitor on growth and metabolite production in plants

S. No	Name of Plant	Elicitor (*Aspergillus niger*)	Metabolite present	Metabolite content**(µg/g)**	Dry weight of callus
1.	*Commiphora wightii*	Not added	Guggulsterone	8.1	2.1±0.06
		Added		2.3	1.1±0.05
2.	*Zingiber officinale*	Not added	Zingiberene	2.7	4.1±0.02
		Added		0.9	2.3±0.01
3.	*Daucus carrota*	Not added	Falcarinol	3.5	5.1±0.04
		Added		1.6	3.2±0.05

nor the bioactive molecules targeted increased. Probably the mycotoxins may have hindered the production or the biosynthetic pathway was not affected. The same experiment is repeated with cell suspension cultures.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article Open Access

VAM association in some Ferns of Bhiwandi Maharashtra

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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:
Jain Ritu L (2018) VAM association in some Ferns of Bhiwandi Maharashtra, *Int. J. of. Life Sciences*, Special Issue, A9: 117-119.

ABSTRACT

Studies carried out in Adiantum and Pteris for Arbuscular Mycorrhizal fungal (AMF) colonization. These fern taxa were collected from Bhiwandi, Maharashtra. Investigation reveals that the root of these ferns has Vesicular Arbuscular Mycorrhiza. Mycorrhizal association is also affected by seasons, it is maximum in winter season and minimum in rainy season.

Key words: Adiantum, Pteris, VAM, Ferns

INTRODUCTION

Vesicular arbuscular muycorrizal association between fungi hyphae belonging to glomates (Zygomycota) and roots of plants. The fungi involved are non pathpogenic and now have been reported to enhance growth of the host plant. These fungi help in mineral transport especially phosphorous to the plant and in return gets photosynthetic sugar from the latter (Varma and Schuepp 1995). Aids in soil aggregates formation (Foster and Nicolsen 1981, Clough and Sulton 1978) and provides method for nutrient exchange between roots of non-related plant species.

Though systematic investigation of many Pteridophytic floras have been carried out by Boullard (1957), Cooper (1976), Berch and Kendrick (1982), Harley and Harley (1987) and Gemma *et al*. (1992) but only a little is known about the mycorrhizae in Indian Pteridophytes (Mishra *et al*. 1980, Raghupathy and Mahadevan 1993, Muthukumar and Udaiyan 2000, Prasher *et al*. 2006 Prasher and Baghla 2007).

Thus, the purpose of the present study is to investigate the presence of AMF association in some common lithophytic Pteridophytes of Bhiwandi, Mumbai. These pteridophytes are *Adiantumcapillus-veneris* L., *Adiantumincisum forssk*, *Adiantumphilippense* L and P*terisvittata* L. which

usually grows on derelict house and dilapidated walls. This study was also carried out to note the type of arbuscular mycorrhizal colony formed, seasonal fluctuations in the formation of vesicles and Arbuscules.

MATERIALS AND METHODS

Material for the present study were collected from Bhiwandi Maharashtra during different season of the year. The cleaning and staining of roots was done according to Phillips and Hayman (1970) as follows: Root segments were heated at 90c for about two hours in 10% KOH. Thicker roots were heated for 3 hours. Cleared roots were then rinsed in distilled water and acidified with N/10 HCL. For about 3-5 minutes and stained by simmering for five minutes in 0.05% Trypan blue in Lactophenol. After staining root segments were mounted in lactophenol. A slight pressure on converslip flattened the roots for investigation. The colonization levels in roots was studied under the microscope and present VAM colonization was calculated following Nicolson (1960).

$$\text{\% of root colonization} = \frac{\text{No. of Segments with VAM}}{\text{Total No. of Segments}}$$

Mycorrhizal infections were scored according to the percentage of root length containing vesicles and arbuscular.

RESULTS AND DISCUSSION

Formation of vesicles, arbuscules and spores within the roots. The number of these mycorhizal structures varied for different species in different seasons and shown in Table-I. The vesicle was large in size and oval to round in shape. The arbuscules were fully matured. Percentage of root colonization for any species was maximum in winter and minimum in rainy season.

AMF root colonization of Pterisvitatta was 79% in winter 40% in summer and 15% in rainy season and this variable trend of AMF *root* colonization were also noted in other members highest Percentage of arbuscules was noted in *Pterisvitatta L* followed by *Adiantumcapillus-veneris* L in winter month while lowest record of arbuscules was met in A. capillus-veneris L (6%) followed by *A. incisum* (7%) in rainy season. Colonization Percentage of vesicles was higher in winter season but there was no vesicle in *A. phillipense* during Rainy season. and in *Pterisvittata* during summer season. The presence of VAM colonization suggests that majority of vascular plant in a natural Ecosystem have Mycorhizal association. The presence of Mycorhizal association is depend on various Factors like Seasons (Muthukumar and Udaiyan, 2000).

Table-1: Percentage infection of VAM and formation of Vesicles and Arbuscles in roots of different species of Ferns.

Sr. No	Plants	% Infection	*Seasons*	*% of Arbuscle*	*% of Vesicle*
1	Adiantumphillipense	81	Summer	42	2
			Rainy	14	0
			Winter	45	8
2	A.capillusveneris	80	Summer	14	13
			Rainy	6	8
			Winter	51	16
3	A.incisum	75	Summer	26	7
			Rainy	7	2
			Winter	45	10
4	Pterisvittata	79	Summer	28	0
			Rainy	15	4
			Winter	50	18

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article | Open Access

Evaluation of Phytotoxic Activity and Antagonism of *Trichoderma koningii*

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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:
Rajkonda JN and Bhale UN (2018) Evaluation of Phytotoxic Activity and Antagonism of *Trichoderma koningii*, *Int. J. of. Life Sciences*, Special Issue, A9: 120-124.

ABSTRACT

Trichoderma species are important biocontrol agent plasticized in present agriculture for the management of crop plant diseases. In the present research study, phytotoxic activity of *T. koningii* on crop plant seeds and antagonism of *T. koningii* against plant pathogenic fungi were tested. Treatment of seeds of crop plants with culture filtrate of *T. koningii* indicated the germination potentiality of treated seeds were increased. There was significant increase in germination of seeds of rice(96%), jowar(94%), and wheat(91%) while moderate increase in germination of seeds of mung(82%), groundnut(81%), sunflower(77%). *Trichoderma koningii* was highly antagonistic over plant pathogenic fungi such as *Alternaria alternata* (71.1%), *Geotrichum candidum* (67.7%), *Rhizoctonia solani* (67.7), *Fusarium proliferatum* (64.4%) and *Aspergillus niger* (57.7%). *Trichoderma koningii* is not phytotoxic in nature for crop plants but it is antagonistic against plant pathogenic fungi.

Key words: *Trichoderma koningii* phytotoxic activity, antagonistism.

INTRODUCTION

Trichoderma species are used wprldwide as a most effective biocontrol agent against number of crop plant pathogens. It reduces growth, survival of plant pathogens by different mechanisms like competition, antibiosis, mycoparasitism and enzyme secretion. The soil borne fungus *T. koningii* is a biocontrol agent with ability to produce enzyme, parasitize pathogenic fungi and induce systemic resistance to crop plants. Antagonistic ability of *Trichoderma* species is very effective nd it inhibits the colony formation of plant pathogenic fungi. Many workers contributed in researching antagonistic activities of *Trichoderma* species. They are closely related with both biocontrol activity and plant growth

(Chet et al., 2006; Howel, 1998).The effect of *T. harzianum* and *T. viride* on cumin seeds after priming resulted increased germination, shoot: root ratio and plant height (Sherma et al., 2009).

Trichoderma species stimulated the growth of tomato plants (Ozbay et al., 2004). Treatment with *Trichoderma* species not only checked the disease but it helps to agriculture in many ways such as soil improvement biological activity of soil, soil fertility and mobilization of minerals (Kulisler, 1997). The *Trichoderma* species can bebeneficial to the plants causing growth stimulation (Kleifield and Chet, 1992; Ousley et al, 1994). *Trichoderma harzianum* was found to be antagonistic against many soil borne pathogens (Papavizas, 1985; Pan et al., 2001; Jash and Pan, 2004). In modern period of plant pathology and agriculture, number of formulations were available in market are made by the application of *T. viride* and *T. harzianum*. But it is necessary to determine the ability of other species of *Trichoderma* for the management of crop plant diseases. Therefore in the research paper, performance of *T. koningii* with respect to phytotoxic activity and its antagonistic potentiality over plant pathogenic fungi was considered.

MATERIALS AND METHODS

1. Isolation of *Trichoderma koningii*:
Trichoderma koningii was isolated from the rhizosphere soil of various crop fields. Isolated *Trichoderma koningii* was maintained on potato dextrose agar (PDA) medium.

2. Phytotoxin activity:
Isolated *T. koningii* was grown on Richard's solution (broth). Twenty five ml of Richard's broth was poured in 100 ml conical flasks. The flasks along with medium were incubated at 15 lbs for 20 minutes. The flasks were allowed to cool and inoculated with 1 ml spore suspension of *T. koningii* from 7 days old culture. The flasks were incubated for 9 days at 27±2^{0}C. after the flaks were harvested by filtration through Whatman filter paper No. 1. The filtrate were collected in pre sterilized conical flaks and considered as crude toxin preperations. These were tested for their toxicity.

For the determination toxicity the crude toxin was treated with seeds of wheat, rice, jowar, gram, mung, moth, groundnut, soybean, sunflower, methi, spinach and shepu. Seeds were sterilizes by treating with 0.1% Mercury Chloride solution and followed by repeated washing with sterilized distilled water. The seeds were soaked in crude toxin for 24 hours. Then they were placed in moist blotter paper in sterilized petriplates. Seed soaked in similarly in uninoculated medium served asa control. Percent germination of seeds was observed and data were recorded (Haikal, 2008)

3. Isolation of pathogenic fungi:
Similarly pathogenic test fungi such as *Alternaria alternata, Rhizoctonia solani, Geotrichum candidum*, *Aspergillus niger, Fusarium oxysporum f. sp. Spinacae, Macrophomina phaseolina, Pythium* spp, *Alternaria tenuissima* and *Fusarium proliferatum* were isolated from naturally infected crop plant. These isolated pathogenic fungi were maintained on PDA slants.

4. Dual culture technique (Morton and Stroube, 1955)**:**
Twenty five ml of sterilized melted PDA was poured in sterilized petriplates and allowed to solidify. Seven days old culture of *Trichoderma koningii* was maintained and inoculated with test fungi about 6 cm away from each other and incubated at 28 ± 1°C. Three replications were maintained for each treatment. Observations of the antagonistic effects of *Trichoderma koningii* were recorded after 7 days. When the growth of pathogenic fungi became static the inhibition over control was calculated (Vincent, 1947).

RESULT

Production of phytotoxin
The obtained results indicated that culture filtrate of *Trichoderma koningii* proved to be stimulatory for the germination of crop plant seeds (Table 1). Seed germination was increased in case of rice (96%), jowar (94%), methi (92%) and wheat (91%) followed by mung (82%), groundnut (81%), and moth (80%). Less increase in germination percent was observed in sunflower (77%), soybean (72%), gram (68%), shepu (67%) and spinach (64%).

Table 1: Effect of culture filtrate of *Trichoderma koningii* on seed germination of crop plants.

Crop plants	Seed Germination (%)		
	control	Treatment	Difference
Wheat (*Triticum aestivum*)	65	91	26
Rice **(***Oryza sativa*)	76	96	20
Jowar **(***Sorghum vulgare*)	80	94	14
Gram **(***Cicer arietinum*)	62	68	06
Mung (*Vigna radiata*)	61	82	21
Moth **(***Phaseolus aconitifolius*)	82	80	02
Groundnut(*Arachis hypogeal*)	75	81	06
Soybean (*Glycine max*)	70	72	02
Sunflower(*Helianthus annus*)	65	77	12
Methi(*Trigonella foenum graecu*)	83	92	09
Spinach *Spinacea oleracea*	62	64	02
Shepu *Anethum graveolens*	60	67	07
Mean	70.08	80.33	10.25

Table No.2: Antagonistic nature of *T. koningii* against phytopathogenic fungi

Sr. No.	Test Fungi	Colony diameter (mm)	Inhibition (mm)	Inhibition (%)
1	Control	90	-	-
2	*Alternaria alternata*	26	64	71.1
3	*Rhizoctonia solani*	29	61	67.7
4	*Aspergillus niger*	38	52	57.7
5	*Geotrichum candidum*	30	60	67.7
6	*Fusarium oxysporum f. sp. Spinacae*	35	55	61.1
7	*Macrophomina phaseolina*	40	50	55.5
8	*Pythium* spp	37	53	58.9
9	*Alternaria tenuissima*	38	52	57.7
10	*Fusarium proliferatum*	32	58	64.4

Antagonistism against pathogenic fungi

Results in the table. 2 indicated that antagonistic potentiality of *T. koningii* against pathogenic fungi. *Trichoderma koningii* inhibited the mycelial growth and sporulation of all tested pathogenic fungi. Its antagonism is maximum in case of *Alternaria alternata* (71.1%), followed by *Rhizoctonia solani* (67.7%), *Geotrichum candidum* (67.7%) and *Fusarium proliferatum* (54.4%). Minimum inhibition on colony formation was observed in case of *Macrophomina phaseolina* (55.5%).

In the present research paper phytotoxic effect of *T. koningii* was tested and results indicated that in case of germination of seeds of crop plants were increased without any adverse effect. However the effect was variable but increase in germination percent is a important for the crop plants that should be advantage for their establishment in the field. *Trichoderma koningii* inhibited the sporulation and mycelial growth of plant pathogenic fungi. The antagonistic nature of *T. koningii* is useful in disease management strategy as its application reduces the use chemical based fungicides which caused many hazards such as pollution problems and induction of physiological resistance in pathogens to fungicides. The use of *T. koningii* can be practiced as supplementary method for disease control. Reports on phytotoxicity by *Trichoderma* species were published

by many workers but *Trichoderma* was not exhibiting phytotoxic effect on crop plants. *Trichoderma harzianum* was found enhanced the growth of lettuce (Caporel et al., 2014). Maka and Alimova (2008) also reported that *T. harzianum* decreased the phytotoxic effect in the soil caused by the use of agrochemicals. *Trichoderma* species secreted metabolites in the soil those were found to be antibacterial as well as antitoxidants of mycotoxins in the soil ecosystem (Zhang et al., 2017). In this study, *T. koningii* investigated as an important antagonistic soil fungus having ability to reduce disease incidence caused by the phytopathogenic fungi. Biological control in present times have been acceptable alternative to the existing chemical treatments (Elad, 2000; Eziashi et al., 2007; Shalini and Kotasthane, 2007). Rajkonda and Bhale (2011) reported effect of *T. harzianum* on seed germination and vigour index in pigeon pea was significant. Bhale et al., (2013) reported that *Trichoderma* species were promising biocontrol agents against post harvest disease in fruits. *Trichoderma viride, T. harzianum* and *T. hamatum* were antagonistic over *Fusarium oxysporum* and *F. proliferatum* (Bahareh et al., 2014). *Trichoderma viride* showed antagonistic effects on *Botrytis cinera, F. oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* (Sridhar et al., 2015).

CONCLUSION

From the results, it is concluded that *T. koningii* exhibited effective antagonism against phytopathogenic fungi. Its antagonism was observed as low as 55.5% in case of *Macrophomina phaseolina* therefore the biocontrol activity was opportunistic to control various fungal diseases. The germination of treated crop plant seeds was better than control indicating that *T. koningii* was useful for promoting growth of crop plants because it does not exhibited phytotoxic effect. From the results two statements can be cleared firstly the bioagents *T. koningii* was highly antagonistic against plant pathogenic fungi and second it decreases phytotoxicity in the soil and makes the soil more fertile. Thus with the disease management of crop plant diseases the *T. koningii* helps the establishment of seedlings.

Acknowledgement:
The authors are thankful to principal, Arts, Science and Commerce College, Naldurg and principal, Yeshwantrao Chavan College, Tuljapur for providing necessary laboratory facilities and also thankfully acknowledged to UGC (WRO), Pune and UGC, New Delhi for providing financial assistance.

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

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Original Article | Open Access

Biochemical changes in Sapota Pulp (*Achrassapota*L.) Due to Post Harvest Fungi

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

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

Editor: Dr. Arvind Chavhan

Cite this article as:
Wagh PM and Bhale UN (2018) Biochemical changes in Sapota Pulp (*Achrassapota*L.) Due to Post Harvest Fungi, *Int. J. of. Life Sciences*, Special Issue, A9: 125-129.

ABSTRACT

In this present investigation, three dominant post-harvest sapota fungi were chosen for artificial infection to the three varieties of sapota fruits i.e. Kalipatti, Kutchh and Cricket ball which are normally cultivated in Thane district was studied in detail for the biochemical changes via post-harvest fungi. When pathogen interacts with the fruits either in the field or in storage, this interaction is responsible for qualitative and quantitative biodeterioration of fruits. *In vivo* studies of biochemical changes revealed increase or decrease in organic components of sapota fruits. Study in relation with the predominant three post-harvest fungi viz. *Aspergillusniger, Geotrichumcandidum* and *Rhizoctoniasolani* were used. The quantitative losses of biochemical changes in fruit pulp studied for ten different parameters. It is observed that there was considerable loss in biochemicals in some extent fruits of all three varieties due to the fungi.

Key words: Sapota, Varieties, post-harvest fungi, biochemicals

INTRODUCTION

Sapota (*Achrassapota*L.) belongs to the family sapotaceae and is an economically important edible fruit crop cultivated in tropical and subtropical regions of the world. Fruits are the essential requirement of human diet. Being soft textured sapota fruits are highly sensitive to exogenous agencies specially fungi, that affects physiology, morphology and biochemistry of fruits and thus ultimately causes loss to the fruit seller. And exposure on consumption of these spoiled fruits may be responsible for serious health hazards. It is native of Southern Mexico and Central America (Popenoe, 1974). In India it ranks 5thposition in production and consumption next to mango, banana,

citrus and grapes. India is the largest producer of sapota in the world, with an area of 160 million hectare with production of 1363 million tons.

Maharashtra, Gujarat, Andra Pradesh, Tamil Nadu, Karnataka, W. Bengal, Kerala, Uttar Pradesh, Punjab, Assam and Haryana states were produced major fruit crops. In India the first commercial sapota cultivation from Maharashtra was taken up in Gholwad area in1898 (Sulladmath and Readdy, 1990). Sapota fruit is rich source of sugar, protein, ascorbic acid, phenols, carotenoids and minerals like Fe, Cu, Zn, Ca and K (Kulkarni et al., 2007). Sapota pulp is used for making sweets, halwas, salads and milk shakes. It is also used for manufacture of industrial glucose and pectin. Seeds can be used to prepare different kind of beverages (Toral- Jarquin, 1988). The post-harvest losses are high in tropical countries particularly in India and it ranges between 25-30% (Sudha et al., 2007). The fruits are very susceptible to diseases caused by many microorganisms especially fungi as they are rich in moisture and nutrients (Sankat and Maharaj, 1997). At postharvest stage, many diseases greatly reduce the storage life, fruit contents and quality of sapota. Postharvest diseases of fruits represent a very important source of wastage andmainly economic losses.

Chaudhary et al. (1980) reported that the considerable decrease in total sugar and an increase in reducing sugars of both healthy and infected tissues of apple. The decrease was more pronounced in non-reducing sugars as compared to reducing and total sugars in peach and plum fruits infected with *Rhizopusstolonifer* (Singh and Prashar, 1981). Majumdar and Pathak (1989) reported that contents of ascorbic acid, sugars and proteins declined in the fruits of guava infected by fungi.Several reports have been reported that fungal diseases cause changes in biochemical composition of sapota pulp (Srivastava, 1969; Gadgile et al., 2010).

MATERIALS AND METHODS

Biochemical studies

Fungi were isolated from fruits of three varieties (cultivars) of chikoo collected from different regions of Thane District of Maharashtra state. Spore (0.01 ml) suspension of *Aspergillusniger, Geotrichum candidum* and *Rhizoctonia solani* isolates were separately inoculated in same aged sapota fruits of three varieties i.e. Kalipatti, Cricket ball and Kutch in aseptic condition. After 8 days of incubation of fruit,100g pulps from each variety were collected in aseptic container. Pulp without inoculation served as control and biochemical changes were estimated by standard biochemical methods (AOAC, 1970, Mungikar, 1999; Sadasivam and Manickam, 1992). Estimation of dry matter (DM) (Mungikar, 1999), total sugar, reducing and non-reducing sugar (Miller,1959), total soluble solids (TSS) (Rangana, 1979). Ash, ascorbic acid (Sadasivam and Theymoli, 1987), protein (Lowry et al., 1951), phenols and Tannin (Malick and Singh, 1980; Schanderi, 1970).

RESULTS AND DISCUSSION

The quantitative loss in fruit pulp contents studied for ten parameters in detail (Table 1).

Dry weight

Three dominant post-harvest sapota fungi were chosen for artificial infection to the three varieties of sapota fruits i.e. Kalipatti, Kutchh and Cricket ball separately which were incubated for a period of 7 days at room temperature, a loss in dry weight was calculated by comparing with healthy fruits. It is observed that there was considerable loss in dry weight of fruits of all three varieties due to the fungi. In Kutchh variety maximum loss in dry weight was found due to *Geotrichumcandidum* (11.5gm⁄100ml) while in case of Kalipatti it was found due to *Aspergillusniger* (12.9gm⁄100ml) and *Rhizoctoniasolani* deteriorated maximum dry weight in Cricket ball (11.6gm⁄100ml) variety.

Total sugar content

It was found that all fungi reduced the total sugar in all varieties. It was also observed from the result that maximum of total sugar of Kalipatti variety was reduced due to *Aspergillusniger* (12.0gm⁄100gm pulp). While in case of Cricket ball maximum loss of total sugar was observed due to *Rhizoctoniasolani* (12.1 gm⁄100gm pulp) and *Aspergillusniger* (12.0 gm⁄100gm

Table 1: Biochemical changes in sapota due to post harvest fungi.

Sr. No	Parameters	Variety / Fungi											
		Kalipatti				Cricket ball				Kutch			
		An	Gc	Rs	C	An	Gc	Rs	C	An	Gc	Rs	C
1	Dry weight (g/100ml)	12.09	14.2	13.4	14.1	12.8	12.2	11.6	12.8	14.3	11.5	12.6	15.1
2	Total sugar (g/100g)	12.0	13.2	12.4	13.5	12.0	12.8	12.1	13.2	11.8	12.9	12.2	13.1
3	Reducing sugar(g/100gm)	11.2	12.4	11.3	12.6	11.2	11.9	11.0	12.0	10.3	12.1	11.0	12.2
4	Non reducing sugar(g/100gm)	0.8	0.8	1.1	0.9	0.8	0.9	1.1	1.2	1.5	0.8	1.2	0.9
5	TSS (g/100g)	11°	15.4°	15.4°	16.5°	12.2°	11.2°	15.7°	16°	14°	14.8°	13.4°	16.4°
6	Ash (mg/100ml)	298	317	299	336	245	293	310	345	243	340	295	366
7	Asorbic acid(mg/100ml)	9.5	8.8	8.9	10.8	8.1	9.7	8.2	10.1	5.8	9.2	8.1	10.4
8	Protein(mg/100ml)	42.8	51.23	52.3	59.2	48.9	56.8	56.1	60	43.1	43.3	53.3	58.8
9	Phenol (mg/100ml)	120.5	125.8	125.8	135.2	122.7	118.1	121.5	134.6	124.5	125.5	120.2	136.6
10	Tannin (mg/100ml)	0.14	0.18	0.18	0.2	0.13	0.17	0.15	0.19	0.15	0.12	0.13	0.18

Legends:An-*Aspergillusniger*Gc- *Geotrichumcandidum*Rs – *Rhizopussolani* C – Control

pulp). *Aspergillusniger* reduced maximum total sugar in Kutchh variety (11.8 gm/100gm pulp).

Reducing sugar content

It was found that all fungi reduced the reducing sugar in all varieties of sapota fruits. It is also found that in Kalipatti, Cricket ball, Kutchh varieties showed maximum depletion of reducing sugar due to *Aspergillusniger.*

Non-reducing sugar content

Three selected post-harvest fungi were selected for artificial infection to the three varieties of sapota fruits separately which were incubated for a period of 7 days at room temperature, loss in non-reducing sugar was calculated by comparing with non-infected fruits and the results are summarized in the table 1. It was noticed that all fungi reduced the non-reducing sugar in all varieties of sapota fruits. It was observed from the results that in Kalipatti variety, non-reducing sugar was declined more due to *Aspergillusniger* (0.8gm/100gm pulp) and *Geotrichumcandidum* (0.8 gm/100gm pulp) while in case of Cricket ball maximum loss of non-reducing sugar was caused by *Geotrichumcandidum* (0.9gm/100gm pulp). It was also found that in Kutchh variety maximum loss of non-reducing sugar was caused by *Geotrichumcandidum* (0.8 gm/100gm pulp).

Total soluble solids content

Total soluble solids (TSS) content of the pulp was determined and found that all fungi reduced the TSS in all varieties of fruits. It was observed from the results that in Kalipatti variety TSS was found more decreased due to *Aspergillusniger* (11°) while in case of Cricket ball maximum loss of TSS was caused by *Geotrichumcandidum* (11.2°). It was also found that *Rhizoctoniasolani* (13.4°) reduced more TSS in Kutchh variety as compared with control (healthy).

Ash content

It was observed, that all fungi reduced the ash contents in all varieties as compared with control. It was found that *Aspergillusniger* (298mg/100ml) depleted maximum ash contents in Kalipatti variety while *Geotrichumcandidum* caused maximum loss of ash contents in Cricket ball (293 mg/100ml) variety. It was also reported that in Kutchh variety,

Rhizoctoniasolani was responsible for maximum depletion of ash contents (295 mg/100ml).

Ascorbic acid content

It was found that all fungi were responsible to reduce the ascorbic acid contents in all varieties of fruits as compared with control. It was found that *Geotrichumcandidum* (8.8 mg/100ml) deteriorated maximum ascorbic acid in Kalipati variety while *Aspergillusniger* (8.1 mg/100ml) and *Rhizoctoiasolani* (8.1 mg/100ml) depleted maximum ascorbic acid content in Cricket ball and Kutchh variety respectively.

Protein content

It was found that all fungi reduced the protein contents in all varieties of fruits. It was found that *Aspergillusniger* (42.8,8.9 & 43.1mg/100ml) and *Rhizoctoiasolani* (52.3, 56.1 & 53.3mg/100ml) depleted maximum protein content in Kalipatti, Cricket ball and Kutchh variety respectively as compared to control**.**

Phenol content

It was observed that all the tested fungi reduced the phenol contents in all varieties as compared with control. It was found that *Aspergillusniger* depleted maximum phenol contents in Kalipatti (120.5 mg/100ml) variety while *Geotrichumcandidum* caused loss of maximum phenol content in Cricket ball (118.1 mg/100ml) variety. *Rhizoctoniasolani* caused loss of maximum phenol content in Kutchh variety (120.2mg/100ml).

Tannin content

It was observed that all the tested fungi reduced the tannin contents in all varieties as compared to control. It was found that *Aspergillusniger*caused maximum loss of tannin in Kalipatti (0.14 mg/100ml) and Cricket ball varieties (0.13mg/100ml) while *Geotrichum candidum* (0.12 mg/100ml) and *Rhizoctonia solani* (0.13 mg/100ml) caused maximum loss of tannin in Kutchhvariety.

Sawant and Gawai (2011) reported that the nutritional content of healthy fruit was found to be significantly higher than the infected fruits. *Sapotemamey* is high in vitamin and mineral content, compared to other tropical fruit such as papaya and jobo or red mombin (Alia et al., 2007). Kulkarni et al. (2007) reported chemical constituents of sapota as: - Total sugar (%) - 11.06 -1.9, Protein (mg/100 g)-312.5 - 5.6, Ascorbic acid (mg/100 g) - 10.52 - 1.2, Carotenoids

(mg/100 g) - 0.92- 0.06, Totalphenolics (mg/100 g) - 134.6- 4.5, Iron (ppm) - 0.11- 0.01, Copper (ppm) - 0.09 -0.01. Khillare et al. (2006) reported total sugars, total amino acids, crude protein DNA and RNA contents increased in their quantity due to infection by both the isolates of fruit rot of grape. Schovánková and Opatová (2011) reported that the apples inoculated with *Moniliniafructigena* demonstrated higher concentration of total phenols in the healthy pulp than in the area surrounding the rotten part. Ruth et al. (2009) reported that *A. niger* GH1 degraded 90% of tannin content of "creosotebush" after 72 h. and there was a considerable increase in the total protein content during this period. Mahattanatawee et al. (2006) reported that the phenolic composition of mango, sapodilla and longan pulp have been previously reported to contain hydrolysable tannins and conjugated hydroxycinnamic, allagic and other phenolic.

CONCLUSION:

Sapota is a climacteric fruit that requires careful handling after harvest in order to maintain quality, extend shelf life and allow transport to markets outside the area of production.Therefore it is concluded that fungalinfection ofsapota pulp decreases biochemical contents. Biochemical changes showed that there was significant variation between artificially inoculated sapota and healthy sapota fruit which served as control.

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

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ISSN: 2322-0015

INTERNATIONAL RESEARCH JOURNAL OF
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An International Peer Reviewed Open Access Bi-Monthly Online Journal
http://www.irjse.in

UGC Approved Journal No. 63628)
SJIF Impact Factor-4.115
Cosmos Impact Factor-3.662

Published in: February, April, June, August, October & December

EDITOR-IN-CHIEF:
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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 9: January 2018
UGC Approved Journal No 48951

Abstracts Open Access

Urban Mushroom Farming

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Nowadays, mushrooms are gaining popularity in metropolitan cities due to its high nutritional value and awareness about health. There are several varieties of edible Mushrooms under cultivation but still **Button Mushroom'** is dominating in terms of production & consumption. People are interested to cultivate button mushrooms on their own but the constraint is heavy initial investment & more space needed for compost making. In the present study mushroom composting is carried out by many Mushroom units across India. But only few supplies spawned ready to grow bags the urban population for button mushroom growing at their houses. **Mushroom Club** has initiated this unique kind of concept in Maharashtra in recent years. Necessary training is provided to them so as to acquaint them with the technology. It was found that from a bag of 10 Kg compost which costs Rs.100/- to the grower and in return they were able to harvest around 2 Kg of fresh Button mushrooms in just two months time period and earn Rs.260/-. One grower can easily place 600 bags in 300 sq.ft. room. There is a tremendous potential for button mushroom farming in urban areas especially in Societies provided if they can get ready to grow Compost bags through centralized composting facility made to be available in nearby area. It is concluded that the unemployed youth can raise **'Centralized Composting Unit'** (CCU) through Prime Minister Mudra loan scheme and provide compost bags to urban population to fulfill their need of quality protein rich food and earn handsome income.

Key words : Button Mushrooms, CCU, Spawned bags.

***

Isolation and Characte-rization of Secondary Metabolites From Endo-phytic Fungi Isolated From Mangrove

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Every plant in the world is reservoir of one or more number of endophytes. Endophytic fungi has attracted many scientist as they have ability to synthesize several novel bioactive compounds are important for Pharmaceutical, agricultural and industrial sector. Mangroves endophytic a fungus has gained more metabolites with unique and novel structures have been isolated. In different collection area mainly four fungal specimens were found namely *Phomopsis sps., Xylaria sps., Colletotrichum sps., Fusarium sps.* species were selected for further study. The endophytic fungi isolate was identified up to species level based on the morphological features from National Fungal Culture Collection of India (NFCCI) Pune. The fungus authenticated as *Fusarium solanii* (Mart.) Sacc. Family- Nectriaceae. Nucleotide sequences of *Fusarium* sps. showed maximum homology (100%) with other *Fusarium* sps. from nucleotide database. Pure culture was isolated on Potato dextrose agar and transferred the culture onto PD broth for 21 days. Maximum biomass and exopolysaccharides obtained at a temperature 26°C and pH 5.0. Yield obtained was 8.2 g/l of biomass and 2.7 g/l exopolysaccharides at day 21 incubation. Literature survey and present investigation showed that an endophytic fungus *Fusarium solanii* extracts from mangroves plant shows production of taxol by HPLC as well as terpenoids present in fungal species. In the present study also endophytic fungi *Fusarium solanii* isolated from mangroves plant shows presence of bioactive compound taxol.

Production and Electrospinning of pullulan extracted from *Aureobasidium pullulans*

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Pullulan is an extracellular microbial polysaccharide consisting of maltotriose units that are connected to each other by an α-1,6glycosidic bond. Pullulan is mainly produced from starch by the fungus *Aureobasidium*species. Pullulan helps the cells to resist desiccation and predation and also facilitates diffusion of molecules both into and out of the cell. Recently, there is an increasing interest in use of for production of nanofibres from pullulan for food grade applications.In this work, pullulan producing fungal cultures were isolated and cultured for production pullulan for further use in electrospinning. Isolates of *Aureobasidium pullulans* were obtained from *Acacia* tree phylloplane gathered in Mumbai, India. The standard fungal culture of *Aureobasidium pullulans* and the isolates were evaluated for pullulan production using sucrose, wheat bran and cotton stalk dust as carbon sources. Electrospinning is a complex process that produces fibers with diameters on the micrometer tonanometer scale from an electrified jet of a polymer solution. Food grade polysaccharide, pullulan purified from the isolate was electrospun with fluorescent and non-fluorescent dyes to form nanofibers that would have multiple applications. Various parameters like polymer concentration, flow rate, applied voltage and spinneret tip to collector distance were optimized.Imaging techniques such as Optical microscopy and Atomic Force Microscopy (AFM) were performed to evaluate the structure and surface morphology of the nanofiber films. Further characterization was done using UV-vis spectroscopy.

Key words: *Aureobasidium pullulans*, Wheat bran, Cotton stock dust, Exopolysaccharide, Pullulan, Electrospinning, Nanofiber, AFM

***

Impact of fungal association on the extracts of bioprospecting lichen *Roccella montagnei*

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Lichens are one of the best example of symbiotic association where two dissimilar organisms, a fungus and an alga or a cyanobacterium live together physiologically synchronized and well intermixed so as to form a single biological unit. Lichens with its intricate shape, have the ability to grow in diverse climatic conditions and on diverse substrates. Owing to its wide range of utility (food and fodder, perfumes, brewing and distilling and dying industry etc), lichens have been described as "treasure chest of natural products" and are regarded as one of the most important bioresource. Lichens are also known as "litmus for air pollution" and also as the "permanent control system" for air pollution assessment as they are very responsive to air pollution and hence, used as an ideal biomonitoring tool. The lichen substances also do have great biological potentials including antibiotic, antimycobacterial, antiviral, antioxidant, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxin. At this hour, where threat is utmost, it is also crucial to disseminate the knowledge of bioprospecting lichen wealth as such knowledge would help in maintaining a sustainable balance between economic growth and ecological permanence. This present work was promulgated to explore the lichen *Roccella* with special reference to its bioprospection with respect to fungal association. This will definitely provide a new base and ray of light for the future perspectives and highlight the need for further studies of this promising source to harvest more beneficial in the field of bioprospection.

Keywords : symbiotic, cyanobacterium, antimycobacterial, antiproliferative, bioprospecting.

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Morphological, Micromorphological and Phylogenetic study of Two *Calvatia* species reported from Pune, MH, India

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During the macrofungi investigation in Pune region, authors have collected two species of *Calvatia* from family Lycoperdaceae viz., *Calvatia candida* and *Calvatia craniformis* were collected from Savitribai Phule, Pune University campus. They were identified based on morphological features like Basidiomata and basidiospore. The Scanning Electron Microscopy (SEM) of basidiospores was done. The Basidiospore of *Calvatia candida* is ovate, 3.4 x 2.4 µm and pedicellate. The Basidiospore of *Calvatia cranifromis* is globose-subglobose, 3.0 x 2.9 µm and pedicellate. The two specimens were identified based by using ITS4-ITS5 gene sequence analysis.

Keyword: *Calvatia*, SEM, ITS etc.

***

Isolation of *Aspergilusflavus* from marketed rice samples in Bhiwandi

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Aflatoxins are natural contaminants of cereals, oilseeds, spices, and tree nuts as well as milk, meat, and dried fruit. Acute and chronic exposures to aflatoxins have deleterious health effects such as hepatotoxicity, liver cancers, infertility, malnutrition, growth retardation and immunosuppression. Aflatoxins are naturally occurring toxic metabolites of fungi, *Aspergillus* species particularly *Aspergilusflavus* and *Aspergillusparasiticus* and most common types are aflatoxins are B1, B2, G1 and G2 butatleast 16 different types of aflatoxins are known to be produced in nature .In the present investigation, the marketed rice sample of three different variety are selected and studied for presence of *Aspergilus* contamination in by standard plate technique on PDA(Potato dextrose agar) .It was found that all the three sample showed the growth of *Aspergilusflavus* which was confirmed by plating and staining.

Key words: Aflatoxin, Aspergilusflavus, malnutrition, PDA , Plating

***

Study of outdoor mycoflora of extension building of G.M. Momin Womens College, Bhiwandi.

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Micro flora found in air, water and soil in nature and take food from organic substances and they are complete own life cycle on a specific host. The present investigation was undertaken to study the outdoor aeromycoflora of the Extension building of G.M.Momin Women's college, Bhiwandi. (Thane) Maharashtra, India. Air sampling was done by using petri plate method for isolation of air borne mycoflora of all the four floor of the building between 20th November to 30th November 2017. In this investigation 15 air borne mycoflora types were observed during the study period, in which *Cladosporiumcladosporioides*, *Aspergillusniger,Penicillium sp.*, *Cladosporium sp.,Alternaria sp*. and *Aspergillusflavus* were prominently observed along with other species. Investigation of aeromycoflora is a key to open the information of sensitivity towards aerodroplet in this atmosphere and our findings may be useful with

regard to the investigation of corrective measures to save the library materials and staff and students from fungal damage and diagnosis and prophylaxis of allergic diseases resulting from aeromycoflora composition of this environment.

Key words: aeromycoflora, aerodroplet, fungal damage, prophylaxis, allergic.

***

Mycorrhizal Association with Asparagus racemosus

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An attempt has been made to survey of Arbuscular Mycorrhizal fungi associated with Asparagus plant . Rhizosphere soil and roots samples of Asparagus plant were collected from two locality of mahad area. Two genera with Eight species were reported from above localitie. The genus Glomus was most common with six species with two species of Scutellespora were reported . The average number of AM propgules per 100 gram soil, was Ranging between 20 to 50 the percentage root infection was ranging between 20 to 60 percent.

Key words : Survey , Arbuscular Mycorrhizal fungi, Asparagus.

***

Trends of Button Mushroom Business in India

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Button Mushroom Cultivation Units are setting up in India at a rapid rate due to ever increasing demand and its popularity among health conscious people. The production of fresh mushroom varies from 500 kg/day to 10 tons/day. In earlier days, button mushroom growers in India were dependent on wheat or paddy strawas a base raw material for compost making. But due to its acute shortage and setting up new unitsthere is a phenomenal shift to Baggage as an alternate raw material. In the present scenario, getting baggage is also difficultas the Sugar industries areusing their baggage for power generation. Button Mushroom industry is now searching for alternative source of cheaper raw material. Another important area which needs attention is non availability of quality button mushroom spawn as there is a dearth of quality spawn production laboratories in India. The third major problem facing by this industry is skilled manpower having industrial exposure. There area limited number of experts available in India which shifts from one industry to another. In the present all these factors are discussed in detail. It is concluded that there is a great scope for capacity building in Button mushroom cultivation technologies. Unemployed youth especially from biotechnology background can enter into spawn production as a full time profession. There should be organized and structured arrangements for collection of agricultural waste direct from the farmers clubs so as to reduce the cost of raw material.

Key words: Bagasse, Spawn, Skilled manpower.

***

Mycorrhizal Inoculum Potential of *Glomusmosseae* in*Sorghum bicolour, Triticumaestivum*&*Zea mays*in Red-laterite Soil

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Mycorrrhizal Potential or Inoculum potential(IP) is a term used as an indicator of propagules density and mycorrhizal activity in the soil. It allows the quality

and infectivity of soils inoculums to be evaluated and is used as biological indicator. Mycorrrhizal Inoculum potential is also a parameter to check the quality of inoculum or the viability of inoculum.
The aim of the work was to find out MIP (Mycorhhizal Inoculum Potential) of *Glomusmosseae* in *Sorghum bicolor*, *Triticum aestivum* & *Zea mays* plants. The experiment was carried out in pots and sterilized red soil was used for the experiment. Pure inoculum of *Glomu smossae* was procured from TERI, New Delhi. The plants were inoculated 15 days after sowing. The root infection was checked after 20 days of inoculation. The percentage infection was found to bet 30% in *Sorghum bicolor*, 50% in *Triticuma estivum* and 20% in *Zea mays*.The vegetative growth of the plants was also observed. There was significant difference between vegetative growth of inoculated and non-inoculated plants. Mycorrhizal plants showed increased height, shoot biomass and root biomass compared to non-mycorrhizal plants.

Key Words; Mycorrhizal Inoculum Potential, *Glomus mosseae, Sorghum bicolour , Triticum aestivum* & *Zea mays*

***

Diversity of Xylariaceae in Pune, Maharashtra, India

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The main aim of the present study is to document the diversity status of Xylariaceae from the Savitribai Phule, Pune University campus, Pune. Members of *Xylariaceae* (*Ascomycota*) are recognized and classified mainly based on the distribution, habitat, morphological and microscopic features of their sexual state. The measurement or dimensions of stromata, perithecia, asci and ascospores were taken. Total fifteen fungal taxa were collected which are belonging to 4 genera *viz*. *Daldinia* (1 species), *Daldinia concentrica*, *Hypoxylon* (3 species), *Hypoxylon multiforme*, *H. heamatostroma*, *H. rubiginosum*, *Rhopalostroma* (1 species), *Rhopalostroma indicum* and *Xylaria* (10 species) *Xylaria anisopleura*, *X. apiculata*, *X. arenicola*, *X. aristata*, *X. filiformis*, *X. grammica*, *X. hypoxylon*, *X. longipes*, *X. multiplex*, *X. nigripes*. *Xylaria* species occur worldwide from arctic to tropic regions where they are especially abundant and occupy ecological diverse habitats.

Keyword: Xylariaceae, *Xylaria*, *Daldinia*, *Hypoxylon*, *Rhopalostroma*, Diversity etc.

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Use of fish compost leachate against plant pathogenic fungi

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Considering total fish landing in the coastal area and the quantum of freshly marketed fish, it is appearant that managing the fish waste is a severe problem which mainly includes fillets, scales and viscera of fishes. The decomposition process forms lots of leachate since fishes themselves have large quantities of moisture. This leachate may support the growth of human and plant pathogens. Present work focuses on decomposing fish waste anaerobically and test it's efficacy against plant pathogenic fungi. It was found that fish waste leachate inhibits the growth pathogenic fungi.
Key words: Curvularia sp., Fusarium sp. Nitrogen, potassium.

Effect of homeopathic drugs on seed germination and antifungal activity

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Abstract
Homeopathic drugs are used as alternative medicines and have potential to resist disease without causing destruction to environment. The objective of present work is to evaluate whether such defence mechanism is activated in plant system so that these drugs can be used in building disease resistance in plants.*Cuprum metallic* (copper), Sulphur and mercury of 30, 200, 1000 potencies were prepared in alcohol and sterile distill water was used as an diluent. According to the initial experiments carried out maximum germination of seeds and inhibition of fungal growth was obtained in the seeds which were treated with lower potencies of *cuprum* (Cu).

Key words: *Cuprum metallicum*, Defense mechanism, Seed germination, Inhibition of fungus.

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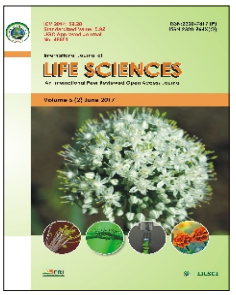

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