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उत्तिष्ठत जाग्रत प्राप्य वरान्निबोधत।

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From the Editor's Desk

The Presidential address by Prof. T. Satyanarayana during the 41st annual meeting of the Mycological Society of India held at the Department of Botany, Punjabi University, Patiala on February 23, 2015 is a welcome call to the mycological fraternity to work for the growth and rejuvenation of the subject of mycology at all levels so that it becomes the subject of preference for study by the youngsters. This can be done by inculcating interest in the field of mycology at school, college and university level for which course curricula have to be designed by including socially relevant applied topics of the subject in the syllabus. To generate interest in the subject and to make it more attractive, there is a need to assign small projects in the identification and characterization of fungi affecting our surrounding environment including multipurpose plants, crops, food stuffs, fruits, vegetables, etc. and on those growing in lawns, along road sides and forested areas during monsoon season. This will definitely help the youngsters in understanding the role of fungi in nature and create interest in them to uptake further studies on various aspects of these microbes when such students join Post Graduate Programmes in the Colleges and Universities.

Mycologists need to play a proactive role at all levels especially in the University Departments and Research Institutes to make the researches in mycology more attractive and application based so that bright students are attracted to nurture the field. It is essential to undertake collaborative projects with various scientists specialized in different branches of science in general and biology in particular. This will definitely help to produce a new generation of mycologists well versed in basic and applied aspects, which is the need of the day to take the field of mycology to its pristine glory that once it enjoyed in India

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Reviving intrest in mycology to regain the pristine glory that once it enjoyed in India

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ABSTRACT

Among the estimated 1.5 million fungal species present on the Earth, approximately 100,000 species have been described so far, which accounts for 6.7% of the estimated fungal diversity. Extensive efforts have been made in India to understand the diversity of culturable fungi that led to the discovery and description of several new fungal genera and species in the last 50-60 years. The fresh efforts must continue to cultivate and describe fungal species yet to be described. Since we are able to cultivate only a small fraction of microbes from the extant microflora in any environmental sample, non-culturable metagenomic approaches must also be employed to understand the diversity of fungi. The students of mycology must be conversant with the modern approaches such as molecular techniques to unravel the diversity of fungi in natural environments. In order to strengthen MSI, we must welcome and enroll biologists working on various aspects of fungi such as physiology and biochemistry, genetics and molecular biology, genomics, metagenomics and metabolomics, and molecular systematics. The work that has been going on in our laboratory on thermophilic fungi and yeasts and their enzymes with multifarious applications are very briefly discussed

Keywords: Thermophilic fungi, diversity, enzymes, applications.

Prof. T. Satyanarayana
President MSI 2014-15

INTRODUCTION

I deem it a matter of immense pleasure to be the President of the prestigious Mycological Society of India during 2014-2015. I wish to place on record my gratitude for all the members for electing me to be the president of MSI. I wish to pay my humble respect to all the past presidents and office bearers of MSI who have contributed significantly for the growth and sustenance of the society. Thanks are also due to Prof. M.I.S. Saggoo (Head & Coordinator, Department of Botany) and Prof. N.S. Atri (Organising Secretary) for hosting the symposium and 41st Annual meeting of MSI at Punjabi University, Patiala during Feb. 23-24, 2015 where the presidential address was presented.

According to Hawksworth (1991; 2001), a conservative estimate of fungal diversity extant on the Earth is 1.5 million species, although this may range between 0.5-9.9 million based on different approaches (Mueller and Schmit, 2007). Nonetheless Hawksworth recommended that the working figure of 1.5 million species be retained until new and independent data sets become available. This figure is conservative because the estimate of species is based on the observed ratio between plant diversity and fungal diversity. Approximately 100,000 species of fungi have been described that accounts for about 6.7% of the estimated fungal species, suggesting that there is a scope for discovering new fungal species. We are able to culture only 0.1-1.0% of the extant microbial diversity in any of the environmental sample, the recently evolved molecular metagenomic and culture-independent methods must, therefore, be employed for understanding fungal diversity, besides the culture-dependent approaches.

Significant and praiseworthy attempts have been made in India by C.V. Subramanian, C. Manoharachari, K.G. Mukherjee, K.S. Thind, A.K. Sarbhoy, S.B. Saxena, M.J. Thirumalachar, K. Natarajan, G.P. Agarwal, to mention a few among several others, and their students to understand the diversity of culturable fungi in soil, water, atmospheric and other environments. Despite the limited government funding, the efforts to retrieve new and yet to be discovered fungal species and their exploitation must continue. Novel organisms could be sources of novel genes/gene clusters that encode useful enzymes, primary and secondary metabolites, and disease causing and/or biocontrol organisms. It is essential to go for collaborative and joint research efforts to understand various aspects of fungi, therefore, we must welcome scientists working in different branches of biology such as physiology and biochemistry, genetics and molecular biology, genetic engineering and genomics [structural and functional (transcriptomics and proteomics)] and metabolomics to join MSI for generating interest and attract young students to mycology. The experienced mycologists must organize training courses to provide theoretical as well as practical hands-on training to young PG students and lecturers. We must do everything that revives interest in mycology in order to regain the pristine glory that it once enjoyed in India.

NEED TO STUDY THE DIVERSITY OF EXTREMOPHILIC FUNGI

Most of the work done in India has been on the culturable mycoflora from normal/moderate environments with pH around neutral, temperatures between 20 and 40 °C, air pressure 1.0 atm, and adequate levels of available water, nutrients and salts. There are many extreme environments in India such as acidic or hot springs, saline and/or alkaline lakes and soils, deserts and the ocean beds, which are too harsh for normal life to exist. Any environmental condition that is perceived to be beyond the normal acceptable range is considered as an extreme condition. The fungi that survive and grow in such harsh environments are known as extremophilic fungi; these organisms not only tolerate specific extreme condition(s), but usually require them for their survival and growth. Most extremophiles are found in the microbial world. Besides natural extreme environments, there are also man-made extremes such as in cool houses, steam heated buildings, laundry machines, acid mine waters and industrial effluents. Very little work has been done on the diversity, adaptations and applications of extremophilic fungi in India. There is a scope for discovering new fungal species from extreme environments which could be sources of novel metabolites like antibiotics and other bioactive compounds (Satyanarayana, 2014).

OCCURRENCE, BIOLOGY AND SIGNIFICANCE OF THERMOPHILIC MOULDS

There are fewer than 50 species of thermophilic fungi, among approximately 100,000 species described so far, which thrive at relatively elevated temperatures (Mouchacca, 1997; 2000; Johri *et al*., 1999). These are common in soils and in habitats wherever organic matter heats up due to decomposition. Thermophilic fungi have been isolated from manure, compost, industrial coal mine soils, beach sands, nuclear reactor effluents and desert soils. In these habitats, thermophiles occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions. Generally there is an inverse relationship between biological diversity and the adaptation required to survive in a specific habitat. Thermophilic fungi are a small group in Eukaryota, which have evolved strategies for growing at elevated temperatures up to 60 to 62 °C. During the last 50-60 years, many new species of thermophilic fungi sporulating at 45 °C and above have been reported. Cooney and Emerson (1964) defined thermophilic fungi as 'those capable of growth at or above 50 °C and a minimum temperature for growth at or above 20 °C.

Thermophilic fungi such as *Aspergillus fumigatus*, *Chaetomium thermophile*, *Sporotrichum thermophiles* and others had been isolated from nesting materials of Indian birds such as crow, crow peasant, sparrow, pipit and bee-eater (Satyanarayana *et al*., 1977). The decomposing plant materials in bird's nests provide suitable habitat for thermophilic fungi. During an investigation on the colonization and composting of paddy straw, the length of straw pieces and C:N ratio of paddy straw influenced decomposition, the most suitable being 2.5 cm and 20, respectively favoured high rate of decomposition (Johri and Satyanarayana, 1984; Satyanarayana and Johri, 1992). Thermophilic fungi are active in the decomposition of plant organic matter by their high colonizing ability, thermal tolerance and by secreting polysaccharide-degrading enzymes like cellulases, xylanases, proteases, amylases, lignin-degrading enzymes, lipases and others (Satyanarayana *et al*., 1985; 1988; Satyanarayana and Johri, 1981a; 1983a; b; Banerjee *et al*., 1995; Kamra and Satyanarayana, 2004). The intracellular mycelial extracts of *Malbranchea pulchella* var. *sulfurea* contained xylose isomerase, xylose reductase and xylitol dehydrogenase (Banerjee *et al*., 1994). This is the first report on the occurrence of three enzymes in a thermophilic mould, which play a role in xylose metabolism. Xylose isomerase of the mould is less thermostable than this from other microbes.

Sporotrichum thermophile produces high titres of xylanase, pectinase and cellulase in solid state fermentation (SSF) in 4 days (Kaur and Satyanarayana 2004a). The production of polygalacturonase was higher in submerged fermentation than in SSF (Kaur *et al*., 2004). On treatment of banana, grapes and apple pulps with the mixture of enzymes, an increase in the juice yield was recorded.

Thermomucor indicae seudaticae produces neutral glucoamylase in solid state as well as submerged fermentations, and by alginate immobilized sporangiospores (Kumar and Satyanarayana, 2004; 2007a; b; Kaur and Satyanarayana 2004b; Kumar *et al*., 2007). The pure enzyme has a molecular mass of 42 kDa and optimally active at pH 7.0 and 60 °C with $T_{1/2}$ of 7 hours at 80 °C (Kumar and Satyanarayana, 2003). Improvement in glucoamylase production was attained using a mutant generated by gamma irradiation (Kumar and Satyanarayana, 2009a; b). Suitability of the enzyme in ideal starch saccharification process has been confirmed (Satyanarayana *et al.,* 2004).

Sporotrichum thermophile produces Histidine Acid Phosphatase (HAP) phytase in submerged and solid state fermentations and also by alginate-immobilized conidiospores (Singh and Satyanarayana, 2006a; b; 2008a; b). The purified phytase is a homopentameric glycoprotein of molecular mass 456 kDa and pI of 4.9 (Singh and Satyanarayana, 2009), and it is active optimally at pH. 5.0 and 60 °C with $T_{1/2}$ of 1.5 h at 80 °C. The enzyme has been shown to play a role in plant growth promotion (Singh and Satyanarayana, 2010), bread making and dephytinization of oil cakes (Singh and Satyanarayana, 2010; 2011a; b; 2015). The gene that encodes phytase has been cloned and expressed heterologously in *Escherichia coli* and *Pichia pastoris* (unpublished). The unconventional yeast *Pichia anomala* produces cell-bound phytase in submerged fermentation (Vohra and Satyanarayana, 2001; 2002; 2004; Kaur and Satyanarayana, 2005). The enzyme is a homohexameric molecule of 384 kDa which is optimally active at pH 7.0 and 60 °C with a $T_{1/2}$ of 5 min at 80 °C (Vohra and Satyanarayana, 2002; 2003). This phytase has the

requisite properties for application as an animal feed additive and useful in dephytinization of soymilk (Vohra *et al*., 2006; Kaur and Satyanarayana, 2010). Phytase gene from the yeast was cloned and expressed heterologously in *Hansenula polymorpha* (Kaur *et al*., 2010) and *Pichia pastoris* (Joshi and Satyanarayana, 2014); in the former, recombinant phytase remains intracellular, while in the latter extracellular.

Myceliophthora thermophila produces endochitinase extracellularly in an inducible manner (Rohatgi, 2012). The enzyme is a monomeric glycoprotein of molecular mass of 43 kDa which is active optimally at pH 4.0 and 55 °C with a $T_{1/2}$ of 3 h at 70 °C. The enzyme generates N-acetyl glucosamine and chitobiose from chitin. The enzyme inhibits phytopathogenic fungi such as *Fusarium oxysporum* and useful in the biocontrol of nematode *Meloidogyne incognita*, mealy bug *Maconellicoccus hirsutus* and mosquito *Aedes aegypti*.

Thermophilic fungi of paddy straw compost have been shown to produce volatiles which play a role in fungistasis in composts as in soils (Satyanarayana and Johri, 1981b). Ammonium was detected in the volatiles from thermophiles growing on paddy straw which affected mycelial growth. The volatiles of *Chaetomium thermophile*, *Sporotrichum thermophile* and *Thermoascus aurantiacus* inhibited spore germination of *Aspergillus fumigatus*, *Humicola lanuginosa* and *Torula thermophila*, suggesting that this phenomenon might be playing a decisive role in establishing thermophilic moulds as the dominant flora in composts.

None of the thermophilic moulds tested was able to breakdown the aliphatic side chains of sterols (cholesterol, sitosterol, lanosterol) [Satyanarayana and Chavan, 1987]. In *Acremonium alabamensis* and *Talaromyces emersonii*, cholestenone was detected in the fermented medium containing cholesterol, where as the former yielded stigmastadienone from stigmasterol and sitosterol. Lanosterol was resistant to bioconversion. All thermophilic moulds displayed avidity for binding sterols to the mycelium, which depended on the nature of the mould and sterol.

The total lipid content of *Acremonium alabamensis* and *Thermomucor indicae-seudaticae* ranged 2.6-7.3 and 8.5-13%, respectively during development (Satyanarayana *et al*., 1987; Satyanarayana and Johri, 1992). Neutral lipid fraction increased while polar and phospholipids declined during growth. The lipids of both moulds contained palmitic, oleic, linoleic and palmotoleic acids as the major fatty acids in lipids. Degree of unsaturation of the lipids of *Acremonium alabamensis* was higher than that in *Thermomucor indicae-seudaticae.* Neutral lipids were more unsaturated than the polar lipids. The ratio of unsaturation index of polar lipids to neutral lipids was either one or less than one. The principal phospholipids in two molds are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidic acid.

Putrescine, spermidine and spermine are widely distributed in thermophilic molds (Singhania *et al.,* 1991). The level of free polyamines is high in growing mycelium of *Humicola lanuginosa* than in the old stationary phase mycelium. Polyamine levels declined at temperatures above and below the optimum for growth (45 °C). Difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC), strongly inhibited mycelial growth of *H. lanuginosa*, *Talaromyces emersonii* and *Mucor pusillus*, suggesting that ODC pathway is present in these molds. Difluoromethyl-arginine (DFMA), an inhibitor of arginine decarboxylase (ADC) did not inhibit thermophilic fungal species except *M. pusillus*, where mycelial growth was considerably reduced. This could possibly be due to either conversion of DFMA to DFMO by arginase or the presence of ADC. Based on the observations, Singhania *et al*. (1991) concluded that polyamines are required for normal growth and development of thermophilic moulds.

CONCLUSION

It is amply clear from our work on thermophilic fungi that there are immense possibilities of carrying out research on various aspects of extremophilic fungi and their useful products. The fungi play an important and critical roles in the environment and sustainable development, and their products are immensely useful to mankind.

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Two decades of endophyte research in VINSTROM results in more questions than answers

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ABSTRACT

Studies on endophytes, although initially lacking in impetus, have increased appreciably in the last few years. To begin with, endophytes of tropical plants were studied with the hope that they could be used as a reporter group to estimate global fungal diversity given the expectation that they would be harbouring many hitherto unknown species of fungi. However, the work undertaken at Vivekananda Institute of Tropical Mycology (VINSTROM) in different types of forests of the Western Ghats show that many endophytes in the dry tropical forests are multi-host forms and colonize trees of different lineages; thus we concluded that perhaps due to a high degree of host acquisition by some multi-host endophytes, taxonomically disparate tree hosts harbour endophyte assemblages overlapping in species composition such that the high plant species diversity in tropics may not mirror endophyte diversity. This leads to questions such as (i) does a group of polyphagous endophytes in the leaves render them resistant to infection by other endophyte taxa leading to reduced endophyte species diversity in the leaves? (ii) how multi-host endophytes have evolved to tackle the different defense metabolites found in plants of different lineages? and (iii) does environment have a greater influence than host factor in determining the endophyte assemblages? A partial answer to the first of these questions was obtained by us in our study on foliar endophytes of mango. Treatment with a systemic fungicide eliminates some native endophytes allowing some non-native endophytes to colonize. Since endophytes are known to produce antifungal compounds, it remains to be seen if a situation similar to that obtained in antibiosis in the soil milieu exists in plant tissues with regard to endophyte colonization. Our studies and those of others have shown that some foliar endophytes also survive in fallen litter as pioneer litter degraders. We found that the endophytes of the fire-prone forests in the Western Ghats have suitable adaptations for such a biphasic lifestyle; their spores are highly thermotolerant, they produce cell wall destructuring enzymes, they utilize furaldehydes - which are the most abundant volatiles produced during biomass burning and are toxic to fungi - as carbon source and survive as saprotrophs in fallen leaves. This leads to the questions (i) is endophytism a phase in the life cycle of some fungi? and (ii) does environmental selection leads to evolution of multi-host endophytes? It is well known that endophytes elaborate many bioactive metabolites. In collaboration with other national and international labs, VINSTROM has found that endophytes produce anti-cancer, anti-fungal, anti-algal and anti-malarial metabolites. Some of these are novel metabolites. Furthermore, our studies indicate that endophytes are a novel source of industrial enzymes such as chitin modifying enzymes, xylanases and asparaginase. It has to be discerned if this extraordinary synthetic ability of endophytes in a reflection of their coevolution with their hosts. The tripartite interaction between endophyte, leaf and phytophagous insect is another facet of endophyte biology that has been barely studied. In one of the early studies, we reported that painted grasshopper does not discriminate between endophyte-infected and endophyte-free leaf as food but may play a role in disseminating the endophytes. Our investigation using Bt cotton plants confirm that insect visitations increase the density of colonization of plants by endophytes. A few recent studies in other labs have shown that endophytes could deter insect pests from feeding on plants. This has raised the hopes of using endophytes as biocontrol agents. Caution is needed however in this regard as yet another study from VINSTROM shows that some effective biocontrol agents could survive as endophytes and also produce mycotoxins. Some plant pathogenic fungi survive as benign endophytes. We have shown that the pepper spot fungus which is a serious peanut pathogen survives as asymptomatic foliar endophyte in the leaves of many varieties of peanuts grown in Tamilnadu state. It is not known if endophytes evolve into pathogens or pathogens have evolved as endophytes and what are the clues which induce latent pathogens that survive as endophytes to express disease symptoms in plants. Simultaneous transcriptome profiling of endophyte-free leaf and leaf with a given suite of endophyte species may help unravel the evolution of this cryptic lifestyle among fungi.

Keywords: Endophytes, bioactive metabolits, multitrophic interactions

INTRODUCTION

I thank the MSI for bestowing on me the honour of delivering the Prof. Agnihothrudu Memorial lecture. I take this opportunity to identify certain gaps in our knowledge of the ecological group of fungi called the endophytes.

It is now well established that plants are constantly in association with non-pathogenic, endosymbiotic fungi - the endophytes. Although significant strides have been made in the study of endophytes in the last two decades, most of these are concerned with the isolation of fungi from individual plants and the discovery of novel metabolites from these fungi. The latter studies have firmly established the biotechnological potential of endophytes (Suryanarayanan *et al*., 2009; Aly *et al*., 2010; Kharwar *et al*., 2011; Kusari *et al*., 2009) thus galvanizing more investigations on this aspect. However, our knowledge regarding the basic aspects of endophyte biology is inchoate. I underscored some of these untouched facets in a recent review (Suryanarayanan, 2013). In this memorial lecture, I deal with a few more such aspects of endophyte biology needing attention by drawing evidence from the 2 decade long studies conducted in Vivekananda Institute of Tropical Mycology (VINSTROM).

ENDOPHYTES ARE NOT PASSIVE RESIDENTS OF PLANT TISSUES

The ubiquitous presence of endophytes within plant tissues and the fact that this association between plant and fungus existed even as early as the early Devonian period (Strullu-Derrien *et al*., 2014) go to show that endophytism is an evolutionarily successful life strategy among fungi. A recent report says that endophytes are more than 'symptomless' residents of plant tissues; infection by an endophyte induces changes in the expression of many genes of the host plant and specifically up regulates genes involved in defence (Mejia *et al*., 2014). This information emphasises the need for focussed investigations at the gene level to unravel various facets of endophyte-plant interactions. Apart from this interaction between a plant and one of its endophyte associates, the interactions between a plant

and a suite of endophytes supported by it and the interactions among the different species of endophytes in the plant tissue have to be discerned. Many endophytes are known to produce antifungal, anti bacterial and cytotoxic metabolites *in-vitro* (Gunatilaka, 2006; Suryanarayanan *et al*., 2009; Rai *et al*., 2014; Kharwar *et al*., 2011; Liu *et al*., 2015; Huzefa *et al*., 2015). We have reported the production of novel bioactive compounds as well as potential anti malarial drugs by endophytes (Geetha *et al*., 2011; Kaushik *et al*., 2014); it is not clear if endophytes produce such compounds *in-vivo* and if so, their interplay with the plant tissues and other endophytes present in them have to be investigated. Elimination of native endophytes from the leaves of mango led to their colonization by alien endophytes due to competitive release thus indicating the production of some antifungal compounds *in-vivo* by the native endophytes (Mohandoss and Suryanarayanan, 2009). Furthermore, it is not known if association with a plant silences or induces certain genes of the endophytes. Another question that remains unanswered is 'does the synthetic ability of a fungus change when it is inside the plant tissue?' Metabolomics studies using endophyte-infected and endophyte-free plants could report the bioactive compounds produced as a result of the symbiosis. Genomic and transcriptomic analysis show that an endophytic *Pestalotiopsis* has a high potential for synthesising many different natural products and the gene families coding for detoxification and virulence have undergone expansion aiding it to lead an endophytic life (Wang *et al*., 2015). Such studies with more endophyte genera would help understand the evolution of this cryptic life style among fungi.

ENDOPHYTES AS PRODUCERS OF PLANT METABOLITES

A very appealing aspect of endophyte research is concerned with their putative ability to elaborate the secondary metabolites of plants. Taxol (paclitaxel), one of the most effective and widely used anticancer drugs is obtained from the pacific yew tree. This tree is scarce and slow growing and many trees have to be destroyed to get a small quantity of taxol (Zhou *et al*., 2010). Hence, a frantic search was made for alternative sources of the compound. Stierle *et al.* (1993) reported that *Taxomyces andreanae*, an endophyte isolated from the yew plant produces taxol in culture. Following this report, many other endophytes (of yew and even other plant) such as *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium* and *Peastalotiopsis* were reported to produce taxol in culture (see Heinig *et al*., 2013). Many endophytes also produce other metabolites of plants such as camptothecin, podophyllotoxin, vincristine, vinblastine, hypericin, diosgenin and rohitukine (see Sachin *et al*., 2013). A common observation for all these endophytes is the gradual attenuation of the production of the metabolites upon subculturing the fungi. It is believed that a horizontal gene transfer (HGT) event of secondary metabolites pathway genes from the plant host to the endophytes is responsible for this (Tan and Zou, 2001; Chandra, 2012). At least with reference to taxol, however, HGT between plant and endophyte is not possible as taxol biosynthesis in plants is complex and involves nearly 20 enzymatic steps, different organelles and the genes controlling the synthesis do not occur as a cluster but are scattered on different chromosomes (Heinig *et al*., 2013). Using phytochemistry, molecular biology and genome sequencing methods they failed to find any 'evidence for independent taxane biosynthesis in any endophyte including *T. Andreanae* (Heinig *et al*., 2013). We surveyed the bioinformatic approaches to investigate the ability of endophytes to produce various secondary metabolites of plants and also to possibly explain the common observation of attenuation of the production of such compounds by endophytes upon subculturing (Sachin *et al*., 2013). We chose the terpenoidindole alkaloid (TIA) pathway of plants as it produces a very large number of alkaloids (>1880) in plants including catharanthine, strychnine, gelsemine, camptothecin, vincristine, vinblastine, and ajmalin - some of which are reported to be produced by endophytes too. The TIA synthesis involves two different pathways - (1) the shikimate pathway and (2) mevalonate/ deoxyxylulose 5-phosphate pathway **(Fig.1)**. By going through several intermediate steps, the two pathways synthesise tryptamine and secologanin, respectively. The enzyme strictosidine synthase (STR) located in the vacuole then catalyses the condensation of tryptamine and secologanin to form strictosidine, the precursor of many TIAs. Analysis of whole genome sequences of 53 fungal species failed to show the presence of STR gene or the genes downstream of the STR step in the pathway. This adds credence to the report that endophytes may not have acquired the plant genes for producing plant metabolites by direct gene transfer (Heinig *et al*., 2013). We further proposed that testing the ability of select endophytes to convert secologanin and tryptamine to srtictosidine would provide some answer to this enigma. We also proposed a few hypotheses to explain the attenuation of plant metabolite production by endophytes. These include the silencing or methylation of the STR gene equivalent in the fungus, or by the steady loss of plasmids or bacteria carrying the plasmids if such a gene is housed in an extra chromosomal element in the

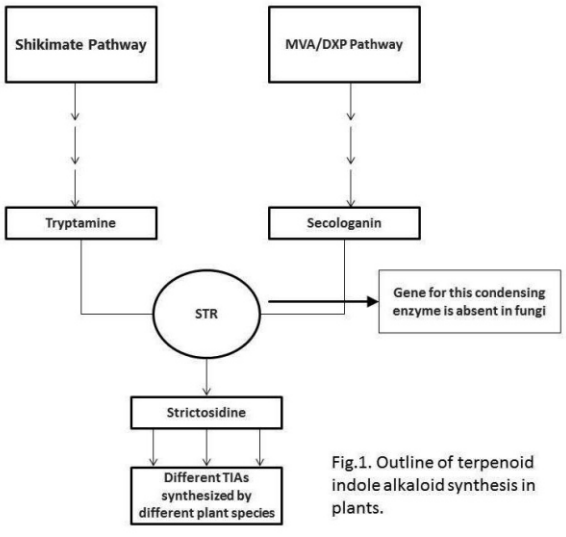

Fig.1. Outline of terpenoid indole alkaloid synthesis in plants.

fungal cytoplasm or in the endohyphal bacteria (see Sachin *et al*., 2013 for details). Thus, the findings regarding plant metabolite production by endophytes are very intriguing but many questions have to be answered before the successful exploitation of endophytes for production of such compounds. Another line of investigation is to find methods to improve the quantity of plant metabolites produced by endophytes in culture (Gond *et al*., 2014). Invariably, the amount produced is too low to be a commercially viable option. A clear understanding of the exact pathways involved in these fungi to synthesise the compounds would help manipulate conditions for overproduction of them. Screening more plants for hyperproducing isolates of endophytes, strain modification by selection and mutation, metabolic engineering and genome mining for silent gene clusters and methods to activate such genes would go a long way in realizing this goal (Brakhage and Schroeckh, 2011).

Many endophytes are closely related to phytopathogenic fungi (Hyde *et al*., 2009; Zhang *et al*., 2009; Slippers *et al*., 2013). In fungi of the order *Phaeomoniellales*, several strains which are endophytic in gymnosperms are pathogenic in angiosperms (Chen *et al*., 2015). Similarly, *Leptospherulina crassiasca* which causes the pepper spot disease of peanut in many peanut growing areas of the world survives as asymptomless endophyte in the leaves of many varieties of peanut cultivated in the state of Tamilnadu (Suryanarayanan and Murali, 2006). There is no information regarding the course of evolution of this dual life style exhibited by some endophytes. Since, endophyte infection upregulates many defence genes in plants, it is likely that colonization by endophytes increase the resistance response of the plant to the pathogenic strain of the same fungus - a situation akin to systemic acquired resistance exhibited by plants in the presence of avirulent pathogens (Cao *et al*., 1994). The use of symptomless endophytes to increase the resistance of plants to pathogenic strains of the fungi would be a natural option if this is proved.

INFLUENCE OF ENVIRONMENT IN SHAPING ENDOPHYTE ASSEMBLAGES IN PLANT TISSUES

A few earlier studies in the wet tropical forest recorded a high diversity of endophytes (Lodge *et al*., 1996; Polishook *et al*., 1996; Arnold *et al.,* 2000; Hyde and Lones, 2002; Shipunov *et al*., 2008). This led to the expectation that the high plant diversity in the tropics would support high endophyte diversity and thus endophytes of the tropics could serve as a focal group for estimating global fungal diversity. Our decade long work on foliar endophytes of the tree species of the Nilgiri Biosphere Reserve proved otherwise. We studied 75 dicotyledonous tree species belonging to 33 families and concluded that endophyte diversity in these dry tropical forests is limited due to loose host affiliations among endophytes. Some endophytes here have a wide host range and colonised taxonomically disparate but co-occurring hosts; additionally such polyphagous endophytes also dominate the endophyte assemblages in these trees (Pandey *et al*., 2003; Suryanarayanan *et al*., 2003; Murali *et al*., 2006; Murali *et al*., 2007; Suryanarayanan *et al*., 2011). It is now well known that certain genera such as *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta* and *Xylaria* occur usually as endophytes in taxonomically unrelated tropical tree species growing in unconnected geographical locations (Bayman *et al*., 1998; Okane *et al*., 2001; Baayen *et al*., 2002; Davis *et al*., 2003; Pandey *et al*., 2003; Lu *et al*., 2004; Jeewon *et al*., 2004; Murali *et al*., 2006; Wei *et al*., 2007; Tejesvi *et al*., 2009; Suryanarayanan *et al*., 2011; Govinda Rajulu *et al*., 2013). Generally, closely related plant species host same fungal species (e.g. foliar pathogens or endophytes in the case of gymnosperms) and host sharing among fungi declines as a function of phylogenetic distance between the plant hosts (Webb *et al*., 2008). Our results with endophyte of the forests of the Western Ghats run contrary to this generalization. Elsewhere, we present explanation for this observation (Suryanarayanan *et al*., 2011). Such host range expansion of a few endophyte species leads to a low host-fungus ratio impacting on the use of endophytes as reporter group for tropical fungal diversity **(Fig. 2)**. It is necessary to estimate the real endophyte diversity of different tropical ecosystems taking recourse to culture independent methods and high-throughput-sequencing since many endophytes cannot be cultured (Impullitti and Malvivk, 2013) and, even if culturable, may not produce the structures which help discern their taxonomy. It is also important that the methods used for molecular studies be refined (U'Ren *et al*., 2014) and the sequence data bases for endophytes should be increased to facilitate meaningful comparisons. Specific phylogenetic analyses should be done for endophytes as has been attempted by Chen *et al.* (2015) which revealed a new, endophyte-rich order, the *Phaeomoniellales* under *Eurotiomycetes*.

Our further studies on endophytes helped us to explain the low diversity of endophytes in the forests we studied and highlighted the role of environment in determining the endophyte assemblage of such ecosystems. As early as 2002, we provided evidence for foliar endophytes surviving in leaf litter and functioning as pioneer litter degraders (Kumaresan and Suryanarayanan, 2002). Many later studies confirm such a switch in the life style of endophytes (Promputtha *et al*., 2007; Zhang *et al*., 2009; Lantz *et al*., 2011; Chaverri and Samuels, 2013). Years later, we reported that the spores of some of these fungi

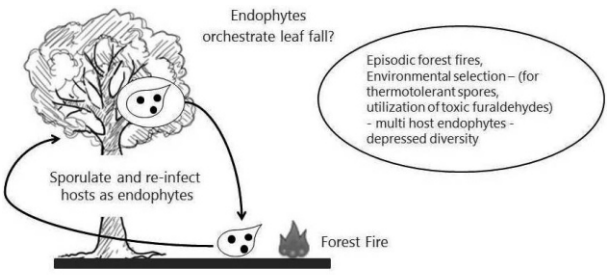

Fig. 2. A hypothesis to explain the role of environment in shaping endophyte diversity.

which grew both as endophytes and as litter fungi in the leaves of many tree species found in the fire-prone forests of Mudumalai, are thermotolerant and could survive exposure to temperatures above 100 °C (Suryanarayanan *et al*., 2011). Furthermore, we showed that these fungi are able to utilize the toxic furaldehydes (the most abundant and most common volatile hydrocarbons released during plant biomass burning) as carbon source (Govinda Rajulu *et al*., 2014). Many of these fungi also produce plant cell wall degrading enzymes (Prakash *et al*., 2015). All these traits together make the fungi ecologically fit to survive in a fire-prone ecosystem. It is not surprising then to note that fungi with such adaptations, through expanded host acquisition, become dominant endophytes in such an ecosystem and consequently depressing the species diversity of the endophytes. A few other studies also show that environment plays a role in shaping the endophyte communities (McGuire *et al*., 2012; Zimmerman and Vitousek, 2012). Endophytes isolated from mangrove plants are salt tolerant (Kumaresan and Suryanarayanan, 2002) and endophytes from high altitude plants elaborate cold-adapted enzymes (Li *et al.,* 2012). More plant communities with distinct ecological conditions have to be studied to know more about the influence of environment in determining endophyte occurrence in them. If the environment shapes the endophyte community much more than the taxonomic affinity of the plant hosts, then precise methods have to be used to trace the host range of a single species of the ubiquitous endophytes and appropriate corrections have to be made in the estimated figures of fungal species currently being purported.

ENDOPHYTES AND MULTITROPHIC INTERACTIONS

Endophytes have largely been viewed as isolated entity so far, and hence their role in the ecosystem remains obscure. Recently, studies focusing on two trophic levels (between endophytes and plants) have been initiated (see Schulz *et al*., 1999; Mejia *et al*., 2014). Investigations involving multitrophic interactions are expected to inform more about the biology of endophytes. According to the tritrophic level hypothesis, interactions between two trophic levels will affect the third trophic level (Price, 1986). Therefore, such cascade effects of trophic interactions have to be discerned with reference to endophytes. In this regard, the interaction between endophytes, plants and phytophagous insects is a case in point. It can be expected that the association of endophytes with plants affects qualitatively and quantitatively the nutrients available to the consumers at higher level in the food chain as opined by Omacini *et al.* (2001) who showed that grass endophytes influence plant-insect interactions. With the horizontally transmitted endophytes, there are very few studies on these lines (Van Bael *et al*., 2009). Work in VINSTROM showed that the tritrophic interaction could be complex. In one study (Devarajan and Suryanarayanan, 2006), we observed that the painted grasshoppers fed equally well on leaves with moderate or heavy load of endophytes in them. The endophytes passed through the gut of the insect undigested and sporulated on the frass suggesting that phytophagous insects could help in the spread of endophytes. We also compared the endophyte status of different tissues of wild and Bt variety of cotton and found that though there was no difference in the species composition of endophytes between the two, the Bt tissues had significantly lower endophyte frequency (Suryanarayanan *et al*., 2011). We argue here that the frequency of insect visitation is directly related to endophyte colonization of plants. More work is needed to understand the role of phytophagous insects as passive and active disseminators of endophytes. The work of Van Bael and her colleagues on leaf cutter ants and endophytes illustrate how intricate the tritrophic interactions could be. They reported that ants avoid endophyte infected leaves. Thus, supporting endophytes could be beneficial to a plant as leaf cutter ants are one of the most active defoliators in the wet tropics (Van Bael *et al*., 2012). This reiterates the need to explore the cost-benefit angle of endophyte-plant association. They also showed that endophytes are not preferred in the ant nest and they are prevented by both the activities of the ants and the inhibition exhibited by the fungus that the ants cultivate in their garden (Van Bael *et al*., 2009). Another interesting work is that of Behie *et al.* (2013) which showed that the insect parasitic fungus *Metarhizium robertsii* which infects an insect, also establishes an endophyte association with a plant and consequently transfers nitrogen directly from the insect to the plant. The occurrence of entomopathogic fungi as endophytes has kindled the notion of using these as biocontrol agents (Vega, 2008). Although this is appealing, several questions need to be addressed before endophytes could be successfully used for insect control. We have observed that an entomopathogenic endophyte isolated from a given plant may not be useful in insect control in a non-host plant as its colonization frequency reduces in the introduced plant with time (Suryanarayanan, 2013). Furthermore, the suitability of the effective endophytic fungus has to be evaluated before it could be used in any biocontrol programme. *Fusarium pallidoroseum* (a non-endophyte) has been advocated for the bio-control of water weed *Eichhornia crassipes* (Naseema *et al*., 2004); it also could function as a bio fertilizer (Srivastava *et al*., 2011); we observed that this fungus also survives as an endophyte and produces mycotoxins (Thirumalai *et al*., 2013) thus questioning its ability to be used in the field either for biocontrol or as a biofertilizer.

CONCLUSION

With the current knowledge that plants invariably have endophytic association in them, reporting the presence of endophytes in yet another plant species would be redundant. Investigations aiming to provide answers to the basic questions discussed above and other related aspects of the biology of endophytes would serve to unravel the mysteries about this cryptic guild of fungi and pave way for better utilization of their potential.

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Forty years with Corticoid Fungi

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ABSTRACT

In the study of taxonomy of corticoid fungi macroscopical examination was a routine during earlier years. However, with the passage of time newer tools including macroscopic examination, microscopical studies, ecological studies, study of cultural aspects and DNA sequencing studies have become handy so as to analyse, delimit and charaterise the corticoid fungi resulting in emergence of many new genera and species. Use of crossing tests in the delimitation of biological species and study of their geographical distribution pattern are yet other aspects of systematic significance.

Key words: Corticoid fungi, systematics, crossing test

INTRODUCTION

Systematics of basidiomycetous fungi relied for a long time on morphological characters, as observed in the field and complemented with odour, taste and consistency. When the microscope came into common use it was mainly for characterization of spores and easily observed cystidia. Already in the beginning of the 20th century, species recognition of corticioid fungi was challenging because of the sparse macro-features. The real breakthrough came with the phase contrast microscope and observations on the far more delicate micro-structures observed here.

During 1950-ies and 60-ies several important contributions on corticioid fungi were published. The method to recognize species had so far been to compare herbarium material in the microscope and to use the short-handed descriptions and simplified drawings which were available in "Hyménomycètes de France" (Bourdot and Galzin, 1928). Now, combination of microscopic characters came into use when delimiting important corticioid genera, like *Hyphodontia*, *Hyphoderma*, *Phanerochaete*, and *Gloeocystidiellum*. The architects behind this new development was John Eriksson and Marinus Donk.

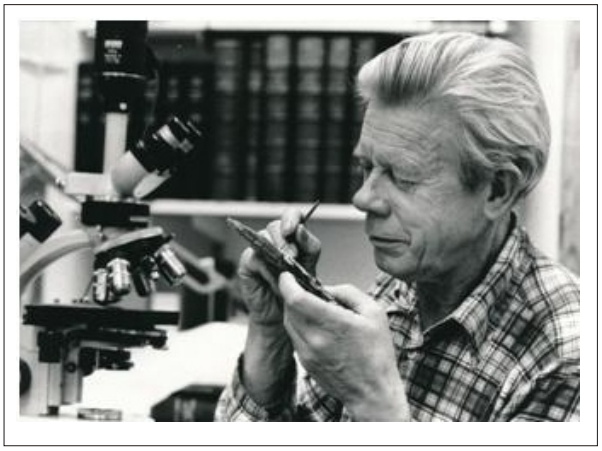

Fig.1 John Eriksson preparing a sample at the microscope.

THE BEGINING

At the beginning of the seventies, when I started as a young student to John Eriksson, he started the work with a well illustrated flora on corticioid fungi. All known species from the Nordic countries were carefully illustrated with line drawings of basidioma sections and magnified illustrations of spores and other details. This was Prof. Eriksson´s speciality. To transmit the structures hidden behind the hymenial surface to our minds in a way that could inform on structure, consistency and hyphal arrangement in a single plate. Few followers have been able to copy his artistic talent, but on the other hand all his drawings are now freely available on Mycobanks website (www.mycobank.org) to the benefit of all followers, although reproduced at a low resolution (Eriksson and Ryvarden, 1973; 1975; 1976; Erikkson and Hallenberg, 1985; Erikkson *et al*., 1978; 1981; 1984; 1987; 1988).

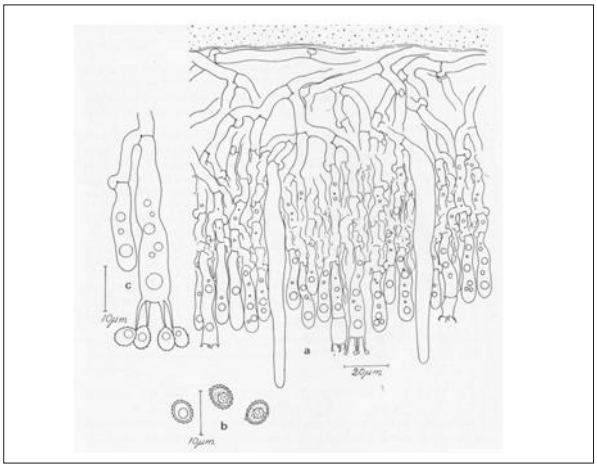

Fig. 2 Section of *Hypochnicium cremicolor*. Drawing by John Eriksson.

At beginning of seventies the technical development of scanning electron microscopy had begun to be an important tool. In particular, spore surfaces could exhibit a great variation in ornamentation. In certain genera this has been found to be of great importance for systematics. Such delicate differences in ornamentation structure was also interpreted by Eriksson in his drawings.

The interest among students was now growing. The flora became a great tool in species identification and now it became fruitful to develop the field of corticioid ecology. After all, this group of fungi were ecologically important wood decayers and thereby essential to all forest ecosystems. Together with Prof. Ryvarden´s polypore

floras we now had some really good tools for students working with natural decay of wood, not only in the Nordic countries but also elsewhere. It became very clear, that the descriptions of corticioid species could not be done trustworthy without this very detailed approach. Even though the *Corticiaceae* flora by Eriksson & Ryvarden was never reprinted, it soon appeared as photocopy all over the world. New geographical areas were investigated and contributed to general mycogeography.

Still, however, species and genus delimitations were based on morphology and thus highly affected by personal opinions. Already during the forties and fifties, John Eriksson and Jaques Boidin had begun to study fungal cultures for the delimitation and characterization of species. They found that wood fungi in general were usually easy to culture on artificial media. Basidiospores from fruitbodies were isolated as haploid cultures and in combination with similar cultures from other specimens could yield clamp connections on their fused hypha. This has been taken as a strong indication of conspecifity.

Fungal cultures were also studied morphologically as fruitbodies and schemes were set up for identification of species obtained as cultures only (Nobles 1965; Stalpers 1978; Nakasone 1990).

DELIMITATION OF BIOLOGICAL SPECIES

During the eighties Nils Hallenberg brought this kind of research further and used extensive crossing tests for delimitation of biological species and investigating their geographical distributions. Until that time it was common belief that all species could be recognized by their morphology, although this difference could be minimal. By the use of crossing tests and comparative morphology it became obvious that a great part of corticioid species recognized so far, were in fact species complexes, composed of two or more biological species, i.e. species which were not possible to distinguish from each other by morphology. Often but not always the different biological species could be distinguished by their substrate preferences or ecology (Hallenberg, 1991). Results from the extensive crossing tests during the eighties could now be tested using DNA sequences and no wonder that the biological species concept was fully supported by these analyses.

SCOPE FOR POTENTIAL USE IN DRUG PRODUCTION

During this period, big pharmaceutical companies intensified their hunting for new, potential drugs and fungi (including the corticioids) was one of their targets. Easy to isolate and propagate and later to test for medical properties. However, little scientific knowledge from theses efforts became common knowledge or reached the free research at universities. At the National Symposium on Mycology in Patiala (2015) lots of activities were exposed where the potential use of fungi for drug production was in focus, from universities all over the country. This is all very promising.

MOLECULAR APPROACH

The great revolution in research on corticioids, as well as other fungi, came with PCR and DNA sequencing, from the beginning of nineties and onwards. Systematics on all levels could be analysed and lots of new genera emerged. For the first time it became possible to recognize higher ranks in systematics (Binder *et al.,* 2005). A remaining delimitation is, however, that less than10% of the estimated number of species known so far are described. In pace with more species and more DNA regions being sequenced, the systematics of fungi will continue to develop but also to change. This is not a problem but merely part of research itself and systematics will never be a final product.

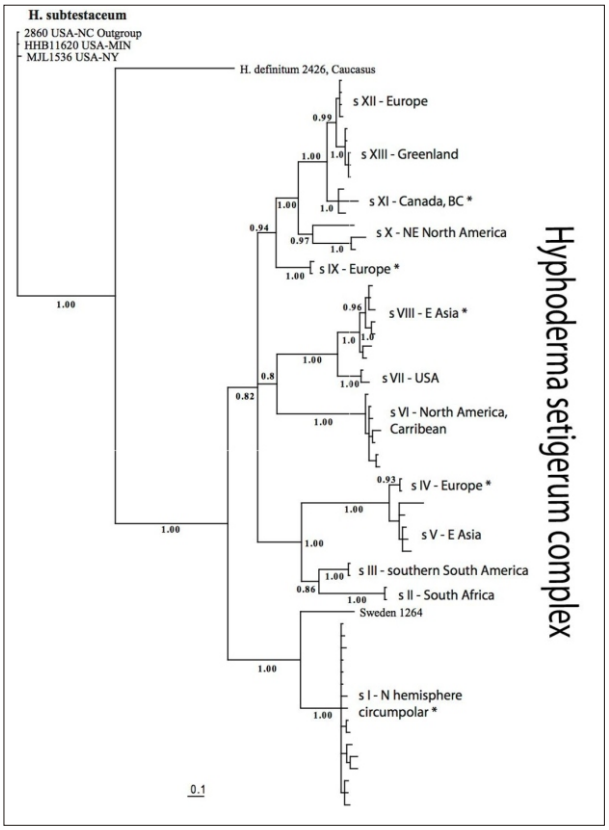

Fig. 3 Phylogenetic analyses based on ITS sequences from *Hyphoderma setigerum* species complex. Specimen numbers are omitted to facilitate a better overview. Crossing tests have confirmed compatibility within terminal clades, incompatibility between them.

From the figures it is obvious that there is a certain geographic differentiation between clades, which obviously has taken place at different biohistorical periods most certainly as a consequence of climatic changes and the associated changes in zonal vegetation on earth. From comparisons with other, clock-calibrated changes in ITS sequences, it can be estimated that even the closely located terminal clades in the figure above are separated by millions of years.

Our common knowledge on species distribution on earth has been highly dependant on the collection of fruitbodies

in nature, from which the DNA was extracted. Species are, however, not only present as spores or fruitbodies but successful fungal establishments are also occurring as simple mycelia - completely hidden from our eyes. Even corticioids and polypores may occur asymptotically as endophytes in trees, other than their natural hosts (Crozier *et al*., 2006). As the databases with fungal DNA sequences become more extensive, environmental samples will show us that species occurrences in nature is far more extensive than their fruitbodies.

CONCLUSION

Researchers have to accept that morphology alone cannot distinguish the true species of Nature. Nevertheless, to identify a specimen as belonging to a certain species complex is also an important step towards a deeper knowledge. Biodiversity research has to go on in all parts of the world and gradually we well get deeper insights on how our biota changes by time and the connections with climate changes. The last forty years in mycology have revealed a dramatic scientific development and we can not even speculate over what the future will bring. I myself can just feel humility for having the great fortune to have witnessed this period in my own work, and I am well aware that far more fascinating stories can be told by other researchers from other parts of then world not least from India.

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A comparative study of metal tolerance and sorption capacities of varied fungal genera from metal - polluted estuarine environments for potential in metal bioremediation

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ABSTRACT

Metal contaminated sites are known to support the development of metal resistant microorganisms, which can serve as biomonitors of a particular pollutant, and as agents for bioremediation. Fungal isolates were obtained from various metal-contaminated estuarine environments and assessed for their tolerance levels and sorption of the different heavy metals and transition metals. A comparative analysis of metal tolerance and sorption capability by fungi with respect to the various sites, the genera or species isolated, and their response to different metals showed that despite of high levels of a particular metal in a given econiche, a fungal organism may or may not have a high tolerance and/or sorption to the metal, and may therefore not necessarily be an indicator of metal pollution. Furthermore, fungal-metal interaction, while being more pronounced in the genera of *Penicillium* and *Aspergillus* also did not show uniformity within a given genus or species. Moreover, metal tolerance and its sorption by a given isolate had no definite correlation, as metal tolerance by a given species was not indicative of the sorptive capacity of the fungus, and could therefore be genus or species specific, as well as metal specific.

Keywords: Fungi, estuarine habitats, metal pollution, metal tolerance, metal sorption.

INTRODUCTION

Metals are normal constituents of the earth's crust. Anthropogenic activity with constantly increasing industrialization has resulted in their release into the surroundings. Metals are not degradable, and therefore accumulate, resulting in ever increasing levels of pollution in the environment. Aquatic water bodies are increasingly being polluted by the disposal of effluents. Metal pollution in the marine ecosystem has become a serious threat, as they accumulate throughout the food chain, leading to serious ecological and health problems (Laws, 1993; Rehman *et al.*, 2008; Banik *et al.*, 2013).

Bioremediation is an ecofriendly and cost-effective alternate technology that uses metabolic processes to degrade or transform contaminants, so that they remain no longer in harmful form. Fungi are preferred microorganisms for bioremediation processes because of their large biomass production and ease of biomass separation. They are a versatile group, able to adapt and grow under extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations, with the advantage of excellent metal-binding properties of their cell wall and capacity for efficient metal sequestration (Gadd, 1994; Volesky and Holan, 1995; Kapoor *et al.*, 1999; Baldrian and Gabriel, 2002; Singh, 2006; Wang and Chen, 2006; Yan and Viraraghavan, 2008; Gazem and Nazareth, 2013; Fomina and Gadd, 2014).

Organisms in metal contaminated sites, develop resistance to metals, and serve as agents for bioremediation (Gadd, 1994). In this context, a study was made on natural systems exposed to the metal contamination from iron ore mining industry. About two-thirds of the mining activities in Goa are located in the Mandovi basin, on the West Coast of the Indian peninsula. The Mandovi estuary connects with the Arabian Sea, and serves as a waterway for transport of the iron ore to the Mormugao harbor for the off-loading and export to eastern countries, particularly China, Japan, South Korea and Eastern European Countries. The estuary is also subjected to dumping of waste, including mine tailings (Alagarsamy, 2006; Mesquita and Kaisary, 2007; Ratnaprabha and Nayak, 2011).

In this work, it was sought to examine the capacity of fungi for metal tolerance and/or metal removal, whether is acquired in the presence of a pollutant metal in the environment, or is an inherent function of the biomass; whether it is enhanced in presence, or is independent of metal concentrations; and whether it is genus/species specific, or is definitive of a particular isolate.

MATERIALS AND METHODS

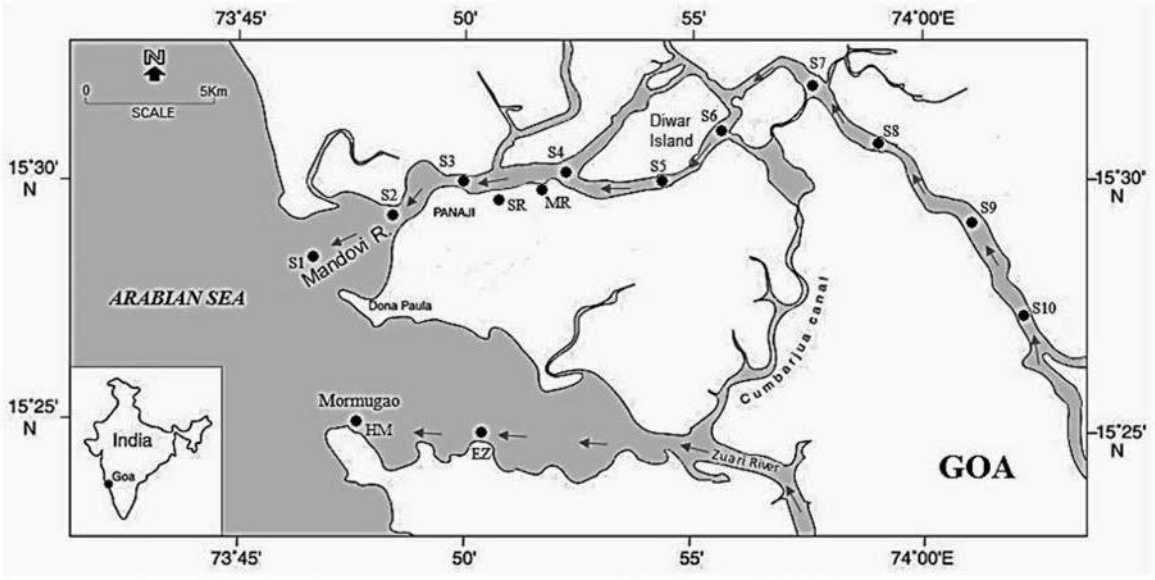
1) Sample sites and collection: Water and sediment samples were collected from five locations in Goa - India (**Fig. 1.**), during pre-monsoon season in the summer month of May.

Sampling was done from Mangroves at Ribander (MR) 15° 30'20.2"N, 73° 53.41"E. Water (w) (MRw) and sediment (s) samples (MRs) were collected aseptically from five sites, for microbial analysis, then pooled together. Similarly, brine water and sediment samples (SRw and SRs) were collected from solar Salterns at Ribander (SR), 15° 30'08.8"N, 73° 51.77"E, pooled together from five different saltpans at the time of salt harvesting.

Sampling was also carried out along the Mandovi estuary (EM), at ten stations: S1 to S10, beginning at the mouth and moving hinterland between 15° 28'34.1"N, 73° 46.65"E to 15° 27'59.8"N, 74° 02.05"E. Surface and bottom water samples, w_s and w_b, respectively, were collected with a Niskin water sampler and sediment using Grab sediment sampler; samples were labeled EM1w_s, EM1w_b and EM1s at station 1; and samples from stations 2-10 were labeled in similar manner.

Water and sediment samples were also obtained from the Zuar dock at Zuari Estuary (EZ) 15° 24'07.8"N, 73° 50.65"E, (EZw and EZs). Likewise, water samples were collected from the harbor at Mormugao (HMw) 15° 24'16.7"N, 73° 47.58"E, before and after the monsoons.

Water samples from the above mentioned sites were also collected in acid-washed polypropylene bottles for



MR : Ribander Mangrove
SR : Ribander Saltern
EM : Estuary, MandoviS1,S2..S10
EZ : Estuary, Zuar Dockyard
S3 : Panjim ferry point (EM3)
HM : Mormugao Harbor
S5 : Diwar ferry point (EM5)
:Barge movement

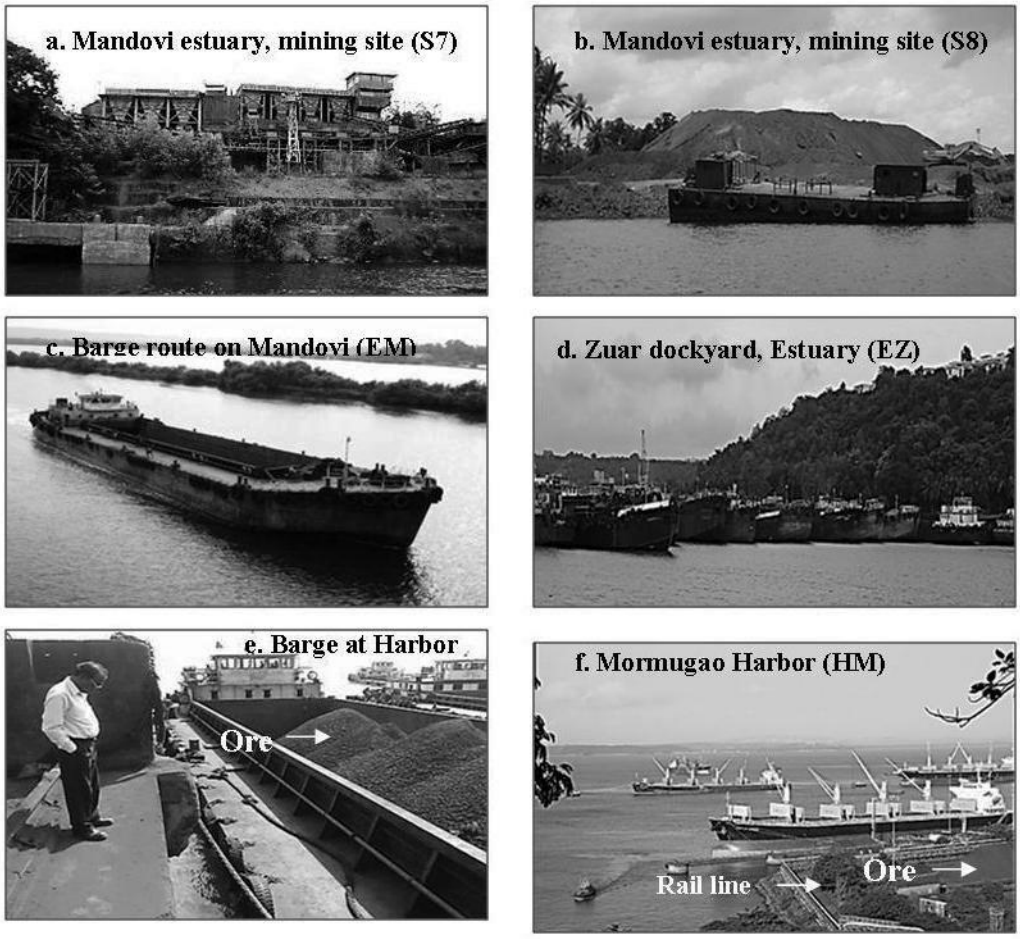


Fig.1. Sampling sites in Goa, India

analysis of metal content, and acidified with 5 ml 1% HNO_3 to eliminate reaction of any organic matter present. Samples were stored at 4 °C till they could be processed.

2) Assessment of levels of metals present in water samples: Metal concentrations in the water samples were measured by a modified solvent extraction method (Satyanarayanan, 2007). Water samples in duplicates, 100 ml each, along with a blank of deionized water, were acidified with 0.5 ml of 0.02 M HNO_3, and the pH adjusted to 4.1 for Fe and 6.4 for Pb, Cu, Cd, Mn using 3 M ammonia / 2 M acetic acid buffer. To this, 1 ml of 1% Ammonium pyrolidine dithiocarbamate (HIMEDIA®) and 5ml of Methyl-isobutyl ketone (Qualigens®)were added, which serves to extract a wide range of dithiocarbamate-metal chelates; the mixture was shaken vigorously for 5 min and then allowed to stand for 20-30 min. To the upper organic phase, 20 ml of 4N HNO_3 was added and shaken for acid back-extraction. The lower aqueous phase was transferred to polypropylene tubes for further analysis by atomic absorption spectrophotometry (AAS), (Shimadzu AA-6300).

3) Isolation and identification of fungi: Water and sediment suspensions in 2% saline were spread plated on Czapek-Dox Agar (HIMEDIA®) + 2% NaCl termed as S-CzA, incubated at 30 °C for 2-4 days. The isolates were picked based on the dissimilarity in the colony characteristics, purified and maintained on S-CzA at 4 °C. The isolates were identified to the genus level on the basis of colony and microscopic characteristics with reference to standard fungal identification keys (Raper and Fennell, 1965; Ellis, 1971; Domsch *et al.*, 1980).

4) Metal tolerance: The isolates were screened for metal tolerance by spot inoculation of spore suspensions on S-CzA plates, each containing a single metal ion: 10 mM Pb^{2+} as $Pb(NO_3)_2$, 5 mM Cu^{2+} as $CuSO_4$, 2 mM Cd^{2+} as $Cd(NO_3)_2$ or as $CdSO_4$, 5 mM Fe^{3+} as $FeCl_3$, 10 mM Fe^{2+} as $FeSO_4$ and 50 mM Mn^{2+} as $MnSO_4$, and incubated at 30 °C for 4 days.

Tolerance index (TI) was determined as the ratio of the growth obtained on medium containing metal to that on medium without metal. The maximum tolerance concentration (MTC) was determined by spot-inoculating, in triplicate, on S-CzA containing metal in the range of 0-25 mM Pb^{2+} or Cu^{2+}; 0-6 mM Cd^{2+} as nitrate and as sulphate; 0-10 mM Fe^{3+}; 0-25 mM Fe^{2+}; 0-250 mM Mn^{2+}. The plates were incubated at 30 °C and growth was recorded in terms of colony diameter after 4 days, and examined visually up to 7 days for changes in growth pattern, sporulation and pigment production, compared against the control of isolates grown in the absence of the metal.

5) Metal Biosorption: Isolates were selected based on the criterion of belonging to different genera, isolated from different econiches and possessing a high tolerance to the metal(s). Spore suspension containing 10^6 spores, was inoculated in 100 ml of Czapek-Dox Broth + 2% NaCl (S-CzB) and incubated for 3 days at 30 °C, 150 rpm. The biomass was harvested by filtering through double layered muslin cloth and washed with 100 ml saline, then washed twice with deionized water. Aliquots of the biomass were taken to obtain the wet weight and then dried to constant weight at 60 °C to obtain the dry weight. Mycelial biomass, 1 g wet weight, approx. 0.1 g dry weight, was homogenized with a mortar and pestle, suspended in 20 ml of 1 mM of individual metal solution with pH adjusted to 6.0. Control flasks of only metal solution were maintained. The flasks were incubated at 150 rpm for 1 h at 30 °C and the biomass then filtered off through Whatman no. 1 filter paper.The filtrates, 10 ml, were digested with 50% concentrated HNO_3, 0.5 ml, for 10 min and then made up to original volume with deionized water. The metal concentration was analyzed by AAS. All the experiments were carried out in duplicates.

The amount of metal sorbed by the fungal biomass was calculated using the following equation: $Q\ (mg\ l^{-1}) = V\ (C_i - C_f)\ /S$, where Q = mg of metal ion sorbed g^{-1} dry weight biomass; C_i = initial metal ion concentration (mg l^{-1}); C_f = final metal ion concentration (mg l^{-1}); S = biosorbent (g), V = volume (l) of the reaction mixture (Volesky and Holan, 1995).

6) Statistical analysis of metal tolerance and sorbing capacity: The metal tolerance and sorption levels of the selected isolates were statistically analyzed for similarity or difference in sorption capacity between the genera and/or species. Statistical analysis was done using the online software Web Agri Stat Package-2.0 (WASP) designed by Indian Council of Agricultural Research, Goa, India.

RESULTS

Levels of metals present in water samples: The concentrations of heavy metals: lead, copper and cadmium as well as transition metals: iron and manganese in the water at the various sites of sampling are shown in **Fig. 2.** The levels of metals in the Mandovi estuary was examined at two Stations, Station 3, the Diwar ferry point (EM3) and Station 5, the Panjim ferry point (EM5). It was observed that iron concentrations at EM5, was only 0.16 ppm, while it was very high 5.4 ppm at EM3. In contrast, manganese was fairly high at 1.82-1.86 ppm in all the samples tested. At mangroves, lead and copper concentrations were not significantly high, but cadmium was high at 0.17 ppm. Saltern brine sample registered high concentration of 0.97 ppm cadmium, 9.91 ppm iron and 8.26 ppm manganese, but lead and copper being at lower concentrations. Zuar dock and Mormugao harbor showed extremely high levels of 4.34 ppm and 1.22 ppm, respectively, the highest lead contamination amongst the sites examined; cadmium levels were also high at these sites, at 0.26 ppm and 0.05 ppm, respectively.

Fungal isolates: Isolates obtained belonged predominantly to *Penicillium* and *Aspergillus*; the other genera obtained included *Fusarium* and the dematiaceous fungi, *Alternaria* and *Cladosporium*. The isolates, selected for study on the basis of their metal tolerance and representative species from different locations (**Table 1**), were tentatively identified as *Penicillium*

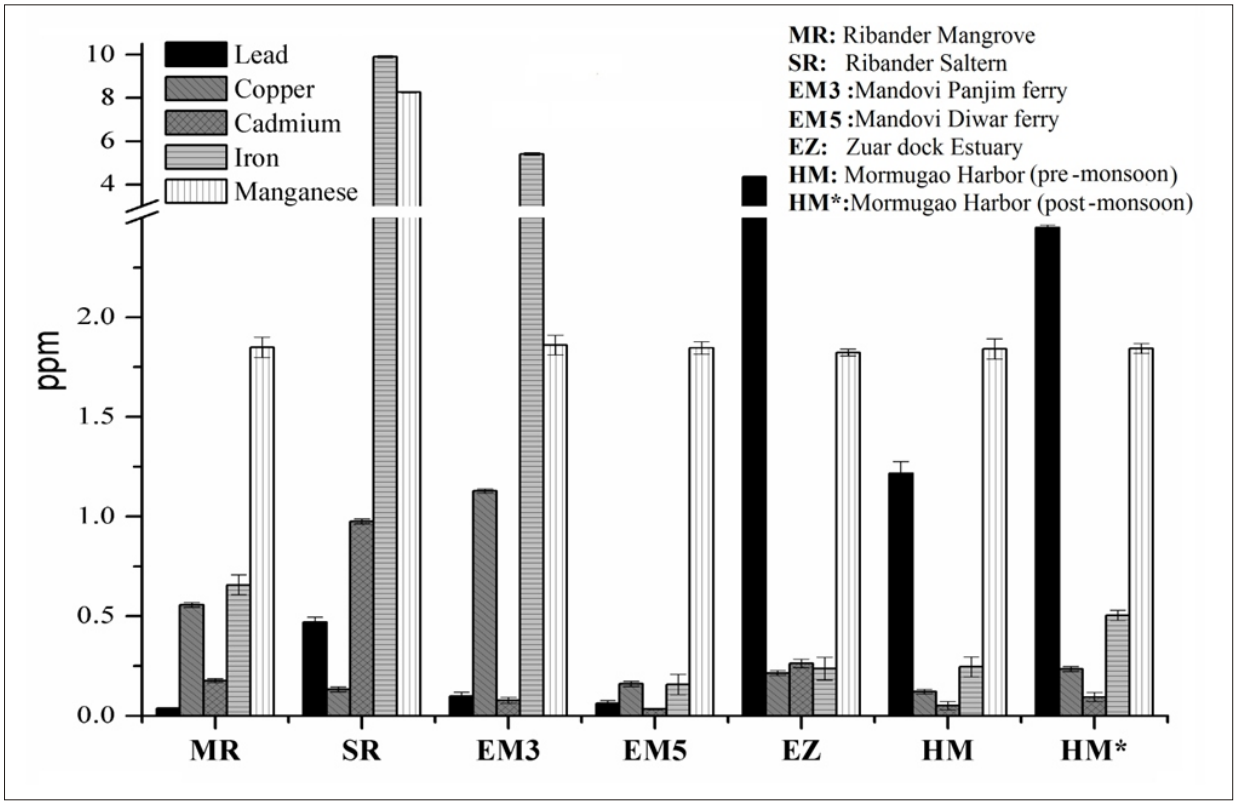


Fig.2. Levels of metals in water samples

brevicompactum, *P. citrinum*, *P. corylophilum*, *P. glabrum*; *Aspergillus flavus*, *A. flavus* var. *flavus*, *A. flavus* var *columnaris*, *A. niger*, *A. oryzae*, *A. terreus*, *A. versicolor*, *A. fumigatus*, *A. awamori*; *Fusarium chlamydosporum*, *F. oxysporum*, *F. solani*, *F. sporotrichioides*; *Alternaria alternata*, *A. citri*, *A. sonchi*; *Cladosporium cladosporioides*, *C. chlorocephalum*, *C. oxysporum*, *C. cucumerinum* and *C. spongiosum.*

Metal Tolerance

I) Screening for metal tolerance: A total of 147 isolates were screened for tolerance to selected metals: fifteen isolates from Ribander mangroves (MR) and fifteen from salterns (SR), fifty from Mandovi Estuary (EM), thirty-seven from Zuar-dock (EZ) and thirty from Mormugao harbor (HM). Tolerance index (TI) obtained (**Fig. 3.A, 3.B**) indicated that most of the isolates from MR and SR showed significant tolerance towards 10 mM lead, with most isolates of *Penicillium* having an index ≥ unity and *Aspergillus* index close to 1, whereas isolates from EM, EZ and HM had TI ≤ 1. Most isolates from all sites showed a moderate tolerance, with TI ≤ 1 for 5 mM copper and iron, and for 50 mM manganese. Many isolates showed TI < 0.5 for 2 mM cadmium with a few penicilli having TI ≤ 1. *Penicillium* species had a lower tolerance to cadmium nitrate as compared to cadmium sulphate. It was noted that all aspergilli from salterns that were examined, could not grow in presence of even 0.5 mM cadmium salts.

Ii) Maximum metal tolerance concentrations: Seventy two isolates were selected based on their ability to grow in presence of high concentrations of heavy metals and transition metals, ten each from MR and SR, a total of thirty two from all the ten stations of EM, and ten each from EZ and HM, for determination of MTC of metals. The highest MTC of each metal by each genera (**Table 2.A, 2.B**) was shown by *Penicillium* and *Aspergillus* at 15 mM Pb^{2+}, 10 mM Cu^{2+}, 5 mM Cd^{2+} as nitrate, 7.5 mM Cd^{2+} as sulphate, 7.5 mM Fe^{3+}, 25 mM Fe^{2+} and 250 mM Mn^{2+}, while *Fusarium* and the dematiaceous fungi *Alternaria* and *Cladosporium* showed MTC values ranging from 7.5-10 mM Pb^{2+}, 7.5 mM Cu^{2+}, 2-4 mM Cd^{2+} as nitrate, 4-5 mM Cd^{2+} as sulphate, 5 mM Fe^{3+}, 7.5-20 mM Fe^{2+} and 100-200 mM Mn^{2+}. The statistical analysis indicated that the genera of *Penicillium* and *Aspergillus* have the maximum tolerance capacity as compared to the other genera.

There was dissimilarity in metal tolerance between the different species of *Penicillium*: *P. corylophilum, P. brevicompactum* and *P. glabrum*. *P. corylophilum* $EM2w_b256$ and *P. glabrum* EM1s239 possessed the maximum tolerance to lead, copper and ferrous ions, while *P. citrinum* SRw119 showed the highest tolerance to cadmium sulphate and both ferric and ferrous salts. In addition, while *P. corylophilum* showed the highest MTC of lead, *P. brevicompactum* showed the lowest MTC. This was also seen amongst different species of aspergilli, with *A. Oryzae* EM3s281 exhibiting the highest tolerance to copper, cadmium nitrate and manganese; however, similar metal tolerance levels by the isolated strains of *A. niger* were observed.

There was a dissimilarity in metal tolerance seen also

Table 1. Fungal isolates obtained from the various sampling sites

Site	Genus	Species	Isolate number
Ribander Mangrove	*Aspergillus*	*A. flavus* var. *flavus*	MRw4
	Penicillium	*P. brevicompactum*	MRw17, MRw19
		P. corylophilum	MRw1,MRw2,MRw3,MRw5, MRw6, MRw7, MRw9
Ribander Saltern	*Aspergillus*	*A. niger*	SRw108, SRw109, SRw110, SRw111, SRw112, SRw113, SRw114
		A. terreus	SRw115
	Penicillium	*P. citrinum*	SRw119
	Cladosporium	*C. chlorocephalum*	SRw120
Mandovi Estuary	*Aspergillus*	*A. flavus* var. *flavus*	EM5w_s317
		A. flavus var. *columnaris*	EM7w_b363
		A. niger	EM3s285
		A. oryzae	EM3s281, EM9w_s390
		A. terreus	EM1w_b234, EM9w_b398
		A. versicolor	EM4w_s294
		A. fumigatus	EM2w_b253
		A. awamori	EM10w_s408
	Penicillium	*P. corylophilum*	EM2w_b256, EM2s260, EM5w_s315, EM7s373, EM8w_s377, EM9s404
		P. glabrum	EM1s239, EM3w_s264, EM6w_b346
	Fusarium	*F. chlamydosporum*	EM9w_s392
		F. solani	EM1w_s210
		F. sporotrichioides	EM3w_s278, EM4w_s295
	Cladosporium	*C. cladosporioides*	EM1w_b232, EM8s385
		C. herbarum	EM4w_s288
		C. oxysporum	EM4w_s300, EM6w_s338
		C. cucumerinum	EM6w_s332
		C. spongiosum	EM10s423
	Alternaria	*A. alternata*	EM7s367
		A. sonchi	EM8s388
Zuar dock Zuari Estuary	*Aspergillus*	*A. flavus*	EZs526
		A. terreus	EZw501
	Penicillium	*P. brevicompactum*	EZs530
		P. glabrum	EZw514
	Fusarium	*F. oxysporum*	EZw520, EZs534
	Cladosporium	*C. chlorocephalum*	EZs531
		C. oxysporum	EZw507
	Alternaria	*A. alternata*	EZw524
		A. citri	EZw523
Mormugao Harbor	*Aspergillus*	*A. niger*	HMw605
		A. versicolor	HMw602
	Penicillium	*P. brevicompactum*	HMw617
		P. corylophilum	HMw626
	Fusarium	*F. oxysporum*	HMw603, HMw611
	Cladosporium	*C. cladosporioides*	HMw610, HMw621
	Alternaria	*A. alternata*	HMw601
		A. citri	HMw615

Table 2.A. Statistical analysis of tolerance and sorption of heavy metals by the isolates

Isolate No.	Species	MTC Pb^{2+} mM	Q (mg g^{-1}) Pb^{2+}	MTC Cu^{2+} mM	Q (mg g^{-1}) Cu^{2+}	MTC Cd^{2+} $(NO_3)_2$ mM	Q (mg g^{-1}) Cd^{2+}	MTC Cd^{2+} SO_4 mM	Q (mg g^{-1}) Cd^{2+}
MRw1	*Penicillium corylophilum*	^{c}10.0	^{k}32.29±0.20	^{f}2.5	^{k}2.24±0.07	^{c}2.0	^{nopq}0.38±0.10	^{f}2.0	^{s}4.15±0.02
MRw2	*P. corylophilum*	^{a}15.0	^{h}36.28±0.01	^{c}5.0	^{k}2.09±0.18	^{c}2.0	^{mnop}0.50±0.06	^{c}5.0	^{v}1.02±0.05
MRw3	*P. corylophilum*	^{b}12.5	^{z}12.17±0.20	^{c}5.0	^{mn}1.50±0.08	^{c}2.0	^{k}1.57±0.03	^{c}5.0	^{u}1.66±0.03
MRw4	*Aspergillus versicolor*	^{a}15.0	^{k}32.28±0.07	^{f}2.5	^{h}3.74±0.08	^{e}0.5	^{l}0.91±0.24	^{e}3.0	^{u}1.33±0.04
MRw5	*Penicillium corylophilum*	^{c}10.0	^{a}45.03±0.04	^{c}5.0	^{k}2.30±0.24	^{d}1.0	^{h}3.54±0.01	^{c}5.0	^{x}0.15±0.05
MRw6	*P. corylophilum*	^{b}12.5	^{z}16.17±0.10	^{c}5.0	^{j}2.86±0.23	^{c}2.0	^{t}0.02±0.24	^{f}2.0	^{op}5.99±0.01
MRw7	*P. corylophilum*	^{b}12.5	^{q}27.24±0.14	^{c}5.0	^{j}2.97±0.02	^{e}0.5	^{r}0.18±0.02	^{e}3.0	^{x}0.08±0.01
MRw9	*P. corylophilum*	^{b}12.5	^{c}40.05±0.05	^{c}5.0	^{i}3.00±0.03	^{c}2.0	^{r}0.19±0.04	^{a}7.5	^{x}0.08±0.04
MRw17	*P. brevicompactum*	^{d}7.5	^{r}26.81±0.81	^{f}2.5	^{h}3.83±0.04	^{e}0.5	^{s}0.15±0.03	^{e}3.0	^{x}0.23±0.03
MRw19	*P. brevicompactum*	^{e}5.0	^{y}18.32±0.60	^{f}2.5	^{h}3.80±0.04	^{h}0.0	^{lm}0.80±0.08	^{a}7.5	^{x}0.14±0.02
SRw108	*Aspergillus niger*	^{e}*5.0*	^{v}22.86±0.61	^{c}5.0	^{g}4.02±0.12	^{h}0.0	^{k}1.50 ±0.04	^{h}0.0	^{x}0.23±0.01
SRw109	*A. niger*	^{e}*5.0*	^{x}20.92±0.14	^{c}5.0	^{lm}1.76±0.09	^{h}0.0	^{lmn}0.73±0.01	^{h}0.0	^{x}0.19±0.04
SRw110	*A. niger*	^{e}*5.0*	^{i}34.64±0.01	^{c}5.0	^{lm}1.67±0.06	^{h}0.0	^{l}0.89±0.01	^{h}0.0	^{u}1.43±0.02
SRw111	*A. niger*	^{e}5.0	^{s}24.95±0.11	^{c}5.0	^{l}1.83±0.07	^{h}0.0	^{l}1.06±0.07	^{h}0.0	^{w}0.69±0.04
SRw112	*A. niger*	^{e}5.0	^{n}30.06±0.30	^{c}5.0	^{j}2.87±0.01	^{h}0.0	^{opq}0.34±0.08	^{h}0.0	^{x}0.11±0.07
SRw113	*A. niger*	^{e}5.0	^{z}13.60±0.17	^{c}5.0	^{n}1.39±0.01	^{h}0.0	^{l}1.00±0.03	^{h}0.0	^{x}0.11±0.06
SRw114	*A. niger*	^{c}10.0	^{b}40.66±0.12	^{c}5.0	^{mn}1.78±0.19	^{h}0.0	^{l}1.04±0.08	^{h}0.0	^{u}1.56±0.09
SRw115	*A. terreus*	^{c}10.0	^{e}38.02±0.06	^{f}2.5	^{j}2.81±0.14	^{h}0.0	^{g}4.02±0.06	^{h}0.0	^{r}4.52±0.07
SRw119	*Penicillium citrinum*	^{b}12.5	^{a}45.10±0.01	^{c}5.0	^{b}6.24±0.01	^{a}4.0	^{f}5.18±0.01	^{a}7.5	^{q}5.02±0.01
SRw120	*Cladosporium chloro-cephalum*	^{e}5.0	^{j}33.81±0.09	^{f}2.5	^{d}4.91±0.15	^{b}3.0	^{j}2.50±0.06	^{c}5.0	^{t}3.04±0.07
EM2w_b253	*Aspergillus fumigatus*	^{b}12.5	^{op}28.69±0.2	^{a}10.0	^{op}0.95±0.03	^{b}3.0	^{e}7.71±0.2	^{e}3.0	^{e}39.3±0.1
EM2w_b256	*Penicillium corylophilum*	^{a}15.0	^{k}32.69±0.01	^{a}10.0	^{ef}4.24±0.06	^{a}4.0	^{l}2.72±0.08	^{b}6.0	^{l}31.1±0.07
EM4w_s288	*Cladosporium herbarum*	^{c}10.0	^{m}30.58±0.04	^{d}4.0	^{ef}4.49±0.06	^{b}3.0	^{k}1.82± 0.04	^{a}7.5	^{h}36.26±0.04
EM6w_s332	*C. cucumerinum*	^{c}10.0	^{z}8.58±0.19	^{b}7.5	^{s}0.49±0.05	^{b}3.0	^{k}1.82±0.07	^{c}5.0	^{h}36.26±0.35
EM8s388	*Alternaria sonchi*	^{e}5.0	^{b}40.69±0.07	^{c}5.0	^{c}5.57±0.04	^{c}2.0	^{ij}2.92±0.13	^{f}2.0	^{j}33.44±0.22
EM9w_s392	*Fusarium chlamy-dosporum*	^{e}5.0	^{f}37.44±0.04	^{c}5.0	^{s}0.40±0.01	^{e}0.5	^{b}15.39±0.01	^{g}1.0	^{a}46.76±0.06
EZw514	*Penicillium glabrum*	^{b}12.5	^{z}12.58±0.09	^{c}5.0	^{e}4.40±0.03	^{c}2.0	^{k}1.74±0.41	^{c}5.0	^{k}32.36±0.68
EZw520	*Fusarium oxysporum*	^{e}5.0	^{t}23.65±0.14	^{e}3.0	^{r}0.65±0.03	^{d}1.0	^{b}15.8±0.38	^{e}3.0	^{c}42.24±0.27
EZw524	*Alternaria alternata*	^{d}7.5	^{gh}36.46±0.04	^{e}3.0	^{e}4.53±0.04	^{d}1.0	^{f}5.29±0.57	^{e}3.0	^{g}37.28±0.27
EZs526	*Aspergillus flavus*	^{c}10.0	^{u}23.22±0.81	^{d}4.0	^{s}0.44±0.03	^{d}1.0	^{k}1.91±0.37	^{d}4.0	^{k}32.68±0.1
EZs531	*Cladosporium chloro-cephalum*	^{e}5.0	^{z}14.73±0.61	^{e}3.0	^{t}0.31±0.03	^{d}1.0	^{d}11.46±0.43	^{d}4.0	^{d}39.58±0.84
HMw601	*Alternaria alternata*	^{c}10.0	^{d}39.27±0.61	^{c}5.0	^{a}7.73±0.06	^{c}2.0	^{f}5.27±0.29	^{c}5.0	^{i}35.91±0.19
HMw602	*Aspergillus versicolor*	^{d}7.5	^{z}17.33±0.14	^{c}5.0	^{t}0.34±0.03	^{d}1.0	^{j}2.3±0.48	^{e}3.0	^{m}26.22±1.14
HMw610	*Cladosporium cladosporioides*	^{e}5.0	^{l}31.04±0.01	^{e}3.0	^{s}0.47±0.03	^{d}1.0	^{f}5.63±0.12	^{e}3.0	^{f}38.12±0.06
HMw611	*Fusarium oxysporum*	^{d}7.5	^{w}21.37±0.11	^{c}5.0	^{q}0.51±0.03	^{c}2.0	^{a}16.78±0.07	^{e}3.0	^{b}42.76±0.61
HMw615	*Alternaria citri*	^{d}7.5	^{g}36.61±0.06	^{c}5.0	^{e}4.55±0.03	^{c}2.0	^{f}5.26±0.08	^{c}5.0	^{g}37.25± 0.05
HMw626	*Penicillium corylophilum*	^{c}10.0	^{r}26.47±0.29	^{c}5.0	^{fg}4.14±0.07	^{c}2.0	^{h}3.16±0.01	^{c}5.0	^{n}25.76±0.65

$^{a-z}$: denotes statistical variation; ± : Standard Error

amongst species of each of the other genera, *Fusarium, Alternaria* and *Cladosporium*:*Fusarium oxysporum* HMw611 showed the highest tolerance to 3 metals, namely, lead, copper and ferrous ions; *Alternaria alternata* HMw601 had the greatest tolerance to lead, copper and cadmium salts, and *Alternaria citri* HMw615 to copper, cadmium salts and ferrous ions; *Cladosporium herbarum* EM4w_s288 was most tolerant of lead, copper, cadmium sulphate and ferrous sulphate. However, a similarity in metal tolerance was also observed between two different species of *Alternaria,* namely *A. alternata* HMw601 and *A. citri* HMw615, obtained from the same site at harbor.

Table 2.B. Statistical analysis of tolerance and sorptionof transitional metals by the isolates

Isolate No.	Species	MTC Fe^{3+}	Q (mg g^{-1}) Fe^{3+}	MTC Fe^{2+}	Q (mg g^{-1}) Fe^{2+}	MTC Mn^{2+}	Q (mg g^{-1}) Mn^{2+}
MRw1	*Penicillium corylophilum*	^{b}5.0	^{hi}10.47±0.02	^{f}5.0	^{e}16.54±0.18	^{e}75.0	^{i}32.15±0.01
MRw2	*P. corylophilum*	^{a}7.5	^{ef}10.76±0.04	^{f}5.0	^{a}20.12±0.07	^{e}75.0	^{f}35.56±0.05
MRw3	*P. corylophilum*	^{b}5.0	^{hi}10.44±0.06	^{b}20.0	^{a}20.11±0.06	^{d}100.0	^{g}34.18±0.08
MRw4	*Aspergillus versicolor*	^{b}5.0	^{fg}10.65±0.01	^{b}20.0	^{a}20.08±0.02	^{e}75.0	^{i}32.23±0.08
MRw5	*Penicillium corylophilum*	^{b}5.0	^{hi}10.46±0.01	^{a}25.0	^{b}19.99±0.02	^{f}50.0	^{a}43.70±0.05
MRw6	*P. corylophilum*	^{b}5.0	^{d}10.93±0.08	^{d}10.0	^{a}20.58±0.02	^{f}50.0	^{b}42.35±0.03
MRw7	*P. corylophilum*	^{a}7.5	^{hi}10.41±0.01	^{d}10.0	^{b}19.94±0.03	^{e}75.0	^{f}35.73±0.06
MRw9	*P. corylophilum*	^{a}7.5	^{l}10.09±0.04	^{d}10.0	^{a}20.28±0.01	^{e}75.0	^{c}41.36±0.02
MRw17	*P. brevicompactum*	^{a}7.5	^{gh}10.50±0.02	^{c}15.0	^{a}20.38±0.02	^{d}100.0	^{h}33.87±0.04
MRw19	*P. brevicompactum*	^{a}7.5	^{jk}10.29±0.05	^{c}15.0	^{a}20.53±0.04	^{d}100.0	^{f}35.91±0.03
SRw108	*Aspergillus niger*	^{a}7.5	^{ef}10.73±0.01	^{c}15.0	^{a}20.69±0.02	^{e}75.0	^{e}36.47±0.02
SRw109	*A. niger*	^{a}7.5	^{kl}10.14±0.09	^{b}20.0	^{b}19.65±0.01	^{d}100.0	^{d}37.21±0.01
SRw110	*A. niger*	^{a}7.5	^{gh}10.56±0.04	^{c}15.0	^{a}20.31±0.07	^{e}75.0	^{h}33.30±0.12
SRw111	*A. niger*	^{b}5.0	^{s}6.62±0.06	^{f}5.0	^{a}20.65±0.02	^{f}50.0	^{a}43.70±0.02
SRw112	*A. niger*	^{b}5.0	^{c}11.26±0.02	^{d}10.0	^{c}18.84±0.02	^{f}50.0	^{g}34.84±0.08
SRw113	*A. niger*	^{a}7.5	^{de}10.84±0.02	^{d}10.0	^{a}20.57±0.08	^{d}100.0	^{h}33.68±0.06
SRw114	*A. niger*	^{a}7.5	^{c}11.14±0.03	^{b}20.0	^{a}20.52±0.02	^{d}100.0	^{a}43.00±0.01
SRw115	*A. terreus*	^{b}5.0	^{m}9.82±0.03	^{c}15.0	^{e}16.66±0.37	^{f}50.0	^{e}36.31±0.70
SRw119	*Penicillium citrinum*	^{a}7.5	^{a}12.56±0.15	^{a}25.0	^{d}17.77±0.38	^{d}100.0	^{a}43.93±0.02
SRw120	*Cladosporium chlorocephalum*	^{f}2.0	^{c}11.19±0.01	^{f}5.0	^{e}16.84±1.02	^{g}25.0	^{b}42.82±0.01
EM2w_b253	*Aspergillus fumigatus*	^{b}5.0	^{ij}10.3±0.01	^{b}20.0	^{m}6.57±0.07	^{d}100.0	^{m}9.82±0.02
EM2w_b256	*Penicillium corylophilum*	^{c}4.0	^{b}11.48±0.01	^{a}25.0	^{l}7.77±0.03	^{a}200.0	^{q}8.84±0.03
EM4w_s288	*Cladosporium herbarum*	^{c}4.0	^{nop}9.19 ± 0.04	^{e}7.5	^{h}10.98±0.04	^{e}75.0	^{mn}9.74± 0.04
EM6w_s332	*C. cucumerinum*	^{d}3.0	^{nop}9.19±0.07	^{f}5.0	^{gh}10.98±0.01	^{f}50.0	^{mn}9.74±0.04
EM8s388	*Alternaria sonchi*	^{f}2.0	^{c}11.48±0.01	^{g}2.5	^{m}6.07±0.03	^{e}75.0	^{m}9.69±0.06
EM9w_s392	*Fusarium chlamydosporum*	^{g}0.5	^{r}6.97±0.07	^{g}2.5	^{kl}7.61±0.01	^{g}25.0	^{jkl}10.74±0.02
EZw514	*Penicillium glabrum*	^{a}7.5	^{b}11.42±0.03	^{a}25.0	^{l}7.26±0.06	^{b}150.0	^{q}8.96±0.06
EZw520	*Fusarium oxysporum*	^{e}2.5	^{r}7.02±0.003	^{g}2.5	^{op}2.78±0.03	^{f}50.0	^{kl}10.58±0.02
EZw524	*Alternaria alternata*	^{b}5.0	^{c}11.48±0.01	^{g}2.5	^{I}9.40±0.01	^{c}125.0	^{l}10.66±0.01
EZs526	*Aspergillus flavus*	^{a}7.5	^{n}9.24±0.01	^{c}15.0	^{j}8.40±0.03	^{c}125.0	^{opq}8.86±0.01
EZs531	*Cladosporium chlorocephalum*	^{b}5.0	^{q}8.13±0.01	^{d}10.0	^{I}9.44±0.01	^{g}25.0	^{l}10.67±0.02
HMw601	*Alternaria alternata*	^{b}5.0	^{b}11.48±0.03	^{g}2.5	^{f}11.14±0.01	^{e}75.0	^{j}11.14±0.02
HMw602	*Aspergillus versicolor*	^{a}7.5	^{r}7.03±0.03	^{b}20.0	^{n}5.42±0.07	^{d}100.0	^{nop}9.25±0.03
HMw610	*Cladosporium cladosporioides*	^{e}2.5	^{t}5.90±0.01	^{f}5.0	^{jk}8.16±0.03	^{f}50.0	^{kl}10.77±0.07
HMw611	*Fusarium oxysporum*	^{e}2.5	^{op}9.1±0.01	^{c}15.0	^{l}7.73±0.04	^{g}25.0	^{jk}10.92±0.04
HMw615	*Alternaria citri*	^{b}5.0	^{b}11.46±0.04	^{d}10.0	^{i}9.20±0.07	^{e}75.0	^{kl}10.36± 0.05
HMw626	*Penicillium corylophilum*	^{a}7.5	^{b}11.44±0.01	^{b}20.0	^{fg}11.10±0.01	^{c}125.0	^{q}8.86±0.02

$^{a-z}$: denotes statistical variation; ± : Standard Error

Iii) Morphological changes in growth in response to metals: Growth of the fungal isolates was inhibited to a very slight extent in presence of lower concentrations of metals, other than the cadmium salts examined, cadmium nitrate being most inhibitory. However, when grown in presence of higher metal concentrations, the isolates grew slower, and showed striking variations in colony appearance as compared to the controls, becoming increasingly compact in presence of copper and cadmium, or with mycelia thinning out at the growing edge in presence of lead, iron and manganese, and a delayed sporulation. The colony of *Penicillium*, *Alternaria* and *Cladosporium* isolates grown in presence of copper and manganese, showed an undulate surface, while all isolates grown in presence of cadmium nitrate had an irregular margin and a crenate surface.

Aspergillus and *Fusarium* species yielded a zone of clearance around the colony, in response to lead, copper, cadmium and manganese but not to iron salts.

Pigment production in *Penicillium*, *Alternaria* and *Cladosporium* was enhanced in response to metals other

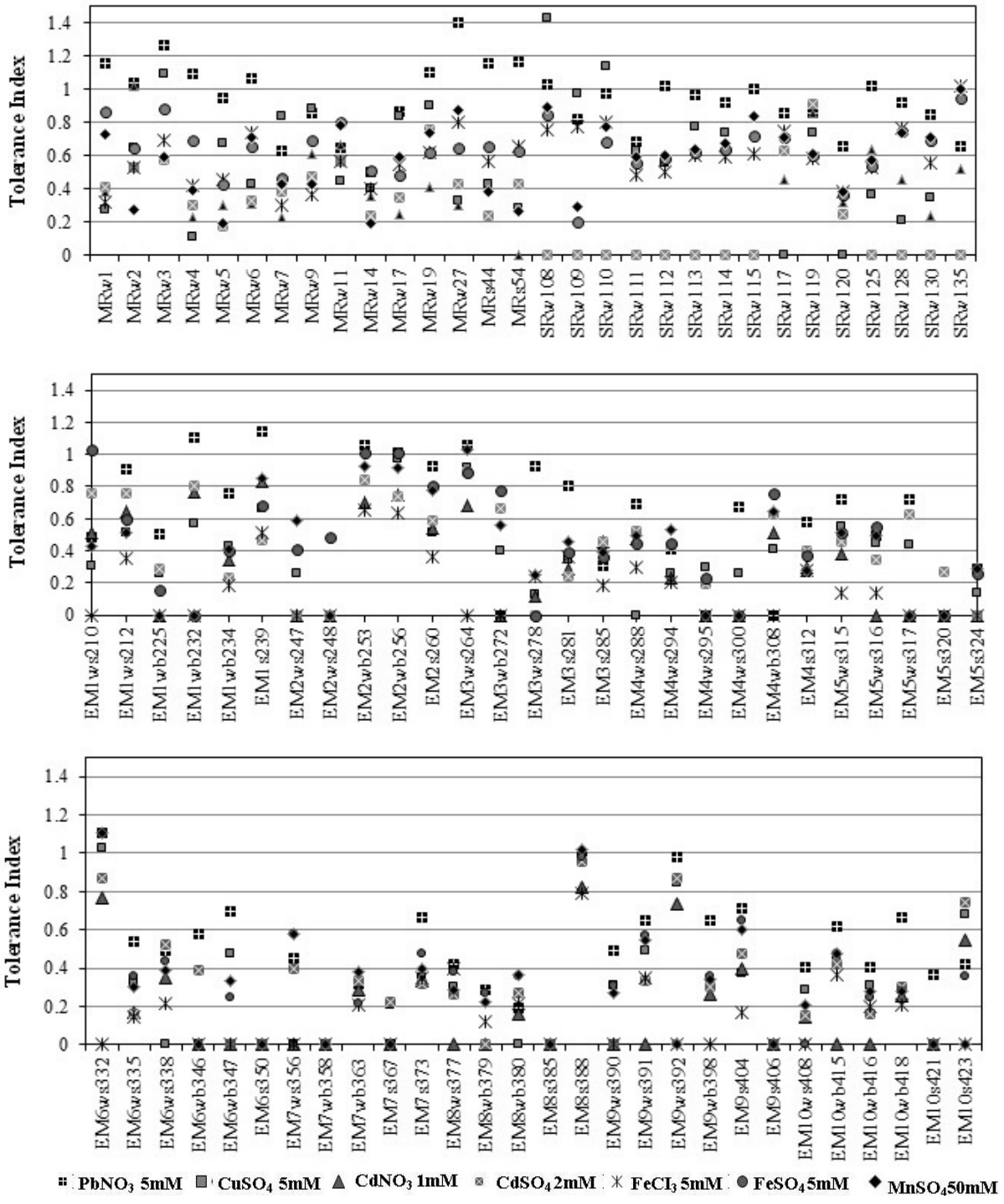


Fig.3.A. Tolerance index of fungal isolates screened for metal tolerance from MR, SR and EM

than cadmium, while in *Aspergillus* and *Fusarium* it was inhibited by all metals. A change in pigment coloration was noted when isolates were grown in presence of iron, becoming orange in *Penicillium* and dark violet to wine red in *Alternaria*.

Metal Sorption

Thirty seven isolates were selected based on high metal MTC values and representative of each genus from different econiches to examine the capacity for metal sorption. Amount of each of the metals sorbed in terms of the sorption coefficient (*Q*) for metal sorbed by the fungal biomass (mg metal g^{-1} dry weight), along with a statistical analysis of their sorption values with respect to the various genera and their species are given in **Table 2.A** and **2.B.** Most of the species of *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* showed good sorption ability.

The average range of *Q* values for *Penicillium* species was 26-45 mg Pb^{2+}, 3-6 mg Cu^{2+}, 2-5 mg Cd^{2+} as nitrate, 10-12

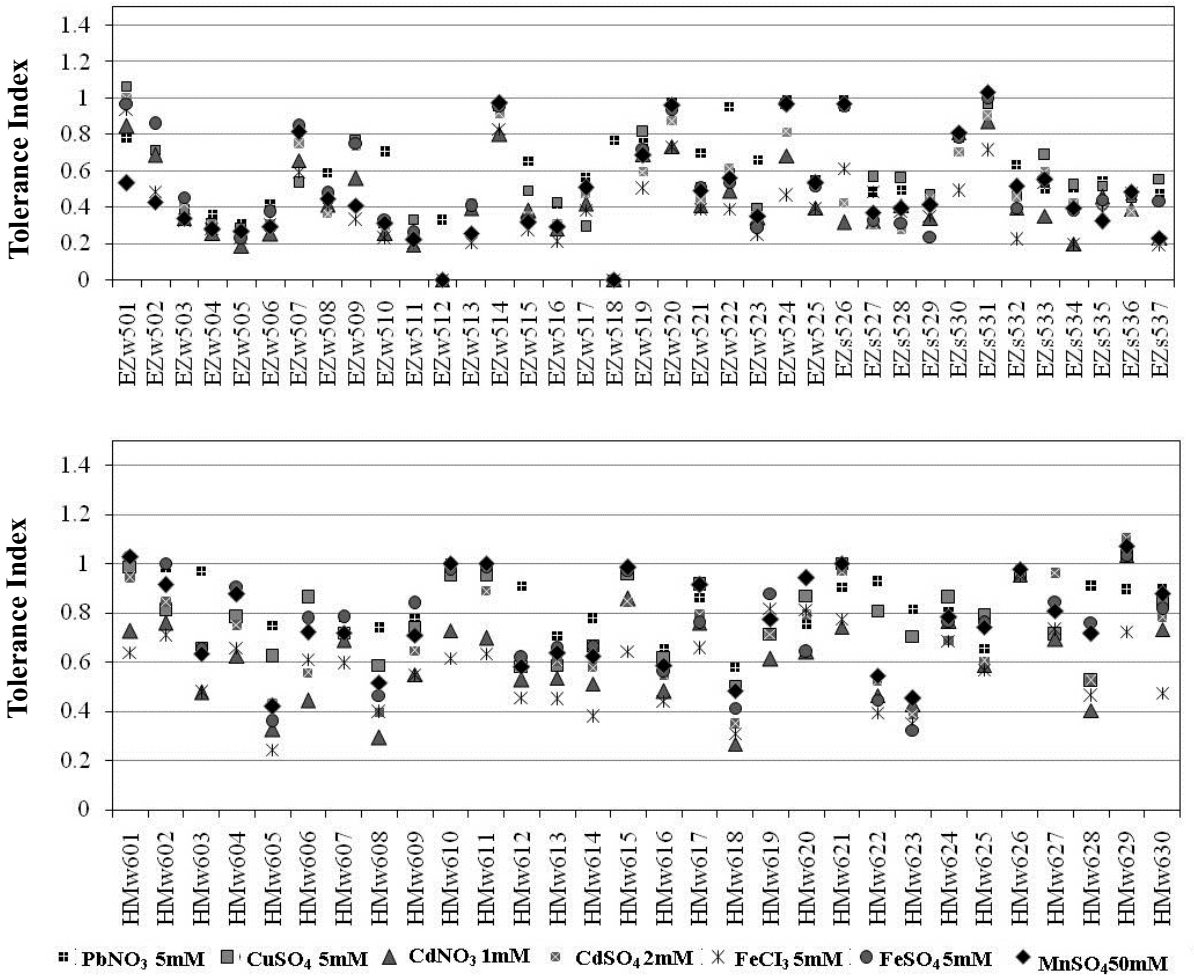


Fig.3.B. Tolerance index of fungal isolates screened for metal tolerance from EZ and HM

mg Fe^{3+}, 16-20 mg Fe^{2+}. In case of Cd^{2+} as sulphate, the isolates from EM, EZ and HM sorbed as much as 25-30 mg Cd^{2+}, while isolates from MR and SR sorbed about 1-5 mg Cd^{2+}. Likewise, isolates from MR and SR sorbed 32-43 mg Mn^{2+} as compared to the isolates from EM, EZ and HM which sorbed an average of 8.5 mg Mn^{2+}. *Penicillium citrinum* Srw119 showed the best sorption capacity, with Q value of 45.22 mg Pb^{2+}, 6.29 mg Cu^{2+}, 12.53 mg Fe^{2+}, 17.57 mg Fe^{2+} and 43.46 mg Mn^{2+} g^{-1} biosorbent.

Similarly, *Q* values for aspergilli were recorded as 20-40 mg Pb^{2+}, 2-4 mg Cu^{2+}, 2-7 mg Cd^{2+} as nitrate, 6-10 mg Fe^{3+}. In case of Cd^{2+} as sulphate, the isolates from EM, EZ and HM sorbed around 32-39 mg Cd^{2+}, with isolates from MR and SR sorbing much less at 1-4 mg Cd^{2+}. Isolates from MR and SR sorbed 18-20 mg Fe^{2+} while those from EM, EZ and HM sorbed only 5-8.5 mg Fe^{2+}. In case of Mn^{2+}, isolates from MR and SR sorbed 32-43 mg Mn^{2+} as compared to the isolates from EM, EZ and HM which sorbed merely 8-10 mg Mn^{2+}.

Q values obtained for *Fusarium* species were 27-37 mg Pb^{2+}, 0.4-0.6 mg Cu^{2+}, 1-6 mg Cd^{2+} as nitrate, 42-46 mg Cd^{2+} as sulphate, 6-9 mg Fe^{3+}, 3-7 mg Fe^{2+} and 10.5 mg Mn^{2+}.

Among the dematiaceous fungi, *Alternaria* species showed a *Q* value of 36-40 mg Pb^{2+}, 5-8 mg Cu^{2+}, 3-6 mg Cd^{2+} as nitrate, 35-37 mg Cd^{2+} as sulphate, 11.5 mg Fe^{3+}, 9-11 mg Fe^{2+} and 10-11 mg Mn^{2+}, while *Cladosporium* species showed 23-33 mg Pb^{2+}, 2-5 mg Cu^{2+}, 5-10 mg Cd^{2+} as nitrate, 35-40 mg Cd^{2+} as sulphate, 6-11 mg Fe^{3+}, 11-16 mg Fe^{2+}. Isolate SRw120 was able to sorb as high as 42 mg Mn^{2+} whereas other isolates could only sorb up to 9-10 mg Mn^{2+}.

There was dissimilarity in heavy metal sorption by the different genera, as well as by the different species within a given genus. However, there was a similarity in sorption of transition metals by the different genera, as well as by the different species within a given genus. In contrast, *Fusarium* and *Alternaria* species yielded similarity in sorption values for both heavy metals and transitional metals, with marginal variations.

DISCUSSION

The levels of heavy metals lead, copper and cadmium, as well as iron, in the Mandovi estuary water samples were found to be within permissible levels, while manganese was present above toxic limits of 0.05 ppm (ATSDR,

2011). The comparatively lower concentration of iron versus manganese could be explained as a result of a greater sedimentation of the iron fines as compared to manganese. This is corroborated by earlier findings that iron in the sediment in these areas, was much higher than manganese (Ratnaprabha and Nayak, 2011) and that almost all of iron but only 7% of manganese gets deposited (Alagarsamy, 2006). The exception in the high iron concentrations at the Mandovi Estuary - Station 3, the Panjim ferry site, could be due to continuous movement of ferries across the estuary as a mode of transport and large recreational yachts, thus slowing down the sedimentation of iron.

The mangrove water sample also registered concentrations below toxic levels of lead and copper; however, cadmium was seen to be well above the permissible limit of 0.1 ppm (ATSDR, 2011). Mangroves serve to entrap not only nutrients, but also pollutants, and their sediments act as a cache (Kathiresan *et al*., 2013); the source of cadmium pollution could be vehicular exhaust from the heavy road traffic adjacent to the mangroves. The contrastingly low level of lead could be due to settling of the heavier lead metal onto the sediment. Alagarsamy (2006) has likewise reported sediments to be primary sinks for lead in aquatic environments. Manganese was also in excess, in keeping with levels of the estuary waters.

Levels of metals in the brine of the salterns were high, as a result of concentration of the water together with the solutes or suspended fines. The source of water to the salterns being from the mangroves, the toxic levels of cadmium and manganese in the mangroves yielded correspondingly higher levels in the brine.

Zuar dockyard water samples contained heavy metals lead and cadmium beyond the toxic limit of 1 ppm for lead and 0.1 ppm for cadmium. The cause of high levels of these heavy metals at the Zuar shipbuilding yard would be from their use as an antifouling agent in paints, the use of lead having been phased out only after 1990s, coupled with ongoing repairs and painting of old barges and consequent scrapings washed into the waters.

The metal contamination at the Mormugao harbor is contributed by the offloading of iron ore, its open air storage and subsequent export. It was observed that the amount of all the metals in the sea water, post monsoon, was doubled as compared to that in pre monsoons, which would be due to the washing of metals into the sea water as a result of rainwater runoffs.

The intense barge movement along the Mandovi and Zuari estuaries, carrying a total of 30 million tons of iron ore per year, or over 82 thousand tons of ore per day at the period of sampling, and the maintenance or building of the barges, would account as the most important factor for the introduction of metals in the estuary.

In this study, the MTC of metals by *Penicillium* and *Aspergillus* species for lead and copper was higher as compared to reported values of 5-12.5 mM Pb^{2+} and 1-5 mM Cu^{2+} (Gopal *et al.*, 2002; Al-kadeeb, 2007; Marbaniang and Nazareth, 2007; Zafar *et al.*, 2007; Iskandar *et al.*, 2011; Gazem and Nazareth, 2013); however, it was less than that of 25 mM Pb^{2+} and 7.5-15 mM Cu^{2+} (Ezzouhri *et al*., 2009). MTC of 2-5 mM cadmium salts by a few *Aspergillus* species were comparable to values reported (Iqbal *et al*., 2006; Zafar *et al*., 2007); that of iron salts at 5-20 mM Fe^{2+} was much higher than earlier reports of 300 mg l^{-1} Fe^{2+} (Osaizua *et al*., 2014).

The isolates showed a higher MTC of lead, than that obtained with the other heavy metals copper and cadmium salts. This corroborates earlier findings with various fungal genera and species (Gopal *et al.*, 2002; Taboski *et al.*, 2005; Iqbal *et al.*, 2006; Zafar *et al.*, 2007; Nazareth and Marbaniang, 2008; Leitão, 2009; Nazareth *et al.*, 2012). It is possible that the mechanism of tolerance to lead by the isolates involved a cell-surface sorption of metal through a mechanism of ion-exchange, with little or no entry into the cell, thereby reducing the metal toxicity to the cell and increasing the tolerance level, as has been recorded earlier (Gadd, 1994; Volesky and Holan, 1995; Akhtar *et al.*, 1996; Kowshik and Nazareth, 1999; Gopal *et al.*, 2002; Taboski *et al.*, 2005; Akar and Tunali, 2006; Yan and Viraraghavan, 2008; Gazem and Nazareth, 2012; Gazem and Nazareth, 2013). Few isolates showed stimulation of growth at lower concentrations of lead and of copper corroborating earlier reports (Al-kadeeb, 2007; Gazem and Nazareth, 2013).

The tolerance levels of different salts of a given metal also varied: cadmium nitrate was seen to be relatively more toxic than cadmium sulphate. Similarly, the isolates grew at much high concentrations of ferrous salts than that of the ferric salt. Ferric as well as the chloride ions are known to be more toxic than the ferrous sulphate salt (Nissim, 1953; Albretsen, 2006). It has been shown that the type of metal salt can affect the nature of fungal response to the metal (Nazareth and Marbaniang, 2008).

A comparative analysis of metal tolerance levels by the different genera of fungi indicated that isolates of *Penicillium* and *Aspergillus* tolerated higher metal concentrations than those of *Fusarium, Alternaria* and *Cladosporium*, with *Penicillium* being the more tolerant genus. However, *Penicillium* species had a lower tolerance to cadmium nitrate and aspergilli from salterns showed no tolerance to both the cadmium salts. These findings confirm earlier reports (Baldrian and Gabriel, 2002; Iqbal *et al.*, 2006; Nazareth *et al.*, 2012).

The variation in metal tolerance among species of each of the genera *Penicillium*, *Aspergillus* and *Alternaria* corroborates earlier findings in species of *Penicillium* (Marbaniang and Nazareth, 2007; Leitão, 2009), *Aspergillus* (Akar and Tunali, 2006; Iqbal *et al.*, 2006; Zafar *et al.*, 2007; Ezzouhri *et al.*, 2009; Gazem and Nazareth, 2013), *Piptoporus betulinus* (Baldrian and Gabriel, 2002) and other genera (Gadd, 1994; Nazareth *et al.*, 2012). This was in contrast with the similarity seen in metal tolerance among species of the genus *Fusarium* and of *Cladosporium*. However, it was also noted that different species of the genus *Alternaria* from the harbor

site, showed similarity in their tolerance levels, an observation also recorded using *Aspergillus* species (Gazem and Nazareth, 2013).

Higher tolerance to heavy metals by isolates from the Mandovi estuary, Zuar dock and Mormugao harbor over that by isolates from the mangroves and salterns, paralleled the relative differences in levels of metal concentration in these areas. This indicates that the isolates developed an increased tolerance to the metals by constant exposure (Gadd, 1994; Akhtar *et al.*, 1996; Ezzouhri *et al.*, 2009; Banik *et al.*, 2013; Fomina and Gadd, 2014).

The reduction in growth in presence of increasing concentration of metal corroborates earlier reports (Baldrian and Gabriel, 2002); this may be due to the internal accumulation of toxic heavy metals, or probably due to the decrease or inhibition of spore germination possibly caused by the metal entering the spores, associating with the particulate insoluble cytoplasmic component and reacting with cytoplasmic receptor sites (Babich and Stotzky, 1982). The change in growth pattern from a loose nature on control medium without metals to a more dense compact growth with narrower colony diameter in presence of cadmium salts corroborates earlier reports (Nazareth and Marbaniang, 2008). Lead ions showed a lesser effect on the nature of growth of the isolates, in comparison with copper, cadmium, iron and manganese; this has also been observed in isolates of *Penicillium* (Nazareth and Marbaniang, 2008).

The presence of a clear zone around the colony, *Aspergillus* and *Fusarium* species, in response to lead, copper, cadmium and manganese salts, has also been reported for *Aspergillus* species and *Trichoderma asperellum*, as being due to an acidification of the medium caused by active fungal growth (Osaizua *et al*. 2014); this acidification could also be responsible for mineral dissolution (Fomina *et al.*, 2005). This phenomenon was particular to only two of the five genera studied, thus affirming that the response of the isolates to metals was specific to a given genus.

The enhanced pigment production correlating with a greater metal tolerance indicated the role of pigment in protection of the fungus against metal stress, through a possible pigment-metal interaction and consequent lowering of available metal and its toxicity to fungus (Gadd, 1994), in keeping with earlier reports on *Penicillium* (Nazareth and Marbaniang, 2008), *Aspergillus* (Gadd, 1994; Gazem and Nazareth, 2013) and *Fusarium* (Kowshik and Nazareth, 2000).

Metal sorption by the isolates showed that the average range of 20-40 mg g^{-1} Pb^{2+} sorption, be the same as reported values of 22 mg g^{-1} by *Aspergillus versicolor* and *A. flavus*, (Cabuk *et al.*, 2005; Akar and Tunali, 2006) and 30-40 mg g^{-1} for various *Aspergillus* species (Gazem and Nazareth, 2013), although less than the 52-76 mg g^{-1} obtained for some species of *Penicillium* and *Aspergillus* (Iskandar *et al.*, 2011; Iram *et al.*, 2013) and for *Corollospora lacera* (Taboski *et al.*, 2005), it was more than that of 6-10 mg g^{-1} obtained for marine fungi *Monodictys pelagica* (Taboski *et al.*, 2005). The sorption of Fe^{2+} and Mn^{2+} at 6-20 mg g^{-1} and 8-43 mg g^{-1} respectively by the isolates, was much higher than values reported of 1.5 mg g^{-1} for *Aspergillus* species and *Trichoderma asperellum* (Osaizua *et al.*, 2014). The *Fusarium* isolates gave very promising results for sorption of Cd^{2+} as nitrate salt, at 16.78 mg g^{-1}, higher than reported results of 5.5-11 mg g^{-1} of *Trichoderma* species (Mohsenzadeh and Shahrokhi, 2014), or in the case of Cd^{2+} as sulphate salt, a *Q* value as high as 42-46 mg g^{-1}, in comparison to a mere 19 mg g^{-1} of *Rhizopus nigricans* earlier reported (Volesky and Holan, 1995). The dematiaceous fungi, *Alternaria* and *Cladosporium*, also sorbed Cd^{2+} as sulphate to a high degree of 33-40 mg g^{-1} Cd^{2+} hitherto unreported. While most of the reports record cadmium sorption of any one salt, the authors have attempted herein to show the difference in sorption of the cadmium ion, as influenced by the salt composite.

A comparison of sorption between the ferrous and ferric ions showed that *Penicillium* and *Aspergillus* species sorbed ferrous ions to a greater extent than ferric ions, while sorption of both ions by *Fusarium* and the dematiaceous fungi *Alternaria* and *Cladosporium* were near equal.

It was observed that most of the isolates of all the genera obtained could sorb lead significantly, with *Penicillium* and *Aspergillus* also sorbing copper, ferrous, ferric and manganese ions to a considerable degree, but sorption of cadmium was poor. The dematiaceous fungi *Alternaria* and *Cladosporium* also sorbed copper and ferric ions substantially, as well as cadmium as sulphate to a high degree. In contrast, *Fusarium* sorbed cadmium as both nitrate and as sulphate to a large extent, but was less effective in the removal of copper, iron and manganese ions from solution.

Analysis of metal tolerance versus sorption by the various genera showed that, *Aspergillus* and *Penicillium* had high levels of tolerance towards copper ions, however, their sorption capacity was not consistently high, and although *Penicillium citrinum* SRw119 had the highest tolerance to cadmium ions and *Aspergillus fumigatus* $EM2w_b253$ to copper, amongst all the isolates, their sorption capacity for cadmium and copper, respectively was moderate or low. In contrast, those species which had a low tolerance of a given metal could sorb the metal efficiently as was seen in the case of *Fusarium*, and some species of *Alternaria* and *Cladosporium*, in presence of cadmium salts; it was also seen that although a fungal species lacked tolerance to a metal, it was capable of sorbing the same, as exemplified in *Aspergillus* isolates with cadmium salts. This has also been shown in *Rhizopus* and *Aspergillus* isolates (Zafar *et al.*, 2007; Gazem and Nazareth, 2013; Iram *et al.*, 2013). In yet other instances, some species of *Cladosporium* had a low tolerance towards copper, as well as a poor sorption capacity of the metals, while other species such as *Penicillium citrinum* SRw119, possessed a high tolerance to lead, copper, iron and manganese ions, as well as displayed a high efficiency in sorption of these metals. Hence, some isolates had a high maximum metal

tolerance level but low or moderate capacity for metal sorption, while some showed moderate tolerance levels but appreciably good sorption with respect to a given metal; some showed low tolerance limits coupled with a low sorption while others had a high tolerance as well as high sorption capacity. This showed that metal tolerance by a species to a given metal during growth, was independent of its sorption capacity and did not necessarily parallel its sorption of the metal, thus indicating that tolerance and its sorption by a given isolate had no definite correlation, or that metal tolerance is not indicative of the sorptive capacity of a fungus.

The development of resistance by a microorganism to a toxic compound is indicative of the compound or pollutant existing in excess in that environment (Gadd, 1994; Zafar *et al.*, 2007; Rehman *et al.*, 2008; Banik *et al.*, 2013). However, it was observed that the degree of metal tolerance and sorption by the isolates, when compared against metal concentrations in the environment, did not necessarily parallel each other. Observations revealed that while lead levels in the saltern brine, the Zuar docks and the Mormugao harbor were high, the tolerance by the isolates from these econiches was low or moderate, and the sorption was good by isolates from salterns, but low to moderate by those from the docks and harbor. Similarly, copper was high in the Mandovi estuary, Station 5, but copper tolerance by the isolates was low. Cadmium was high in the saltern brine, and while *Penicillium* isolate had a good tolerance and sorption, the aspergilli showed no tolerance, and had a poor sorption capacity for cadmium. This indicated that metal in the environment did not always induce metal tolerance in the fungal organisms, nor did it have a bearing on their sorption capacity, the process of fungal-metal sorption involving functional groups on the cell wall, which are specific to a given genus and/or species (Gadd, 1994; Akhtar *et al.*, 1996; Kapoor *et al.*, 1999; Wang and Chen, 2006; Gazem and Nazareth, 2013).

CONCLUSION

Studies on metal tolerance and sorption by fungi obtained from metal-contaminated econiches were carried out with different genera or species, and with various metals, including different salts of a given metal and/or different valencies. It has been shown that metal tolerance and sorption is affected by the nature of its salt and/or valency. Furthermore, although a genus may have an overall greater capacity for metal tolerance and sorption, as in the case of *Penicillium* and *Aspergillus*, a high tolerance to metals by a fungal species need not correspond to a high metal sorption capacity. Similarly, a fungus having a low growth in presence of metals, may display high sorption ability, due to its inherent characteristic functional groups on its cell wall. Furthermore, fungi existing in a metal-polluted environment did not necessarily develop a high metal tolerance and/or sorption capacity, or may also have been isolated as a chance environmental contamination in the econiches. It therefore appears that the ability of a fungal species for effective interaction with metals is distinctive of a particular species in response to a given metal.

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Mushroom research-emerging trends and prospects

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ABSTRACT

Scientific cultivation of mushrooms started in the beginning of 20th century. In the first half of the century, button mushroom was the most commonly cultivated mushroom and its cultivation was done only in few temperate countries. Today 20-30 types of mushrooms are cultivated in more than 100 countries. The world's two most popular mushrooms are white button mushroom and shiitake. The growth of mushroom production was slow initially and has picked up in the last 25 years. China contributes around 80% of the world's mushroom production and produces about 22 million tonnes mushroom from about 60 different types of mushrooms. The production of all mushrooms in the last four decades has increased by 20 times. The current trend is towards diversified uses of mushroom as a result of which around 30% market share in terms of money is that of medicinal mushrooms and their products. Our country produces around 700 million tonnes of agro-wastes in addition to horticultural and forest wastes. Currently, we are using only 0.03% of these for cultivating mushrooms. Producing mushrooms by using agrowastes not only address the issue of production of quality food and environmental issues but can also address the health aspect as mushrooms are neutriceuticals and many of these have medicinal value. There has been rapid increase in trade of medicinal mushrooms. One area that has drawn global attention towards mushrooms is their role in cancer and reducing side effects of chemotherapy and radiotherapy. For any mushroom to be used commercially for medical use it is important to identity the strains with higher output of desired chemical, to standardize the cultivation and environmental conditions for maximizing the production of such constituents, to ensure adequate availability of raw materials and to have testing and evaluation procedures in place for proper labeling and traceability. There is also need for generating greater information on stimulation of immunological systems and exact mode of action. Till then the status of medicinal mushrooms will be same as of numerous medicinal plants available in our country. Considering the natural resources available in the form of agro-wastes, a predominant rural based economy, prevalence of educated youth looking for suitable vocations, and also the increased demands for quality food/functional foods, there is ample scope for growth of mushroom industry in India.

Keywords: Mushrooms, current trend, cultivation, mushroom medicines, agro-waste, quality food.

INTRODUCTION

Mushrooms are fruit bodies of fungi, the second largest group of organisms after insects and are neither plants nor animals. Instead these have been categorized as a different group. Out of estimated 1.5 million fungi only 75,000 have been described. Out of these about 15,000 produce visible fruit body (mushroom). Mushrooms like wood ear, winter mushroom and shiitake were cultivated in China on wood logs more than a thousand year ago while button mushroom was cultivated in France about four centuries ago. But the scientific cultivation of mushrooms started in the beginning of 20th century that commercial production of mushrooms started picking up. In the first half of the century, button mushroom was the most commonly cultivated mushroom and its cultivation was done only in few temperate countries. Today mushrooms are cultivated in more than 100 countries. We have succeeded in cultivating more than 100 types of mushrooms, of which only 30 are cultivated on commercial scale in one or other part of the world. Of these, 5-6 species are grown at industrial scale. The major share of cultivated mushroom is of button, shiitake, oyster, paddy straw, winter and wood ear mushroom.

WORLD MUSHROOM PRODUCTION SCENARIO

The world's two most popular mushrooms are white button mushroom and shiitake. The latter is hardly under cultivation in our country. Two types of mushroom production data are available. One is by FAO and the other by Chinese Association of Edible Fungi. The former treats button mushroom, morels and truffles as mushrooms whereas the Chinese data possibly covers all types of mushrooms including tropical and medicinal

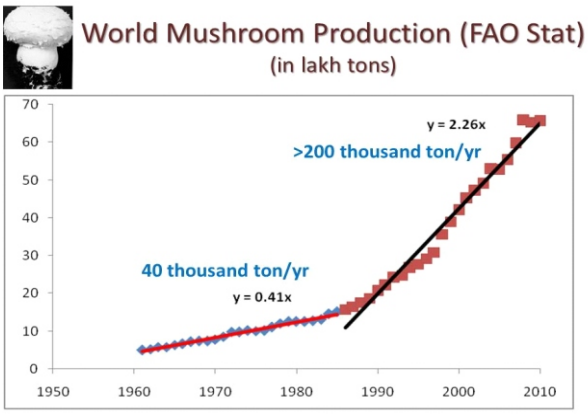


Fig 1 . World mushroom production in last five decades (FAO stat)

mushrooms. If we look at the FAO statistics of last fifty years, we can see that growth of mushroom production was slow initially and has picked up in the last 25 years (**Fig 1**). Said simply, there has been exponential growth in mushroom production in the world. If we consider the production of all mushrooms and figures by Chinese Association, the mushroom production in the world five decades ago was less than one million tonne that has now increased to over 27 million tonnes. China contributes around 80% of the world's mushroom production and produces about 22 million tonnes mushrooms and cultivates about 60 different types of mushrooms. Three decades ago, the mushroom production in China was less than 0.1 million tonnes (**Fig. 2**).

In the last decades there has been an exponential increase in the production as well as consumption of mushrooms in different parts of the world including India. The production of all mushrooms in the last four decades has

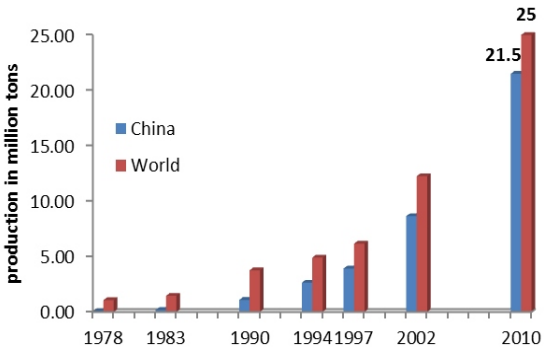


Fig.2 Production of Mushrooms in World and China

increased by 20 times. The mushroom consumption per person was less than 0.25 kg per person in mid seventies and it has increased to 4 kg per person (**Fig. 3**).

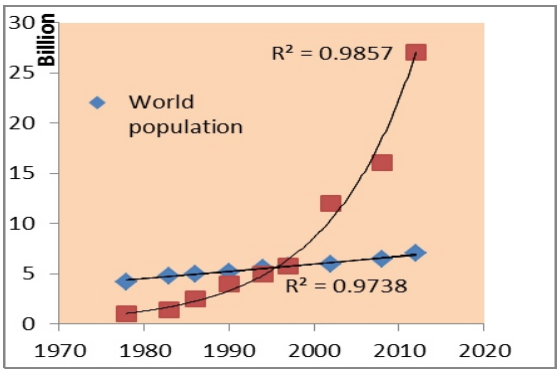


Fig.3 Trends in world population and mushroom growth

The current trend is towards diversified uses of mushroom as a result of which around 30% market share in terms of money is that of medicinal mushrooms and their products (**Fig. 4**). In the earlier part of 20th Century, button mushroom was the dominant mushroom. However, in the past decade the number of other mushrooms have become popular and *Agaricus* at present contributes about 30% of world production. *Pleurotus* is second with 27% and *Lentinula* 3rd with 17%.

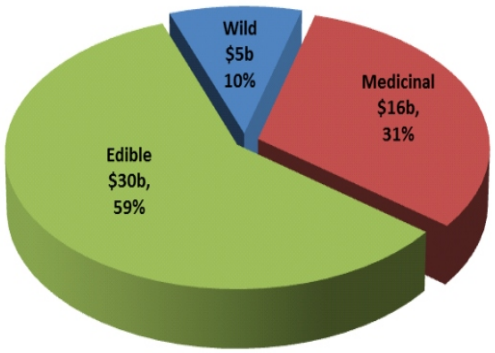


Fig.4 Relative contribution of edible, wild and medicinal mushroom to the world economy

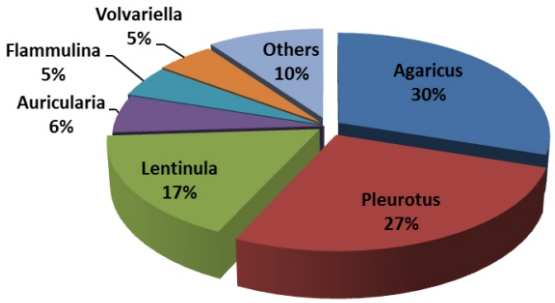


Fig.5 Relative contribution of different edible mushrooms to the world mushroom production

Auricularia, Flammulina and *Volvariella* contribute 5-6% each. These six mushrooms account for about 90% of the world mushroom production (**Fig.5**).

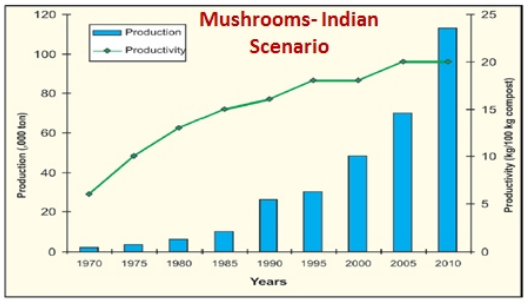
Our country produces around 700 million tonnes of agro-wastes in addition to horticultural and forest wastes. Currently, we are using only 0.03% of these for cultivating mushrooms. There is a global shift towards consumption of fresh mushrooms. For example, out of the total mushrooms consumed in US in late 60s, about 74% were in the form of canned products. At present, of the total mushroom consumption in US, only 15% is in the form of canned mushrooms. Mushrooms are a quality food having number of unique properties that include high content of vitamin B_3, the only vegetable source of Vitamin D_2, zero cholesterol, very high potassium and low sodium, rich in minerals including copper and selenium. These attributes put the mushroom in the category of functional foods. The 19th century was a survival food age, the 20th century has been the convenience food age and the 21st century is going to be a functional food age. The demand for mushrooms is going to increase over time. An awareness generation about their nutritional and medicinal values is important to popularize mushroom among the masses and break consumer resistance observed in many pockets due to misinformation about its edibility and nature. Even though, some of the mushrooms collected from forest can be poisonous but the ones that are cultivated are perfectly edible and mushrooms are considered a vegetarian food.

INDIAN SCENARIO

In India at present four different types of mushrooms viz., button, oyster, milky and paddy straw mushroom are under commercial cultivation (**Fig 6**). Few growers are showing interest in cultivation of other mushrooms like shiitake, wood ear mushroom, etc. The production in initial years was low and many units that came up with the help of foreign technology got closed down due to various reasons. Now there is a new surge and number of small units are coming up in all parts of the country. At present the mushroom production of the country is over 1.2 lakh tonnes (**Fig 7**).

After rains many mushrooms appear from nowhere, especially in grassland, near manure heap, dung or rotting straws/ wood. All of these are not edible. About 5000 of

Fig. 6. Mushrooms under cultivation in India (L to R) Button, Milky, Pink Oyster and Paddy Straw Mushroom



Button	Oyster	Milky	Paddy Straw, etc	Total Production
1,00,700	6,400	900	10,300	1,18,300

Fig. 7. Mushroom Production in India

these are considered edible. Fungi have been classified into number of phyla and mushrooms mainly belong to the phylum *Basidiomycota* and few of the mushrooms belong to the phylum *Ascomycota*. At present there is no simple method to differentiate an edible mushroom from non-edible type. A few of these are poisonous. There have been attempts to cultivate different types of mushrooms.

Mushrooms are commonly considered tools for converting waste into wealth as these are cultivated on agrowastes. The total agricultural wastes in our country are over 1500 million tonnes of which over 600 MT are crop residues (**Fig.8**).

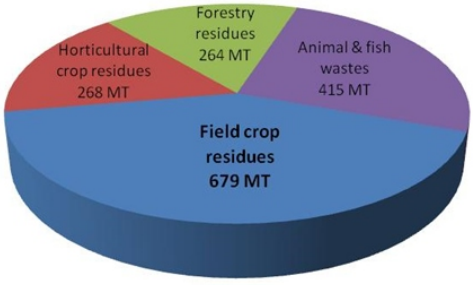


Fig. 8. Estimated agrowaste production in different sectors in India (NAAS 2010)

The second most important requirement for success in mushroom cultivation is manpower. The demographic pattern is going to change across the globe with rapid urbanization. However, in India despite rapid urbanization, majority in our country will continue to be in villages. In addition to this, India in 2020 will be young nation with average age of 29 years as compared to 48 years of Japan (**Fig.9**).

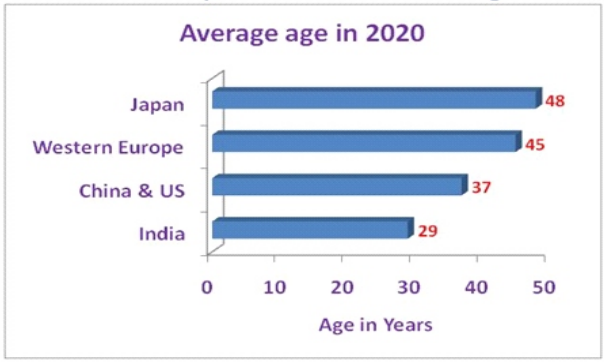


Fig. 9. Average age in 2020 in India and other countries

Producing mushrooms by using agrowastes not only address the issue of production of quality food and environmental issues (like pollution due to burning of straws, agri-residues), but can also address the health aspect as mushrooms are neutriceuticals and many of these have medicinal values. In fact depending on the type of mushrooms, these can serve as food, act as supplement, or serve as medicine.

MUSHROOMS FOR TOTAL RECYCLING OF AGRO-WASTES

Mushrooms are not only a quality food but also a way of utilizing agricultural wastes and generating wealth from the waste. The material left after growing mushrooms, commonly referred as spent mushroom substrate, can be processed into manure. Thus, mushroom cultivation is an important method to promote sustainable manure based farming. Addition of spent mushroom substrate is also reported to improve the soil health. The very fact that mushrooms can be cultivated on paddy straw and number of other agricultural wastes, many of which are just burnt, is sufficient reason to grow mushrooms. By growing mushrooms, we are not only creating a quality food but are also generating a healthy environment. More importantly, it leads to employment generation and women empowerment. With increasing population the land is shrinking and mushroom cultivation utilizes vertical space and requires minimal land making it possible to promote mushrooms in peri-urban and urban areas also. Mushrooms are considered to be the highest protein producers per unit area per unit time.

MUSHROOMS AS FOOD

With increasing population, urbanization and awareness, demand for quality foods like mushrooms is going to increase in coming decades. Mushrooms are a rich protein source having essential amino-acids and high

digestibility. Mushrooms are also good for heart as they have got low fat, no cholesterol, has more of unsaturated fatty acids and some of the mushrooms have compounds like lovastatin that is known to lower the cholesterol in the blood. Moreover, mushrooms have low-sodium and high potassium content making it a suitable food for persons suffering from high blood pressure. The mushrooms are also considered delight of diabetics as it is a low calorie food with no starch and has also number of anti-oxidants. These are also rich in fibres and are also a very good source of vitamins especially vitamin B complex. Mushrooms are the only vegetarian source of vitamin D.

MUSHROOMS AS DIETARY SUPPLEMENTS

Dietary supplements have been described as ingredients obtained from foods, plants and mushrooms that are taken, without further modification, separately from foods for their presumed health-enhancing benefits. Mushroom derived preparations have variety of names like dietary supplements, tonics, functional foods, nutraceuticals, phytochemicals, food supplements, nutritional supplements, mycochemicals, bio-chemopreventives, designer foods, etc. (Chang and Wasser, 2012). There has been rapid increase in trade of medicinal mushrooms.

There are various regulations for dietary supplements in different countries like China, Taiwan US, etc. The standards vary in different countries and so do the claims of safety and quality. Any good product requires a uniform quality from batch to batch that is possible only when a uniform material is produced (which will require specific strains grown under controlled conditions) and is processed by following standard practices. The Newton's third law that for every action there is equal and opposite reaction applies every where also when we tend to claim too many benefits of medicinal mushrooms. This leads to criticism, especially in the light of variable quality of products labelled identically. Thus, there is need for quality standards to have better market.

MUSHROOMS AS MEDICINE

Many mushrooms that are used as food also have medicinal value. For example, oyster mushroom is a source of the drug class of statins (Lovastatin) used for lowering cholesterol and so preventing cardiovascular disease. Similarly, shiitake has Lentinan that is considered to have anti tumor, anti thrombosis, anti asthma, anti virus and anti cholesterol activity. Some of the mushrooms are still collected for medicinal use. The important example is *Cordyceps sinensis* that has Cordycepin (3'-deoxyadenosine, a derivative of the nucleoside adenosine) considered to provide energy and endurance. One area that has drawn global attention towards mushroom is their role in cancer and reducing side effects of chemotherapy and radiotherapy (Petrova, 2014). Mushroom polysaccharides like β-d-Glucans linked to proteins have been tested on humans, as these are considered to enhance immunity. Immunoceuticals isolated from 30 mushrooms species have demonstrated anti tumor activity in animal treatments (Chang and Wasser, 2012). Some of the medicinally important mushroom species are: *Lentinula edodes, Grifola frondosa, Schizophyllum commune, Ganoderma lucidum, Trametes versicolor Inonotus obliqus, Flammulina velutipes, Phellinus linteus, Cordyceps sinensis,* etc.

Modern medical practice relies on highly purified pharmaceutical compounds whose activity and toxicity show clear structure-function relationships. Herbal medicines contain mixtures of natural compounds, for which detailed chemical analyses is not always available and mechanism of action is not fully known. How to translate traditional Eastern practices into acceptable evidence-based Western therapies is an important step to promote mushroom as medicine. There is a long list of claims of medicinal benefits which need to be substantiated scientifically. A number of mushrooms are still collected from the wild and thus variations in chemical constituents are bound to be there. For any mushroom to be used commercially for medical use it is important to identify the strains with higher output of desired chemical, to standardize the cultivation and environmental conditions for maximizing the production of such constituents, to ensure adequate availability of raw materials and to have testing and evaluation procedures in place for proper labeling and traceability. There is also need for generating greater information on stimulation of immunological systems and exact mode of action. Till then the status of medicinal mushrooms will be same as of numerous medicinal plants available in our country. Drugs, particularly those related to cancer treatment, are required in quantities that cannot be met by mushrooms quantity available. Many of these compounds are polysaccharides and their chemical synthesis is not easy. Many of the mushrooms are parasitic and their cultivation can lead to other problems. Commercialization will require growing fungal mycelia or fruit bodies under controlled conditions where we get uniform high quality product in large quantities.

CONCLUSION

Considering the natural resources available in the form of agro-wastes, a predominant rural based economy, prevalence of educated youth looking for suitable vocations, and also the increased demands for quality food/functional foods, there is ample scope for growth of mushroom industry in the country. Considering that our per capita consumption is miniscule as compared to that of other countries like China, Europe and USA, there is ample scope for growth of mushroom cultivation and its diversification. Greater effort is needed in generating awareness about its nutritive values, health/medicinal benefits.

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Podaxis pistillaris- A common wild edible mushroom from Haryana (India) and its Sociobiology

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ABSTRACT

This paper deals with an illustrated account of *Podaxis pistillaris* (L.)Fr., a wild edible mushroom commonly found growing in Haryana. It is being used traditionally for nutritive and medicinal purposes by the local people. Information in this regard was collected by interviewing people of the area.

Key Words: *Agaricaceae*, edible, gastroid, sociobiology, taxonomy

INTRODUCTION

Podaxis pistillaris belongs to family *Agaricaceae*. The genus *Podaxis* includes 10 species (Kirk *et al.,* 2008), out of which *P. pistillaris* is most commonly found in India (Bilgrami *et al*., 1979; Jamaluddin *et al.,* 2004). It is a gastroid agaric and many earlier workers have described it under a separate class *Gasteromycetes* (Nair and Patil, 1978; Thind and Thind, 1982). It is found worldwide mainly in subtropical dry areas (Arora, 1986). As per the earlier reports, *P. pistillaris* is collected by the people of rural areas in various parts of the world including India because of its nutritional and medicinal value (Arora, 1986; Batra, 1983). It is known to have antimicrobial properties (Panwar and Purohit, 2002a) and also used for the treatment of skin diseases (Gupta and Singh, 1991) and inflammation (Mao, 2000).

The examined specimen was collected from sandy soil near the bank of river Yamuna and dry roadside areas in Tajewala, district Yamunanagar (Haryana) at an altitude of 294m above mean sea level and from grassy lawn along the road side in Mansa Devi Complex, Panchkula at an altitude of 305m above mean see level during monsoon season in the Year 2014-15.

MATERIAL AND METHODS

Taxonomy: Fresh sporophores were collected from their natural habitat. Field characters were noted down, spore print and field photographs were taken in the field as per standard methodology given by Atri *et al.* (2005). For noting the color tone of various sporophore parts the color terminology given by Kornerup and Wanscher (1978) was followed. The specimen was preserved wet as well as dry. Microscopic drawings were made with the aid of camera lucida and microscopic photographs were taken with the Leica DFC295 microscope. Terminology used to designate tissue types is given after Korf (1973). Exosporal details were studied with the help of Scanning Electron Microscope. The examined collection has been deposited in the Herbarium of Department of Botany, Punjabi University, Patiala (Punjab), India under PUN.

Sociobiology: A questionnaire was prepared containing questions regarding vernacular names, edibility, season of occurrence, methods of collection, culinary and medicinal properties of mushroom as per the guidelines provided by Kumari *et al.* (2012) in this regard. The local people in the area of collection were asked these questions and the data based upon interviews and personal observations was compiled.

TAXONOMIC DETAILS

***Podaxis pistillaris* (L.)Fr. *Systema Mycologicum* 3:**63. 1829

Figs. 1 (A to H) & 2(A to E)

Sporophores 6 - 15 cm in height. Pileus 3 - 8 cm long, 1.5 - 5 cm broad, cylindrical to ovate, does not expand at maturity, whitish ($2A_1$); margin irregular, remains attached to the stipe in early stages, become free at maturity; exoperidium whitish ($2A_1$) when young, turning to flesh color ($6B_3$) at maturity; scaly, scales

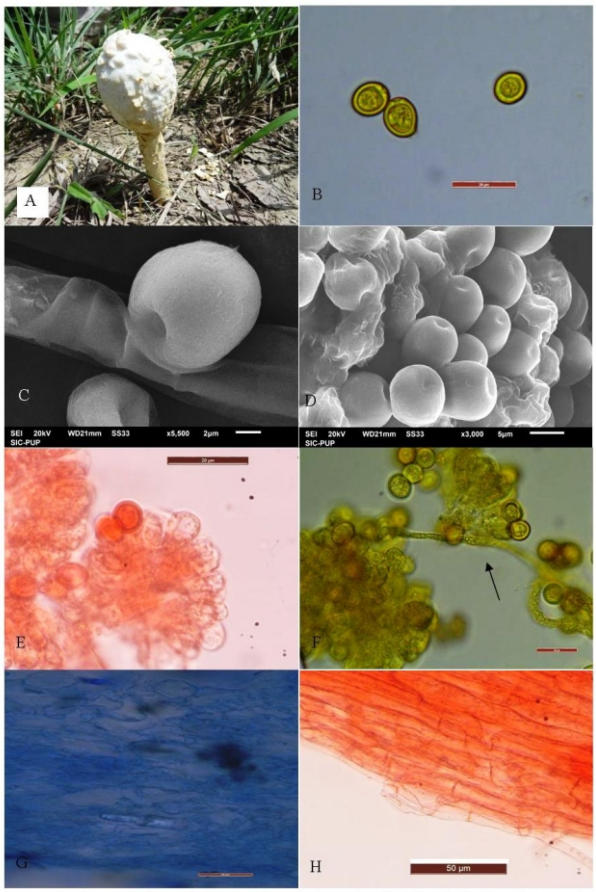

Fig. 1. *Podaxis pistillaris* (A) Sporophore in its natural habitat, (B) Microphotograph of Basidiospores, (C) SEM photograph of Basidiospore with Capillitial thread, (D) Basidiospores in cluster under SEM, (E) Microphotographs of fasiculate Basidia and Basidioles, (F) Capillitial thread, (G) Peridium, (H) Stipe surface tissue (Porrecta type)

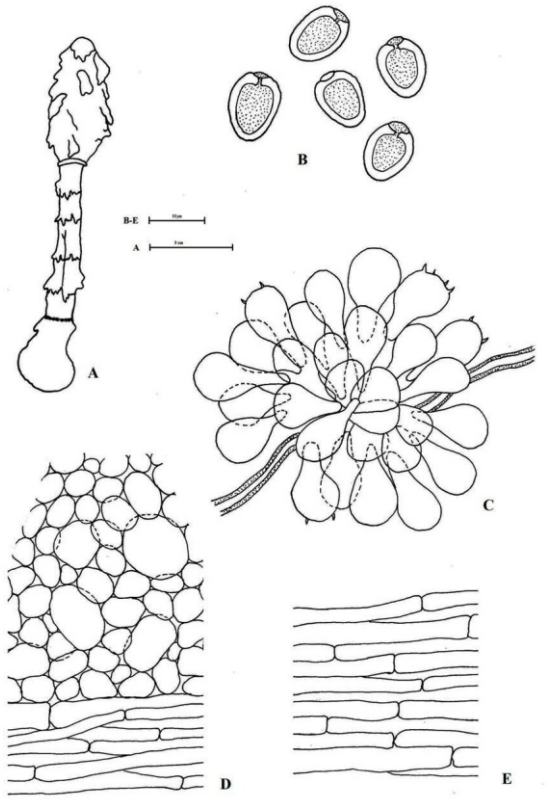

Fig. 2. *Podaxis pistillaris* A. Sporophore B. Basidiospores C. Fasiculate Basidia and Basidioles with Capillitium thread D. Peridium E. Stipe cuticle

membranous, evanescent; endoperidium tough, fleshy, silky-fibrillose. Gills replaced by pulverulent white gleba when young which changes to yellowish, then reddish brown to blackish and finally powdery at maturity. Stipe central, 4 - 10 cm long, 0.5 - 1 cm thick, equal in diameter throughout, abruptly bulbous at the base, often twisted, concolorous with the pileus, extending into the pileus, hollow, fibrillose to scaly; scales shed off with age. Spore print white ($2A_1$).

Basidiospores 9.6 -12.8 µm x 8.0 - 9.6 µm (11.2 x 8.8 µm) (Q= 1.13 - 1.20), subglobose to broadly ellipsoid or even globose, smooth, hyaline, double walled, outer wall thick, with an apical germ pore which appears truncate under immersion oil (100x) but depressed rounded under SEM. Rounded apical depression and equidistant, radiating thin lines emerging from the apical pore region are apparent in the SEM details. Basidia 12.8 - 19.2 x 8.0 - 11.2 µm (16.0 x 9.6 µm), clavate, bisporic to tetrasporic; sterigmata up to 1.6 µm long, fasiculate. Basidioles present. Capillitium threads present, thick walled, sparsely branched, 3.2 - 4.1 µm in diameter. Exoperidium 36 - 48 µm broad, exoperidial tissue porrecta type, formed of 3.2 - 6.4 µm broad hyphae. Endoperidium formed of globulosa type tissue with cells measuring 4.8 - 19.2 µm in diameter. Stipe made up of regularly arranged hyphae measuring 3.2 - 8.0 µm in diameter forming porrecta type tissue arrangement. Clamp connections not observed.

Habitat: Solitary to scattered, found on sandy soil, on bank of river, roadsides and dry pastures. Growing commonly in large number.

Distribution: Worldwide, India, Pakistan, Australia, China, Africa, North America, South America, Hawaiin islands.

Collection Examined: Haryana, Yamunanagar, Tajewala (294m) growing scattered on sandy soil, Mridu, PUN 7151, April 23, 2015. Panchkula, Mansa Devi complex (305m) growing solitary on grassy lawn along the road side, Mridu, PUN 7192, July 28, 2015.

Remarks: *Podaxis pistillaris* is commonly known as "Desert Shaggy Mane" due to its morphological resemblance with *Coprinus comatus* (Arora, 1986). However, it differs in having pulverulent gleba and non-deliquescent nature unlike the presence of gills and deliquescent nature in *C. comatus.*

This mushroom was first collected and described by Linnaeus (1771) as *Lycoperdon pistillare* Linn. from Tamil Nadu, India. Later, Persoon (1801) transferred this to *Scleroderma pistillare* (L.) Pers. and Fries (1829) described it as *Podaxon pistillaris.* Morse (1933, 1941) gave a detailed account of this species under the name *Podaxis pistillaris.* It has been reported from many parts of the world mainly hotter areas. From India, it was described by various workers including, Ahmad (1939) from Punjab plains, Hennings (1901) from U.P., Thind and Thind (1982) from Shivalik foothills, Chandigarh, Patel and Tiwari (2012) from Madhya Pradesh and Patil *et al*. (1995) from Maharashtra.

The presently examined collection is a small spore variant of *P. pistillaris.* In its gross morphology and other taxonomic details it agrees closely with the description given by Thind and Thind (1982) except in having appreciably smaller spore size measuring 9.6 - 12.8 µm x 8.0 - 9.6 µm in comparison to 14 - 20 x 11 - 14 µm described by Thind and Thind (1982). However, these are well within the spore size range (10 - 16 x 9 - 15 µm) given by Arora (1986) for the species.

SOCIOBIOLOGY

The rural people of district Yamunangar and Panchkula in Haryana collect *Podaxis pistillaris* commonly as a traditional practice. It is locally known as 'Khumbi'. Since ages they are using it to make dishes and for medicinal purposes too. Most of the people of rural areas especially elders as well as children are aware about its benefits and applications. Following personal information was collected by asking a set of questions from 6 - 10 persons of different ages from 12 years- 60 years of age:

Q: Do you eat wild mushrooms? : 70-75% rural people said they eat wild mushrooms.

Q: By what name do you call this mushroom (*Podaxis pistillaris*)? : 'Khumbi' is a common term used by people there for edible mushrooms, and poisonous or inedible mushrooms they call as 'Saanp ki chhatri' (umbrella of a snake or snake's cap).

Q: When and from where do you collect it? : During monsoon season from nearby rivers or roadsides where soil is sandy and when they are in unopened stage.

Q: Who in the family collects the wild mushroom and from where did you learn this? : Any elder man or

Fig.3. Sociobiology (A) Asking questions from local villager. (B) Vegetable (Recipe) prepared with *Podaxis pistillaris*

woman who goes to the field for farming collects it early in the morning. They learnt the method of collection from their parents and grand parents.

Q: How does it taste? : It tastes good like any other edible mushroom

Q: Do you know about its medicinal uses? : People considered it as beneficial for health. They also use it externally on wounds as a healing agent as well as for the treatment of skin diseases.

Q: How do you use it for medicinal purpose? : Both fresh and dried sporophores are used as medicine. Fresh sporophores are mashed and mixed with mustard oil while dried sporophores are powdered and mixed with mustard oil and applied on wounds.

Q: Do you sell these mushrooms in the market and at what price? : Only if found in sufficient quantity, otherwise it is used for domestic consumption. It is generally sold at the rate of Rs. 120/- to 170/- per Kg depending upon the availability by the local vendors from vegetable stalls.

Q: Do you preserve them? : Yes, only for two-three days in refrigerator.

Q: How do you prepare recipe? : Mushroom sporophore in unopened stage are washed with lukewarm water and chopped into small pieces followed by shallow frying with usual ingredients including tomatoes, onion, peas, ginger and garlic. Salt and spices are added according to taste requirement and individual preference and water is added to prepare gravy. It is considered as a royal seasonal cuisine in weddings [Fig. 3(B)].

Q: Are you aware of any myths or beliefs regarding mushrooms? : People believe that poisonous mushrooms grow where snakes reside.

It was also observed that many persons were unable to distingiush between *P. pistillaris* and *Coprinus comatus* that is why they avoided eating it. The deliquescent nature of *C. comatus* is confused for its poisonous nature.

DISCUSSION

Podaxis pistillaris, commonly referred as 'Khumbi' in North India, is one of the most preferred edible mushrooms in Punjab, Haryana, Himachal Pradesh and Rajasthan (Purkayastha and Chandra, 1985). Many earlier workers have described this as secoitiod fungus under the class *Gasteromycetes* and many times it is confused with *Coprinus comatus* due to morphological resemblance (Kierle *et al*., 2004). But recent molecular and phylogenetic studies have proved that *Podaxis* is a separate small clade under family *Agricaceae* (Vellinga, 2004). A lot of variation has been noticed in morphological and microscopic characters of sporophores collected from various regions of the world.

As was observed during the present investigation, *P. pistillaris* is known to have various applications among villagers like in Rajasthan the spore mass of this mushroom is dusted over the wounds to heal them and also given to people with bone-cracks and to pregnant women due to high calcium content (Panwar and Purohit, 2002a). In Australia, it is reported to be used as a fly repellent and and also by old men to darken the white hair (https://www.anbg.gov.au/fungi/aboriginal.htm). In Sindh (Pakistan) people fry this mushroom for consumption while in North India it is cooked as a vegetable. Besides this, like any other edible mushrooms there are many other formulations to cook this mushroom (Jordan and Wheeler, 1998; Verma and Rai, 2005). In Pakistan these are preserved dry in plastic or glass containers for almost one year for use during off season. It is also recommended for diabetic and heart patients by the traditional herbal doctors (http://psf.gov.pk/blog/?cat=17). Recent studies have shown that *P. pistillaris* exhibits very strong antibacterial activity against human pathogenic bacteria *Pseudomonas aeuginosa* and *Proteus mirabilis* (Panwar and Purohit, 2002a) and anti fungal activity agianst *Aspergillus niger* (Panwar *et al*., 2002b). It is sold in the market at various prices like, in Sindh (Pakistan) 1 kg has been documented to cost about 100 to 150 Pakistani Rupees (about 1 to 1.5 US$) (http://mushroaming.com/_Mushroom_Markets). In India its cost ranges from Rs. 120/- to 170/- per Kg (Personal information). There are reports of its bulk collection and consumption from other parts of the country including Rajasthan (Personal information).

FAO(http://www.fao.org/docrep/007/y5489e/y5489e06.htm) has documented conflicting reports about the edibility of *P. pistillaris* as it is reported to be edible from India and Pakistan (Batra, 1983) and many other countries but considered to be poisonous in Nigeria (Walleyn and Rammeloo, 1994). The present observations with respect to its edibility and medicinal utility from rural areas of Yamunanagar and Panchkula in Haryana corroborates the earlier reports in this regard from other parts of the world.

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Assessment of air microflora of air conditioned working environments in software companies of Bangalore

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ABSTRACT

A study was undertaken on the air microflora of three air conditioned software companies, such as Company A, Company B and Company C located in the south of Bangalore city. The study was conducted for a period of June 2014 to September 2014. The indoor air was compared with that of the outdoor, while the outdoor air served as a control. Plate Exposure Method was used to enumerate the numbers and kinds of respirable bacteria and fungi inside the companies. There were significant differences in microbial air contaminants between the indoor and the outdoor. Gram Positive cocci were found to be significantly higher than the Gram Positive rods in the indoor air-conditioned environment. The air inside air-conditioned companies was found to have significant numbers of *Alternaria, Cladosporium* and *Aspergillus*. The aim of the present study is to compare and evaluate the impact of air-conditioning systems on indoor air quality with that of outdoor.

Key words: Indoor, microflora, outdoor, software company

INTRODUCTION

Bioaerosols containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Fracchia *et al.,* 2006). Fungi and bacteria are common in indoor and outdoor environments and the presence of bioaerosols can compromise normal activities with making efficient monitoring crucial (Stetzenbach, 2007; Okafor and Opuene, 2007). Indoor microorganisms adherent to walls, floors and fast growing species, attaching itself to building materials and producing microbial products and ultimately causing adverse health effects (Madukasi *et al.,* 2010). The relative humidity and moisture content of the materials determines that to what extent different microorganisms are able to grow on indoor materials (Dhanasekaran *et al.,* 2009). In the present investigation, work was undertaken to understand the quality of air in the working environment of software companies in Bangalore which are fully air conditioned, by studying the airborne microorganisms (fungi and bacteria) over a period of time. The aim of the present study is to compare and evaluate the impact of air conditioning systems on indoor air quality with that of outdoor.

MATERIALS AND METHODS

Sampling site

The study of air microflora in three software companies such as Company A, Company B and Company C was chosen for our study. The Air Conditioner (A.C) in the software companies A, B, C had two types of filters such as High Efficiency Particulate Air Filters (HEPA Filters) and Electrostatic Precipitators. Company A had centralized A.C system, Company B had split type of A.C system and Company C had centralized and split A.C system. The study involved three air conditioned software companies located in the south of Bangalore.

Sampling technique

The Settle Plate Technique (Aneja, 2001) was used for the assessment of air flora. Nutrient agar and Sabouraud's Dextrose Agar were used as culture media for isolation of bacteria and fungi, respectively. The medium was autoclaved and under aseptic conditions poured into sterile disposable plastic petri dishes.

Indoor and outdoor

Indoor air sampling was done inside the working place, which was fully air conditioned. The sterile petri plates were exposed for 15 minutes duration every day from 11am. Outdoor air sampling was done just outside the building, but within premises limits of the company and this served as a control.

Incubation

After the exposure the plates were carefully placed back in a sterile cover. The plates were immediately brought to the laboratory and were incubated at 37 °C for 2 days for bacteria and 4 days at 28 ºC for fungi. After incubation of plates, the bacterial colonies were characterized by gross appearance and microscopic examination using Grams stains and results were tabulated. Fungal colonies were identified by gross appearance and microscopic examination of their spore and hyphal characteristics by using scotch tape mounts with Lacto Phenol Cotton Blue as stain. Fungal colonies were identified following Barnett and Hunter (1998).

Questionnaire

A questionnaire was distributed to the source of population. Information about personal factors, symptoms of any allergy, respiratory disorders, perception of indoor air quality experienced during work, smoking habits was gathered. Based on this data the quality of air was determined along with the bacteria and fungi obtained from the outdoor and indoor plate exposure.

RESULTS

The present study has clearly demonstrated that the microflora in the indoors of the air-conditioned office were significantly lower than the outdoor environment.

Bacterial and fungal population of Company A

The classification of bacteria in the study was limited to

Gram-positive bacilli, Gram-positive cocci and Gram-negative bacilli. Bacterial counts in the indoor were comparatively lower than the outdoors (**Table 1**). Furthermore, there were differences in the distribution of bacteria, most notably few rods and more Gram-positive cocci in air-conditioned rooms. Gram-positive coccobacilli were also present, but in a small number. Percentage contribution of Gram-positive cocci was found to be highest with Gram-negative bacilli being the least. Similarly in the outdoor environment percentage contribution of Gram positive rods was the highest followed by Gram positive cocci and the least by Gram positive tetrads.

Table 1. Percentage contribution of bacteria in Company A

Indoor percentage		Outdoor percentage	
Gram positive cocci	28.7	Gram positive cocci	21.95
Gram positive rods	27.3	Gram positive rods	17.3
Gram-positive coccobacilli	6.2	Gram positive tetrads	13.73
Gram positive tetrads	10.9	Gram positive rods in chains	24.2
Gram positive rods in chains	23.2	Gram positive cocci in clusters	22.76
Gram positive cocci in clusters	2.73		

In fungi eighteen genera were observed in the study (**Table 2**). *Alternaria* sp., *Cladosporium* sp. and *Aspergillus* sp. were found to be abundant in both indoor and outdoor environment. Fungal distribution in the indoor with AC was comparable with the outdoors. *Alternaria* accounted for the highest percentage contribution in both indoor and outdoor locations. *Cladosporium* sp., which is the predominant fungi in the outdoor air, is present in much smaller numbers in the indoor environment which was found to contain more of *Aspergillus* sp.

Table 2. Percentage contribution of fungi in Company A

Indoor percentage		Outdoor percentage	
Cladosporium sp.	16.66	*Cladosporium* sp.	20.48
Alternaria sp.	21.43	*Alternaria* sp.	85.13
Acremonium sp.	4.76	*Mucor* sp.	17.83
Syncephalastrum sp.	2.38	*Penicillium* sp.	12.28
Mucor sp.	16.66	*Rhizopus* sp.	17.83
Penicillium sp.	14.28	*Aspergillus* sp.	16.38
Rhizopus sp.	4.76		
Aspergillus sp.	19.04		

Bacterial and fungal population of Company B

The bacterial counts in all air conditioned rooms of indoor were comparatively higher than that of outdoor (**Table 3**). There were differences in the distribution of bacteria, most notably fewer rods and many more Gram-positive cocci. The bacterial counts in the indoor were higher than the outdoor, mostly due to substantial increase in the number of Gram-positive cocci.

Table 3. Percentage contribution of bacteria in Company B

Indoor percentage		Outdoor percentage	
Gram positive cocci in singles	12.86	Gram positive cocci in singles	8.28
Gram positive rods in chains	5.8	Gram positive rods in chains	5.32
Gram positive cocci in clusters	57.97	Gram positive cocci in clusters	65.68
Gram positive bacilli	10.12	Gram positive bacilli	3.55
Gram positive coccobacilli	13.19	Gram positive coccobacilli	8.87
		Gram negative bacilli	8.28

Altogether five genera of fungi were observed in the study (**Table 4**). *Cladosporium* sp. and *Aspergillus* sp. were found to be abundant in both, indoor and outdoor environments. *Cladosporium* accounted for the highest percentage contribution in all the air-conditioned buildings.

Table 4. Percentage contribution of fungi in Company B

Indoor percentage		Outdoor percentage	
Cladosporium sp.	57.93	*Cladosporium* sp.	62.2
Aspergillus sp.	23.61	*Aspergillus* sp.	26.77
Mucor sp.	18.45	*Alternaria* sp.	11.02

Bacterial and fungal population of Company C

Again, similar to company A, bacterial counts in indoor were comparatively less compared to the outdoor (**Table 5**). Gram-positive cocci in clusters accounted for highest percentage contribution in both indoor (26.66%) and outdoor (29.09%) locations. Gram-positive cocci in pairs, singles, etc were dominant.

Table 5. Percentage contribution of bacteria in Company C

Indoor percentage		Outdoor percentage	
Gram positive cocci in clusters	26.66	Gram positive cocci in singles	18.18
Gram positive cocci in singles	15.55	Gram positive rods in chains	12.72
Gram positive cocci in tetrads	10.22	Gram positive cocci in clusters	29.09
Gram positive cocci in pairs	13.33	Gram positive cocci in pairs	7.27
Gram positive cocci in triplets	4.2	Gram positive bacilli	21.81
Gram positive bacilli	12.2	Gram positive coccobacilli	10.9
Gram positive coccobacilli	18.22		

Total nine genera of fungi were observed in the study (**Table 6**). *Cladosporium* sp. was the dominant fungus followed by *Mucor* sp. *Aspergillus* sp. and finally *Acremonium* sp. with the least proportion. In the outdoor environment *Rhizopus* sp. showed 38.5% and *Acremonium* sp., 16.14%. A comparative account of the bacterial and the fungal counts was also done which revealed that the bacterial population in Company A, B, C in the indoor was much lower when compared to the outdoor population. There was significant increase in the Gram Positive cocci in the indoor locations followed by

Gram Positive rods. The predominant fungi in Company A were found to be *Alternaria* sp**,** where as in Company B and C *Cladosporium* sp. was dominant. *Aspergillus* sp. was the second most predominant fungi in all the three companies.

Table 6. Percentage contribution of fungi in Company C

Indoor percentage		Outdoor percentage	
Cephalosporium sp.	6.4	*Rhizopus* sp.	38.5
Alternaria sp.	6.2	*Acremonium* sp.	16.14
Penicillium sp.	9.2	*Mucor* sp.	22.36
Fusarium sp.	12.2	*Fusarium* sp.	22.98
Cladosporium sp.	34.8		
Mucor sp.	16		
Aspergillus sp.	14		
Acremonium sp.	1.6		

Clinical studies

A health status survey was conducted by distributing questionnaires to the employees of Company A, B and C. The survey from all the companies revealed that 85% of the working population showed no signs and symptoms of major respiratory ailments, while the other 15% showed minor symptoms like dry cough, sore throat, sneezing, skin dryness and general head ache.

DISCUSSION

The results of the three companies taken up for our study in general, demonstrated that the total microflora in air conditioned offices were significantly lower than the outdoor environment. This was further supported by our health survey of the workers of the companies, majority (85%) of whom reported no signs of major respiratory ailments within the working environment. This suggests that the filters of air-conditioning systems effectively screen out many of the microbes found in the air. However, the study carried out in Company B showed higher fungal counts in the indoor than the outdoor. A basic principle in creating allergic disorders is that of avoidance of the offending allergens. Although aeroallergens originating in nature are difficult to avoid, exposure to these agents may be reduced, often substantially, within man-made structures. There are many reports available, which have described clinical benefits as well as reduced particle levels within air conditioned environments (McDonald *et al*., 1993; Dhanasekaran *et al.,* 2009; Cuthbertson *et al.,* 2010; Madukasi *et al.,* 2010).

The fungus *Cladosporium* sp., that is ubiquitous in air showed the highest percentage ranging from 55 to 80% in the indoor when compared to the outdoor (62.2%). Common activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological particulate matter. In addition food stuffs, house plants and flower pots, house dust, pets and their bedding, textiles, carpets, wood material and furniture stuffing, occasionally release spores of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* into the air (Maeir *et al.,* 2002). The bacterial counts in the indoor were also significantly higher. American Society of Heating, Refrigerating and Air Conditioning Engineers (2000) reported, the health effects caused by microorganisms that are in indoor environments with air conditioning systems can be infective or immunological and lack of cleaning and checking out of the ventilation and air conditioning systems may allow microbial growth, in 20% or more users of such environments they may causes rhinitis, bronchitis, pharyngitis, pneumonia, conjunctivitis and keratitis. It must be stressed that several factors such as outdoor air quality, air insufficiency or bad air distribution, inefficient control of air temperature and humidity, improper project or changes in the original building project and lack of monitoring, checking out and cleaning the air conditioning systems, etc. results in variety of such ailments in the exposed population due to poor air quality.

Such human health concerns about the chemical and biological contaminants in air, water and food have been highlighted by Almond (2006). It was concluded that although the numbers and types of microbial population in air conditioned software companies were high, yet they have little adverse effects on human health. Overcrowding of workers within the limited working area could be taken care in order to increase the quality of air within the air conditioned environment.

CONCLUSIONS

Our study clearly indicates that there is significant population of the indoor and outdoor airborne bacteria and fungi. It has been shown that other parameters like ventilation, temperature and humidity also play a major role as an indoor contaminant agent. This data brings out a need to adopt strict remedial measures in the air conditioned settings so as to provide clean and fresh environment to the working population.

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KAVAKA 44:42-44 (2015)

Three hitherto unreported macrofungi from cold arid region of Ladakh Province, Jammu and Kashmir, India

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ABSTRACT

Three species of *Thelephora*, namely *T. japonica* Yasuda, *T. regularis* Fr. and *T. vialis* Schwein. associated with angiospermic taxa were collected, identified and described in detail. All the three species are new records for India.

Key words: Ladakh, new record, taxonomy, *Thelephora*

INTRODUCTION

The genus *Thelephora* Ehrh. ex Willd. (*Thelephorales*, *Thelephoraceae*) is characterized by leathery, coralloid or fan shaped sporophores with split or torn margins having coloured spores that are usually warted or spiny (McKnight and McKnight 1987; Bessette *et al*., 1997). Well known to form mycorrhizal association with several conifers (Iwanski *et al*., 2006; Hilszczanska *et al*., 2011; Davis *et al*., 2012), worldover 50 species of *Thelephora* have been described (Kirk *et al*., 2008) and currently Index Fungorum (2014) shows 879 records of the genus while as about 23 species of this genus have been reported from North America that predominantly inhabit tropical and sub tropical regions (Burt 1914; Ramirez-Lopez *et al*., 2013). In India, several species of *Thelephora* viz., *Thelephora atra* Weinm., *T. aurantiaca* Pers., *T. caryophyllea* (Schaeff.) Pers., *T. gelatinosa* Sauter., *T. palmata* (Scop.) Fr., *T. pusilla* Currey., *T. ramarioides* Reid., *T. sowerbyi* Berk., *T. sparassoides* P. Henn., *T. terrestris* Ehrh. etc. are known to exist (Bilgrami *et al*., 1979; 1991; Jamaluddin *et al*., 2001, Prasher and Ashok 2013). Out of these, only two species namely *T. caryophyllea* (Schaeff.) Pers. and *T. palmata* (Scop.) Fr. have been reported from Kashmir region of Jammu and Kashmir (Berkeley 1856; Currey 1874; Pala *et al*., 2012). The present communication describes the macro- and microscopic details of the three species of *Thelephora* collected from Phey and Gonpa villages of district Leh, Ladakh (Jammu and Kashmir). All these species were found associated with angiospermic taxa and constitute new records for India.

MATERIALS AND METHODS

Collection of macrofungal specimens were made from Phey and Gonpa villages of Leh district of Ladakh, during July-September 2012-2013. Macroscopic features including shape, colour and dimensions of pileus and stipe were observed and recorded from fresh specimens in the field and the photograph of fresh sporocarps were taken in their natural habitat. Microscopic characters were observed on dried material using hand cut sections after reviving a part of the dried specimen in 3% KOH solution, and stained with 1% Congo red and Melzer's reagent (iodine 0.5 g + potassium iodide 1.5 g + chloral hydrate 20 g + distilled water 20 ml). Microphotography was accomplished using a Nikon Eclipse E400 microscope. Specimens cited have been deposited in the Herbarium of Botany Department (HBJU) University of Jammu.

TAXONOMIC DETAILS

1. *Thelephora japonica* Yasuda, *Mycol. Writ.* **5** (43): 597, 1916. Fig. 1

Fig. 1 *Thelephora japonica*. a. Sporophores showing both ventral and dorsal view. b. Basidiospores. c. Basidioles. d. Basidia showing sterigmata. e & f. Pileus and stipe hyphae showing clamp connections.

Sporophores 1.7-2.2 cm high, rosulate. Pileus 2.0-3.2 cm wide, spathulate to flabelliform, velvety-soft, margin paler; abhymenial surface tomentose, dark greyish; hymenial surface pale greyish, papillate, turning blackish brown in 3% KOH solution. Stipe 0.5-0.6 cm, short, greyish. Odour not perceptible. Basidiospores 4.0 - 8.0 × 4.0-6.4 µm, a_vL= 6.0, a_vW= 5.2, Q= 1.0-1.2, Q_m= 1.1, brown (in Congo red), angularly-ellipsoid, echinulate, thick-walled, uniguttulate, inamyloid. Basidia 38.4-68.8 × 4.8-10.4 µm, clavate to cylindrical, hyaline, thick-walled, multiguttulate, 2-4 spored, with basal clamp;

sterigmata up to 4.0 µm long. Hyphal system monomitic composed of generative hyphae; pileus hyphae 2.4-4.8 µm wide, hyaline, septate, clamped, branched, thick-walled; stipe hyphae 2.4-4.8 µm wide, hyaline, thick walled, septate, clamped, branched.

Habit and habitat: Ectomycorrhizal, humicolous, gregarious in mixed forest of *Populus nigra* and *Salix alba*.

Edibility: Not edible in the study area.

Collection examined: India, Jammu and Kashmir, Leh, Phey village, R. Yangdol and Y. P. Sharma, HBJU 320, August 9, 2012.

Remarks: The macroscopic and microscopic details of the present collection are in conformity with the description given by Corner (1968) except for some minor differences in the length of basidia which was larger (40.0-80.0 µm) in the earlier collection in contrast to the present collection (38.4-68.8 µm). In addition, pileus margin was more yellowish as compared to Ladakh collection where it was whitish.

2. *Thelephora regularis* Schwein., *Schriften der Naturforschenden Gesellschaft zu Leipzig* **1**: 105, 1822. Fig. 2

Fig. 2 *Thelephora regularis*. a. Sporophores in the natural habitat. b. Ventral view of a sporophore. c. Basidioles. d. Basidia with attached basidiospores. e & f. Pileus and stipe hyphae showing clamp connections.

Sporophores 2.5-6.0 cm high, flabelliform, uplifted, coriaceous, infundibuliform. Pileus 3.0-5.5 cm wide, brittle; abhymenial surface fibrillose, ochre (yellowish orange); hymenial surface dark greyish, rugose; margins paler. Stipe 1.0-1.5 cm, clavate, short, mesopodial, tomentose, brownish red. Odour not perceptible. Basidiospores 5.6-8.0 × 4.8-8.0 µm, a_vL= 6.8, a_vW= 6.4, Q= 1.1-1.0, Q_m= 1.0, globose to subglobose, angular, minutely echinulate, more or less lobate, light olive brown (in Congo red), thick-walled, uniguttulate, inamyloid. Basidia 35.2-102.4 × 6.4-9.6 µm, clavate to cylindrical, hyaline, thick-walled, multiguttulate, 4-spored, with basal clamp; sterigmata up to 7.2 µm long. Hyphal system monomitic composed of generative hyphae; pileus hyphae 2.4-4.8 µm wide, hyaline, septate, clamped, branched, thick-walled; stipe hyphae 1.6-5.6 µm wide, hyaline, septate, clamped, branched, thick-walled.

Habit and habitat: Ectomycorrhizal, humicolous, scattered under *Salix excelsa, S. alba* and *Populus nigra.*

Edibility: Not edible in the study area.

Collection examined: India, Jammu and Kashmir, Leh, Gonpa village, R. Yangdol and Y. P. Sharma, HBJU 321, August 24, 2012.

Remarks: This fungus was described in the literature as *Thelephora regularis* by Corner (1968). However, it was subsequently shifted to genus *Hyphodontia* as *H. spathulata* (Parmasto, 1968). The present collection is somewhat different in having comparatively larger basidia (35.2-102.4 µm) as compared to the earlier described specimens (40.0-90.0 µm). It also differs in having wrinkled hymenial surface in contrast to smooth in the earlier reports.

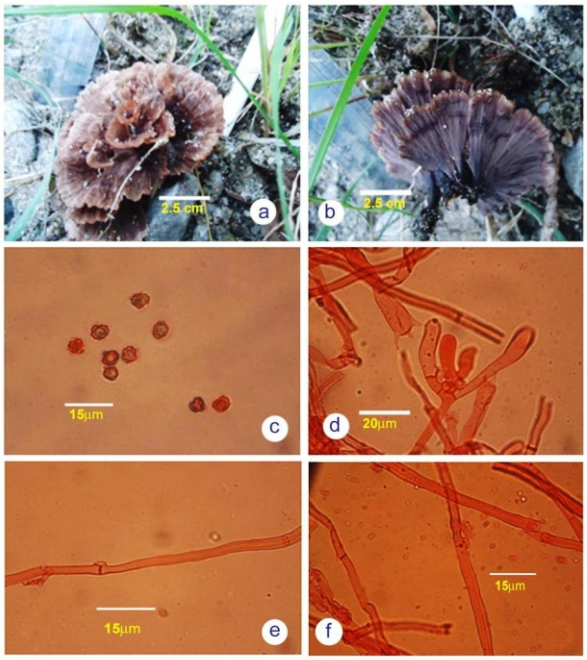

Fig. 3 *Thelephora vialis*. a. Sporophores in the natural habitat. b. Ventral view of a sporophore. c. Basidiospores. d. Basidioles and Basidia. e & f. Pileus and stipe hyphae showing prominent clamp connections.

3. *Thelephora vialis* Schwein., *Trans. Am. phil. Soc.* **4** (2): 165, 1832. Fig. 3

Sporophores 2.5-3.0 cm high, erect, rosulate, infundibuliform, coriaceous. Pileus 3.0-6.4 cm broad, spathulate to flabelliform; margins paler; abhymenial surface fibrillose, fawn colour, becoming pale dull brown on drying; hymenial surface grey brown, wrinkled. Stipe 0.4-0.9 cm bulbous, central, short, solid, blackish. Odour

mushroomy. Basidiospores 4.8-8.0 × 4.8-6.4 µm, a_vL= 6.4, a_vW= 5.6, Q= 1.0-1.2, Qm= 1.1, angular, olive-buff (in Congo red), warted, thick-walled, uniguttulate, inamyloid. Basidia 26.4-64.0 × 5.6-10.4 µm, clavate to cylindrical, hyaline, thick-walled, multiguttulate, 4-spored, with basal clamp; sterigmata up to 6.4 µm long. Hyphal system monomitic composed of generative hyphae; pileus hyphae 3.2- 4.8 µm wide, hyaline, thick-walled, septate, clamped, branched; stipe hyphae 3.2-7.2 µm wide, hyaline, thick- walled, septate, clamped branched.

Habit and habitat: Ectomycorrhizal, humicolous, scattered to gregarious in the mixed forest of *Populus nigra*, *Salix alba* and *Hippophae rhamnoides*.

Edibility: Not edible in the study area.

Collection examined: India, Jammu and Kashmir, Leh, Phey village, R. Yangdol and Y. P. Sharma, HBJU 322, August 28, 2012.

Remarks

The taxonomic details of *Thelephora vialis* are in conformity with the description given by Corner (1968) except for basidiopores which are somewhat broader than the earlier examined specimens.

ACKNOWLEDGEMENTS

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Optimization of culture condition for amylase production by *Penicillium frequentans AVF2*

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ABSTRACT

Amylases hydrolyze starch and release several products including dextrines and small polymers of glucose units. Amylases have considerable importance in biotechnology with a wide spectrum of applications in various industries, including food and pharmaceutical industries, chemical, textile, paper and cellulose, leather, detergent, beer, liquor, bread and children cereals, liquefaction and conversion of starch to sugar, animal chow and fermentation industries. Considering these facts, endophytic fungi isolated from *Pinus rouxburghii* were screened on glucose yeast extract peptone agar (GYP) medium for amylolytic activity. Among 17 isolates of endophytic fungi, *AVF2* showed the highest amylolytic activity and was chosen for further study. The fungus was characterized by Forest Research Institute, Dehradun as *Penicillium frequentans*. Influence of various physical and chemical factors such as pH, temperature, carbon and nitrogen sources on amylase and biomass production by *P. frequentans* in liquid media was studied. At 30 ºC incubation temperature, the maximal amylase activity of 0.28U/mL and maximum fungal biomass productivity of 5.618g/25mL were achieved. At pH 7.0 of the cultural media, the observed maximum amylase activity and fungal biomass are 0.461U/mL and 5.511g/25mL, respectively. Among the various carbon sources, 1.5% maltose showed the highest amylase production. Among different nitrogen sources 0.3% sodium nitrate was found to be optimal for fungal biomass.

Key Words: Endophytic fungi, *Penicillium frequentans*, amylase activity, fungal biomass

INTRODUCTION

Endophytes are the microorganisms which live in symbiotic relationship with living plant tissues. A major portion of the endophytic population constitutes fungi (Strobel *et al*., 2004). Endophytic fungi spend their whole lifecycle in colonizing inside the healthy tissues of host plants (Zhao *et al*., 2010). This kind of symbiotic relationship protects the host from predators and in return endophytes receive nutrients and space for living (Zaferanloo *et al*., 2012). Endophytic fungi synthesize enzymes which hydrolyze several plant-derived macromolecules and secondary metabolites. Fungal enzymes are used in textile and leather industries, food, beverages and confectionaries where they simplify the processing of raw materials. They are more stable as compared to the enzymes derived from other sources. Enzymes derived from the endophytes degrade polysaccharides which are available in the host plants. There is a growing demand in many industries for new sources of enzymes with various thermo-stability and pH profiles for different applications (Zaferanloo *et al*., 2013). This demand has driven the exploitation of endophytes as enzyme sources for promising industrial applications in biotechnology, agriculture and pharmaceuticals (Laird and Schamp, 2006).

Amylases are ubiquitous enzymes, being widespread in animals, fungi, plants, unicellular eukaryotes and prokaryotes. Amylases are those industrial enzymes which have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. Amylases have completely replaced chemical hydrolysis of starch in starch processing industry. Due to many advantages such as consistency, cost effectiveness, less time and space required for production and ease of process modification and optimization, fungal amylases have been widely used for industrial production. These enzymes account for 30% of the world's enzyme production (Vander Maarel *et al*., 2002). These are starch degrading enzymes which catalyze the hydrolysis of internal glycosidic bonds in polysaccharides with the retention of anomeric configuration in the products. Most of these are metalloenzymes, which require calcium ions for their activity, structural integrity and stability (Bordbar *et al.,* 2005). There is considerable interest in developing these enzymes with better properties such as raw starch degrading amylases, which are suitable for industrial applications and their cost effective production techniques, because of their increasing demands. Most important amylases for industrial and biotechnological applications are glucoamylases and α-amylases (Sivaramakrishnan *et al*., 2006). The latter have the most diverse range of industrial applications including brewing, baking, textiles and detergents. Each of these applications requires unique enzymatic properties with respect to temperature, pH, specificity and stability (Gupta *et al.,* 2010; Varalakshmi *et al*., 2009). Mostly amylases are produced from soil fungi such as *Aspergillus*, *Penicillium* and *Rhizopus* (Pandey *et al.,* 1999; 2000). Only a very few reports are available on amylases produced from endophytic fungi, and mainly explored for beneficial secondary metabolites with different bioactivities (Bhardwaj and Agrawal, 2014). The characteristics of the amylase indicate that it has the potential to meet specifications of various industrial applications, especially the detergent industry. Zaferanloo *et al.* (2014) reported a strain of endophytic fungi *Preussia minima* which was isolated from an Australian native plant, *Eremophila longifolia* for the purification and characterization of a α-amylase. Sunitha *et al*. (2013) also reported an endophytic fungus C*ylindrocephalum* sp. which is isolated from medicinal plant *Alpiniacal carata* for amylase production.

The aim of the present study was to optimize amylase production from *Penicillium frequentans AVF2*, endophytic fungus isolated from spikes of *Pinus rouxburghii,* and to study the capability of hydrolyzing a broad range of carbon source as substrates which can be used further for industrial purposes.

MATERIAL AND METHODS

Plant material as source of endophytic fungi

Healthy (showing no visual disease symptom) and mature plants of *P. rouxburgii* were carefully chosen for sampling. Spikes of *P. rouxburgii* from different sites of Pauri, Gharwal region of Uttarakhand, are randomly collected for study. The material was brought to the laboratory in sterile bags and processed within a few hours of sampling. Fresh plant materials were used for isolation work to reduce any chances of contamination (Bhardwaj *et al*., 2014).

Isolation of endophytic fungi

The plant material was rinsed gently in running water to remove dust and debris. After proper washing spikes were cut in small pieces, and further processed under aseptic conditions. The spikes were cut in 0.5-1cm of length. The isolation of endophytic fungi was performed according to the method described by Petrini (1986). They are further treated with 1% sodium hypochlorite for 1-2 min and then washed with autoclaved water. Afterwards, they are again sterilized by treating with 70% ethanol for 1 min and washed with autoclaved water for 2-3 times. The sterilized explants are then inoculated in potato dextrose agar (PDA) plates. Control plates of PDA medium containing unsterilized sample spikes are also run. The hyphal tips growing out from each inoculated sample are transferred to the other plates containing PDA which is used as a maintenance medium. The Petri plates were supplemented with antibiotic streptomycin 100µg/mL to suppress bacterial growth. PDA plates were incubated at 28 °C for 2-3 weeks. The fungi were identified based on the cultural characteristics and the morphology of the fruiting bodies and spores using standard manuals (Barnett and Hunter, 1972). All the isolates were maintained on PDA slants.

Identification of endophytic fungi

Endophytic fungi were further characterized by Pathology Division, Forest Research Institute, Dehradun (Uttarakhand).

Screening for secretion of amylase

To test for the production of amylase, endophytes were inoculated on glucose yeast extract peptone (GYP) agar medium (glucose 1g, yeast extract 0.1g, peptone 0.5g, agar 15 g, and distilled water 1000 mL, pH 6) containing 1% soluble starch. After incubating for 5 days, 1% iodine in 2% potassium iodide was flooded in the fungal colony plates. A clear zone appearance surrounding the colony was considered positive for amylase enzyme (Maria *et al*., 2005).

Optimization of culture conditions for amylase production

Out of 17 taxa of endophytic isolates screened for amylase production on solid media 12 of them were shown positive. Among these isolates, only *Penicillium frequentans AVEF-2* was selected for the optimization of amylase activity in liquid media as it showed the maximum activity. The organism was first grown in 25 mL of basal media g/L (KCl 5, $MgSO_4 7H_2O$ 0.5, $NaNO_3$ 3, KH_2PO_4 1, $CaCl_2$ 0.1, $FeSO_4 7H_2O$ 0.01) and then supplemented with 1.5% starch in 150 mL Erlenmeyer flasks and autoclaved at 121 ºC (15 lbs) for 15 min. After sterilization process, the flasks were cooled to room temperature and 0.1 mL spore suspension of the fungal strain was inoculated and incubated for 10 days at different parameters as described below by taking one parameter at a time. An uninoculated flask served as control.

Effect of pH, temperature, carbon and nitrogen sources on amylase activity

The study was carried out at different pHs (5, 7, 9 and 11) and temperatures (25, 30, 37 and 45 ºC). Different sources of carbon such as lactose, glucose, maltose, sucrose at 1.5% (w/v) and nitrogen sources such as ammonium nitrate, sodium nitrate, beef extract and yeast extract at 0.3% (w/v) were used to determine their effect on the production of amylase activity.

Determination of fungal biomass

The fungal culture biomass was expressed as dry weight by drying the mycelium in hot air oven at 80 ºC for 16h (Sunitha *et al*., 2012).

Enzyme assay

The culture broth was filtered by Whatman filter paper no.1. Then the filtrate was centrifuged at 3000 rpm for 8 min at 4 ºC and the resulting supernatant was used for enzyme assay. Amylase activity was determined at room temperature in a reaction mixture containing 1mL of 1mol/L sodium acetate buffer (pH 6.0), 0.5 mL of the crude enzyme extract and 0.5 mL 1% starch (w/v). After an incubation period of 30 min, the liberated maltose was estimated by dinitrosalicylic acid (DNS**)** (Miller, 1959). One unit of amylase activity (U) is defined as the amount of enzyme releasing 1µmol of reducing sugar mL/min, with maltose as standard under the assay conditions mentioned above. The denatured culture filtrate served as control.

RESULTS

All endophytic fungi were metabolically characterized on the basis of amylase enzyme secretion on solid media. Out of 17 endophytic isolates screened for the amylolytic activity on solid media, 12 showed positive results (data not shown). Among these the isolate *P. frequentans AVF2*, which showed maximum zone of clearance, was selected for the optimization of amylase activity in liquid media.

Optimization of culture conditions

Effect of pH of the culture media on amylase activity and fungal biomass production

In this study at different pH the maximum amylase activity was 0.461U/mL at pH 7 and the maximum fungal biomass production is 5.511g/25mL and at pH 11 the amylase activity was 0.001U/mL and fungal biomass production was 0.248g/25 mL **(Fig. 1)**.

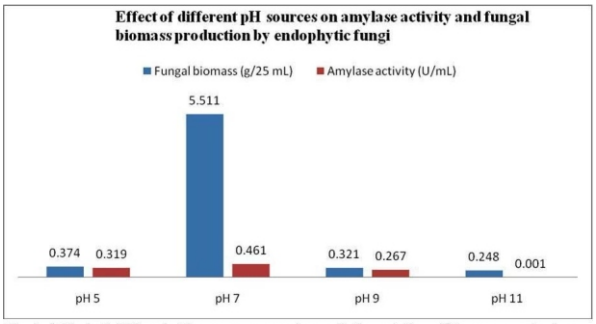

Fig. 1. Effect of different pH sources on amylase activity and fungal biomass production

Amylase activity and the biomass were shown at both the lower (25 ºC) and higher incubation (45 ºC) temperatures. For amylase production, the optimum incubation temperature was found to be 30 ºC and biomass is also correlated with the results **(Fig. 2)**. At different temperatures maximum amylase activity of 0.28U/mL is observed at 30 ºC and the maximum fungal biomass production is 5.618g/25mL which is also at 30 ºC. No fungal activity is observed at 45 ºC and the fungal biomass is 0.201g/25mL.

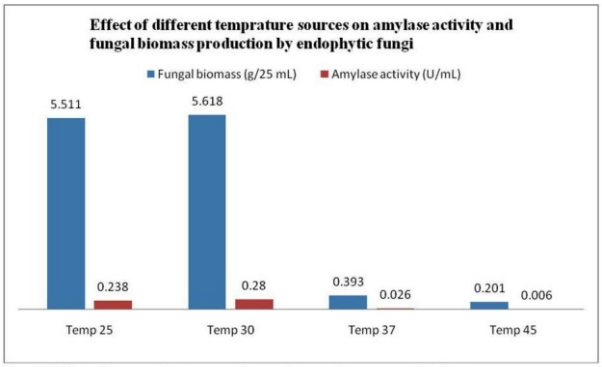

Fig. 2. Effect of temperature on amylase and fungal biomass production

Effect of different carbon sources on amylase and fungal biomass production

The fungus was able to grow on all the tested carbon sources. There were significant differences in biomass yield and amylase production. Among the various substrates screened, maltose showed the highest enzyme activity for amylase production, followed by starch. The order of usability of substrate was maltose>starch> lactose>glucose. There was significant increase in the yield in case of maltose and glucose **(Fig. 3)**. This result indicates that the amount and nature of carbon source in culture media are important for biomass growth and extracellular amylase production (Hegde *et al*., 2011). So carbon source of the medium plays a very important role in inducing enzyme secretion. In this study, the maximum amylase activity among different carbon sources was shown by maltose. Maximal fungal biomass obtained was 0.508g/25mL and maximum amylase activity was 0.44U/mL.\

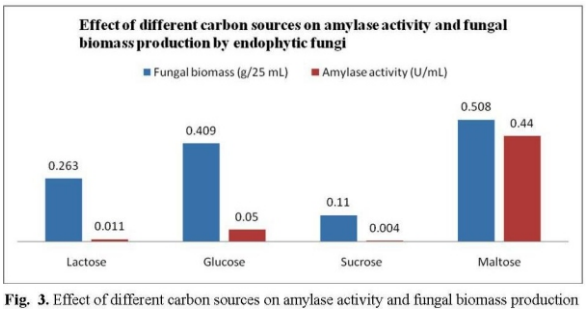

Fig. 3. Effect of different carbon sources on amylase activity and fungal biomass production

Effect of various nitrogen sources on amylase and fungal biomass production

However, in this study, there was no significant increase in the yield of enzyme in case of supplementation with either inorganic or organic nitrogen sources. A maximum increase was noted in the amylase activity with the addition of sodium nitrate which is about 0.411g/25mL in fungal biomass production and 0.214 U/mL in amylase activity **(Fig. 4)**. A minimum activity was observed when yeast extract was used as nitrogen source. Beef extract indicates that any of these sources can be used alternatively (Akcan, 2011).

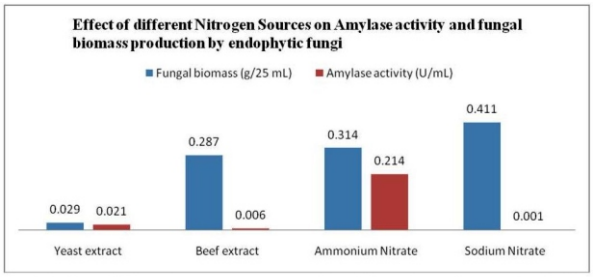

Fig.4. Effect of different nitrogen sources on amylase activity and fungal biomass production

DISCUSSION

The extracellular enzyme production profiles of endophytic fungi often indicate about their ecological roles as endophytes/latent pathogens or saprobes in their natural environment (Faeth and Fagan, 2002). They further stated that the ability to produce enzymes may be related to the lifestyle abilities of the endophytic fungi being tested. The knowledge of enzyme production by endophytic fungi may provide insights into their possible biotechnological applications and also provide an idea about their life cycles within the host plant. The endophytic fungal isolates studied in the present investigation revealed potential prospects for enhanced production of industrially relevant metabolites such as enzymes. From an evolutionary perspective, these endophytic fungal strains may have adapted to the respective metabolic machinery of the host tissues to produce biomolecules, which are not only important for their own physiology, but also for the adaptation of host plant. Moreover, the genetic machinery required to produce starch degrading enzymes such as amylase may be already present in endophytic fungi prior to the establishment of relationship with the host plant.

The pH is one of the most important factors that determine the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium. The pH is known to affect the secretion of α-amylase and its stability. Optimum pH for amylase production was 7.0 and similar findings were reported by Hegde *et al*. (2011) in

Calophyllum inophyllum. The biomass yield was found to be higher in case of pH 5.0 which was contradictory as reported by Olama and Sabry (1989). While the amylase activity and the biomass yield were maximal at pH 7.0 in *Aspergillus flavus* and *Penicillium purpurescence, Aspergillus oryzae, A. ficuum* and *A. niger* were found to give significant yields of α-amylase between pH 5.0 and 6.0 (Carroll, 1988; Chandra *et al*., 1980).

For amylase production, the influence of temperature is related to the growth of the organism. Hence, optimum temperature is based on whether the culture is mesophilic or thermophilic (Ramachandran *et al.*, 2004; Pandey *et al*., 1999). Mostly amylase production studies have been carried out with mesophilic fungi within the temperature range of 25-37 ºC (Ramachandran *et al*., 2004; Francis *et al*., 2003). An optimum temperature of 30 ºC has been reported by Kathiresan and Manivannan (2006) in *Penicillium fellutanum* isolated from mangrove rhizosphere soil and similar finding have been observed by Sunitha *et al.* (2012) in endophytic *fungus Cylindrocephalum* sp. isolated from medicinal plant *Alpiniacal carata*. Similar findings have been reported by Ray (2004) in *Aspergillus oryzae*, *Botryodiplodia theobromae* and *Rhizopus oryzae.*

Amylase is mostly induced in the presence of carbon sources such as starch and its hydrolytic products. Similar findings were reported by Kathiresan and Manivannan (2006) indicating maltose as a best carbon source to enhance the amylase activity in *P. fellutanum*. Compared to defined carbon sources, the biomass yield was higher in undefined carbon sources similar to the findings in case of Rhizobial strains (Oliveira *et al.,* 2007). *Thermoactinomyces vulgaris* produces best yields of α-amylase when starch or maltose is used as carbon source (Kuo and Hartman, 1996).

Nitrogen sources have been reported to have an inducing effect including α-amylase in SSF system (Rahardjo *et al*., 2005) on the various enzyme productions. Earlier reports show that among several inorganic nitrogen sources tested, ammonium chloride, ammonium hydrogen phosphate and ammonium sulphate favored enzyme secretion and growth.

CONCLUSION

An attempt has been made to offer an endophytic fungus as source of enzymes for industrial needs. In the present study for the maximum amylase production, the growth parameter of *Penicillium frequentans* has been standardized, which can effectively be used in large scale production. The maximal amylase productivity and fungal biomass were achieved at 30 ºC of incubation and at pH 7.0 of the cultural media. Among the various carbon sources, 1.5% maltose gave the highest amylase production. Among different nitrogen sources 0.3% sodium nitrate was found to be optimum for fungal biomass. However, a more detailed investigation is required to characterize amylase enzyme, which can be used in large-scale production for commercial purpose in future.

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KAVAKA 44:50-52 (2015)

First report of a lichenicolous fungus *Opegrapha phaeophysciae* from India

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ABSTRACT

The present paper describes occurrence of lichenicolous fungus *Opegrapha phaeophysciae* R. Sant. Diederich, Ertz & Christnach. colonizing thallus of *Phaeophyscia hispidula* from Indian subcontinent, thus raising the tally of this group of fungi to 52 from India. Previously the species was reported from Russia and Far East Asia (Japan and South Korea) growing on various species of *Phaeophyscia*.

Key words: India, lichenicolous, new record, *Opegrapha, Phaeophyscia*

INTRODUCTION

Lichenicolous fungi are a well documented phenomenon (Hawksworth, 1979; 1981; Triebel, 1989; Rambold and Triebel, 1992; Diederich, 1996; Matzer, 1996) and comprise a large and taxonomically diverse group of about 1800 species growing as obligate parasites or saprotrophs on lichens (Lawrey and Diederich, 2003; 2011). They either belong to primarily non lichenized fungi (e.g. *Lichenopeltella* Höhn.) or are secondarily delichenized, as in *Arthonia* Ach., *Opegrapha* Ach. and *Gyalediopsis* Vězda (Lücking and Lücking, 2000). These fungi have not been well studied in India, and so far only 51 species have been reported (Joshi *et al.*, 2015), of which 05 [*Homostegia hertelii* D. Hawksw., V. Atienza and M.S. Cole, *Lichenodiplis lecanorae* (Vouaux) Dyko & D. Hawksw., *Lichenodiplis lichenicola* Dyko & D. Hawksw., *Pyrenidium actinellum* Nyl., *Sphinctrina tubaeformis* A. Massal.] were reported first time from Kumaun Himalaya. During recent (August 2014) lichenological tours in Pithoragarh district of Kumaun Himalaya, one of the authors (SU) collected few patches of *Phaeophyscia hispidula* (Ach.) Moberg heavily infected by *Opegrapha* species.

The genus *Opegrapha*, belonging to family *Roccellaceae*, is a large genus of *ca.* 300 species, with both lichenized and lichenicolous species (Kirk *et al*., 2008). It is generally characterized by crustose noncorticated thallus, sunken or sessile apothecia, black carbonaceous excipuloid tissue, *Opegrapha*-type asci and transversely septate, hyaline, I-ascospores. Lawrey and Diederich (2011) have reported 58 species and 01 variety of this genus as lichenicolous across the globe. In India, the genus in its lichenized state is represented by 22 species (Joseph and Sinha, 2011); while as lichenicolous it is represented by only 02 species *O. tenuior* Stirt. and *O. foreaui* (Moreau) Hafellner and R. Sant. (Awasthi, 1991; Coppins and Kondratyuk, 1998).

Thorough anatomical examination followed by literature survey revealed that the *Opegrapha* collections did not belong to the two previously reported species from India and could be any of the Asian species described in the past. Hence, the purpose of this study was to identify the species and investigate the species diversity of lichenicolous fungi in Kumaun Himalaya. The specimen on its identification turned out to be *Opegrapha phaeophysciae* R. Sant. Diederich, Ertz & Christnach. which is new to India, and thus raises the tally of lichenicolous flora to 52. The species is described here in detail.

MATERIALS AND METHODS

The samples were collected from three localities of Pithoragarh district, Uttarakhand. Anatomical examinations were made using hand cut sections mounted in water and lactophenol cotton blue (LCB). Chemical reactions of the ascomata were investigated in 1% aqueous iodine solution (I), 0.25% aqueous iodine solution (I_{dil}), 1% aqueous iodine solution after pretreatment with 10% aqueous potassium hydroxide solution (K/I), 10% aqueous potassium hydroxide solution (K) and 10% nitric acid (N). All the specimens are housed in the herbarium of SSJ Campus, Kumaun University, Almora (ALM) and under PUN in the herbarium of Department of Botany, Punjabi University, Patiala.

TAXONOMIC DETAIL

Opegrapha phaeophysciae R. Sant., Diederich, Ertz and Christnach., *Biblioth. Lichenol*. **91**: 132 (2005)

Ascomata scattered to crowded on the upper side of the host thallus, immersed first but soon emergent, black, rounded, slightly convex to flattened, basally not constricted, 0.1-0.4 mm diam., with fragments of the host cortex attached to the younger stages; upper stromatic layer irregularly reticulate fissured and/or with a ring fissure along the outer margin, occasionally breaking away in the centre to expose the hymenium. Sterile tissue/Stroma dark brown, 90-120 × 100-250 µm, K+ olivaceous brown, N- (more orange brown), stromatic pigment Atra brown. Hymenium in horizontal section undulate to marginally divided and often with one to three rounded columns of stromatic plectenchyma; in transversal section with one to three subspherical to extensive loci; marginal and apical stromatic layer 20 - 40 µm thick, sometimes missing in the central portions of the ascomata; hyaline, 70 - 120 µm high, I_{dil}+ pale red, I+ red, K/I+ blue. Subhymenium hyaline, 10 - 15 µm high, I_{dil}+ pale blue, I+ pale red mottled with pale blue, K/I+ blue. Epihymenium brown, 15 - 20 µm high, I_{dil}+ blue, I+ blue, K/I+ blue. Paraphysoids abundant, branched and anastomosing, 2 - 2.5 µm thick, apically slightly swollen. Asci of *Opegrapha* type, clavate, with an apical K/I+ blue ring, 4 - spored, 40 - 70 × 12 - 16 µm. Ascospores hyaline, becoming brown and granular warty at maturity, elongate

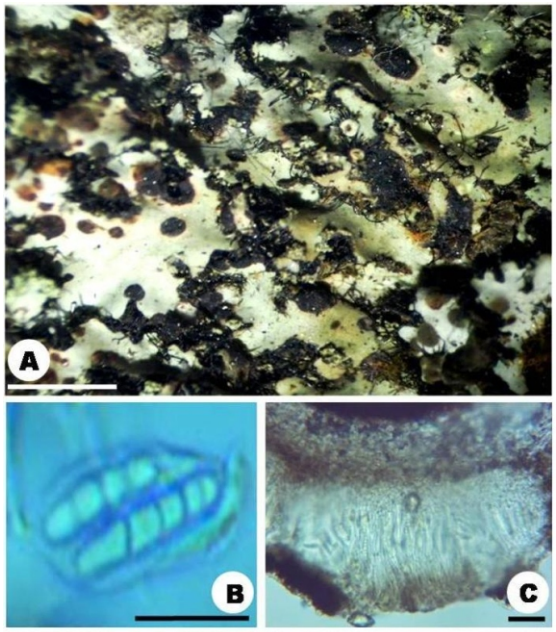

Fig. 1 A) Black dots on the thallus of *Phaeophyscia hispidula* indicating colonies of *Opegrapha phaeophysciae* (Scale bar = 1 mm). B) Spores (Scale bar = 20 um). C) Cross section through ascomata (Scale bar = 100 um).

ellipsoid, (3-)6 septate, slightly constricted at the septa, 21 - 28(32) × 4.0 - 6.0 µm, the median cell often somewhat elongated. Perispore thin, *ca*. 1 µm thick, with a brown granulose pigmentation located on the ascospore wall at maturity. Pycnidia not observed.

Specimens Examined: India, Uttarakhand, Pithoragarh, Thal Ke Dhar, alt. 2082 m, 05 August 2014, 29º30'57.7" N, 80º14'20.5" E, over *Phaeophyscia hispidula*, on bark of Ban oak, Shashi Upadhyay and Sandhya Shukla, *s.n.* (ALM), 7154(PUN); ibid., Chandak Pashupati Nath forest, Mosta Manu temple, alt. 1800 m, 07 August 2014, 29º36'53.60" N, 80º11'48.31" E, over *Phaeophyscia hispidula*, on the bark of *Quercus leucotrichophora*, Shashi Upadhyay and Sandhya Shukla, *s.n.* (ALM), 7152(PUN); ibid., on way to Asurchula temple (Chana village), alt. 2000 m, 06 August 2014, 29º37'47.42" N, 80º10'38.66" E, over *Phaeophyscia hispidula*, on the bark of Ban oak, Shashi Upadhyay and Sandhya Shukla, *s.n.* (ALM), 7153(PUN).

Ecology and Distribution: The species is found parasitizing thallus of *Phaeophyscia hispidula* growing on bark of *Quercus leucotrichophora* A. Camus (Ban oak) from three localities in Kumaun Himalaya Chandak, Asurchula and Thal Ke Dhar, all lying in Pithoragarh district of Uttarakhand. Previously, it was reported from the Primorskii Region of eastern Russia (Ertz *et al*., 2005), Japan (Frisch and Ohmura, 2013) and South Korea (Kondratyuk *et al.*, 2013) (**Table 1**) growing on various species of *Phaeophyscia* Moberg.

Remarks: *Opegrapha phaeophysciae*, placed in the parasitic *O. anomea* Nyl. group, is the third parasitic (i.e. lichenicolous) *Opegrapha* species known to India besides *O. tenuior* and *O. foreaui.* Both these species differ from *O. Phaeophysciae* in having different hosts and geographical distribution: *O. tenuior* reported from Eastern Himalaya used to parasitize members of *Thelotrema* Ach., while *O. foreaui* reported from Western Ghats parasitizes members of *Heterodermia* Trevis.

Table 1. Geographical distribution and host specificity of *Opegrapha phaeophysciae*

S. No.	Country	Host	Reference
1	Russia	*Phaeophyscia hispidula* (Ach.) Moberg	Ertz *et al.* (2005)
2	South Korea	*Phaeophyscia* aff. *squarrosa* Kashiw., *P. adiastola* (Essl.) Essl., *P. exornatula* (Zahlbr.) Kashiw.	Kondratyuk *et al.* (2013)
3	Japan	*Phaeophyscia limbata* (Poelt) Kashiw.	Frisch and Ohmura (2013)
4	India	*Phaeophyscia hispidula* (Ach.) Moberg	Present Collections

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Genus *Amanita* section *Lepidella* from North Western India

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ABSTRACT

Present paper deals with four species of genus *Amanita* belonging to sub-genus *Lepidella*, section *Lepidella*. Out of these *A. cinereocarpa* is a new species, *A. polypyramis* (Berk & Curt.) Sacc. is being reported for the first time from India while *A. griseofarinosa* Hongo and *A. vittadinii* (Moretti) Vitt. are being reported for the first time from Himachal Pradesh and Punjab, respectively.

Keywords: *Amanita,* section *Lepidella*

INTRODUCTION

Amanita Pers. is well known mushroom genus with mostly poisonous and some edible species (Semwal *et al.* 2005). This genus is commonly found growing in temperate region under some angiospermous and gymnospermous trees and is less common in tropical region. Of the seven sections known under this genus, the present paper deals with the taxa falling under *A.* sect. *Lepidella* which is characterized by cap with appendiculate margin and surface often whitish, sometimes with grayish, brownish, pinkish, yellowish or greenish tinges, more rarely brown or gray, very rarely brightly colored. Annulus membranous to friable. Stipe cylindrical or with small to very large marginate bulb. Remnants of volva are normally found scattered over whole stipe or concentrated at middle part, rarely concentrated near base. Basidiospores small to large, globose to bacilliform, thin-walled to slightly thick-walled, amyloid. In the present paper four species of this section namely *A. cinereocarpa* sp. nov., *A. polypyramis* (Berk. & Curt.) Sacc., *A. griseofarinosa* Hongo and *A. vittadinii* (Moretti) Vitt. are included.

MATERIALS AND METHODS

The material was collected from Himachal Pradesh and Uttrakhand in North Western India. The morphological details were recorded from fresh sporophores. The field characters pertaining to gross morphology, shape, color and size of this pileus, stipe and lamellae, presence or absence of annulus, etc were noted down on the 'Field key' provided by Atri *et al*. (2005) and the colour terminology used is that of Kornerup and Wanscher (1978). The specimens were hot air dried and packed in cellophane paper bags containing 1-4 dichlorobenzene. The microscopic details were studied by cutting free hand sections of revived part of the dried specimen and staining them in 1% Cotton Blue or 2% Congo red. The spores were studied from the spore print as well as from the crush mounts of the lamellae, amyloid reaction was checked in Melzer's Reagent. The dried specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala, (Punjab), India under PUN for further reference.

TAXONOMIC DESCRIPTIONS

***Amanita cinereocarpa* Yadwinder Singh & Munruchi Kaur sp. nov. Figs. 1(A-B) & 2(A-G)**

Mycobank - MB 812270

Diagnosis: *A. cinereocarpa* is characterised by its subcylindrical to slightly bulbous stipe with greyish brown, floccose, fibrillose scales all over; brownish grey, striated annulus; brownish grey volva with floccose-fibrillose patches forming incomplete rings.

Fig. 1 (A-B) ***Amanita cinereocarpa* sp. nov.:** A) Carpophore in its natural habitat. B) Under view of cap showing free, white lamellae and stipe covered with grayish brown scales having grey striate annulus.

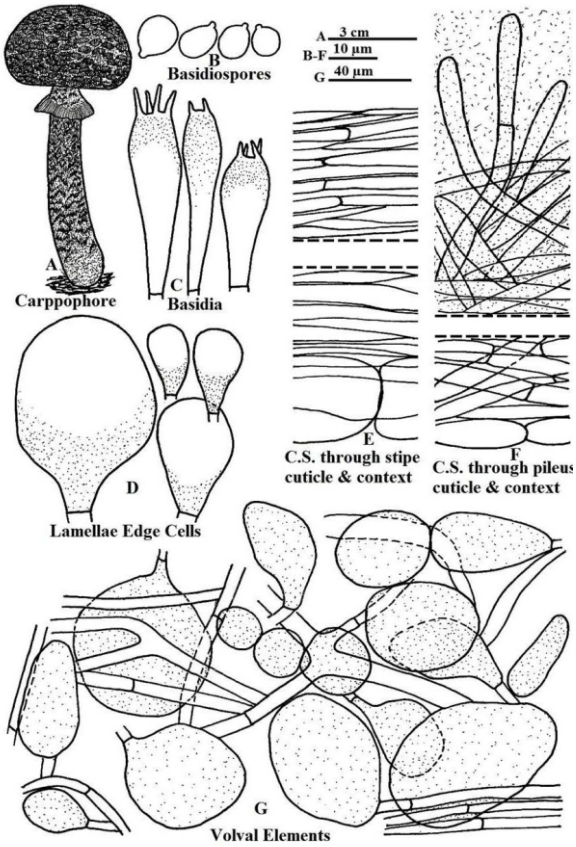

Fig. 2 (A-G). Internal details of *A. cinereocarpa* sp. nov.

Holotype: Himachal Pradesh, Churdhar (2,700 m), growing solitary, in coniferous forest, under *Cedrus deodara*, Yadwinder Singh, PUN 6427 (holotypus), September 7, 2009.

Etymology: Epithet name refer to color of the sporophore

Sporophore up to 9.5 cm high. Pileus up to 4.5 cm wide, hemispherical; lacking umbo; margin regular, non-appendiculate, nonstriate, decurved; surface greyish brown ($7E_3$) to brownish grey ($7D_2$) (metallic); covered with dark brown ($7F_3$) to blackish brown, squarrose, appressed fibrillose volval remnants; cuticle fully peeling; flesh up to 0.5 cm thick, white, unchanging; odor mild. Lamellae free, with decurrent line, close, broad (up to 0.5 cm), white, unchanging; gill edges serrate, grey; lamellulae attenuate. Stipe excentric, up to 9.0 cm long, up to 1.1 cm broad above, up to 1.3 cm broad at the base, subcylindrical, with slightly bulbous base, greyish white background, covered with greyish brown ($7E_3$), floccose, appressed fibrillose, flat scales; solid; annulate, annulus single, brownish grey ($7D_2$) above and brownish below, skirt like, striated, persistent; volva as brownish grey, floccose-fibrillose patches, arranged as scattered, incomplete rings.

Basidiospores [35/1/1] (4.8-) 5.6-8.0 x 4.8-7.2 µm (**L** = 5.6-7.2 µm; **L'** = 6.6 µm; **W** = 4.8-6.4 µm; **W'** = 5.9 µm; Q = 1.0-1.33; **Q'** = 1.11); globose, subglobose to broadly ellipsoid; amyloid, hyaline, thin walled, smooth; apiculate, apiculus up to 0.8 µm long. Basidia 24.0-35.2 x 6.4-9.6 µm, clavate, granular above, without clamp connections, tetra-sterigmate, occasionally bi-sterigmate; sterigmata up to 4.8 µm long. Lamellae edge cells abundant, granular in lower half, thin walled, clavate to ellipsoid, 12.8-38.4 x 6.4-29.0 µm. Pileus cuticle hyphal, gelatinized, made up of thin walled, granular, subradially tangled, septate 1.6-6.4 µm broad hyphae; pileus context composed of irregularly arranged, septate, thin walled, 3.2-8.0 µm broad hyphae. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, smooth, made up of longitudinally and compactly arranged, septate, 1.6-11.2 µm broad hyphae; stipe context composed of loosely interwoven, septate, thin walled, 3.2-12.8 µm broad hyphae; acrophysalides thin walled, abundant, up to 32.0 µm. Volval remnants on pileus surface similar as on stipe base, composed of subglobose, broadly ellipsoid, claviform, elongated, 20.4-86.0 x 14.3-69.5 µm inflated cells, arranged in rows of three or four, abundant, intermixed with branched, thin walled, septate, 2.0-12.3 µm broad hyphae. Clamp connection absent throughout.

Distribution and Ecology: The present specimen was collected growing solitary, in coniferous forest, on soil, under *Cedrus deodara* at 2,700 m from Himachal Pradesh.

Remarks: This species has been assigned to subg. *Lepidella* (E. J. Gilb.) Vesely emend. Corner & Bas, sect. *Lepidella* (E. J. Gilb.) Corner & Bas, subsec. *Vittadiniae* Bas and stirps *Thiersii* bacause of the presence of amyloid basidiospores, elongated stipe base, basidia clampless and globose to subglobose basidiospores measuring less than 10 µm in length. The present collection is distinct in having medium sized sporophores, greyish brown to brownish grey (metallic) cap, covered with dark brown to blackish brown, squarrose, floccose volval remnants, free closely spaced lamellae with decurrent line, closely placed, white with grey serrate gill edges, excentric, subcylindrical stipe, with slightly bulbous base, stipe surface greyish white in background, covered with greyish brown, floccose, fibrillose scales, annulus brownish grey, striated; volva in the form of brownish grey, floccose-fibrillose patches, arranged as scattered, incomplete rings, basidiospores amyloid, globose - subglobose to broadly ellipsoid and basidia without clamp connections. With these combination of characters the present collection did not match with any of the known species of strips *Thiersii*, also all the known species of this stirps are whitish, pinkish yellowish or bright orange yellow colored (Bas, 1969). Thus, a new species *Amanita cinereocarpa* sp. nov. has been proposed to accommodate this collection. In due course, molecular studies may also be undertaken with more collections to secure its phylogenetic position.

Amanita polypyramis (Berk. & Curt.) Sacc. *Syll. Fung.* **5:** 18, 1887. **Figs. 3 and 4(A-F)**

Sporophores 18.0 - 20.0 cm high. Pileus 12.0 - 14.0 cm broad, convex to flattened applanate; broadly umbonate; margin regular, appendiculate, non-striate, splitting at maturity; surface white ($1A_1$), volval remnants breaking up into small, white, conical to irregular warts which cover the entire pileus surface, with a glabrous centre; moist; cuticle fully peeling; flesh up to 1.5 cm thick, white, unchanging; odor disagreeable. Lamellae adnexed, with a decurrent line on the stipe, close, forked, broad (up to 1.7 cm), yellowish white ($4A_2$), unchanging; gill edges serrate; lamellulae attenuate. Stipe central, 16.2-18.5 cm long, up to 2.5 cm broad above, up to 2.8 cm broad in the middle and up to 4.5 cm broad at the base which is bulbous, narrowing upward; white, unchanging; decorated with white powdery, conical warts, glabrescent at maturity; solid; annulate, annulus superior, single, fragile, evanescent, skirt like, with fringed margins, decorated with large and small pieces of conical warts underneath, smooth above, white; stipe base large, subglobose to sub-napiform, covered with small conical persistent warts, arranged in rings.

Fig. 3. ***Amanita polypyramis*****:** A mature carpophore in its natural habitat.

Basidiospores [44/1/1] 8.0-12.8 x (5.6-) 6.4-8.0 µm (**L** = 8.0-11.2 µm; **L'** = 9.8 µm; **W** = 6.4-7.2 µm; **W'** = 6.7 µm; Q = (1.22-) 1.25-1.67 (-1.87); **Q'** = 1.43); broadly

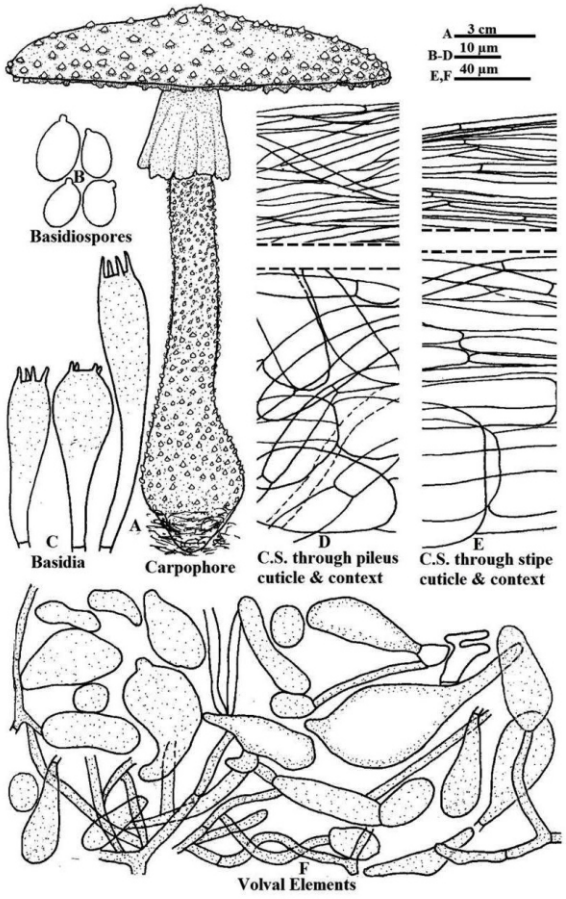

Fig. 4(A-F). Internal details of *A. polypyramis.*

ellipsoid, ellipsoid to elongate; amyloid, hyaline, thin walled; apiculate, apiculus up to 1.6 µm long. Basidia 30.4-59.2 x 9.6-13.6 µm, clavate granular, without clamp connections, tetra-sterigmate; sterigmata up to 4.8 µm long. Gill edges fertile. Pileus cuticle hyphal, made up of thin walled, 1.6-4.8 µm broad, septate hyphae, pilocystidia absent; pileus context composed of loosely interwoven, septate, thin walled, 4.8-12.8 µm broad hyphae; acrophysalides broadly ellipsoid, broadly clavate, thin walled up to 38.4 µm. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, smooth, made up of longitudinally and compactly arranged, septate, 4.1-8.2 µm broad hyphae; stipe context composed of loosely interwoven, septate, thin walled, 4.1-12.3 µm broad hyphae; acrophysalides thin walled, abundant, narrowly clavate, up to 33.0 µm broad. Volval remnants on stipe base composed of claviform, subpyriform, elongated to subglobose, 16.4-123.0 x 12.3-49.0 µm, inflated cells, abundant, thin walled, granular, intermingled with branched, granular, thin walled, septate, 4.1-8.2 µm broad hyphae. Clamp connection absent throughout.

Collection examined. Uttarakhand, Chakrata, Korba (2,300 m), growing scattered on soil in broad leaved forest, under *Quercus leucotrichophora*, Yadwinder Singh, PUN 6428, July 21, 2010.

Distribution and Ecology: Bas (1969) found *Amanita polypyramis* from mixed forest of *Pinus taeda*, *Quercus falcata*, *Cornus florida*, and *Liriodendron tulipifera* interspersed with *Kalmia latifolia of* South Carolina, U.S.A. and also collected it from tropical Oak forest of Costa Rica, associated with *Quercus oleoides,* at 750 m altitude during October and November. The present collection was found growing scattered on soil in a broad-leaved forest, under *Q. leucotrichophora* in late July from Uttarakhand.

Remarks: The above examined collection belong to *A.* subg. *Lepidella* (E. J. Gilb.) Vesely emend. Corner & Bas and sect. *Lepidella* (E. J. Gilb.) Corner & Bas bacause of the presence of amyloid basidiospores, appendiculate pileus margin and lacking robust saccate volva. The morphological and internal details of above examined collection completely match with the descriptions given for *Amanita polypyramis* (Berk. & Curt.) Sacc. by Bas (1969). This species is distinctive in large, robust sporophore, pileus white, appendiculate, nonstriate, small volval remnants, white, conical to irregular shaped warts which cover the entire pileus surface sometimes glabrous in the centre; odour disagreeable; lamellae adnexed, yellowish white, stipe narrowing upward, with large, subglobose to sub-napiform bulb at the base, decorated with white powdery, conical warts, glabrescent at maturity; annulus apical, smooth above, decorated with large and small pieces of conical warts below, white, evanescent and a bad odor as reported by Bas (1969). Presently, this species is reported for the first time from India.

Amanita griseofarinosa Hongo. *Mem. Fac. Liberal Arts Shiga Univ., Nat. Sci.* 11: 39, 1961. **Figs. 5 and 6(A-F)**

Collection Examined: Himachal Pradesh, Solan, Kala Ghat (1,600 m), growing solitary on humicolous soil, in coniferous forest, in mycorrhizal association with *Pinus roxburghii*, Yadwinder Singh and Munruchi Kaur, PUN 3852, August 20, 2008.

Distribution and Ecology: Hongo (1959) found *Amanita griseofarinosa* growing gregariously among mosses in woods in Japan. Mao (1991) and Yang (1997) reported it from China. Semwal *et al*. (2005) reported it as growing solitary or scattered under *Quercus glauca*, *Cinnamomum jeylanicum* and *C. tamala* trees in Uttarakhand, India. The present collection was found growing solitary in mixed coniferous forest under *Pinus roxburghii*.

Remarks: Due to the presence of amyloid basidiospores, appendiculate pileus margin and lacking robust saccate volva the presently worked out collection belongs to *A.* subg. *Lepidella* (E. J. Gilb.) Vesely emend. Corner & Bas and sect. *Lepidella* (E. J. Gilb.) Corner & Bas. Macroscopic and microscopic details are in conformity with the description given for

Fig. 5 ***Amanita griseofarinosa*****:** Carpophore in its natural habitat.

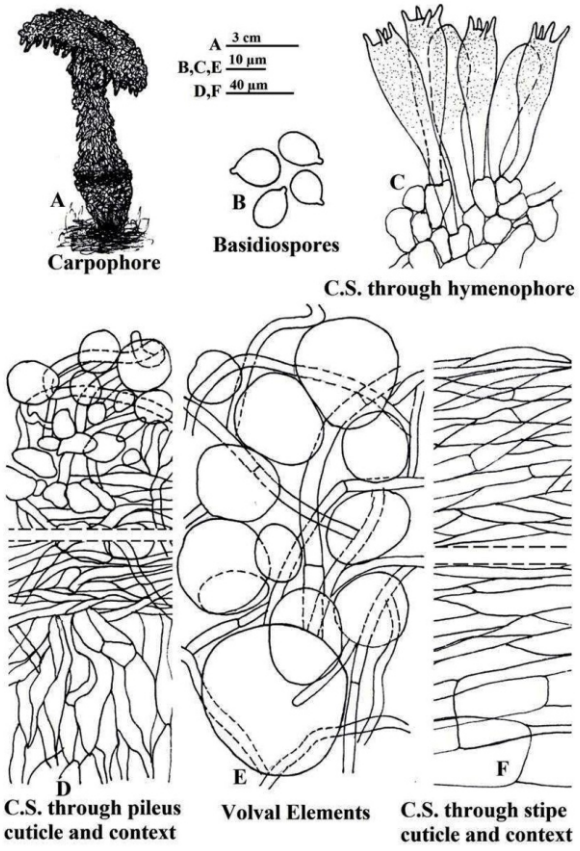

Fig. 6 (A-F). Internal details of *A. griseofarinosa*.

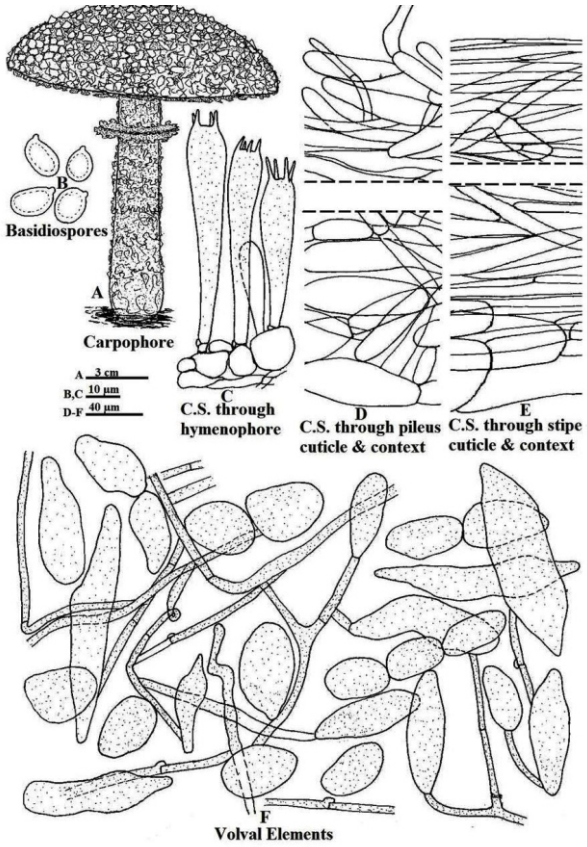

Fig. 8(A-F). Internal details of *A. vittadinii*.

Amanita griseofarinosa Hongo by Bas (1969). Semwal *et al*. (2005) has already described this species from Uttarakhand (India). It is a first time report of this species from Himachal Pradesh.

Amanita vittadinii (Moretti) Vitt. *Tent. Mycol. Amanita*, Ill. 31, 1826. **Figs. 7 and 8(A-F)**

Collections examined: Punjab, Patiala, Bahadurgarh (250 m), growing scattered in small groups, Open grassy lawn, among grasses, Yadwinder Singh, PUN 6421, August 18, 2009; Bathinda (210 m), growing solitary, on humicolus soil, Yadwinder Singh, PUN 6422, July 31, 2009; Faridkot, Baja Khana (196 m), growing scattered, on humicolus soil, Yadwinder Singh, PUN 6423, September 8, 2009; Faridkot, Dod (196 m), growing scattered, on humicolus soil, Yadwinder Singh, PUN 6424, September 16, 2009; Bathinda, Bhagta Bhaika (196 m), growing scattered in small groups, on sandy soil, Yadwinder Singh, PUN 6425, July 31, 2010; Faridkot (196 m), growing scattered in small groups, on humicolus soil, Yadwinder Singh, PUN 6426, August 23, 2010.

Fig. 7 ***Amanita vittadinii*****:** Underside of cap showing adnexed lamellae.

Distribution and Ecology: Bas (1969) recorded *Amanita vittadinii* growing solitary or in rings, in the fields, open woods, parks, etc. Presently studied collections were collected solitary to scattered on humicolous soil or rarely on sandy soil at an altitude varying from 196 m - 250 m during late July to mid September.

Remarks: The presently examined collections are assigned to *A.* subg. *Lepidella* (E. J. Gilb.) Vesely emend. Corner & Bas, sect. *Lepidella* (E. J. Gilb.) Corner & Bas, subsect. *Vittadiniae* Bas and stirps *Vittadinii* Bas in having amyloid spores, elongated stipe base, basidia with clamp connections at the base and subglobose to broadly ellipsoid to ellipsoid basidiospores, generally more than 10 µm in length. It was easily keyed to stirps *Vittadinii* by following the keys provided by Bas (1969). In the macroscopic field characters and microscopic details the above examined collection match well with the description of *Amanita vittadinii* (Moretti) Vitt. as provided by Bas (1969). This species is similar to *A. theirsii* Bas by possessing similar sporophore color, appendiculate pileus margin, elongated stipe base and amyloid basidiospores, but differs in having cap with pyramidal, subpyramidal, fibrills at the centre and appressed fibrillose, recurved squamules at the margin, white or orange white or light brownish volval remnants, lamellae free to adnexed, white to yellowish white, stipe decorated with appressed to recurved, fibrillose, flat scales and basidia with clamp connection. The basidiospores in the present collections are slightly smaller varying from 8.0-11.2 (-12.0) x (5.6-) 6.4-8.0 µm in comparison to (9) 10-13 (15) x (6.5) 7.5-10 (11) µm given by Bas (1969) for this species. Abraham and Kachroo (1989) and Pandotra (1997) reported this species from Jammu and Kashmir.

Presently, it has been collected from Punjab plains. Hence, it is a first time record for Punjab.

ACKNOWLEDGMENTS

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Some new reports of genus *Peniophorella* from Jammu Division (J&K)

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ABSTRACT

An account of five resupinate, non-poroid taxa (*Peniophorella clavigera*, *P. pallida*, *P. praetermissa*, *P. pubera* and *P. tsugae*) has been given in this paper. All except *P. pubera* are being reported for the first time from Jammu division (J&K).

Key words: *Basidiomycota, Rickenellaceae*, clamped hyphae

INTRODUCTION

The members of genus *Peniophorella* are characterized by resupinate, adnate, effused, membranaceous to ceraceous basidiocarps, branched, septate, clamped generative hyphae, presence of echinocysts or stephanocysts, clavate, 4-sterigmate basidia, cylindrical to ellipsoid to allantoid, inamyloid, acyanophilous basidiospores with oily contents. An account of five species (*Peniophorella clavigera*, *P. pallida*, *P. praetermissa*, *P. pubera* and *P. tsugae*) has been given. Out of these five species, *Peniophorella clavigera*, *P. pallida* and *P. tsugae* are also new records for the state of Jammu and Kashmir. All species except *P. pubera* reported here are new reports for the Jammu division (J&K) which was earlier reported by Thind and Rattan (1970) from Ramban district of Jammu division. The material of all specimens has been deposited at the Herbarium, Botany Department, Punjabi University, Patiala (PUN). The color standards used are as per Methuen's Handbook of colors by Kornerup and Wanscher (1978).

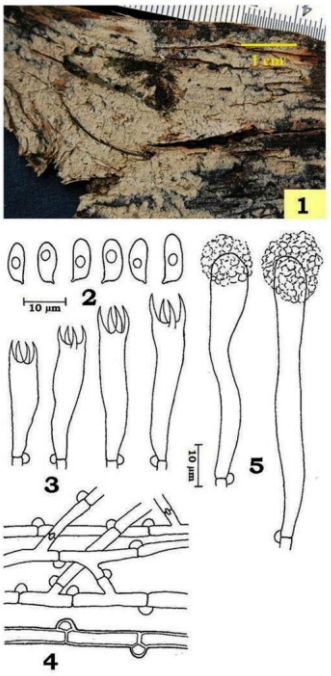
Key to the species

1. Stephanocysts usually present…..... *P. praetermissa*
1. Stephanocysts usually absent 2
2. Cystidia clavate..... *P.clavigera*
2. Cystidia subfusiform, tapering towards apex..... 3
3. Cystidia encrusted, basidiospores narrowly ellipsoid to subcylindrical…..... *P. pubera*
3. Cystidia naked..... 4
4. Basidiospores allantoid..... *P. pallida*
4. Basidiospores ellipsoid..... *P. tsugae*

TAXONOMIC DESCRIPTIONS

***Peniophorella clavigera* (Bres.) K.H. Larss.**, *Mycol. Res.* **111**(2): 191, 2007. ***Kneiffia clavigera* Bres.**, *Annls Mycol.* **1**(2): 103, 1903. **Figs. 1-5**

Basidiocarp resupinate, adnate, effused, up to 180 µm thick in section; hymenial surface smooth to rough with some cracks, to somewhat tuberculate under lens, pale yellow to grayish orange 6 B4 to brownish orange 6 C4 when fresh, somewhat darkening on drying; margins thinning, grandinioid, paler concolorous, to indeterminate. **Hyphal system** monomitic. Generative hyphae up to 3.6 µm wide, branched, septate, clamped; basal hyphae loosely interwoven, parallel to the substrate, thin-to somewhat thick-walled; subhymenial hyphae denser, vertical, thin-walled. **Cystidia** 52-67 × 7.5-8.5 µm, clavate to subclavate, somewhat capitate, with brownish resinous encrustation at the apex, with basal clamp, thin-walled. **Basidia** 22-33 × 6-7.5 µm, clavate, 4-sterigmate, with basal clamp; sterigmata up to 6.8 µm long. **Basidiospores** 9-12 × 3.5-5 µm, cylindrical to suballantoid, apiculate, thin-walled, smooth, inamyloid, acyanophilous.



Figs. 1-5. ***Peniophorella clavigera*:** 1. Basidiocarp showing hymenial surface; 2. Basidiospores; 3. Basidia; 4. Generative hyphae; 5. Clavate to subclavate cystidia.

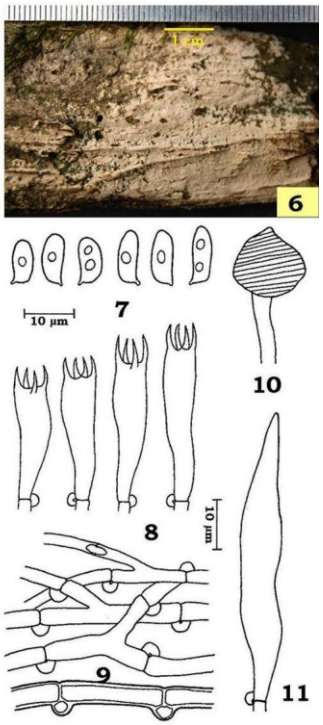
Collection examined- J&K: Ramban, Sanasar, on the under surface of log of *Pinus wallichiana*, Jyoti 5097 (PUN), September 11, 2012.

Remarks: This species is peculiar in having clavate to subclavate, somewhat capitate cystidia with brownish resinous encrustation at the apex, clavate basidia and cylindrical to suballantoid basidiospores. Singh (2007) was the first to report it from India from district Chamba (H.P.) in the North Western Himalaya followed by Priyanka (2012) from the same area. Both described it as *Hyphoderma clavigerum* following Donk (1957), but Larsson (2007) placed it in genus *Peniophorella* mainly on the basis of results from molecular phylogenetic analysis (Larsson 2007). Later, Dhingra *et al*. (2014) listed it as a species in genus *Peniophorella* following Larsson (2007). However, it is the first report of this species from J&K.

***Peniophorella pallida* (Bres.) K.H. Larss.**, *Mycol. Res.* **111**(2): 192 (2007). ***Corticium pallidum* Bres.**, *Fung. trident.* **2**(1113): 59, 1898. **Figs. 6-11**

Basidiocarps resupinate, adnate, effused, up to 240 µm thick in section; hymenial surface smooth to somewhat

tuberculate with few cracks, grayish yellow 3 B4 when fresh, not changing much on drying; margins thinning, paler concolorous, to indeterminate. **Hyphal system** monomitic. Generative hyphae branched, septate, clamped; basal hyphae up to 4.5 µm wide, parallel to the substrate, thin- to thick-walled; subhymenial hyphae up to 4 µm wide, vertical, thin-walled. **Cystidia** 46-54 × 6.8-8.5 µm, subfusiform, tapering towards the apex, with basal clamp, thin-walled; cystidia like capitate hyphal ends with apical patches of brownish resinous matter present in the hymenium and context. **Basidia** 21-30 × 6-7.5 µm, clavate, 4-sterigmate, with basal clamp; sterigmata up to 5.5 µm long. **Basidiospores** 9-11.5 × 4-4.5 µm, suballantoid, apiculate, thin-walled, smooth, with oily contents, inamyloid, acyanophilous.



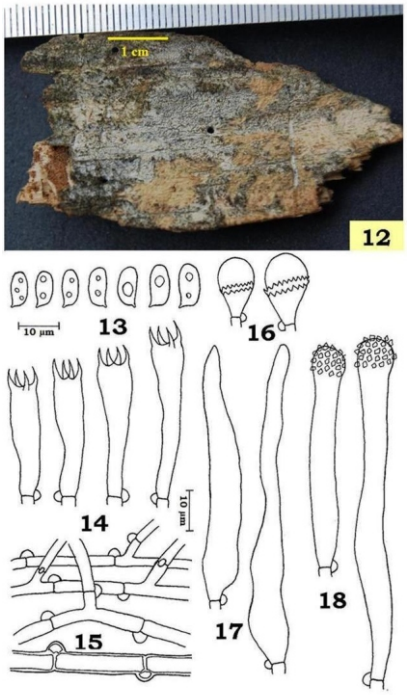
Figs 6-11. ***Peniophorella pallida*****:** 6. Basidiocarp showing hymenial surface; 7. Basidiospores; 8. Basidia; 9. Generative hyphae; 10. Subfusiform cystidium; 11. Capitate hyphal ends.

Collections examined-J&K: Ramban, about 15 km from Patnitop towards Sanasar, on stump of *Cedrus deodara*, Jyoti 5098, 5099 (PUN), September 10, 2012.

Remarks: This species is peculiar in having subfusiform cystidia, clavate basidia and suballantoid basidiospores. From India, Rattan (1977) was the first to report it from district Chamba (H.P.), followed by Singh (2007) from district Kullu (H.P.) in the North Western Himalaya and Dhingra (1989) from Meghalaya and W. Bengal in the Eastern Himalaya. This species has also been described by earlier workers under genus *Hyphoderma* following Donk (1957), presently it is placed by Larsson (2007) under *Peniophorella* on the basis of results from molecular phylogenetic analysis and presence of echinocysts (though these are difficult to find). Dhingra *et al.* (2014) listed it as a species in genus *Peniophorella* following Larsson (2007). Here it is the first report of the species from J&K.

***Peniophorella praetermissa* (P. Karst.) K.H. Larss.**, *Mycol. Res.* **111**(2): 192, 2007. ***Peniophora praetermissa* P. Karst.**, *Bidr. Känn. Finl. Nat. Folk* **48**: 423, 1889. **Figs. 12-18**

Basidiocarp resupinate, adnate, effused, up to 200 µm thick in section; hymenial surface smooth to rough, appearing cracked due to such substrate, grayish white 1 B1 with an orange 6 B7 tint, not changing much on drying; margins thinning, paler concolorous, to indeterminate. **Hyphal system** monomitic. Generative hyphae septate, clamped; basal hyphae up to 5.3 µm wide, less branched, loosely interwoven, parallel to the substrate, thin-to thick-walled; subhymenial hyphae up to 4 µm wide, much branched, denser, vertical, thin-walled. **Sterile structures of 3 types: I. Cystidia** 53-83 × 7.3-8 µm, subcylindrical, more or less capitate, with apical crystalline encrustation and basal clamp, thin-walled; projecting up to 60 µm out of the hymenium. **II. Gloeocystidia** 61-78× 8.5-10 µm, abundant, subfusiform, apically tapering, smooth, thin-walled, with basal clamp; enclosed, with oily contents negative to sulphovanillin. **III. Stephanocysts** 14-18 × 9-10 µm, bladder shaped, surrounded by a whorl of small teeth, with basal clamp. **Basidia** 26-38 × 6.8-8.5 µm, subclavate to clavate, 4-sterigmate, with basal clamp; sterigmata up to 5 µm long. **Basidiospores** 8.5-10.5 × 4-5.5 µm, ellipsoid to subcylindrical, apiculate, thin-walled, smooth, with oily contents, inamyloid, acyanophilous.



Figs 12-18. ***Peniophorella praetermissa*****:** 12. Basidiocarp showing hymenial surface; 13. Basidiospores; 14. Basidia; 15. Generative hyphae; 16. Subcylindrical cystidia; 17. Gloeocystidia; 18. Stephanocysts.

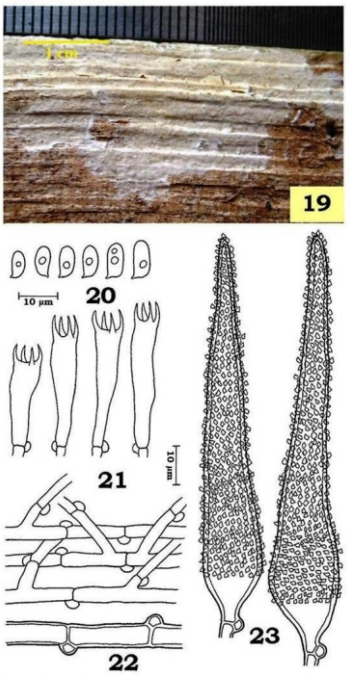
Collection examined-J&K: Ramban, about 18 km from Patnitop towards Sanasar, on the undersurface of *Cupressus sempervirens*, Jyoti 5100 (PUN), September 11, 2012.

Remarks: This species is marked by the presence of three types of sterile structures and has also been placed in *Peniophorella* by Larsson (2007) on the basis of presence of stephanocysts, and from results of molecular phylogenetic analysis. From India, Rattan (1977) was the first to report it from districts Chamba, Kullu and Shimla (H.P.), Dehradun (U.K.) and Baramula (J&K) followed by Singh (2007) from district Sirmaur (H.P.) and Priyanka (2012) from district Solan (H.P.) in the North Western Himalaya; Dhingra (1989) from the Eastern Himalaya; and Bhosle *et al*. (2005) from the Western Ghats (Maharashtra). All the earlier Indian workers kept it in genus *Hyphoderma.* Later, Dhingra *et al*. (2014) listed it as a species in genus *Peniophorella* following Larsson (2007). However, it is the first report of this species from

district Ramban (J&K).

***Peniophorella pubera* (Fr.) P. Karst.**, *Bidr. Känn. Finl. Nat. Folk* **48**: 427, 1889. ***Thelephora pubera* Fr.**, *Elench. fung.* **1**: 215, 1828. **Figs. 19-23**

Basidiocarps resupinate, adnate, effused, ceraceous, up to 350 µm thick in section; hymenial surface smooth to rough, mealy under lens, yellowish white to pale yellow when fresh, becoming orange gray 5 B2 to pale orange 6 A3 on drying; margins thinning, paler concolorous, to indeterminate. **Hyphal system** monomitic. Generative hyphae septate, clamped; basal hyphae up to 6 µm wide, loosely interwoven, less branched, parallel to the substrate, thin- to thick-walled; subhymenial hyphae up to 4 µm wide, much branched, denser, vertical, thin-walled. **Cystidia** 90-106 × 15-17.5 µm, subfusiform with acute apex, thin- to somewhat thick-walled, strongly encrusted; projecting up to 42 µm out of the hymenium. **Basidia** 21-33 × 6-7.5 µm, clavate, 4-sterigmate, with basal clamp; sterigmata up to 6 µm long. **Basidiospores** 6.5-10 × 3.3-4.5 µm, narrowly ellipsoid to subcylindrical, apiculate, thin-walled, smooth, with oily contents, inamyloid, acyanophilous.



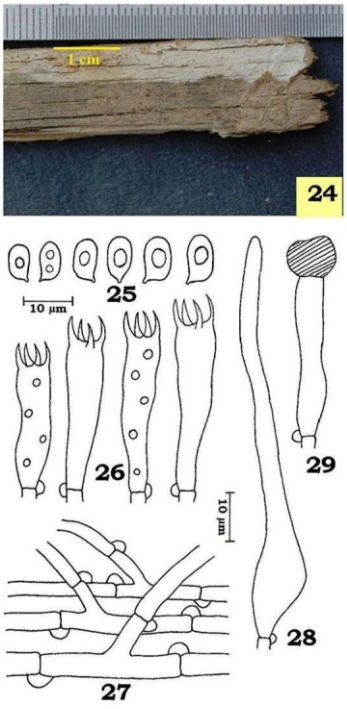
Figs 19-23. *Peniophorella pubera*: 19. Basidiocarp showing hymenial surface; 20. Basidiospores; 21. Basidia; 22. Generative hyphae; 23. Subfusiform encrusted cystidia.

Collections examined- J&K: Ramban, Nathatop, Ladhadhar, on gymnospermous stump, Jyoti 5101 (PUN); Batote, Shampa, on stump of *Pinus roxburghii*, Jyoti 5102 (PUN); on angiospermous log, Jyoti 5103 (PUN); on log of *P. roxburghii*, Jyoti 5104 (PUN); on log of *Cedrus deodara*, Jyoti 5105 (PUN); on gymnospermous stump, Jyoti 5106 (PUN), October 11, 2011; about 2 km from Patnitop towards Batote, on stump of *C. deodara*, Jyoti 5107 (PUN), October 11, 2011; Karlah, Nag Mandir Road, on gymnospermous stump, Jyoti 5108 (PUN); on stump of *Cedrus deodara*, Jyoti 5109 (PUN), September 10, 2012; about 18 km from Patnitop towards Sanasar, on log of *Cupressus sempervirens*, Jyoti 5110 (PUN), September 10, 2012.

Remarks: A common species, earlier reported from India by Thind and Rattan (1970) from district Chamba (H.P.) and Ramban (J&K), followed by Singh (2007) from districts Kullu and Shimla (H.P.), Priyanka (2012) from district Sirmaur (H.P.) in the North Western Himalaya; Dhingra (1989) from the Eastern Himalaya; and Bhosle *et al*. (2005) from the Western Ghats (Maharashtra) as *Hyphoderma puberum.* But now this species is being recognized as *Peniophorella pubera* as per Karsten (1889). Dhingra *et al*. (2014) listed it as a species in genus *Peniophorella* following Karsten (1889).

***Peniophorella tsugae* (Burt.) K.H. Larss.**, *Mycol. Res.* **111**(2): 192, 2007. ***Corticium tsugae* Burt**, *Ann. Mo. bot. Gdn, St. Louis* **13**(3): 276, 1926**.** **Figs. 24-29**

Basidiocarp resupinate, adnate, effused, up to 180 µm thick in section; hymenial surface smooth to rough, yellowish white 4 A2 to pale yellow 3 A3 when fresh not changing much on drying; margins thinning, paler concolorous, to indeterminate. **Hyphal system** monomitic. Generative hyphae up to 5 µm wide, branched, septate, clamped; basal hyphae loosely interwoven, parallel to the substrate, thin- to somewhat thick-walled; subhymenial hyphae denser, vertical, thin-walled. **Sterile structures of 2 types: Leptocystidia** 53-80 × 9.8-10.5 µm, basally widened, apically tapering, with basal clamp, thin-walled; projecting up to 20 µm out of the hymenium **Cystidia** 22-40 × 7.8-10 µm, subcylindrical, somewhat capitate; encrusted with brownish resinous matter at the apex. **Basidia** 24-34 × 6.5-8 µm, clavate, 4-sterigmate, with basal clamp; sterigmata up to 5.5 µm long. **Basidiospores** 7-10 × 4-5.5 µm, ellipsoid, apiculate, thin-walled, smooth, inamyloid, acyanophilous.



Figs 24-29. *Peniophorella tsugae*: 24. Basidiocarp showing hymenial surface; 25. Basidiospores; 26. Basidia; 27. Generative hyphae; 28. Subfusiform leptocystidium; 29. Subcylindrical cystidium.

Collection examined-J&K: Ramban, about 18 km from Patnitop towards Sanasar, on log of *Cupressus sempervirens*, Jyoti 5111 (PUN), September 11, 2012.

Remarks: This species is characterized by the presence of two types of sterile structures along with clavate basidia and ellipsoid basidiospores. Dhingra (1989) was the first to describe it from India from W. Bengal in the Eastern Himalaya followed by Dhingra and Singla (1993) from district Chamba (H.P.), Singh (2007) from district Kullu (H.P.) and Priyanka (2012) from district Sirmaur (H.P.) in the North Western Himalaya. Earlier workers reported it as *Hyphoderma tsugae,* but now it has been placed in *Peniophorella* by Larsson (2007). Dhingra *et al*. (2014) listed it as a species in genus *Peniophorella*

following Larsson (2007). It is the first report of this species from J&K.

ACKNOWLEDGEMENTS

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KAVAKA 44:62(2015)

Stirtonia punctiformis (*Ascomycota*: *Arthoniaceae*) : new to Indian Lichen flora

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ABSTRACT

***Stirtonia punctiformis* Aptroot & Sipman is discovered from Assam as a new record for India lichen flora. The species is characterized by its inconspicuous ascigerous zones, linear or round or consisting of individual 6-8 spored asci, and transversely septate, brown ascospores.**

Key words: Assam, lichen, new record, taxonomy.

INTRODUCTION

The lichen genus *Stirtonia* A.L. Sm. (*Arthoniaceae*) was recently revised by Aprtoot *et al*. (2014) with a worldwide key of 21 species. Of these 7 species viz. *Stirtonia alboverruca* Makhija & Patw., *S. dubia* A.L. Sm., *S. indica* Makhija & Patw., *S. macrocarpa* Makhija & Patw., *S. obvallata* (Stirt.) A.L. Sm. *S. ramosa* Makhija & Patw. and *S. santessonii* Makhija & Patw are known from India (Singh & Sinha, 2010). During studies on the lichen flora of Assam, *Stirtonia punctiformis* Aptroot & Sipman has been discovered as a new record for Indian lichen flora thus raising the number of species within this genus to 08 in India. A brief taxonomic description of the species is provided to facilitate its identification.

MATERIALS AND METHODS

Identification was carried out in the Lichenology Laboratory, Botanical Survey of India, Central Regional Centre, Allahabad (BS). The images displayed in **Fig. 1** were obtained with a stereozoom disecting microscope (Olympus SZ61) and compound microscope (Nikon Eclipse 50*i)*. The hand cut sections of thalli and ascomata were mounted in water, 10% KOH, and Lugol's iodine solution and all the measurements were made in water. Chemical constituents were identified by thin-layer chromatography in solvent C (170 ml toluene, 30 ml glacial acetic acid) as per Orange *et al.* (2001).

TAXONOMY

Stirtonia punctiformis Aptroot & Sipman, *Lichenologist* **46**(5): 675-676. 2014. **(Fig. 1)**

Thallus crustose, ecorticate, continuous, whitish grey, smooth, calcium oxalate crystals present; prothallus thin, black; photobiont *Trentepohlia*. Ascigerous part dispersed, rounded to irregular, immersed in thallus, slightly elevated with brown spots; asci abundant, often almost superficial, globose to ovoid, brown, 6-8 spored; ascospores brown, ellipsoid-fusiform with rounded to subacute ends, with larger middle locule and narrower locules at the ends, transversely 9-11 septate, 67-82 × 27-36 µm.

Chemistry: All spot tests of thallus and ascigerous part negative, UV^{-}; no substances detected by TLC.

Specimen examined: ASSAM: Cachar district, Kalain, Kalain Tea Garden, on the bark of a tree, 24° 58′N, 92° 34′E, c. 200 m alt., 09 February 2008, G.P. Sinha & A.M. Dhore 4182 (BSA).

Distribution: The species is so far known from Costa Rica (Puntarenas) and Guyana (Takutu). It is a new record for Indian Lichen flora and is currently reported from Assam.

Remarks: *Stirtonia punctiformis* is so for the only known species in the genus which is characterized by the brown ascos-pores (Aptroot *et al.,* 2014). Morphologically it resembles *S. Mcrocarpa* Makhija & Patw. but latter differs in heaving 2′ -*O* -methylperlatolic acid (Makhija and Patwardhan, 1998).

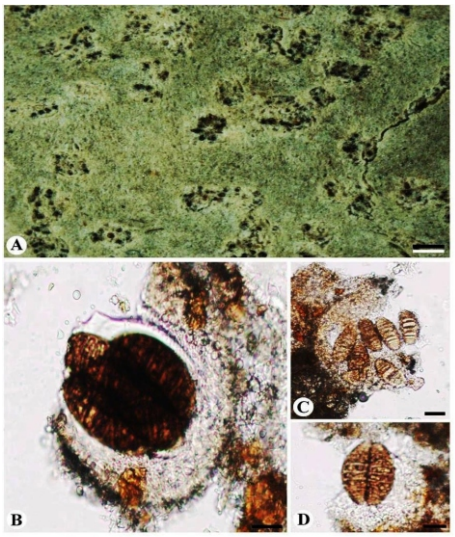

Fig. 1. *Stirtonia punctiformis* **A**. habit; **B**. section through ascigerous area; **C & D**. ascospores. Scales **A**. 0.1 mm; **B**, **C** & **D**. 25 µm.

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KAVAKA 44:63-65 (2015)

Two new lichen species of the genus *Coenogonium* (*Ostropales*: *Coenogoniaceae*) from the Western Ghats in India

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ABSTRACT

***Coenogonium kiggaense* and *C. dattatreyense* are described as new species to science from the Shola forests of the Western Ghats, India. The genus *Coenogonium* is being recorded for the first time from the Western Ghats. Key to the *Coenogoium* species in India is also given.**

Key Words: *Dimerella*, foliicolous, *Trentepholia*, *taxonomy*

INTRODUCTION

The lichen genus *Coenogonium* Ehrenb. Consists of more than 80 species. It belongs to the family *Coenogoniaceae* (*Ostropales*) and includes chiefly tropical lichens (Xavier-Leite *et al.*, 2014). It is characterized by biatorine (rarely zeorine), yellow to orange colored apothecia. Other important characters include the hemiamyloid hymenium, thin-walled unitunicate asci, 1-septate or rarely non-septate ascospores, and a trentepohlioid photobiont (Rivas Plata *et al.*, 2006).Twenty one species of Neotropical foliicolous species of this genus were keyed out by Lücking (2008), including seven filamentous species and fourteen crustose species.

Awasthi (1991) reported five species of *Coenogonium* [*C. himalayense* Pant &Awasthi, *C. implexum* Nyl., *C. leprieurii* (Mont.) Tuck., *C. moniliforme* Tuck. and *C. retistriatum* Leighton] and six species of genus *Dimerella* which now stands merged with genus *Coenogonium* [*C. isidiatum* (G. Thor & Vezda) Lücking, Aptroot & Sipman; *C. dilucidum* (Kremp.) Kalb & Lücking; *C. epiphyllum* (Mull. Arg.)Vain.; *C. luteum* (Dicks.) Kalb & Lücking.; *C. nepalense* (G. Thor & Vezda) Lücking, Aptroot & Sipman and *C. pineti* (Schrad. ex Ach.) Lücking & Lumbsch] from the Indian subcontinent. Lücking and Kalb (2000) merged the genera *Coenogonium* and *Dimerella* due to the fact that the filamentous genus *Coenogonium* and the crustose taxa of *Dimerella* have exactly the same type of apothecia, while the morphological differences are due to the photobiont characters. While revising the taxonomic and nomenclatural status of *Coenogonium*; Rivas Plata *et al.* (2006) pointed out *C. himalayense* as illegitimate name and this species is most probably related to *Lecanorales*. Hence,we can say ten species of *Coenogonium* are currently known from India, Nepal and Sri Lanka region. Among them only four species, namely *C. dilucidum, C. luteum*, *C. subluteum*, and *C. Zonatum* are known from Indian subcontinent (Awasthi, 1991). *Coenogonium luteum* was found on bark as well as leaves, whereas, remaining three species were reported as corticolous.

This genus is well studied in the Neotropics but more research is needed in other parts of the world especially in the palaeotropics. During a recent field trip to the Western Ghats, we found two species of *Coenogonium* in Shola (montane evergreen) forests and report them here as a new species to science.

MATERIALAND METHODS

Specimens were collected from the montane evergreen forest (Shola) of Chikmagalur district, Karnataka, India. A stereo microscope (Carl Zeiss Stemi 2000C) was used for morphological studies and a compound microscope (Carl Zeiss Primo Star) for studying the anatomy of thalli and fruiting bodies. Photographs were taken using an AxioCamERc5s camera and the images were analyzed using Axio Vision LE (AxioVs40 V 4.8.2.0) software. Anatomical characteristics were observed on hand cut sections mounted in water and 10% KOH (K); all measurements were taken in water. The hemiamyloid reaction of the ascus was studied in Lugol's solution (0.2% I and 0.6% KI) after pretreatment with KOH. Voucher specimens are deposited in LWG.

TAXONOMY

Coenogonium kiggaense Shravan Kumar S. & Y.L. Krishnamurthy sp. nov. **Fig. 1.**

MYCOBANK MB 809528

Diagnosis: Thallus filamentous, corticolous or foliicolous, prostrate pannose. Photobiont *Trentepohlia* contributing to the filamentous nature. Apothecia with smooth margin. Asci 8 spored, each ascospore 1-septate. Paraphysis clavate.

Type: India, Karnataka, Chikkamagaluru, Kigga, evergreen forest on the way to Narasimha Parvatha, 13º25'N, 75º10'E, 858m alt., corticolous on *Syzygiumcumini* (L.) Skeels., September 14, 2013. Shravan Kumar S. & Y.L. Krishnamurthy (Holotype LWG 17033); on *Artocarpus heterophyllus* (Paratype 5578); foliicolous on *Memecylon malabaricum* (C.B. Clarke.) Cogn., September 14, 2013, Shravan Kumar S. & Y.L. Krishnamurthy (Paratype 5579).

Thallus filamentous, prostrate-pannose, glabrous, yellow-green; corticolous or foliicolous; isidia absent; pycnidia not seen; photobiont *Trentepohlia,* cells cylindrical, in distinct filaments, not constricted at the septa, 40-50 x 25−30 µm; apothecia pale yellow-orange, white in summer, substipitate - stipitate, some young ascocarps are abconical, biatorine, disc concave to flat, 0.5-0.75 mm diam., 0.2-0.25 mm high, margin thin,

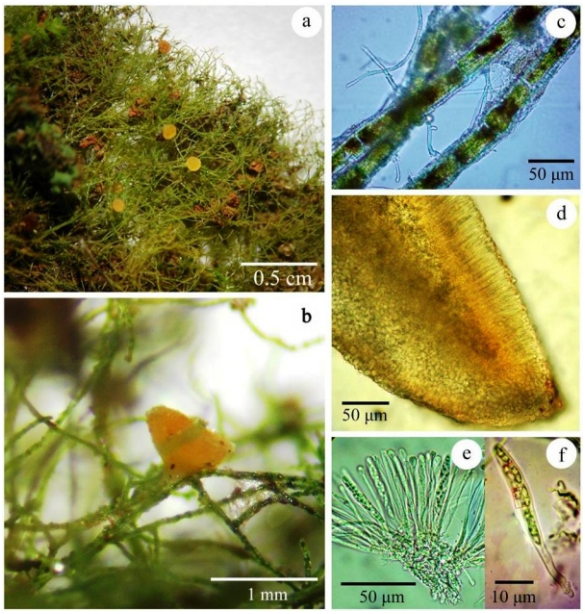

Fig. 1. *Coenogonium kiggaense* (holotype): a & b - microscopic view of filaments with apothecia, c- algal and fungal filaments, d- cross section of apothecia, e- asci and paraphysis, f- ascospores in a ascus.

smooth, often evanecent; excipulum pachydermatous cells with large lumina, very thin-walled ultraleptodermatous cells at margins; hymenium 60-70 µm high; asci 50-65 x 5-8 µm, 8-spored; ascospores broadly ellipsoid, hyaline, acicular, 7-7.5 x 2-2.5 µm, 1-septate; paraphyses unbranched, tips clavate, 2.5-3.5 µm wide, with or without yellow pigments.

Chemistry: K-, I-, C-, P-, TLC not done.

Etymology: The species name refers to the place from where the holotype was collected.

Distribution and Ecology: Found in wet patches (near streams) of Shola forest. On the bark of *Syzygium* spp., *Artocarpus heterophyllus* trees and on the margin or cut portion of the leaves of *Memecylon* spp. in humid and shady places. Usually found in understory trees. It is a facultative foliicolous species.

Although the apothecial characters play the major role in the classification of the species, the filamentous character is also considered important in delititation of species in the *Coenogonium*. About 29 taxa with filamentous thallus has been identified under the genus. The mycobiont play a major part in the formation of the filaments by holding the filament of the algae together as bundles. The cladistic analysis of phenotype characters of apothecia creates two major groups moniliforme and linki/epiphyllum (Rivas Plata *et al*., 2006). The species in the moniliforme groups are distinguishd by the moniliform filaments of algae and the filamentous nature is only visible under lens. Hence, our species belongs to the linki/epiphyllum group where the filaments can be seen without any magnification. The prostrate filamentous thallus and 1-septate ascospores narrow down the species to the *C. interplexum* group. Within this group it resembles *C. interplexum* aggr. by having algae in solitary filaments and non-pruinose apothecia. It differs from *C. interplexum* in having a more compact thallus with loose vertical filaments, pachydermatous excipulum with large lumina, ascospores are three times as long as broad. The ascocarps of *C. kiggense* are abconical in shape when young and mature apothecia turns white during summer. Some of the specimens of Costa Rica showed denticulate / uneven apothecial margin but in our case all specimens showed smooth margin forming clear conical structure. The cells of the photobionts in our species are significantly larger than in *C. interplexum* group. The paraphysis of our species is clavate shaped and sometimes the tips are filled with yellow pigments. Conidia are not observed in our species whereas in *C. interplexum* it is present.

Coenogonium dattatreyense Shravan Kumar S. & Y.L. Krishnamurthy sp. nov. **Fig.2**

MYCOBANK MB 809529

Diagnosis: Thallus foliicolous crustose with white prothallus. Photobiont *Trentepohlia* froming rounded cells near apothecia having persistent margin; asci 8 spored with broadly ellipsoid ascospores. Pycnidia wart shaped.

Type: India, Karnataka, Chikkamagaluru, Chandra-Drona Parvatha, Shola forest, on the way to Mullaiahnagiri Peak, 13º23'N, 75º42'E, 1693m alt., foliicolous on *Memecylon malabaricum* (C. B. Clarke.) Cogn., December 4, 2013, Shravan Kumar S. & Y.L. Krishnamurthy (Holotype LWG 17034, Paratype 5551).

Thallus crustose, green and shiny, with white prothallus, glabrous; foliicolous; isidia absent; pycnidia 0.1 mm, wart shaped; conidia non-septate, 3-3.5 x 1.5-2 µm; photobiont *Trentepohlia*, rounded cells near apothecia, 6-10 µm diam., angular cells in rest of the thallus, 5-7 x 20-25 µm diam.; prothallus white; apothecia yellow, biatorine, sessile, 0.5-1.25 mm diam., margin smooth, irregular, persistent; hypothecium 50-60 µm high;

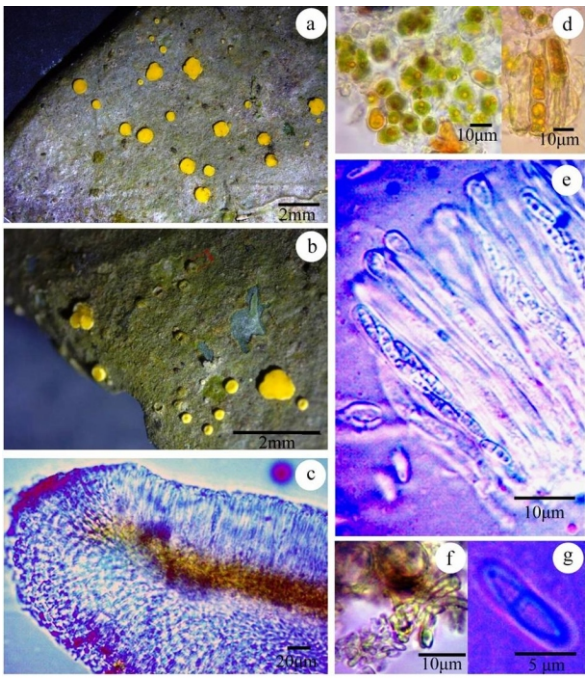

Fig. 2. *Coenogonium kiggaense* (holotype): a- habit, b- close view showing apothecia and pycnidia, c- cross section of apothecia, d- algal filament, e- asci and paraphysis, f-condiospores, g-ascospore.

hymenium 80-100 µm high; asci 8-spored, uniseriate; ascospores broadly ellipsoid, hyaline, 1-septate, 7-7.5x (2.5)3-3.5 µm, 2-3 times long as broad; paraphyses usually unbranched, tips clavate, 2.5-4 µm wide.

Chemistry: K-, I-, C-, P-, TLC not done.

Etymology: The species name refers to the place from where it was first collected.

Distribution and Ecology: This species is found in Shola forest of Mullayanagiri, Karnataka usually on slopes of the streams, on the leaves of *Memecylon malabaricum*.

The diameter of the apothecium (0.5 to 1.25 mm), and the ascospores size (7-7.5x (2.5)3-3.5 µm) places the new species in the *C. luteum* (large apothecia) group (Rivas Plata *et al.*, 2006). Within this group our species *C. dattatreyense* is similar to *C. fallaciosium* but can be distinguished by the size of the ascospores and the persistent apothecial margin. Our specimen does not have thick setae and has pycnidia hence it differs from *C. strigosum*. Although *C. luteolum* has most of the characters of our species it lacks prothallus and ascospores are 3-4 times long as broad (9-13 x 2.5-3.5 µm). In case of *C. luteum* the thallus is matt, grey coloured, without prothallus and the apothecia are orange yellow to orange coloured. *C. dattatreyense* is also similar to *C. subzonatum* but the latter has smaller, denticulate, and marginally hypophyllous apothecia.

Key to the *Coenogonium* species found in India

Seven species of *Coenogonium* known from India can be distinguished by the following key;

1a. Thallus filamentous ..... *C. kiggaense*

1b. Thallus crustose.....2

2a. Thallus with white prothallus 3

2b. Thallus lacking white prothallus 4

3a. Conidia 1-septate, 12-18 x 2-3 µm ..... *C. zonatum*

3b. Conidia non-septate, 3-3.5 x 1.5-2 µm .... *C. dattatreyense*

4a. Apothecia small, 0.1-0.25(0.4) mm diam. .... *C. dilucidum*

4b. Apothecia medium-sized to large, 0.3-2 mm diam. ...5

5a. Apothecia medium-sized, 0.3-0.8 mm diam., pale orange-yellow; pycnidia common ..... *C. subluteum*

5b. Apothecia large (0.5) 0.8-2 mm diam., orange; pycnidia rare or absent.....*C. luteum*

ACKNOWLEDGEMENT

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Taxonomic notes on two species of *Kretzschmaria* from the Eastern Himalayas

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ABSTRACT

This paper provides an account of two *Kretzschmaria* species (*K. mauritanica* and *K. microspora*) collected from different localities of Eastern Himalayas. The taxonomic details are given along with illustrations of their morphological and anatomical features. *Kretzchmaria mauritanica* forms a new record for India and *K. microspora* has been reported for the first time from Meghalaya.

Key words: Ascomycota, perithecium, ascal plug, systematics

INTRODUCTION

The genus *Kretzschmaria* Fr. is characterized by erect, short stalked stromata, terminated by a clavate or flat topped umbonate clava, simple to branched, closely aggregated in swarms. Ectostroma brown to black, persistent or cracking irregularly into scales; inner entostroma white to creamish white within and dark, carbonaceous outside. Perithecia completely immersed, subglobose to globose with minute ostiolar papillae. Asci cylindrical with obtuse apex; stalk narrow, filiform. Ascospores inequilateral-ellipsoid or slightly curved. It is distinguished from genus *Xylaria* on the basis of its vertically oriented perithecia and excentric development of the growing axis.

It is a small genus represented by 17 species (Hawksworth *et al.,* 1983; Rogers *et al.,* 1987). In India, only three species of the genus have been previously described by Thind and Dargan (1980) and Kar and Gupta (1981) [*Kretzschmaria heliscus* (Mont.) Massee., *K. clavus* (Fr.) Sacc. and *K. microspora* P. Henn.]. However, reference has also been made in Indian literature for few more species like *K. micropus* (Fr.) Sacc and *K. phoenicis* Kale & Kale (Tunstall 1922; 1929), *K. rugosa* and *K. benghalensis* Rawla & Narula (Narula and Rawla, 1987) and *K.caryotae* and K. *diopvre* (Wangiker 1988; Sharma and Dargan, 1989). These workers merely listed the species without giving adequate description. Hence, based on collections made from Eastern Himalayas, two species of *Kretzschmaria* (*K. mauritanica* Pat. and *K. microspora* P. Henn.) are described with illustrations.

MATERIALAND METHODS

Preparations of crushed mounts in water or 3% KOH was the first part in the microscopical study to observe the colour of asci, ascospores and appendages. Separation in paraphyses and hyphae of the stroma was studied by preparing crush mounts in 1% aqueous phloxine solution. Colour reaction of ascal plug was studied by preparing crushed mounts of the specimen in Melzer's reagent. Sections were studied in cotton blue and then mounted in lactophenol. The drawings of microscopic details were made with the aid of camera lucida under an oil immersion lens. All the collections examined have been deposited in the Herbariμm of Botany Department, Punjabi University, Patiala (Punjab), India. [(PUN) 1444, (PUN) 1435]

KEY TO THE SPECIES

1. Stromata simple but aggregated, terminated by fertile clava with pointed apex; umbonate. ascospores 17.5-25.5 × 9.6-12.8 μm inequilateral ellipsoid with longitudinal germ slit……..... .....*K.mauritanica*
2. Stromata simple; closely gregarious; terminated by fertile clava, non-umbonate, ascospores 9.6-14.5 × 3.2-4.8 μm apiculate.....*K. microspora*

TAXONOMIC DESCRIPTIONS

Kretzschmaria mauritanica Pat., *Bull. Soc. Mycol. France* **20**: 120, 1905 **Figs. 1(A-E)**

Stromata erect, 0.5-1.5 × 0.5-0.1 mm and up to 1.5 mm high, simple, scattered, individual stroma sometimes confluent, terminated by fertile clava, fertile clava

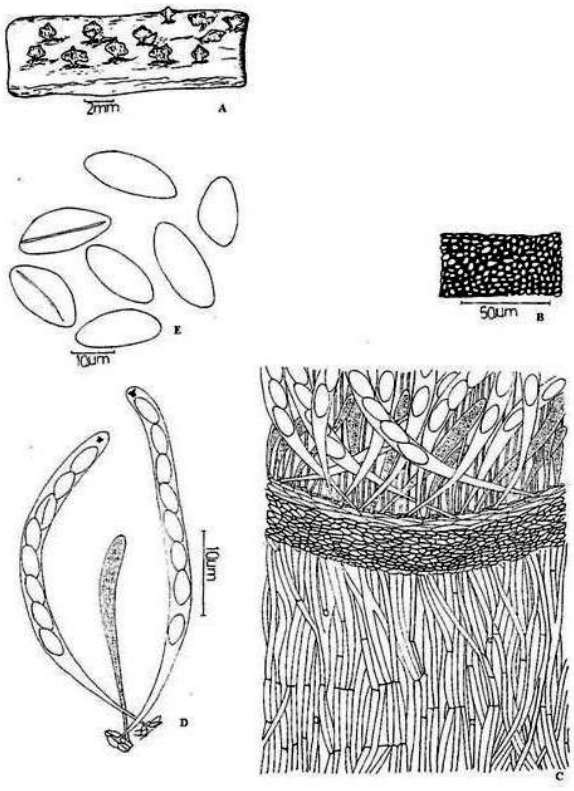

Fig. 1. (A-E) *Kretzschmaria mauritanica* A. Stromata general habit B. V.S. part of stroma showing detail structure of outer entostroma C. V.S. part of stroma showing detailed structure of perithecial wall and inner entostroma D. Asci E. Ascospores.

obconical to flat topped, up to 1.5 mm in diameter, umbonate, apex finely pointed, stalked, stalk sharply defined, small, narrow-cylindrical and black. Ectostroma absent; entostroma dark-brown to black above and whitish beneath, solid, distinguished into two zones; outer entostroma 60-80 µm wide, textura angularis, cells 3.2-9.6 × 2.4-8.0 µm. thick walled, dark brown; inner entostroma textura intricate; hyphae 3.2-4.0 µm wide, simple to branched, septate, light brown; hyphae in the stalk region, thin walled, hyaline. Perithecia 150-320 × 250-440 µm, globose to subglobose, ostiolate, ostiole papillate, papilla minute, black; perithecial wall 25-30 µm wide, textura angularis, composed of two zones; outer zone 20- 22 µm wide. Cells 1.6-4.8 × 0.8- 2.4 µm, thick walled, dark brown, inner zone 8-10 µm wide, cells 6.4-12.8 × 1.6-3.2 µm, thin walled, hyaline, elongated. Asci 160.0-200.0 × 10-12 µm, sporiferous portion 120-140 µm long, cylindrical, apex obtuse, ascal plug cylindrical, small and staining deep blue with Melzer's reagent. Ascospores 17.5-25.5 × 9.6-12.8 µm, elliptical-fusoid to inequilateral-ellipsoid, dark brown with a longitudinal germ slit. Paraphyses filiform nµmerous, thin and aggregate.

COLLECTION EXAMINED: Darjeeling; on way to Menibhanjyong, on fallen dead angiospermous twigs. Ashwani Kumar 241 (PUN) 1444. July 6, 1987.

The above fungus is characterized by simple, small, scattered, black, stroma with umbonate, finely pointed apex, small perithecia and large sized ascospores with longitudinal germ slit. It resembles *Kretzschmaria mauritanica* Pat. in all the essential features as described by Rogers *et al*. (1987) except for small sized asci and longitudinal germ slit in ascospores. The species is of rare occurrence and was not previously reported from India.

Kretzschmaria microspora P.-Henn., *Hedwigia* **43**: 261,1904 **Figs. 2: (A-E)**

Stromata erect, 1.0-3.5 × 1.0-3.0 mm and up to 3.5 mm high, simple, rarely forked at base, closely gregarious in dense swarms, individual stroma sometimes confluent, terminated by fertile clava, fertile clava flat topped, non umbonate, up to 3.0 mm in diameter, stalked, stalk sharply defined, narrow-cylindric and black. Ectostroma not observed; entostroma dark-brown to black above the perithecia and creamish white beneath, solid, differentiated into two zones; outer entostroma 80-120 µm thick, textura angularis, cells 3.2-8.0 × 1.6-4.8 µm, thick walled, dark brown; inner entosrtoma textura intricate, hyphae 3.2 µm wide, simple to branched, thin walled and hyaline. Perithecia completely immersed, vertically oriented 230-475 µm in diameter, globose to subglobose,ostiolate, ostioles papillate, papilla small; Perithecial wall 12-16 µm wide, textura intricate, not differentiated into zones and hyphae 2.4 µm wide, simple to branched, thick walled, slightly pigmented. Asci 108-120 × 4-6 µm, cylindrical, apex obtuse, ascal plug cylindrical and staining deep blue with Melzer's reagent. Ascospores 9.6-14.5 × 3.2-4.8 µm, elliptic-fusoid to inequilateral-ellipsoid, hyaline to dark brown, apicule present on one or both sides, longitudinal germ slit, guttulate. Paraphyses filiform, nµmerous, thin, aseptate.

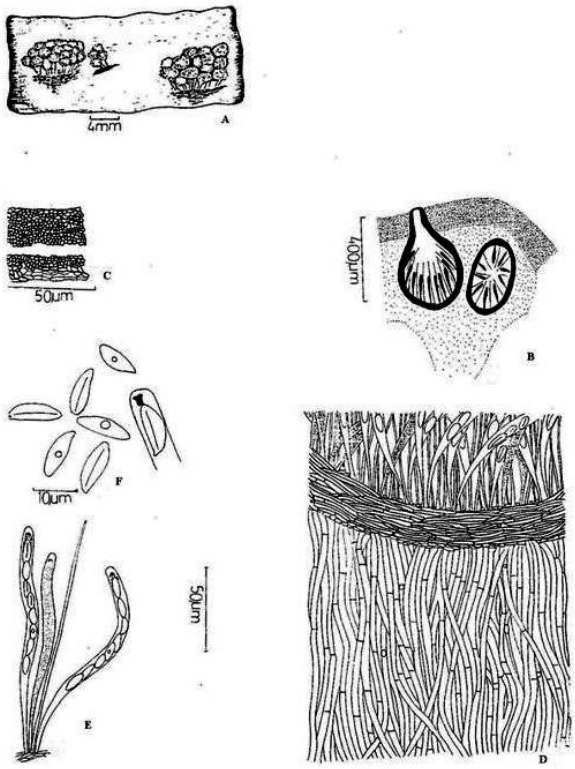

Fig. 2. (A-E) *Kretzschmaria microspora* A. Stromata general habit B. V.S. part of stroma showing various zones. C. V.S. part of stroma showing detailed structure of outer entostroma D. V.S. part of stroma showing detailed structure of perithecial wall and inner entostroma E. Asci and Paraphysis F. Ascospores and ascal plug.

COLLECTION EXAMINED: Shillong, Botanical garden, on dead angiospermous twigs, Ashwani Kumar 171 (PUN) 1435, September 24, 1986.

The above fungus resembles *Kretzschmaria microspora* P. Henn. as described by Rogers *et al*. (1987) in all essential features of stromata, perithecia and ascospores. Previously the taxon was reported in India by Kar and Gupta (1981) growing on unidentified rotten wood from Jalpaigudi (West Bengal). The species however also resembles *K. microspora* as described by Kar and Gupta (1981) in all essential features but stalk is well defined and stromata are comparatively larger in this collection as compared to West Bengal species.

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Effect of Plant Growth Promoting *Rhizobacteria* (PGPR) and Arbuscuar Mycorrhizal (AM) Fungi as bio-inoculants on the growth enhancement of *Gmelina arborea* seedlings in nursery

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ABSTRACT

Production of healthy and quality planting stock are highly important to meet the plantation target by the state forest departments, wood based industries and tree growers. In the present study, an experiment was conducted to test the efficacy of different bio-inoculants *viz.*, Plant Growth Promoting *Rhizobacteria* (PGPR) and Arbuscular Mycorrhizal (AM) fungi individually and in combinations on growth enhancement of economically important fast growing indigenous tree species, *Gmelina arborea* in nursery. It is evident from the data that all the bio-inoculants have greatly improved seedling growth and biomass of *G. arborea* in nursery on par with DAP application. Dual application of bio-inoculants was found to be better than single inoculations indicating the synergistic effect. Further, dual combinations involving a N fixer and a P solubilizer/ mobilizer were found to be highly effective.

Key words: Rhizosphere, arbuscular, mycorrhiza, growth promoting microbes, growth parameters, transplantation.

INTRODUCTION

The National Forest Policy, 1988 stipulates that one-third of geographical area should be under forest or tree cover. This can be achieved by rehabilitating the degraded forests and by promoting the plantation forestry. The plantation forestry was initiated mainly for the production of industrial raw materials as well as fodder and fuel wood. The exotic plants like species of *Eucalyptus*, *Casuarina* and *Acacia* are the major ones having larger area under the plantations compared to other tree species. However, in the recent years, much emphasis is being given to raise the plantations of fast growing indigenous tree species to meet the industrial raw material needs. Hence, the private and corporate entrepreneurs are taking up planting of the fast growing native tree species on commercial scale. To meet this planting demand, quality seedlings of these tree species are very much essential for better productivity in the field.

Gmelina arborea Roxb. commonly known as Gamhar (Hindi), is a deciduous tree (family: *Verbanaceae*) occurring naturally in most parts of India. It grows in different localities and prefers moist fertile valleys with 750-4500 mm rainfall. It does not thrive on ill drained soils and remains stunted on dry, sandy or poor soils. It is a light demander, tolerant of excessive drought but moderately frost hardy. The tree attains height of 30 m and girth of 1.2 to 4.5 m with a clear bole of 9-15 m and thick foliage forming a conical crown on the top of the tall stem. The wood of this tree is yellowish brown and soft to moderately hard, light to moderately heavy, usually straight to irregular grained and medium coarse textured. The timber is reasonably strong for its weight. It is used in constructions, furniture making, plywood industry, sports goods, musical instruments, artificial limbs and in boat building. The wood is also used for picture and slate frames, turnery articles and handles of brushes, chisels, files, saws, screw drivers, sickles, etc. In instrument industry, timber of this tree is widely used for the manufacture of drawing boards, plane tables, instrument boxes, thermometer scales and cheaper grade metric scales. The leaf is a good fodder for cattle. The bark and flowers of *G. arborea* have several medicinal properties and roots are used in preparation of Ayurvedic medicine, *Dasamularishtam*.

The rhizosphere is a dynamic soil environment formed by living plant roots and their associated microorganisms and fauna. Among different microbes, Plant Growth Promoting *Rhizobacteria* (PGPR) and Arbuscular Mycorrhizal (AM) fungi are present in rhizosphere soil and they are able to exert a beneficial effect upon plant growth. These microbes have multiple functions like nitrogen fixation, phosphorus solubilization and mobilization (Sen and Paul, 1957) and stimulating effect on root development by producing metabolites like IAA and other growth hormones (Lynch, 1990). They are eco-friendly, renewable, cost effective and pollution free. Therefore, the use of these beneficial microorganisms as bio-inoculants provides an effective alternative to the chemical fertilizers. The effects and potentialities of various beneficial micro-organisms as bio-inoculants in agriculture have been well documented (Subba Rao, 1993; Dash and Gupta, 2011; Brahmaprakash and Sahu, 2012).

They can also serve as important tools in forestry programmes including reclamation of degraded lands, mined overburdens and other problematic areas so as to make these programmes more effective (Grove and Le Tacon, 1993). The success or failure of a plantation can be forecasted by the quality of the nursery that has been maintained. Hence, to establish a good plantation, raising of high quality elite seedlings is necessary (Durvey and Landis, 1984). The apparent result of the beneficial microorganisms may not be evident under all natural conditions because of insufficient population naturally occurring in the soil (Powell and Daniel, 1978). Therefore, application of most suitable beneficial microorganisms becomes imminent. Bio-inoculants are capable of making afforestation programmes successful by improving planting stock quality and ultimately increasing productivity. The use of bio-inoculants in forestry appears to be more important than in agriculture because, firstly, in countries like India no large scale

provisions exist to irrigate, fertilize and protect the plantations and secondly, the chemical fertilizers are costly and are in short-supply. Therefore, inoculating nursery seedlings with selective bio-inoculants holds promise for improving seedling quality, out planting performance and increased resistance to root diseases and climatic stresses in the field. Also the use of these bio-inoculants would reduce the cost of chemical fertilizers involved in plantation programmes. The effective utilization of bio-inoculants for trees will not only provide economic benefits but also improve and maintain the soil fertility and sustainability in natural soil ecosystem. Since these bioresources represent a great diversity in chemical, physical and biological characteristics, their efficient use depend on, among others, identification of suitable type of bio-inoculants. Since very limited reports are available on use of AM fungi, *Azospirillum, Azotobacter* and Phosphobacteria on forest tree species, especially the economically important fast growing native tree species, the study with an aim of elucidating the information on the efficacy of these bio-inoculants on the growth improvement of *G. arborea* plants in the nursery.

MATERIALS AND METHODS

Seed source: Healthy seeds of *G. arborea* were obtained from Genetics Division, Tamil Nadu Forest Department (TNFD), Coimbatore, Tamil Nadu.

Pre-treatment of seeds: The seeds of *G. arborea* were pre-treated by soaking in cool water overnight.

Potting medium: Potting medium used in the present study was a mixture of solar sterilized sand: soil: farmyard manure in the ratio 1:2:1. Potting medium was analyzed for its physico-chemical parameters such as pH, Electrical Conductivity (EC), available Nitrogen (N) (Jackson, 1973), available Phosphorus (P) (Jackson, 1973), available Potassium (K) (Sankaram, 1966) and micronutrients such as copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) (Lindsay and Norvell, 1978) following standard procedures. The physico-chemical properties of the potting medium are presented in the (**Table 1**).

Bio-inoculants: The bio-inoculants used in the present study were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. The soil based inoculum of Arbuscular Mycorrhizal (AM) fungi consortium consisted of *Glomus mosseae* and *G. fasciculatum* @ 12-15 spores/gram of soil. Peat soil based inocula of *Azospirillum, Azotobacter* and phosphobacterium consisted of *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megaterium,* respectively, each with a population count of 10^8 bacterial cells/gram of peat soil. The commercial chemical fertilizer, Diammonium Phosphate (DAP) used in the study was procured from the market.

Table 1.Physico-chemical properties of potting medium used in the nursery experiments

Texture	pH	E.C. ($dS\ m^{-1}$)	Macro Nutrients ($kg\ ha^{-1}$)			Micro Nutrients (ppm)			
			N	P	K	Cu	Zn	Fe	Mn
Sandy loam	7.3	0.14	180	19	225	1.8	1.54	10.8	11.08

Species and Treatment structure

Number of Treatments : 13
Number of Replicates/treatment : 3
Number of plants per replicate : 50
Design : CRD

Bio-inoculants and DAP dosage

AM fungi- *Glomus mosseae* + *G. fasciculatum* : 10g/polybag
Azospirillum - A. brasilense : 5g/polybag
Azotobacter - A. chroococcum : 5g/polybag
Phosphate solubilizing bacterium - *(Bacillus megaterium)* : 5g/polybag
Diammonium Phosphate (DAP) : 5g/polybag

Experimental design

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum*
T4 *Azotobacter*
T5 Phosphobacterium
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

Raising and transplantation of seedlings in poly bags: Seeds of *G. arborea* were sown in nursery bed containing solar sterilized sand for raising seedlings. The bio-inoculants/ DAP were applied to polythene bags (10 x 20 cm size) already filled with the potting medium up to 5cm below the top surface as per treatment schedule in completely randomized design by layering inoculation technique. On the bio-inoculants/DAP layer, the potting medium was again filled up to the surface. Seedlings of uniform size from mother bed were immediately transplanted into the bags. The seedlings were maintained under nursery conditions and watered twice daily for further studies.

OBSERVATIONS

Observations were taken at regular interval i.e., 90 and 180 days after inoculation (DAI). The growth data such as shoot height, collar diameter, total dry weight (shoot + root) were recorded after harvesting the seedlings. Growth indices like absolute growth rate (AGR), relative growth rate (RGR), leaf area ratio (LAR) and net assimilation rate (NAR) were subsequently calculated. Further, quality indices like root:shoot ratio, sturdiness

index, volume index and Dickson's quality index were also calculated. Microbial Inoculation Effect and Benefit Cost Ratio were worked out.

STATISTICAL ANALYSIS

All data were subjected to analysis of variance and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) at P=0.05 level using SPSS (Version 10.0, SPSS Inc.) statistical software to determine the effects due to treatments.

RESULTS

Data on the growth parameters of *G. arborea* seedlings inoculated with the selected bio-inoculants *viz*., AM fungi (*Glomus mosseae* + *G. fasciculatum*), *Azospirillum brasilense* (Azo), *Azotobacter chroococcum* (Azoto) and Phosphate solubilizing bacterium (*Bacillus megaterium*) individually and in combinations as well as chemical fertilizer *viz*., Di-ammonium Phosphate (DAP) alone is presented in **Tables 2-4** and **Figs. 1** and **2**.

Shoot height

Data on shoot height of *G. arborea* seedlings inoculated with selected bio-inoculants individually and in combinations and DAP (alone) is presented in **Table 2**. It was observed that the seedlings treated with DAP (alone) followed by combined application of all the bio-inoculants (AMF + Azo + Azoto + PSB) and dual application of AMF + Azo showed significantly greater shoot height at 90 DAI over uninoculated control and other treatments, but among them they were found at par. These were followed by the seedlings treated with dual application of Azoto + PSB and AMF + Azoto, which were at par between them. However, at 180 DAI, all the above five treatments in that order showed significantly greater shoot height, which were found at par among them. In general, all the bio-inoculants-inoculated seedlings had significantly greater shoot height when compared to the uninoculated control seedlings during both ages of the observation.

Collar diameter

Data on the collar diameter of *G. arborea* seedlings inoculated with different bio-inoculants individually and in combinations is presented in **Table 2**. There was a gradual increase in collar diameter with age. The collar diameter was more in all the bio-inoculants and DAP treated plants when compared to the uninoculated control. There was a significant variation among the treatments. The collar diameter was found greater and highly significant in seedlings treated with DAP (alone) followed by combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB) at 90 DAI. Among the other treatments, seedlings with dual inoculations of AMF + Azo, AMF + Azoto, Azoto + PSB and Azo + PSB showed more collar diameter, but all the five treatments were found to be at par. However, at 180 DAI, seedlings treated with dual application of AMF + Azo followed by DAP (alone), combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB) and dual application of AMF + Azoto showed more collar diameter, but were significantly at par among them. Among the other treatments, seedlings with dual inoculation of Azoto + PSB showed higher collar diameter value. At both age levels, the seedlings with individual application of bio-inoculants showed higher collar diameter than dual inoculations, but they had significantly greater values than the uninoculated control seedlings.

Table 2. Effect of bio-inoculants on the shoot height, collar diameter and total dry weights of *G. arborea* seedlings

Treatment	Shoot Length (cm)		Collar diameter (mm)		Total Dry weight (g)	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T1	18.07 a	30.27 a	2.23 a	4.23 a	3.083 a	4.203 a
T2	23.73 b	36.37 b	3.37 b	5.67 bc	5.217 b	7.034 c
T3	22.70 b	35.73 b	3.27 b	5.33 bc	5.184 b	6.900 c
T4	23.27 b	34.07 b	3.17 b	5.23 b	4.767 b	6.433 b
T5	23.53 b	34.80 b	3.27 b	5.40 b	5.193 b	6.933 c
T6	34.27 g	45.60 c	4.70 ef	7.20 f	6.393 c	8.400 de
T7	32.40 f	43.70 c	4.63 ef	6.93 ef	6.290 c	8.300 de
T8	27.77 d	35.93 b	4.27 c	5.97 cd	5.336 b	7.217 c
T9	26.30 c	36.40 b	3.80 c	6.03 cd	4.947 b	7.234 c
T10	29.13 e	36.56 b	4.43 de	6.03 cd	5.313 b	7.234 c
T11	32.90 f	42.13 c	4.63 ef	6.50 de	6.103 c	8.090 d
T12	34.70 g	45.60 c	4.83 f	7.07 f	6.283 c	8.450 de
T13	35.07 g	45.90 c	4.87 f	7.17 f	6.410 c	8.600 e

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI-Days after inoculation

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum*
T4 *Azotobacter*
T5 Phosphobacterium
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

Total dry weight

Data on total dry weight (shoot + root) of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 2**. There was significant variation in total dry weight of seedlings among the treatments. It was observed that the seedlings inoculated with DAP (alone); combination of all the four bio-inoculants (AMF + Azo + Azoto + PSB); dual inoculations of AMF + Azo; AMF + Azoto and Azoto + PSB resulted in higher significant total dry weights at both ages, which were at par among themselves. These were followed by the seedlings treated with dual inoculations of Azo + PSB, Azo + Azoto and AMF + Azoto which showed higher values than seedlings treated with single inoculations. All the seedlings treated with bio-inoculants as well as DAP (alone) showed significantly higher total dry weight values than uninoculated control seedlings.

Growth indices

Absolute Growth Rate (AGR) : Data on the absolute growth rate (AGR) of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 3**. There was significant difference between the control and all other treatments. It was found that seedlings treated with dual inoculation of Azo + Azoto showed highest AGR value followed by DAP (alone) and combined inoculation of all the four bio-inoculants (AMF + Azo + Azoto + PSB). These were followed by dual inoculations of AMF + Azoto, AMF + Azo and Azoto + PSB. Uninoculated control seedlings showed the lowest AGR.

Relative Growth Rate (RGR): Data on the relative growth rate (RGR) of *G. arborea* seedlings inoculated

Table 3. Effect of bio-inoculants on the AGR, RGR, LAR and NAR of *G. arborea* seedlings

Treatment	AGR (mg/g/day)	RGR (mg/g/day)	LAR (cm^2/g)	NAR (mg/cm^2/day)
T1	12.444 a	3.443 ab	9.973 a	0.345 a
T2	20.189 b	3.320 ab	8.712 a	0.381 a
T3	19.067 b	3.177 ab	8.941 a	0.355 a
T4	18.511 b	3.330 ab	8.471 a	0.393 a
T5	19.333 b	3.211 ab	7.526 a	0.427 a
T6	22.300 b	3.034 a	6.580 a	0.461 a
T7	22.333 b	3.081 a	7.735 a	0.398 a
T8	20.900 b	3.355 ab	8.659 a	0.387 a
T9	25.422 b	4.225 b	8.520 a	0.496 a
T10	21.344 b	3.429 ab	7.753 a	0.442 a
T11	22.078 b	3.132 ab	8.507 a	0.368 a
T12	24.078 b	3.292 ab	6.832 a	0.482 a
T13	24.333 b	3.266 ab	7.316 a	0.446 a

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI-Days after inoculation

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum*
T4 *Azotobacter*
T5 Phosphobacterium
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 3**. It was observed that seedlings treated with dual inoculation of Azo + Azoto showed the highest RGR value followed by uninoculated control. There was no significant difference between control and all other treatments.

Leaf Area Ratio (LAR): Data on the leaf area ratio (LAR) of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 3**. It was found that seedlings treated with dual inoculation of AMF + Azo showed the best LAR values followed by combined inoculation of all the four bio-inoculants (AMF + Azo + Azoto + PSB) and DAP (alone). In general all the treatments showed better LAR values than uninoculated control seedlings, though there was no significant difference between them.

Net Assimilation Rate (NAR): Data on the net assimilation rate (NAR) of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 3**. It was observed that seedlings treated with combined inoculation of all the four bio-inoculants (AMF + Azo + Azoto + PSB) showed maximum NAR. This was followed by dual inoculation of AMF + Azo, DAP (alone) and Azo + PSB. In general all the treatments showed better NAR values than uninoculated control seedlings, though there was no significant difference between them.

Quality indices

Root shoot ratio: Data on the root- shoot ratio of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 4**. There was a significant variation among different treatments and control at both the ages studied. It was found that the seedlings inoculated with dual application of bio-inoculants *viz*., Azo + PSB and Azo + Azoto had maximum values at 90 DAI and the seedlings inoculated with PSB at 180 DAI. In general, all the treatments showed higher root shoot ratio values than uninoculated control seedlings at both the ages. Also, the seedlings showed higher root shoot ratio values at180 DAI than at 90 DAI.

Sturdiness quotient: Data on the sturdiness quotient of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 4**. The uninoculated control seedlings showed highest sturdiness quotient values at both the ages of observation. It was observed that there was significant difference between uninoculated control seedlings and other treatments at 90 DAI whereas, the significance levels between the uninoculated control seedlings and other treatments was reduced at 180 DAI. However, the seedlings treated with bio-inoculants/DAP showed lesser and better values when compared with uninoculated seedlings at both the ages. In general, the sturdiness quotient values of the seedlings were improved towards the desired value of <6.00 at 180 DAI than at 90 DAI. Seedlings inoculated with dual combination of AMF + PSB, Azo + Azoto and Azo + PSB showed better sturdiness values at 180 DAI.

Volume index: Data on the volume index of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 4**. When the volume index of the seedlings was calculated, it was observed that a significantly high value was obtained in seedlings treated with DAP (alone) followed by combined inoculation of all four bio-inoculants and dual inoculation of AMF + Azo at 90 DAI, but were at par among them. These were followed by Azoto + PSB and AMF + Azoto, which were at par between them. However, at 180 DAI, seedlings treated with DAP (alone) followed by dual inoculations of AMF + Azo, combined inoculation of all four bio-inoculants, AMF + Azoto showed highly significant volume index values than other treatments, but were found at par among them. These were followed by dual inoculation of Azoto + PSB. It was also found that dual inoculated seedlings showed better values than single inoculated seedlings at both the ages. All the treatments

Table 4. Effect of bio-inoculants on the volume index and quality index of *G. arborea* seedlings

Treatment	Root Shoot ratio		Sturdiness Quotient		Volume Index		Quality Index	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T1	0.939 a	0.964 a	8.102 d	7.155 b	2.022 a	9.776 a	0.160 a	0.301 a
T2	0.949 a	1.029 a	7.042 bc	6.414 ab	5.527 b	20.286 bcd	0.337 b	0.581 bc
T3	0.975 ab	0.971 a	6.942 abc	6.704 ab	5.012 b	17.841 bcd	0.337 b	0.539 bc
T4	0.972 ab	0.990 a	7.340 c	6.514 ab	4.812 b	16.804 b	0.295 b	0.504 b
T5	0.952 a	1.020 a	7.197 c	6.444 ab	5.254 b	18.060 bc	0.323 b	0.557 bc
T6	0.996 ab	1.000 a	7.291 c	6.333 ab	13.387 ef	38.630 f	0.460 d	0.740 e
T7	0.989 ab	0.992 a	6.998 abc	6.306 ab	12.541 e	34.458 ef	0.461 d	0.731 e
T8	1.107 ab	1.058 a	6.503 a	6.019 a	9.693 d	22.097 cd	0.400 c	0.637 cd
T9	1.186 b	1.028 a	6.921 abc	6.035 a	7.429 c	22.808 cd	0.344 b	0.636 cd
T10	1.134 ab	0.973 a	6.576 ab	6.063 a	10.761 d	23.414 d	0.401 c	0.618 cd
T11	0.994 ab	1.092 a	7.106 bc	6.482 ab	12.769 e	29.998 e	0.440 cd	0.683 de
T12	0.976 ab	0.996 a	7.184 c	6.450 ab	14.386 f	37.272 f	0.456 d	0.732 e
T13	0.997 ab	1.015 a	7.201 c	6.402 ab	14.807 f	38.711 f	0.464 d	0.749 e

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI-Days after inoculation.

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum*
T4 *Azotobacter*
T5 Phosphobacterium
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

showed significantly higher values than the untreated control seedlings at both the ages.

Dickson's Quality Index: Data on the quality index of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 4**. It was found that seedlings treated with DAP (alone) showed highest quality index value followed by dual inoculations of AMF + Azoto, AMF + Azo, combined inoculation of all the four bio-inoculants (AMF + Azo + Azoto + PSB) and Azoto + PSB at 90 DAI, but among them they were at par. However, at 180 DAI, it was observed that seedlings treated with DAP (alone) showed highest quality index value followed by dual application of AMF + Azo, combined inoculation of all the four bio-inoculants (AMF + Azo + Azoto + PSB), dual inoculations of AMF + Azoto and Azoto + PSB, but among them they were at par. In general, the seedlings treated with dual inoculations showed better quality index values than the seedlings treated with single inoculations. It was interesting to note that all the treatments with bio-inoculants and DAP (alone) showed significantly higher quality index values than the uninoculated control seedlings at both the ages.

Microbial Inoculation Effect (MIE): Data on microbial inoculation effect (MIE) of *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Fig.1**. It was observed from the study that MIE varied with the treatment. Seedlings inoculated with combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB) showed the highest value of microbial inoculation effect, followed by AMF + Azo, AMF + Azoto and Azoto + PSB inoculated seedlings when compared to all other treatments. In general, the seedlings treated with dual inoculations of bio-inoculants showed higher values when compared to single inoculations at both the ages of observation.

Benefit cost ratio (B C ratio): Data on the benefit cost ratio for *G. arborea* seedlings inoculated with different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Fig. 2**. It was observed from the study that the BC ratio varied with the treatment. Seedlings inoculated with DAP showed the highest BC ratio followed by dual inoculation of Azoto + PSB. This was followed by dual inoculation of AMF + Azo, AMF + Azoto and then by combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB). In general, the seedlings treated with bio-inoculants showed higher BC ratios when compared to un-inoculated control seedlings.

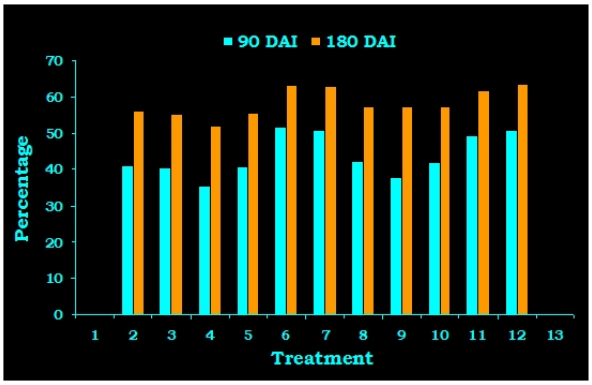

Fig.1. Microbial inoculation effect (MIE) of bio-inoculants on *G. arborea* seedlings

DISCUSSION

Plant growth response: The shoot height, collar diameter and total dry weight were higher in *G. arboea* seedlings inoculated with AM fungi and PGPRs than uninoculated control (**Table 2**). The present findings were quite in agreement with the results reported by earlier researchers on different indigenous tree species. Kandasamy *et al.* (1987) while working with *Ailanthus excelsa* and two other species reported that inoculation with AM fungus, *Glomus fasciculatum* increased the shoot and root dry weights *(*62.08% and 43.18%, respectively). Balasubramanian and Srinivasan (1995) have also reported that inoculation with four AM fungi significantly increased the total biomass, leaf area and total chlorophyll of *Ailanthus excelsa, Tectona grandis* and *Dalbergia sissoo* seedlings. Madan *et al*. (1995) reported that AM fungal inoculated *Ailanthus excelsa, Pongamia glabra* and *Cassia siamea* seedlings showed

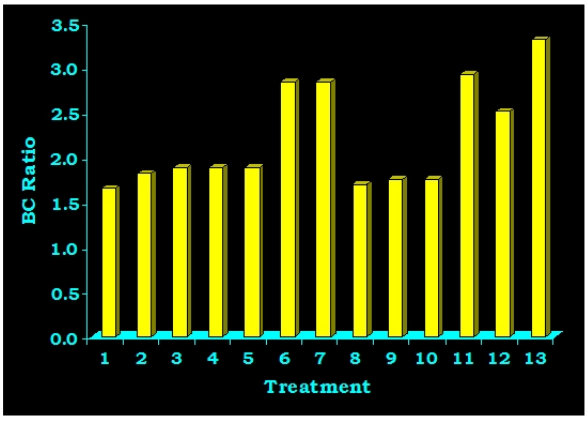

Fig. 2. Benefit cost ratio of bio-inoculants on *G. arborea* seedlings

Better shoot height, shoot and root dry weight in highly alkaline-saline soils. In the subsequent years, Rahangdale and Gupta (1998) reported that all the twelve AM fungi have contributed to significant increase in shoot height, root and shoot biomass in all the six tree species tested including *Gmelina arborea.*

Talukdar and Thakuria (2000) also reported that AM fungi inoculated *G. arborea* and *Tectona grandis* with organic amendments gave better yield in terms of shoot and root biomass in degraded soils. Chandra and Ujjaini (2002) reported that when *Gmelina arborea* and four other species were inoculated with AM fungi + organic matter, the plant biomass increased up to 36.85%. Barua *et al*. (2010) have found that length of shoot and root, collar diameter, fresh and dry weight of shoot and root were improved in AM fungi inoculated *G. arborea* plants. They also observed that the mycorrhizal seedlings had as much as 40% higher increment in total growth and 2.4 times higher increment in biomass compared to non-

mycorrhizal seedlings. The significant effect of AM fungi and PGPR on different growth parameters of *G. arborea* seedlings in the current study may be due to the increased production of auxins and gibberellins by the selected bio-inoculants of PGPR and AM fungi. The findings are also in conformity with the studies conducted by De La Cruz *et al*. (1988) and Dubey *et al*. (1997). The increase in seedling biomass production may be strongly correlated with improved accumulation of N due to *Azospirillum* and *Azotobacter* and P due to AM fungi and PSB inoculation (Ratha Krishnan *et al*., 2004).

In general, seedlings inoculated with combination of all bio-inoculants showed maximum values for the growth parameters at both 90 DAI and 180 DAI in *G. arborea* plants. Further, among the treatments involving bio-inoculants, dual combinations, especially the ones involving a Nitrogen fixer and a Phosphorus solubilizer/ mobilizer were found to be better than single inoculations. These findings are in accordance with the findings of Seema *et al*. (2000) who reported that AM fungi and *Azotobacter chroococcum* inoculation on *Gmelina arborea, Bambusa arundinacea, Tectona grandis* and *Dalbergia sissoo* resulted in improved growth and biomass. Zambrano and Diaz (2008) have also reported a positive correlation between mycorrhization and plant height and a synergic effect between AM fungi, *Glomus* spp. and *Azospirillum brasilense* on *Gmelina arborea* with two potting media.

The effect of bio-inoculants on the growth performance (shoot height, collar diameter and total dry weight) varied with the treatment and the host plant species. Balasubramanian and Srinivasan (1995) also reported that different AM fungi responded differently to different tree species with *Gigaspora margarita* on *Tectona, Glomus mosseae* on *Dalbergia* and *G. monosporum* on *Ailanthus* showing maximum beneficial effect. Rahangdale and Gupta (1998) reported that twelve different AM fungi produced different growth response on six tree species tested. Seema *et al*. (2000) also reported variations among treatments and species while working with AM fungi and *Azotobacter chroococcum* and three tree species. The greater increase in shoot height, collar diameter and total dry weight in treatment with combination of all the plant growth promoting microbes and dual inoculations involving AM fungi/PSB a

nd Azo/Azoto, in *G. arborea* plants may be due to improved accumulation of both N by *Azospirillum* and *Azotobacter* (Wong and Stenberg, 1979) and P by AM fungi and PSB inoculations (Habte and Manjunath, 1987; Saravanan, 1991).

Growth indices: Aggregate Growth Rate (AGR) and Net Assimilation Rate (NAR) were higher in *G. arborea* seedlings inoculated with bio-inoculants than uninoculated control. No significant difference was recorded in case of Relative Growth Rate (RGR). However, Leaf Area Ratio (LAR) was found to be lower in inoculated seedlings of *G. arborea,* which indicates that lesser leaf area in inoculated seedlings is able to produce more dry matter. These findings are in accordance with the findings of earlier researchers on other tree species. Huante *et al*. (1993) reported that AM fungal inoculation resulted in increased biomass production and RGR in *Caesalpinia eriostachys, Cordia alliodora* and *Pithecellobium mangense.* Lovelock *et al*. (1996) reported that inoculation with AM fungi increased RGR of the tropical tree, *Beilschmiedia pendula* seedlings. Shishido and Chanway (2000) reported that hybrid spruce (*Picea glauca* × *Picea engelmannii*) seedlings pre-inoculated with PGPR (*Pseudomonas* sp. and *Bacillus* sp.) exhibited significantly higher shoot and root RGRs (increased by 10-234%) four months after outplanting, at all the four sites tested. Ratha Krishnan *et al.* (2004) reported that inoculation of *Azospirillum, Azotobacter, PSB* and AM fungi on *Simarouba glauca* seedlings resulted in increased RGR. Recently, Zapata *et al.* (2009) reported that AM fungi increased RGR of *Desmoncus orthacanthos* (*Arecaceae*) seedlings at low P level.

Increase in growth rates of different bio-inoculants applied plants may be due to alteration of morphology (more leaf area) and physiology (improved nutrient accumulation and photosynthesis) of the seedlings. NAR and LAR are correlated with RGR of plants. The higher the NAR the more efficient the species, which usually translates into higher growth rates (Larcher, 2003). Inoculation with AM fungi and PGPR increased both total plant growth, as measured by increased AGR, and the efficiency of growth, as measured by increased NAR. This increased growth efficiency allowed the plant to have a smaller shoot system (decreased LAR), which is the source, while still enhancing the size of the root system which is a sink (Leopold and Kriedemann, 1975).

Quality indices: The root: shoot ratio is an important measure for seedling survival. It relates the water absorbing area (roots) to the transpiring area (shoot). A good ratio- one which indicates a healthy plant is 1:1 to 2:1 root:shoot mass (Jaenicke, 1999). No significant difference was found between treated and untreated seedlings of *Gmelina arborea.* A less rigorous, but non-destructive, index is the 'sturdiness quotient' which compares height (in cm) over root collar diameter (in mm). A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. A sturdiness quotient higher than 6 is undesirable (Jaenicke, 1999; Gregorio *et al*., 2007). The seedlings of *G. arborea* showed sturdiness quotient values of >6.0 which indicates that these seedlings were basically lanky.

The volume index and the quality index were higher in *G. arborea* seedlings inoculated with different bio-inoculants (AM fungi and PGPRs) than uninoculated control. These findings are in accordance with the findings of earlier researchers on native and related tree species like *Azadirachta indica* (Sumana and Bagyaraj, 2002), *Tectona grandis* (Ayswarya, 2008; Rajeshkumar *et al*., 2009) *Melia azedirach (*Rajeshkumar *et al*., 2009) as well as exotic ones like *Eucalyptus hybrid* (Sastry *et al.,* 2000), *Simarouba glauca* (Ratha Krishnan *et al.,* 2004) and *Acacia auriculiformis* and *A. mangium* (Tamilselvi,

2005). Sumana and Bagyaraj (2002) also reported that dual inoculation of *Glomus mosseae* and *Azotobacter chroococcum*, resulted in maximum volume index and quality index of neem seedlings while Rajeshkumar *et al*. (2009) reported that triple inoculation of *G. geosporum, A. chroococcum,* and *Bacillus coagulans* resulted in maximum volume index and quality index of *Melia azedarach* seedlings.

Microbial Inoculation Effect (MIE): It was observed from the study that the Microbial Inoculation Effect (MIE) varied with the treatment and the seedlings inoculated with combined application of all four bio-inoculants (AMF + Azo + Azoto + PSB) showed the highest value of MIE, followed by dual inoculations when compared to all other treatments in all the four tree species tested (**Fig. 1**). This indicates that these organisms act synergistically when inoculated in combinations. Similar findings were reported by Muthukumar *et al*. (2001) in neem.

BC ratio: In the present study, it was observed that the bio-inoculants treated plants especially, combined application of all bio-inoculants and dual inoculations involving N fixer and P solubilizer/ mobilizer having good quality index and attaining good height and collar diameter gave better benefit cost ratio than other. In terms of benefit cost analyses of the bio-inoculants, it is well known that the beneficial effect of the bio-inoculants is multifaceted and does not result solely from improved nutrient uptake. Thus it may not be appropriate to compare the cost of inoculation directly with the cost of chemical fertilizer additives that achieve equivalent growth response in target plants. Indeed, the benefits of chemical soil additives are usually short lived, unless slow release formulations are used, and hence are not sustainable in low input or natural ecosystems. If the bare economics of the relative costs of chemical fertilizers versus bio-inoculants are compared, the latter will be little more costly in the short term. However, in terms of overall plant health and sustainability, the benefits of establishing an effective AM symbiosis and PGPR are much wider and more long lasting.

In the present study, combined application of all bio-inoculants was found better in plant growth response, growth indice, quality indice, microbial inoculation effect and BC ratio of *Gmelina arborea* plants. It was also recorded that dual application of bio-inoculants was found to be better than single inoculations indicating the synergistic effect. Further, dual combinations involving a N fixer and a P solubilizer/ mobilizer were found to be highly effective. Application of plant growth promoting microbes as bio-inoculants will not only give desired benefits in terms of good seedling quality and out planting performance in the field, but also ensure the maintenance of soil health which is essential for sustainable and eco-friendly forestry. Use of superior AM fungi and PGPR bio-inoculants in seedling and transplanting stage may be an excellent solution to strengthen seedlings to withstand dry land conditions and support the Trees Outside Forest (TOF) efforts, thereby promoting farmers, tree growers and wood based entrepreneurs of the country.

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Diversity of fungal endophyte communities in lemon grass (*Cymbopogon* spp.)growing in areas of Central Western Ghats, Karnataka, India

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ABSTRACT

***Cymbopogon* grass produces many bioactive compounds, which are used for various medicinal purposes. In this study, endophytic fungi were isolated from 2000 samples of *Cymbopogon* grass species collected from different regions of Central Western Ghats, Karnataka. Healthy leaf and root segments of *Cymbopogon citratus, C. flexuosus and C. caesius* were cultured on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) medium. From surface sterilized tissues, overall 65.9 per cent colonization rate of fungi was observed. A sum of 30 different fungal species were isolated, in these 19 were identified up to species level and remaining 11 were considered as morpho species. These morpho species isolates were identified by sequencing ITS-rDNA gene. Leaf segments yield a lower number of isolates when compared with root segments. *Curvularia* sp. and *Fusarium* sp. were frequently isolated endophytic fungi with the high colonization rate.**

Key words: *Cymbopogon,* grass, ITS-rDNA gene, kemmannugundi

INTRODUCTION

The Western Ghats or Sahyadri hills are well known for their rich diversity of flora and fauna. There are diversity of vegetation types like evergreen forests, semi evergreen forest, deciduous forests, scrubby forests, and grasslands (Champion and Seth, 1968). *Cymbopogon* grass is an aromatic perennial tall grass of the plant family *Poaceae* growing for its aromatic volatile oil. They also grow naturally in high altitude areas. This grass is a fast growing scented perennial grass reaching a height of 1-2 meters with green leathery leaves. There are nine species of *Cymbopogon* grass which occur in Karnataka (Gamble, 1915-1936). *C. citratus* is available only under cultivation for its aromatic volatile oil. Other species like *C. flexuosus, C. caesius* and *C. martini* are found in natural habitat along the Western Ghats of Karnataka. *Cymbopogon* grasses grow luxuriantly in the iron rich soil. The volatile oil extracted from these species is commonly used in cosmetics, pharmaceutical industries, flavouring agents and in perfumery products.

Endophytes are microorganisms, which live inside the host without causing any negative effect by their presence (Petrini, 1986). Recent researches have revealed the mutualistic association between the grasses and the fungal endophytes (Suryanarayanan *et al*., 1998; Arnold *el al*., 2000; Schulz and Boyle, 2006 and Rodriguez *et al.,* 2009). These endophytes affect the physiology, ecology and reproductive biology of the host plants (Clay and Schardle, 2002). The fungal endophytes of tropical plants are among the groups of fungi that have been studied to attain at the predicted figure of 1.5 million fungal biodiversity (Hawksworth, 1991).Plant protects and feeds the endophyte, which in return produces bioactive secondary metabolites to enhance the growth and competitiveness of the host in nature (Carroll, 1988; Lu *et al.*, 2000). Endophytes are widely investigated for their bioactive metabolites and potential compounds having therapeutic uses (Clovis and Langer, 2012).

We examined the different culturable endophytic fungi in lemon grass collected from Kemmannugundi, Kudremukh and Sringeri regions of Central Western Ghats, Southern India. Despite the wide distribution of *Cymbopogon* grass along Central Western Ghats of Karnataka, only limited work has been done for their association with fungal endophytes. Even though oil extracted from the grass is used in medicine, presently there is no adequate information regarding diversity of fungal endophytes with the species. Hence, we have selected *Cymbopogon caesius, C.citratus* and *C. flexuosus* for exploring diversity of the fungal endophytes by employing classical and molecular sequencing methods.

MATERIALS AND METHODS

Sampling: *Cymbopogon* grasses are growing abundantly along the hill ranges of Kemmannugundi, Kudremukh, Sringeri, Kigga and Hariharapura in Central Western Ghats, Karnataka. *C. citratus* was collected from Sringeri whereas *C. flexuosus* from Kudremukh and Kemmannugundi and *C. Caesius* was collected from Hariharapura and Kigga regions of Chikkamagaluru District (**Fig. 1**). Sampling was performed for a period of six months, representing two seasons viz., summer and monsoon. The samples were collected from healthy growing culms with the help of disinfected knife and placed in separate zip lock polythene bags. To avoid damage, roots were collected with a ball of soil. The

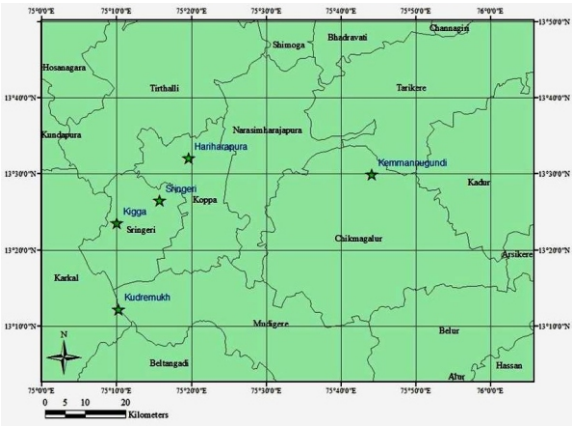

Fig. 1: Location of sampling sites in Central Western Ghats.

samples were brought to the laboratory placing inside the ice box. The samples were washed thoroughly in running tap water within 24 hours of collection, root, stem and leaf samples were processed for isolating fungal endophytes.

Isolation of fungi: For the isolation of fungal endophytes, the washed plant materials were separated into root, stem, and leaves. They were surface sterilized by immersing the samples in 70 per cent Ethyl alcohol for 2 min followed by immersion in 3 per cent Sodium hypochlorite for 1 minute and again washed twice with distilled water (Suryanarayanan *et al*., 1998). The excess water content was removed by placing inside the laminar airflow. The efficacy of surface sterilization was confirmed by inoculating surface sterilized water collected from last wash of the sample onto a nutrient medium. Surface sterilized small pieces were cut into 0.3-0.5cm segments. Two hundred segments of each sample (root and leaf) from all the collection sites were inoculated on Potato Dextrose Agar (PDA) medium (PDA, Hi Media Laboratories, Mumbai, India) and in MEA medium by gently pressing on it which is supplemented with Amoxillin (250 mgL-1) to suppress the growth of bacteria. Inoculated Petri plates were wrapped with Petriseal and incubated at laboratory temperature. After three days of inoculation, the plates were observed daily for growth of fungi from cultured segments up to one month. The fungi that were grown very fast was discarded and slow growing were retained for further study. The emerged fungal endophytes from cut ends were transferred to new Petri plates containing PDA medium for further work.

Identification of endophytic fungi: For the identification of endophytic fungi, slides were prepared from pure cultures and were stained with Lacto phenol cotton blue stain and observed under 40x and 100x magnification under Karl Zeiss Primo star® microscope. Morphological characteristics such as growth pattern, colour of colony, mycelium texture, spore production type and characters of the spore were observed and identified using standard taxonomic keys (Subramanian, 1983; Barnett and Hunter, 1998; Ellis, 1976). Most of the endophytes isolated were sterile forms (Morpho species) and were identified by the molecular tools by sequencing ITS-rDNA gene.

Fungal DNA extraction, Amplification and Sequencing: Sequencing of the morpho species has been done and some morphologically identified fungal species carried out to identify the fungal species. The genomic DNA was extracted from fresh purified mycelia using standard Qiagen® DNA isolation plant mini kit according to manufacturer's protocol. PCR of the isolated DNA was carried out using Eppendorf Nexus gradient PCR machine. The ITS was amplified using ITS1F and ITS4R primers (White *et al*., 1990). Amplification was carried out in 25 µl reactions containing 2.5 µl of 10× PCR buffer, 16.8 µl of PCR H_2O, 1 µl of 200mMdNTP, 0.2 µl of Taq polymerase,1 µl of each primer and 2.5 µl fungal DNA. The thermal cycling process is as follows: 5 min Initial denaturation at 96ºC followed by 30 cycles of 1 min denaturation at 95ºC, 1 min primer annealing at 58ºC, 1 min extension at 72ºC, with a final elongation for 10 min at 72ºC. The PCR products were sequenced at Chromos Biotech Pvt Ltd. Bangalore by next gen sequencing method. The sequenced data were observed with the NCBI database and the fungal species were identified.

Statistical analysis: The ratio of the total number of segments yielding endophytes and the total number of segments inoculated were used to calculate the colonisation rate (Petrini *et al*., 1982).

$$\text{Colonization Rate} = \frac{\text{Number of fragments with endophytes}}{\text{Total number of fragments analysed}} \times 100$$

The diversity indices were calculated using methods from standard literature (Simpson, 1949; Shannon and Weaner, 1963).

RESULTS

Out of 2000 segments of grasses, 1318 segments yield 30 fungal endophytes from their cut ends**.** Microphotographs of some of these are shown in **Fig. 5.** Most frequently found endophytes were *Fusarium oxysporum* (8.25%), *Curvularia lunata* (7.95%) and *Aspergillus clavatus* (6.55%) as is evident from the data in **Table 1**. Many of the isolated endophytes belongs to the group *Hyphomycetes, Basidiomycota*, *Ascomycota* and sterile morpho species.

The root segments of *Cymbopogon* grass showed more number of isolates of endophytes (88.7%) as compared to leaf segments (65.9%) (**Table 2**). In our study, eleven unidentified fungi were numbered, categorized and stored as morpho species. The colonization frequency (%) of endophytic fungi differed significantly between leaves and roots. Maximum colonization rate was observed in roots when compared to leaves. Molecular sequencing of some fungal cultures was done to identify sterile fungi and the result showed 11 different forms of fungi with over 98 per cent identity by NCBI BLAST search (**Table 3**). Among 11 fungal endophytes, 07 were identified up to species level and two up to genus level. Most of the endophytes belong to *Ascomycota* group (86%) and only few belongs to *Basidiomycota* (3%). Even though some are not identified and were kept as morpho species (11%) (**Fig. 2**).

Fungal endophytes recovered from grass samples of all the study sites showed almost similar diversity with a slight

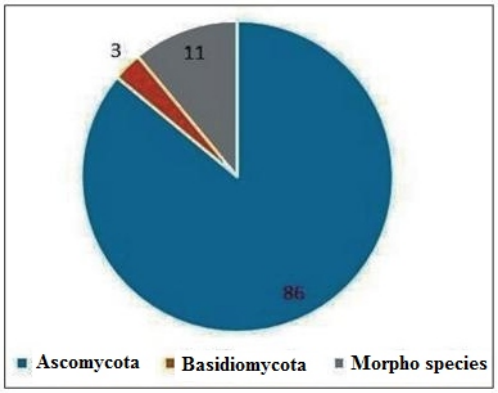

Fig. 2. Per cent of different groups of fungi isolated from lemon grass collected from Central Western Ghats, Karnataka.

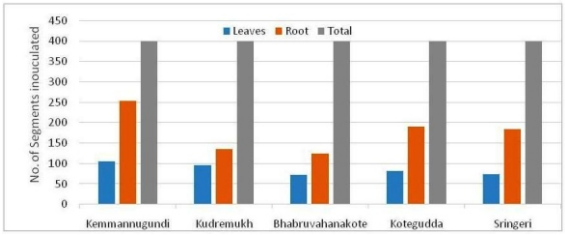

Fig. 3 Showing inoculation ratio between root and leaves of *Cymbopogon* species in different study sites.

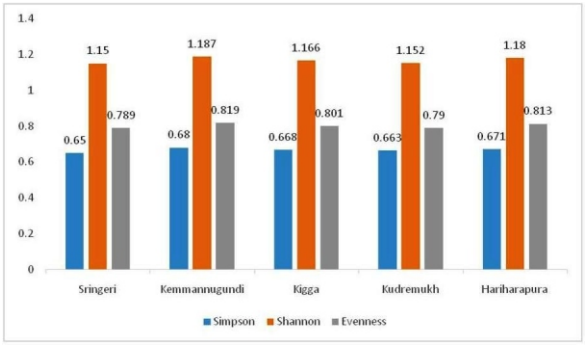

Fig. 4 Showing diversity indices of fungal endophytes isolated from *Cymbopogon* grass species

difference in diversity indices. Shannon diversity of Kemmannugundi and Hariharapura showed almost similar value (1.8 and 1.87, respectively). Simpson diversity index of Sringeri has the value of 0.65, which indicates diversity of fungal endophytes is more when compared to other study sites (**Fig. 4**). The fungal endophytes were evenly distributed among all sampling sites.

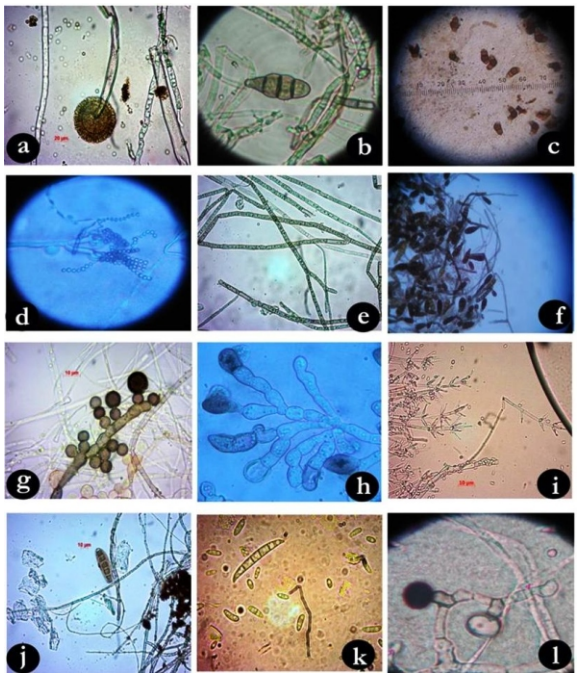

Fig. 5: a. *Aspergillus niger*, b. *Alternaria alternata*, c. *Curvularia lunata*, d. *Penicillium chrysogenum*, e. Non sporulating Fungi, f. *Chetomium* sp., G. *Periconia byssoides*, h. *Wardomyces* sp., i. *Verticillium* sp., J. *Bipolaris* sp. k. *Fusarium oxysporum* spores, l. *Nigrospora oryzae*

DISCUSSION

The grass community provide good host for endophytes. Most of the endophytes isolated from grass were sterile form. Mycelia sterilia (morpho species) was isolated as endophytes in a wide range of plants (Arnold *et al*., 2000;

Table 1. Colonization rate (CR) % of endophytic fungi in different parts of lemon grass collected from some areas of Central Western Ghats, Karnataka.

Sr. No.	Endophyte Name	Colonisation frequency (%)		
		Root	Leaf	Total
1.	*Alternaria alternata*	4.1	-	2.05
2.	*Aspergillus clavatus*	8.3	4.8	6.55
3.	*Aspergillus flavus*	5.6	6.9	6.25
4.	*Aspergillus niger*	8.7	4.1	6.4
5.	*Bipolaris incurvata*	2	-	1.00
6.	*Cephalosporium* sp.	0.8	-	0.4
7.	*Cladosporium herbarum*	0.6	0.2	0.4
8.	*Curvularia andropogonis*	11	1.7	6.35
9.	*Curvularia lunata*	12.2	3.7	7.95
10.	*Curvularia tritici*	6.9	2.3	4.6
11.	*Fusarium oxysporum*	10.1	6.4	8.25
12.	*Hansfordia ovalispora*	0.9	-	0.3
13.	*Oidiodendron* sp.	1.6	-	0.8
14.	*Oidium* sp.	0.9	0.4	0.65
15.	*Phoma exigua*	2.1	-	1.05
16.	*Trichoderma viride*	0.4	-	0.2
17.	*Verticillium* sp.	0.6	-	0.3
18.	*Wardomyces anomala*	0.6	-	0.3

Table 2. Infection frequency and isolation rate of fungal endophytes of *Cymbopogon* species

	***Cymbopogon* grass tissues**		
	Leaves	Root	Total
Number of samples	1000	1000	2000
Number of isolates recovered	431	887	1318
Colonisation rate (%)	43.1	88.7	65.9
Number of morpho species	4	7	11

Naik *et al*., 2008). The plant-associated habitat is a dynamic environment in which many factors affect structure and species composition of microbial communities that colonize roots, stems, branches and leaves (Rubini *et al*., 2005). Endophytic fungi are one of the most unexplored and diverse group of organisms having symbiotic associations with higher plants which are reported to produce beneficial substances for the host (Weber, 1981). In this study, 30 species of endophytes harboured among three different parts of *Cymbopogon* spp. and higher number of endophytes were existent in the roots. Twenty-four different fungal species belonging to 21 genera were reported from the leaves and rhizome of *C. citratus* in an earlier study (Deshmukh *et al*., 2010) which was lower than endophytes isolated in the present study. Colonization rate is reported to increase with the age of the leaves. The dominant fungal species like *Alternaria alternata*, *Aspergillus clavatus, Curvularia lunata* and *Fusarium oxysporum* have been repeatedly reported as endophytes from medicinal plants surveyed from Malnad regions (Naik *et al*., 2014). Shekhawat *et al*., (2010) found that *Hyphomycetes* group largely occurs in all plants which provide protection against a number of pathogens. Suryanarayanan *et al*., (1998; 2000) reported that *Aspergillus niger, Nigrospora sphaerica* and *Colletotrichum* sp. belongs to endophytic fungi group.

Table 3. Sequenced details of fungal endophytes by ITS rDNA gene data with Genbank accession number.

Isolate code	Plant tissue	Endophytes	NCBI accession number	Query coverage (%)	Max. Identity (%)
BLRL19011307	Leaf	*Alternaria alternata*	KP666172	100	98
CDCMR4	Root	*Aspergillus flavus*	KP340003	100	99
BLRL01	Leaf	*Clonostachys pseudochroleuca*	KP666173	99	99
CDCML4	Leaf	*Daldenia eschscholzii*	KP666174	100	95
CDCMR9	Root	Fungal endophyte	KP666175	99	95
BLRL19011312	Leaf	*Fusarium proliferatum*	KP666176	99	99
CDCMR7	Root	*Nigrospora oryzae*	KP666177	99	93
BLRS19011304	Root	*Periconia byssoides*	KP666178	99	99
CDCML2	Leaf	*Phomopsis longicolla*	KP666179	99	97
BLRR19011319	Root	*Trichoderma harzianum*	KP666180	99	99
CDCMR2	Root	Uncultured fungus	KP666181	93	90
CDCML1	Leaf	*Xylaria* sp.	KP666182	99	99

Many endophytic genera described in other grasses such as *Phragmites australis* (Wirsel *et al*., 2000) were also present in *Cymbopogon* grass. Similar study on *Panicum virgatum* (Ghimire *et al*., 2011) documented almost three times more endophytes in roots compared to shoot tissues. Higher colonization rate (CR) of endophytes was reported in leaf and stem tissues, as compared to roots (Siegel and Latch,1991; Clay and Schardle, 2002; Gautam *et al*., 2013) in some grasses. In the study of dicot medicinal plant species, leaves are reported to colonise greater number of endophytes when compared to stem and bark which is contrary to our results (Raviraja, 2005). The variation in the CR depends on the host habitat and soil environment (Naik *et al*., 2014). In this study, the CR varied in different parts of the plant. We came across the high colonization rate from the plants collected from Kemmannugundi region (**Fig. 3**). Therefore, survey for fungal endophytes at different seasons may help in recovery of more species.

CONCLUSION

Present study revealed the diversity of endophytic fungi present in three *Cymbopogon* grass species collected from different parts of Central Western Ghats. We collected the samples during different seasons and came to a conclusion that population of endophytes within host plants varies with season and other environmental factors.

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New and interesting *Hyphomycetes* from North-Western Himalayas

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ABSTRACT

During a routine investigation for hyphomycetes diversity growing on bark of trees of Chandigarh and adjoining areas, thirteen fungi belonging to eleven genera were recovered. These fungi were collected on Parwanoo- Solan highway, Himachal Pradesh. Of the thirteen fungi, three taxa viz: *Hermatomyces tucumanensis*, *Monodictys putredinis* and *Monodictys castaneae* are reported for the first time from North-Western Himalayas. *Moorella speciosa* is a new record for Himachal Pradesh. Whereas the remaining nine taxa: *Curvularia lunata*, *Dictyoarthrinium sacchari*, *Lacellina graminicola*, *Stachybotrys echinata, S. levispora, Myrothecium roridum*, *Periconia byssoides, Spegazzinia tessarthra* and *Stachylidium bicolor* constitute new host records.

Keywords: Anamorphic fungi, host records, Himachal Pradesh

INTRODUCTION

This work is in continuation with our ongoing studies of anamorphic fungi inhabiting the bark of trees of Chandigarh and adjoining areas (Prasher and Kaur 2014; Prasher and Singh 2012; 2013; 2014a; 2014b; 2015; Prasher and Sushma 2014; Prasher and Verma 2012a; 2012b; 2015a; 2015b). During an investigation of anamorphic mycoflora of Parwanoo-Solan region, bark of various trees were collected. Thirteen hyphomycetes were obtained, four of them are reported for the first time from North-Western Himalayas and one species constitutes new record for Himachal Pradesh. Short descriptions, illustrations and remarks about the host and geographical distribution in North-Western Himalayas, especially in Himachal Pradesh are provided for each taxon.

MATERIALS AND METHODS

Bark of different tree species were collected in ziplock plastic bags and taken to the laboratory. The specimens were mounted in 4% KOH, Lactophenol and 0.01 % Cotton blue in lactophenol (Kirk *et al.,* 2008). The specimens were studied microscopically under Matrix stereo trinocular microscope (VL-Z60) and transmission microscope (VRS-2*f*) for macroscopic and microscopic characters. All the measurements were taken with the help of Pro MED software. The specimens were deposited in the herbarium of Botany Department, Panjab University, Chandigarh, India (PAN).

TAXONOMY

Hermatomyces tucumanensis Speg. *Anal. Mus. nac. B. Aires, Ser*. **3 13**: 446 (1911) **Fig. 1C**

Colonies effuse, dark brown, velvety. Conidiophores closely packed together, short, pale brown, smooth, 10-12 × 2-3 µm. Conidia solitary acrogenous, lenticular, elliptical to almost round in one plane, smooth, muriform, with pale peripheral cells surrounding central brown to black cells, 30-40 × 20-28 µm, 13.5-15 µm thick.

Collection examined: Gargi Singh, 30740 (PAN), on bark of *Mangifera indica*, Solan, May 14, 2014.

Remarks: This species has been previously recorded on dead twigs of *Acacia pinnata*, Amboli, Maharashtra (Patil and Thite, 1978). This is the first report of its occurrence in the North-Western Himalayas.

Lacellina graminicola (Berk. & Broome) Petch. *Ann. R. bot. Gdns Peradeniya* **9**: 171 (1924) **Fig. 2 (A-B)**

Setae usually many in each colony but formed singly or in groups of 5-7 on each stroma, nearly black below the middle, paler above, 275-1100 µm long, 10-18 µm thick at the base, tapering to 4-6 µm near the apex. Conidiophores 40-110 µm long, 3-5 µm thick. Conidia spherical, brown, 4-7.5 µm diam, some what flattened.

Collection examined: Gargi Singh, 30741 (PAN), on bark of *Pinus wallichiana*, Solan, May 14, 2014.

Remarks: The genus is characterised by the presence of setae with subhyaline to pale brown, verrucose conidiophores surrounding the bases of setae; conidia catenate, simple, somewhat flattened, smooth to verrucose (Ellis, 1971). This is the first report of this species from Solan district, Himachal Pradesh and constitutes a new host record.

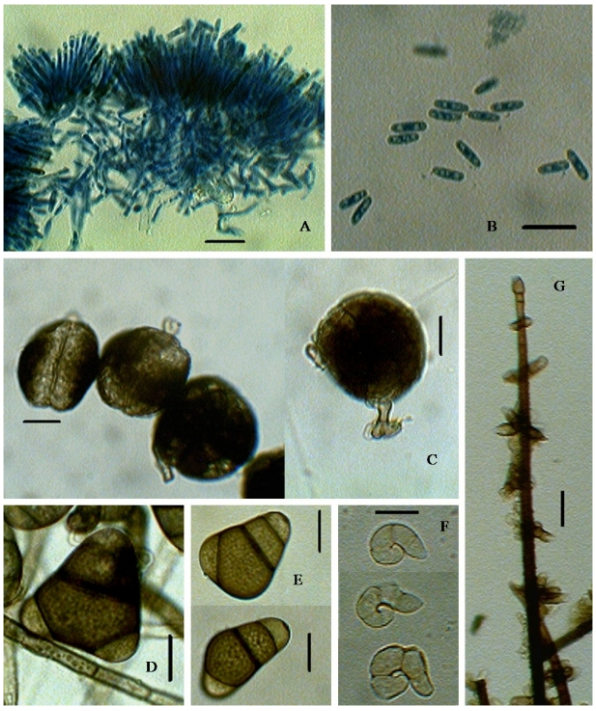

Fig. 1: A, B. *Myrothecium roridum* A. Section of sporodochia showing conidiogenous cells B. Conidia C. *Hermatomyces tucumanensis* C. Conidia D, E. *Curvularia lunata* D-E. Variously shaped conidia F, G. *Moorella speciosa* F. Conidia G. Conidiophore. Bars A-F=10 µm, G=20 µm.

Myrothecium roridum Tode. *Fung. mecklenb. sel. (Lüneburg)* **1**: 25 (1790) **Fig. 1 (A-B)**

Sporodochia sessile, up to 1.5 mm. diam., often confluent, at first green, later black with a white margin, without setae. Mycellium immersed. Conidiophores macronematous, mononematous, closely packed together to form sporodochia, branched, with the branches apical and arranged penicillately, straight, hyaline. Conidiogenous cells monophialidic, descrete, cylindrical. Phialides 10-12 × 1-2 µm. Conidia cylindrical with rounded ends, colourless to pale olive, green to black in mass, mostly 5-7 × 1.5-2 µm.

Collection examined: Gargi Singh, 30743 (PAN), on bark of *Azadirachta indica*, Solan, May 14, 2014.

Remarks: This species has been reported from Himachal Pradesh on leaves and roots of *Antirrhinum majus*, Chambaghat, Solan, on leaves of *Humulus lupulus*, Solan and on leaves of *Morus alba*, Paonta Sahib (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004). Since this species has never been collected on *Azadirachta indica*, it is therefore, a new host record for the fungus.

Moorella speciosa P.Rag. Rao & D. Rao. *Mycopath. Mycol. appl.* **22**: 52 (1964) **Fig. 1(F-G)**

Colonies effuse, dark brown to black, velvety. Conidiophores erect, straight or slightly flexuous, septate, dark brown, smooth, up to 350 µm long, 8-10 µm thick at the base, 5-8 µm at the apex. Conidiogenous cells polyblastic, integrated and terminal, denticulate. Conidia solitary, dry, simple, helicoid, 1-1.5 times coiled, 4-7 septate, hyaline, smooth, 10-16 µm diam.

Collection examined: Gargi Singh, 30742 (PAN), on bark of *Mangifera indica*, Solan, May 14, 2014.

Remarks: This is the first report of *Moorella speciosa* from Himachal Pradesh. It has been previously reported on bark of *Eucalyptus globosus*, Jabalpur, Madhya Pradesh (Sharma, 1979); bark of unidentified trunk, Nizamabad, Andhra Pradesh (Rao, 1964) and on wood of unidentified angiospermous tree, Dehra Dun (Prasher and Kaur 2014).

Curvularia lunata (Wakker) Boedijn. *Bull. Jard. bot. Buitenz*, 3 Sér. **13**(1): 127 (1933) **Fig. 1(D-E)**

Colonies effuse, brown, hairy. Mycelium immersed in natural substrata. Stromata absent. Conidiophores up to 6.2 µm wide, macronematous, mononematous, straight or flexuous, often geniculate, brown, smooth. Conidiogenous cells polytretic, integrated, terminal, sometimes later becoming intercalary, sympodial, cylindrical. Conidia 17.2-33.1 × 9.3-24.8 µm, solitary, acropleurogenous, simple, often curved, clavate, broadly fusiform, obovoid or pyriform with 3 and rarely four transverse septa, pale or dark brown, often with some cells, usually the end ones, paler than the others, sometimes with dark bands at the septa, smooth.

Collection Examined: Gargi Singh, 30744 (PAN), on bark of *Azadirachta indica*, Solan, May 14, 2014.

Remarks: There have been many reports of this species from India occurring on variety of hosts. This species has been reported from Himachal Pradesh on seeds of *Dolichos biflorus*, Solan and Kangra; on *Corchorus* sp., Palampur; on seeds of *Cichorium intybus*, Solan; on seeds of *Abelmoschus esculentus* (Bilgrami *et al*., 1991, Jamaluddin *et al*., 2004). Reported here on *Azadirachta indica,* it constitutes a new host record.

Dictyoarthrinium sacchari (J.A. Stev.) Damon. *Bull. Torrey bot. Club* **80**: 164 (1953) **Fig. 2(C-D)**

Colonies black, up to 1 mm diam. Conidiophores straight or flexuous, cylindrical, pale brown except for the thick brown or dark brown transverse septa, smooth to minutely verrucose, up to 170 µm long, 4-5.5 µm thick. Conidia solitary, dry, square, spherical or subspherical, flattened in one plane, cruciately septate, 4-celled, mid to dark brown, verruculose, 9-18 µm diam in face view, 7-9.5 µm thick.

Collection Examined: Gargi Singh, 30746 (PAN), on bark of *Pinus wallichiana*, Solan, May 14, 2014.

Remarks: In the North West Himalayan region, it has been reported from Bhawarna, Himachal Pradesh on *Dendrocalamus strictus* (Jamaluddin *et al*. 2004). This species is reported here on bark of *Pinus wallichiana*, which is a new host record for the fungus.

Stachybotrys echinata (Rivolta) G. Sm. *Trans. Br. mycol. Soc*. **45**(3): 392 (1962) **Fig. 2(E-F)**

Colonies effuse, black, powdery. Mycelium immersed. Stroma none. Setae and hyphopodia absent.

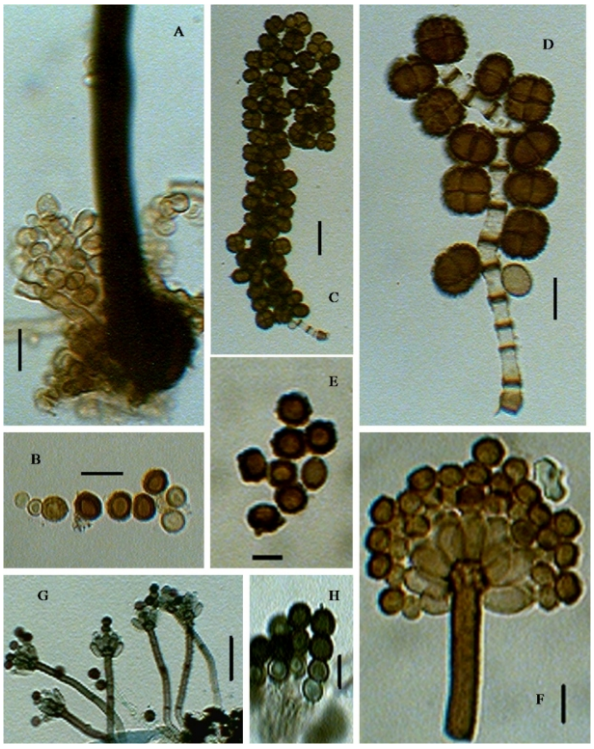

Fig. 2: A, B. *Lacellina graminicola* A. conidiophores surrounding the base of setae B. Conidia C, D. *Dictyoarthrinium sacchari* C-D. Conidiophore bearing conidia E, F. *Memnoniella echinata* E. Conidia. F. Conidiophore, phialides and conidia in chain G, H. *Memnoniella levispora* G. Conidiophores bearing phialids H. Conidia arising in chains Bars C, G= 20 µm, B-D=10 µm, E=5 µm.

Conidiophores macronematous, mononematous, erect, hyaline at the base, 68.80-99.84 × 3.02-3.95 µm. Phialides mostly in groups of 4-9, 6.5-8.1 × 3.1-4.8 µm. Conidia spherical or flattened dorsiventrally, 3.8-5.1 µm in diameter.

Collection examined: Gargi Singh, 30745 (PAN), on bark of *Azadirachta indica*, Solan, May 14, 2014.

Remarks: This is probably the most common species of the genus. This species have been reported from almost every part of the country on different hosts (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004). In this study this species is reported on *Azadirachta indica,* which is a new host record.

Stachybotrys levispora (Subram.) Yong Wang bis, K.D. Hyde, McKenzie, Y.L. Jiang & D.W. Li, in Wang, Hyde, McKenzie, Jiang, Li & Zhao. *Fungal Diversity* **71**: 57 (2015) **Fig. 2(G-H)**

Colonies effuse, black, velvety. Conidiophores unbranched, swollen at the apex, light brown to dark brown, often with scattered dark granules, up to 50 × 3.1-3.6 µm. Phialides 5.8-7.1 × 3.5-4.7 µm. Conidia smooth, often hemispherical, 4.2-6.5 µm in diameter.

Collection examined: Gargi Singh, 30747 (PAN), on bark of *Pyrus pyrifolia*, Solan, May 14, 2014.

Remarks: This fungus was collected on bark of *Pyrus pyrifolia*. A review of literature (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004) indicated that it has not been reported on *Pyrus pyrifolia*. Therefore, it is a new host record for this fungus.

Monodictys castaneae (Wallr.) S. Hughes. *Can. J. Bot*. **36**: 785 (1958) **Fig. 3A**

Colonies lavender to dark grey or black. Mycelium superficial. Stroma none. Conidia oblong rounded at the ends, pyriform, clavate, subspherical or irregular, mid to dark reddish brown, usually verrucose, basal cell sometimes paler than the others, 14-40 × 10-25 µm.

Collection examined: Gargi Singh, 30748 (PAN), on bark of *Psidium guajava*, Solan, May 14, 2014.

Remarks: *Monodictys castaneae* have been reported from Mt. Abu, Rajasthan on dead bark, from Pune, Maharashtra on *Acacia sphaerocephala* (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004) and from Chandigarh on angiospermous wood (Prasher and Singh, 2012). This is the first report of this species from North-Western Himalayas.

Monodictys putredinis (Wallr.) S. Hughes. *Can. J. Bot*. **36**: 785 (1958) **Fig. 3(B-C)**

Colonies effuse, blackish brown to black. Mycelium superficial. Stroma none. Conidiophores micronematous, irregularly branched, hyaline to brown, smooth and not markedly swollen. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical. Conidia pyriform, ellipsoidal or subspherical, multicellular, sometimes slightly constricted at the septa, dark reddish brown to almost black, smooth, 20-30 × 15- 25µm.

Collection examined: Gargi Singh, 30749 (PAN), on bark of *Pyrus japonica*, Parwanoo, May 14, 2014.

Remarks: This species has been previously reported from Anantgiri, Andhra Pradesh on dead wood (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004) and from Chandigarh on angiospermic wood (Prasher and Singh, 2012). This species constitutes a new record for North-Western Himalayas.

Periconia byssoides Pers. *Syn. meth. fung. (Göttingen)* **1**: 18 (1801) **Fig. 3(D-E)**

Colonies effuse, dark brown to black, hairy. Conidiophores up to 200-1000µm, 14-20 µm at base, 14-19 µm immediately below the head. Conidia catenate, spherical, brown, verrucose 8-15 µm in diameter.

Collection examined: Gargi Singh, 30751 (PAN), on bark of *Pyrus pyrifolia*, Parwanoo-Solan highway, May 14, 2014.

Remarks: *Periconia byssoides* is a very common species which has been reported on a number of substrata from different parts of the country (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004) but has never been reported on *Pyrus pyrifolia*. Therefore, it is a new host record for this species.

Spegazzinia tessarthra (Berk. & M.A. Curtis) *Sacc. Syll. fung. (Abellini)* **4:** 758 (1886) **Fig. 3(F-G)**

Colonies orbicular, black, up to 2 mm in diam. Conidiophores with '*a'* conidia up to 200 µm long, 2 µm thick at the base, up to 4.5 µm at the apex, becoming dark

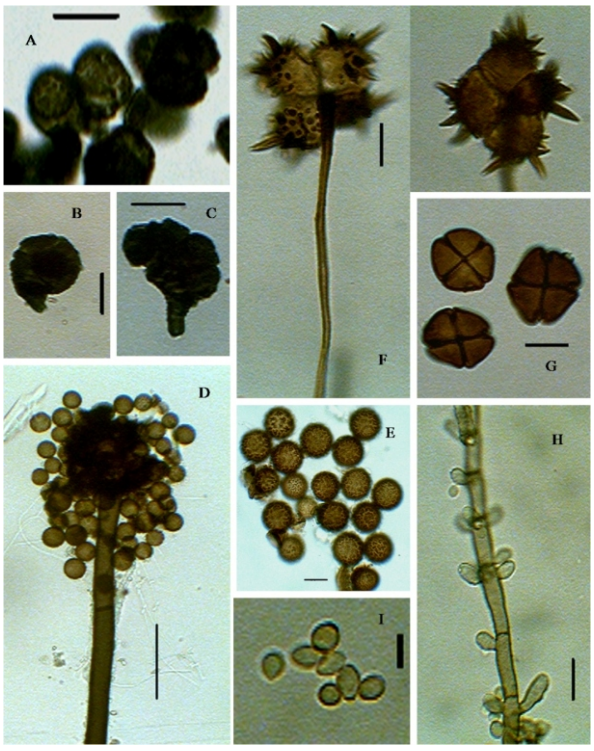

Fig. 3: A. *Monodictys castaneae A*. Verrucose conidia B, C. Monodictys putredinis B-C. conidia D, E. *Periconia byssoides* D. Conidiophore with conidia arising in chains E. Conidia F, G. *Spegazzinia tessarthra* F. Conidia with spines G. Cruciately septate smooth conidia H, I. *Stachylidium bicolor* H. Conidiophores with verticillately arranged phialides I. Conidia Bars A-C, E-H= 10 µm, D= 50 µm, I= 5 µm.

brown, upper part often verrucose; those with '*b*' conidia up to 16 µm long. Conidia of two types: '*a*' conidia 12-20 µm in diam excluding spines which are up to 10 µm long, '*b*' conidia flattened, cruciately septate, smooth, 13-18 µm wide, 7.5-9 µm thick.

Collection examined: Gargi Singh, 30750 (PAN), on bark of *Mangifera indica*, Parwanoo-Solan highway, May 14, 2014.

Remarks: This species agrees well with the description of *Spegazzima tessarthra* (Ellis, 1971) with slightly longer conidiophores of '*a*' conidia. A review of literature (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004) reveals that *S. tessarthra* has never been reported on *Mangifera indica*. Thus, it constitutes a new host record for this fungus.

Stachylidium bicolor Link. *Mag. Gesell. naturf. Freunde, Berlin* **3**(1-2): 15 (1809) **Fig. 3(H-I)**

Colonies effuse, olivaceous brown. Mycelium immersed. Conidiophores brown becoming paler towards the apex, branched, with verticillately arranged phialides, up to 700 µm, 4-7 µm thick at the base, 2.5-4 µm at the apex. Conidiogenous cells monophialidic, discrete, determinate, smooth, hyaline, 8-18 × 3-4 µm. Conidia aggregated in slimy heads, simple, smooth, narrowly ellipsoidal with rounded ends, hyaline, aseptate, 4-8 × 3-5 µm.

Collection examined: Gargi Singh, 30752 (PAN), on bark of *Pinus wallichiana*, Parwanoo, May 14, 2014.

Remarks: This is the third report of *Stachylidium bicolor* from Himachal Pradesh (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004). But the fungus has never been reported on *Pinus wallichiana*, thus, it is a new host record for the fungus.

ACKNOWLEDGEMENTS

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OBITUARY

Professor S. S. Saini
(01.07.1938 - 19.07.2015)

Dr. Satnam Singh Saini, popularly known as S. S. Saini in literature, was born on July 1, 1938 at Village Fateh Pur in Hoshiarpur District of Punjab. He did his post graduation in first class first from Panjab University, Chandigarh. He was an illustrious student of an illustrious teacher Professor K. S. Thind with whom he did his doctorate on "Trace Element Studies on some Pathogenic Fungi" in 1967 from Panjab University, Chandigarh. He joined as a Lecturer at Botany Department, Punjabi University, Patiala in 1967, became Reader in 1974, Professor in 1983 and superannuated in 1998. During his service at Patiala he served as Head of the Botany Department, Head Biotechnology Department, Dean Faculty of Life Sciences, Chairman Post-graduate and Undergraduate Board of studies in Botany, Agriculture, Food and Nutrition and Home Science. He also remained member of Punjabi University Syndicate, Sennate, Academic Council and various other governing bodies. He also served as a local Secretary for Agriculture section of 83rd Indian Science Congress held at Punjabi University, Patiala in 1996. He was a versatile researcher and a devoted teacher. During his initial years he worked on Physiology of fungi, Plant diseases survey and Post-harvest diseases of fruits and vegetables. In early eighties he switched over his research interest to the study of mushrooms. With his unilateral persuit along with his students, he developed a full fledged centre of research on Mushroom Systematics at Patiala for which he established International collaboration with Dr. Rolf Singer of Field Museum of Natural History Chicago, Dr. P. Heinemann of Faculte Des Sciences Agronomiques, Gembloux, Belgium, Dr. D. N. Pegler of Royal Botanic Gardens, Kew and many more mycologists of International repute. As a part of his research program Professor S. S. Saini visited Rijksherbarium Leiden, Herbarium of Royal Botanic Gardens, Kew and CAB International Mycological Institute, Kew, England. He participated in large number of National and International Conferences including International Mycological Congress held at Regensburg, Germany in 1990. Like me there are over 40 M. Sc., M. Phil, and Ph. D. students who were benefitted with his close association as a devout teacher and a Research guide. Personally speaking with his demise I lost a life time mentor who touched my soul and shaped my destiny. But for him, I could not have been what I am today. He was from amongst the rare breed of teachers, who use to sit with his students on stool in the laboratory for hours together to impart practical training. A hard task master indeed, who was honest to the core and has left indelible mark during his tenure as a teacher in the Department. He has three original books in Punjabi and two books edited along with more than 100 research publications in the National and International journals of repute to his credit. He was a Life member of number of prestigious scientific societies. Professor S. S. Saini left to his heavenly abode on 19-07-2015 and with his demise mycology fraternity has lost a devoted researcher and a guide. Professor Saini is survived by his two daughters and a son. One of his daughters, Dr. Munruchi Kaur, a budding mycologist herself, is working as Associate Professor in the Department of Botany, Punjabi University, Patiala, younger daughter Mrs. Akal Rachna Kaur is happily settled in Canada and son Mr. Harnoor Singh at Patiala. May Almighty give enough strength and preservance to the family members, his close associates and friends to bear this irreparable loss and grant eternal peace to the departed soul.

N. S. Atri
Professor of Botany
Additional Dean Research and
Dean Life Sciences
Punjabi University, Patiala

BOOK REVIEW
Title: Fundamentals of Plant Pathology
Authors : R.S.Mehrotra and Ashok Aggarwal
Pblishers :McGraw Hill Education (India) Private Limited, New Delhi.
Year of Publication: 2013
Price: Rs. 440/-

This book has been primarily written for the undergraduate students of Plant Pathology in the universities and colleges. The book has been divided into 26 chapters. The Introductory chapter deals with the nature and concept of disease and discusses various terms associated with disease development in addition to discussing briefly classification of Plant diseases. The second chapter is devoted to the history of Plant Pathology, dwelling over the evolution of the subject and its application from herbalists to the recent development in the field of plant pathology, including bacteria, viruses, Phytoplasmas and establishment of disease clinics. The chapters, 3-11 are devoted to the Principles of Plant Pathology and include chapters on: Pathogenesis; Enzymes and Toxins in Plant diseases; Alteration in Plant Physiological Function due to Plant Pathogen Interaction; How Plants Defend Themselves against Infection; Genetics of Plant-Pathogen Interaction; Effect of Environmental Factors and Nutrition on Disease Development; Dispersal of Plant Pathogens; Plant Disease Epidemiology and Plant Disease Forecasting and Management of Plant Diseases. The next chapter (12) deals with the general characteristics of fungi and classification of fungi. The authors have given outline of classification proposed by Alexopolous *et al.,* (1996) and Kirk *et al.,* (2008). The chapters, 13-19 deal with important plant diseases caused by fungi belonging to *Chytridiomycota*, *Zygomycota*, *Ascomycota*, *Basidiomycota*, Anamorphic fungi and *Oomycota*. The information on different diseases discussed in these chapters includes Symptoms, Causal organism, Disease cycle and Predisposing factors and Disease Mangement. The written text is supplemented with line drawings of the pathogen and the black and white photograph of the disease symptoms. The chapters, 20-22 deal with the diseases caused by Mollicutes, Bacteria and Viruses. In the chapters on diseases caused by bacteria and on viruses, a brief account of structure, reproduction and classification is also provided. The 23rd chapter deals with the diseases caused by nematodes along with the brief discussion regarding their characteristic features, ecology, nematode-fungal interaction as well as general methods of control. The next chapter deals with the Abiotic diseases of plants. The 25th chapter pertains to the application of biotechnology in Plant Pathology which includes: use of tissue culture techniques, recombinant DNA technology, use of monoclonal antibodies, somaclonal variants as source of disease resistance and innovative tools for detection of plant pathogenic microbes. The last chapter deals with the phytopathological techniques used in the Plant pathology. It includes: isolation of fungi, nutrient requirements of fungi, special media for cultivation of pathogens in addition to discussion on Koch's Postulates. All the chapters are written in a concise manner. At the end of each chapter review questions and multiple-choice questions have been provided. The text has been written, keeping in view the UG curricula of different universities and also covers specialized topics related to physiological and molecular aspects of plant pathogens. The information provided is pertinent to Indian scenario. In summary, the orientation of the book is well suited to the undergraduate students of Indian universities and colleges. The text is also accompanied by an online learning center available at https://www.mhhe.com/mehrotra/fpp1 which contains 2 sample question papers, extra reads and web references. This book is a welcome addition to literature for the undergraduate students of Agriculture Universities as well as traditional universities.

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INSTRUCTIONS TO THE AUTHORS

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