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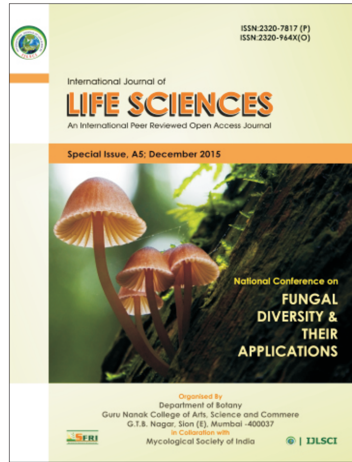

National Conference on

FUNGAL DIVERSITY & THEIR APPLICATIONS

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in Collaration with
Mycological Society of India

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SANJAY DESHMUKH PhD
VICE-CHANCELLOR

No.VC/ECD/2015-16/~~574~~ 578 November 17, 2015

MESSAGE

I am glad to know that the Department of Botany of Guru Nanak College of Arts, Science and Commerce, Mumbai is organizing a National Conference on 'Fungal Diversity and their Applications' on December 11-12, 2015. The theme of the Conference covers a broad range of topics related to fungi. I am sure the sub themes of the Conference will provide an opportunity to student and teacher researchers to learn some recent developments in the field. Deliberations on such topics would update the advancements in the field of Mycology.

Guru Nanak College plays a unique role of catering to the educational needs of the community with humble financial background in the vicinity. The Conference will provide a platform for exchange of latest information among scientists, teaching faculty and research students from various institutes.

I congratulate the Management, Principal, staff and students of this Institution on this occasion and extend my good wishes for the event.

I wish the Conference a grand success.

SANJAY DESHMUKH

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FROM DESK OF CHAIRPERSON MYCOLOGICAL SOCIETY OF INDIA

Fungi are important organisms and so distinct from plants and animals that they have been allotted a 'kingdom' of their own in our classifications of life on earth. They are the most important link between plants and animals. They are the silent workers which support the life on earth. Multifaceted as they are useful as food, in industries, medicines and mycoremediation. Some can even be the indicator species of the habitat. Lot of work has been reported on their diversity and their applications by eminent mycologists but lot more is yet to be uncovered. There are millions of them which have not been discovered and are being lost due to habitat destruction. The need of the hour is the conservation of these important organisms.

The role of Mycological Society of India Mumbai unit has been always to encourage young researchers and scientist to explore and showcase their work through workshops and seminars. It also gives opportunities to the young researchers to interact with the eminent scientists. Since 2003 MSI Mumbai unit has covered varied topics such as Mychorrhiza, Medicinal Mycology, Fungal Biotechnology, Fungi and Health care, Bioremediation, Human welfare, Agriculture etc.. The objective is mainly to reach out to students of different classes and inspire them to study and understand fungi. So each year the seminars are conducted in different colleges of Mumbai. We are grateful to all the eminent scientists who have shared their experiences and been the resource for the success.

The National Seminar on Fungal Diversity and their applications in Guru Nanak College on 11 and 12 December will surely make an impact on the intellectual gathering enough to inspire them to take interest in the biodiversity of fungi and their conservation.
I wish the Organizing committee best wishes for a successful conduction of the seminar.

Dr. Sashirekha Suresh Kumar
Chairperson
Mycological Society of India Mumbai unit

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GURU NANAK VIDYAK SOCIETY

MESSAGE FROM PRESIDENT

I am delighted to know that the department of Botany of Guru Nanak College of Arts, Science and Commerce, is organizing a National Conference on 'Fungal Diversity and their Applications'.

The college believes in innovation and adaptation to the changing global world. It continuously strives to live up to the principles of Shri Guru Nanak Devji – 'Service to Society'. The Management of Guru Nanak Vidyak Society which has been instrumental in setting up more than 27 educational institutions in the city of Mumbai has proved that the College and the Society have been doing exemplary services in the area of higher education. I wish the college reaches great heights through the efforts of the teachers, students and all others who are associated with it.

I congratulate the Principal and organizing committee on this occasion and give my best wishes in their future endeavors.

S.Manjit Singh Bhatti
President
Guru Nanak Vidyak Society

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FROM THE DESK OF PRINCIPAL

It is a great privilege and pride to host a National Conference on 'Fungal Diversity and their Applications' in our college in association with Mycological Society of India, Mumbai Unit on December 11th and 12th, 2015.

Fungi have been the source of several useful chemical substances starting from ethyl alcohol to antibiotics to anti-cancerous compounds. Many industrial processes such as production of enzymes, vitamins, polyhydric alcohols, etc are based on fungi. A conference which covers such topics is undoubtedly the need of this era of Biotechnology.

The Guru Nanak Vidyak Society has been in the field of education since 1947. The aim of the society is to provide quality, value based education to students. Guru Nanak College an affiliate of the society is one of the prestigious colleges which offers various courses keeping in mind the needs of the time. It encourages students to achieve high standards of education and inculcates in them the confidence to shape their future.

I congratulate the entire organizing committee for putting in all efforts required to make this event successful.

I wish all the participants, research scholars a pleasant learning time along with motivating intellectual deliberations.

Dr. Vijay Dabholkar,
Principal

GURU NANAK COLLEGE OF ARTS, SC. & COM. G. T. B. Nagar, M-37.

FROM THE DESK OF CONVENER

On behalf of the organizing committee, it gives me immense pleasure to present this Special Issue of the International Journal of Life Sciences based on the National Conference on 'Fungal Diversity and their Applications' in our college in Association with Mycological Society of India, Mumbai Unit on December 11th and 12th, 2015. The organizing committee extends a warm welcome to all.

Fungi being a major component of ecosystem have various roles of useful and destructive kinds. They play an important role in conservation of ecosystem. Fungal diversity helps mankind for various beneficial aspects like health, food, environment etc. Use of fungi to remediate environmental pollution is a recent approach. Research on plant specific mycorrhizal fungi and its application to crop plants can increase the yield. Besides simply spoiling foods, certain fungi also produce mycotoxins particularly ochratoxins or aflatoxins produced on cereals and nuts. Aflatoxins have been found to be most potent carcinogenic compounds. In recent years, effective immunosuppressant compounds like cyclosporin, drugs for cancer treatment- taxol, cortisone, ergosterol, various enzymes and organic acids, plant growth regulators etc are obtained from fungi. A number of fungi particularly yeasts are important model organisms for studying problems in genetics and molecular biology.

With wide array of speakers, participants will get new insights into the recent approaches towards the different themes of the conference.

I believe the conference will give excellent opportunity and platform for interactions, deliberations and scientific collaboration for academicians, research scholars and scientists.

I take this opportunity to thank and express my deep sense of gratitude to our kind donors and well wishers. I extend sincere thanks to our Management, Principal, teaching and support staff and students.

Dr. Charuta S. Vaidya
Convenor

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FUNGAL DIVERSITY& THEIR APPLICATIONS

11th and 12th December, 2015

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KEYNOTE ADDRESS
&
INVITED TALK

KEY NOTE ADDRESS

Utilization of Fungi in Human Endeavours

Dr. Bhat Jayarama

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Traditionally, fungi have been put into use in the making of bread and alcoholic beverages. In case of bread, yeast raises the dough and enhances aroma whereas wine is an alcoholic fermentative product of grape fruits mediated by yeast. People in the Oriental countries used edible mushrooms since long as an important culinary food item. People in Asian countries also used bracket and caterpillar fungi in the making variety of medicines. However, by and large in rest of the world, fungi have been thought of not so good organisms for human utilization.

Cook and Berkeley in 1888 wrote '....except those which are employed for human food [mushrooms, yeasts], very few [fungi] are of any practical utility value [in art and medicine]'. Truly, fungi have largely been known as causative agents of crop diseases and human infections. Rust of wheat, smut of maize, powdery mildews on vegetable crops, downy mildews on cereals, post-harvest deterioration of perishable fruits and ergot toxins are all due to fungi. Fungi are known to cause superficial, subcutaneous and systemic infections on humans.

Perception of fungi however has changed as time passed. C.V. Subramanian, the doyen of Indian mycology, wrote in 1992 'recent examples of harnessing fungal power for human needs highlight the massive potential and future bright possibilities from fungi'. Another giant of the mycological field, Bryce Kendrick from Canada, write in 1993, 'There is a paradigm shift in our understanding of fungi that they are not bad but indeed good organisms'. Why they said so....?

Advances in studies on fungi in the last fifty years have shown that these microorganisms have much to offer to us. They are the best degraders of all kinds of organic materials. Edible fungi such as button or oyster mushrooms are undoubtedly good food. Penicillium-like fungi are producers of life-saving antibiotics. Mycorrhizal fungi are used as bio-fertilizers in agriculture. Certain entomogenous fungi are in use as bio-control agents of crop pests. Fungal enzymes are in high demand as precursors of plant-based bio-fuels. Needless to say that all alcoholic beverages are yeast-mediated produces. Worldwide commercial market for fungi in 2008 in healthcare (pharmaceuticals), foodstuffs and beverages, agriculture and waste utilization, industrial enzymes, etc. has been so huge. All these point out that there is a great deal that fungi can offer to us.

This lecture will detail out the utilization of fungi in various human endevaours.

***

INVITED TALK

Fungal biodegradation of PVC

Dr. Mishra RL

Principal LSPM's Sr. College of Arts, Science and Commerce, Chondhi-Kihim, Alibag

WHAT IS PVC?

Polyvinyl chloride (PVC) is a kind of thermoplastic resin (Plastic) that can be re-softened by heating and comes in two basic forms i.e. rigid and flexible. It is white brittle solid polymer insoluble in alcohol but soluble in tetrahydrofuran and third most widely produced synthetic plastic, after Polyethylene and Polypropylene causing global environmental and health problems.

WHAT MAKE PVC FOR SEVERAL APPLICATIONS?

Toughness, strength, eases of blending and processing; flame, fire, grease, oil and chemical resistant and excellent electrical insulation properties etc. make PVC appropriate for several applications.

DISPOSAL OF PVC WASTE MATERIALS:

It includes: Landfill (most useful method) causing numerous contamination problems; Incineration/Combustion though reducing stress on landfills up to 20% to 30% but causing a lot of air pollution and Recovering and Recycling to reduce energy usage, reduce volume of landfills, reduce air and water pollution, reduce green house gas emissions and preserve natural resources for further use, however, to overcome all the hurdles, there is need of bioremediation of these hazardous but useful material.

FUNGAL BIODEGRADATION OF PVC:

PVC is supposed to be a non-biodegradable material. Nevertheless, the hope exists for disposal of PVC waste materials by use of fungal forms. Researchers all over the globe make efforts to overcome the problems of disposal of these hazardous materials.

The present lead lecture is attributed to the success towards isolation and application of fungal forms for degradation of PVC materials on experimental basis and further investigations on disposal of PVC waste materials on commercial basis.

The experiment involves:

a) Isolation of fungi from soil possessing PVC degradation capacity.

i) 28 fungal forms were isolated from soil by use of synthetic medium.

b) Screening and Identification of fungi:

i) Screening of plastic degradating fungi involves culturing of the fungi on synthetic culture medium containing PVC as carbon source than glucose.

ii) After two weeks experiments, out of 28 fungal forms, 10 fungal forms were found to be growing well on culture medium containing PVC. These forms were identified by use of manuals and confirmed by Agarkar Research Institute Pune. Further screening has been carried out to find the high potential for PVC degradation.

iii) Fungal forms include 1(one) species of each genus viz. *Mucor*, *Penicillium, Chaetomium, Phoma*

and *Chrysonilia,* 2 (two) species of *Fusarium* and 3 (three) species of *Aspergillus*. Out of these fungal forms *Aspergillus flavus* found to be most efficient mycobiodegradator of PVC.

iv) The degrading ability of fungi has been tested in laboratory by different methods such as determination of weight loss, gravimetric analysis (CO_2 estimation), structure analysis by use of FTIR –Imaging System, Thermal analysis (TG & DTA) and FEG-SEM to confirm the structural changes in polymer, time period required and to compare the activity of individual fungal form.

Future Prospects:

When experiment was continued for period of two weeks to two months, significant increase in CO_2 production noticed suggesting that complete polymer degradation is possible if we provide adequate conditions for longer duration. However, mycoremediation of PVC on the commercial basis can be achieved by genetic manipulation of PVC degradating fungi that needs further investigation as genetic manipulation in asexually reproducing fungi is a tedious job due to parasexual cycle in them.

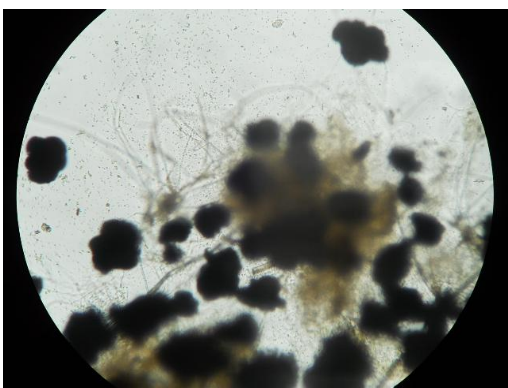
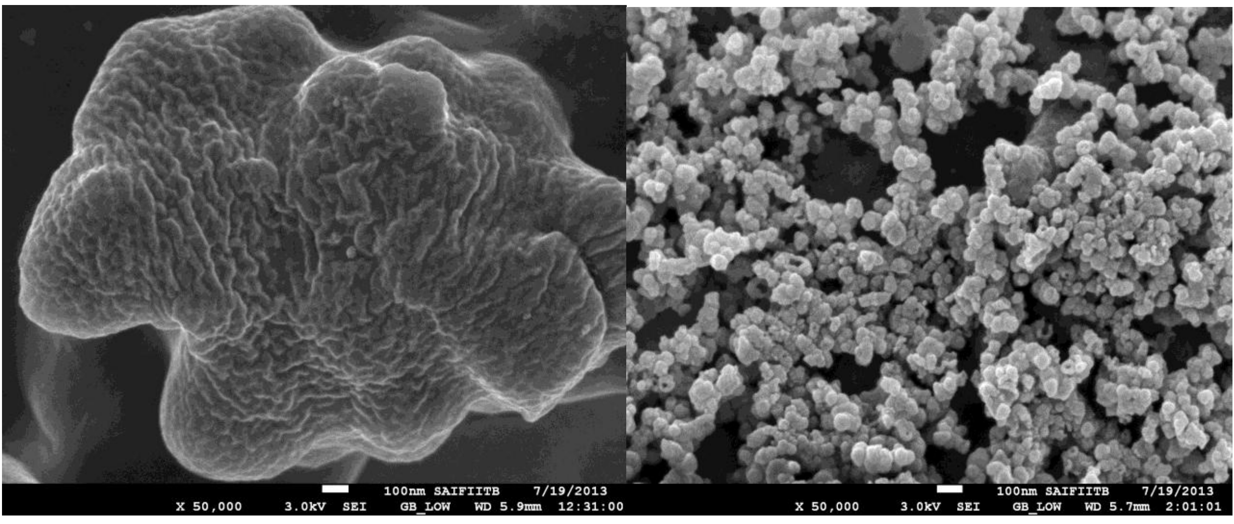


Fig. 1: Fungal mycelia adhered to the PVC granules



Before Treatment **After Treatment**

Fig. 2: SEM Images of PVC

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INVITED TALK

Role of National Fungal Culture Collection of India (NFCCI) in Conservation of Mycological Heritage of India for Future Generation

Singh Sanjay K

National Fungal Culture Collection of India (NFCCI), MACS'Agharkar Research Institute, GG Agarkar Road, Pune 411004

MACS' Agharkar Research Institute has a great legacy of scientific advancement, particularly of mycological research started soon after the establishment of this institute under the great leadership of late Prof. S.L. Ajrekar. Prof. M.N. Kamat and several others pioneer mycologists whose more than 40 years of contributions had laid down a strong foundation for the study of Mycology and Plant Pathology at this Institute. During seventies need of a mycological herbarium was greatly felt by researchers in Western India for carrying out comparative taxonomic, monographic, and plant pathological studies, and a mycological herbarium was established and named in the honor of pioneer mycologist, Prof S.L. Ajrekar as Ajrekar Mycological Herbarium (ACRONYM-AMH), and became internationally recognized. Soon after establishment, acquision, verification, deposit and accession of exsiccate specimens, exchange of type materials with other world recognized herbaria, like HCIO (New Delhi, India), CMI (England), Stockholm (Sweden), Argentina, Padova (Italy), Royal Botanic Gardens (England), etc. has been the regular activities, and school of mycology at this institute became internationally known for mycological work.

Later on in recognition of the mycological expertise available at this Institute, DST, Govt of India sett-up a National Facility for Culture Collection of Fungi in 2008, which principally act as service collection performing basic functions as core activities, viz., acquisition, verification, preservation & maintenance, deposit & accession, distribution of authentic fungal strains and associated information and cataloguing. The facility is well equipped and offers various knowledge based services, like morphological and sequence based identification, deposit & accession, and supply of authentic fungal strains to academia and industry. This facility is a unique blend of research, repository, and Services. About 4000 germplasm of indigenous fungi received from distant geographic locations, various habitats and substrates of 28 states in India, are preserved and maintained in NFCCI using different methods of preservation. In addition, hundreds of Indian institution/university, colleges and private centres are annually benefited out services rendered by NFCCI. As such NFCCI plays vital role in conservation of diverse fungi forming bed-rock for applied research. Since dimension of fungal biology is changing tremendously and fungi are considered as model organism for detail study from biology and biotechnology perspectives, necessitates long term preservation in pure and live form by applying various techniques, which range from continuous growth/serial sub-culturing to the method that halt/suspend the metabolism of the strain under ultra low temperature. Even though fungi have been integral part of biodiversity, they have rarely been considered part of conservation biology. However, this scenario is changing now especially after fungi has resumed the

independent status separated from other microorganisms, plants and animals considering their invaluable ecosystem services. However, conservation strategy of fung*i* will get impetus under the frame work of CBD already implemented in most of the countries. It is now responsibility of mycological fraternity of each country to make proper awareness among the citizens about fungi and need of their conservation for future generation. Therefore, various strategies for long term preservation of fungi in India and other related issues will be discussed during the conference.

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INVITED TALK

Emerging nanotechnologies for detection of mycotoxin production during fungal growth and morphogenesis

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Mycotoxins are secondary metabolites produced by fungi that cause widespread problems. The Food and Agriculture Organization has estimated that 25% of the world's cereals harvests are contaminated with mycotoxins. Consumption of such contaminated food cause health problems for animal and humans such as abdominal pains, hepato-, nephro-toxicity and carcninogenecity. Mycotoxicogenic fungi such as *Aspergillus ochraceus, A. parasiticus, Penicillium verrucosum, Fusarium graminearum* produce aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisins etc. These secondary toxic compounds are formed during exponential growth phase of the fungi. In field, biotic and abiotic stresses lead to fungal infestation of plants while contamination is influenced by conditions of moisture, temperature etc. during grain storage. The "Food Security" initiative from Government of India assures wholesome, quality food grains for the Indian population. Therefore, effective prevention, detoxification and detection of mycotoxins are the need of the hour for reduction in the contamination levels. Detection of fungi and mycotoxins face problems due to time consuming conventional tests. Further, antibodies based serodiagnostic kits are not readily available in India. HPLC detection, LC- or GC-MS characterization of microbial metabolites are equipment and cost intensive. Molecular methods such as real time PCR require skilled personnel and are unsuitable for on-site application. Nanoparticles, due to their size and shape dependent physical and chemical properties have potential for development in various colorimetric, fluorometric, enzymatic, and electrochemical diagnostic assays. Semiconductors, noble metals, and metal oxides nanoparticles are used in various imaging and sensing applications. Conjugation of nanoparticles to different biomolecules such as DNA, RNA, antibodies, enzymes, etc allows for promising applications in signal amplification and detection. Therefore, the development of nanomaterials based diagnostic assays for rapid, sensitive detection of mycotoxins from farm to table is the need of the hour for proper food safety in a country like India.

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INVITED TALK

Prebiotic activity of polysaccharides extracted from some Pleurotus sp. from Konkan region of Maharashtra, India

Verekar Shilpa

St. Xavier's Autonomous College, Mumbai

Pleurotus is an edible mushroom commonly found in the Western Ghats of Maharashtra, India. We getaround 16 species of *Pleurotus* in the Konkan region of Maharashtra, of which *P. citrinopileatus* Singer, *P.cytidiosus* Miller, *P. djamor* (Fr.) Boedeijn, Kummer, *P. eous* (Berk) Sacc. *P. floridanus* Singer, *P.ostreatus* (Jacq.) P. Kummer, *P. sajor-caju* (Fr.) Singer are very common. These mushrooms are easilycultivated at low cost hence the polysaccharides obtained from fruit body of some of these mushroomswere evaluated for their prebiotic activity. Polysaccharides were extracted from fruit bodies by hot waterand alkali extraction. Some of the crude polysaccharides of *Pleurotus*species exhibited potential prebioticactivity when tested against four probiotic strains of *Lactobacillus* viz., *L. lactis* (ATCC No. 8000),*L. acidophilus* (ATCC No. 4963), *L. plantarum* (ATCC No. 8014) and *L. bulgaricus* (ATCC No. 8001) as a carbon source. These polysaccharides exhibited significant increase in the growth of *Lactobacillus* ascompared to 1% (w/v) Lactulose (Duphalac, Abbott) used as a positive control.

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INVITED TALK

Trichoderma from field to Lab., and back again

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Trichoderma species are, biotechnologically important, filamentous fungi that are widely used in agriculture as biofungicides, and in industry as sources of enzymes. Apart from these, *Trichoderma* spp. are useful as sources of secondary metabolites, transgenes and also as agents of bioremediation. The immense importance of these fungi has been the driving force behind the whole genome sequencing of more than seven species. We have isolated a strain of *Trichoderma virens* that has been demonstrated to be highly active as a biofungicide under the field conditions across several crops and pathogens. This strain has been developed by us as a model genetic system for gene function analysis. Recently, we have completed the whole genome sequencing of this strain. A comparative analysis of whole genome sequences of two strains of *Trichoderma virens* resulted in the discovery of a novel gene cluster for secondary metabolism. We have also isolated several novel mutants of this strain and one of them, with improved biocontrol potential has been formulated into a purely organic seed dressing formulation that has shown excellent biocontrol potential under the field conditions.

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INVITED TALK

Developing the consortia for degrading Phalate and other aromatic compounds using fungal model

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Phthalates are synthesized in massive amounts to produce various plastics and have become widespread in environments following their release as a result of extensive usage and production. This has been of an environmental concern because phthalates are hepatotoxic, teratogenic, and carcinogenic by nature. Numerous studies indicated that phthalates can be degraded by bacteria and fungi under aerobic, anoxic, and anaerobic conditions. The biodegradation of phthalates and includes the following aspects:

1. The relationship between the chemical structure of phthalates and their biodegradability,
2. The biodegradation of phthalates by pure/mixed cultures,
3. The biodegradation of phthalates under various environments, and
4. The biodegradation pathways of phthalates.

The basic chemical structure of phthalates is benzene dicarboxylic acid with two side chains, which can be alkyl, benzyl, phenyl, cycloalkyl, or alkoxy groups. Studies have demonstrated that phthalates ith shorter ester chains like dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), diphenyl phthalate (DPP), dipropyl phthalate (DPrP), and butyl-benzyl phthalate (BBP) can be readily biodegraded and mineralized. On the other hand, phthalates with longer ester chains, such as dicyclohexyl phthalate, dihexyl phthalate (DHP), dioctyl phthalate (DOP), and di-2-ethylhexyl phthalate (DEHP) are less susceptible to biodegradation. In this presentation we will dealing will the capabilities of some fungi viz. Aspergillus niger, Penicillium lilacinum, Fusarium oxysporum, Trametes versicolor, Daldinia concentrica Sclerotium rolfsii and Polyporus brumalis which were isolated from various dumpling grounds Fusarium sp. to degrade the Phthalates and their mechanism of action in detail.

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RESEARCH ARTICLES

REVIEW ARTICLE

Mushrooms of Immortality: Anti cancerous use of mushrooms in Chinese medicine- A Review

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ABSTRACT

Traditional Chinese Medicine has used mushrooms for thousands of years. There are over 200 species of mushrooms in China that are used to practice healing. The last decade has witnessed the over-whelming interest of western research fraternity in pharmaceutical potential of mushrooms. Medicinal mushrooms and mushroom extracts are used worldwide to fight cancer and enhance and modulate immune response. The Chinese were among the first people to appreciate the value of the mushroom. About 100 species are being studied for their health-promoting benefits. More than 50 mushroom species have yielded potential immunoceuticals that exhibit anticancer activity in vitro or in animal models and of these, six have been investigated in human cancers which are rich in polysaccharides and beta glucans, the primary active immune-enhancing constituents. Research, including 4 Nobel Prizes, shows that glycoproteins can help cellular communications.

Key Words: Mushroom, Anticancer, Immunoceuticals, Chinese, Glycoproteins.

INTRODUCTION

Mushrooms have been used in traditional chinese medicines for many centuries over past two millenia. They have been used in both nutritional and herbal medicine applications. (Aung, 2005). The chinese have included many mushroom species in their cooking and also healing purposes. Mushrooms have long been regarded as a great delicacy and are often included in the main daily meal (Hobbs, 1995). Mushrooms have been utilized in folk medicine since ancient time (Wasson and Wasson, 1957; Wasson, 1968; Hobbs, 1995). Mushrooms are gaining popularity as a beneficial super food worldwide. Not only are mushrooms a healthy addition to the diet, they also have important medicinal properties (Lee *et al*., 2003).

Traditional Chinese Medicine has used mushrooms for thousands of years. There are over 200 species of mushrooms in China that are used to practice healing. An amazing 25 percent of these mushrooms are credited with tumor-fighting capabilities (Wasser and Weis, 1999). All varieties of mushrooms possess cleansing capabilities. Another amazing property of mushrooms is a compound named Polysaccharides. This enables mushrooms to boost the immune system and fight the growth of tumors. Medicinal mushrooms also contain other compounds that further enhance tumor-fighting capabilities (Kidd, 2000). Long-chain polysaccharides, particularly alpha- and beta-glucan molecules, are primarily responsible for the mushrooms beneficial effect on immune system (Sakagami and Aoki, 1991; Wasser, 2002). Other compounds in mushrooms such as fungal proteins, lectins, peptides and laccases have also been reported to have significant effects on immune function. A protein-bound polysaccharide extract from turkey tail mushrooms is also being used to boost cancer patients' immune function. An extract from maitake mushrooms has also been shown to stimulate the immune system in breast cancer patients (Lull *et al*., 2005 and Lindequist *et al*., 2005).

Mushrooms in Cancer Treatment:

From long time mushrooms have been valued by humankind as a culinary wonder and folk medicine in Oriental practice (Patel and Goyal, 2012; Sharma *et al.,* 2013). Here are six of the most well-researched anti-cancer mushrooms rich in polysaccharides and beta glucans, the primary active immune-enhancing constituents.

1. ***Reishi (Ganoderma lucidum (W.Curt:Fr.) P.Karst)**:*

Commonly called the **Ling-Zhi** in China and **Reishi** in Japan. This species is one of the bracket fungi, but unlike most species, typically has a long slender stalk that attaches to the side of the "cap" of the fruiting body. This is one of the great longevity tonics of Chinese Medicine used in cancer treatment (Sanodiya *et al.,* 2009). Reishi enhances immune response, alleviates chemotherapy side effects such as nausea and kidney damage. A number of anti-cancer constituents have been isolated from *G. lucidum*. These compounds have demonstrated antitumor and immunostimulating activities. Various studies showed that Reishi, restricted blood vessels to prostate cancer tumours and stopped cancer cell proliferation (Gao *et al.*, 2002). Anticancer effect of *Ganoderma lucidum* has been studied alone or in combination with chemothereupatic drugs as well as radio therapy effectively (Sharma *et al.,* 2013). Ganoderic acids A and C from *G. lucidum* are inhibitors of farnesyl protein transferase, an enzyme that participates in Ras-dependent cell transformation. Inhibitors of this enzyme represent a potential therapeutic strategy for the treatment of cancer (Lee *et al*., 1998). The proliferation, maturation and activities of both T and B lympocytes, NK cells and dendritic cells improved significantly during both lab and animal tests when taking Reishi. Beta-glucan is the most constituent for immune support (Zhou *et al*., 2011).

2. ***Turkey tail mushroom (Coriolus versicolor (L.er.Fr)):***

Coriolus versiciolor is one of the most clinically studied botanical in the world with over 400 studies. It is a biological response modifier. Turkey Tail has been used in Chinese Medicine as a tonic for centuries. Studies show that it improves survival rates and acts as an immune modulator with immune stimulating and anti-tumor properties. Some studies show that it can enhance the effects of chemotherapy cancer treatment and reduce the side effects of radiation therapy (Monro, 2003). Reports showed that gastric cancer, lymphoma, leukaemia and lung cancer cell lines being suppressed by extracts of *Coriolus*. *Coriolus versicolor* also increases Natural Killer cells, and CD8+ T-cells (a type of white blood cell) that can kill cancer cells (Eliza *et al*., 2012). *Coriolus Versicolor* has been shown to

activate T-lymphocyte production, macrophage activity and other immune system functions. The *Coriolus* extract, PSP, has been shown to have immune boosting and modulating effects and anticancer properties in both pre-clinical experiments and clinical trials (Torkelson and Sweet, 2012). The protein bound polysaccharide that was isolated from the mushroom is called Polysaccharide–K (Krestin, PSK). Their research found that PSK is effective as an immune system boosting agent (Kidd, 2000). In the 1980s, Chinese researchers improved upon the Japanese finding a decade earlier and introduced a more potent extact called Polysaccharide-Peptide or PSP. Both PSK and PSP have been successfully used in Japan, China, Hong Kong, and some European countries for medical treatments of various types (Tomochika *et al*., 1989).

3. *Caterpillar mushroom (Cordyceps sinensis (Berk.) Sacc):*

In the wild, this mushroom is parasitic on caterpillar with an elongated, cylindrical fruiting body with the mycelium invading and completely covering the caterpillar. This caterpillar fungus or "mushroom" has been used in Chinese medicine for centuries. Because of the scarcity of this mushroom in the wild, the mycelia of this mushroom is now cultivated on solid substrate such as brown rice or in liquid medium for use as a dietary supplements (Holliday and Cleaver, 2008). *Cordyceps* acts an immune stimulator by raising cancer- and virus-fighting T Cells and Natural Killer Cells and prolongs the life of white blood cells, improving resolution of infections. It has demonstrated anti-tumor properties and also protects the kidneys from chemotherapy side effects. It is one of the most widely used tonics in anti-cancer formulas in Chinese Medicine (Khan *et al*., 2010). Some studies have shown that CS prevents metastasis by inhibiting angiogenesis; the process by which tumor cells make new blood vessels, allowing tumors to grow in size, allowing cancer cells to enter the blood stream and travel to other parts of the body (Li *et al*., 2009).

4. *Brazilian mushroom (Agaricus blazei Murill):*

Brazilian mushroom is an edible mushroom native to Brazil and is cultivated widely. A cold-water extract of this mushroom is consumed traditionally in Brazil. Delmanto *et al* (2001) studied immunomodulatory, anti-carcinogenic and anti-mutagenic effects of *Agaricus blazei* Murrill extracts on clastogenicity induced by cyclo-phosphamide (CP) in mice. KA_{21} is the polysachharide fraction of this mushroom with immune-modulating activity containing a β-glucan content of about 12%. Several studies have shown that beta glucans from these mushrooms can alter cytokine and T- and B-cell activity (Liu *et al*., 2008). This prompted the view that the mushrooms would not just be applicable to fighting solid tumour cancers, but also in fighting blood cancers where the white cells themselves were in trouble like leukaemia.

Fig. 1: Reishi *(Ganoderma lucidum)*

Fig. 2: Turkey tail mushroom (*Coriolus versicolor)*

Fig.3: Caterpillar mushroom (*Cordyceps sinensis*)

Fig. 4: Brazilian mushroom (*Agaricus blazei*)

Fig. 5: Shiitake mushroom (*Lentinus edodes*)

Fig. 6 Maitake mushroom (*Grifola frondosa*)

Research showed that *Agaricus blazei* has anti-angiogenic (inhibits the formation of new blood-vessels to the tumour) properties. It inhibits the enzyme aromatase, which is associated with the development of breast cancer. Liver protective and detoxifying properties of *Agarics blazei* extracts have a detoxifying, blood purifying effect that can help rid the body of dangerous toxins and restore a healthful balance (Fujimiya *et al*., 1998). In addition, other health benefits including normalized liver function and decreased blood cholesterol levels, were also observed. Mushroom extract also was beneficial in reducing the chemotherapeutic side effects such as nausea, hair loss, loss of appetite, insomnia and other symptoms (Ahn *et al*., 2004).

5. *Shiitake mushroom (Lentinus edodes (Berk) Sing):*

Lentinula edodes is an antiproliferative mushroom widely known as Shitake mushroom an edible fungus native to Asia, and cultivated for food in many countries. The fresh and dried forms of the mushroom are commonly used in East Asian cooking. Extracts from the mushroom, and sometimes the whole dried mushroom, are used in herbal remedies. Shiitake mushroom is used for boosting the immune system, lowering blood cholesterol levels, treating prostate cancer, and as an anti-aging. One shiitake extract called lentinan is a beta glucan. This is a type of complex sugar compound. Beta glucan is believed to stimulate the immune system and trigger certain cells and proteins in the body to attack cancer cells. In laboratory studies, it seems to slow the growth of some cancer cells (Ritz, 2011). Lentinan, a compound found in Shitake, is used as an intravenous anti-cancer drug with antitumor properties (Yang *et al*., 2008). Both α and β-glucans derived from shiitake are used as adjuvants in various clinical studies. Clinical studies have associated lentinan with a higher survival rate, higher quality of life and lower recurrence of cancer (Shah *et al*., 2011). From a clinical standpoint, AHCC has generally been administered as an adjuvant in combination with surgery and chemotherapy or radiation (Gu and Belury, 2005).

6. *Maitake mushroom (Grifola frondosa (Dicks) Gray):*

Maitake (Japanese for “dancing mushroom”) is an edible mushroom that grows in clusters at the foot of oak trees. Maitake (Grifola frondosa) contains grifolan, a beta glucan polysaccharide. This activates macrophages which search and engulf foreign invaders in the body. Another ingredient, termed d-fraction, stimulates the immune system at the cellular rather than blood stream level. D-fraction can be used on its own, but seems capable of enhancing the effect of cancer drugs whilst reducing side-effects such as nausea and hair loss. For medicinal purposes, maitake is used as a dried powder, hot water extracts, or isolated fractions and compounds. β-glucan is the active polysachharide extracted or isolated from fruiting bodies. This is used in traditional Chinese and Japanese medicine to

enhance the immune system (Nanba *et al*., 1987). Studies have shown that it can enhance both the innate immune response to fight infections as well as adaptive immune response conferring long-term immune enhancement. Maitake also protects cells with its antioxidant properties and decreases the inflammatory factor COX2 enzyme so common in cancer physiology. Studies have also shown that Maitake has potential anti-metastatic properties inhibiting the proliferation and spread of cancer.

CONCLUSION

Mushrooms have long been used in medicine, the earliest records go back over 4,000 years in China. There are thousands of species of mushroom growing in the wild, but most studies have focused on six main varieties discussed here. In the last few decades, large number of mushroom fungi has been progressively used as a source of medicinal compounds and therapeutic adjuvants or health food supplements. Recently, the anticancer and anti-proliferative activities of polysaccharides or polysaccharide-protein complexes derived from mushrooms have received much attention in cancer treatment. Medicinal Mushrooms contain high levels of glycoproteins and polysaccharides. A trend toward integration of immunopotent agents with the extant cancer regimens of surgery, chemotherapy, and radiation therapy is now considerably advanced in Japan and China – countries where mushroom preparations have been an anticancer resource for centuries. Glucan and proteoglycan mushroom immunoceuticals offer hope for cancer patients. These substances as dietary supplements, they are safe, clinically proven, and exhibit near-perfect benefit-risk profiles have also been successfully studied in humans.

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REVIEW ARTICLE

Electron Beam Irradiation - An environmentally safe method of fungal decontamination and food preservation: A review

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ABSTRACT

Electron Beam Irradiation (EBI) is an effective and environmentally safe method ofsterilization and fungal decontamination of a variety of food commodities. This irradiation allows decontamination of many food and agricultural commodities, which usually suffer from fungal contamination and mycotoxin interference during post-harvest processes. Since EBI uses less energy thanconventional techniques, it may replace dry sterilization techniques in future.

Key words: Electron BeamIrradiation, agricultural produce, food commodities.

INTRODUCTION

Providing safe and adequate food supplies for all has been a challenge since time immemorial for all Governments. The Food and Agriculture Organization (FAO) has estimated the annual loss of the world's food supply to be about 25% due to microbial contamination, improper handling and storage. The European Union (EU) and several other countries have imposed stringent laws on the quality and safety of imported materials. This has encouraged the introduction of non-conventional alternatives for food preservation andhave opened up possibilities of commercializing food irradiation on a large scale (Sridhar and Bhat, 2008).

Radiation is one of the latest methods in food preservation.Over 42 countries in the world including USA, UK, Canada and France have given clearance for radiation processing of food. The Government of India has permitted the use of Gamma radiation technology in preservation of food items such as potato, onion, rice, semolina wheat flour, mango, raisins, dried dates, ginger, garlic, shallots (small onions) as well as meat and meat products including chicken (Balakrishnan, 2015).

The objective of this review is to highlight the importance of Electron Beam Irradiation technology, to prevent fungal contamination and thus enhance the quality of food.

Radiation processing Technology

Three principal types of radiation source can be used in food irradiation according to the Codex Alimentarius General Standard (Food and Agriculture Organization, WHO, 1984). Radiation processing involves precise exposure of food and agricultural commodities to ionizing radiations such as gamma rays (cobalt-60 and caesium-137) or machine generated X-rays (5 Mev) and high-energy electrons (8-10 Mev). Ionizing radiation in Electron Beam Irradiation (EBI) technology is provided through electrons from a linear particle accelerator, while mainly by photons in X-rays.The amount provided by the source is known as dose and measured in kilo Grays (kGy) (1 kGy = 1,000 kJ). Radiation processing is broadly classified into three categories:

1. Low dose (<1 kGy), mainly used for sprout inhibition of vegetables, for disinfestation of storedgrains, dry fruits and spices and for delayed ripening in fruits.

2. Medium dose (1-10 kGy), for reducing microbial load of whole and powdered spices, and for elimination of spoilage microbes in fruits and sea food to extend shelf life.

3. High dose (10-45 kGy), necessary to make foods sterile, wherein no refrigeration is required. Some spices recommended for export has been given clearance for this dose range. This dose produces sterile foods in hospital diets for patients with compromised immune systems and for foods used by astronauts during space flight.

Packed foods or agricultural commodities are allowed to pass through a radiation chamber on conveyor belts in such a way that they will not come in direct contact with radioactive materials. Nutritionists are of the opinion that radiation processing produces no greater nutritional loss than other food processing methods like cooking or canning. Nutrient losses can be minimized by irradiation of foods in anoxic conditions or while freezing.

Gamma irradiation (Co-60) is a well-established technology while Electron Beam Irradiation technology is relatively new.

The available accelerators for radiation processing with varying beam power of 10-200 kW include: LINAC, RHODOTRONS, CYCLOTRONS, MICROTRONS and DYNAMITRONS. (Sridhar and Bhat, 2008; Kalyani and Manjula, 2014).

The energy from electrons or X-rays has to target and inactivate fungal nucleic acids. This damage occurs directly as a result of electron and photon interactions with DNA and RNA and indirectly through the radiolytic products of water, which further react with nucleic acids. Microorganisms with large genomes are usually more susceptible to radiation than those of smaller genomes. When ionizing radiation reaches microbes, its high energy breaks chemical bonds in molecules that are vital for cell growth and integrity, and this results in microbial death. EBI technology has been shown to be more effective on fungal spores as D-10 value (the minimal dose required to kill an organism) is usually higher in gamma irradiation. Being a cold process, radiation can be used to pasteurize and sterilize foods without causing changes in freshness and texture of food unlike heat. Further, unlike chemical fumigants, radiation does not leave any harmful toxic residues in food and is more effective and can be used to treat packaged commodities too (Sridhar and Bhat, 2008)

Current status of Irradiation technology

Food, feed and silage: Improper storage of consumed food grains, fruits and beverages worldwide include rice, wheat, barley, sorghum, rye, sugar (sugar cane and sugar beets), grapes, spices, cocoa, coffee, wine and beer, can become contaminated with Mycotoxins like like Aflatoxin

and Patulin which are known for teratogenic, mutagenic and carcinogenic effects in livestock and humans. The efficacy of EBI treatment in combination with other methods to prevent the growth of molds will be of immense value. EBI may also serve as an effective means of decontamination and preservation of fresh sea foods.

Animal feed and fodder borne fungal contamination and aflatoxins produce by fungi in humid conditions in groundnuts and oilseed meal affect the livestock as well as human health through animal products (Bastianelli and Le-Bas, 2000). Mycotoxin contamination of fodder due to*Aspergillus flavus, A. ochraceus* and *A. parasiticus* which proliferate in fodder in tropical warm humid conditions, while *Penicillium expansum* and *P. verrucosum* predominate in temperate conditions (Chhabra and Singh, 2005).

Growing consumption of minimally processed vegetables for nutritional benefits has raised questions about their safety. Fungal diseases during post-harvest storage are well known. Rhizopus spp. are common and cause considerable loss of fruits and vegetables. Raw sprouted seeds consumed as nutritional supplement are prone to be contaminated by toxigenic molds as they are wet and nutritionally rich. A low-dose EBI on fresh-cut cantaloupe combined with modified atmospheric packaging promises to extend shelf life. Similarly, the impact of EBI on packaged fresh blueberries at greater than 1.0 kGy on the quality has been evaluated by Moreno *et al*. (2012).

Table 1: Effect of Gamma rays andEBI on the fungal contaminants- a few examples

Fungus	**Food**	**Dose**	**Effect**
Aspergillus flavus & Osmophilic molds	black pepper, turmeric, rosemary and coriander	4 kGy EBI	Eliminated or reduced to below detectable limit most of the contaminant microflora (Ito and Islam (1994)
Aflatoxins of the molds*Aspergillus flavus*, *Aspergillus parasiticus*and *Aspergillus nomius*	Peanut seeds	10 kGy. Gamma irradiation 20 kGy	complete inhibition of Fungi at irradiation dose 10kGy 83-100% inactivation of Aflatoxin B (Prado *et al*., 2003)
Fungus	Food	Dose	Effect
Spores of *Aspergillus* and *Penicillium and* Yeasts	Food Packaging material, yogurt cups, containers, bottles and seal caps	EBI 5-7 kGy	Inactivation of spores and vegetative cells (Mittendorfer *et al*., 2002).
Aflatoxin Initial microbial load including fungi	Coconut, Dry parsley leaf, parsley root, carrot, celery leaf and root, red beet and dry mushrooms	0- 5.0 kGy 5 kGy	Inactivation (Prado *et al*., 2003; and Rogovschi *et al*., 2009). 5 kGy was sufficient to reduce the initial microbial load by 10^5 organisms (Migdał and Maciszewski, 1995)
Initial microbial load	Soyabeans	Soft electrons up to 7.5 kGy	reduce the microbes in soybeans so high temperature sterilization 120°C not required (Todoriki *et al*., 2007)

Harvested mushrooms also undergo deterioration by fungal contaminants during storage in spite of packing and refrigeration. EBI is a promising approach for the decontamination of raw materials for mushroom cultivation, and to prevent fungal growth during andafter production of mushrooms (Sridhar and Bhat, 2008).

CONCLUSION

EBI is an effective method for mold decontamination and sterilization. In future, it may replace conventional dry sterilization techniques, high dose EBI sterilization may allow energy saving compared to the conventional techniques.

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RESEARCH ARTICLE

Isolation and screening of wild yeasts for maximum xylitol production

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ABSTRACT

The sugar xylitol is a five carbon sugar alcohol that has beneficial health effects. Xylitol represents an alternative to current dominant sweeteners for diabetic people. The microbial production of xylitol is an alternative for the catalytic hydrogenation of xylose in wood hydrolysates. The bioconversion of xylose to xylitol is efficiently brought about by yeasts. A total of 20 yeasts strains form natural environments were screened to check their capacities for3% xylose utilization within 72hours by DNSA method. The best xylose utilizing isolate of all was thus selected for xylitol production from 5% xylose as substrate. A garden soil isolate (Sx) appeared to be a promising strain producing xylitol with good yield in a optimized medium containing Yeast Nitrogen Base (0.58g of xylitol per gram of xylose consumed). This isolate was identified to be Candida tropicals strain. Xylitol yield by the immobilized cells was also studied for this isolate. The percentage efficiency of xylose to xylitol conversion was significant. All the samples were analyzed by HPLC for determination of xylitol production. Xylitol yield in immobilized condition was also studied. The present work deals with experimental investigation for the production of xylitol from natural isolates.

Keywords: HPLC, Candida tropicals, xylitol, xylose, yeast nitrogen base.

INTRODUCTION

Xylitol was first popularized in Europe as a safe sweetener for people with diabetes which would not affect insulin levels. This tolerance is attributed to the lower effect of xylitol on a person's blood sugar compared to that of regular sugar. Its dental significance was researched in Finland in the early 1970s when scientists at Turku University showed it had significant dental benefits. Thus xylitol production gains importance (Tom *et al.*, 2007).

MATERIAL AND MATERIALS

Presently, xylitol is manufactured by reducing pure xylose, obtained from hard-wood hydrolysates, in the presence of a Raney nickel catalyst. However the overall xylitol yield is relatively low from the total xylan content of the wood hemicelluloses. Thus microbial xylitol production is an alternative to chemical method of xylitol production. The use of metabolically engineered yeasts, *Saccharomyces cerevisiae* or *Candida*, has been studied as an alternative for industrial production of xylitol (Tom *et al*., 2007). *Candida species* are an excellent set of model organisms for xylitol production, and can grow on xylose as a sole carbon and energy source. *Candida* yeasts have been extensively studied with regards to their biotechnological application in the production of xylitol. This is due to the fact that they have an advantage over the metabolically engineered *S. cerevisiae* for being natural D-xylose consumers and maintaining the reduction–oxidation balance during xylitol accumulation (Raluca *et al*., 2010).

Screening of naturally occurring xylose utilizing yeast is an effective method for obtaining xylitol producing yeast with industrial applications (Guo and Zahao, 2005).The fermentation process that produces xylitol in yeasts is controlled by a series of factors such as substrate concentration, carbon source, inoculum, aeration degree, temperature or pH. Although high xylitol yields from D-xylose have been frequently reported, a lots of research work has been devoted to optimization conditions, with particular concern to the effect of oxygen level (Deigo *et al*., 2007)(Walter*et al*., 2002).Among nitrogen sources, the yeast extract, urea and the yeast nitrogen base are the nutrients preferred by the yeasts producing xylitol(Raluca *et al*., 2010).

The present work deals with experimental investigation for the isolation, screening, optimization and production of xylitol from natural yeast isolate (Kock

2.1 Sample collection for isolation of xylose utilizing yeasts (Guo and Zahao, 2005)
Several soil and fruit samples were collected which are source of xylose themselves. These samples included black grapes, garden soil, honey, strawberries, dates, sugarcane juice and stem washings etc. These samples were cleanly washed and processed.

2.2 Enrichment of 3% xylose utilizing yeast (Kumar *et al*., 2007; Kumar *et al*., 2007; Srivani and Setty, 2007)
All the above samples were selected for enrichment of xylose utilizers. One g of each sample was inoculated in 100ml sterile 3% xylose broth containing xylose 3 %, peptone 0.25% , 100 ml distilled water (pH 6.5) and incubated at 30^0C for 48h on rotary shaker at 100 rpm.

2.3 Isolation of 3% xylose utilizers
Serial dilutions of the enriched sample were made in sterile saline. An aliquot of 0.1ml of each dilution was spread on 3% xylose agar medium and the cultured plates were incubated at 30^0C for 48h. All the colonies of yeasts were selected and examined under microscope .The cultures were re-streaked and pure cultures were maintained on 3% xylose agar medium.

2.4 Screening of xylose utilizing yeasts
(Guo and Zahao, 2005)**,**
The ability to utilize 5% xylose was checked for all the 35 yeast isolates obtained from various sources. Each isolate was grown for 24h in broth containing xylose 1%, glucose 1%, peptone 0.5% (pH6.5) on shaker and incubated at 30^0C.Ten ml of pre-grown cultures were inoculated in medium containing xylose 5%, peptone 1.2%, NH_4Cl 0.4%, $MgSO_4$ 0.0075%, $ZnSO_4$ 0.00075%, KH_2PO_4 1.2% (pH 6.5) and incubated on rotary shaker at 30^0C for 72h.

The samples were withdrawn periodically and the concentration of xylose was estimated by standard DiNitro Salicylic Acid (DNSA) method.

2.5 Optimization of fermentation medium.
(Srivani and Setty, 2007; Timothy, 2003; Timothy, 2003; Solange *et al*., 2006; Rodrigues, 2003) Out of all 35 isolates, the garden soil isolate (Sx) which showed rapid utilization of xylose within 72 was selected for shake flask fermentation.

Fermentation was carried out in 500ml flasks containing 100ml sterile fermentation medium. Two Fermentation media were optimized for enhancing xylitol production. Medium A was containing xylose 5%, peptone 0.5%, yeast extract 0.5%,NH_4Cl 0.4%, $MgSO_4$ 0.0075%, $ZnSO_4$ 0.00075%, KH_2PO_4 1.2% (pH 6.5) and Medium B was containing xylose 5%, peptone 0.5%, yeast extract 0.5%,NH_4Cl 0.4%, $MgSO_4$ 0.0075%, $ZnSO_4$ 0.00075%, KH_2PO_4 1.2% , glycerol 0.5g% ,biotin 2µg/L (pH 6.5) (Maria *et al*., 1988). Yeast nitrogen base was filter sterilized using a 0.22µ sterile syringe filter, glycerol was separately autoclaved and used. The culture was pre-grown and a 10% inoculum was used. These flasks were incubated at 30^{0}C on rotary shaker at 100rpm. Samples were withdrawn periodically after every 24h up to 48h. Samples were centrifuged at 10,000rpm and supernatant was used for estimating xylose concentration by DNSA method (Miller, 1959). The 48h samples were analyzed for xylitol production by HPLC.

The xylose utilization and xylitol production was studied in these two fermentation media under shaker condition. Also, the effect of various nitrogen sources on xylose utilization was studied (Deigo *et al*., 2007; Walter *et al*., 2002).

2.6 Identification of the isolate by standard bio-chemicals and CHROMagar Medium
Aragao and Azeredor , 1998; Walter , 1994)
The soil isolate (Sx) producing xylitol was identified by its cultural and morphological characteristics. Gram staining and wet mount were performed and observed under microscope .The CHROM agar *Candida* medium, majorly contributed towards the identification of the isolates.

A loopful of saline suspension of the culture was isolated on sterile CHROMagar *Candida* medium and incubated at 30^{0}C for 48h. The colony appearance and colour on CHROMagar was compared with the Pantone Colour Guide, thus helped in identification of this isolate (Kock *et al*., 2008; Frank and Chromagar, 1994).

2.7 Production of xylitol by immobilization of selected strains in calcium-alginate gel capsules (Deigo *et al*., 2007; Walter*et al*., 2002)
The yeasts cells were immobilized by entrapment in calcium alginate bead method. The concentrations of sodium alginate 20g/L, and calcium chloride 11g/L was used. An adequate volume of cell suspension of 0.1 OD was added to a solution of sodium alginate previously heated at 121^{0}C for 15min. Cell-gel beads were produced by dripping this suspension in calcium chloride solution, using 1ml pipette. The beads were maintained in calcium chloride solution at 4^{0}C for 2h. Afterwards they were washed with sterile distilled water and 10g of beads were introduced into the fermentation flasks.

Under same parameters as free cells, fermentation was carried out. Samples were withdrawn periodically, centrifuged and DNSA test was performed. The 72h samples were analyzed by HPLC for xylitol production.

RESULTS AND DISCUSSION

In all 20 different yeast isolates were obtained from all the sources. These isolates were purified and maintained on sterile xylose agar slants and refrigerated for further studies. Their ability to utilize xylose was screened in xylose peptone medium. The garden soil isolate was able to utilize 5% xylose within 72h as compared to other isolates. This soil isolate was named as “Sx”. This isolate was further employed for xylitol production. Identification of this isolate was carried out by morphological (Fig No.1) cultural (Fig No.2) biochemical tests (Table No. 1).

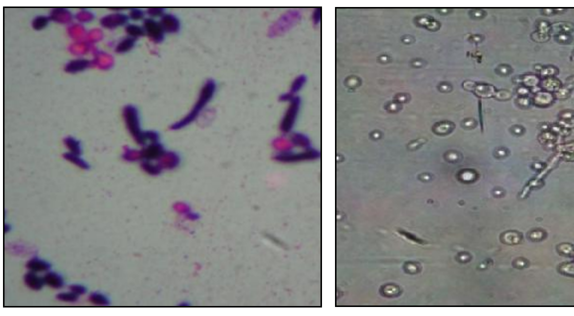


Fig. 1: Gram staining (100X) and wet mount (40X) of Soil isolate (Sx) from 3% xylose agar plate

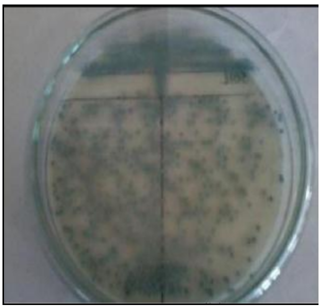
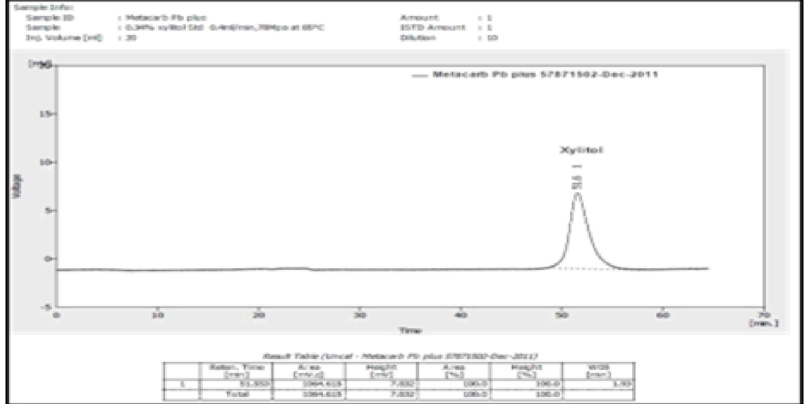
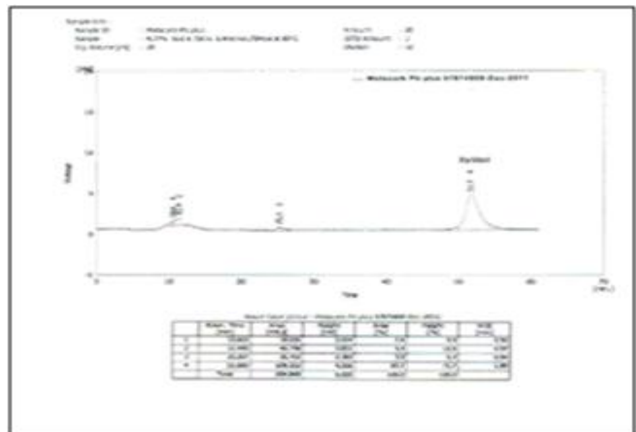


Fig. 2: Isolation on CHROMagar medium. Blue-gray colonies, reverse and front view of Sx.





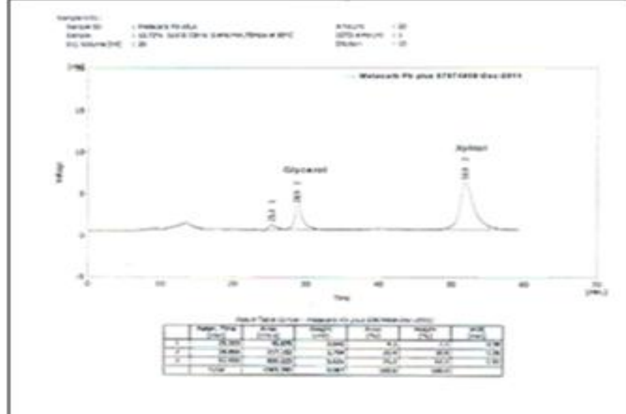
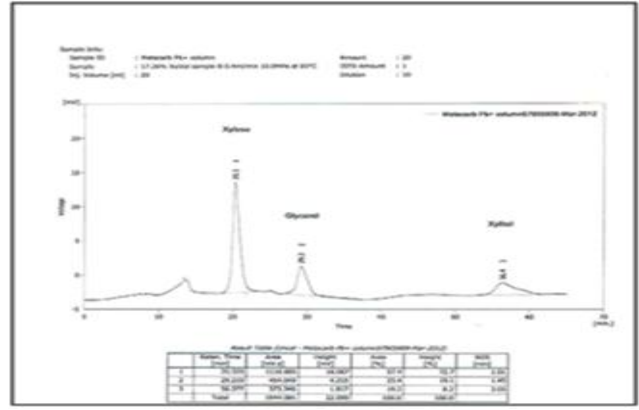


Fig. 3: HPLC analyses: Standard Xylitol



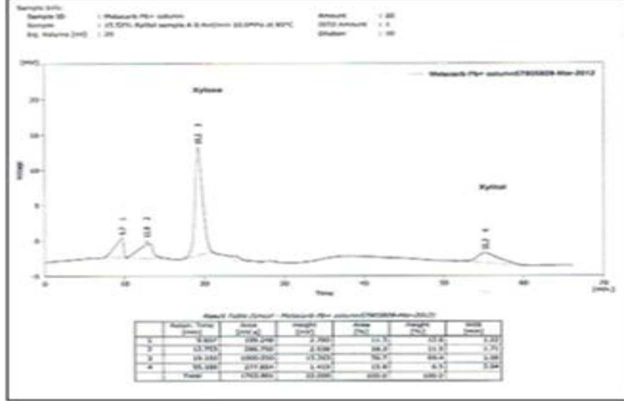


Fig.4: HPLC analyses Fermentation Medium A, Medium B of free cells.

Fig.5: HPLC analyses Fermentation Medium A, Medium B of immobilized cells.

Table 1: Biochemical identification of Sx isolate.

Test	P. anmola	C. Succiphila	C. guilliermon	C. tropicalis	S. cerevesiae	C. intermedia	Isolate (Test) Sx
Dextrose	+	+	+	+	+	+	+
Xylose	+	-	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Maltose	+	+	-	+	+	+	+
Lactose	+	-/+	-	-	-	-	-
Mannitol	+	-	+	+	-	+	+
Raffinose	+	-	-	-	+	+	-
Starch	+	-	-	-	+	-	-
Inositol	+	-/+	-	-	-	-	-
Citrate	+	+	+	+	-	+	+

Table2: Xylitol yield by free cells and immobilized cells

Parameters	Sx (immobilized cells)			Sx (free cells)	
	Media A	Media B		Media A	Media B
€ (gL^{-1})	50	50		50	50
Time (hr)	48	48		48	48
Xylose Consumption (%)	80.56	79.65		100	100
ά (%)	16.35	13.95		64.12	51.90
$Y_{p/s}$ (gg^{-1})	0.157	0.128		0.58	0.47
B (g)	-	-		Shaker : 3.9 Static :0.195	Shaker : 2.8 Static :0.17

This isolate was identified to be a *Candida tropicalis* strain on the basis of morphological cultural and biochemical tests. Strains of this genus can carry out the xylose to xylitol conversion rapidly and efficiently.

Furthermore, there was a rapid utilization of xylose in both the optimized media by Sx isolate. It utilized 5% xylose completely within 48 hour in both the optimized media. The HPLC analyses of samples are depicted in Fig No. 3, 4 and 5 as follows.

This indicates that xylitol production is a relatively common feature among xylose utilizing yeasts, as suggested also by other workers (Raluca *et al*., 2010; Kumar *et al*., 2007; Kumar *et al*., 2007). The xylose to xylitol conversion was sensitive to nitrogen source.

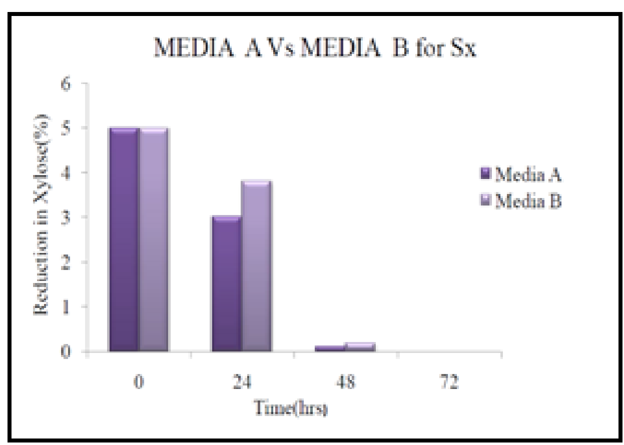
Key : € (gL^{-1}): initial xylose concentration, ά (%): percentage efficiency of xylose to xylitol conversion
$Y_{p/s}$ (gg^{-1}): yield of xylitol, B (g): Biomass, cell dry weight.

Also a notable aspect of the present study was that a relative high yield was obtained in the Medium A specifically 0.58gg^{-1} of xylose with yeast nitrogen base as nitrogen source (Table 2: free cells).

Medium A containing yeast nitrogen base gave higher yields of xylitol as compared to medium B containing glycerol and biotin. This could be because yeast nitrogen base (YNB) contains all essential nutrients and vitamins necessary for the cultivation of yeasts (Maria *et al*., 1988). Although in medium B, xylitol was produced but the yield

was comparably low. Complete xylose consumption was shown by the Sx within just 48 hours. The highest xylose to xylitol conversion efficiency was for soil isolate which was 64.12% (free cells). On immobilization of the soil isolate by calcium-alginate bead method, it was observed that the highest yield of xylitol was 0.157 gg^{-1} of xylose in medium A (Table 2: immobilized cells). Also the overall yield was maximum in the medium A as compared to medium B. The immobilization strategy using calcium- alginate beads gave a very low yield as compared to the free cells. The immobilization of *Candida guilliermondii* for xylitol production gave a yield of 0.53gg^{-1} of xylose in the work carried out by (Walter *et al*., 2002) by using sugarcane bagasse as biomaterial for immobilization. Thus this strategy could be applied to the present study for improving the yield.

The soil isolate showed higher production of biomass in shaker condition in comparable with static conditions in the 5% xylose medium. Under aerobic conditions, there was rapid utilization of 5% xylose. The biomass production was high under shaker condition as compared to static condition (Table 2: free cells). There was rapid xylose utilization by free cells than immobilized cells in the fermentation media. Therefore, there was very slow utilization of xylose in both the media with altered nitrogen sources by immobilized soil isolate as compared to free cells of soil isolate (Fig 6).



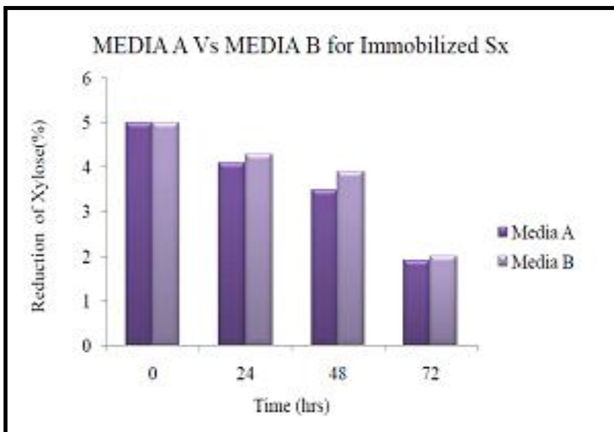
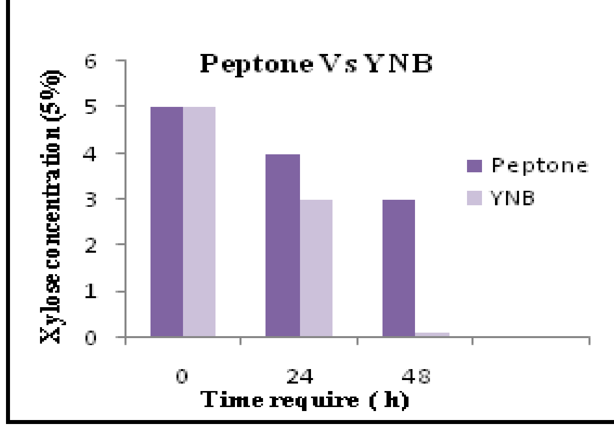


Fig. 6: Xylose utilization by free cells and immobilized cells in Medium A and Medium B

Key: Sx- Soil isolate, Media A- xylose medium containing yeast nitrogen base; Media B – xylose medium containing glycerol and biotin



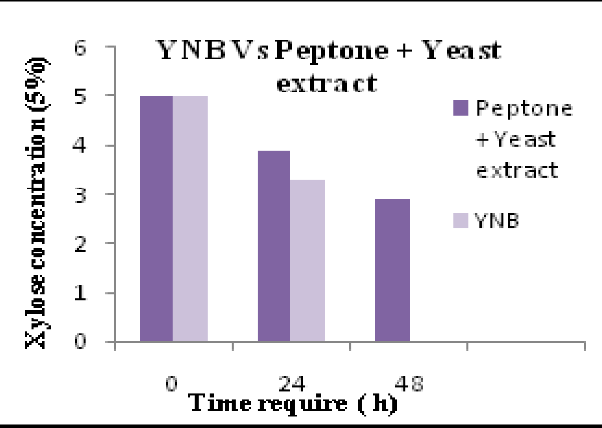


Fig. 7: Xylose utilization pattern with different nitrogen source by free cells.

Key: Sx- Soil isolate, Peptone- xylose medium containing peptone as nitrogen source, YNB – xylose medium containing yeast nitrogen base as nitrogen source,Peptone + YE– xylose medium containing peptone and yeast extract as nitrogen sources

In the above figure (Fig7), it can be observed that in presence of peptone as nitrogen source, the soil isolate did not completely utilize xylose within 48h. Also it can be observed that in presence of peptone+ YE as nitrogen sources, the soil isolate slowly utilized 5% xylose.

Whereas, when YNB was used as the nitrogen source there was almost complete 5% xylose utilization within 48h. Thus, YNB proved to be a better nitrogen source than peptone, yeast extract and it clearly enhanced xylose utilization of this isolate.

CONCLUSION

The maximum xylitol yield was 0.58gg^{-1} of xylose, by the soil isolate in medium A with yeast nitrogen base, which also showed a slight increase in final xylitol concentration as compared to medium B containing glycerol and biotin. Thus this study clearly indicates yeast nitrogen base to be efficient nitrogen source for xylitol production as compared to peptone and yeast extract.

The highest percentage efficiency of xylose to xylitol conversion in this study is 64% of the soil isolate This study was carried out under lab scale conditions, shake flask method was used for fermentation rather than a fermentor which restricted the exact conditions required for the fermentation process i.e. baffles, sparger, impeller etc for maintaining homogeneity, equal distribution and maintaining dissolved oxygen content. Thus the percentage efficiency of xylose to xylitol conversion and the yield for this isolate could be enhanced by improvising necessary parameters. Also the yield of xylitol could be worked upon by optimizing several parameters including various concentrations of yeast nitrogen base, glycerol and biotin.

The maximum yield given by the isolates on immobilization is 0.157gg^{-1} with 16% xylose to xylitol conversion efficiency. The calcium-alginate concentrations could be optimized for more excretion of xylitol into the medium. Also the matrix used for immobilization can be altered with sugarcane baggase etc. Five percent xylose was rapidly and efficiently utilized under aerobic conditions than the static conditions by this isolate. Also this isolate grew well under aerobic conditions. Thus it indicates that these isolates appear to be aerobic natural xylose utilizers.

For improving the xylitol yield, the enzymes responsible for xylose to xylitol conversion can be extracted and this enzyme solution can be employed for direct xylose to xylitol conversion.

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RESEARCH ARTICLE

Rice Cropping in Urban Farming with Special Reference to AM Fungi

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ABSTRACT

Urban farming is no more new to Mumbai and it's suburban. Being a metropolitan city, its expansion is a common trend in every possible direction. A railway is a life line of this city. This local service is divided into Central railways, Western railways, Harbour railway and Trans Harbour railway. From which Trans Harbour railway is comparatively new development connecting Thane to Vashi (New Mumbai). Near the railway tracks some area of land might be available as a free space. On this land various vegetables like Okra (*Abulmoschus esculantus* L.), Methi (*Trigonella longiceps* F.), Bathua or Mayalu (*Chenopodium album* L.) Chawali (*Vigna unguiculata*.), Palak (*Spinacia oleracea* L.). are cultivated during pre and post monsoon period. Whereas in monsoon season, Paddy cultivation (Rice, *Oryza sativa* L.) is a common practice. Such rice cultivation is largely based on stagnant water which gets collected during heavy monsoon.

In present study, one of the urban farms which are near Koparkhairane station on the trans - harbour train route was selected. From the urban farm *Oryza sativa* L. (rice) plants were collected at specific intervals throughout the monsoon period and percent root colonization of arbuscular mycorrhizal fungi from roots was analyzed. Rhizosphere soil was analysed for pH, mineralizable nitrogen along with spore density of arbuscular mycorrhizal fungal chlamydospore. Very high level (as high as 100 %) root colonization by various arbuscular mycorrhizal fungi could be observed. A positive correlation between spore density and percentage root colonization was drawn from the results.

Keywords: Urban farming, mycorrhiza, per cent root colonization, abiotic stresses.

INTRODUCTION

Urban farming is no more new to Mumbai and it's suburban. Being a metropolitan city, its expansion is a common trend in every possible direction. New routes for approach have been developed in recent past. A railway is a life line of this city. Thousands of people use local trains as a convenient mode of transport every day. This local service is divided into Central railways, Western railways, Harbour railway and Trans – Harbour railway. From which Trans – Harbour railway is comparatively new development connecting Thane to Vashi (New Mumbai). New big stations with broad platforms have been constructed for easy use by passengers. Since this line is comparatively new and have been constructed in near past, stations are well maintained, but local tracks pass from area which are under developing stages. Earlier to the development of this local route, this area use to be either an open fields or industrial area. Near the railway tracks some area of land might be available as a free space. Such areas are generally utilized by Indian citizens who are migrated from other parts of India to Mumbai in search of their daily needs and some work. Such people conveniently develop slums for their accommodation and use such areas for garbage disposal and as their backyards. Along with such slum development, many of people often use free space along the tracts for farming. On this land various vegetables like Okra (*Abulmoschus esculantus* L.), Methi (*Trigonella longiceps* F.), Bathua or Mayalu (*Chenopodium album* L.) Chawali (*Vigna unguiculata*.), Palak (*Spinacia oleracea* L.).are cultivated during pre and post monsoon period. Whereas in monsoon season, Paddy cultivation (Rice, *Oryza sativa* L.) is a common practice. Such rice cultivation is largely based on stagnant water which gets collected during heavy monsoon. Along with monsoon as a source, nearby water bodies are used as a source of water fir paddy cultivation. Often these water bodies are polluted with heavy metals, sewage from nearby area, garage effluents etc.

In present study, one of the urban farms which is near Koparkhairane station on the trans - harbour train route was selected. This selection was done on the basis of easy collection of samples from the field and friendly approach of urban farmers from the field. This is because these urban farming practices are totally illegal. Majority of these urban farmers are from very poor families and migrants from North India. Since it is illegal, it becomes very difficult to get samples in enough number and other information as well, as these farmers refuse to give any relevant information. It was very difficult to get photographs as well of the cultivation in month wise manner. Authors could manage to get some few photographs with very great difficulties.

Mycorrhizal biodiversity of petro effluent – irrigated fields were investigated by Kothari *et al* (1997). Petro effluent of Indian Petrochemicals Corporation Limited (IPCL) has been tested for irrigation in the ecoform at IPCL for recycling millions of tonnes of water. Rhizosphere of 6 crops raised in these fields has been evaluated for mycorrhizal biodiversity along with mycorrhization status of these crops. Authors claim this as the first report of mycorrhizal biodiversity of land distributed by petro effluent in India.

Exceptionally wide range of plants in different ecosystems shows association with AM fungi. The latter play a major role in better nutrition, species diversity and survival. Amongst the Angiosperms, about 90 per cent of the families develop AM association. The occurrence of AM fungi differs qualitatively and quantitatively with the change in edaphic factors and type of vegetation (Smith and Smith, 1996). Ecological factors which influence the colonization of AM fungi in the soils are soil pH, temperature, moisture, organic matter content in the soil and soil pollutants. Changes in the soil pH greatly affect the development and functioning of AM fungi. Different species and strains of AM fungi show different responses to soil pH. Higher fungal spore density in acidic soil, while in alkaline soil

the spore density is low has been reported by Dalal and Hippalgaonkar (1995). Higher temperature results into high root colonization in temperate zone whereas it can be just a reverse in tropical region. AM fungi prefer optimum soil moisture for sporulation and growth. Because of it, during winter and summer spore density is low as comparing to rainy season in which it is quite high. Organic matter and soil fertility also play an important role in growth and sporulation of AM fungi. In low fertile soil, AM fungi spore density is higher to that of high fertile soil where it is quite low. Changes in soil fertility due to amendments with mineral fertilizers or organic matter markedly affect the activity and survival of AM fungi. Nitrogen can stimulate or suppress root colonization by AM fungi and sporulation by changing the soil pH. In the presence of sufficient amount of Nitrogen in the soil, addition of Phosphorus suppresses root colonization. More amount of AM colonization is observed in P deficient soil (Sylvia and Neal, 1990).

A systematic study was carried out by Gupta (2001) to determine the effect of AM fungi on growth and phosphorus content in plant tissue of rice. Plant height in inoculated conditions was increased significantly. An improvement in dry biomass production upon inoculation was also observed. Phosphorus content of the plant system was found to be significantly higher in inoculated plants. A positive symbiotic association between rice and AMF inoculated was clearly evident under different soil treatment conditions. Among the AM inoculants, *Sclerocystic dussii* performed best way in increasing growth of rice in pot culture.

In present investigation, roots of *Oryza sativa* L. (Rice) were collected from Koparkhairane from Trans Harbour Route (Thane to Turbhe) railway tracks from urban farms and screened for per cent colonization by mycorrhiza fungi. Similarly, rhizospheric soil samples were also collected and detail investigations were carried out like physical parameters such as soil pH, % nitrogen of soil, Samples were screened for Arbuscular Mycorrhizal Fungi (AMF) spores also and spore density was calculated for each sample.

MATERIAL AND MATERIALS

For the present investigation sample collection was the main fundamental aspect. As discussed earlier, sampling was done throughout the monsoon period at random time (when it was possible as permission from farmers was use to be a chance). So whenever it use to be possible 10 to 15 number of plants from random spots in farms were collected. Plants were collected during the range of 25 to 40 days after sowing, 85 to 95 days after sowing, 115 to 130 days of sowing. The reason behind is same as farmers were some time cooperative where as sometime they use to refuse permission to enter in farms. The plants with root system along with rhizospheric soil were carefully collected in clean, unused plastic bags of suitable size. The samples were carefully labeled showing the records like date of collection, time, etc and were brought in the laboratory. After bringing the samples in laboratory, roots were carefully separated from entire plants, tapped gently to separate soil particles which were adhered to it (which was put in respective soil samples). The root samples were washed under tap water to clean and stored in 70 per cent of alcohol until further use. Soil samples were dried under sunlight and stored in well labeled, sterilized plastic bags.

A. Percentage root colonization was calculated after staining procedure given by Koske (1989), Carol and Stribley (1991). The roots of collected plants were washed thoroughly with running tap water for removal of alcohol which was used as preservative. The properly cleaned roots were subjected to staining procedure. Roots were subjected to 10 per cent of potassium hydroxide and heated in water bath at 90^0 C for one hour to clear the tissue. Such cleared roots were washed with distilled water several times to remove the traces of alkali. In case of dark pigmentation of roots, they were

treated with three per cent hydrogen peroxide for bleaching. It took 5 to 30 minutes depends upon the degree of pigmentation. This step was eliminated in the case of roots which are white or non-pigmented. Once again roots were washed thoroughly for several times with distilled water. Washed roots were kept in 1 per cent hydrochloric acid for about 18 hours to neutralize the effect of alkali used for cleaning the root tissue. Acidification was followed by washing several time the treated roots to remove traces of acid. The roots were then kept in acid glycerol containing 0.05 per cent aniline blue in test tube. Then it was autoclaved for 15 minutes under the pressure of 15 labs at 121^0 C. After that, excessive aniline blue was drained off and excessive stain was removed from stained roots by using acid glycerol. The stained roots were stored in amber coloured bottle in acid glycerol until further use to avoid destaining. The roots were cut into small pieces approximately 1 cm in length and mounted on micro slides using glycerine as mounting medium. After placing the cover slips, the slides were observed under low power of objective of 10 X magnification to confirm the presence of mycorrhizal hyphae and high power objective of 40 X magnification to observe the presence of vesicles, spores etc. The slides were sealed with nail polish. The per cent root colonization was calculated by Nicolson's formula.

$$\text{Per cent root colonization} = \frac{\text{No. of root pieces showing colonization}}{\text{Total no. of root pieces observed}} \text{ X 100}$$

B. Isolation and quantification of AMF spores was carried out by wet sieving and decanting method (Gerdeman and Nicolson, 1963). 25 g of sundried rhizospheric soil of different soil of different plants collected at different localities was taken in separate beakers. Half a litre of water and a pinch of soap powder were added to this. The solution was stirred and was allowed to stand for half an hour. The soil solution was then filtered through sieves of 500, 250, 150, 105 and 55 mm mesh which were kept one above the other. The spores and soil particles which settled on the surface of 150, 105 and 55 mm mesh sieve were washed and collected in separate beakers. The water was again filtered through whatman filter no. 1. This paper was observed under stereoscopic binocular microscope to count the number of spores. Different spores were examined for their taxonomic status by using standard key (Schenk and Perez, 1989).

Rhizospheric soil samples were analyzed for its various physico – chemical properties were estimated.

C. Soil pH was determined by using pH meter model number EQ 614.

D. Mineralizable Nitrogen of soil sample was determined by Jackson's method (1967). 5 g of air dried soil was taken in 500 mL Kjeldahl flask. 5 mL of water was added followed by addition of 15 mL of conc. H2SO4. The setup was kept undisturbed for 30 minutes. To this mixture was added 0.1 g of selenium powder. The digestion was first started over small flame and gradually increased until fumes of sulphuric acid were produced. The flask was removed immediately and added 5 g of potassium sulphate. The flask was againreplaced over the flame and digestion was continued for 1 to 2 hours till the digest has become colourless. The flask was allowed to cool and then 50 mL of water was added. The solution was left for 30 minutes to allow the soil particles to settle down. With the help of pipette the top layer of clear soil extract filtered out and stored in plastic bottles. From the stock 10 mL of soil extract was poured in 500 mL distillation flask. This was diluted by 100 mL of water and 10% NaOH was added to make the mixture in the digestion flask neutral. The mixture was well shook and distillation was commenced. The liberated ammonia was collected in 25 mL of 0.1 N HCl containing 2 to 3 drops of methyl red indicator. The distillation was carried out until the distillate was about 1/3rd of the liquid has passed over. When the distillation was over, the condenser tube was rinsed with distilled water to remove any traces of nitrogen if trapped into the

0.1N HCl. A blank without soil extract was carried out in exactly the same manner. The percentage of nitrogen in the soil was calculated on the basis of 5 g soil by using the following formula:

$$\%Nitrogen = (B-T) X N X \frac{0.14}{\text{Weight of Soil}} \frac{X}{100}$$

Where:

B = Blank titration (ml of alkali used)

T = Actual titration

N = Normality of the standard alkali (0.1)

RESULTS AND DISCUSSION

Oryza sativa L. (Rice) plants were screened for AM fungi association in terms of per cent root colonization and no. of AMF spores per 25 g of rhizospheric soil. These observations are tabulated in table no.1 similarly; rhizospheric soil samples were analyzed for physico – chemical properties such as pH of soil, and mineralizable nitrogen, shown in table no. 2. Table no. 3 shows different genera with species of AMF observed in *Oryza sativa* L. (Rice) plants. Average percent colonization and AMF spore count for *Oryza sativa* L. (Rice) plants (at intervals of collection days) are presented in Figure No. I. During the present investigation AMF spores from 4 genera with 7 species have been encountered which is shown in Figure No. II. Figure No. III shows generic level distribution of AMF of investigation.

All soil samples were acidic in pH ranging in between 4.9 to 5.8 which is fairly acidic range. This might be because of very poor quality of water used along with rain water to maintain water level. Abiotic stress can be lower down by mycorrhizal colonization [Zuccarini and Savé, (2015), Dahmosh, (2002)]. Very high (as high as 100%) root colonization has been found out during present investigation. Zero to 43 % root colonization in *Oryza sativa* L. (Rice) plant has been reported by Hajiboland *et al* (2009) where as Rajeshkannan *et al.* (2009) reported it as 37.18%. He also reported that during 35 to 42 days after sowing shows increment in root colonization whereas root colonization percentage gets decreased during 42 to 70 days after sowing in *Oryza sativa* L. (Rice) plants. According to Maiti *et al* (2006) root colonization

Table1: Per cent colonization and spore density in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Days after sowing	Percent colonization	Spore density
30 days (25 to 40 days)	89.2	224
90 days (85 to 95 days)	93.8	289
120 days(115to130days)	100	304

Table2: Range of physic-chemical parameters (P. C. P.) of rhizosphere soil of *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Days after sowing / P. C. P	pH	Mineralizable nitrogen
30 days (25 to 40 days)	5.8	2.82
90 days (85 to 95 days)	5.0	2.58
120 days (115 to 130 days)	4.9	1.40

Table3: AM fungi associated with *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Plants / Spot	Airoli
30 days (25 to 40 days)	*Glomus mosseae , Glomus fasciculatum*
90 days (85 to 95 days)	*Glomus mosseae, Acaulospora laevis, Glomus macrocarpum*
120 days (115 to 130 days)	*Glomus mosseae, Glomus macrocarpum, Gigaspora margarita, Acaulospora foveata, Sclerocystis clavispora*

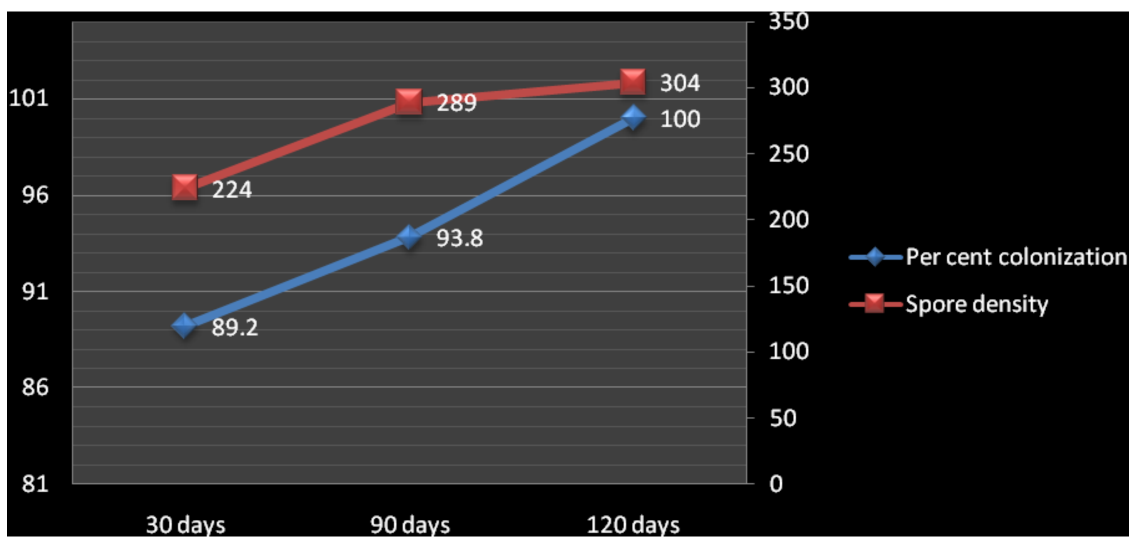

Fig.1: Percent colonization and spore density in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

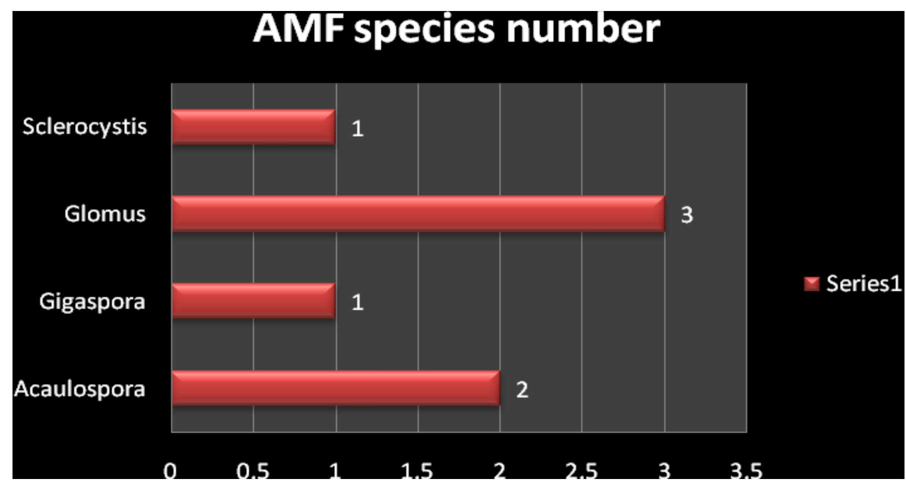

Fig.2: AMF genera with species number in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

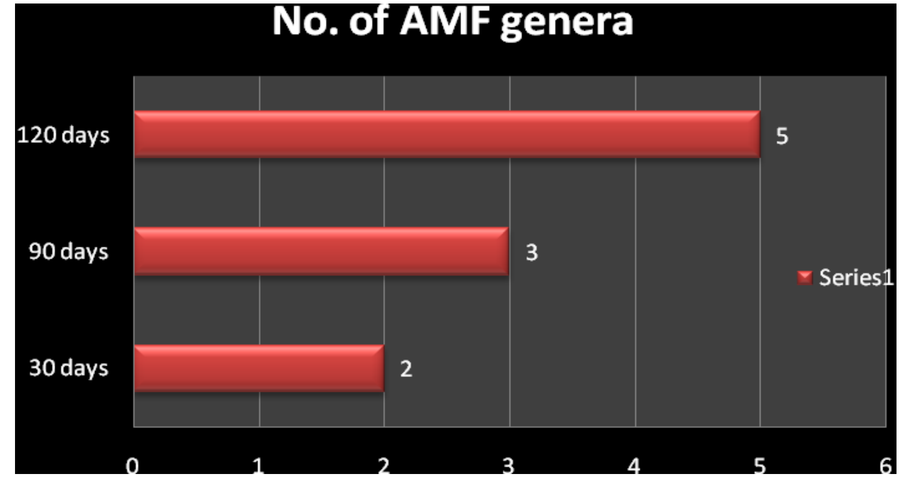

Fig.3: AMF diversity at generic level on days of collection in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

percentage in *Oryza sativa* L. (Rice) plant can be 5 to 65 %. Sadhana (2015) reported a gradual increment in percent root colonization from 44.67 to 58.0% from 15 days to 60 days after sowing. Gradual decrease in percent root colonization from 55 to 28% from 1 day to 105 days after sowing has been reported by Solaiman and Hirata (1996). Xu *et al* (2010) reported it as 14%.wher as according to Fernandez *et al* (2011) it ranges from 2 to 44% in rice plant. Muhammad Ali (2008) reported 100 % root colonization in basamati rice but it is in experimental conditions where AM fungal culture was added.

Bhattacharjee and Sharma (2011) reported decrease in percent root colonization up to 90 days after sowing where as increased percent colonization in the period of 90 days to 135 days after sowing. Which ranges from as low as 54 % to as high as 80 %. In this investigation the percentage root colonization in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts) is very high ranging from 89.2 to 100 %. *Glomus mosseae* is the most common in occurrence in soil samples analysed. *Glomus* with 3 species, *Acaulospora* with 1 species,*Gigaspora* with 2 species and *Sclerocystis* with single species observed in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts). Remarkable fact came in light that the farmers apply high amount of urea (a nitrogenous fertilizer) in urban farming for fast growth of the plants. It is well accepted fact that high nitrogen content and low phosphate content of soil increases root colonization of AM fungi. This might be one of the contributory facts to get middle range to high number of AMF spore count and very high per cent colonization (almost 100 %). Generally, a negative correlation is observed in between percent root colonization and spore density. In present study a positive correlation could be observed in them.

CONCLUSIONS

Based on the above observations and results, conclusions can be drawn that mycorrhiza can be used in urban farming for sustainability. This research work is throwing light on scenario of mycorrhiza in such lands where abiotic stress (like polluted land, poor quality of water used for irrigation) is very high. So if local farmers are made available with mycorrhizal cultures for the use in such urban farming, it may give very good results in terms of high yield and rapid growth (as it is well established fact that, mycorrhiza enhance the growth in host).

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RESEARCH ARTICLE

Effect of Arbuscular Mycorrhizal interactions on chlorophyll content and per cent productivity of *Pisum sativum* L.

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ABSTRACT

The mutually beneficial relation between feeder roots of plants and fungi is called ‘Mycorrhiza’. The term ‘Mycorrhiza’ was coined by Frank in 1885 to describe symbiotic association of plant roots and fungi. The word ‘Mycorrhiza’ originate from two greek words ‘Mycos’ meaning fungus and rhiza meaning root. Arbuscular mycorrhizae (AM) are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that association with AM fungi facilitates better nutrient uptake enhancing plant growth. Hence to exploit these biological tools, pot experiments were carried out and response on growth and yield of *Pisum sativum* L. was studied. AM inoculums brought from Tamil Nadu Agricultural university containing the mixture of *Glomus* species was directly used as an inoculum to study the effect of AM on *Pisum sativum* L. Pot experiments were conducted in mixture of sterilized garden soil and sterilized sand in the ratio 3:1. The experiment was conducted with AM (treated) and non AM (control) plants of *Pisum sativum* L. Soluble protein content, alpha amino nitrogen content, nitrate content and nitrate reductase activity in the leaves of treated and control plants of *Pisum sativum* L. were estimated at an interval of 15 days after sowing the seeds (DAS), 30DAS, 45DAS and at 60DAS. The association of AM fungi enhances the growth in all the treated plants. The significantly high growth rate and yield was observed in treated plants than control plants. Significantly higher amount of soluble protein content, alpha amino nitrogen content, nitrate content and nitrate reductase activity was observed in the leaves of treated plants of *Pisum sativum* L. than that in control ones.

Key word: Arbuscular mycorrhizae (AM), chlorophyll content, *Pisum sativum* L, symbiotic association, growth rate, *Rhizobium,*

INTRODUCTION

Arbuscular mycorrhizae (AM) are symbiotic association formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that association with AM fungi facilitates better nutrient uptake enhancing plant growth. The association of AM fungi enhances the ability of leguminous plants to withstand the various stresses to some extent. When the nutrient uptake levels and growth rate were estimated in AM and control leguminous plants in drought and saline stresses, the AM associated leguminous plants showed more growth rate and nutrient levels than the ones without AM association. It was found that percentage variation in growth rate (i.e. root and shoot length and root and shoot dry weight) and nutrient uptake in leguminous plants under drought and different levels of salinity stress condition were directly proportional to the percentage of mycorrhization (Kumar and Muraleedhara, 2003*)*. Am symbiosis can affect stomatal behaviour and photosynthesis of host leaves and have been shown to increase both transpirational and photosynthetic rates as well as chlorophyll concentration (Devi and Reddy, 2004). They observed that innoculation with AM fungus alone or in combination with *Rhizobium* brought about significant increase in chlorophyll “a”, chlorophyll “b” and total chlorophyll content in ground nut, thereby increasing the rate of photosynthesis.

MATERIALS AND METHODS

AM inoculums

AM inoculums was brought from Tamil Nadu Agricultural university which contained the mixture of *Glomus* species (*Glomus fasciculatum, Glomus aggregatum, Glomus multicaule, Glomus dimorphicum, Glomus microcarpum*, etc) was directly used as an inoculum to study the effect of AM on *Pisum sativum* L.

Preparation of control and treated pots:

Twelve large sized plastic pots with holes at the bottom having an internal diameter of 18 cm were used for the experiment of which six were maintained as control and six were used for treatment with ‘Mycorrhiza’. Garden soil was obtained in bulk from nursery suppliers. Similarly sand was procured from sea shore and was washed thoroughly in running water for several hours to remove soluble salts. Both garden soil and sand were mixed in proportion of 3:1 by volume in large trays. Sand help in improving aeration in pot and thereby help AM fungi to grow as mycorrhizae are aerobic microorganisms. This soil sand mixture was sterilized at a temperature of 200^0c for 2 hours in hot air oven, to kill soil microorganisms and insects. This sterilized mixture was used as a growth medium for pot experiments. Out of 12 pots six were maintained as treated and remaining six as control. Initially ¾th part of each pot was filled up with sterilized soil mixture. 10 g of AM inoculums was added to each treated pot the inoculum was distributed evenly in the pot and was covered with a layer of 4 cm. of sterilized soil mixture. Twelve water soaked seeds were sown in each pot and covered with a layer of soil. The pots were watered with watering can having small pores to avoid the disturbance of the soil surface.

Following physiological parameters from the leaves of the plants of *Pisum sativum* L., both control and treated were studied.

1)Chlorophyll content

2) Phosphate content

3) Per cent productivity.

The method of Arnon (1949) was used for estimating the chlorophyll content of the leaves. Phosphate in the oven dry leaf material was extracted by the method of wet digestion byJackson, (1967).

All the parameters were studied on 15th, 30th, 45th and 60th day after sowing the seeds. The roots of *Pisum sativum* L. were screened to obtain percentage of AM colonization at 15, 30, 45 and

60 DAS. Isolation and quantification of spores from rhizosphere soil of *Pisum sativum* L. was also carried out before sowing the seeds and at 60 DAS.Screening of the roots was carried out to study the per cent of root association by AM fungi in treated pots by the method described by Grace and Stribley (1991). The percent of root infection was calculated by using Nicolson's formula (1955).

RESULTS AND DISCUSSION

Chlorophyll content, phosphate phosphorus content in the in the leaves of treated plants of *Pisum sativum* L. was higher than the control ones throughout the period of experiment and per cent productivity of the treated plants was higher than the control ones.

Table 1: Chlrophyll 'a' content in the leaves of treated and control plants of *Pisum sativum* L. (mg per 100 g fresh leaf)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	9.276	9.732	9.651	9.305
Control	9.001	9.629	9.531	9.182
Calculated 't'	13.75	6.058	8.571	12.3
Level of significance	+++	+++	+++	+++
Standard error (S.E.)	± 0.02	± 0.017	± 0.014	± 0.01

Table 2 –Chlorophyll 'b' content in the leaves of treated and control plants of *Pisum sativum* L. (mg / 100 g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	9.518	10.807	10.674	9.626
Control	9.239	10.605	10.344	9.414
Calculated 't'	3.065	9.181	13.75	10.6
Level of significance	+	+++	+++	+++
Standard error (S.E.)	± 0.091	± 0.022	± 0.024	± 0.02

Table 3: Total chlorophyll content in the leaves of treated and control plants of *Pisum sativum* L. (mg / 100 g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	23.029	20.169	16.691	15.736
Control	20.831	18.958	15.855	15.489
Calculated 't'	6.820	6.465	4.146	2.523
Level of significance	+++	+++	+++	+
Standard error (S.E.)	± 3.220	± 1.871	± 2.016	± 0.977

Table 4: Phosphate content in the leaves of treated and control plants of *Pisum sativum* L. (mg / g fresh leaf.)

	15 D A S	30 D A S	45 D A S	60 D A S
Treated	0.625	1.325	1.666	0.791
Control	0.466	0.533	1.158	0.583
Calculated 't'	4.009	8.020	5.907	2.518
Level of significance	++	+++	+++	+
Standard error (S.E.)	± 0.0392	± 0.0980	± 0.085	± 0.0274

Table 5 –Analysis of fruits of treated and control plants of *Pisum sativum* L.

	Fruit length in cm.	No. of seeds	Fresh wt. of seed	Dry wt. of seed	Per cent of production
Treated	6.533	2.166	0.687	0.181	71.11%
Control	4.466	1.166	0.250	0.071	53.333%
Calculated 't'	3.256	2.301	2.364	2.322	2.410%
Level of significance	++	+	+	+	+
Standard error (S.E.)	± 0.634	± 0.433	± 0.184	± 0.0473	± 7.379

Level of significance 'o' = Difference of mean not significant, '+' = Difference of mean significant (P=0.05), '++' = Difference of mean significant (P=0.01), '+++' = Difference of mean significant (P=0.001), DAS = Days after sowing. Each value is a mean of six replicates

Table 6 – Results of the screening of roots of *Pisum sativum* L. to obtain percentage of AM colonization

Days after sowing	% colonization of AM roots	AM structures observed in the root cortex
15	16	Mycelium
30	45	Mycelium + vesicles
45	72	Mycelium + vesicles
60	87	Mycelium + vesicles + spores

Before sowing the seeds 10 g of AM inoculums were found to contain 57 AM spores while at 60 DAS it showed presence of around 78 spores. The results are tabulated in following tables.

Chlorophyll chlorophyll 'b' and total chlorophyll content in terms of mg per 100 g fresh leaf, has seen to be significantly higher in the leaves of treated plants than that in the control ones throughout the period of experiment.

Similar results has been reported by Devi and Reddy (2004) while working with *Arachis hypogaea* L., incoulation with AM fungus, either alone or in combination with *Rhizobium,* brought about significant increase in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. This increase may be due to the increase in stomatal conductance, photosynthesis, transpiration, enhanced plant growth (Levi and Krikun, 1980;) or due to the presence of larger and more numerous bundle sheath chloroplasts present in AM inoculated leaves (Krishna and Bagyaraj, 1984).

Growth of the mycorrhizal plant is usually high when the potential for active photosynthesis is high. The high rate of photosynthesis by mycorrhizal plants may be evoked by a number of changes such as an increase in plant hormones (Miller, 1971). Stomatal opening, enhanced ion transport, and regulation of chlorophyll level (Johnson, 1984). Increased chlorophyll accumulation was observed in AM inoculated papaya plant as compared to that in control papaya plants by Shivaputra *et al*. (2004). Higher phosphorus levels in tissues as a result of root colonization by the AM can be expected to increase the chlorophyll content, as phosphorus is one of the important component of chlorophyll. Increased uptake of nitrogen, phosphorus, potassium, copper, manganese, iron and zinc and increased tolerance to biotic stresses may be contributed to the production of more leaves and greater leaf area, and thereby higher chlorophyll, higher photosynthetic capacity even during reproductive phase and translocation of carbohydrates from other plant parts to reproductive parts might have resulted in increased yield and yield attributes (Sabarad *et al*., 2007).

Khare *et al*. (2008) working with *Cyamopsis tetragonoloba* observed that absorption of phosphorus and its supply to the root system of the AM plants is a major contribution of AM fungi. Mycorrhizal inoculation also enhances magnesium uptake and reduces sodium concentration in plants. This in turn helps in increasing the chlorophyll content and improves the overall growth performance of mycorrhizal plants.

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RESEARCH ARTICLE

Epicoccum nigrum link. as a potential source of Mycoremediation against oil spill

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ABSTRACT

Incident of 2,00,000 barrels of crude oil spill in Alaska fetched attention of the world. The problems caused due to oil spill are environmental pollution along with risk to the marine life and human beings. The global use of crude oil, mainly derived from various fossils throughout the world, is estimated to be average 5.5 metric tons per year. Biodegradation of such complex hydrocarbons is very challenging task. Use of microorganisms viz. bacteria and fungi to degrade these hydrocarbons is a primary mechanism to eliminate the pollutants from the environment. Effect of environmental parameters on the microbial activities in terms of metabolic pathway and its impact on the genetic bases was always been an area of intense curiosity and reviews. In case of fungi, spp. of *Aureobasidium, Candida, Rhodotorula*, *Sporobolomyces, Trichoderma* and *Mortierella* are commonly used as source of mycoremediation for degradation of hydrocarbons. In the present studies, we have tested *Epicoccum nigrum*, as a potential source of mycoremediation against oil spill. The effective degradation of hydrocarbons from the oil and petrol was measured in terms of titrimetric estimation of phytosterols from the samples.

Keywords: Crude Oil, Biodegradation, Mycoremediation, Primary Mechanism

INTRODUCTION

Petroleum hydrocarbons are usually divided into four groups such as saturated, aromatic, asphaltenes and resins (Colwell and Walker, 1977). Depending upon their structures, the hydrocarbons are susceptible to microbial attack. It has been studied that n-alkenes are more prone to microbial degradation that the cyclic alkenes (Perry, 1984). Biodegradation rates are highest for the saturated hydrocarbons, followed by the light aromatics. The high-molecular weight aromatics and polar compounds exhibiting extremely low

rates of degradation (Jobson *et. al*., 1972; Walker *et. al*., 1976; Fusey and Oudot, 1984). Cooney *et. al.* (1985) reported greater degradation losses of naphthalene than hexadecane in case of freshwater lake while Jones *et. al.* (1983) observed extensive biodegradation of alkylaromatics of the crude oil in marine sediments. Fedorak and Westlake (1981) also reported rapid degradation of aromatic hydrocarbons during the degradation of crude oil by marine microbial populations. Formation of emulsions through the microbial production and release of biosurfactants is an important process in the uptake of hydrocarbons by bacteria and fungi (Singer and Finnerty, 1984).

Fungi have been reported to be important inhabitants of specialized niches such as sub-merged wood (Kirk and Gordon, 1988). They are also components of the surface film of water, decomposing algae, and the surface of tarballs (Ahearn and Crow, 1986). Few studies have directly compared the degrees of hydrocarbon degradation accomplished by bacteria and fungi in the marine environment. Hydrocarbon utilizing fungi are readily isolated from soil (Llanos and Kjoller, 1976; Pinholt *et. al*., 1979; Atlas *et. al*., 1980) and the application of oil or oily wastes to soil results in increased numbers of fungi (Jensen, 1975; Llanos and Kjoller, 1976; Pinholt *et. al*., 1979). Hydrocarbon degradation by microbial communities depends on the composition of the community and its adaptive response to the presence of hydrocarbons (Song *et. al*., 1986). The factors mainly responsible for process of degradation are pH, temperature, light intensity, pressure, surface area, salinity and availability of nutrients (Atlas, 1981).

Epicoccum nigrum Link. is a common pathogenic fungal organism isolated from the soil. Morphological, cultural and metabolic characters of *Epicoccum nigrum* are similar to the spp. of *Aureobasidium, Candida, Rhodotorula, Sporobolomyces, Trichoderma* and *Mortierella*, which are commonly used as source of mycoremediation for degradation of hydrocarbons in oil spill (Kirk and Gordon, 1988). Hence, in the present studies, the authors have tested *Epicoccum nigrum* Link. as a potential source of mycoremediation against oil spill.

MATERIAL AND MATERIALS

1. Isolation of *Epicoccum nigrum*:

Epicoccum nigrum was isolated from the soil in the College campus by using **Agar plate method:**

a) Preparation of Potato Dextrose Agar medium (PDA) –

Peeled potatoes - 200 gms.
Dextrose - 20 gms.
Agar agar - 20 gms.
Distilled water - 1000 ml.

200 gms of peeled potatoes were boiled in 500 ml of distilled water till the solution becomes sticky by dissolution of the potato pieces. The solution was filtered and 20 gms of dextrose was added in it. 500 ml of distilled water was taken in the other beaker, it was boiled and 20 gms of agar agar powder was dissolved in it. Both the solutions were mixed and final volume was made to 1000 ml.

b) Sterilization of the glass ware

Required glass wares i.e. Petri plates, conical flasks; beakers etc. with PDA Medium were sterilized in an autoclave at 120º C at pressure of 15 lbs (pounds) for about 30 minutes. The glass ware and medium were preserved for the further use.

c) Incubation

The plates were incubated at 28º C (± 2) in the incubator.

2. Preparation of pure culture of *Epicoccum nigrum*:

Colonies of various fungal organisms obtained in Agar Plate Method were identified on the basis of study of colony characters, sporulation and spore specifications. Colonies of *Epicoccum nigrum* were selected and pure culture was obtained by serial purification method by using Agar Plate Method as mentioned above.

3. **Treatment of various sources of hydrocarbons with *Epicoccum nigrum*:**

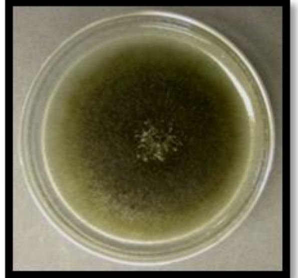
1 ml sample of Petrol, Til oil, Castor oil, Clove oil and Parachute oil (Coconut oil) each was treated with a loopful pure culture of *Epicoccum nigrum* and 4 sets were prepared. The sets were incubated for 7, 14, 21 and 28 days respectively at 28º C (± 2) in the incubator. At the end of decided period, each set was estimated to find out the amount of phytosterols present in the sample.

Estimation of phytosterols:

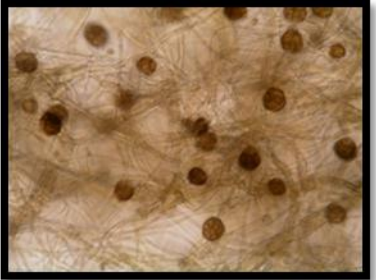
Lipids in the oils in the form of phytosterols were estimated by method given by Tomita *et. al.* (1970). According to this method, 1 gm oil was extracted with 10 ml 80% alcohol and the mixture was warmed slightly. The homogenate was allowed to cool for 10 minutes and filtered through Whatman No. 1 filter paper. The filtrate was collected and used for estimation of phytosterols. 0.5 ml of the extract was taken in a test tube and 2ml glacial acetic acid and 2 ml coloured reagent were added to it. The total volume was adjusted to 5 ml by adding 80% alcohol in it. The test tubes were incubated in an ice bath at 0 ^{o}C for 10 minutes and absorbance was read at 440 nm. The values were calculated by using 0.1 as the multiplication factor.

RESULTS AND DISCUSSION

Table 1of Estimation of Phytosterols clearly indicates decrease in the amount of phytosterols in various types of oils and petrol during incubation with *Epicoccum nigrum* indicating decrease in the amount of hydrocarbons. Clove oil showed presence of highest amount of phytosterols i.e. 0.096 mg in the control followed by 0.095, 0.094, 0.092 and 0.043 mg of phytosterols during successive incubation of 7, 14, 21 and 28 days. Parachute (Coconut) oil was in the second place with presence of 0.094 mg of phytosterols in control degrading to 0.086, 0.075, 0.069 and 0.023 mg during 7,14, 21 and 28 days of incubation respectively. Til oil showed presence of 0.069 mg of phytosterols in control; which degraded to 0.063, 0.026, 0.022 and 0.011 mg respectively during range of incubation from 7 to 28 days. Petrol showed presence of 0.068 mg of phytosterols in control decreasing to 0.051, 0.017, 0.013 and 0.006 mg during successive incubation for 7, 14, 21 and 28 days. The lowest amount of phytosterols (0.053 mg) were present in castor oil and degraded to 0.049, 0.042, 0.021and 0.008 mg of phytosterols in respective period of incubationfrom to 28 days.



Pure Colony of *Epicoccum nigrum*



Fruiting bodies of *Epicoccum nigrum*

Control Set

Experimental Set

Table 1: Estimation of Phytosterols (Milligrams per Gram)

Sr. No.	Category	Control	7 Days	14 Days	21 Days	28 Days	Consumption
1	Petrol	0.068	0.051	0.017	0.013	0.006	91.18%
2	Til Oil	0.069	0.063	0.026	0.022	0.011	84.06%
3	Castor Oil	0.053	0.049	0.042	0.021	0.008	84.91%
4	Clove Oil	0.096	0.095	0.094	0.092	0.043	55.21%
5	Parachute Oil	0.094	0.086	0.075	0.069	0.023	75.53%

The highest amount of consumption of hydrocarbons by *Epicoccum nigrum* was seen in case of petrol (91.18%) follwed by castor oil (84.91%), til oil (84.06%) and parachute oil (75.53%). The lowest amount of consumption was shown by clove oil which was 55.21 %. Hence, *Epicoccum nigrum* supposed to be very effective in degradation of hydrocarbons from petrol.

CONCLUSION

Biodegradation of petroleum and other hydrocarbons in the environment is a complex process, whereby quantitative and qualitative parameters are dependent upon nature and amount of hydrocarbons along with environmental conditions and type of the microbes. Microbial degradation of oil occurs due to attack on aliphatic or light aromatic components in the oil while high-molecular-weight compounds exhibit very low rates of biodegradation. The microbial degradation of hydrocarbons is limited due to various factors such as nutrient concentrations, pressure, temperature, salinity, moisture and pH. Hydrocarbon degradation by microbial organisms is depending on the type and adaptive response of the microorganisms to the presence of hydrocarbons. Fungi are one of the key agents of degradation becoming more important in freshwater and terrestrial environments. In this scenario, apart from the mentioned fungi; *Epicoccum nigrum* can be looked upon as a potential source of mycoremediation in future because it has worked effectively in degradation of hydrocarbons from various samples of oil and petrol. It needs further investigation based upon various parameters.

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RESEARCH ARTICLE

Studies of the fungi *Lenzites acuta* Berk. from Western Maharashtra, India

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ABSTRACT

Wood rotting fungi are an important component of forest ecosystems. Especially white rote fungi belong to the order basidomycetes that participates in the biodegradation of lignin in nature, which is essential for global carbon recycling. In present paper an attempt was made to isolate the wood rotting fungi from different host plants from the forest of Western Maharashtra, India. Out of sixty five samples examined the white rot fungi, *Lenzite sacuta* Berk. of genus Lenzites Fr. were isolated for further studies. The morphological, macro chemical and cultural features of the species are described in this paper.

Keywords: Wood rotting fungi, Western Maharashtra, morphological and cultural features, *Lenzites*

INTRODUCTION

The genus Lenzites Fr. was first described by Fries in 1835 with *Lenzites betulina*(Fr.) as type species. It is important wood rotting fungi, acosmopolitan genus, causing white rot. The study of wood rotting fungi is fundamental to understand the fungal diversity in forests.Wood rotting fungi are those fungi that have the ability to decompose wood causing rot. A good number of these fungi produce large and conspicuous fruiting bodies. They comprise 10% of total fungal diversity, of which 16-41% have been described to date (Rossman 1994, Muller et al., 2007). Polyporoid and corticoid fungi are some of the most common and important wood inhabiting fungi in forest. These species account for the majority of the fruiting bodies found on wood debris (de Vries 1990). Some fungi attack living trees, otherinvades dead or felled timber and slash on the forest floor.

Wood decaying basidiomycetescolonize and degrade wood using enzymatic and mechanical processes (Lyngdoh and Dkhar, 2014). Brown rot fungi preferentially attack and rapidly depolymerize structural carbohydrates (celluloses and hemicelluloses) in the cell wall leaving the modified lignin behind. White rot fungi can progressively utilize all major cell wall components, including both carbohydrates and lignin (Jasalavich et al., 2000).

Wood rotting fungi are an important component of forest ecosystem (Wang, et al., 2011). In the last decade these fungi emerged as an important component of forest ecosystem due to their decomposition role in the recycling of wood and wood debris, and attracted the attention of research community towards their potential application in pollutant purification, soil remediation and antibiotic production. Hence our current study is focused on isolation of such wood rotting fungi from the forest of Western Maharashtra, India.

MATERIAL AND MATERIALS

For present study, specimens (fruiting bodied) of Lenziteswhere collected from different sites of the Western Ghats and Satpura ranges, in the state of Maharashtra. The specimens where conveniently collected in the paper bags, noting the host, locality, colour of the material and date of the collection as suggested by Gilbertson and Ryvarden(1986).From the collection few specimens were used for spore prints, sporocarp culture and a few for macro and micro morphological characters of the basidiocarp. Micro structure has been studied from the sections of fruiting body. Martin's (1934) staining method was used. Lactoglycerin with 1% cotton blue were used for semi permanent slides, which were sealed with a nail polish (Beneke, 1958). Melzers reagent (IKI) prepared as per the method of Singer (1982) was used for testing the amyloidity and dextrinoidity.

Sporocarp culture was obtained by aseptically transferring a piece of fruiting bodies into to the sterile 2% Malt Extract Agar (MEA) Medium containing 10 ppm Novobiocin and incubated at 25^0c for 4-6 weeks in B.O.D. Isolates were sub cultured and transferred to the fresh slant for every fortnight. The pure cultures were obtained and stored on 2% MEA slant. Culture characteristics of the specimens were described using the terminology of Rayner (1975) and Stalpers (1978), on the basis of characters such as chemical tests for detection of enzymes; growth rate; characteristics of mat; other macroscopic characters; hyphal characters; propagative structures etc. The species were identified with their species code on the basis of a key proposed by Stalpers (1978).

The type of rot was identified by spraying 1% benzidine solution in 90% ethanol (Hintikka and Laine, 1970), on decaying wood sample. Oxidase reactions in culters were determined by growing fungi on malt agar medium containing Gallic acid and Tannic acid separately (Gilbertson and Ryvarden, 1986).

RESULTS AND DISCUSSION

For the present study the Specimens collected from different sites were critically examined with respect to their external and internal Morphological characters of basidiocarp, cultural charecters and macro chemical tests. The observations of the study are as discussed as follows,

Lenzites acuta **Berk.** Journ. Bot. 1: 146, 1842.(Plate No.4.1.5,4.1.7 and 4.2.3; Fig. No.7)

1. Morphological Characters.

FRUITBODY annual to perennial, broadly attached, dimidiate with a contracted base, in some cases almost stipitate, semicircular to flabelliform, single or imbricate brown to gray, hard, woody to corky coriaceous when fresh, flexible when dry; strongly attached 13-15 cm

long x 9-10 cm broad x 1-1.5 cm thick at the base; PILEUS semicircular more or less angulate, dimidiate, flat, upper surface usually whitish, uneven, finely velutinate concentrically zonate, slightly sulcate, distinctly radially wrinkled, dotted warty, fine nodulate, nodules usually scattered near the base more rough than the margin with asperulate of agglutinated hyphae, zones of cream to brown and grey colour alternating with each other first white, cream, pale ochraceous to clay or tan coloured, then leather or dirty brownish coloured; MARGIN sharp, wavy, sometime folded bent downwards. PORE SURFACE flat to oblique orange buff, yellowish creamy to brown coloured, mostly with a yellowish tint, this colour seems to persist even when the upper surface has become white and dirty grey, pore surface extremely variable in some specimens poroid 2-4 mm wide, mostly angular mixed with daedaloid to sinuous lamellae up to 3.5 mm wide, in other specimen purely lamellate 3.5 mm wide, 10-11 lamellae per cm, lamellae straight or wavy especially towards the base where they are deeper tubes of lamellae up to 7-9 mm deep; CONTEXT white cream to yellowish coloured, 3-5 mm thick.

HYPHAL SYSTEM trimitic; Generative hypahe hyaline, thin walled, with clamps, 1.5-3.0 µm in diameter; Skeletal hypahe, straight thick walled to solid upto 5-7 µm in diameter; Binding hyphae hyaline thick walled solid highly branched, sword like, long side branches up to 4.5-5.5 µm in diameter; CYSTIDIA absent but thick-walled skeletal hyphae project into the hymenium; HYPHAL PEGS present conical to cylindrical; BASIDIAclavate 4 sterigmata 14.7-16.4 x 5.8-6.4 µm BASIDIOSPORES hyaline cylindrical, smooth, thin walled and non-amyloid 6-9 x 2-3 µm (L/B 3).

SPECIMEN EXAMINED Benzidine test positive, white rot on dead wood. Two specimens collected from Karnala RPO-128 on Mangiferaindica L and RPO-40 Garugapinnata one specimen RPO-07 from Toranmal on Pongamiaglabra.

Fig.-1 Pileus and Hymenial surface of *lenzites acuta*

Fig.-2 Upper and Lower surface of the culture of *lenzites acuta*

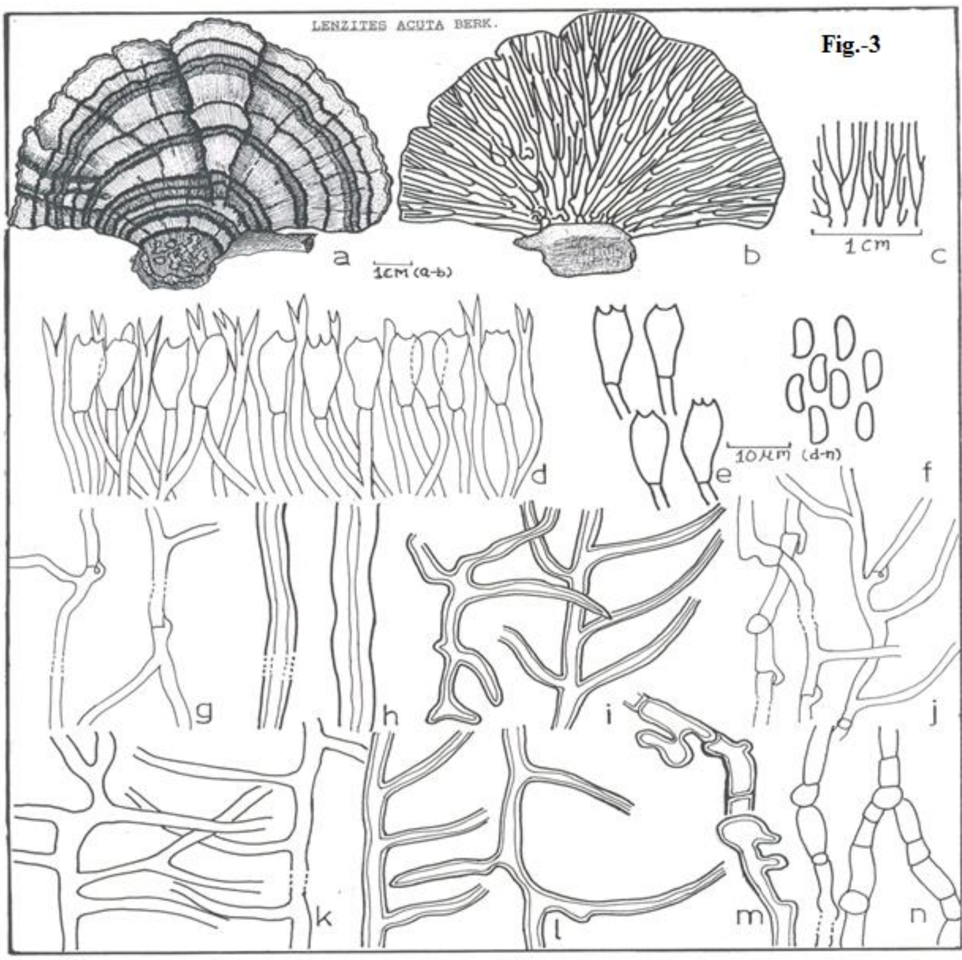

Fig.- 3 Lenzitesacuta
e. Upper surface **b**. Lower surface**c.** Nature and number of pores per cm.
d. Hymenium Basidia **f**. Basidiospores**g.** Generative hyphae **h.** Skeletal hyphae
i. Binding hyphae **j.** Generative hyphae in culture. **k**. Aerial hyphae in culture
l. Submerged hyphae in culture; **m.** Interlockinghyphae inculture;
n. Swellings (in culture)

2. CULTURE CHARACTERS

GROWTH CHARACTERS Growth rapid 45-50 mm in two weeks; Advancing zone white even apprised to raised; Mat pure milky white, silky, cottony to wooly, velvety on upper surface mat zonatehyphal growth radial margin circular equal, hyphal growth distinctly radial; Reverse bleached to creamy to yellowish coloured, fruitbody formation occurs after three weeks, odour none. Tests for extracellular oxidase are strongly positive on Gallic acid and Tannic acid agars diffusion zones strongfor α–napthol, Guaicol, Syringaldazine, P-cresol reactions strong.

HYPHAL CHARACTERS Advancing zone hyphae hyaline thin walled, branched, septate with clamps 1.5-2.5 µm in diameter; Aerial hyphae hyaline thinwalled branched 2.5-4 µm in diameter, submerged hyphae hyaline, thick walled to semisolid, branched 3.7-5 µm in diameter, some are irregularly swollen with intercalary and terminal swellings up to 5.5 µm.

SPECIES CODE:1,2,6,7,12,13,14,15,20,21,22,25,26,(29),30,37,(38),39,45,(47),52,53,64,75,89.

CONCLUSION

The observations of the present study reveals that the species of the Polyporaceae family are more common wood inhabiting fungi than the other families in the forest.As white rot fungi *lenzitesacuta* can be used for degrading lignin and wide range of environmental pollutants, so it can be used for bioremediation. Further study should be undertaken to prove its efficiency as biological delignification agent in commercial industries.

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RESEARCH ARTICLE

Management of *Alternaria alternata* causing fruit rot of Strawberry using various plant extracts

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ABSTRACT

Strawberry (*Fragaria ananassa* L.) is an important fruit in Maharashtra. Twenty five isolates of *Alternaria alternata* were isolated from rotted strawberry fruit and their sensitivity was tested against Mancozeb. The MIC was ranged between 680.0- 2040.0µg/ml *in vitro*. Isolate *Aa*-10 (680µg/ml) was sensitive while isolate *Aa*-08 was resistant showing maximum MIC (2040µg/ml). In present investigation total thirty six plant extracts were used to manage Mancozeb resistant mutant of *A. alternata* (*Aa*-EMS-2) individually as well as in mixture with Mancozeb. The individual PCE of *Zingiber officinale* Rosc., *Mimusops elengi* (L.), *Aloe vera* (L.), *Lantana camara* (L.), *Mentha arvensis* Benth., *Catharanthus roseus* (L.), *Eucalyptus globules* (Labill.), *Allium sativum* L.(Leaf and bulb), *Calotropis gigantea* (L.) R. Br. ex Schult. and *Cymbopogon citrates* DC. Stapf. gave fruitful results in individually. While mixture with Mancozeb, *Allium sativum* L. (leaves) (91.11), *Allium sativum* L. bulb (89.91), *Mimusops elengi* L. (86.67), *Lantana camara* L. (74.45), *Polyalthia longifolia* Benth. & Hook. f. (74.45), *Catharanthus roseus* L. (73.33), *Eucalyptus globulus* Labill. (72.22), *Ficus benghalensis* L. (72.22), *Datura inoxia* Mill. (70.00) and *Aloe vera* L. (68.89) shows fruitful results in controlling Mancozeb resistant mutant of *A. alternata* (*Aa*-EMS-2).

Keywords: *Strawberry rot, Alternaria alternata, Plant extract.*

INTRODUCTION

Strawberry (*Fragaria ananassa* Dutch.) is highly perishable fruits due to their extreme tenderness, vulnerability to mechanical damage and their susceptibility to fungal spoilage (Maxie *et. al.* 1959; Dennis, 1978). Fresh strawberries, therefore, have a very limited postharvest life and cannot be stored except briefly (Dennis and Mountford 1975).

Post-harvest losses are typically more severe, especially when conditions are favorable for disease development; in some cases 80-85% of a crop may be lost (Hong *et. al.* 1998; Larena *et. al.* 2005). Strawberry fruits infected by various fungal pathogens viz. *Alternaria alternata, Colletotrichum acutatum, C. gloeosporioides C. fragariae, Rhizopus nigricans, Phytopthora paracitica, P. cactonum, Botrytis cinerea, Fusarium solani, Aspergillus niger, Aspergillus flavus, Penicillium expansum* (Michel Dignand, 2004) out of which Alternaria rot caused by *Alternaria alternata* is severe. Strawberry growers heavily rely on the use of fungicides for control of fruit diseases in strawberries. But due to adverse effects of fungicides, growers are using integrated disease management methods for controlling various diseases. Several higher plants and their constituents have been successfully used in management of plant diseases and have proved to be harmless and nonphytotoxic, unlike chemical fungicides.

In present, investigated that the mechanisms of disease suppression by plant products have suggested that the active constituents present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of the disease. In this sense plant extract presently used as an alternative for plant disease management. Wongkaew and Sinsiri (2014) evaluated *C. longa* extract against *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotium rolfsii*, *Phytophthora infestans* and *Pythium* sp. in comparison to commercial fungicides such as copper oxychloride and Mancozeb. Kantwa *et. al.,* (2014) reported that garlic clove extract was found most effective in inhibiting the mycelial growth of *A. alternata* (46.60%) followed by neem (43.30%) and datura (40.30%) leaf extract. Harison *et. al.,* (2014) studied the aqueous leaves extract of *Pongamia pinnata*, *Calotropis procera*, *Nerium indicum* and *Curcuma longa* against *Alternaria solani* shows zone of inhibition of 20, 22, 21, 30mm respectively.

MATERIALS AND METHODS:

A total 36 *viz. Hyptis suaveolens* (L.), *Ricinus communis* (L.), *Syzygium cumini* (Lam.), *Allium cepa* (L.), *Allium sativum* (L.), *Eclipta alba* (L.), *Calotropis gigantea* (L.) R. Br. ex Schult., *Lantana camara* (L.), *Mentha arvenses* (Benth.), *Zingiber officinale* (Rosc.), *Aloe vera* (L.), *Vernonia spp.*(Schreb.), *Carica papaya* (L.), *Anethum graveolens* (L.), *Coriandrum sativum* (L.), *Murraya koenigii* (L.), *Ficus globosa* (Blume.), *Ficus religiosa* (L.), *Ficus benghalensis* (L.), *Azadirachta indica* (A. Juss.), *Plumaria alba* (L.), *Psidium guajava* (L.), *Mangifera indica* (L.), *Jasminium grandiflorum* (Dumort), *Datura inoxia* (Mill.), *Mimusops elengi* (L.), *Polyalthia longifolia* (Benth. & Hook. f.), *Catharanthus roseus*(L.), *Ocimum sanctum* (L.), *Ocimum basilicum* (L.), *Cymbopogon citratus* (DC. Stapf.), *Nerium indicum* (L.), *Eucalyptus globulus* (Labill.), *Citrus limonum* (Risso.) and *Hibiscus rosasinensis* (L.) medicinal plants were collected from Dombivali Shikshan Prasarak Mandal's, K. V. Pendharkar College campus and nearby college area for experiment.

The part of the plant v*iz*. leaves; rhizome and root were washed under the running tap water and finally rinsed with sterilized distilled water. 100 gm of plant parts were cut into small pieces and minced with the help of grinder by adding 100 ml sterilized distilled water. These leaf extracts were filtered through double-layered muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 15 lbs pressure for 20 minutes. The plant extracts were tested against mycelial growth of Mancozeb resistant mutant of *A. alternata* (*Aa*-EMS-2) by poisoned food technique (Nene and Thapliyal, 1992). Each plant extracts were tested at four different concentrations *viz;* 25, 50, 75 and 100% individually and in mixture with Mancozeb. Each plate was inoculated with 5 mm disc of mycelial bit taken from the periphery of 7days fresh culture of *A. alternata* (*Aa*-EMS-2) growing on PDA. The inoculated petriplates were incubated at $27\underline{+}2^0$C. Petri-plates were used for each treatment serving as three replications.

Medium without extract was served as control. Similar set was prepared using plant extract in mixture with Mancozeb (680µg/ml). Colony diameter was noted after 7 days of incubation. Percentage Control Efficacy was calculated by Baviskar and Suryawanshi (2014).

$$\text{Percentage Control Efficacy} = \frac{C-T}{C} \times 100$$

Where,

C = Diameter of the colony in control

T = Diameter of colony in treatment

RESULTS AND DISCUSSION

The results are revealed in table 1. Individually, plant extracts showed PCE ranges from 06.67-61.67. The individual PCE of *Zingiber officinale* Rosc., *Mimusops elengi* (L.), *Aloe vera* (L.), *Lantana camara* (L.), *Mentha arvensis* Benth., *Catharanthus roseus* (L.), *Eucalyptus globules* (Labill.), *Allium sativum* L.(Leaf and bulb), *Calotropis gigantea* (L.) R. Br. ex Schult. and *Cymbopogon citrates* DC. Stapf. ranges (61.67 to 48.89) gave fruitful results in individually at @ of 25, 50, 75 and 100 percent and mixture with Mancozeb PCE ranges 91.11-43.33% gave fruitful results followed by *Allium sativum* L. (leaves) (91.11), *Allium sativum* L. bulb (89.91), *Mimusops elengi* L. (86.67), *Lantana camara* L. (74.45), *Polyalthia longifolia* Benth. & Hook. f. (74.45), *Catharanthus roseus* L. (73.33), *Eucalyptus globulus* Labill. (72.22), *Ficus benghalensis* L. (72.22), *Datura inoxia* Mill. (70.00) and *Aloe vera* L. (68.89).

Table1: Efficacy of fresh plant extracts against Mancozeb resistant mutant isolate of *A. alternata* (*Aa*-EMS-2) *in vitro*.

Sr. no.	Scientific name	Family	Part used	Individual/ Mixture	Percentage Control Efficacy			
					25%	50%	75%	100%
1.	*Hyptis suaveolens* (L.)	Lamiaceae	Leaves	Individual	13.33	21.11	27.78	32.22
				Mixture	43.33	47.78	53.33	62.22
2.	*Ricinus communis* (L.)	Euphorbiaceae	Leaves	Individual	28.89	34.44	41.11	46.67
				Mixture	64.44	65.56	66.67	68.89
3.	*Syzygium cumini* (Lam.)	Myrtaceae	Leaves	Individual	38.33	42.78	44.44	48.89
				Mixture	46.67	49.90	56.67	68.89
4.	*Allium cepa* (L.)	Liliaceae	Bulb	Individual	18.89	20.56	23.89	25.56
				Mixture	43.33	49.91	53.33	62.22
5.	*Allium sativum* (L.)	Liliaceae	Bulb	Individual	38.89	41.11	42.78	52.78
				Mixture	56.67	66.67	85.56	89.91
6.	*Allium sativum* (L.)	Liliaceae	Leaves	Individual	31.11	37.78	47.78	52.22
				Mixture	57.78	65.56	84.44	91.11
7.	*Eclipta alba* (L.)	Asteraceae	Leaves	Individual	06.67	35.00	46.11	47.78
				Mixture	42.22	50.00	54.45	64.45
8.	*Calotropis gigantea* (L.)	Apocynaceae	Leaves	Individual	23.89	29.45	50.00	51.67
				Mixture	62.23	62.23	61.12	64.45
9.	*Lantana camara* (L.)	Verbenaceae	Leaves	Individual	29.44	31.11	36.67	57.78
				Mixture	62.78	67.22	69.90	74.45
10.	*Menthaarvenses* (Benth.)	Lamiaceae	Leaves	Individual	05.56	22.22	36.67	56.67
				Mixture	47.22	52.78	58.33	63.33
11.	*Zingiber officinale* (Rosc)	Zingiberaceae	Rhizome	Individual	31.67	46.11	55.56	61.67
				Mixture	47.11	50.56	59.44	66.11
12.	*Aloe vera* (L.)	Liliaceae	Leaves	Individual	41.11	47.78	52.22	59.98
				Mixture	49.90	52.33	61.11	68.89
13.	*Vernonia spp.* (Schreb.)	Asteraceae	Leaves	Individual	10.00	12.22	18.89	32.22
				Mixture	43.33	46.67	50.00	52.22

Table 1: Continued…

Sr. no.	Scientific name	Family	Part used	Individual / Mixture	Percentage Control Efficacy			
					25%	50%	75%	100%
14.	*Carica papaya* (L.)	Caricaceae	Leaves	Individual	12.22	13.33	13.33	30.00
				Mixture	41.12	42.22	45.56	49.90
15.	*Anethum graveolens* (L.)	Apiaceae	Leaves	Individual	07.78	15.56	17.78	28.89
				Mixture	41.11	44.44	47.11	50.00
16.	*Coriandrum sativum* (L.)	Apiaceae	Leaves	Individual	0.0	0.0	04.44	07.78
				Mixture	51.11	54.44	62.23	65.56
17.	*Murraya koenigii* (L.)	Rutaceae	Leaves	Individual	0.0	0.0	04.44	06.67
				Mixture	60.33	62.23	64.44	69.90
18.	*Ficus globosa* (Blume.)	Moraceae	Leaves	Individual	13.33	21.11	26.67	32.22
				Mixture	43.33	47.78	50.00	60.00
19.	*Ficus religiosa* (L.)	Moraceae	Leaves	Individual	18.89	13.33	11.11	08.89
				Mixture	57.78	54.44	46.67	43.33
20.	*Ficus benghalensis*(L.)	Moraceae	Leaves	Individual	18.89	24.44	34.44	41.11
				Mixture	56.67	60.00	68.89	72.22
21.	*Azadirachta indica* (A. Juss.)	Meliaceae	Leaves	Individual	11.11	15.56	21.11	28.89
				Mixture	54.44	60.00	64.44	66.67
22.	*Plumaria alba* (L.)	Apocynaceae	Leaves	Individual	13.33	15.56	24.44	28.89
				Mixture	47.78	50.00	52.22	55.56
23.	*Psidium guajava* (L.)	Myrtaceae	Leaves	Individual	15.56	18.89	21.11	22.22
				Mixture	53.33	55.56	57.78	60.00
24.	*Mangifera indica* (L.)	Anacardiacea e	Leaves	Individual	15.56	17.78	22.22	24.44
				Mixture	54.44	57.78	60.00	62.23
25.	*Jasminium grandiflorum*	Oleaceae	Leaves	Individual	14.45	17.78	20.00	25.56
				Mixture	51.11	54.44	57.78	64.44
26.	*Datura inoxia* (Mill.)	Solanaceae	Leaves	Individual	28.89	34.44	41.11	46.67
				Mixture	64.44	60.00	66.67	70.00
27.	*Mimusops elengi* (L.)	Sapotaceae	Leaves	Individual	47.78	52.22	57.78	61.11
				Mixture	75.56	77.22	80.00	86.67
28.	*Polyalthia longifolia*	Annonaceae	Leaves	Individual	31.11	34.44	40.00	43.33
				Mixture	64.44	68.89	72.22	74.44
29.	*Catharanthus roseus* (L.)	Apocynaceae	Leaves	Individual	28.89	40.00	48.89	54.44
				Mixture	60.00	64.44	71.11	73.33
30.	*Ocimum sanctum* (L.)	Lamiaceae	Leaves	Individual	06.67	08.89	13.33	33.33
				Mixture	53.33	57.78	56.67	60.00
31.	*Ocimum basilicum* (L.)	Lamiaceae	Leaves	Individual	07.78	13.33	17.78	24.44
				Mixture	46.67	44.44	46.67	48.89
32.	*Cymbopogon citratus*	Poaceae	Leaves	Individual	18.89	25.56	32.22	48.89
				Mixture	43.33	48.89	51.11	54.44
33.	*Nerium indicum* (L.)	Apocynaceae	Leaves	Individual	14.44	17.78	34.44	36.67
				Mixture	44.44	46.67	57.78	64.44
34.	*Eucalyptus globulus*(Labill.)	Myrtaceae	Leaves	Individual	32.22	41.11	51.11	53.33
				Mixture	53.33	57.78	64.44	72.22
35.	*Citrus limonum* (Risso.)	Rutaceae	Leaves	Individual	22.22	25.56	33.33	34.44
				Mixture	53.33	55.56	56.67	60.00
36.	*Hibiscus rosasinensis* (L.)	Malvaceae	Leaves	Individual	07.78	15.56	17.78	28.89
				Mixture	41.11	44.44	47.11	50.00
37.	Mancozeb 680µg/ml			Individual	41.11	41.11	41.11	41.11
				Mixture	--	--	--	--

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RESEARCH ARTICLE

Analysis of cellulase systems from some fungi

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ABSTRACT

Although a variety of fungi are capable of growth on cellulosic substrates, only a dedicated handful of them can effectively hydrolyse native cellulose through production of cellulases. In the present study, the cellulolytic capabilities of seven cellulolytic fungal organisms viz. *Aspergillus niger* van Tieghem, *Chaetomium crispatum* Fuckel, *C. globosum* Kunze (2 isolates), *C. olivaceum* Cooke and Ellis (2 isolates) and *C. mollicellum* Ames were determined in terms of activities of enzymes, Endo- and Exo-1, 4 β glucanase. *Chaetomium olivaceum* demonstrated maximum activity of Endo- as well as Exo-1,4 β glucanase, followed by *C. crispatum*, *C. globosum* (isolate # g1), *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Endo-1,4 β glucanase; and *C. globosum* (isolate # g1), *C. crispatum*, *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Exo-1,4 β glucanase respectively. Enzyme activities were compared with those demonstra- ted by *Aspergillus niger*, a commercially exploited and well known cellulolytic biodeteriogen.

Keywords: *Chaetomium*, Exo-1,4 β glucanase, Endo-1,4 β glucanase, celluloses

INTRODUCTION

Cellulose, the most abundant natural organic compound on earth, makes up an integral part of the plant cell wall, and thus, a major constituent of plant matter. It is a well acknowledged fact that several microorganisms can grow on cellulosic material, however, only a few of these constitute an elite group that can extensively hydrolyse native cellulose by production of extra-cellular enzymes viz. cellulases. Plenty of research attention has been focused on increasing our understanding on the exact mechanisms by which fungi affect cellulose; several concepts and views having been put

forward to explain the mechanism from time to time (Streamer *et al*., 1975; Chen, 2014); the phenomenon also having attracted some excellent overviews and reviews (Wood and Garcia-Campyo, 1994: Leschine, 1995).

The enzymatic hydrolysis of cellulose is known to involve synergistic action of three different groups of enzymes viz., endoglucanases (endo 1,4-β-glucanases), exoglucanases (exo 1,4-β-glucanases) and β- glycosidases. Endoglucanase is acknowledged to first act randomly on amorphous cellulose, causing reduction in degree of polymerization, forming cellobiose and glucose; exoglucanase is known to hydrolyse crystalline cellulose, starting from the ends of the chains, whereas cellobiase finally acts by separating the β- 1,4 glycosidic bond of cellobiose and small oligosaccharide molecules accompanied by formation of monomeric sugers (Bhat, 2000).

Recent research has been devoted to understanding the role of these enzymes in saccharification, identification and addition of new, superior fungal organisms in the restricted group of cellulolytic organisms (Jung *et al*., 2015), finding alternative and innovative techniques for higher yields of cellulases (da Silva *et al*., 2014; Hansen *et al*., 2015), experimenting with novel substrates (Singh, *et al*., 2015); and the innovative trend continues. Cellulases have diversified applications and many environment-friendly uses. Recent years have witnessed steps towards enhancement of their efficiency and cost efficacy (Dubey *et al*., 2014). A survey of literature on the subject revealed extensive but scattered and piecemeal data. In the present investigation, cellulose degrading abilities of 7 cellulolytic test organisms were determined in terms of activities of enzymes, Exo 1,4-β-glucanase (C_1 cellulase) and Endo 1,4-β-glucanase (Cx cellulase).

MATERIALS AND METHODS

Seven fungal isolates viz. *Chaetomium crispatum* Fuckel, *C. globosum* Kunze (2 isolates), *C. olivaceum* Cooke and Ellis (2 isolates), *C. mollicellum* Ames and *Aspergillus niger* van Tieghem, obtained from various cellulosic sources (Kolet, 2009; 2011) and characterized using standard literature (Gilman, 1967; Arx *et al*., 1986; Tzean *et al*., 1990) were used for the current study. Cellulase enzyme was obtained from the isolated fungi by shake flask fermentation. Reese liquid medium (Mandels and Weber, 1969) was utilized to determine the amount of production of cellulases by the fungal isolates and methodology as suggested by Bagool (1982) was adopted for the enzyme assay. Soluble proteins were determined as described by Lowry *et al*. (1951). The enzyme activities were determined by estimating reducing sugars, using DNSA reagent (Mandels *et al*.,1976). Activity of Endo-1,4 β glucanase was monitored as expressed in terms of reducing sugars released/mg protein/30 minutes; while that of exo-1,4 β glucanase was expressed as reducing sugars released /mg protein/24 hours. Enzyme activities were compared with that of *Aspergillus niger*, a commercially exploited and well known cellulolytic biodeteriogen.

RESULTS AND DISCUSSION

Analysis of enzyme activities revealed that *Chaetomium olivaceum* (isolate #o1) showed maximum activity of Endo-1,4 β glucanase, articulated in terms of reducing sugars released /mg protein/30 minutes, followed by *C. crispatum*, *C. globosum* (isolate #g1), *C. olivaceum* (isolate #o2), *C. mollicellum*, *C. globosum* (isolate #g2) and *Aspergillus niger*. The maximum activity with respect to Exo-1,4 β glucanase, expressed in terms of reducing sugars released /mg protein/24 hours, was demonstrated by *Chaetomium olivaceum* (isolate #o1), followed by *C. globosum* (isolate #g1), *C. crispatum*, *C. olivaceum* (isolate #o2), *C. mollicellum*, *C. globosum* (isolate #g2) and *Aspergillus niger*. The results, depicted in Fig. 1, are in agreement with those of El-Said *et al*. (2014). Lee (2015) hinted at C_1 cellulase as the entity determining the ultimate

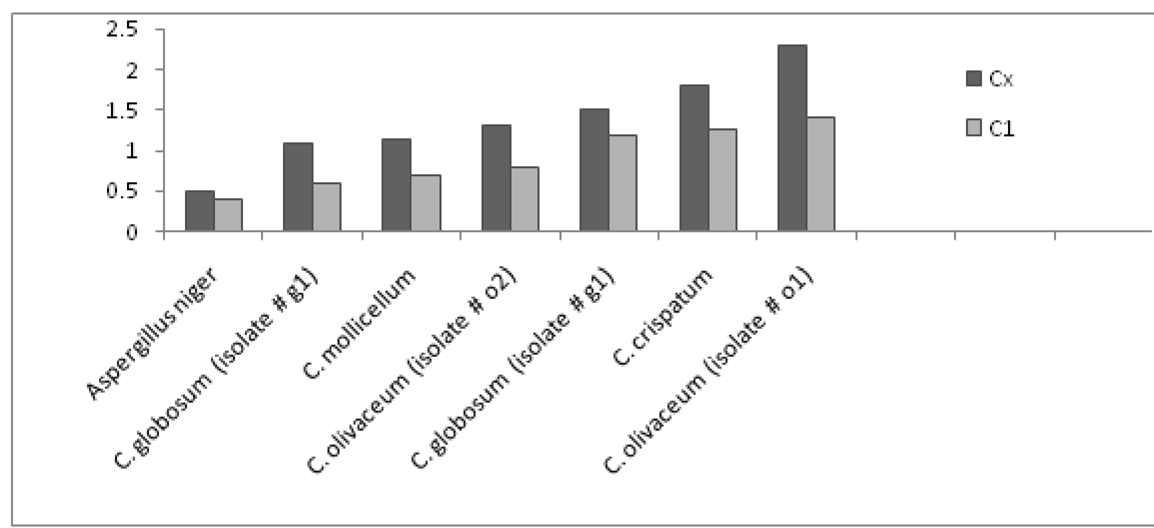

Fig. 1. C_1 and C_x enzyme activities (mg reducing sugars released /mg protein/ 24 hrs and mg reducing sugars released /mg protein/ 30 min) of *Aspergillus niger* and *Chaetomium* sps.

rate of hydrolysis. Earlier, the ratio of activities of enzymes C_1 and Cx cellulases was shown to indicate stability of the enzyme systems in respective organisms (Mandels and Weber, 1969), a key characteristic for their commercial exploitation. In accordance with this criterion, the maximum stability of cellulase complex, in the current investigation, was observed in *Aspergillus niger*. The ratios of activities of the enzymes from isolates studied in the current investigation are indicative of stable enzyme systems which could also be commercially utilized.

CONCLUSION

Seven cellulolytic fungal isolates were utilized for determining activity of cellulases. *Chaetomium olivaceum* (isolate # o1) demonstrated maximum activity of Endo-1,4 β glucanase as well as Exo-1,4 β glucanase, followed by *C. crispatum*, *C. globosum* (isolate # g1), *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Endo-1,4 β glucanase; and *C. globosum* (isolate # g1), *C. crispatum*, *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Exo-1,4 β glucanase respectively. The cellulase enzyme complex of *Aspergillus niger* was observed to demonstrate maximum stability.

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RESEARCH ARTICLE

Protective effect of *Butea monosperma* leaves extract against Aaflatoxin induced haemolysis

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ABSTRACT

The present investigation is an attempt to evaluate the possible ameliorative effect of *Butea monosperma* leaves extracts on aflatoxin induced haemolysis. Blood sample were collected from healthy adult human being (25-30 years old) in EDTA vials and were used for preparation of RBC suspension in saline. Saline suspension of RBC was treated with aflatoxin (0.5-2.0 µg/mL) with and without *Butea monosperma* leaves extracts (1-100 µg/mL). The results revealed that addition of aflatoxin (0.5-2.0 µg/mL) to RBC suspension caused significant dose-dependent increase in the rate of haemolysis. However, concurrent addition of aflatoxin (2.0µg/mL) and extracts of *Butea monosperma* leaves caused concentration dependent retardation in aflatoxin induced hemolysis.

Keywords: aflatoxin, , *Butea monosperma*, retardation, haemolysis

INTRODUCTION

Aflatoxins are secondary toxic metabolites produced by molds of *Aspergillus flavus* and *Aspergillus parasiticus* which are found to be growing on grains, groundnuts and other food stuffs. Occurrence of aflatoxin in various food commodities have been widely reported from various countries, being most prevalent in tropical and subtropical countries where environmental conditions are more favorable for moldy growth and toxin production. (Shank *et al*.,1972; Stoloff, 1977; Busby and Wogan, 1984). Aflatoxins have been implicated in acute hepatitis, hepatocarcinogenesis and mutagenesis. (Busby and Wogan, 1984; Groopman *et al*., 1988; Verma and Raval, 1992). The decreased RBC count during induced chronic aflatoxicosis in rabbits (Verma and Raval, 1992). Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin treated animals.(El-Gibaly *et al*., 2003).

Butea monosperma is a medium sized deciduous tree which belongs to family Fabaceae. It is a commonly used Ayurvedic plant with important medicinal values and is widely used by tribal and rural people in different parts of India to cure many disorders. The presence of triterpenes, flavonoids, butein, butin, stigmasterol, ί-carotene, ί-sitosterol, myristic, palmitic, stearic, oleic and linolenic acids. Sindhia and Bairwa (2010). The plant provides vast pharmacological potential. It has been reported to have anticonvulsive, antidiabetic, (Ahmed *et al*., 2012) anti-inflammatory, hepatoprotective, antihelmintic, antioxidant, anti-stress, antimicrobial activities (Kumar and Samant, 2012). Moreover, the leaves of *Butea. Monosperma* possess antioxidant and anticancer activity which is a prerequisite for anticlastogenic activity. The present investigation was an attempt to evaluate ameliorative effects of aqueous, alcoholic and flavonoids extracts on aflatoxin induced haemolysis.

MATERIAL AND MATERIALS

Aflatoxin was produced by growing *Aspergillus sps* on medium (sucrose, 20 g; magnesium sulfate, 0.5 g; potassium nitrate, 3 g; yeast extract, 7 g; and distilled water, 1000 mL) for 10 days at 28 ± 2^{0}C as described by(Diener and Davis,1966). Obtained culture filtrates were extracted with chloroform. *Butea monosperma* leaves were collected from Bhavan college campus and was used to make aqueous, alcoholic and flavonoid extracts. The flavonoid extract was preparedas described by Subramanian and Nagarajan (1969). The extract was subjected to qualitative chemical tests to determine the nature of the phytoconstituents. (Horbone, 1998).Blood samples were collected into EDTA vials intravenously from healthy humans (25-30 years age group) having normal RBC counts. After dilution with saline, the samples were centrifuged at 1000rpm for 10 min. Supernatant was discarded and the RBC pellet was further washed twice with saline by centrifugation. Final RBC suspension was prepared in saline. For examining the haemolysis due to aflatoxin on RBC and its amelioration by antioxidants.

First sets of test tubes were prepared to check toxic level of aflatoxin.

Second sets of the tubes were prepared as follows:

1. Control tubes containing 2.0 mL of RBC suspension.

2. Antioxidants control tubes containing 100μg/mL *Butea monosperma* leaves extracts added to 2.0 mLof RBC suspension.

3. Treated tubes containing different concentrations(0.5 μg/mL to 2 μg/mL) of aflatoxin added to 2.0 mL of RBC suspension.

4. Tubes containing different concentrations of alcoholic/aqueous/flavonoid extracts (1μg/mL to 100 μg/mL)of *Butea monosperma* leaves added to RBC suspension treated with 2 μg/mL of aflatoxin. Aflatoxin solutions and extracts of *Butea monosperma* leaves were prepared in normal saline (0.9 % NaCl). Total volume of each tube was made up to4.0 mL by adding saline. All the tubes were incubated at 37^{0}C for 4 h. Morphological alterations in RBC were observed after staining with Leishmanís Stain. Tubes were centrifuged at 1000 rpm for 10min and color density of supernatant was measured spectrophotometrically at 540 nm.

$$\text{Percent haemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100 \% haemolysis}} \text{X100}$$

Percent retardation with different concentration of antioxidants was calculated with the following formula (Raval and Verma,1993):

$$\text{Percent Retardation} = \frac{A - B}{A} \text{ X100}$$

Where,

A = aflatoxin-induced haemolysis;

B = haemolysis caused by concurrent addition of aflatoxin and antioxidant.Studentís t-test was used for statistical analysisof the data.

RESULTS AND DISCUSSION

The phytochemical screening of extracts revealed the presence of bioactive constituents like alkaloid, carbohydrate, phytosterols, flavonoids, proteins and diterpenes. In case of control, it was observed that normal RBC appears as flattened indented spheres or biconcave discs. The cell pellets remained settled in the bottom of the tube and the ambient supernatant remained clear. The addition of 2 µg/mL of aflatoxin to a RBC suspension caused a significant rise in haemolysis and swelling of the cells. The cell pellets in the bottom of the tubes reduced with reddish colored supernatant indicating haemolysis due to bursting of the cells due to excess swelling. It could be due to the direct action of aflatoxin on the plasma membrane causing lipid peroxidation, membrane permeability alterations and cell lysis (Verma and Nair, 1999). The concurrent addition of aqueous and alcoholic extracts of *Butea monosperma* leaves and flavonoid extracts (1 µg/mL to 100 µg/mL) to the RBC suspension significantly reduced aflatoxin induced haemolysis. An almost concentration-dependent effect was observed. flavonoid extracts was found to be most effective, followed by alcoholic extract; aqueous extract was comparatively less effective. The mechanism of action of *Butea monosperma* leaves aqueous, alcoholic and flavonoid extracts on aflatoxin induced hemolysisis could be due to antioxidative property of *Butea monosperma* leaves and other compounds in case of aqueous extracts ansd alcoholic extracts.

Table1: Aflatoxin induced haemolysis

Aflatoxin µg/mL	Haemolysis
0	0.08
0.5	3.02
1	10.01
1.5	16.01
2	23.12

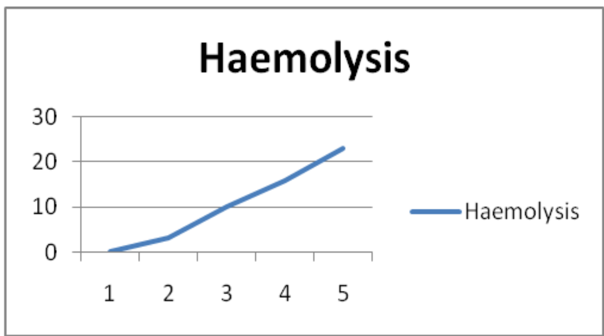

Fig.1: Aflatoxin induced haemolysis

Table 2: Retardation of aflatoxin-induced hemolysis by aqueous and alcoholic extracts and flavonoid extract of *Butea monosperma*(µg/ml)

Aflatoxin (µg/mL)	Aqueous extract/ Alcoholic extract/ flavonoid extract of *Butea monosperma* (µg/mL)	Hemolysis (%)		
		Aqueous extract) (µg/mL)	Alcoholic extract/ (µg/mL)	Flavonoid extract (µg/mL)
0	0	0.07±0.005	0.07±0.005	0.07±0.005
0	100	2.31±0.01	1.22±0.01	0.62±0.05
0	0	23.12±0.009	23.12±0.009	23.12±0.009
2	1	21.03±0.005	20.03±0.005	18.01±0.005
2	2)	20.06±0.005	18.03±0.005	16.01±0.005
2	3	18.02±0.009	16.01±0.009	14.01±0.009
2	4	16.01±0.009	14.02±0.005	10.02±0.005
2)	5)	15.03±0.009	12.01±0.005	8.01±0.005
2	10	13.01±0.005	10.02±0.005	6.03±0.005
2)	25)	11.05±0.01	9.01±0.01	4.0±0.009
2	50)	7.06±0.009	6.08±.0.01	3.23±0.01
2)	75	5.23±0.01	4.04±0.01	2.45±0.005
2	100	4.59±0.01	2.05±0.01	0.55±0..1

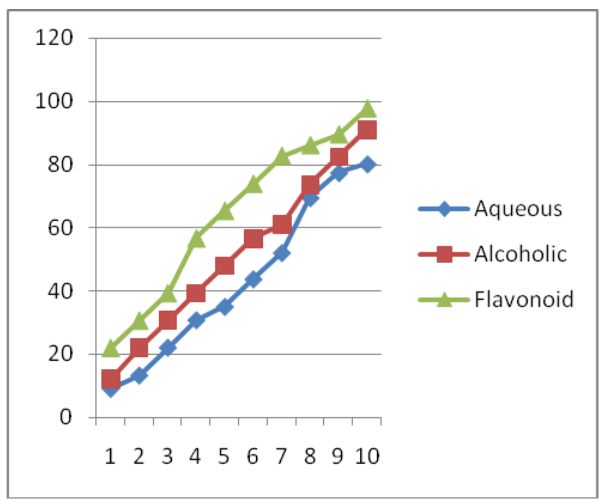

Fig.2: Retardation of aflatoxin

It should be noted that other plant products such as flavonoids, lignans, citric acid, lactic acid etc., reduce aflatoxin toxicity by modifying the bioactivation process of aflatoxin, in which microsomal mixed function oxidase plays a major role (Souza *et al*., 1999). So it can be said that these micronutrients can the restriction aflatoxin production, they can also reduce cytotoxicity in the body.

CONCLUSION

It can be concluded that as compared to other extracts of *Butea monosperma* leaves the flavonoid extract is the most active compound and plays an important role in ameliorating the aflatoxin-induced haemolysis.

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RESEARCH ARTICLE

Dictyostelium discoideum, strain AX2, a novel model system for studying Autophagy

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ABSTRACT

Dictyostelium is an eukaryote, a protist, and a slime mold. *Dictyostelium* shows developmental vacuolated cell death (autophagy) in the stalk. Macroautophagy is an intracellular degradative process for cytosolic components at the lysosome. Derailing of this mechanism is implicated in several neurodegenerative diseases, as it leads to accumulation of mutant aggregate proteins. *Dictyostelium* lacks the required machinery for apoptosis, and undergoes cell death only by autophagy or necrosis. Starvation in *Dictyostelium* sets in the mode of multicellular development in which stalk cells enlarge and altruistically die by autophagy which can be mimicked i*n vitro*. In the present work, Lithium and Valproic acid were used to observe autophagy in the AX2 strain. Monolayer cultures of AX2 strain of *Dictyostelium* was standardized to observe the percentage of vacuolated autophagic cells using microscopic and staining techniques. Our studies established that AX2 strain can be a new model system to study autophagic cell death in neurodegeneration and can be used as a pharmacological model for putative drugs as well.

Keywords: Macroautophagy, AX2 strain, Monolayer cultures, *Dictyostelium*

INTRODUCTION

Macroautophagy is an intracellular lysosomal degradative pathway. It is implicated in neurodegenerative states as a major mechanism in the clearance of mutant aggregate proteins and as a mechanism of neuronal cell death (Hochfeld *et al*., 2013). So far apoptosis and necrosis have been given much attention but macroautophagy has not been well probed for its role in cell death and neuronal damage. In mammalian systems, the study of its mechanism, is often complicated by interfering apoptotic machinery.

A unique model to study autophagic cell death is *Dictyostelium discoideum*. A simple eukaryote, which goes through two stages during

its lifetime; a unicellular amoeboid stage and a multicellular fruiting body, consisting of stalk cells and spore cells. The formation of stalk cells is in part achieved by macroautophagy (henceforth referred to as autophagy) (Otto *et al*., 2003). The signals required for autophagic cell death are starvation, cAMP (cyclic AMP) and DIF1 (differentiation inducing factor 1). The major advantage of the system is that it lacks apoptotic machinery, thus making the system ideal for the study of autophagic cell death (Giusti *et al*., 2008).

The present study has used this simple model system to study autophagy and the effects of the neuroprotective agents Lithium and Valproic acid on it. The stalk length was measured to infer the changes in autophagic process brought about by these agents. The formation of autophagic stalk cells is also studied by inducing vacuolisation invitro in monolayers. By observing the changes in cell morphology as compared to healthy controls and induced autophagic cells, the effect of Lithium and Valproic acid on autophagy may be inferred.

MATERIALS AND METHODS:

Maintenance

D.discoideum AX2 vegetative cells, courtesy [Dr Malik's Lab, TIFR (Mumbai)] were grown in flask, suspended in liquid HL5 media (Ashworth and Watts,1970) Media and Buffers; HL5 media, KK2 buffer, Sorensons buffer (SB). Reagents; 3 mM cAMP (cyclic AMP; 3',5'adenosine cyclic monophosphate, sodium salt, Sigma A6885), 100 nM DIF-1 {DIF-1: differentiation-inducing-factor 1,1-(3,5-dichloro-2, 6-dihydroxy- 4 – methoxy-phenyl) - hexan-1-one; DN1000, Affiniti Research Products, Exeter, UK } , LiCl2 (Lithium chloride), VPA (Valproic acid).

In vivo Stalk length measurement:

For stalk length measurements *D.discoideum* AX2 spores were collected from fruiting bodies growing axenically on SM agar (Sussman and Sussman, 1967). Approximately 15-20 spores were suspended in KK2 buffer supplemented with 2mM, 5mM and 10mM LiCl2 and were placed on SM agar plates. Controls were maintained by suspending the spores in KK2 buffer. Plates were incubated at 22°C in BOD incubator for 5 days. Sets were run in duplicates, imaged and the stalk length measured by Image J software [40X Axioscope].

Stalk cell differentiation in vitro:

For inducing stalk cell differentiation in monolayers (Fey *et al*., 2007) 30µl overnight culture of *D. Discoideum*-AX2 (Axenic) cells in liquid HL5 media (cell count 3x105/ml) were placed on the coverslip and allowed to adhere for 24 hours. Media was removed and the cells were washed with Sorensons Buffer (SB buffer), chemical treatments were then added to the cells for 24hours: SB buffer, 3mM cAMP, 10mM lithium chloride, 10mM VPA. Cells were then washed in SB buffer, fixed in 4% pfa for 20mins, mounted in glycerol and observed using phase contrast and confocal imaging. The percentage of vacuolated cells was calculated.

RESULTS AND DISCUSSION

At organismal level: (i*n vivo* conditions):

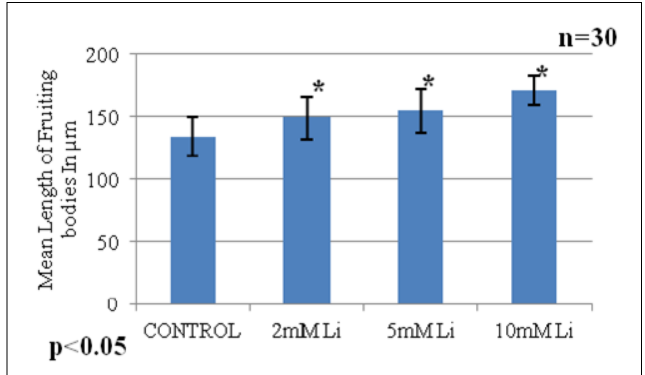
Our results suggest that lithium and VPA enhances the stalk length of the fruiting bodies (Fig.1& 2). *Dictyostelium* stalk cells are dead by autophagy, adding lithium and VPA caused an increase in length of the stalk, which could mean the number of cells being differentiated into stalk cells increases. These increased proportions of stalk cells indicate more number of cells undergoing autophagic cell death on treatment with lithium.

Lithium's inhibition on the spore cell differentiation is mimicked by a mutation in the gene gskA which encodes a homologue of the signaling molecule glycogen synthase kinase 3 (GSK-3). These molecules are conserved signaling molecules mediated in various GSK-3 pathways. The neuroprotective effects of lithium are

mediated, at least in part, by the inhibition of GSK-3β activity in neurons (Diniz *et al*., 2013). Hence studying the pathways mediated by GSK-3 in a simple model might help in elucidating the possible targets of lithium on these pathways.

Autophagy was successfully induced *in vitro*:
There was increase in autophagic activity in cells treated with cAMP as compared to the control (Fig.3). Despite the fact that cAMP is a natural chemoattractant and facilitates attraction of the cells during starvation, intracellular cAMP has been shown to induce autophagy via recruiting components of the cell cycle. In order to become autophagic, *Dictyostelium* cells require two stimuli a combination of starvation and cAMP. Cells treated with cAMP were compared with unstarved cells and cells which were only starved (not treated with cAMP). The former showed a greater percentage of cells with autophagic vacuoles. Starvation was used as an initial stimulus to successfully induce autophagy induction in the monolayer of *Dictyostelium discoideum* AX2 cells. Thus the conditions present in vivo due to starvation secretion of cAMP were successfully mimicked in vitro inducing autophagy in the monolayer culture.

Lithium induces macroautophagy in *D. Discoideum*.
Dictyostelium cells treated with Lithium showed vacuolation comparable to that of the cAMP treated positive control. On treatment with 5mM Lithium, AX2 cells showed an increase in the number of cells showing vacuoles comparable to cyclic AMP treated cells. Furthermore, cAMP and lithium when added together, greatly enhanced vacuolation showing an additive effect (Fig. 3). Lithium acts by competing with the cofactor Mg 2+, thus inhibiting intracellular enzymes. It induces autophagy by inhibiting the enzyme inositol monophosphatase (Sarkar *et al*., 2005). Lithium has been shown to induce autophagy by inhibiting the enzyme inositol monophosphatase. This inhibition causes a depletion of intracellular inositol triphosphate (IP3) which has been shown to induce autophagy and protein clearance (Sarkar *et al*. 2005). By inducing the formation of autophagic vacuoles in *Dictyostelium* we have shown that even in a simple system Lithium is a potent inducer of autophagy. In order to check whether Lithium had the ability to enhance autophagy even in the presence of cAMP, 5mM Lithium was added along with cAMP. Results indicate a large increase in the number of cells vacuolated showing that Lithium enhances autophagy in a monolayer of *Dictyostelium* cells. In *Dictyostelium* , DIF1 induces autophagic cell death and this activity has been found to be mediated by the IP3R, i.e. the receptor and calcium fluxes are also important for cell death signalling (Ganley *et al*.,2009). Thus inhibition of the IP3R by lithium could prevent autophagic cell death.



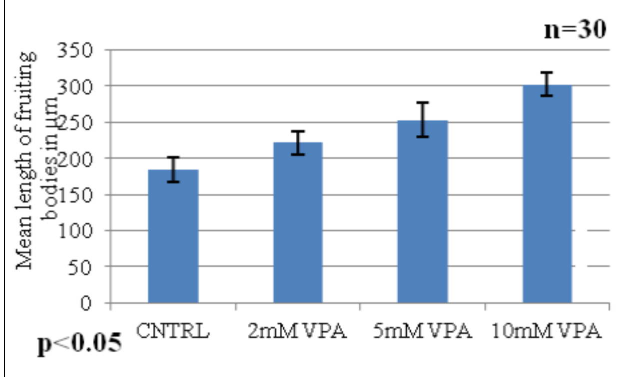


Fig.1&2: Figures 5 and 6 show quantitative data of the increase in stalk length with both Lithium and Valproic acid. Fig.1with Lithium, Fig.2 with Valproic acid

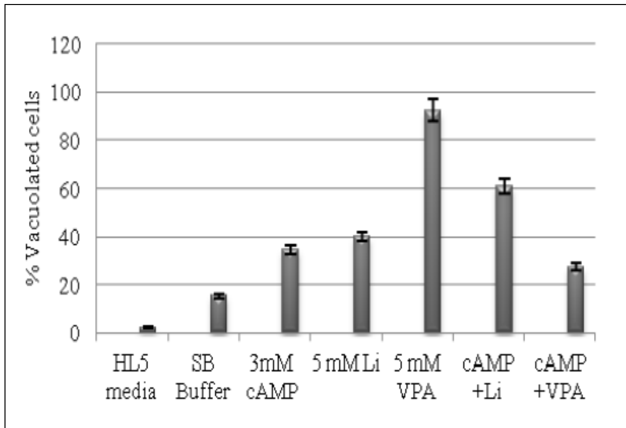


Fig. 3: Graph of percentage vacuolated cells. Lithium and cAMP show comparable results, Li and cAMP have a synergistic effect. **indicates $p<0.005$

Although poles apart both mechanisms involving the IP3R point to an important role in autophagy and autophagic cell death, in which intervention with lithium could lead to cell protection. Thus the timing of intervention with lithium could be an interesting way to either enhance autophagy as well as a method to prevent cell death. Further, lithium's protection in mammalian cells which do not use DIF would be interesting to investigate.

Valproic acid enhances autophagy:

On treating the cells with 5mM VPA a significant increase in autophagic vacuoles was observed as compared to the control (Fig.3). However when added together, vacuolization was shown to be decreased. Theoretically this can be postulated to be caused by a decrease in the intracellular levels of IP3 and an upregulation in the Erk 1/2 pathway due to increase in ROS (Cuervo and Dice, 2000). The Erk 1 (extracellular-signal-regulated kinases) pathways has been shown to decrease IP3 levels via the disheveled protein in the non-canonical Wnt-1 pathway (Ding, & Shen, 2008). This will further lead to an increase in autophagy. The increase in IP3 levels could lead to an increase in Ca2+ levels in the cell which will lead to an upregulation in autophagy. cAMP has also been shown to increase intracellular cAMP levels. Thus if the upregulation of Erk 1/2 leading to a decrease in IP3 levels is countered with an increase in IP3 levels due to cAMP expression then at a particular concentration range the net change in IP3 levels is zero/negligible. This would lead to no change in autophagy induction in the cells. Thus autophagy may occur at higher (as seen in our *in vivo* results) and lower concentrations of VPA but will not lead to a significant increase in autophagy within a certain concentration range. However, this needs to be confirmed by a systematic study of the cellular and molecular effects of valproic acid in *D. discoideum*.

CONCLUSION

In most neurodegenerative diseases where autophagy becomes dysfunctional, upregulating the process of autophagy to degrade the aberrant proteins would be a potential therapeutic. However, the complex roles of autophagy in survival and death should be considered when designing pharmacological therapeutics for disease. Here we have used lithium and valproic acid to test its effect on the process of autophagy in a simple model. The molecular markers involved in autophagy and autophagic cell death would help us understand the switch from autophagy for survival to a mechanism of death. Hence *Dictyostelium* may prove to be a beneficial model to test the effect of neuroprotective drugs in enhancing autophagy and protecting the cell from autophagic cell death and could serve as primary screen to study the effect of pharmacological inducers on autophagy and autophagic cell death.

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RESEARCH ARTICLE

Evaluation of benzimidazole fungicides on *Penicillium expansum* causing blue mold of apples

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ABSTRACT

The present study assayed the effect of the certain fungicides viz. dithane Z-78, polyram, acrobat, kocide, dithiocarbmate, thiobendazole and diphenylamine on blue mold of apple caused by *Penicillium expansum* Link. Twenty isolates of *P. expansum* were isolated from infected apples collected from various fruit markets of Maharashtra. All these isolates were tested their sensitivity against carbendazim on PDAmedium.MIC of carbendazim was determined against isolates.*Pe*-9 MIC-750.6µg/ml was sensitive while isolate *Pe*-15 MIC-970.3µg/ml was tolerant. Pe-9(MIC-1900.2µg/ml) was sensitive and Pe-15 MIC-2470.3µg/ml was tolerant i*n vivo*. Carbendazim resistant mutant (Pe-EMS-10, MIC-4850.6µg/ml) is equally important to control using different fungicides. *P.expansum* was tested against carbendazim and other fungicides. *In vitro* the fruitful results were observed that the (PCE) values at 50 %Conc. (44.02 - 49.28) while at 100 %Conc. (50.89-61.39) individually and in mixture with Carbendazim the PCE value increased at 50 % Conc.(55.39 - 68.98) while at 100% (68.72 -73.57). PCE value at 50% Conc. (51.62 - 56.95) individual and mixture (57.25 - 59.26) while at 100 from alone (58.32 - 69.36) and in mixture (71.63-79.82) PCE value increased as compared with individual value(*In vivo*). Without fungicide served as control.

Key word: *Penicillium expansum*, Blue mold, Apple and Carbendazim fungicide.

INTRODUCTION

Blue mold of apple (*Pyrus malus* L.) caused by *Penicillium expansum* Link. Postharvest apples are stored in order to provide market with quality of fruits. Fungal diseases occur regularly during storage and can cause severe yield and economic losses. Postharvest losses on

apples were up to 80 - 90% (Anderson, 1956). In 2004 report was published suggesting that deterioration during storage caused 5 to 25% losses of total yield (Jijakli and Lepoivre, 2004). The major postharvest pathogens of apple according to literature are *Penicillium expansum, Botrytis cinerea*, *Monilinia fructigena* (Snowdon 1990; Konstantinou *et al.,* 2011). Fungal pathogens were isolated from rotten apple viz. *Venturia inaequalis*, *Colletotrichum acutatum*, *Mucor piriformis*, *Rhizopus arrhizus*, *Alternaria alternata*, *Aspergillus flavus* and *A. fumigatus* (Baviskar and Suryawanshi 2015). Therefore its management is equally important to blue mold of apple caused by *Penicillium expansum.* Very few reports have been available on blue mold control using fungicides. Therefore, post harvest diseases of apple strongly recommended Carbendazim and thiobendazole. (Wicks, 1977; Staub and Sozzi, 1984; Dahiwale *et al.*, 2009; Dahiwale and Suryawanshi, 2010). However, there are few reports suggested the emergence of carbendazim resistance in this pathogen (Nene and Thapliyal, 1992; Gangawane 1981, Abdelfettah *et al.,* 2007; Dahiwale and Suryawanshi, 2011, Suryawanshi 2015).

MATERIALS AND METHODS

During 2013-2014 survey of infested apples collected from various markets in Maharashtra. Seven fungicides were selected for this investigation. Twenty isolates of *P.expansum* isolated from infested apples were tested against carbendazim on agar plates (*in vitro*) and *in vivo.* Fungicides viz. dithane Z-78, polyram, acrobat, kocide, dihtiocarbamate, thiobendazol, diphenylamine and carbendazim were mixed with 2X PDA medium with equal quantity of fungicide pour in the petriplates by food poisoning method. The carbendazim conc. was also adjusted along with other fungicides conc. at 50 and 100μg/ml. Individual and combine effect of fungicides was observed. After solidify the medium, plates were inoculated with resistant mutant of *Penicillium expansum* (*Pe*-EMS-10) at the center and incubated at 27±2°C. After 8 days growth was measured and Percentage Control Efficacy (PCE) was determine. *In vivo* studies apple were dipped in various fungicides at 50 and 100μg/ml. The fruits were inoculated with resistant mutant and wrapped with tissue paper and incubated for 15 days at 27±2°C temperature. PCE was calculated (Cohen, 1989).

RESULTS AND DISCUSSION

Seven fungicides viz. dithane Z-78, polyram, acrobat, kocide, dihtiocarbamate, thiobendazole and diphenylamine were used individually and in mixture with carbendazim. The Conc. was used at 50μg/ml and 100μg/ml in PDA medium and PCE was determined. *In vitro* results are indicated in (Table 1), that the individually all the fungicides showed less PCE against *P. expansum*. The PCE was higher i.e. dithane Z-78(61.39) and kocide (57.33) when compared with carbendazim at 100μg/ml, diphenylamine was least effective. When the fungicides were used in mixture with carbendazim, PCE was highly increased with all the fungicides. It went up to 55.39-68.98at 50μg/ml Conc., use of acrobat with carbendazim PCE appeared to be more fruitful than other fungicides and at the Conc. of 100μg/ml PCE was more than that of lower Conc. Thiobendazole was more fruitful PCE (73.57) followed by dithiocarbamate, dithane Z-78, acrobat and kocide in decreasing manner.

The *in vivo* results are reveled in (Table 2) that treatment of dithiocarbamate gave lowest PCE (51.62) at 50μg/ml. whereas higher Conc. dithane Z- 78 PCE was 69.36 and followed by acrobat, thiobendazole, dihtiocarbamate and polyram PCE range (63.42-67.49). A mixture of carbendazim with thiobendazole gave higher PCE (59.26) at 50μg/ml., whereas higher Conc. acrobat gave very fruitful PCE at 100μg/ml (79.82) followed by thiobendazole, dithane Z-78, diphenylamine, polyram, kocide and dihtiocarbamate PCE range (77.50-71.63) in decreasing order. Similar results were observed by(Forster and Staub, 1996) at

600mg/L of boscalid and 60mg/L of cyprodinil in combination with 40mg/L fludioxonol against *B. cinerea* on table grapes and other fruit crops (Blacharski *et al.,* 2001; Latorre *et al.,* 2001; Wedge *et al.,* 2007). Cyprodinil plus fludioxonol effectively controlled *P. expansum*, *R. stolonifer*, and *A. niger* and also the results compared with earlier studies have reported that tolerance of benzimidazole fungicides and calcium chloride on *Alternaria alternata* and *Penicillium expansum* rot during storage of pears (Wicks, 1977; Abdelfettah *et al.,* 2007).

Table 1: Percentage Control Efficacy (PCE) of Carbendazim individually and in mixture with other fungicide against resistant isolate of *Penicillium expansum* on agar plate

Sr. No.	Fungicide(µg/ml)	PCE individual	PCE mixture with Carbendazim
1.	Dithane Z-78		
	50	47.39	55.39
	100	61.39	72.85
2.	Polyram		
	50	48.12	63.67
	100	50.89	68.72
3.	Acrobat		
	50	48.47	68.98
	100	53.89	72.62
4.	Kocide		
	50	46.14	61.88
	100	57.33	70.71
5.	Dithiocarbamate		
	50	49.28	55.40
	100	56.15	72.98
6.	Thiobhandazole		
	50	45.98	59.27
	100	54.28	73.57
7.	Diphenylamine		
	50	44.02	56.19
	100	53.29	71.81
8.	Carbendazim	56.84	---
	(µg/ml)		
	SE	3.276	5.333
	CD (P= 0.05)	6.784	11.51
	(P= 0.01)	7.993	15.99

Table 2: Percentage Control Efficacy (PCE) of Carbendazim individually and in mixture with other fungicide against resistant isolate of *Penicillium expansum* on apple.

Sr. No.	Fungicide(µg/ml)	PCE individual	PCE mixture with Carbendazim
1.	Dithane Z-78		
	50	53.37	59.12
	100	69.36	75.32
2.	Polyram		
	50	51.62	59.04
	100	63.42	73.44
3.	Acrobat		
	50	56.95	58.27
	100	67.49	79.82
4.	Kocide		
	50	54.95	58.48
	100	58.32	73.00
5.	Dithiocarbamate		
	50	51.62	57.25
	100	64.21	71.63
6.	Thiobhandazole		
	50	54.78	59.26
	100	65.26	77.50
7.	Diphenylamine		
	50	52.52	56.09
	100	63.92	74.21
8.	Carbendazim	72.82	---
	(µg/ml)		
	SE	1.875	2.449
	CD (P= 0.05)	3.883	5.089
	(P= 0.01)	4.575	6.009

CONCLUSION

Use of chemicals is an important tools in management of blue mold. Fungicides continue to be most effective means of controlling blue mold of apple caused by *P. expansum.* Easily available and convenient to use the fungicide. For effective management we need to educate one and all familiaring with various brands and formulation regarding their proper and rational use to achieve effective control of apple diseases. To minimize the use of fungicides and avoid hazards to man and environment. The most people have shown great reliance on one or more fungicides which often has lead to many problems.Lastly we conclude with this fungicides (dithane Z-78, polyram, acrobat, kocide, dihtiocarbamate, thiobendazol, diphenylamine and carbendazim)

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RESEARCH ARTICLE

Potential of biocontrol agents against basal rot of onion caused by *Fusarium oxysporumf. sp. Cepae*

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ABSTRACT

Onion (*Allium cepa* L.) is an important vegetable crop in India and as well in Maharashtra. There are various pathogens attacking on onion and basal rot caused by *Fusarium oxysporum f. sp. cepae.* Schlecht. Emend. Snyder & Hansen is dominant in Nasik district. The disease management is equally important using biocontrol agents to increase productivity of this crop. Therefore, evaluated the ability of biocontrol agents viz. *Aspergillus niger, A. flavus, Trichoderma viride, T. harzianum, T. koningii*, *Curvularia lunata, Penicillium expansum, Alternaria alternata, Xanthomonos axonopodis, Bacillus subtilis,* and *Pseudomonas fluorescens* against *Fusarium oxysporum*. All these biocontrol agents observed significantly reduction in the growth of the pathogens. *Trichoderma viride* was more fruitful PCE value (78.88) against *Fusarium oxysporum* followed by *A. niger*, *T. harzianum*, *A. flavus*, *T. koningii*, *Curvularia lunata*, *Alternaria alternata*, *Penicillium expansum*, *Xanthomonas axonopodis*, *Bacillus subtilis* and *Pseudomonas fluorescens* (12.85) by dual culture technique *in vitro.* In the nature microbial interactions involve competition, hyper parasitism and these phenomena play vital role in striking ecological balance and keeping several plant pathogens in check. In recent times biological control of plant pathogenic fungi has received a considerable attention, as it has several advantages such as possibility of multiple pathogen suppression, low cost and promotion of soil fertility.

Key words: Basal rot, onion, biocontrol agents, *Fusarium oxysporum*.

INTRODUCTION

Onion (*Allium cepa*L.) is an important vegetable crop grown in almost all part of country. It is popularly due to condiment and spices value and diversified use soups, meat dishes, salads and nutritional significance as a source of and is also vital role in traditional medicine

as a diuretic for the treatment of chicken pox, common cold measles and rheumatism (Schwartz and Mohan, 1995). The antimicrobial characters of the onions are likely the result of the effect of sulphur compounds. (Schwartz and Mohan, 1995). It is affected by several diseases, reflecting negatively on plant growth and the produced yield.Among, pathogenic fungi especially, the basal rot of onion caused by *Fusarium oxysporum f. sp. cepae* remain to be challenging task in terms of management. *F.oxysporum f.sp.cepae* is a highly destructive pathogen that causes basal rot disease in onion resulting in significant yield losses of the crop growing areas of the world (Ozer and Koycu, 2004). The pathogen infects the basal stem plate of the onion bulb and degrades it, ultimately kills the whole plant (Crammer, 2000). The main sources of inoculums are contaminated seeds and soil (Ozer and Koycu, 1997).

Under field conditions, early disease symptoms are yellowing of leaves and tip dieback, and the whole plant may collapse with the development of the disease. Disease development is optimized when soil temperature ranges from 25^0 to 28^0C (Sumner, 1995). If pathogen attacks the host plant late in the season, the symptoms may not appear until onion bulbs are in storage (Ozer *et al*., 2003). Control of plant diseases by the use of antagonistic microorganisms can be effective means (Cook and Baker, 1983). Interaction between biocontrol agents and plant pathogens has been studied extensively and application of biocontrol agents to protect some commercially important crops is promising (Vesseur *et al*., 1990). A large number of plant diseases have been successfully controlled through fungal and bacterial antagonists (Cook and Baker, 1983; Federico *et al*., 2007; Sahebani and Hadavi, 2008). Phytopathogens play major role in causing diseases to many agriculturally important crops, resulting in loss of plant yield. Fungicides and other pesticides accumulate hazardous toxic compounds which posses threat to human life and the surrounding environment. Pathogens are also found to develop resistance against several pesticides. Biological control was introduced that uses microorganisms, which interferes with pathogens and pests of various crops to overcome the problems caused by chemical means of plant protection (Anand and Reddy, 2009).

Present study found that biological agents inhibit the growth of *F. oxysporum*. Biological control can limit the instances of basal rot of onion caused by *F. oxysporum* and have specific advantages over synthetic fungicides, including fewer non-target and environmental effects, efficacy against fungicide-resistant pathogens and reduced probability of resistance development.

MATERIAL AND MATERIALS

Onion showed typical symptoms of basal rot caused by *Fusarium oxysporum* was collected from Nasik district of Maharashtra and brought to the K.V.P.C. Research Laboratory. *Fusarium oxysporum* was isolated using PDA media and identified on the basis of pure culture, mycelial growth with conidiophores and conidia (micro and macro) using literature. Pure culture of biocontrol agents was collected from our research laboratory and effect of biocontrol agents *viz; Aspergillus niger, Aspergillus flavus, Trichoderma viride, Trichoderma harzianum, Trichoderma koningii*, *Curvularia lunata, Penicillium expansum, Alternaria alternata, Xanthomonos axonopodis, Bacillus subtilis, Pseudomonas fluorescens,* against *Fusarium oxysporumf. sp. cepae* by dual culture method (Dennis and Webster, 1971). A mycelial disc of 5 mm diameter of the *Fusarium oxysporumf. sp. cepae* was placed at one end of the agar plate with PDA and opposite to biocontrol agent was placed. In case of bacteria, a loopful of bacteria was streaked at one end of the agar plate and 5 mm diameter mycelial disc of *Fusarium oxysporumf. sp. cepae* was placed on the opposite end. Replicate three times and radial growth of *Fusarium oxysporumf. sp. Cepae* was measured as served as control. PCE was determined using formula.

$$\text{Percentage Control Efficacy} = \frac{C-T}{C} \times 100$$

Where, C- control growth of *Foc*
T- Treatment

RESULTS AND DISCUSSION

There are various pathogens isolates from onion and basal rot of onion was dominant caused by *Fusarium oxysporumf. sp. cepae.* Similarly 30 isolates were tested their sensitivity against thiophanate methyl. *Foc*-25 was sensitive while iasolate *Foc*-23 was resistant. For further studies Foc-23 was used to control using biocontrol agents. Results are presented in the (Table.1 and Plate 1-2)reveals that eleven biocontrol agent more or less significantly, inhibited the mycelial growth of *Fusarium oxysporumf. sp. cepae.* causingbasal rot in onion. Out of eleven microorganisms tested *Trichoderma viride*was most effective against *Fusarium oxysporumf .sp. cepae.* reduced the radial growth of *Fusarium oxysporumf. sp. cepae.* by (78.88) followed by *Aspergillus niger* and *Trichoderma harzianum* (73.33), *Aspergillus flavus* (67.77), *Trichoderma koningii* (61.47), *Curvularia lunata* (45.55), *Alternaria alternata* (40.00), *Penicillium expansum* (30.00), *Xanthomonas axonopodis* (26.17), *Bacillus subtilis* (23.75), *Pseudomonas fluorescens* (12.85) was observed.Similar results was also reported and correlated with present investigation. Sivan and Chet (1987) reported that *Trichoderma spp.* successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon. Sesame seeds treated with three isolates of *T.viride* reduced the pre-and post–emergence damping off caused by *R.solani* and *F. oxysporumf. sp. sesami* under pot culture and field conditions. Rauf and Javaid (2013) noticed that antifungal activity of different plant extracts of *Chenopodium album* against *Fusarium oxysporumf. sp. cepae.*, the cause of onion basal rot. Farooq and Nasreen (2014) observed that the evaluation of biocontrol agents in vitro against *Fusarium oxysporum* causing *Fusarial*rot of *Pleurotus* spp. all the biocontrol agents more or less inhibit the growth of *Fusarium oxysporum*. The *Bacillus subtilis* more efficient antagonistic activity against *Fusarium oxysporum* and followed by *Pseudomonas fluorescens.*

Table 1:- Evaluation of biocontrol agents against *Fusarium oxysporumf. sp. cepaein vitro*.

Sr. No.	Biocontrol Agents	PCE*
1.	*Aspergillus niger* van Tieghem.	73.33
2.	*Aspergillus flavus* Link.	67.77
3.	*Trichoderma viride* Pers.	78.88
4.	*Trichoderma harzianum* Rifai.	73.33
5.	*Trichodermakoningii*Oudem.	61.47
6.	*Curvularia lunata* (Wakker) Boed.	45.55
7.	*Penicillium expansum* Link.	30.00
8.	*Alternaria alternata* (Fr.) Keissl.	40.00
9.	*Xanthomonos axonopodis* Hasse.	26.17
10.	*Bacillus subtilis* Cohn.	23.75
11.	*Pseudomonas fluorescens* Migula.	12.85
12.	Control	80.00
	SE	16.896
	CD at (0.05)	35.449
	(0.01)	39.321

* Values are mean of three replicates.

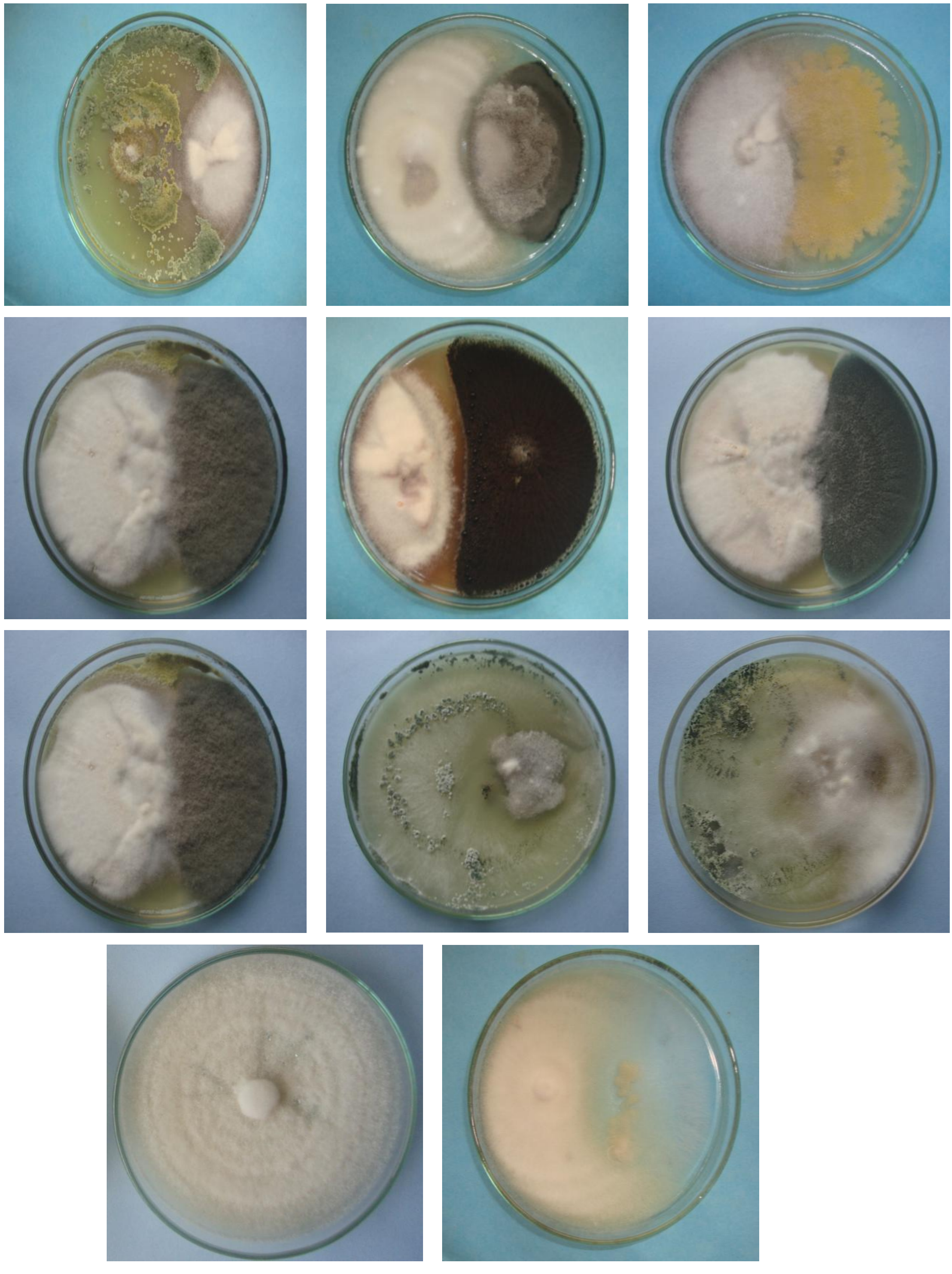

Fig.1: Biological control agents against *Fusarium oxysporum*(*in vitro)*.

CONCLUSION

The present investigation envisions the preliminary results of *in vitro* evaluation of both fungal and bacterial microorganisms are one of the potential bio-control agents against basal rot of onion caused by *Fusarium oxysporumf. sp. cepae.* Further studies are promising enough to reveal and characterize the antagonistic agent responsible for bio-control activity. While use excess amount of biocontrol agent's for the management of pathogens maintained eco-friendly relationship. Therefore the management of plant diseases using fungicides in excess amount was dangerous to host as well as environment. For minimize the usage of pesticides and chemical fungicides use biological origin products.

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RESEARCH ARTICLE

Evaluation of antifungal activity of chemically synthesized Chalcone derivatives against *Candida albicans*

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ABSTRACT

Three Chalcone derivatives of biological interest were prepared by Claisen-Schimdt condensation of freshly distilled acetophenones with aromatic aldehydes in the presence of 10% sodium hydroxide and ethanol at room temperature.The purity of these compounds were checked by Thin Layer Chromatography method.These derivatives were evaluated for their antifungal activities against yeast *Candida albicans* by Agar Well Diffusion method.All the three derivatives are found to exhibit antifungal activity against *Candida albicans.*

Keywords: Chalcone, Antifungal, Claisen-Schimdt Condensation, *Candida albicans*.

INTRODUCTION

At present there is a growing interest in the discovery of new antimicrobial agents to bate against pathogenic microorganisms especially those that are resistant to antibiotics (Thanh-Dao *et al*., 2011). So, Chalcones constitute an important group of natural products having a wide variety of properties such as anti-inflammatory, anti-ulcer, anti-cancer, anti-bacterial, anti-fungal, anti-mitotic activity (Vibhute and Baseer, 2003). Chalcone serve as precursors for the synthesis of classes of flavanoids which are common substances in plants (Kumbhar, 2014). Chalcones are also key precursors in synthesis of many biologically active compounds useful in medicines (Habib and Kulkarni, 2013; Tiwari et a., 2010; Kulkarni *et al*., 2009). Depending upon the substitution of aromatic ring, the chalcone exhibit different antimicrobial activity (Osman *et al*. 2012; Sato *et al*., 1997). Recently the anti-candidal activity of phytochemicals with a

chalcone skeleton (1,3-diphenyl -2-propene-1-one) was studied (Martina *et al*., 2015). Design and synthesis of new compound with appropriate therapeutic importance is a major challenge in medicinal chemistry (Bhale *et al*., 2013). Thus, the present study aims to evaluate for the antifungal activity of Chalcone derivatives of biological interest so that they can be used as a potent antifungal agents against pathogenic *Candida albicans*.

MATERIAL AND MATERIALS

All the chemicals used for the synthesis of chalcone derivatives were obtained from Loba Chemie, India. The media components used were from Hi-media, India. All other chemicals used were of analytical grade and were prepared in distilled water.

***1) General procedure for synthesis of Chalcone derivatives*:**
The Chalcone derivatives were synthesized by taking a mixture of 10%Sodium Hydroxide solution (25ml) in a 250ml round-bottomed flask by shaking well and keeping the flask in an ice-bath. Then freshly distilled Acetophenone (5ml) and Benzaldehyde (4.5ml) were added with constant stirring continuously for 2-3 hrs at 15-30^{0}C and then was kept overnight in a refrigerator. The solid obtained was filtered in a Buchner funnel,under suction by washing with cold water, dried in air and crystallized from rectified spirit.

***2) Test Microorganism*:**
The test Microorganism used was a pathogenic *Candida albicans*.

***3) In-Vitro Antifungal Activity*:**
The antifungal activity of chalcone derivatives were evaluated by Agar Well Diffusion method using a culture density of 1.5x10^{8}cfu/ml by visually comparing the density with 0.5Mcfarland standard. The 20ml of Sterile Sabouraud Dextrose Agar was poured into each sterile petri plate and the plates were swabbed with 100uL of the test fungi and were kept for 20 minutes at room temperature for adsorption. Then using sterile cork borer of 8mm diameter, wells were bored in seeded agar plates and these were loaded with a 100uL volume with concentration of 1000ug/ml of each of the three compound reconstituted in ethanol. All the plates were incubated at Room Temperature of 25^{0}C for 72hrs. Antifungal activity indicated by an inhibition zone surrounding the well containing the compounds was recorded if the zone of inhibition was greater than 8mm. DMSO was used as a negative control and Clotrimazole (1000ug/ml) was used as a positive control.

RESULTS AND DISCUSSION

In all 20 different yeast isolates were obtained from all the sources. These isolates were purified and maintained on sterile xylose agar slants and refrigerated for further studies. Their ability to utilize xylose was screened in xylose peptone medium. The garden soil isolate was able to utilize 5% xylose within 72h as compared to other isolates. This soil isolate was named as “Sx”. This isolate was further employed for xylitol production. Identification of this isolate was carried out by morphological (Fig No.1) cultural (Fig.2) biochemical tests (Table No. 1).

Table 1.Antifungal activity of synthesized Chalcone Derivatives

Chalcone Derivative	*Candida albicans*-Zone of Inhibition(mm)
PRODUCT-1	12
PRODUCT-2	15
PRODUCT-3	9
POSITIVE CONTROL	22
NEGATIVE CONTROL	NA

NA-Not Applicable, Zone of inhibition was measured in mm

CONCLUSION

The Three Chalcone Derivatives were synthesized to check for their anti-fungal activity against *Candida albicans* by Agar Well Diffusion method.It was found that all the three derivatives showed antifungal activity. Hence we can conclude that these derivatives are active against pathogenic *Candida albicans* and can be used as potent antifungal agent.

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RESEARCH ARTICLE

Field examination of *Glomus*, a mycobiont

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ABSTRACT

Biofertilizers are the boon to the agricultural sector. The search for the proper living organisms to be supplemented to the crop plants gave fuel to the findings of mycobionts of the symbiotic association of Mycorrhiza. Field examination along the coastal line of Mumbai and suburbs reveal the abundance of the genus *Glomus*, one of the most important fungal partner in these localities. The current study deals with the findings of these microbes in specific geographical area, their presence in higher plants and identification.

Key words: Mycobiont, Mycorrhiza, *Glomus*

INTRODUCTION

The study gave the status of Arbuscular Mycorrhizal association in the geographical area from Mira Road to Bandra, the Western Suburbs of Mumbai. The Western Coast is generally sandy and stony. In The soil cover is largely sandy because of its proximity to the sea. Its geography states that the underlying rock of the expanse is made of Black Deccan Basalt flows and their acid and basic variants. Soil cover in the suburbs is largely alluvial and loamy. Mycorrhizal association is an integral component of soil micro flora. Mycorrhiza is an assemblage of a fungal partner and the roots of higher plants. This association proves to be beneficial to the host plants as it improves the mineral uptake there by the productivity of the host plant. Present study reveals the presence of a mycobiont, *Glomus* in various edaphic conditions. Identification of Arbuscular Mycorrhizal Fungi is based on their morphological characteristics namely, Hyphal mantle, Arbuscules, Vesicles and Spores.

The spores collected from pure lines of Vesicular Arbuscular Mycorrhizal Fungi are maintained on certain host plants cultivated in soil, pot culture or in nutrient solutions, hydroponic culture, under

sterile conditions. These spores are very useful in identifying Vesicular Arbuscular Mycorrhizal Fungal genera as they are definite structures with unique morphological characters (Maggirwar et al., 2013; Gerdemann and Trappe, 1974; Hall, 1977; Schenck and Smith, 1982; Morton, 1985; Walker, 1983). In the soil, the spores are generally produced singly on the hyphae and in living as well as dead roots (Koske, 1985; Morton and Walker, 1984; Mehrotra and Baijal,1993). The morphological characteristics of spore which are used in their identification are Colour, Size, Shape, Wall structure, Number, width, position and ornamentation of wall layers.

MATERIAL AND MATERIALS

Sampling:
The wild plants were used as samples, collected from ten different locations of the Western Suburbs of Mumbai. The locations were Mira Road East, Dahisar East, Gorai, Malad, Goregaon, Andheri, Juhu, Santacruz East, Bandra East and Bandra West.

Screening of roots:
This was done as per the method suggested by Koske (1985). Screening of both the roots and the rhizosphere soil of all the plants was done to determine the Arbuscular Mycorrhizal colonization in the roots, rhizosphere soil or in both. The roots were cut and washed thoroughly with water. The roots were kept in running water overnight to separate the adherent soil particles. The screened roots were subjected to pretreatment for staining. The sequential steps for pretreatment are as follows. Fixation and preservation, clearing of tissue, rinsing and bleaching, acidification washing and staining.

Isolation and quantification of Arbuscular Mycorrhizal spores:
Isolation and quantification of Arbuscular Mycorrhizal spores was carried out from hundred grams of oven-dried rhizosphere soil of plants collected from ten different locations by the wet-sieving and decanting method (Gerdemann and Nicolson, 1963).

Taxonomy of Arbuscular mycorrhizal Fungi:
Arbuscular mycorrhizal spores were identified using the manual described by Schenk and Perez (1989).

RESULTS AND DISCUSSION

The bulk of known species belong to the family Glomaceae (Pirozynski and Dalpé, 1989) which includes the genera *Glomus* and *Sclerocystis*. The spores are single-celled structures, of generally globoid shape, with thick walls made up of several layers of different textures, connected to the filamentous network by a suspensor Hypha of varied morphology.

Five species of *Glomus*, namely *Glomus aggregatum Glomus Mossae, Glomus macrocarpum, Glomus fasciculatum, Glomus multicaule* were observed in the wild plants collected from various locations of Mumbai and its suburbs. This is in confirmation with findings of Mulani & Prabhu (2002) and Mulani *et al.* (2004). Most species of the genus *Glomus* produce spores singly in the soil. One to several spores develop on aseptate hyphae, sometimes flared towards the point of attachment. At maturity, the spores get detached from the hyphae. Spores of *Glomus* species develop basically at the end of a sporogenous hypha. The surface of spores of *Glomus* species appeared smooth (Pawaar and Kakde, 2012). This field study highlight the abundance of the genera, *Glomus* which can enhance the productivity in Crop plants when introduced as they are not host specific like the Leguminocean Plants and can easily form the natural biofertilizer, Mycorrhiza.

CONCLUSION

The plants in the western suburbs and the soil thereby are rich in spores of genus *Glomus*.

Table 1: Comparison of Arbuscular Mycorrhizal association for the same Genus at different locations

Sr. No.	Host	Location	pH	% colonization	Arbuscular Mycorrhizal fungi
1	*Physalis minima*	Mira road E Juhu Bandra E.	6.2 7.1 6.6	56 0 58	*Glomus aggregatum* *Nil* *Glomus aggregatum*
2	*Eclipta alba*	Mira road E Goregaon	6.2 6.2	47 0	*Glomus aggregatum* *Nil*
3	*Vernonia cineria*	Mira road E Gorai Bandra W	6.2 6.4 6.5	62 0 58	*Glomus mossae* *Nil* *Glomus macrocarpum*
4	*Tridax procumbens*	Mira road E Bandra W	6.2 6.5	40 45	*Glomus fasciculatum* *Glomus aggregatum*
5	*Urena lobata*	Dahisar E Andheri Bandra W	6.4 6.5 6.5	42 72 33	*Glomus mossae* *Glomus fasciculatum* *Glomus aggregatum*
6	*Euphorbia hirta*	Dahisar E	6.4	50	*Glomus macrocarpum*
7	*Boerhaavia diffusa*	Goregaon Gorai Andheri	6.2 6.4 6.5	66 36 40	*Glomus mossae* *Glomus mossae* *Glomus fasciculatum*
8	*Cyperus rotundus*	Gorai Andheri Bandra W	6.4 6.5 6.5	43 75 0	*Glomusmulticaule* *Glomus fasciculatum* Nil
9	*Achyranthes aspera*	Malad, Santa cruz E Bandra E Bandra W	6.3 6.8 6.6 6.5	0 0 0 0	Nil Nil Nil Nil
10	*Aegeratum sps.*	Goregaon Bandra W	6.2 6.5	0 59	*Nil* *Glomus macrocarpum*
11	*Sida acuta*	Andheri Santa cruz E	6.5 6.8	0 52	*Nil* *Glomus mossae*
12	Cynodon dactylon	Juhu Bandra (W)	7.1 6.5	55 71	*Glomus aggregatum* *Glomus macrocarpum*

Table 2: Taxonomy and Morphology of *Glomus* species

Mycobiont	**colour**	**shape**	**size** (μm)
Glomus aggregatum	pale yellow to yellow brown	globose to sub-globose, obovate, to irregular in shape	40-120
Glomus Mossae	pale yellow to golden yellow	Globose to subglobose	80-280
Glomus macrocarpum	yellow brown to brown	sub-globose to globose	90-180
Glomus fasciculatum	Pale yellow to pale yellow-brown	ellipsoidal Spore wall	60-100
Glomus multicaule	Dark brown	Ellipsoid, broadly ellipsoidal, sub-globose, or occasionally triangular	140 -160

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RESEARCH ARTICLE

In vitro antifungal activity of the bacterial Biosurfactant

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ABSTRACT

Research in the area of biosurfactant has expanded quite a lot in recent years. Biosurfactants are amphiphilic compounds i.e., they contain both hydrophilic and hydrophobic moieties. Their low toxicity and eco-friendly nature and the wide range of potential industrial applications in health care, bioremediation, and food processing makes them a highly useful compound. They are produced by a variety of microorganisms as extracellular compounds. Biosurfactants are superior to the chemical surfactants with respect to their biocompatibility, lower toxicity, and higher biodegradability. The present study was focused on in vitro antifungal activity of rhamnolipid biosurfactant produced by bacteria *Pseudomonas aeruginosa* using olive oil as substrate. Biosurfactant was extracted by solvent extraction method using equal volume of Chloroform and n-butanol (65:15). The extracted biosurfactant was evaluated for its potential antifungal activity by using agar cup method against yeast *Candida albicans* and was compared with commercial chemical surfactant Sodium lauryl Sulphate. Extracted rhamnolipid Biosurfactant showed antifungal activity against *Candida albicans*. Thus, the work suggests that after further purification it could be used as therapeutic agent in Biomedical and pharmaceutical applications.

Key Words: Rhamnolipid Biosurfactant, Antifungal activity, olive oil, therapeutic agent.

INTRODUCTION

It is well known that the emergence of fungal infections is most common. Even though there are over 200 kinds of antibiotics, the problem of resistance to antibiotics is observed (Ahimou et al., 2000). Hence finding new therapeutic agents becomes important. Biosurfactant is a highly useful compound that significantly works as antifungal agents (Banat et al., 2010).

In the recent past, attentions have been paid to alternative, environmental friendly, surface active products synthesized by microorganisms known as biosurfactants. Biosurfactants are amphiphilic molecules mainly produced by microorganisms as a secondary metabolite. They possess both hydrophilic and hydrophobic moieties and are able to display a variety of surface activities and help to solubilize hydrophobic substrates Bacteria are the main group of biosurfactant-producing microorganisms, although it is also produced by some yeasts and filamentous fungi (Bodour et al., 2004; Desai and Banat, 1997). *Pseudomonas* species is well known for its ability to produce rhamnolipid biosurfactants with Potential surface active properties when grown on different carbon substrates and therefore is a promising candidate for large scale production of biosurfactants (Karsa et al., 1999). The present study is aimed at producing biosurfactant using olive oil as substrate by bacteria *Pseudomonas aeruginosa* and investigating it's in vitro antifungal activity against yeast *Candida albicans.*

MATERIAL AND MATERIALS

Organism

Pseudomonas aeruginosa is used in the present study for Biosurfactant production and was maintained on nutrient agar slant.

Confirmation of biosurfactant production by *Pseudomonas aeruginosa*

***Media and cultivation conditions*:**
After 24 hrs of incubation the microbial growth from slant was scraped out and suspended into 50ml of Sterile Luria Bertani broth in a 250ml Erlenmeyer flask. The flask was incubated on a rotary shaker for 4 days at 28^{0}C. After incubation, the content of the flask was centrifuged at 7000 rpm for 20min.Taking supernatant following tests for identifying the presence of biosurfactant were performed:

Phenol-Sulphuric Acid Method:
In 1ml of cell free supernatant 1ml of 5% phenol was added. To this mixture, 2-5ml of concentrated sulphuric acid was added drop by drop, until characteristic color was developed. Development of orange color indicated the presence of glycolipids.

***Blue agar plate method (BAP)*:**
Basal Mineral salt agar medium was supplemented with (2%) glucose as carbon source and cetyltrimethyl- ammonium bromide (CTAB: 0.5 mg/mL) and methylene blue (MB: 0.2 mg/mL) for the detection of anionic biosurfactant. Wells were punched at the centre of the blue agar plate with alcohol sterilized cork borer to which 30µl of cell free supernantant was added. The plates were incubated at 37°C for 48-72 h. A dark blue halo zone around the well is considered positive for anionic biosurfactant.

***Emulsification test (E24)*:**
Several colonies of pure culture were suspended in test tubes containing 2 mL of mineral salt medium, after 48 h of incubation, 2 mL hydrocarbon (oil) was added to tube. Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 h. The emulsification index (E24) is the height of the emulsion layer (mm) divided by total height (mm), multiplied by 100 (Bodour *et al.,* 2004).

Production and extraction of biosurfactant

***Preculture*:**
loop full microbial growth from the 24 hrs old slant was scraped out and inoculated into 100ml of Sterile Luria Bertani broth in a 250ml Erlenmeyer flask and incubated at 28^{0}c for 24hrs on shaker.

***Production*:**
Five different flasks containing 100ml of Basal Mineral Salt medium containing 2% Oilve oil as carbon source whereas 0.1% of Ammonium Nitrate as Nitrogen source was inoculated with

5ml of preculture and incubated at 28^{0}c for 5days on shaker.

Extraction:

Acid precipitation**:** Remove Cells from the culture broth by centrifugation at 10 000 x g, 4°C for 10 min. acidify the supernatant containing biosurfactant with 6N HCl until pH 2.0 is obtained.

Solvent Extraction:

Extract an acidified supernatant with the equal volume of mixture of the extraction solvents with the following ratio; (chloroform: n-butanol; 65:15). Shake the mixture vigorously for 15 min and allowed to set until the phase separation occurs. White coloured precipitate if seen at the interface between the two liquids proved the presence of biosurfactant. The biosurfactant formed was carefully taken out with the help of micropipette and kept in centrifuge tubes. These were centrifuged at 7000rpm, 4°C for 30 minutes. The supernatant was discarded and the pellet obtained was the crude extract of biosurfactant.

Determination of in vitro antifungal activity of extracted biosurfactant

Sterile Muller Hinton Agar media was prepared and poured into sterile petriplate. Plate was swabbed with *Candida albicans* culture. Three Wells were made using alcohol sterilized cork borer and were named as positive control (1%SDS), Negative control (Sterile Distilled water), Test (Extracted Biosurfactant). Plates were kept in incubation at 28°C for 24-48 hours.

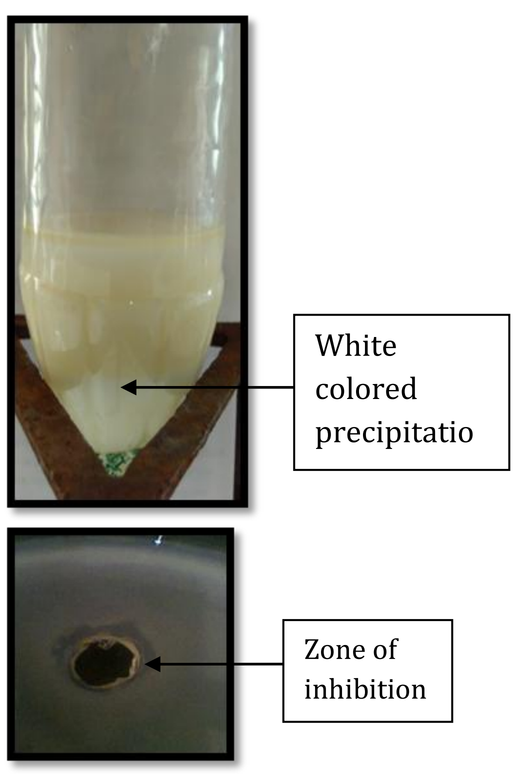
RESULTS AND DISCUSSION

Production and extraction of biosurfactant

After 5 days of incubation medium was dispensed into centrifuge tubes and was centrifuged at 10000g at 4^{0}c for 10 min and the resultant supernatant was extracted using chloroform and n-butanol solvent mixture in the ratio 65:15.White colored precipitation was seen at the interface between the two liquids proved the presence of biosurfactant. The biosurfactant formed was carefully taken out with the help of micropipette.

Table 1: Confirmation of biosurfactant production by *Pseudomonas aeruginosa*

TEST	OBSERVATION	RESULT
Phenol-Sulphuric Acid Method	Orange colour developed indicated the presence of glycolipids.	Positive
Blue agar plate method (BAP	A dark blue zone around the well indicated the presence anionic biosurfactant.	Positive
Emulsification test (E24)	63.75.% emulsion was observed	Positive



Determination of in vitro antifungal activity of extracted biosurfactant

The crude extract of biosurfactant showed antifungal activity against *Candida albicans* after 48hrs. The presence of clear zone marked the antifungal activity of biosurfactant.

CONCLUSION

In conclusion, the study represented surfactant activity of the bacterial strain used. This confirms *Pseudomonas aeruginosa* shows biosurfactant producing ability by utilizing Oilve oil as substrate. Used simple extraction method is efficient in extracting biosurfactant. Extracted biosrfactant is showing antifungal activity in vitro hence study suggests its use as a therapeutic agent. Further study recommends in vivo antifungal activity on animal and human cell lines along with further purification of biosurfactant.

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RESEARCH ARTICLE

Biodiversity of Arbuscular Mycorrhizal fungi in Kaas plateau, Satara, Maharashtra, India

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ABSTRACT

Arbuscular Mycorrhizal fungi, previously known as Vesicular–Arbuscular-Mycorrhizal fungi (VAM) are soil microbes , forming obligate symbiotic association with the roots of 87% of the land plants. The Kaas plateau is situated in the western ghat of Sahyadri ranges 22km from Satara city in Maharashtra. This plateau is famous for its flowering plant diversity. The objective of the present study was to study the biodiversity of AM Fungi in five plants most commonly occurring and flowering in the month of September i.e. *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia* and *Dipcadi montanum.* These plants were screened for arbuscular mycorrhizal (AM) spore number and root colonization. The average mycorrhizal root colonization was found to be 79.2±6.1 percent. *Dipcadi montanum* showed maximum root colonization. The spore number ranged from 210±10 to 618±17.5 per 5 gm. The highest mycorrhizal spore count was found in *Dipcadi montanum* and lowest in *Eriocaulon manoharanii*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. The dominant AM species found were *Acaulospora scrobiculata*, *Glomus albidum* and *Glomus macrocarpum*. The average spore density of AM fungi in Satara Kaas plateu is found to be 330 spores per 5gm. Type of infection found in the roots was in the form of arbuscules, vesicles, spores (intra radical and extra radical spores), hyphae and sporocarps. Another type of potentially beneficial fungi associated with roots was observed, namely, dark septate endophytic fungi (DSEF). Dark septate endophytic fungi were identified in *Pogostemon deccanensiss* and *Senecio grahamii* .

Keywords: Arbuscular Mycorrhizal Fungi, Kaas Plateau, Dark septate endophytic fungi (DSEF).

INTRODUCTION

Many microorganisms form symbiosis with plants that range, on a continuous scale, from parasitic to mutualistic. Among these, arbuscular mycorrhizal (AM) fungi are ubiquitous plant root symbionts that can be considered as 'keystone mutualists' in terrestrial ecosystem, forming a link between biotic and abiotic ecosystem components via. carbon and nutrient fluxes that pass between plant and fungi in the soil (O'Neill *et al*., 1991).It is estimated that about 87% of terrestrial plants form mycorrhizal associations (Stoyke and Currah, 1993). In addition to mycorrhiza, the roots of plants may get frequently colonized by fungi with dematiaceous septate hyphae which are often sterile in culture conditions (Newsham, 1999). Such fungi are collectively called as dark septate endophytic fungi (DSEF). Dark septate endophytic fungi (DSEF) belong to ascomycetous and anamorphic fungi. Once DSEF colonized the roots of the plants, they form characterized inter and intracellular structures like net of hyphae, microsclerotia and occasionally, a partial mantle (Jumpponen and Trappe, 1998). DSEF can be easily distinguished from AM fungal hyphae by their dark red brown to dark brown colour, thick lateral wall and frequent septa. So far DSEF have been reported mostly from arctic (Jumpponen and Trappe, 1998), alpine environments (Read and Haselwandter, 1981), neotropical cloud forests (Rains *et al*., 2003) and grassland ecosystem (Mathew and Malathy, 2007). AM Fungi were not documented from Kaas plateau and hence this study was carried out in some of the dominant plants which were flowering in the month of September 2015 to study the diversity of AM Fungi.

MATERIALS AND METHODS:

Soil sampling: Rhizosphere soil of five plants viz; *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia, Dipcadi montanum* was collected from Kaas plateau, Satara, Maharashtra in the second week of September 2015 and preserved in sterile polythene bags and stored at 4°C until use. Soil samplesupto 20cm depth was collected. Root samples were collected from the rhizospheric soil during sieving and decanting technique as uprooting the plants is prohibited from Kaas plateau.

***Spore Extraction*:** The soil samples were subjected to wet sieving and decanting technique for the isolation of spores by Gerdeman and Nicolson's method,(1963). The isolated spores were picked up with needle under stereo zoom microscope and were mounted in polyvinyl lactoglycerol and observed under compound microscope.

***Taxonomic Identification of spores*:** This was done using the identification manual by Shenck and Perez, 1990, Rodrigues and Muthukumar,2009 and descriptions provided by the international collection of VAM www.invam.in and www.zor.zut.edu.

***Spore Density counting*:**
The quantification of AM spores was done by 'Grid Line Intersect Method of Adholeya and Gaur (1994).

***Root Colonization of AM fungi*:**
Phillips and Hayman, (1970) Root samples isolated from the rhizosheric soil were processed and stained by 'Rapid Clearing and Staining Method' following Phillips and Haymann (1970). Per cent root colonization was observed taking 10 randomly selected root pieces. Qualitative characteristics of AM fungi i.e. external hyphae, vesicles, arbuscules and endospores were observed in stained root samples. The percentage of mycorrhizal root colonization was determined by the following equation:

$$\text{Percentage AM root colonization} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

RESULTS AND DISCUSSION

AM fungi are ubiquitous and ecologically important root symbionts of most terrestrial plants. In the present study, status of AM fungi associated with five plants most commonly occurring in Kaas plateau was studied. The results of the rhizosphere soil assessment of five plant species have been presented in the table. The mycorrhizal root colonization ranged from 70% in *Senecio grahamii* to 86% in *Dipcadi montanum*.

Root samples of all the plant species showed a wide range of variation in terms of AM root colonization. The mycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were reported in the roots. Vesicles of different shapes like elliptical, round, globose, oval and elongated were observed. Paris type (Coiled) of arbuscules were observed in *Senecio grahami* and in the rest four plants it was arum type (Linear).

The AM spore density ranged from 210± 10 to 618.3± 17.5. The highest spore population was recorded in the rhizospheric soil of *Dipcadi montanum* and it was followed by *Pogostemon decanensis* and *Senecio grahamii.* The lowest was recorded in *Eriocaulon manoharani*. We observed highest spore density in family Asparagaceae. Kumar (2013) reported highest spore count in the family Asteraceae followed by Boraginaceae while Liliaceae was observed with least spore count. *Asparagus* is placed in Liliaceae in Bentham and Hookers classification. *Pogostemon* showed maximum species diversity as shown in the table and least was shown by *Impatiens.* Coexistence of DSEF and AMF was detected in two plants *Pogostemon* and *Senecio.*

Table: Status of different structures and spore density of AMF and DSEF in the plants of Kaas Plateau.

Sr. No	Botanical Name	Common name	Family	Type of Infection			AM spore count/ 5gm. of soil	% AM Root Colonization	AM Fungi Identified	Dark Septate Endophytic Fungi
1	*Eriocaulon manoharanii*	Dwarf Pipewort	Eriocaulaceae	+	+	+	210 ±10	80 ±5	*Glomus constrictum, Acaulospora* x, *Acaulospora y*	-
2	*Pogostemon deccanensis*	Jambhli Manjiri	Lamiaceae	+	+	+	318.3 ±16	83.6 ±5.1	*Gigaspora, Glomus albidum, Glomus macrocarpum, Glomus* x, *Acaulospora scrobiculata, Acaulospora* x *Spore in spore syndrome*	+
3	*Senecio grahamii*	Sonki	Asteraceae	+	+	-	260 ±11.3	70 ±5	*Glomus macrocarpum, Acaulospora x*	+
4	*Impatiens oppositifolia*	Lal terda	Balsaminaceae	+	+	+	240 ±19	77.66 ±8.5	*Glomus, Acaulospora* x	-
5	*Dipcadi montanum*	Dalzell	Asparagaceae	+	+	-	618.3 ±17.5	86 ±5.2	*Acaulospora spinosa, Acaulospora* x, *Acaulospora* y, *Glomus glomerulatum*	-

Tripathi (2014) have reported DSEF from the deciduous forests of Central India. Mandyam and Jumpponen (2005) speculated that DSE fungi would be prevalent in various habitats and colonize a substantial proportion of the species present in mixed plant communities. This group of fungi cannot be overlooked while assessing the fungal communities of any ecosystem, as their abundance may equal or even exceed that of the VAM fungi.

We could not find any significant correlation between root colonization and spore density in our study. The variation in spore density and colonization of AMF associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics of rhizosphere under host species, variation in host species, mycorrhizal dependency, host plant-mediated alterationof the soil micro environment,or other unknown host plant traits, as described by Lorgio *et al*. (1999) and Eom *et al*., 2000. However, some researchers found a positive relationship between VAMF colonization and spore density (Sigüenza *et al*., 1996), whereas others found a negative relationship (Fontenla *et al*., 1998).

CONCLUSION

The plants selected for study of AM Fungal diversity were *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia* and *Dipcadi montanum.* The study was carried out in mid September. The spores isolated from the plants belonged to three genera*Glomus, Acaulospora, Gigaspora*.The dominant AM species found were *Acaulospora scrobiculata*, *Glomus albidum* and *Glomus macrocarpum*. *Pogostemon deccanensis* and *Senecio graham* also showed DSEF in the roots . The root colonization showed presence of mycorrhizal structures - Arum type (Linear) of arbuscules in four plants and Paris type(Coiled) of arbuscules in *Senecio grahami. Pogostemon deccanensis* showed maximum species diversity as shown in the table and least was shown by *Impatiens oppositifolia.*

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RESEARCH ARTICLE

Development of anti-fungal herbal Hand wash gel

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ABSTRACT

One of the primary modes of transmission of micro-organisms are our hands. Hand-washing is important in food production, food service and day care preparations. Hence it brings us to the use of antiseptic for hand washing purposes. Many of the antiseptic available in market are alcohol based sanitizers which have some shortcomings or adverse effects. Their frequent use can lead to skin irritation. The present research was aimed to evaluate the anti-fungal efficacy of herb and plant material such as Lemongrass and Bermuda grass by agar-cup method of these plant extracts that were obtained by soxhlet extraction. Also the research was carried out to formulate and evaluate the herbal hand wash liquid containing the above extracts. The anti-fungal activity of the formulated hand wash gel was tested against *Aspergillus niger* by Agar Cup Method. Thus this work suggests and supports the incorporation and utilization of herbs and traditional plant materials in the formulation to give better anti-fungal effect. The further development should be performed to achieve broad antifungal activity with different extracts.

Keywords: herbal hand wash gel, *Aspergillus niger*, antifungal activity, plant extracts..

INTRODUCTION

One of the primary modes of transmission of micro-organisms are our hands. Hand-washing is important in food production, food service and day care preparations (Ravi *et al.*, 2005). Contaminated hands can serve as vectors for the transmission of microorganisms. Pathogenic microorganisms responsible for outbreaks are spread from the hands of the food handler to others when the food handler contaminates his/her hands and then passes these microorganisms to consumers via hand contact

with food or drinks. The consumer is exposed following the ingestion of these microorganisms, which may cause gastrointestinal illness. Hand contact with ready-to-eat foods represents a very important mechanism by which pathogens may enter the food supply (NDSC, 2004). To protect the skin from harmful micro organisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution (Snyder and Paul, 1988).

Many of the chemical antiseptics are now available in market as alcohol based sanitizers, chlorhexidine products etc. These soaps or solutions help to reduce health care associated transmission of contagious diseases more effectively but they have some shortcomings or adverse effects. Their frequent use can lead to skin irritation and also resistant among pathogens (Luby and Agboatwalla, 2005). Organism such as *Aspergillus niger* is one of the causative agents of the skin infections. Since fungal infection are always almost everywhere and are just waiting for an opportunity to strike, maintaining a very hygienic lifestyle and putting extra effort on taking care of yourself will pave a long way. It boils down to treatment and prevention to save you from any kinds of fungal infection. Some studies been conducted have shown that resistance to chemical antiseptic have led to outbreaks. Historically, plants have provided a good source of anti-infective agents. India is a rich country in biodiversity. For millennia, traditional healers have used the rich flora to cure ailments. The same plants are being used today. Traditional plants-as cure continues to be very popular since the large part of the population has either no access to, or no resources to afford western treatments. These plant based antimicrobials represent a vast untapped source for medicines. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Plants containing flavonoids and polypeptides used in traditional medicine have been found to be active against a wide variety of micro-organism.

In the present research**:** *Cymbopogon citrates* and *Cynodon dactylon* are been selected in preparing herbal anti-fungal hand wash gel.

Cymbopogon citrates: Lemongrass is a well known medicinal herb in the east. Lemongrass in skin formulations is used to treat acne, cellulite, and other skin problems. Unlike harsh chemical sprays, the scent of lemongrass is very pleasant.

Cynodon dactylon: Bermuda grass has many health as well as medicinal benefits. Durva grass is loaded with medicinal uses that can be used as home remedies. Durva grass is used as antiviral and antimicrobial. In India, Bermuda grass is considered as sacred plant, which has great significance in ayurveda because of its medicinal as well as clinical properties.

MATERIAL AND MATERIALS

Plant collection

Samples of *Cymbopogon citrates* and *Cynodon dactylon* were collected during july-august (2015) from mumbai market, Maharashtra, India.

Preparation of plant extracts

The plant materials were sun-dried.
After drying, the material is made into fine powder with the help of grinder and each plant material is weighted. 20 gm of coarse powder of plant material to be extracted separately in 200 ml of acetone using soxhlet extractor for 6hrs/sample. Temperature to be maintained till the boiling point of acetone i.e. 56°c. The extract is separated from the solvent with the help of evaporation. The solvent vapourises, leaving the extract which is then oven-dried.

Anti-fungal assay

The anti-fungal activities of the extracts were determined by agar-cup method technique (Rose and. Miller 1939a) against *Aspergillus niger*. Mueller-Hinton agar was used as a culture

medium. The culture was swabbed over the agar plates and then wells were punched with a 0.85cm cork-borer. 0.1 ml of the extract was introduced into the well. The plates were incubated for 48hrs at room temperature.

Formulation of herbal hand-wash gel

KOH-Water mixture was added to a separate beaker which was placed in a boiling water bath. The desired concentration of foaming agent was measured accurately and dispersed in the KOH-water solution with moderate stirrer speed. Desired quantity of the extracts, colorant were added to the formulation along with boiling water. PEG was added to adjust the pH. The paste was then neutralized and diluted. The formulated hand wash was then filled in a suitable container and stored at a cool and dry place.

Anti-fungal studies of the herbal hand wash

The anti-fungal studies of the herbal hand wash gel were studied by agar-cup method technique (Rose and. Miller 1939) against *Aspergillus niger* with Mueller-Hinton agar as a culture medium. The culture was swabbed over the agar plates and then wells were punched with a 0.85cm cork-borer. 0.1 ml of the formulated hand wash was introduced into the well. The plates were incubated for 48hrs at room temperature.

RESULTS

Fig.1: Hand wash showing antifungal activity

The results of the agar cup method of the acetone extracts of lemongrass and Bermuda grass against *Aspergillus niger* showed significant results. Hence it was encouraging to be used in the preparation of herbal hand wash gel. The hand wash showed greater antifungal activity (fig.1). Thus, the potency of the herbal hand wash against *Aspergillus niger* is very remarkable.

CONCLUSION

In conclusion, based on the above findings it is clear that lemon grass and Bermuda grass is active against Aspergillus niger. The results clearly prove that the herbal hand wash gel thus prepared is far more active. It can be stated that the active compounds in the hand wash are more effective in killing or removing organisms than the chemicals that are used. Thus these compounds can be extracted and incorporated in hand wash formulation in order to prepare superior antiseptic herbal hand wash gel with little or no side effects. Thus, a new way can be found to provide safe and healthier living through germ-free hands. Although the removal is not 100% but a major number can be reduced.

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RESEARCH ARTICLE

Biodiversity of mycoflora in mangroves habitat of Mumbri Creek of South Konkan, MS, India

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ABSTRACT

The present investigation is carried out in Mumbri Creek of Sindhudurg district (Lat. 16º 21' N. Long. 73º 25' E). The main aim of this work is to find out the productivity of the Mumbri creek. This abstract consists of major mycoflora such as Phycomycetes, Ascomycetes, *Zygomycetes* and Deuteromycetes. The mycoflora help in making the wetlands highly nutritious which enhances commercially important resource organisms. Pneumatophores of *Avicennia marina* provide excellent media to grow the fungi on large scale. The Deuteromycetes were dominating group.

Keywords: Mycoflora, Mangrove, Deuteromycetes, Mumbri Creek.

INTRODUCTION

The diversity and density of mycoflora associates with root surfaces of one major mangrove species in Mumbri creek, South Konkan, Maharashtra was studied during Jan.-Sept. 2015. The species recorded in the present investigation belonged to *Phycomycetes, Ascomycetes, Zygomycetes* and *Deuteromycetes.* The fungi were observed on one dominant mangrove species namely *Avicennia marina*, which harbours 11 genera and 16 species and some unidentified colonies. Among the species, *Aspergillus spp.* was most dominant species on the mangroves.

From the mycofloral point of view, tannin is an important secretion of mangrove that protects the protoplast against dessicational decay which plays an important role in establishment of mycoflora. The tannin along with, entangled fine silt or sand particles, from a film around the roots in which spores and fungi germinate to establish mycoflora in this interesting microhabitat. The film seen as a nutrient broth or medium in which the fungi are trapped and grow luxurious developing colonies of different classes.

MATERIALS AND METHODS

Regular fortnight samples of the pneumatophores of *Avicennia marina* were collected for a period during Jan.-Sept. 2015. The fungi were obtained by serial washing of roots following Harley and Waid (1955) method.

RESULTS

In all genera, A*spergillus spp*. was the dominating genus with highest average percentage to be followed by *Curvularia*. The percentage distribution was *Deuteromycetes* (40.22), *Zygomycetes* (13.88), Phycomycetes (17.0), Ascomycetes (25.77) unidentified colonies (3.13). *Chaetomium olivaceum* (Ascomycetes) was the percentage wise lowest species. The class percentage of fungi was low in monsoon compared to pre monsoon and post monsoon periods. The maximum number of species was recorded in the months of October to March, while minimum in July and August. The observed variation in density can be attributed to extreme hydrological condition like heavy rainfall, high velocity of water currents, flooding and mechanical stress due to wind and water currents that prevent formation and stability of slime film formation around roots. In the case of *Avicennia marina*, 12 genera and 17 species and some unidentified colonies were recorded of mycofloral diversity (Dekate, 2011). Among the fungal class, *Deuteromycetes* was dominating over the other three and A*spergillus* was the most abundant species.

But due to less rainfall this year, growth of fungal flora has changed slightly. Now only 11 genera and 16 species have been identified in the study area. Due to change of season this year the luxuriant growth in mycoflora has been affected. *Aspergillus niger* or *A. niger* is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called fruit scab on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food (Baviskar and Suryawanshi, 2013).

Table 1: List of fungal species

1)	Phycomycetes
a)	*Absidia ramose*
b)	*Rhizopus nigricans*
c)	*Syncephalastrum olivacum*
2)	Ascomycetes
a)	*Emericella nidulans*
b)	*Cirrenalia tropicalis*
3)	Deutromycetes
a)	*Aspergillus fumigatus*
b)	*A. nidulans*
c)	*A. niger*
d)	*A. terreus*
e)	*A. flavus*
f)	*Cladosporium oxysporum*
g)	*Curvularia oryzae*
h)	*C. tuberculata*
i)	*Fusarium oxysporium*
j)	*Penicillum nigricans*
4)	Zygomycetes
a)	*Mucor racemosus*
5)	Unidentified colonies

Table 2: Check list of fungi species isolated from the mangroves.

Sr. No.	*Avicennia marina*
01	*Absidia ramose*
02	*Mucor racemosus*
03	*Rhizopus nigricans*
04	*Syncephalastrum olivacum*
05	*Aspergillus fumigatus*
06	*A. nidulans*
07	*A. niger*
08	*A. terreus*
09	*A. flavus*
10	*Cladosporium oxysporum*
11	*Curvularia oryzae*
12	*C. tuberculata*
13	*Emericella nidulans*
14	*Fusarium oxysporium*
15	*Penicillum nigricans*
16	*Cirrenalia tropicalis*
17	Unidentified colonies

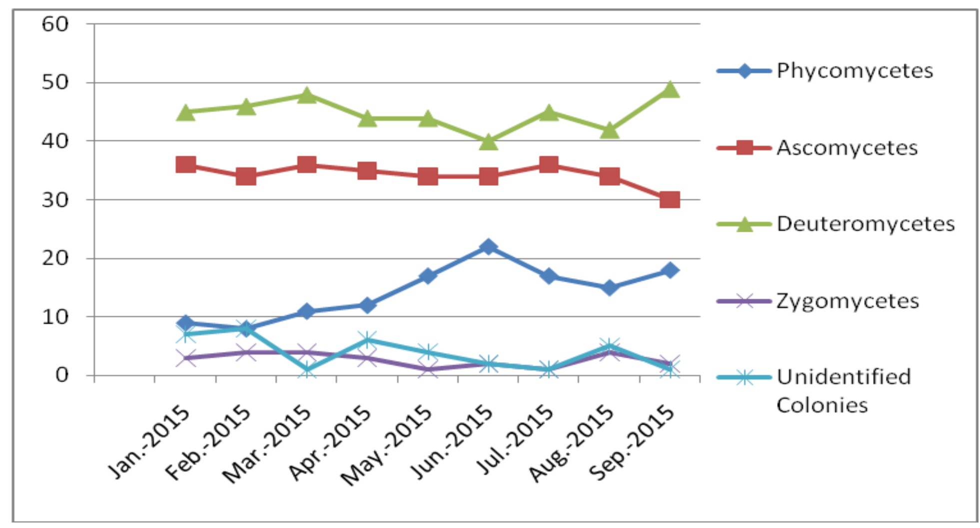

Fig.1: Percente composition of fungal classes isolated from *Avicennia marina*.

Aspergillus fumigatus is a fungus of the genus *Aspergillus,* a saprotroph widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling.

CONCLUSION

Patole (2009) has recorded that the stilt roots are better for the abundant growth of mycoflora than for the pneumatophores of *Avicennia* species. The stilt or prop roots also differentiate that the inner roots show more fungi than those in the outer peripheral region because of comparatively more humidity on the inner side. The humidity is directly proportional to both qualitative as well as quantitative growth of fungi. Babu (1999) noticed that the mycofloral growth is affected in monsoon than in any other season. In monsoon, due to the breaking of the waves on the root surfaces, the freshly developed film gets washed out thus inhibiting the multiplication of mycoflora. Parkinson (1967) described the root surface fungi of rhizoplane fungi. Untawale et al. (1973) have investigated rhizoplane fungi of certain plants and also those colonizing inter-tidal region of the mangrove swamps.

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RESEARCH ARTICLE

In-vitro antimicrobial activity of fungi from extreme environment

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ABSTRACT

Fungi are known to produce a vast array of secondary metabolites that are gaining importance for their biotechnological applications. In the present study, two fungal isolates which can grow at alkaline condition were screened for antimicrobial activity. Antimicrobial activity was studied using cell free extract.One out of two fungi exhibited antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis.* Besidesfungal metabolite,extraction was also carried out using methanol.

Keywords: Fungi, Antimicrobial activity, Extraction, Fungal metabolites.

INTRODUCTION

To survive in the environment and compete with the other microorganisms for resources many microorganisms produce antimicrobial compounds to inhibit or kill other competing strains including human and animal pathogens (Borgave *et al.*, 2012). Fungi are common in nature and considered as good natural sources for antimicrobial agents (Tawfik and Halla, 2012). Secondary metabolites are small molecules that are not directly involved in metabolism and growth of the organism. Both plants and fungi are known for producing a large number of chemically diverse secondary metabolites (Abulwahid *et al.*, 2013). The identification of the microorganisms that produce bioactive compounds is of great interest in the development of new molecules to fight against many pathogens.

MATERIAL AND METHODS

Screening of the fungal isolates

Fungal isolates were inoculated into Sabouraud Dextrose broth (pH-

11) and incubated for 10-12 days at 25⁰C. Cell free extract was prepared by filtration using membrane filtration assembly with 0.45 micron cellulose acetate filter paper. The filtrate was collected by applying vacuum in a flask. This filtrate was used for antimicrobial assay. Antimicrobial assay was carried out by agar well diffusion method.

The 24hr old cultures of the test organisms *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* were streaked on sterile Muller- Hinton (MH) agar with the sterile swab. The wells were made on MH agar. The wells were filled with the cell free extract obtained by filtration as well as with the methanol extract. These plates were incubated at 37⁰C for 24hrs then diameter of zone of inhibition was noted.

RESULTS AND DISCUSSION

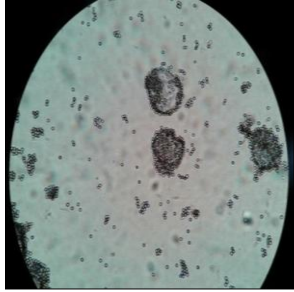
Abdulwahid *et al.* (3) extracted and characterised antibacterial compound from *Aspergillus niger*.They had studied antimicrobial activity of these fungus on organisms isolated from infections of the patients.

Swathi *et al.,* (2013) characterised the secondary metabolites from marine fungi *Microascus sps.* They had studied antimicrobial activity against the *S. aureus, E.coli, S. mutans, Candida albicans.* They found good antimicrobial activity against *E.coli and Candida albicans* ascompared to other pathogens.

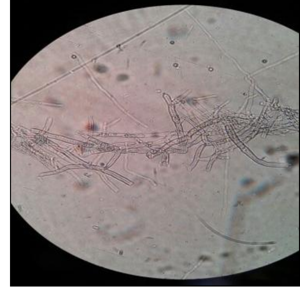
Samuel *et al.* (2014) studied antimicrobial activity of the fungi from Kubah National Park, Kuching, Sarawak, Malaysia. They had tested fungal isolates against *E.coli, Listeria monocytogenes, Salmonella enteriditis and Klebsiella pneumonia*. Six fungal isolates showed strong antimicrobial activity against the test bacteria.

Eric *et al.* (2013) found new effective antimicrobial assay of filamentous fungi.This bioassay also provided a way for studying the production dynamics of antimicrobial compounds of filamentous fungi.

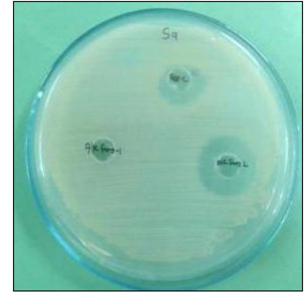
Manimegalai *et al.* (2013) investigated secondary metabolite content of marine fungal strain *Cephalosporium acremonium and P. citrinum,* showed broad spectrum activities.



Species 1



Species 1



Staphylococcus aureus

Bacillus subtilis

Fig. 1: Wet mount of alkaliphilic fungus under 45X

Table No.1: Zone of inhibition results by cell free extract of the alkaliphilic fungi1 and 2

	Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633	*Pseudomonas aeruginosa* ATCC 15442	*Escherichia coli* ATCC 8739
Alkaline fungus-1	0	0	0	0
Alkaline fungus-2	21 mm	18 mm	0	0
Standard culture *Aspergillus niger*	15.5 mm	14 mm	0	0

Table No.2: Zone of inhibition results by methanol extract of the alkaliphilic fungi1 and 2

	Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633	*Pseudomonas aeruginosa* ATCC 15442	*Escherichia coli* ATCC 8739
Alkaline fungus-1	12.5 mm	0	0	0
Alkaline fungus-2	13 mm	0	0	0
Standard culture *Aspergillus niger*	12 mm	0	0	0

CONCLUSION

The isolated alkaliphilic fungi were screened for their antimicrobial activity against pathogens by agar well diffusion method. Antimicrobialassay was carried using cell free extract. Metabolite of the alkaliphilic fungus-2 was showing better antimicrobial activity compared to alkaliphilic fungus-1 against Staphylococcus aureus and spore bearer Bacillus subtilis.

The extraction of the fungal metabolites was carried out with methanol.The study revealed that antimicrobial activity of both alkaliphilic fungi was found to be reduced after extraction with the methanol.Methanol was not suitable for the extraction of these fungal metabolites.

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RESEARCH ARTICLE

Biodiversity and the floristic affinities of the Poroid Aphyllophorales from Karnala (Maharashtra) India

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ABSTRACT

This work comprised a two years study on biodiversity and some qualitative aspects of Poroid Aphyllophoralesin a tropical forest of Karnala in the Western Ghats. More than 200 specimens were collected and the identification revealed 38 genera and 55 species belonging to order Aphyllophorales. Amongst these, 12 genera and 25 species belongs to family Polyporaceae, commonly called Poroid Aphyllophorales. Genera like *Antrodia, Antrodiella, Dichomitus, Junghuhnia, Perenniporia, Rigidoporus, Trametes, Wrightoporia* occurs on live standing trees. *Oxyporus, Incrustoporia,* occurs on dead standing tree. Genera like *Cristelloporia, Oxyporus, Xylobolus* are most abundantly found on fallen branches. Analysis of the WRF was carried out which clearly indicates that majority of them belongs to southern distribution. Some of species belongs to northern distribution while very few are found in both the hemisphere. It was also observed that the distribution of Aphyllophoraleshas close affinities with that of tropical memberswith majority of African species.

Keywords: Aphyllophorales, Biodiversity, Tropical forest, floristic affinities, Karnala.

INTRODUCTION

Aphyllophorales, an order of the Basidiomycetes are roughly characterized by non-septatebasidia, persistent gymnocarpous and non-putresent fruit bodies which usually are not lamellate. Amongst wood rotting mycobiota, Aphyllophorales constitute a major group that attack standing trees and fallen branches. They belong to family Corticiaceaeand family Polyporaceae. The family Corticiaceae is characterized non-poroid fruiting bodies while family Polyporaceae is characterized by poroid fruiting bodies. The corticoid non-poroidAphyllophorales constitute the major group (60%) while poroidAphyllophorales (40%) occur with variety of morphological characters (Gilbertson, 1979). Such fungi play a major role in the

process of decay, resulting in a serious damage to the forest economy.

Two kinds of wood decaying fungi are distinguished, one which degrade lignin and cellulose where wood is bleached, is known as 'white rot', while the other in which cellulose is degraded, leaving lignin more or less intact as brown residue known as 'brown rot'. It is therefore very important to identify wood rotting Aphyllophorales that cause great deal of damage to our valuable forest plantations and structural timber.

The tropical forests of the Western Ghats by and large have rich fungal flora in the context of composition of wood rotting mycobiota. The endemic angiospermic taxa exist in this region. (Kulkarni, 1979; Singh, 1981). It was suggested since a long time that the subtropical forests in the Western Ghats of India are related with that of tropical Africa, South-East Asia and with part of tropical America. It was revealed in the studies of lichen flora (Awasthi, 1965) and in rust flora (Sathe, 1969). During the field visits, it was observed that, there is a rich wood rotting mycobiota in the preserved and protected forest at Karnala which is declared as first bird sanctuary in Maharashtra.

According to Mahabale (1979) the development of deciduous forest is believed to be from evergreen to semi evergreen forest. This exactly occur in Karnalawhich provides a rich angiospermic vegetation of unique type showing a mixture of semi-evergreen and moist deciduous forests with few isolated patches of evergreen vegetation in deep ravines (Mandavgane, 1978). On account of full protection, the vegetation has not been cut down like other forests and also the dead branches are lying on the forest floor. Thus it appears that existing vegetation has reached ecologically climax condition.The climatic conditions in Karnalaalso favours the growth of wood rotting fungi. The present study deals with the study of only poroidAphyllophorales. Because of the unique type of vegetation in Karnala more emphasis was given to the study of the floristic affinities of such fungi. To understand the floristic affinities, the distribution of such fungi in southern and northern hemisphere was studied.

MATERIALS AND METHODS

Collection of fruiting bodies

Field work was carried out along different tracks at - Karnala in different seasons. Field information included data about type of substratum, host plant, date and place of collection, colour and texture of fruiting bodies were recorded.

Identification of WRF

Macroscopic and microscopic features of WRF were analyzed with usual methodology proposed for these type of fungi (Ryvarden and Johanson, 1980; Gilbertson and Ryvarden, 1986). Exact identification was done by using key given by Gilbertson and Ryvarden (1986) for poroid Aphyllophorales. Identification of poroid Aphyllophorales was also done with Bakshi (1971). Measurement of hyphae, basidia, cystidia and spores were taken. Some standard chemical tests, such as benzidine test to confirm the type of rot (Hintikka and Laine, 1970) and Melzer's reagent test to confirm amyloid and non-amyloid nature of basidiospores (Talbot, 1954) were taken.

Analysis of data

The data was analyzed for the distribution of poroid Aphyllophorales.

RESULTS AND DISCUSSION

Collection and identification of WRF

Total 240 specimens were collected from the collection sites. Detailed macroscopic and microscopic analysis of these fungi revealed 25 poroid (PO) species of Aphyllophorales. Climatic and other conditions in Karnala favours the growth of WRF. Out of these 4 species were difficult to allocate to any known species.

Table 1: Different type of Fungi identified

white rot fungi	**White rot fungi**	**Brown rot fungi**
Antrodiella species	*Perenniporia medulla-penis*	*Antrodiaalbida*
Cristelloporiadimitica	*Perenniporiasubasida*	*Antrodiaoleracea*
Incrustoporia species	*Perenniporiavoeltzkowii*	*Dichomitusleucoplacus*
Junghuniacrustacea	*Polyporusgrammocephalus*	*Wrightoporiaafricana*
Junghunianitida	*Rigidoporusmicroporus*	
Loweporus species	*Trametescervina*	
Oxyporus species	*Trametesincana*	
Oxyporuscervino-gilvus	*Trametesmenziezii*	
Oxyporuslatemarginatus	*Trametesscabrosa*	
Oxyporuspellicula	*Trametesvillosa*	
Perenniporiaalbida		

Table. 1 Distribution of poroidAphyllophorales

Name of the species identified from Karnala	**Southern distribution**						**Northern distribution**				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Antrodiaalbida	+			+		+		+			+
Antrodiaoleracea	+		+	+		+	+				
Antrodiellaspecies				+							
Cristelloporiadimitica	+			+							
Dichomitusleucoplacus	+	+		+							
Incrustoporia species				+							
Junghuniacrustacea	+			+		+					
Junghunianitida	+		+	+		+					
Loweporusspecies				+							
Oxyporusspecies				+							
Oxyporuscervino-gilvus		+		+		+			+		
Oxyporuslatemarginatus	+			+	+			+			
Oxyporuspellicula	+			+		+					
Perenniporiaalbida				+	+						
Perenniporia medulla-penis	+			+							
Perenniporiasubasida	+			+			+				
Perenniporiavoeltzkowii	+			+		+					
Polyporusgrammocephalus		+		+	+						
Rigidoporusmicroporus	+		+	+		+					
Trametescervina	+			+			+	+			
Trametesincana				+		+					
Trametesmenziezii	+			+							
Trametesscabrosa	+		+	+							
Trametesvillosa	+			+							
Wrightoporiaafricana	+			+							

(1) Africa ; **(2)** Australia and New Zealand; **(3)** South Africa; **(4)** India and Ceylon
(5) Mediterranean countries; **(6)** South Asia; **(7)** North America; **(8)** Europe **(9)** China
(10) Poland, U.S.S.R., Siberia and Japan; **(11)** Denmark, Sweden and Norway.

The vegetation in Karnala seems to be made up of three canopy layers reaching an average of 20 m. in height which gives wide range of habitats for the luxuriant growth of wood rotting fungi. In Karnala it was observed that from old mango plantation, 5 poroid Aphyllophorales were identified. Therefore *Mangifera indica* is most susceptible host for saprophytic and pertophytic fungi. The mixed vegetation of *Eugenia*, *Garuga, Heterophragma, Pongamia* and *Terminalia* showed 11 poroid species of WRF. These poroid members with effuso-reflexed to *pileate basidiocarp* occur dominantly in first layer canopy trees like *Mangifera indica, Pongamia pinnata, Heterophragma roxburghii, Careya arborea, Bombax malabaricum.*

It was consistently observed that coppiced wood showed luxuriant growth of poroid Aphyllophorales such as *Antrodiella, Incrustoporia, Perenniporia voeltzkowii.* The species of *Cristelloporia, Junghunia, Loweporus, Oxyporus, Perenniporia, Trametes* observed more frequently on the fallen branches of second layer of trees or bushes or climbers like *Ficus racemosa, Murraya paniculata, Bridelia squamosa, Gmelina arborea, Smilax zeylanica, Combretum ovalifolium* etc. The identification at species level revealed following species of the above genera. The study of type of rot showed 21 white rot fungi as compared to 4 brown rot fungi.

Floristic affinities

The analysis of poroid Aphyllophorales was carried out as shown in table 2. It clearly indicates that majority of them belong to southern distribution namely, *Cristelloporiadimitica, Dichomitus leucoplacus, Junghunia crustacean, Junghunianitida, Oxyporus pellicula, Perenniporia albida, Perenniporia medulla-penis, Polyporus grammocephalus, Rigidoporus microporus, Trametesincana, Trametes menziezii, Trametes scabrosa, Wrightoporia africana.* While certain species of northern distribution are located in above region namely, *Antrodiaalbida, Antrodiaoleracea, Ceriporia xylostromatoides, Oxyporus cervinogilvus, Oxyporus latemarginatus, Perenniporia subasida, Trametes cervina.* Some species like *Antrodiella subglobosa, Loweporus microspores, Oxyporusalbidus occur only in India and Ceylon.*

The analysis also shows the affinities of tropical Aphyllophorales have close proximity with majority of African species. Concerning the distribution of species of poroid Aphyllophorales with the other regions of India, it is noteworthy to point out that, *Dichomitus leucoplacus, Perenniporia medulla-penis, Trametes cervina, Rigidoporus microporus* are recorded from Himalayas and *Ceriporia xylostromatoides* from central peninsula.

CONCLUSION

The present study materially adds to our knowledge of biodiversity of poroid Aphyllophorales from Karnala, the part of the Western Ghats. Due to climatic and other conditions, Karnala is rich in fungal flora. On the basis of detailed macroscopic and microscopic observations, 21 species of poroid Aphyllophorales were identified, which occurred on different habitats and host plants. The fallen branches are the most suitable substratum for WRF, followed by live standing tree. Out of 25 species, 21 species are white rot fungi while only 4 are brown rot fungi. The species which were not allocated to any known species, may require further study to confirm the species. Distribution of Aphyllophorales has close affinities with that of tropical members especially with close proximity of African species. All species identified from Karnala are distributed in India and Ceylon. Amongst these, 64% were distributed in Africa and 16% from S. America and Australia each. The Mediterranean countries and S. Asia show 12% and 36% respectively. In the northern distribution, countries like N. America, Europe show still lesser percentage distribution.

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RESEARCH ARTICLE

Effect of petroleum ether extract of different plant parts on seed mycoflora and seed health (seed germination, shoot length and root length) of Green gram (*Vigna radiata* L.) by blotter method

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ABSTRACT

Green gram (*Vigna radiata* L.) is affected by seventeen seed-borne fungi. These fungi case adverse effects on seed health and yield. Application of synthetic fungicides causes damage to consumers and environment. Therefore, petroleum ether plant part extracts of locally available plants are tried to control seed mycoflora of the pulse. Almost all plant extracts showed restrictive effect on seed mycoflora of the test pulse. Significant plants that controlled seed mycoflora in higher percents are *Azadirachta indica* A. Juss., *Cyperus rotundus* L., *Ocimum basilicum* L., *O. americanum* L., *O. sanctum* etc.

Keywords: seed mycoflora, pulses, plant extracts.

INTRODUCTION

Green gram (*Vigna radiata* L.) is important pulse crop in Maharashtra, it is affected by different fungal pathogens as seed mycoflora which is harmful to seed health, seed content and ultimately to yield. Association of the fungi with the seed has found to be harmful to the seed health and seed content. Total seventeen seed-borne fungi (*Alternaria tenuis, A. alternate, Aspergillus carbonarius, A. flavus, A. niger, A. nidulans, A. fumigatus, Cladosporium spp., Colletotrichum truncatum, Chaetomium globosum, Curvularia lunata, Drechslera tetramera, Fusarium moniliforme, Fusarium oxysporum, Penicillium spp., Rhizopus stolonifer, Macrophomina phaseolina*) were isolated from the test pulse, on Agar plates and Moist blotters. Agar plates showed more fungal incidence compared to Moist blotters. Among seventeen fungi isolated and identified, six dominant fungi *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera, Fusarium oxysporum* and *Rhizopus stolonifer* taken for the study. These six dominant seed-borne fungi of Green gram were tested against plant extracts of eighteen commonly and locally available plants.

MATERIALS AND METHODS

Preparation of petroleum ether plant extracts:
Five g powder of each of the plant parts was dissolved separately in mixture of 50 ml petroleum ether and 50 ml distilled water; in 250 ml borosil glass conical flasks. The flasks were kept in oven (Metlab) for 24 hours at 60oC and the content was filtered through Whatman filter paper No.1. The filtrates were used as 5% plant extracts.

Evaluation of seed mycoflora and seed health (seed germination, seedling emergence, shoot, and root length) of pulse.
During present studies, the seeds of Green gram were soaked separately in the leaf, stem, and root petroleum ether extracts (petroleum ether and water 1:1) of the selected plants for 24 hours. The effect of extracts on seed health was studied by placing seeds of test pulse on moist blotter plates and incubated for ten days at room temperature. On eleventh day percent seed mycoflora, seed germination, root and shoot length was recorded. The seeds soaked in sterile distilled water served as control.

For seedling emergence, seeds of Green gram were treated as mentioned above and sown in earthen pots containing sterilized soil for ten days; at room temperature. On eleventh day percent, seedling emergence, root, and shoot length was recorded. The seeds soaked in sterile distilled water served as control.

RESULTS AND DISCUSSION

Three plant parts like leaf, stem, and root were used in the experiments. Extracts prepared from leaf of most of the test plants were found more effective against seed mycoflora and stimulatory for seed germination, seedling emergence, shoot and root length of the test pulse. It is evident from the tabulated results that, plant extract of all test plants showed inhibitory effect on seed mycoflora and supporting or stimulating seed germination, shoot and root length of test pulse, with few exceptions. The plants that caused maximum reduction in seed mycoflora were Ocimum basilicum L. (leaf 9 %, stem 10 %), *Ocimum americanum* L. (stem 10 %, root 10 %, and leaf 15 %), *Azadirachta indica* A. Juss (leaf 10 %, stem 20 %, and root 30 %) and *Cyperus rotundus* L. (leaf 10 % and rhizome 30 %). Plants like *Samania saman* (Jacq.) Merr. (root 60 %, leaf 50 % and stem 50 %), *Melingtonia hortensis* (root 60 % and stem 50 %) and *Croton tiglium* L. (stem 50 %) were found to be less effective in controlling seed mycoflora of test pulse.

Enhanced seed germination and stimulatory effect was reported in plant extract of *Ocimum basilicum* L. (leaf 100 %, stem 100 % and root 100 %), *Ocimum sanctum* L. (leaf 100 %, stem 100 % and root 80 %), *Azadirachta indica* A. Juss (leaf 100 %, stem 80% and root 90 %) etc. Seed germination was inhibited due to the extracts of *Ruelia tuberosa* L. (leaf 20 %) and *Acorus calamus* L. (rhizome 50 % and leaf 60%). Root length was maximum due to *Acorus calamus* L. (rhizome 6.7 cm); *Ocimum basilicum* L. (leaf 6.6 cm and root 6.4 cm) and *Croton tiglium* L. (stem 6.3 cm). Root length was suppressed due to *Tagetis erecta* L. (root 2 cm). In majority of the cases there was less growth in shoot length over the control except in few cases like *Ciba pentandra* (stem 4.7 cm), *Ocimum basilicum* L. (stem 4.6 cm) and Ocimum sanctum L. (root 5 cm) and it was at par with control in *Cyperus rotundus* L. (leaf 4.6 cm).

The plant extracts of *Ocimum sanctum* L., *O. americanum* L., *O. basilicum* L., *Azadirachta indica* A. Juss, *Cyperus rotundus* L., *Eucalyptus lanceolatus*, *Ruelia tuberosa* L. etc. reduced seed mycoflora and stimulated seed germination, seedling emergence, shoot and root lengths of pulses in variable degrees. The results also suggest that, none of the test plant extracts could completely inhibited seed mycoflora and plants like *Samania saman* (Jacq.) Merr., *Melingtonia hortensis*, *Tagetis erecta* L. were less effective on seed mycoflora and seed health as well.

Table 1: Effect of petroleum ether extract (petroleum ether and water 1:1) of different plant parts on Seed mycoflora and seed health (seed germination, shoot, and root length) of Green gram (*Vigna radiata* L.) on blotters (after ten days of incubation).

Sr. No	Source plant	50% petroleum ether + 5gm powder	Seed mycoflora (%)	Seed germination (SG)		
				SG (%)	RL(cm)	SL(cm)
1	*Acorus calamus* L.	Leaf	30	60	5.8	2.7
		Rhizome	40	50	6.7	05
2	*Adenanthera pavonia* L.	Leaf	20	60	3.1	2.1
		Stem	40	70	4.1	4.4
		Root	50	100	5.3	4.5
3	*Azadirachta indica* A. Juss.	Leaf	10	100	5.1	3.2
		Stem	20	80	5.6	3.1
		Root	30	90	5.2	4.1
4	*Butea monosperma* (Lam.) Taub.	Leaf	40	70	5.8	03
		Stem	30	90	5.5	4.1
		Root	20	100	5.6	4.5
5	*Carum copticum* Benth & Hook. f.	Leaf	42	90	5.5	3.1
		Stem	20	100	5.2	02
		Root	30	80	4.2	3.3
6	*Ciba pentandra*	Leaf	17	100	5.1	04
		Stem	21	80	06	4.7
		Root	20	90	5.3	3.1
7	*Croton tiglium* L.	Leaf	30	100	5.3	2.2
		Stem	50	90	6.3	4.3
		Root	40	90	5.6	3.8
8	*Cyperus rotundus* L.	Leaf	10	100	5.8	4.6
		Rhizome	30	70	5.2	3.3
9	*Eucalyptus globulus.* Labill.	Leaf	10	100	05	04
		Stem	10	80	4.5	3.3
		Root	20	90	5.1	4.2
10	*Melingtonia hortensis*	Leaf	42	90	05	4.2
		Stem	50	100	5.3	4.3
		Root	60	80	5.3	4.2
11	*Muntangia calabura* L.	Leaf	36	90	06	05
		Stem	20	90	5.1	4.2
		Root	20	80	5.2	03
12	*Murraya koinigii* (L.) Spreng.	Leaf	40	100	06	2.3
		Stem	27	60	04	2.9
		Root	28	100	5.6	4.3
13	*Ocimum basilicum* L.	Leaf	09	100	6.6	4.2
		Stem	10	100	06	4.7
		Root	30	100	6.4	4.1
14	*Ocimum americanum* L.	Leaf	15	80	5.3	4.1
		Stem	10	90	5.2	4.3
		Root	10	100	5.5	3.2
15	*Ocimum sanctum* L.	Leaf	20	100	06	3.1
		Stem	30	100	06	4.2
		Root	40	80	5.7	05

Table 1: Continued…

Sr. No	Source plant	50% petroleum ether + 5gm powder	Seed mycoflora (%)	Seed germination (SG)		
16	*Ruelia tuberosa* L.	Leaf	38	30	4.3	3.3
		Stem	30	90	3.6	3.1
		Root	40	60	4.2	3.3
17	*Samania saman* (Jacq.) Merr.	Leaf	50	100	5.3	3.2
		Stem	50	90	03	2.9
		Root	60	70	5.1	4.2
18	*Tagetis erecta* L.	Leaf	34	60	05	4.2
		Stem	30	100	5.3	4.3
		Root	40	90	02	03
19	Control	Sterile distilled water	60	70	05	4.6

RL – Root length; SL – Shoot length; SG- Seed germination

Similar findings were recorded on different crops by various workers like Gomati et al. (2000), Ahmed and Aquil (2003), Patni et al. (2005), Oana Rosa-Casian et al. (2007) and Duraipandiyan and Ignacimuthu (2007). Umer et.al. (2014) studied Antifungal potential of twenty antagonistic plants was assessed against the most damaging phytopathogenic fungus Macrophomina phaseolina. All the test plants inhibited the growth of *M. phaseolina* significantly to varying levels. Arshad javed et.al (2012) studied antifungal potential of an allelopathic grass *Sorghum halepense* Pers. for the management of *M. phaseolina* isolated from charcoal rot infected cowpea plants. In laboratory bioassays, different concentrations (0, 0.5, 1.0, 3.0 g/ml) of methanolic extracts of shoot, root and inflorescence of the test grass were evaluated for their in vitro antifungal activity against *M. phaseolina*. Extracts of all the three parts of the grass exhibited variable antifungal activity. Emad M. El-Kholie et.al. (2012) shown antifungal effects of ehanolic and methanolic extracts of *Azadirachta* on different fungi. Manoorkar et.al. (2015) reported antifungal effect of ethanol and aqueous extracts of leaf & latex of *Calatropis procera* (Ait.) against ten seed-borne dominant fungi viz., *Curvularia lunata*, *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium solani*, *Penicillium chrysogenum*, *Aspergillus niger*, *A. flavus*, *A. terrus A. fumigatus*, and *Rhizopus* sp were effective. Zakaria et.al. (2015) found that ethanolic extracts of *Datura strumanium*, *Mentha longifolia* and *Malva parviflora* were effective against *Alternaria alternata*, *Botrytis cinerea*, and *Penicillium italicum*.

CONCLUSION

Out of the 15 different powdered samples, four samples were selected for phytochemical analysis because they occur commonly in the selected area. Out of these selected samples, *Ganoderma* is used as folk medicine whereas *Daedalea* is a common wood inhabiting fungus. One of the shop samples was also selected for phytochemical analysis due to its medicinal importance. The results of phytochemical analysis indicated the presence of amino acids like leucine, phynelalnine, and tryptophan in all five samples. The *Ganoderma* samples having brownish colour with a sweet and pleasant odour showed the presence of terpenoids. The *Daedalea* samples having a cream-yellow colour with a sweet odour

showed the presence of sugar alcohol s like mannitol. All samples showed the presence of polyphenols which on hydrolysis formed catechol and salicylic acid. The presence of organic acids like citric, malic, succinic and tartaric acid was also detected. The results of chemical analysis showed that in the *Ganoderma* samples and the shop sample, the values of amino acids, polyphenols and mannitol were close to each other indicating similarities in their active ingredients. Out of 35 species of wood rotting fungi, five samples were analysed. This indicates that there is enough potential for further studies.

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RESEARCH ARTICLE

Effect of chemically synthesized Coumarin derivative against *Candida albicans* as an antifungal agent

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ABSTRACT

One Coumarin derivative of biological interest was synthesized by green synthesis method chemically. This derivative was evaluated for its antifungal activity against yeast *Candida albicans* by Agar Well Diffusion method. The Coumarin derivative was found to show antifungal activity against *Candida albicans.*

Keywords : Coumarin, Antifungal, Green synthesis, *Candida albicans*.

INTRODUCTION

Coumarins are well-known plant derived natural product that are verified to have antioxidant, anti-inflammatory (Sandhya *et al.*2010)), anti-coagulation, estrogenic, dermal-photosentisizing, vasodilator (Joshi *et al.* 2012) molluscidal, anti-helminthic, sedative, hypnotic, analgesic, hypothermic and antiulcer activities (Monga *et al*., 2012). Coumarins are extremely variable in structure, due to the various types of substitution in their basic skeleton, which can influence their biological activity (Kumaresan *et al.,* 2013; Chaudhary and Datta, 2014). Coumarins are important oxygen containing fused heterocycles used in drugs and dyes and the incorporation of of fused component into parent coumarin converts it into natural coumarin that is found to have antidiabetic activity, anabolic antioxidant and hepato-protective activities (Chitra *et al.,* 2014; Cristina *et al.,* 2008). The synthesis of coumarins and their derivatives has attracted the attention of organic and medicinal chemists, as these are widely used as fragrances, pharmaceuticals and agrochemicals (Helcio *et al.,* 2015; Montagner *et al*., 2008; Kasumbawe *et al*., 2014). The present study aims to evaluate the antifungal activity of chemically synthesized coumarin derivative against pathogenic *Candida albicans* to be used as a potent antifungal agent.

MATERIAL AND MATERIALS

1) General procedure for synthesis of Coumarin derivative: The Coumarin derivative of biological interest was synthesized by mixing Ethyl acetoacetate and Resorcinol in equimolar ratio in a conical flask.To this 30 units of Porcine Pancreatic Lipase was added followed by 5ml water and 5ml ethanol.This was incubated for 12hrs and poured in water.It was filtered washed with water and recrystallised from alcohol.

2) Test Microorganism: The test Microorganism used was a pathogenic Candida *albicans*.

3) In-Vitro Antifungal Activity: The antifungal activity of Coumarin derivative was evaluated by Agar Well Diffusion method using a culture density of 1.5x10^{8}cfu/ml by visually comparing the density with 0.5Mcfarland standard. The 20ml of Sterile Sabouraud Dextrose Agar was poured into each sterile petri plate and the plates were swabbed with 100uL of the test fungi and were kept for 20 minutes at room temperature for adsorption. Then using sterile cork borer of 8mm diameter, wells were bored in seeded agar plates and these were loaded with a 100uL volume with concentration of 1000ug/ml of the coumarin derivative reconstituted in ethanol and DMSO (1:1). All the plates were incubated at Room Temperature of 25^{0}C for 72hrs. Antifungal activity indicated by an inhibition zone surrounding the well containing the compound was recorded if the zone of inhibition was greater than 8mm. DMSO was used as a negative control and Clotrimazole (1000ug/ml) was used as a positive control.

RESULTS

The Coumarin derivative was synthesized by green synthesis method chemically. The derivative was checked for its anti-fungal activity against yeast *Candida albicans* by Agar Well Diffusion method on Sterile Sabouraud Agar plate using Clotrimazole (1000ug/ml) as a positive control and DMSO (Dimethyl Sulphoxide) as a negative control. By measuring the diameter of zone of inhibition in mm, it was found that the product was effective in inhibiting the pathogen *Candida albicans* The results of antifungal activity shown by coumarin is in accordance with the findings of Helcio *et al*. (2015) and Montagner *et al*. (2008). A careful analysis of the data for antifungal activity shows that there may be the presence of hydroxy group in coumarin that may be found to possess antifungal activity against *Candida albicans* (Montagner *et al*., 2008).

Table1: Antifungal activity of synthesized CD.

Coumarin Derivative	*Candida albicans*- Zone of Inhibition(mm)
PRODUCT	20

CONCLUSION

The Coumarin Derivative was synthesized to check for its anti-fungal activity against *Candida albicans* by Agar Well Diffusion method.It was found that the Coumarin derivative showed antifungal activity. Hence, we can conclude that this derivative is active against pathogenic *Candida albicans* and can be used as potent antifungal agent.

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ABSTRCATS

ABS-001

Degradation of diesel by soil fungi

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Abstract

Environmental pollution due to oil spill is one of the common problems along the coastal region. Technologies that involves microbes and plants for the remediation of soils.Groundwater contaminated with organopollutants are widely used for their environmentally friendly impact combined with low cost compared to physical treatments. Fungi grow on organic pollutants and their survival in extreme environmental conditions make them promising bio remediating agent. Present study aims to identify and isolate petroleum hydrocarbon degrading fungi. Soil samples were collected from areas contaminated with oil (garage soil). Selected fungal isolates were identified and screened to assess degradation of petroleum hydrocarbon (diesel). Fungal Isolateswere cultured in Bunshell Haas medium which exhibited decolourization of a redox indicator 2,4-dichrophenol indophenol. 45-72% percent degradation was observed in the isolates.

***

ABS-002

Inhibitory activity of antibodies against plant pathogenic fungi

Nirmalkar Vaishali S

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

Abstract:

Fungi, one of the agents causing diseases in plants, destroy many agricultural crops, fruits and nut plants. They are a major problem in agricultural crops, along with other pests. Many insecticides and pesticides are available in market for various pests. However, they are very costly and also specific towards the agent to be inhibited. Taking these points into consideration, an experiment was designed to produce antibodies against these fungi, another strategy for biocontrol. Antibodies were induced against *A. niger* whole cell antigen. From the antiserum IgG purification was carried out through ion exchange chromatography and purified IgG was used to test the inhibition of all the test plant pathogenic fungi. The antibodies were tested in broth as well as radial growth assay was performed in solid media. It was active for more than fifteen days in inhibiting the spore germination in all test fungi except *A. niger.* Whereas in *A. niger* from seventh day onwards spore germination was observed. Thus these antibodies raised, showed cross reactivity with all the tested fungi. Maximum inhibition was found in *Curvularia sp., Rhizoctonia bataticola, Botryodiplodia theobromae, Alternaria porri, Fusarium oxysporum, Fusarium solani* and *Aspergillus niger,* respectively. This immunological approach may prove to be better in elucidating the pathogenic molecule and its extent, giving us the knowledge of its control measures to be implemented.

***

ABS-003

Fungi in the Indoor Environments

Rambal Kavita

Department of Botany, Maharishi Dayanand College of Arts, Science & Commerce. Parel, Mumbai

Abstract:
Life without air is impossible for all living beings and it needs to be free from chemical, particulate or biological material i.e., free from any pollutants. Indoor air is becoming increasingly a greater health hazard than outdoor air as people spend more time time indoors.Indoor air pollution also refers to air quality within around buildings and structures especially as it relates to the health and comfort of the people there. Indoor air quality issues associated with exposure to moulds and their metabolites are gaining importance. Fungal species because of ecological adaptation exploit the built environment.The present study was undertaken in the indoor environments of a high school. The present study aimed at identifying and isolating the major fungal organism in the school campus. A total of 9 fungal species and 5 genera were recorded in the present study. The most dominant fungi were Rhizopus stolonifer and Aspergillus niger.

***

ABS-004

Pathogenic Mycobiota of Soybean in Marathwada

Bhosale Shrikant B, Jadhav DS and Chavan Ashok M

Shikshan Maharshi Dnyandeo, Mohekar Mahavidyalaya Kallamb, Dist Osmanabad, India.

Abstract
Soybean (*Glycine max (L.) Merr*.) is the today's world leading vegetable oil and protein producing crop, which is cultivated in Marathwada as well as Vidharbha. Soybean crop is infected by bacteria, viruses and fungi all over the world wide. Fungi associated with seed of 14 cultivars of soybean were investigated and 27 species were isolated by PDA method. Pathogenic fungi frequently isolated fungi *viz. Verticillium lecanii, Rhizoctonia solani, Fusarium oxysporum, Fusarium solani, Alternaria alternata, Colletotrichum gloeosporioides, Aspergillus flavus, Trichoderma viride, Helminthosporium* spp*. Penicillium notatum* etc. Literature reports that soybean crops in the region of Marathwada such as districts Aurangabad, Osmanabad, Latur, Beed, Nanded, Hingoli, Parbhani and Jalna infected by over 20 pathogenic fungus. In our investigations the number of established species was lower than the number of isolated species. Based on the results of the literature review and of the research, it could be concluded that significance of the pathogenic fungal fauna connected with soybean has changed over the time. Obtained results indicate that the increase in fungal population and that some existing species became significant of soybean disease. Soybean ecosystems including its varying feeding strategies and needs much additional attention.

***

ABS-005

Diversity of Phyllosphere Mycobiota in Conventional and Organic Cotton Fields

Mane Shrikant B and Chavan Ashok M

Seed Pathology and Fungal Biotechnology Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Abstract

The phyllosphere represents the habitat of aboveground parts of plants, supports a large and complex microbial community. Microbial interactions in the phyllosphere can affect the fitness of plants in natural communities, the productivity of agricultural crops. The structure of phyllosphere communities reflects immigration, survival and growth of microbial colonists, which is influenced by numerous environmental factors in addition to leaf physico-chemical properties. In Indian agriculture scenario Cotton is vital commercial crops playing a key role in the economical, political and social affairs of the country but growers suffering from major losses due to foliar diseases to this crop. For this investigation leaf samples were collected from conventional fields allow to grow on RBA and PDA. Most common fungal species isolated from Cotton *Gosssypium hirsutum* were the qualitative and quantitative fungal occurrence difference was reported from phyllosphere of conventional and organic Cotton fields. The fungal species like *Alternaria macrospora, Colletotrichum gossypii, Fusarium moniliforme, Penicillium chrysogenum, Penicillium notatum, Pythium species, Rhizoctonia solani, Sclerotium rolfsii* were reported more from conventional Cotton fields contrary in organic Cotton *Trichoderma species* were more.

***

ABS-006

Biosorption of Zinc & Lead from water using *Pleurotus sps.*

Jagtap Pratik, Tiwari Pooja, Didwana Vinodkumar, Satvi Vaibhav and Yadav Madhulika

Department of Botany, VIVA College, Virar.

ABSTRACT

Improper management and increasing discharge of comprehensively polluted waste in the water create drastic effect on terrestrial as well as aquatic ecosystem. Remediation of water contaminated with heavy metals received considerable attention in recent years. Mycoremediation integrating the removal of toxic compounds employing the fungi is an efficient tool as it tolerates extreme physical and chemical condition. It is also cost effective and its end products are non-hazardous. In the present study, the white-rot fungi *Pleurotus sps.* was investigated for its ability to remediate. The water containing different concentration of Zinc and Lead were evaluated and mycelium was found to be effective in biosorption of these metals. Thus results indicate *Pleurotus sps.* have potential source for remediating waste water contaminated with heavy metals.

***

ABS-007

Isolation of *Penicillium* species from different seeds of Marathwada

Shaikh Nishat U and Chavan Ashok M

Seed health and fungal biotechnology laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS).

Abstract

Penicillium is one of the most important threaten in seed health and seed quality. After harvesting seeds were stored in store houses, containers etc. Since the harvesting to transportation and storage many microbes get interacted with seeds. When the favorable conditions arises like humidity, moisture; those microbes associated with seeds starts their growth and multiplication. *Penicillium* is the one of most important fungal genus associated with seeds during storage condition. During favorable condition they grown on the seeds; it will reduce the biochemical constituent of seeds. It also secretes some poisonous secondary metabolite in the seed, that seeds are not good for the human consumption. In the present study seeds of different crop plants were collected from different markets, store houses to isolate different *Penicillium* species associated with those seeds. Seeds of Sorghum, maize, Bajra, Groundnut, Soyabean, Safflower were screened for this study.

***

ABS-008

Isolation of Storage fungal Pathogen from (Allium Cepa L.) in Marathwada

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Abstract:

Onion is one of the most important commercial vegetable crop grown in world as well as India. The major onion growing state in India is the Maharashtra, Present study was investigated of the isolation of fungal diseases in the fieldof onion. Onions infected from many fungal diseases, such as white rot, Black mold, Blue mold, Botrytis brown stain, and Smudge etc.Black rot and Blue mold is the major fungal Storage diseases. Complete causes the number of onion are every year by fungi. Diseases werecollected from the different varieties likewise,N-53, Puna fursingi, Panchganga and China red of different locality of Marathwada like, Osmanabad, Latur, Beed, , Jalna, Aurangabad, Nanded and Parbhani districts. Fungi were isolatesfrom the infected storage onions of different pathogen as like, Aspergillums sps, Botrytis sps, Penicillium sps and Colletotrichum sps. etc isolated fungi in Marathwada region for this during study.

***

ABS-009

Pathgenic fungal Fauna and Association of Soybean in Marathwada

Bhosale Shrikant and Jadhav DS

Shikshan Maharshi Dnyndeo Mohekar Mahavidylay, Kallamb, Dist Osmanabad

Abstract:
The importance of soybean (*Glycine max (L.) Merr.*), as today's world leading oil and protein crop, is increasing in marathwada. Soybean crops are attacked by over 180 pests, 150 insects and 30 species from other animal classes all over world among which approximately 25 pest species are the most important .Fungi associated with seed of 14 cultivars comprising 16 samples of soybean were investigated and 27species were isolated. Pathogenic fungi frequently isolated, *Colletotrichum dematium, Diaporthe phaseolorum, Fusarium equiseti, F. fusarioides, F.moniliforme, F. semitectum, Macrophomina phaseolina, Myrothecium roridum, and Phoma sorghina*.Other fungi isolated included species of *Aspergillus, Cladosporium, Curvularia, Nigrospora, odulisporium, Penicillium, Rhizopus, Trichoderma , Zygosporium and rhyzktonia* species. Literature reports that soybean crops in the region where arathwada belongs (Aurangabad, Osmanabad, Latur, Beed, Nanded, Hingoli, Parbhani and Jalna) are attacked by over 20 pathogenic fungus . In our investigations the number of established species was lower than the number obtained by literature review. Based on the results of the literature review and of the research conducted, it could be concluded that significance of the pathogenic fungal fauna connected with soybean has changed over the time. Obtained results indicate that the increase in fungal population has occurred and that some existing species became significant of soybean disease. Soybean ecosystems including its varying feeding strategies and needs much additional attention.

***

ABS-010

Fungal diversity around wetlands of Gad River, Kankavli, District Sindhudurg

Desai Sanjay M
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Abstract:
The lower group of plants that is fungi are capable of degrading organic material into simpler form. This work was undertaken in the area under study i.e. Gad river, Kankavli, District Sindhudurga, Maharashtra. The research revealed that wetland fungi are capable of breaking down the woody tissues of leaves, small woody sticks those are carried along with water and enters wetlands near the river. This helps in degradation of such material, resulting into release of carbon into ecosystem. The work done was an effort to search for such fungi members within the vicinity of area under study.

***

ABS-011

Spore to spore agar culture of *Diachea subsessilis* peck : A Myxomycetes

Phate Preeti V

Department of Botany, J.S.M. College, Alibag, Raigad 402201 (M.S.)

Abstract:
From the known species of myxomycetes only 10% species have been cultured till now of which most of the species fall under two only genus i.e. *Physarum* and *Diderma*, but still do not provide the complete knowledge about the biology of myxomycetes. These eukaryotic organisms were found to be the source of about 100 novel secondary metabolites, thus it becomes the need of the present to culture and explore these organisms so that they can serve the society as they have also shown the potential for the development of drugs for clinical trials. *Diachea subsessilis* is characterized by deep blue globose sporangiate fruiting body, white milky phaneroplasmodium and complete reticulate spores. The said species was grown on wide range of agar medium and found to complete is life cycle in about 30 days. The present paper takes an opportunity of providing first report on agar culture of *Diachea subsessilis*, a member of Physarales. Thus the present research work adds one more species to the available list of cultured species of myxomycetes.

***

ABS-012

Isolation of major Mycotoxins producing fungi from Marathwada

Bhalerao Varsha A and Chavan Ashok M

Seed health and fungal biotechnology laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS).

Abstract
Cereal seed samples collected from 6 districts of Marathwada were investigated for present study. Cereals like Wheat, Bajra, sorghum and Maize, during improper storage conditions interacts with varieties of fungi, bacteria and nematodes, Biodeterioration of seed occurs due to the impact of certain fungi. They affect on the seed quality, shape, size & germinability of the seeds. The major mycotoxins producing fungi are species *Alternaria spp, Aspergillus spp, Fusarium spp* and *Penicillium spp.* Maize seed samples shows maximum incidence of *Alternaria alternate* , *Aspergillus flavus* , *Aspergillus terreus* ,*curvularia lunata, Fusarium spp* and *Penicillium spp*. Followed by Wheat seed as compare to other cereal samples. These are the major toxigenic fungi which contaminate large amount of seeds as well as food product, and that contaminated seeds are not good for human consumption.

***

ABS-013

Occurrence of toxigenic fungi in *Sorghum bicolor* (l.) Moench

Sherkar Dnyaneshwar K and Chavan Ashok M

Seed health and fungal biotechnology laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS).

Abstract
Sorghum is one of most important cereal crop after Rice, Wheat, Maize and Barley. It is also a good source of carbohydrate and minerals. Sorghum crop reduces its yield and nutritive values due to the impact of different diseases. The present investigation was carried out to screen the occurrence of toxegenic and non-toxigenic fungi from sorghum grain, in the field condition as well as in storage condition, and to check their impact on the nutritional status and germinability of sorghum seed. Seeds of different varieties of sorghum where collected from various regions of Marathwada, and brought into the laboratory for further investigation. The results indicated that the fungi of five genera were isolated from the examined seeds. The isolated fungi include *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. fumigatus, A. paraciticus, Curvularia lunata, Fusarium monilliformae, F. oxysporum, Penicillium chrysogenum* and *P. citranum. Alternaria alternata, Aspergillus flavus, Curvularia lunata and Penicillium chrysogenum* reduces the germinability of sorghum seeds, whereas *Curvularia lunata, Penicillium citranum, Fusarium moniliformae* and *F. oxysporum* reduces the nutritive values of sorghum grain.

***

ABS-014

Some interesting Hyphomycetean Fungi From Vidarbha (Maharashtra)

Kaste PS

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Abstract
During the course of mycofloristic studies of Melghat forest and Amravati region, several interesting form-fungi were collected, including seven hyphomycetean members. All the members were growing saprophytically on dead stems and fruits of various angiospermic plants. On microscopic examination and comparison, the present fungi were found to be indistinguishable with that species. All of them are new host reports (Bilgrami. *et.al,* 1992: Subramanian, 1997: Ellis, 1971; Sarbhoy. *et al,* 1982 - 92), except *Pleurothcium recurvatum* (Morgan) Hohnel, found on dead unidentified dicot stem. The specimens were examined and deposited at 'Ajrekar Mycological Herbarium', Agharkar Research Institute, Pune (India) as holotype and the accession numbers were received as AMH No.

***

ABS-015

Mycoremediation of Plastic material-A review

Dubey Pooja[1] and Satnam Singh Sohal[2]

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Abstract

Plastics are one of the synthetic polymers or man-made polymers. The accumulation of the plastic is responsible for the most unique and long lasting changes to the environment. The composition of the plastics consist carbon, hydrogen, silicon, oxygen, chloride and nitrogen. Because of its stable and durable characteristic, plastics are widely used. Mostly used plastics are polyethylene (LDPE, MDPE, HDPE and LLDPE), Poly Ethylene Terephthalate (PET), Polybutylene Terephthalate (PBT), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR). These are the synthetic polymers which accumulate in the environment due to the absence of efficient methods for safe disposal and posing an ever increasing ecological threat to flora and fauna. The most attractive plastic waste treatment method is fungal enzymatic degradation. Many reports have published to emphasize the role of fungi and various enzymes secreted by them in bioremediation of wastes by the process of biodegradation, biosorption and bioconversion. Fungi of the genera *Alternaria*, *Aspergillus*, *Phoma*, *Pennicilium*, *Plectosphaerella*, *Geomyces*, *Nectria*, *Emericella, Fusarium, Trichoderma and Gliocladium* and *Neonectria* are known to degrade PUR samples. *Geomyces pannorum* is the most commonly isolated PUR-degrading organism.Some of the mushrooms have also shown the ability to degrade plastic e;g;*Pleurotus ,Lentinula,Ganoderma* etc. Few organisms have been shown to degrade PUR as a sole carbon source. *Aspergillus niger* and other species has some reported degradation activity. This review paper focuses on role of fungi in degrading plastic material which is a major environmental pollutant today

***

ABS-016

Study of Mycorrhizal association with bottle gourd

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Abstract

An attempt has been made to survey of arbuscular Mycorrhizal fungi (AMF) associated with Bottle guard (*Lagenariasiceraria*) plant.Rhizosphere soil and roots samples of bottle gourd plant were collected from two localities of Sangamner Akole area. Two genera with eight species were reported from above localities. The genus *Glomus* was most common with six species two species of *Scutellospora* were reported.The average number of AM propagules ranged from 38 to 60 per 100 g soil, while the AM fungal root colonization ranged from 20 to 60%.

***

ABS-017

Screening of mycoflora from fruit wastes

Deshpande Ashwini and Srinivas R.

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Abstract

Fruits carry dormant pathogenic epiphytic mycoflora. There is a rapid progress in the field of chemical detection technology. This technology appears to be involved in estimation of remaining shelf-life of fruits and early detection of spoilage process in fruits. Predictive microbiology aims to summerise the probable behavior of specific spoilage organisms and progression of spoilage process in fruits. The quantitative knowledge generated in the field of predictive mycology provides a sound basis for the rational development of devices with which it becomes easy to monitor loss of shelf-life during storage, distribution and retail sale of edible fruits. To predict remaining shelf –life accurately ,it is necessary to consider the microbial ecology of the fruit system. The isolates from ripened mango and guava fruits were studied as tropical seasonal fruits. The Gram's Staining Technique was used. The study revealed presence of mycoflora like *Aspergillus, Fusarium, Mucor, Rhizopus, Botrytis, Gotrichium, Candidum* etc The isolation of such fungal species will help in detection of the type of organism by chemical tests so as to decide which antimycological agents should be used. This may not help only in a small-scale level but also in large pomology industries to delay early spoilage and increasing shelf- life of edible fruits so that proper measures can be adopted by preventing it to become waste.

***

ABS-018

Isolation of alkaliphilic fungi from lonar lake

Bari Kishor P. and Unnati Padalia

Department of Microbiology, K.J. Somaiya College of Science and Commerce, Vidyanagar, Vidyavihar, Mumbai-400077

Abstract

Present study deals with the screening of the alkaliphilic fungi from the water and sediment samples from the LonarLake.The main aim of our work is to isolate the fungi which can grow at alkaline pH. Two alkaliphilic fungi were obtained on Sabouraud Dextrose agar (pH-11) from Lonar lake after enrichment. This screening demonstrates that there exists a population of the fungi which can grow at alkaline pH.

***

ABS-019

Increase in bulbil formation of *Curculigo orchioides* grown in shake flask cultures elicited with *Aspergillus niger* and *Penicillium notatum*

Meeta Mathur* and Manisha Chotrani

Dept. of Botany, Mithibai College Vile Parle (W) Mumbai 400056

Abstract :
Increase in bulbil formation from leaf explants of an endangered monocot herb, *Curculigo orchioides*, grown in shake flask cultures has been achieved using biotic elicitors *viz. Aspergillus niger* and *Penicillim notatum.* The leaf explants were obtained from *in vitro* grown plantlets maintained on MS solid medium containing BA (0.1 mg/L) and IBA (0.1 mg/L) for the past 8 years. The use of this biotic elicitor to note the change in the bulbil formation was significant over other treatments. In the present study the effect of MS-medium supplemented with different concentrations of biotic elicitor *Aspergillus niger* and *Penicillim notatum* on growth parameters was investigated. The number of bulbils per explants doubled during 4 to 8 weeks growth while total yield (fresh biomass) increased four folds. Formation of plantlets from bulbils was 60% and their survival was ~50% in the field conditions. These results will be helpful in developing technology for micropropagation of the plant using bioreactor.

***

ABS-020

Influence of arbuscular mycorrhizal (am) fungi on the growth of Safflower (*Carthamustinctorius*) plants.

Palkarand Shyam S and Heble Mangala S

Department of Botany, D.G. Ruparl College, Mumbai -400016.

Abstract
Vegetables that are produced by using biofertilizers are gaining great importance than those produced by chemical fertilizers. Safflower (*Carthamustinctorius*) ,the crop is grown for its oil seeds and the petals are used to produce dye of commerce. Mycorrhiza is the symbiotic association of fungi with plant roots. Arbuscularmycorrhizal(AM) infection may increase the growth of the host plants, especially when nutrient supply in the soil is low. Arbuscularmycorrhiza was foundto be associated with Safflower plants. There was significant difference between height and biomass of AMinoculated Safflower (*Carthamustinctorius)* plants and the control Safflower (*Carthamustinctorius)* plants during the time period of 90 days of growth.

***

ABS-021

Phytochemical analysis of some wood inhabiting and fleshy fungi used as folk medicine

Pillai Pramoda S and Vaidya Charuta S

Guru Nanak College of Arts, Science and Commerce, GTB Nagar, Mumbai-400037

Abstract

A study of the fungal flora of Yeor Hills and parts of Sanjay Gandhi National Park showed that there were more than 100 specimens belonging to different groups of fungi. Of these 35 species were identified as wood rotting fungi. Literature survey reveals that some of these wood rotting fungi have been used as medicine by the local tribes. Of these, fungi belonging to the order Aphyllophorales have medicinal uses against piles, diarrhoea, dysentery, jaundice, tumours, wounds, etc which has been reported by various workers. The present study deals with the phytochemical analysis of commonly occurring species of *Ganoderma, Daedalea* and *Phellinus.* Detection of active ingredients such as polyphenols, amino acids, organic acids and terpenoids has been carried out.

***

ABS-022

Diversity of Macrofungi and Myxomycetes at Palasdari, Maharashtra

Pathak Anubha and Vaidya Sharda

Smt. C. H. M. College, Ulhasnagar-03

Abstract

Palasdhari is a small village situated in Werstern Ghats 3 km away from Karjat. The place now developing as a picnic spot because of presence of waterfall. The way to waterfall is the area of dense mixed deciduous forest dominated by the trees of *Butea, Bombax, Tectona* and the shrubbry growth of *Woodfordia*. These plants cover the ground completely. Their leaves and branches fall and create organic debris below the plants in the shade. This area then becomes the site of development of diverse types of macrofungi and the slime molds. But due to encroachment of human beings as trekkers and the people visiting for the picnic, this natural habitat is getting disturbed and it may be ruined or lost completely in future. It is a must at this stage to study and protect this biodiversity. With this aim, the study was undertaken and frequent visits were made to the place. The specimen were collected. The identification was done by first taking section of gills and stipes and by observing the spore colours and referring to standard literature. Many species were observed but a few that were in critical condion of survival are described here.

***

ABS-023

Rhizosphere and nonrhizosphere mycoflora of *Rauwolfia serpentina* l.

Vaidya Sharda[1] and Mishra RL [2]

[1] Smt. C. H. M. College, Ulhasnagar-03
[2] J. S. M. College, Alibag.

Abstract
Rhizosphere is the region around the root in the soil with immense activity. The studies in this region are important for studying the relationships of soil organisms with roots and in turn throw the light on the productivity and metabolism of the plant. There are diverse types of microorganisms in this region. In the present investigations only fungi from the rhizosphere of *Rauwolfia* have been studied. *Rauwolfia* is a plant of medicinal importance in high blood pressure, insomnia, etc. The plant is in the threatened category under natural conditions. In the present investigations, the rhizosphere soil of this plant was cultured on PDA, AMA, LEA and MA media. Among the different fungi isolated, *Aspergillus* was a dominant genus.

***

ABS-024

Biosorption of Zinc & Lead from water using *Pleurotus sps.*

Jagtap Pratik *, Tiwari Pooja, Didwana Vinodkumar,
Satvi Vaibhav and Yadav Madhulika

Department ofBotany, VIVA College, Virar.

Abstract
Improper management and increasing discharge of comprehensively polluted waste in the water create drastic effect on terrestrial as well as aquatic ecosystem. Remediation of water contaminated with heavy metals received considerable attention in recent years. Mycoremediation integrating the removal of toxic compounds employing the fungi is an efficient tool as it tolerates extreme physical and chemical condition. It is also cost effective and its end products are non-hazardous. In the present study, the white-rot fungi*Pleurotussps.*was investigated for its ability to remediate. Thewater containing different concentration of Zinc and Lead were evaluated and mycelium was found to be effective in biosorption of these metals. Thus results indicate *Pleurotussps.*have potential source for remediating waste water contaminated with heavy metals.

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ओळख राष्ट्रीय सन्मानाची
प्रतिमा ग्राहक हिताची

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A Chapter in a Book:

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Report:

Makarewicz JC, Lewis T, Bertram (1995) Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference Proceedings:

Chavhan AB (2012) Biomedical Waste Management: Awareness and Practices among Healthcare Providers in Amravati". Proceeding of national conference on Recent Trends and Innovative Development in the Frontiers of Life Science. pp- 148-153. (ISBN-978-93-81733-04-2).

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