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उत्तिष्ठत जाग्रत प्राप्य वरान्निबोधत।

This issue is dedicated to the fond memory of Late Professor B.P.R. Vittal in recognition of his contributions to Indian Mycology

The Mycological Society of India was founded in January 1973 with a view to bring together the mycologists of the country and with the broad objective of promoting the development of Mycology in India in all its aspects and in the widest perspective.

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The name of the journal KAVAKA *is a Sanskrit word which means Fungus.*

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PREFACE

Prof. B.P.R. Vittal
(1944-2018)

This volume of Kavaka is dedicated to the fond memory of Late Professor B.P.R.Vittal, founder member of Mycological Society of India. His immense efforts and contributions in the field of mycology has played a pivotal role in the growth of Mycological Society of India in general and Kavaka in particular. As a part of the preface to this volume of Kavaka, a brief account of his life sketch and recognitions he received during his professional life has been highlighted so as to acquaint the mycological fraternity about the contributions of this great Indian Mycologist whose career flourished under the stewardship of none other than great Late Professor C.V.Subramanian.

Coming from a humble background and belonging to Ranipadu village, Cumbum Mandal, Prakasam district, Andhra Pradesh, Late Prof.B.P.R. Vittal had his schooling from Narasaraopet, Prakasam district, Andhra Pradesh. He obtained a Masters degree in Botany from Andhra University, Visakhapatnam, Andhra Pradesh in 1964 and Ph.D. from CAS in Botany, University of Madras under Late Prof. C.V. Subramanian, the doyen of Indian Mycology on taxonomy and ecology of leaf litter fungi. After his appointment as a faculty member at CAS in Botany, University of Madras in 1976, Prof. B.P.R. Vittal established a research niche on aerobiological studies at this centre, while still pursuing diversity studies on litter degrading fungi. Prof. Vittal supervised the research work of 26 M.Phil. students and 11 Ph.D. students. He has over 80 publications in national and international journals and Proceedings of Symposia. He successfully handled 10 major research projects in the fields of Mycology and Aerobiology with funding from UGC, CSIR, DBT and MOEF.

In the course of his investigations on diversity of leaf litter fungi Prof. B.P.R.Vittal was involved in the description of new genus *Civisubramaniania eucaliptii*, and number of new species such as *Cercosperma longispora, Dactylaria eucalypti, Minimidochium indicum, Kellermania intermedia* and *Stachybotrys ramosa* collected on *Eucalyptus* litter and *Lophiostoma mangrovei* (now *Rimora mangrovei*). In addition to taxonomic studies he also worked on the various aspects of litter fungi, marine fungi in mangroves from east coast of India including Pichavaram in Tamil Nadu and Godavari and Krishna delta mangroves in Andhra Pradesh. Prof. B.P.R. Vittal also established a special niche for himself by initiating Aerobiological studies for the first time in 1976 at the Centre for Advanced studies in Botany, University of Madras. In recognition of his expertise and contribution to aerobiology Prof. Vittal was awarded a project by MOEF, New Delhi, as one of the coordinating centers of aerobiological research in India.

Prof Vittal served different societies in various capacities including the President of Indian Aerobiological Society (1994-1997), Treasurer, Secretary and President of Mycological Society of India (1994-2004). He was also a member of International Aerobiological Society. Prof. Vittal was conferred with several Fellowships, Awards and Honours which included Fellowship of Indian Aerobiological Society; Fellowship of Madras Science Foundation; Lifetime Achievement Award of Indian Aerobiological Society and Lifetime Achievement Award of Mycological Society of India.

His sudden demise in 2018 is an irreparable loss to Indian Mycological fraternity. We in MSI and editorial team of Kavaka felt it not only appropriate but also most befitting and apt that a commemorative volume of Kavaka is released in memory of Late Prof. B.P.R. Vittal as a tribute in recognition of his achievements and contributions to Indian Mycology.

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Expedition with micro- and macro-fungi: New perspectives to bridge the gaps*

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ABSTRACT

Fungi are the most fascinating group of organisms distributed widely in different ecosystems. Strategic geographic location of the Indian subcontinent is a major hub of fungal resources which offers ample scope for their exploration as well as application. My curiosity in mycology initiated journeying freshwater lotic habitats of the Western Ghats and west coast of India. It was soon ascertained that the freshwater hyphomycetes serve as model group facilitating assessment of basic concepts of detritus food chain and aquatic productivity. Second fascinating group attracted my attention was the marine fungi in various ecosystems of the west coast playing significant role in nutrient turnover. Third striking aspect of my interest was macrofungi of the Western Ghats and west coast. The basic function and ecosystem services of all these groups is breakdown and transformation of organic matter. The impacts of decomposition is highly valuable in production of precious metabolites, enrichment of organic matter and pull other groups of organisms to drive the energy flow initiated from detritus ecosystem. All these research areas are highly fascinating in the Indian subcontinent owing to diverse habitats, varied environment, wide range of substrates and numerous fungi. Progress towards basic or applied facets of fungal diversity, significance of metabolites and sustainable ecosystem functions demands collaborative endeavors. Specific strategies and schemes for prospective harness of fungal ecosystem services in the Indian subcontinent have been discussed in this commentary.

Dr. K.R. Sridhar
President, Mycological Society of India (2018)

KEYWORDS: Aquatic fungi, diversity, decomposition, detritus food web, ecoregions, Indian Subcontinent, macrofungi, mangrove fungi, marine fungi, west coast, Western Ghats

INTRODUCTION

The Kingdom Mycota emerged as an independent eukaryotic line about 1 billion years ago (Lücking and Nelsen, 2018). Being devoid of photon trapping ability and gastrointestinal tract, fungi acquired outstanding capabilities to produce enzymes and metabolites. They are morphologically versatile from microscopic structures to giant fruit bodies. Usefulness of fungi in human nutrition, beverage and medicine archaeologically dates back to about 6,000 years (Willis, 2018). Although fungi are commonly viewed as plant and animal pathogens, they play a major role in nutrition, food processing, decomposition of organic matter, production of pharmaceuticals, generation of biofuels and serve as biopesticides or bioprotectants. The lifestyle of fungi is flexible, which is mainly dependent on the ecological niches and competent to perform their functions in terrestrial, aquatic, anaerobic, mutualistic and several extreme habitats. Fungi comprise versatile ability to develop network underneath the soil, process the organic matter, recycle or distribute nutrients, involve in growth promotion, antagonistic to disease causing organisms, offer stress tolerance and detoxify recalcitrant compounds (Suz *et al*., 2018).

Tremendous curiosity has been developed to explore the fungi after Rio convention on biodiversity in 1992, which has become a worldwide priority to understand the ecosystem structures and functions. The global estimate of fungi has become a black box, which needs inventiveness to provide evidence and statistics. The debate on fungal resource although ranges from 0.5 to 9.9 million based on morphological and molecular basis (Cannon, 1997; May, 2000; Blackwell, 2011), rationally accepted estimate is 2.2-3.8 million (Hawksworth and Lücking, 2017), which is more than six-fold of angiosperms (Willis and McElwain, 2013). However, molecular methods of fungal community gave a clue of existence of up to 5.1 million species (median of 0.5-9.9 million) (O'Brien *et al*., 2005). Fungal association with palms in Queensland was 1:26, while fungal association with palms in Australia and Brunei Darussalam was 1:33 (Fröhlich and Hyde, 1999) indicating regional difference in fungal estimation depending on the specific flora. The debate on fungal estimate will continue as and when additional evidences emerge. Currently documented fungi is only 7% (144,000) with the highest members documented in *Ascomycota* (90,000) followed by *Basidiomycota* (50,000) and *Microsporidia* (1,250), with addition at the existing rate of 2,000 per annum (Cannon *et al*., 2018; Niskanen *et al*.,

* Presidential address delivered at 45th Annual meeting of Mycological Society of India on November 19, 2018 held at ARI, Pune, Maharashtra. This contribution has been dedicated to Prof. C.T. Ingold, Prof. E.B.G. Jones and Prof. D.L. Hawksworth.

2018) (**Box 1**). About 2,000 new fungi have been discovered during 2017, wherein the Asian Continent added the highest number (35%), followed by Europe (25%) and Australia (14%) (Niskanen *et al*., 2018). Up to 180 new mycorrhizal fungi were established in Australia, Europe and India. The current statistics broadened our knowledge on the whole genome sequence of about 1,500 species, which is more than those of plants and animals put together. It is evident that up to 90% of plant species on land co-exist mutualistically with fungi as mycorrhizas, which have a history of 400 million years. Among the mycorrhizas, the orchid mycorrhizal fungi were the highest (25,000) followed by ectomycorrhizal fungi (20,000) (Suz *et al*., 2018). This appraisal presents significance of filamentous aquatic fungi (freshwater and marine) and macrofungi with emphasis on the potential of the Indian Subcontinent to fill the knowledge gaps.

Box 1. Estimate of described eight fungal phyla (Total: ~144,140) (Source: Cannon *et al*., 2018)

Ascomycota	~90,000
Basidiomycota	~50,000
Microsporidia	~1,250
Chytridiomycota	~980
Zoophagomycota	~900
Mucoromycota	~760
Blastocladiomycota	~220
Cryptomycota	~30

FRESHWATER FUNGI

Freshwater fungi are phylogenetically diverse assemblage of *Ascomycota*, *Chytridiomycota*, *Cryptomycota* and *Zygomycota* distributed in different habitats worldwide (Jones *et al*., 2011; Raja *et al*., 2018). The lifestyle of freshwater fungi widely differs (e.g. saprobic, planktonic, endophytic, pathogenic and parasitic) and dependent on the ecological niche. Being integral component of aquatic food web, broad ecological functions of freshwater fungi are breakdown of coarse particulate organic matter (COPM) and drive the energy to higher trophic levels. They have also adapted to overcome the impact of one way transport of propagules, overgrazing by the shredders, dominance of other organisms living in the same niche, seasonal periodicity of detritus input and intermittent wet and dry regimes. Diversity of substrates in the lotic habitats supports their perpetuation as well as stability of population. The live (e.g. roots and macrophytes) as well as dead (e.g. leaf litter, flowers, twigs and logs) substrates are colonized by the freshwater fungi.

The life styles of these fungi have been fine tuned based on the longevity of the substrates in lotic ecosystem. Investigations pertain to freshwater fungi which could be divided into three major segments: i) diversity, distribution and phylogenetic studies based on morphological and molecular approaches; ii) studies on the ecology and ecosystem services; iii) production of secondary metabolites. Two dimensions of the aquatic detritus food web include decomposition of CPOM (horizontal) and energy flow (vertical) to the higher trophic levels (**Fig. 1**). The extent of energy flow depends on the nature of detritus, abiotic factors and biotic features of the aquatic habitat. Fungal decomposers have the main role in processing the detritus to transform into fungal biomass and products of decomposition. Depending on the fungal processing of detritus, the detritivores (e.g. shredders) facilitate or hasten the transformation of CPOM into several components. Aquatic fungi and detritivores together drive the energy to higher trophic levels and several products of decomposition serve as source of energy in aquatic habitats.

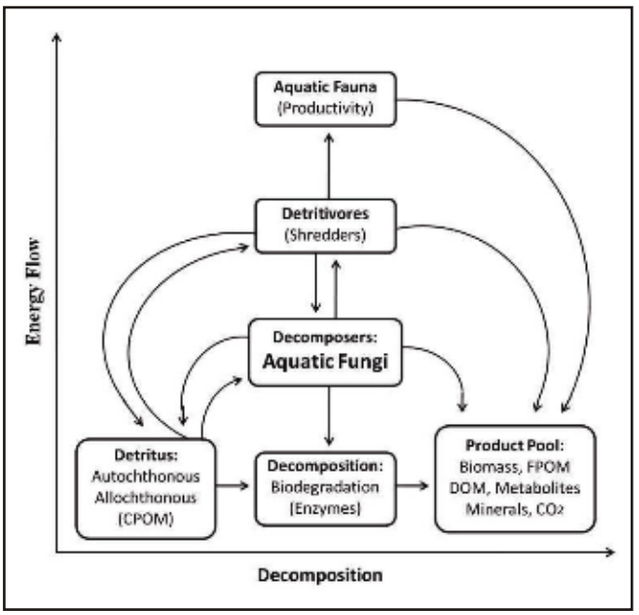

Fig. 1. Features of fungal driven detritus food web in lotic habitats (CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; DOM, dissolved organic matter).

Freshwaters are hit by as many as seven disturbances among the 10 worst perturbations in the world leading to the highest loss of species (Wall *et al*., 2001; Malmqvist and Rundle, 2002; Rockström *et al*., 2009). The human interference which has major influence on species loss in freshwaters include alteration of riparian habitats, extensive water extraction, loading pollutants and invasion of alien species. However, aquatic filamentous fungi have expanded their territory beyond their usual habitats (see Chauvet *et al*., 2016). The **Box 2** records some of the terrestrial and semi-aquatic habitats where aquatic fungi persist. Specific adaptations to such unusual (or stressed) habitats need to be investigated and such habitats are of special interest to follow up transformation of anamorphs into teleomorphs.

Hyphomycetous Fungi: Freshwater hyphomycetous fungi (or Ingoldian fungi) are characterized by production of morphologically distinct conidia especially multiradiate (staurosporus) and sigmoid (scolecosporus) (Ingold, 1975; Marvanová, 1997; Gulis *et al*., 2005). However, some of them also produce conidia of conventional shapes (spherical or

Box 2. Occurrence of aquatic hyphomycetes in selected niches outside the lotic habitats (Source: Sridhar, 2017a)

Terrestrial	**Semi-aquatic**
Forest floors	Stream slopes
Tree holes	Tree holes
Tree canopy	Stemflow
Crown humus	Throughfall
Live leaves/twigs/roots	Crown humus
Epiphytes	

oval or fusiform: e.g. *Dimorphospora, Tumularia* and *Vermispora*). The complex conidial shapes represent functional traits in aquatic habitats like floatation, impaction and sedimentation similar to plankton. Hyphomycetes have worldwide distribution and currently reported up to 300 species (95 genera) with preponderance in freshwaters of mid-latitudes (Wood-Eggenschwiler and Baerlocher, 1985; Gulis *et al*., 2005; Shearer *et al*., 2007; Raja *et al*., 2018; Seena *et al*., 2019).

The autochthonous and allochthonous detritus serve as major nutritional resource in aquatic habitats and several aquatic organisms compete to utilize such resources. Broad range of functions and survival strategies of hyphomycetes might have been evolved through natural selection especially adaptation to drastic variations of habitats (*r*-selection) or adaption to thrive for prolonged periods on stable organic substrates (*K*-selection) (Cooke and Rayner, 1984; Hawksworth and Mueller, 2005). Hyphomycetes, grow on fragile substrates like leaf litter, have to adapt *r*-selection (ruderal strategy) for rapid colonization, growth and reproduction owing to rapid loss of substrate. In order to compete for resource utilization, hyphomycetes follow boom-bust cycle or Baerlocher's effect (Sridhar, 2017b). The sequence of events like drift conidial adherence to detritus (arrival), conidial release from detritus (departure) and mycelial growth in detritus (biomass) happen with time lapse (Baerlocher, 2009). Conidial recruitment, release of conidia and fungal biomass accumulation on fragile substrate decreases owing to less hospitable status of substrate. Such events are dependent on nature of detritus, geographic conditions and human interference. Thus, evaluation of these variables serves as authentic strategy to assess the exponential decay pattern of detritus. To maintain the inoculum in the upstream, escape grazing from shredders and other adverse conditions, hyphomycetes have adapted several strategies: i) colonization on the stable organic substrates like wood (it may aid to produce teleomorphic state); ii) colonize live roots exposed in to water (as endophytes); iii) survive on the moist substrate in stream border or valley; iv) survival in intestine of aquatic fauna (e.g. crabs, fishes, prawns and tadpoles).

Ascomycetous Fungi: Similar to hyphomycetous fungi, ascomycetous fungi also colonize woody and herbaceous detritus in aquatic habitats (Wong *et al*., 1998; Shearer and Raja, 2017). Their reproductive structures mainly consist of flask or bowl shape. The ascospores have undergone modifications to develop sticky gelatinous sheaths as well as appendages as to float, adhere and colonize substrates in water. Recent report reveals that up to 675 species of ascomycetes have been reported in freshwaters worldwide (Shearer and Raja, 2017). Similar to aquatic hyphomycetous fungi, the diversity of ascomycete community differs along the latitudinal gradients with a clue that the highest diversity in the interface of temperate and tropical regions (Shearer *et al*., 2015; Raja *et al*., 2008, 2018).

Enzymes of aquatic ascomycetes (e.g. amylases, cellulases, peroxidases, pectinases and xylanases) aids in causing soft rot in woody debris in aquatic habitats, which is slower compared to fungal white rot or brown rot in terrestrial habitats (Savory, 1954). The extent of fungal decay of wood in aquatic habitats confined to a few millimeters owing to lack of oxygen in interior region (Shearer, 1992). However, ascomycetes could penetrate their hyphae into the deeper regions of wood to cause decomposition by translocation of molecular oxygen through their hyphae similar to fungal activity in deeper zones of anoxic sediments. Although hyphomycetous fungi growing on fragile leaf litter follow *r*-selection, those colonize stable woody litter have the opportunity to switch over to perfect state leading to adapt *K*-selection, which is advantageous to thrive under chaotic situations or disturbances (e.g. unusual niches, dryness, temperature stress, pollution and flood conditions) (Cooke and Ryaner, 1984; Chauvet *et al*., 2016).

Aeroaquatic Fungi: Aeroaquatic (or helicosporous) hyphomycetous fungi occur often in lotic habitats by building vegetative biomass underwater and production of conidia in air-water interface (helicospores). They are also inhabitants of moist forest litter, ponds and semi-aquatic habitats. As saprobes they grow on plant litter, wood, twigs and moist locations in and around aquatic bodies. *Helicoma*, *Helicomyces*, *Helicoön*, *Helicosporium*, *Spirosphaera* and related genera are characterized with two or three dimensional coiled spores as special adaption for flotation (Zhao *et al*., 2007). Helicosporous *Cirrenalia* and *Zalerion* exist in marine habitats. Other aeroaquatic and aeroaquatic-like fungi include *Candelabrum, Clathorsporium, Clathroconium, Clathrosphaerina, Helicodendron, Helicoubisia, Inesiosporium, Moorella, Pseudoclathrosphaerina, Spirosphaera, Strumella, Sympodioclathra, Trochophora,* and *Xenosproium*. Morphological characters are very important in classifying helicosporous hyphomycetes. Some of the important morphological features necessary for classification include habitat, substrate, conidial coiling pattern, hygroscopic/non-hygroscopic features of conidial filaments, colour/hyaline nature of conidial filaments, denticulate conidiogenous cells and pattern of conidiogenesis (Zhao *et al*., 2007). According to a recent literature, from the aquatic habitats up to 90 species (19 genera) of aeroaquatic fungi have been reported worldwide (Raja *et al*., 2018).

Studies in the Western Ghats and West Coast: The first report from India (Kambakkam Hills, north of Chennai, Tamil

Nadu) on the occurrence of four aquatic hyphomycetes in a streamlet was published by Ingold and Webster (1973). **Table 1** provides selected literature on aquatic fungi of the Western Ghats and west coast of India. Different aspects evaluated on freshwater fungi include diversity, distribution, endophytes, occurrence outside the usual habitat, decomposition, palatability to fish, enzymes, impact of pollutants and techniques followed. A few reviews, checklists and monographs consolidated the studies carried out in the Western Ghats and west coast of India. Most of the literature emphasized on the occurrence, diversity and distribution of aquatic hyphomycetes. Among several surveys carried in the Western Ghats and west coast, Sampaje stream in the Western Ghats at about 500 m asl (mid-altitude) possesses the highest number of aquatic hyphomycetes. Including studies outside the stream habitats (e.g. tree holes, stemflow and through fall) nearly 25% of globally known aquatic hyphomycetes have been recorded in a few samples during post-monsoon season. In any freshwater streams of the Western Ghats and west coast, single sample (e.g. water, foam and leaf litter) assessment provide at least 10% of globally known aquatic hyphomycetes. A few new genera and species of aquatic hyphomycetes have been described from the Western Ghats and west coast of India (e.g. *Kumbhamaya jalapriya, Synnematophora constricta, Trinacrium indica, Triscelophorus konajensis* and *Vermispora cauveriana*) (Sridhar, 2010; Borse *et al*., 2017). Diurnal periodicity of aquatic hyphomycete spores have been studied in the streams of the Western Ghat and west coast (Sridhar and Sudheep, 2010; Ghate and Sridhar, 2016d).

Many aquatic hyphomycetes have been reported as endophytes in riparian trees and ferns (Raviraja *et al*., 1996a) (**Table 1**). Similar to occurrence of more hyphomycetes in the mid-altitude of the Western Ghats (Raviraja *et al*., 1998a), endophytic fungi were also high in the mid-altitude region (Ghate and Sridhar, 2017b). Aquatic hyphomycetes have extended their niches within (sediments, aquatic roots and fish intestine) (Sridhar and Sudheep, 2011b; Sudheep and Sridhar, 2012; Ghate and Sridhar, 2015a) and outside their usual ecological niches (e.g. tree holes, stemflow, throughfall, bark, epiphytes and canopy) (Sridhar, 2009c; Chauvet *et al*., 2016). Aquatic and aeroaquatic hyphomycete spores were also found in the street runoff of urban habitats of a southwestern Indian city (Ghate and Sridhar, 2018). Besides aquatic hyphomycetes, several aeroaquatic hyphomycetes were also reported from the rainwater dripping from the tree species (Ghate and Sridhar, 2015c). Along with aquatic hyphomycetes, occurrence of aeroaquatic hyphomycetes, terrestrial hyphomycetes and aquatic ascomycetes have also been reported in leaf and woody substrates (e.g. Sridhar and Kaveriappa, 1989; Sridhar and Sudheep, 2011a; Sudheep and Sridhar, 2011, 2013a). Comparison of leaf litter decomposition in stream locations and tree holes in the west coast revealed involvement of several aquatic hyphomycetes with double the duration of half-life of decomposition in tree holes than stream locations (Sridhar *et al*., 2013).

Besides studying the colonization of aquatic hyphomycetes and allied group of fungi on leaf litter and woody litter in streams, a few studies are involved in assessing the pattern of decomposition (**Table 1**). Decomposition is the functional phase of aquatic fungi, which results in improvement of palatability, energy flow and probably facilitates the bioremediation process. Native and exotic tree leaves have been investigated to understand the pattern of decomposition in streams, rivers and dam sites (Raviraja *et al*., 1996b, 1998b; Sridhar *et al*., 2011a; Sudheep and Sridhar, 2013a). Studies on wood decomposition are relatively limited and these studies in addition follow the mass loss, changes in leaf chemistry as well as extracellular enzymes are assessed (Sridhar *et al*., 2011a; Sudheep and Sridhar, 2013b). Chandrashekar and Kaveriappa (1988, 1991, 1992) evaluated the capacity of production of extracellular enzymes by pure cultures of aquatic hyphomycetes.

Table 1. Selected literature on freshwater fungi of the Western Ghats and west coast of India.

Diversity and distribution	Subramanian and Bhat (1981), Sridhar & Kaveriappa (1984, 1989, 1992), Chandrashekar *et al*. (1986, 1990), Rajashekhar & Kaveriappa (1993, 2003), Raviraja *et al*. (1998a), Maddodi *et al*. (2009), Sridhar *et al*. (2010a, 2013), Sudheep & Sridhar (2011, 2012, 2013a, b), Ghate & Sridhar (2015a), Karun *et al*. (2016a)
Endophytes	Raviraja *et al*. (1996a), Ghate & Sridhar (2017b)
Outside the habitat	Sridhar & Kaveriappa (1987), Sridhar *et al*. (2006, 2013), Karamchand & Sridhar (2008), Sridhar & Karamchand (2009), Sridhar (2009c), Ghate & Sridhar (2015c, 2016b, 2018)
Decomposition	Raviraja *et al*. (1996b, 1998b), Sridhar *et al* (2011, 2013), Sudheep & Sridhar (2011, 2013a, b)
Palatability	Chandrashekar *et al*. (1989)
Enzymes	Chandrashekar & Kaveriappa (1988, 1991, 1992)
Pollution	Sridhar & Kaveriappa (1986), Chandrashekar & Kaveriappa (1989, 1994), Raviraja *et al*. (1998b), Raghu *et al*. (2001), Ghate & Sridhar (2018)
Technique	Chandrashekar *et al*. (1990), Sridhar & Sudheep (2011b), Sudheep & Sridhar (2012), Ghate & Sridhar (2015b)
Checklist	Patil & Borse (2015)
Review	Sridhar *et al*. (1992), Sridhar (2009a, c, 2010)
Book/Monograph	Borse *et al*. (2016, 2017)

Although there are no specific studies on the bioremediation of pollutants using aquatic fungi, some studies dealt with occurrence of aquatic hyphomycetes in polluted habitats and impact of pollutants on growth, sporulation and spore germination of aquatic hyphomycetes (**Table 1**). In addition, occurrence of aquatic fungal spores in urban runoff has been investigated recently (Ghate and Sridhar, 2018). A few new techniques have been developed to study the aquatic hyphomycetes (**Table 1**). Among them, indirect evaluation of fungi in aquatic sediments, fish intestine and spore trap using plant latex are interesting (Sridhar and Sudheep, 2011b; Sudheep and Sridhar, 2012; Ghate and Sridhar, 2015b). Reports on aquatic hyphomycetes on woody litter are limited, but applying bubble chamber incubation technique similar to the leaf litter revealed several aquatic hyphomycetes on woody litter from the Western Ghats and west coast (e.g. Sridhar *et al*., 2010a; Sudheep and Sridhar, 2011; 2013b). Available studies on the aquatic fungi in streams and rivers of the Western Ghats and west coast indicate that there are several major gaps in our knowledge on the aeroaquatic fungi, aquatic ascomycetes (including *Dothideomycetes*) and

aquatic lichens. Recently, many freshwater lichens have been reported by Thüs *et al*. (2014) and no such reports from the freshwater habitats of the Western Ghats and west coast of India. Habitat destruction and pollution are the major threats for functioning of aquatic fungi. Aquatic fungi being the major link between detritus and aquatic fauna (e.g. crabs, fishes, prawns and tadpoles), rehabilitation tasks are of immense significance to harness their ecosystem services and valuable biomolecules.

MARINE FUNGI

Marine mycology embodies broad groups of fungi belonging to *Ascomycota*, *Basidiomycota*, *Blastocladiomycota*, *Chytridiomycota*, yeasts and fungus-like organisms. Pang *et al*. (2016) have broadened the definition of marine fungi to encompass those which could grow and or sporulate in marine habitats, develop mutualistic association with marine living beings in marine habitats and genetically adapted or metabolically dynamic in marine habitats. Marine fungi are accessible to a diverse substrata as well as niches (sand, soil and sediment; neritic, oceanic, deep sea and mangrove waters; dead algae, seagrass, mangrove vegetation and animal substrates; live algae, seagrass, mangrove vegetation and live animals). Similar to freshwater fungi, frequent question needs answer is on the estimation of marine fungi. Roughly the current known marine fungi are about 1,200 (10%) against the predicted estimate of 10,000-12,000 (Jones, 2011).

The functions and survival attributes of marine fungi depends on the substrata and the ecological niche (e.g. planktonic, saprophytic, mutualistic, pathogenic and parasitic). As an important component of marine food web, marine fungi mainly involve in decomposition and nutritional enrichment of organic matter to transfer energy to the higher trophic levels. They have several adaptations to lead planktonic life and strategies to prevail under adverse effect on their vegetative and reproductive phases. Marine fungi flourish on wood (drift, jammed and panels), algae (microalgae and macroalgae), sediment (coast, continental shelf and deep-sea) and rocks/corals (endolithic). Usually, the sea foam composed of spores of many marine and marine-derived fungi. The current research on marine fungi involves understanding: i) the diversity, distribution and phylogeny; ii) ecological consequences; iii) production of natural products of industrial and medicinal significance.

Diversity: Up to 943 ascomycetes have been reported in marine habitats (Jones *et al*., 2015). The members belonging to the orders *Eurotiales* and *Saccharomycetales* are known to have wide association with water, sand, sediment, plant and animal substrates. Similar to freshwater fungi, marine ascomycetes have appendaged ascospores for dispersal and to hold the substrates for growth (e.g. *Halosphariaceae* and *Lulworthiales*) (Jones, 1995; Campbell *et al*., 2005). *Lulworthiales* are obligate marine fungi associated with macroalgae and corals (Campbell *et al*., 2005). Although representation of basidiomycetous fungi is least in marine habitats, Jones *et al*. (2015) listed 21 filamentous and 75 basidiomycetous yeasts occurring in oceanic, mangrove and brackish water habitats. Up to 213 marine basidiomycetes and ascomycetous yeasts have been reported by Jones *et al*. (2015). Marine chytrids are also underestimated like marine basidiomycetes and reported 27 species (Jones *et al*., 2015). They are the most abundant group in the Arctic and sub-Arctic regions (Comeau *et al*., 2016) and also known as parasites on diatoms (Ohtsuka *et al*., 2016). Recent update on filamentous fungi included 300 species occurring in marine habitats (Jones *et al*., 2015). Studies on mangrove plant species yielded as many as 637 species of endophytic fungi (Sakayaroj *et al*., 2012b). Marine fungi are also composed of several pathogens on plants (mangrove and salt marsh), seaweeds, marine animals and diatoms.

Endophytic Fungi: Interest on ecological studies in different habitats of marine ecosystems resulted in documentation of a variety of marine fungi (Jones and Pang, 2012; Sakayaroj *et al*., 2012a). One such potential field of ecological interest is marine endophytic fungal studies. Endophytic fungi are generally known to confer fitness to establish in a specific niche by overcoming the negative impacts of abiotic and biotic stresses (e.g. resistance against herbivory, prevention of pathogen attack and drought tolerance). Some studies confined to mangrove ecosystems, coral reefs, islands and coastal sand dunes. Studies are available on endophytic fungal association with salt marshes, mangrove plants, mangrove associates, coastal sand dune plants, seaweeds and seagrass. However, many studies in marine habitats have documented endophytic fungi up to genus level indicating several new species. Endophytic fungal studies have not only added new fungi occurring in marine habitats, it has projected the potentiality of many marine-derived endophytic fungi in production of medicinally and industrially valuable bioactive compounds of bioprospect interest. Some marine endophytic fungi have bioremediation potential against stress as well as pollutants.

Food Web: Organic matter constitutes the major hub of transformation of energy into the marine food web. Input of CPOM to the ocean will be of autochthonous or allochthonous origin. The facets of organic matter breakdown by fungi in the marine ecosystem have been conceptualized in **Fig. 2.** The CPOM composed of stable (e.g. wood, root and animal remains) and fragile (e.g. leaf litter, seaweed and seagrass) material. The habitats that provide substantial input of CPOM include mangroves, salt marshes, coastal sand dunes, islands and coral reefs. The CPOM accumulates in sand, soil, sediment, intertidal, oceanic, continental shelf, pelagic and benthic habitats. Fungi being major components of life in marine habitats involve in degradation of organic matter leading to energy flow to the higher trophic levels. Decomposition of organic matter by fungi in marine habitats depends on the nature of CPOM and many abiotic factors. The fungal structure and function on CPOM leading to energy flow to the higher trophic segments via several products like fine particulate organic matter (FPOM), dissolved organic matter (DOM), metabolites, minerals and carbon dioxide (see Sridhar, 2012). During the transformation of organic matter by fungi, the physical as well as nutrient status of CPOM modifies leading to create habitats for colonization of other organisms. Besides, the fungal biomass accumulated in the

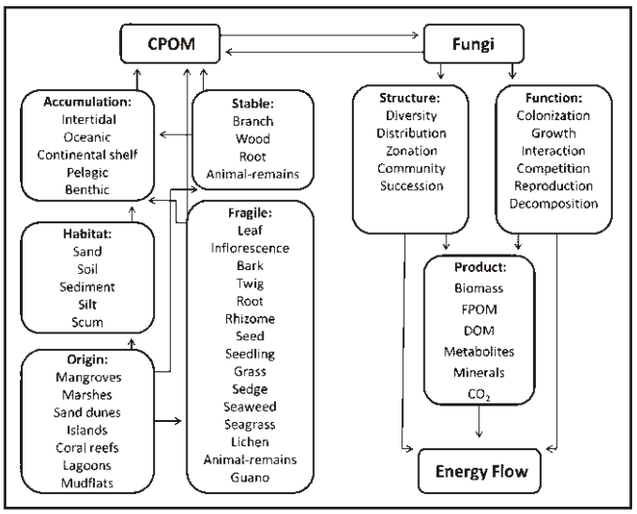

Fig. 2. Elements of fungal driven food web in marine habitats (CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; DOM, dissolved organic matter).

organic matter itself attractive to marine fauna. It is likely, several fungal keystone species and consortium of fungi involve in fine-tuning the CPOM transformation, which may also control the rates of turnover.

Petersen and Curtis (1980) performed a comparative study of energy budgets from subarctic, temperate and tropical areas (Greenland, North Sea and West Thailand). This study compares the incident solar energy, energy budgets of organic matter, phytoplankton, zooplankton, benthos and filter feeders. Such comparisons are not available for the decomposer food chain by fungal decomposers in spite of fungi serve as energy signatures especially in the detritus food chain. However, estimation of ergosterol helps up to some extent to understand the contributions to energy budgets by only filamentous fungi. Data are available on the decay coefficient and mass loss of wood, roots, leaf, sedge and seagrass in marine environments of different geographical locations (see Sridhar, 2012). In marine ecosystems, mangroves and coral reefs serve as productive regions of interest to study the interaction of fungi with biota and energy flow (Yap *et al*., 1994; Twilley, 1995). Information is available on the productivity of mangroves (e.g. carbon, nitrogen and phosphorus budget), global litter accumulation and annual range of litter production in mangroves (see Sridhar *et al*., 2012). Various animal populations depend on mangrove habitats for food and survival. Other than those inhabiting the floors (e.g. crabs, sea cucumbers and snails), birds, insects and bats are also important components of mangrove ecosystems in terms of detritus production. For example, the caterpillars of moth (*Hyblea purea*) are known to consume substantial leaf biomass of *Avicennia germinans* in the Caeté estuary in Brazil within a few weeks (Koch and Wolff, 2002). Such processing leads to accumulation of feces and leaf particles, which are the major energy source for transport. Similarly, bat and bird guano constitutes major organic matter input in many mangroves. In the Man-of-War Cay (Belize), shipworm borer (*Teredo bratschi*) activities are high especially on the stakes of mangroves owing to nutrient enrichment by guano deposition (ammonia, nitrate and phosphate) (Kohlmeyer *et al*., 1995). Such unusual situation in marine habitats may have different food web complexity and the dynamics of energy transfer by fungi needs further exploration.

Studies in the Arabian Sea: Occurrence of lignicolous filamentous marine fungi (12 ascomycetes and 6 mitosporic fungi) from India (Tamil Nadu coast) was the first report from India by Raghukumar (1973). Subsequently, studies on marine fungi continued in the east coast and west coast simultaneously. **Table 2** provides selected contributions on marine fungi from the west coast of India. Different facets of marine fungi studied include diversity, distribution and occurrence in deep-sea, endophytes, decomposition, enzymes and bioremediation. A few reviews, books and monographs consolidated the studies carried out in the west coast of India. Raghukumar (2017) contributed a book dealing with world marine fungi in the coast and oceanic regions, which consists of several studies carried out in India. Recently, Indian marine fungal database has been constructed by Kiran Ramachandra Ranadive and Neta Jagtap from Maharashtra (http://www.fungifromindia.com/fungiFromIndia/databases/IMFD/nextPage.php?id=references.php).

Similar to freshwater hyphomycetes, studies on the diversity and distribution of marine fungi in the west coast of India dominates other studies (**Table 2**). These studies include diversity of fungi in several marine habitats like coastal sand dunes, mangroves and small islands of the west coast. Several new species of marine fungi have been described from the west coast of India (Sridhar, 2013; Borse *et al*., 2017). Most commonly studied substrates to assess marine fungi are the woody litter, while some studies also assessed the animal remains in mangroves and beaches. The woody litter and animal substrates needs long-term incubation to assess the colonized marine fungi. Assessment of deep-sea for fungi is a new venture, which has expanded our knowledge on the diversity, occupation of extreme habitats and their

Table 2. Selected literature on marine fungi of the west coast of India.

Diversity and distribution	Prasannarai & Sridhar (1997, 2000-2001, 2001, 2003), Ananda *et al*. (1998), Prasannarai *et al*. (1999), Maria & Sridhar (2002, 2003a, 2004), Ananda & Sridhar (2004), Sridhar & Maria (2006), Karamchand *et al*. (2009), Jebaraj *et al*. (2010), Raghukumar *et al*. (2010), Khan & Manimohan (2011), Singh & Raghukumar (2014)
Deep-sea	Damare *et al*. (2006), Jebaraj *et al*. (2010), Singh & Raghukumar (2014)
Endophytes	Ananda & Sridhar (2002), Maria & Sridhar (2003b), Anita & Sridhar (2009), Anita *et al*. (2009), Shreelalitha & Sridhar (2015)
Decomposition	Maria *et al*. (2006), Ananda *et al*. (2008), Sridhar *et al*. (2010b)
Enzymes	Raghukumar *et al*. (1994, 1999, 2004, 2008), Damare *et al*. (2006),
Bioremediation	D'Souza-Teilo *et al*. (2006), Raghukumar *et al*. (2008), Verma *et al*. (2010)
Checklist	Borse *et al*. (2013)
Review	Sridhar (2009b, 2013), Sridhar *et al*. (2012), Singh & Raghukumar (2014)
Book/Monograph	Raveendran & Manimohan (2007), Borse *et al*. (2012, 2017), Raghukumar (2017)
Database	http://www.fungifromindia.com/fungiFromIndia/databases/IMFD/nextPage.php?id=references.php

biotechnological potential (Damare *et al*., 2006; Raghukumer *et al*., 2010). Small islands provide diverse intertidal and marine habitats suitable for colonization of fungi owing to their specific topography and vegetation, which are ideal to test several mycological hypotheses.

Studies on the endophytic fungi in the west coast mainly concentrated on mangrove vegetation (mangrove and mangrove associates), which provide a variety of substrates (leaves, bark and roots) and zonation for fungal colonization (canopy, tidal zones and roots in sediments). The endophytic fungi in mangrove vegetation composed of a mosaic of fungi (terrestrial, mangrove and marine) and some are also plant pathogens (e.g. Ananda and Sridhar, 2002). There are no studies on the fungi occurring in stemflow, throughfall and tree holes of mangrove tree species.

Several enzymes have been assessed from the marine fungi of the west coast (e.g. lignin modifying enzymes, xylanases and laccases) (**Table 1**). Many such enzymes besides biotechnological potential (paper, pulp and textile industries) they are useful in bioremediation (e.g. dye degradation and treatment of industrial effluents). Decomposition of organic matter in marine environment is crucial for energy flow to higher trophic levels. A few studies are available on the degradation of leaf litter, sedge and woody litter in the mangroves (e.g. Maria *et al*., 2006; Ananda *et al*., 2008; Sridhar *et al*., 2010b). These studies in addition to document the fungal diversity, recorded differences in the dynamics of mass loss, chemical changes and fungal colonization during the exposure period. Another study has evaluated the fungal association on the intertidal wood and introduced wood panels in a harbour of the southwest coast (Prasannarai *et al*., 1999). However, so far decomposition of seaweeds and seagrass has not been studied. Another area of immense importance is the study of diversity of marine lichen-forming fungi as reported by Hawksworth (2000).

Even though marine fungi are diverse in the west coast and Arabian Sea, their metabolites, biotechnological and bioremediation potential have been less explored. Some of the important areas attracted meagre attention are the studies on association of fungi with marine fauna, deep-sea mycology, endophytic association and decomposition of organic matter. Human interference in marine habitats resulted in increased plastic input to the mangroves, coast and ocean ecosystems. There is a wide scope to isolate and harness the power of plastic degrading fungi from the marine environments.

MACROFUNGI

Macrofungi constitute an important non-timber forest resource worldwide. They consists of mainly *Ascomycota* and *Basidiomycota*, in addition a few *Zygomycota* have also been recognized (Mueller *et al*., 2007). Their prime functions to stabilize ecosystem are crucial through decomposition of organic matter, turnover of nutrients, restoration of soil productivity and develop mutualistic association (Deighton, 2003; Schmit, 2005). The functional role of macrofungal communities investigated in forest ecosystems include association with woody materials, mutualistic relationship as ectomycorrhizas and decomposition as saprophytes (Winterhoff, 1992). The macrofungal communities in the forest ecosystem are mainly controlled by a number of climatic, abiotic factors and biotic factors (Kutszegi *et al*., 2015). Macrofungi are valuable source of nutrition, food supplements and medicine (Wani *et al.*, 2010; De Silva *et al*., 2013; Donnini *et al*., 2013). Even though nearly 1,000 species of ectomycorrhizal fungi are edible, a few species have been commercially harnessed (Hall and Zambonelli, 2012; Donnini *et al*., 2013). Macrofungi have attracted the attention of mycologists owing to their edibility, mutualistic association and production of bioactive metabolites (e.g. enzymes, toxins, metabolites, hallucinogens, pharmaceuticals and plant growth promoters).

Diversity: Based on the flowering plant species/macrofungus ratio, Mueller *et al*. (2007) estimated global macrofungi ranging from 53,000 to 110,000, which is close to the macrofungal estimate by Hawksworth (2001). However, Rossman (1994) and Hawksworth (2019) are of the opinion that 10% of all fungi exists (2.2-3.8 million) as macrofungi, which is ranging between 220,000 and 380,000. The major input on macrofungal research comes from the European and North American continents (Kutszegi *et al*., 2015). According to Mueller *et al*. (2007), about 35,000 species are unknown based on the published and unpublished species lists worldwide (North America; Mexico, central America and Caribbean; tropical South America; temperate South America; Antarctica; Temperate Asia; Africa; Europe; museums; botanical garden of Oslo; tropical Asia; Hawaii; Australia; New Zealand; New Caledonia; New Guinea). Certainly, the known and unknown number of macrofungi further shoot up as several regions of the world are unexplored or underexplored. The diversity of macrofungi in a given region depends on the availability of different substrates (**Box 3**) as well as suitable ecological conditions.

Mutualistic Association: Up to 90% of plant species are in association with mycorrhizal fungi (Suz *et al*., 2018). The ectomycorrhizae (EM) is one of the principal groups among macrofungi which engage in root colonization of tree species worldwide. Three important groups of fungi composed of EM fungi are *Ascomycota*, *Basidiomycota* and *Mucoromycota*. The EM fungi are well known to develop external mantle in root surroundings, on penetration of hyphae develop the Hartig net in the cortex and epidermal intercellular spaces (Smith and Read, 2008). The main tasks of EM fungi are to augment more surface for absorption, acquisition of nutrients

Box 3. Diverse substrates support the macrofungi

Soil:	Humus, lateritic, sandy, loamy, termite mound, anthill and compost
Root:	Below ground and exposed
Wood:	Coarse, medium, fine and bark
Leaf litter:	Petiole, midrib, veins and lamina
Dung:	Monogastic and polygastric
Insect:	Adult, larva, dead, carapace and nest

and develop resistance against pathogens in the rhizosphere (Agerer, 2006). Such association with host plant species facilitates the mycorrhizal fungi to absorb organic compounds as well as energy sources (Bonfante and Genre, 2008). According to an estimate, 20,000 to 25,000 EM fungi are associated with 6,000 tree species (Rinaldi *et al*., 2008; Tedersoo *et al*., 2010). The major studies have been performed on EM fungi in the temperate and subarctic ecosystems (Smith and Read, 2008). A largest number of EM fungi have been reported from the Holarctic regions compared to Austral and tropical regions (Tedersoo *et al*., 2010). There is a postulation that EM fungi are Gondwanan origin and they are not capable to disperse from the endemic tree species as well as owing to their host-specificity.

Mutualistic association between fungi with termites is confined to Africa and Asia. It is highly fascinating and such association results in degradation and enrichment of plant materials required for termites. In Africa and Asia up to 330 species belonged to the subfamily *Macrotermitinae* involved in cultivation of termitomycetes (Müller *et al*., 2005). About 40 species of *Termitomyces* are known to symbiotically associate with termites (Kirk *et al*., 2001). Termitomycetes are represented by tiny *Termitomyces microcarpus* (2 cm pileus) to giant *T. titanicus* (1 m pileus) (Tibuhwa *et al*., 2010). Termitomycetes serve as alternative source of human nutrition against plant and animal source. In addition, they are also known for their antioxidant properties and production of extracellular enzymes (serve as additives in food, bread leavening, silage processing and clarification of fruit juices) (Ghorai *et al*., 2009). Termites are also in association with the ruminant dung, which result in tripartite relationship among termites, ruminants and termitomycetes (Karun and Sridhar, 2013).

Unlike lignocellulosic materials, which are relatively poor in nutritional quality will be enriched by the herbivores yielding enriched dung, thus attracted by a variety of detritivores like beetles and millipedes. Other than ruminant dung, there seems to be meager or no studies pertaining to the macrofungal relationship with dung or dung-like resources (e.g. non-ruminant dung, bird guano, bat guano, worm casts and insect droppings). Similar to ecology of termites and termitomycetes, further insights are necessary to comprehend the positive and or negative relationship of macrofungi (other than *Cordyceps* and allied species) with insects. For example, 34 species of the attine genus *Apterostigma* are known to cultivate the coral fungi or clavarioid fungi belonging to *Pterulaceae* (Mehdiabadi and Schultz, 2010).

Studies in the Western Ghats and West Coast: Several studies pertaining to the diversity, distribution, taxonomy, ecology, nutritional and bioactive potential of macrofungi have been undertaken in the Western Ghats and west coast of India (**Table 3**). More emphasis has been laid on the diversity, distribution and taxonomy of macrofungi. Several books and monographs facilitate identification of macrofungi in the Western Ghats and west coast of India. However, consolidated information is available through a few reviews. Checklists are available only for *Agaricales* and *Aphyllophorales* (Ranadive *et al*., 2011; Farook *et al*., 2013; Ranadive and Jagtap, 2016). Ranadive *et al*. (2015) have developed a database for wood rotting *Aphyllophorales*.

A few studies have been carried out exclusively on the ectomycorrhizal fungi (**Table 3**). So far, about 150 species belonging to 34 genera of ectomycorrhizal fungi (with known host tree species) associated with native and exotic tree species have been reported from the Western Ghats. Eight host trees belonging to the family *Dipterocarpaceae* harboured up to 80% of ectomycorrhizal fungi reported. The tree species *Vateria indica* harboured the highest number of ectomycorrhizal fungi followed by two *Hopea* spp. and *Diospyros malabarica*. The most dominant genus was *Inocybe*, followed by the genera *Russula* and *Amanita*. A recent study on the impact of fire (moderate) in scrub jungles has diminished ectomycorrhizal fungi from 54% to 15% (Greeshma *et al*., 2016). Interestingly, many ectomycorrhizal fungi are also edible and or medicinal, thus their ecosystem services are manifold. Several typical ectomycorrhizal fungi have been reported without connecting or identifying the host tree species. Evaluation of host-fungus relationship of ectomycorrhizal fungi in the Western Ghats and west coast is very crucial to make progress in sylviculture, polyculture and agroforestry.

Studies on the nutritional properties of wild mushrooms have attracted attention recently (**Table 3**). About 51 species (in 23 genera) have been reported as edible exclusively based on the ethnic knowledge of tribals in the Western Ghats (Karun and Sridhar, 2017). Although several *Amanita* are poisonous, one of the *Amanita* species found in the scrub jungles of west coast form ectomycorrhizal association with several tree species and is reported to be ethnically edible in tender stage

Table 3. Selected literature on macrofungi of the Western Ghats and west coast of India.

Diversity and distribution (see also: Ectomycorrhizae below)	Manimohan *et al* . (1988 , 1995, 2004, 2006, 2007), Manimohan & Leelavathy (1988, 1989a, b), Bhavanidevi (1995), Natarajan (1995), Vrinda *et al*. (1997a, b,c, 1998, 2000, 2003), Thomas *et al* . (2001, 2002), Thomas & Manimohan (2003), Natarajan *et al*. (2005a), Pradeep & Vrinda (2005, 2007), Brown *et al*. (2006), Leelavathy *et al*. (2006), Swapna *et al* . (2008), Deepa *et al* . (2009), Kumar & Manimohan (2009a, b), Bhosale *et al* . (2010), Ranadive *et al* . (2011), Karun & Sridhar (2013, 2014a, b, 2015, 2016, 2017), Pradeep *et al*. (2013, 2016), Karun *et al*. (2014, 2018a), Mohanan (2014), Senthilarasu (2014), Borkar *et al* . (2015), Ghate & Sridhar (2016a, c), Greeshma *et al* . (2016), Senthilarasu & Kumaresan (2016), De Souza & Kamat (2017) , Dattaraj *et al*. (2018), Jagadish *et al*. (2019).
Ectomycorrhizae	Natarajan & Raman (1983), Natarajan *et al* . (2005b), Mohan (2008), Pradeep & Vrinda (2010), Mohanan (2014)
Nutrition	Johnsy *et al* . (2011), Sudheep & Sridhar (2014), Ravikrishnan *et al* . (2015), Ghate & Sridhar (2017a, 2019), Greeshma *et al*. (2018a), Karun *et al*. (2018b), Pavithra *et al*. (2018)
Bioactive properties	Puthusseri *et al* . (2010), Pavithra *et al* . (2016), Karun *et al* . (2016b, 2017), Ghate & Sridhar (2017a), De Souza *et al*. (2018), Greeshma *et al*. (2018b)
Checklist	Natarajan *et al*. (2005c), Ranadive *et al*. (2011), Farook *et al*. (2013), Ranadive & Jagtap (2016)
Review	Riviere *et al*. (2007), Thiribhuvanamala *et al*. (2011), Ranadive (2013), Ranadive *et al*. (2013), Sridhar (2018)
Book/Monograph	Sathe & Daniel (1980) , Sathe & Deshande (1980), Leelavathy & Ganesh (2000), Mohanan (2011), Aravindakshan & Manimohan (2015), Hakimi *et al*. (2013), Latha & Manimohan (2017)
Database	Ranadive *et al*. (2015)

(Greeshma *et al*., 2018a). Similarly, ectomycorrhizal *Astraeus hygrometricus* and *A. odoratus* found in the foothill of the Western Ghats and the west coast are also known for its nutritional and medicinal potential (Pavithra *et al*., 2015, 2018). Termitomycetes are highly preferred edible mushrooms in the Western Ghats as well as west coast. The Western Ghats represent 50% of the species of *Termitomyces* recorded worldwide (40 spp.) (Karun and Sridhar, 2013). Major inventory on termitomycetes was prepared in Goa (35 spp.) followed by Kerala (15 spp.), Karnataka (9 spp.), Maharashtra and Tamil Nadu (2-3 spp.) (see De Souza and Kamat, 2017). *Termitomyces microcarpus* is widely distributed in the Western Ghats. Some termitomycetes of the Western Ghats and west coast (*Termitomyces clypeatus, T. globulus, T. umkowaan*) possess low lipid, high protein, high fibre and many essential amino acids to cater the needs of human nutrition and health (Sudheep and Sridhar, 2014; Karun *et al*., 2018b; Ghate and Sridhar, 2019).

Studies on the bioactive potential of a few wild macrofungi from the Western Ghats and west coast have been carried out (**Table 3**). In addition to nutritional potential, several macrofungi of the Western Ghats and west coast are also known for their pharmaceutical potential (e.g. *Amanita* sp., *Astraeus hygrometricus*, *Lentinus squarrosulus* and *Termitomyces clypeatus*). These mushrooms with nutraceutical potential possess many bioactive components (phenolics, flavonoids, vitamin C, β-carotene, phytic acid, lycopene and trypsin inhibition activity) and showed potent antioxidant properties (Pavithra *et al*., 2016; Ghate and Sridhar, 2017a; Greeshma *et al*., 2018b). Bioactive components and antioxidant potential of four macrofungi of the Western Ghats showed their ability to combat the cardiovascular diseases (Karun *et al*., 2017). It is interesting to note that the elephant dung-inhabiting fungi of the Western Ghats are known for their hallucinogenic potential (Manimohan *et al*., 2007; Karun and Sridhar, 2015). Recently, sulphur rich melanin pigment has been purified and characterized from the edible mushroom *Termitomyces albuminosus* occurring in Goa region (De Souza *et al*., 2018).

There are several gaps in our knowledge on macrofungi of the Western Ghats and west coast of India. There is ample scope to evaluate the macrofungal association with different habitats and with different tree species. Ethnic knowledge is valuable in identifying edible and poisonous macrofungi. The basis of ethnic identification of edible mushrooms rests on the host tree species supporting the growth of a specific mushroom. Recently, several *Cordyceps* and allied species have been reported from the Western Ghats and west coast of India (see Dattaraj *et al*., 2018). There is a wide scope to protect habitats supporting the growth of *Cordyceps* in the Western Ghats and west coast of India to harness their biomedical potential.

THE INDIAN SUBCONTINENT

The Indian Subcontinent (66º-98ºE, 8º-36ºN) as seventh largest country in the world possesses an area of 3.3 m k^2 with 100 m ha of mountains, 30 m ha of arid zones and 8,000 km coastline (Singh and Chaturvedi, 2017). The geographic and climatic diversity ranged from tropical to arctic with mountains, plains and wetlands as major ecosystems. It encompasses 24.2% forest cover (17,500 angiosperms), inland waters (rivers: 14 major and 44 medium rivers with several tributaries; natural lakes: 0.72 m ha; reservoirs: 3.15 m ha; seasonal shallow waters: 1.5 m ha), coastal wetlands (mangroves, estuaries and coast: 8 m ha) and coral reefs (2,400 km^2).

Being one of the 12 megabiodiversity and megagene centers, the Indian Subcontinent possesses 10 biogeographic zones, 25 biogeographic provinces and more than 400 biomes. The trend of increased biological diversity towards the equator has been ascertained repeatedly, which is highly applicable to the Indian Subcontinent owing to its strategic geographic and climatic position. Increased latitudinal gradient of diversity is owing to high resource availability to consumers resulting in coexistence of a large number of species (Frank *et al*., 2018). **Fig. 3** shows 10 biogeographic zones of the Indian Subcontinent with their area in per cent. Although the area of Western Ghats (4%) and Himalayas (5.6-6.4%), which is substantially lower than other geographic zones (e.g. Deccan peninsula, 42%; and semi-arid zone, 46.6%), these are the hotspots of biodiversity due to the existence of endemic and endangered species. **Table 4** provides different ecoregions of 10 biogeographic zones. Interestingly, although the Nicobar Island is tiny, it is one of the hotspots of biodiversity of the Indian Subcontinent. Each of the ecoregions represents uniqueness in aquatic, soil, forest, grassland, mountains and desert ecosystems. The biogeographic classification has not encompassed many ocean resources especially coral reefs and deep sea ecosystems in the boundary of the Indian Subcontinent. Considering the geographical setup, climatic conditions, variety of ecoregions and ecosystems, it is not surprising that the Indian Subcontinent consists of diverse mycota.

ENDEAVORS

Investigations on fungi and allied organisms represent a major way forward of progress in mycology of the Indian Subcontinent. Existing climatic and geographic zones in India provide shelter for almost all types of fungal groups. According to an estimate, based on the association of fungi with vascular plants in India at the ratio of 1:6 yields about 96,000 fungi, however 28% of them are invented

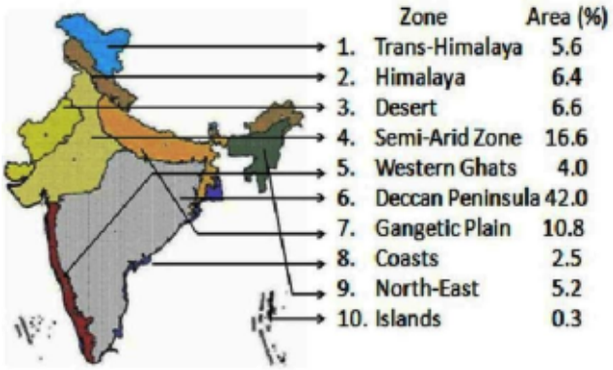

Fig. 3. Ten biogeographic zones of the India Subcontinent.

Table 4. Ecoregions of 10 biogeographic zones of the Indian Subcontinent (see Figure 4 for details of geographic boundaries; *, hotspots of biodiversity).

	Biogeographic zone	Ecoregion
1	*Trans-Himalaya	a) Ladakh Mountains b) Tibetan Plateau c) Sikkim
2	*Himalaya	a) North-West Himalaya b) West Himalaya c) Central Himalaya d) East Himalaya
3	Desert	a) Thar b) Katchchh
4	Semi-arid Zone	a) Punjab Plain b) Gujarat Rajputana
5	*Western Ghats	a) Konkan Region b) Malabar Region
6	Deccan Peninsula	a) Central Highlands b) Chotta Nagpur c) Eastern Highlands d) Central Plateau e) Deccan South
7	Gangetic Plain	a) Upper Gangetic b) Lower Gangetic
8	Coasts	a) West Coast b) East Coast
9	North-East Zone	a) Brahmaputra Valley b) North-East Hills
10	Islands	a) Andaman b) *Nicobar c) Lakshadweep

(Manoharachary *et al*., 2005; Hawksworth, 2019) suggests the wide gap in our knowledge on the Indian mycota. The vascular plants exist in climatic and geographic conditions of India (tropical, sub-tropical and Himalayan arctic weather), the plant/fungus ratio 1:6 may be an underestimate and warrants reassessment. **Box 4** lists some of the mycologically underexplored or neglected ecosystems, food webs and substrates. Attention to explore such gray areas will expand our knowledge on the role of fungi in the ecosystem functions and ecosystem services.

Box 4. Some of the neglected ecosystems, food webs and substrates

Ecosystem	Food web	Substrate
Aero-aquatic	Freshwater	Weeds
Intermittently aquatic	Marine	Insects
Thermal springs	Islands	Humus
Sand dunes	Marshes	Compost
Tree canopy	Mangroves	Dung
Termite mounds	Detritus	Recalcitrants
Anthills		
Nest		
Caves		

The most important challenge in ecological investigations is to establish the connection between the biological diversity with ecosystem functions (Cardinale *et al*., 2000). In the recent past, several global collaborations have been initiated to explore the fungal resources, diversity, distribution, phylogeny, ecology and ecosystem services. Such partnerships or networks are not prominent in the Indian situation, which tends to bridge the gaps in our knowledge on the significance of mycota. **Fig. 4** provides major divisions of mycological investigations undertaken or needs further emphasis in India.

Each division or subdivision needs collaborative efforts to acquire comprehensive knowledge on fungal resources and their significance. Ethnic knowledge (tribal or traditional) has been largely ignored pertain to edible, poisonous and medicinal mushrooms. Strategies followed by the tribals (nutrition, medicine and toxicity) are important to understand the value of mushrooms and future studies need to evaluate the authenticity. Nonetheless, part of the share should be set out to tribal upliftment from the benefit derived by the ethnic knowledge.

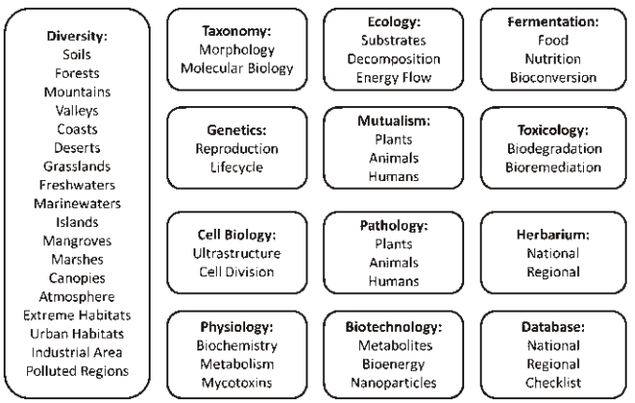

Fig. 4. Segments of mycological research in the Indian Subcontinent.

CONCLUDING REMARKS

One of the most important challenges of the 21st millennium is the assessment of global biodiversity for sustainable exploitation and conservation for future benefits. Similar to plants and animals, understanding the fungal resource and functions are crucial to link or broadcast their diversity with ecosystem services. Evaluation of fungal resources or their roles could be achieved by different approaches such as ecosystem-based or niche-based (e.g. forests, aquatic habitats, soils, islands and coasts), host-based (e.g. trees, crops, weeds and animal species) and substrate-based (e.g. leaf litter, woody litter, dung, soil, humus, compost and insect). Considering the importance of fungi in the ecosystem services, strategies of conservation could be implemented, which may differ from region to region. Collaborative efforts will also pave way to open new doors in mycology of the

Indian Subcontinent. Being diverse in climatic, geographic and ecological conditions, collaborative ventures on mycology in the Indian Subcontinent itself will be highly rewarding by offering more or less global replica of diversity and role of mycota. On comparison of studies on freshwater fungi, marine fungi and macrofungi in the Western Ghats and west coast, the major emphasis has been laid on the assessment of diversity, phylogeny and distribution. In view of exploiting these diverse fungi especially for their metabolites, enzymes and bioremediation potential, it is obvious to have a strong collaboration and network. Besides, the collaborative effort of scientific networks will facilitate to bridge the gaps by precise comparisons (e.g. Himalayas vs. Western Ghats; east coast vs. west coast; Arabian Sea vs. Bay of Bengal; islands of west vs. islands of east; lateritic scrub jungles of western vs. eastern region). Recent advances especially in fungal taxonomy and phylogeny in India resulted in addition of many new species of fungi to the global list (Willis, 2018). Similarly, advances in fungal biotechnology, nanotechnology, pharmaceuticals and metabolomics are also in progressive phase. Attention need to be addressed seriously on the enrichment of repositories, databases and sequences of fungi for future developments. As focused on culture-dependent fungi, culture-independent fungi could also be exposed by molecular tools. The conventionally practiced morphological perspectives with the recent advances in molecular approaches demand further mycological progress in India by working hand in hand with microscopes and PCR machines.

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Taxonomy of Arbuscular Mycorrhizal Fungi

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ABSTRACT

Taxonomy of arbuscular mycorrhizal fungi (AMF) created problems mainly being obligate symbionts. In this review, the history and development of taxonomy of AMF is addressed. In the initial discovery period (1845-1974) the sporocarp-forming species were described and a classification for these fungi was proposed. This was followed by alpha taxonomy period (1975-1989) in which time solid morphological basis for species identification was developed resulting in description of several new species. The next cladistic period (1990-2000) resulted in the first cladistic classification of AMF based on phenotypic characters. The present phylogenetic synthesis period (2001- till date) proposed a new classification based on genetic characters using sequences of multicopy rRNA genes. In conclusion it can be said that the taxonomy of AMF belonging to the phylum *Glomeromycota* has generated considerable confusion and controversy among mycologists working with this important plant symbionts.

KEYWORDS: AM fungi, Classification, *Glomeromycota*, Taxonomy

INTRODUCTION

Arbuscular mycorrhiza (AM) is a symbiosis between fungus and the root, in which the fungus supplies the root with nutrient and the root supplies the fungus with carbon (Harley and Smith, 1983). Intensive research has been carried out throughout the world on the role of these fungi in improving growth and yield in plants. It is now well-demonstrated that these fungi can substantially increase the uptake of mineral nutrients, particularly P, drought tolerance and resistance to soil-borne plant pathogens (Bagyaraj, 2011). Formerly, all fungi forming arbuscular mycorrhizal associations were placed under the genus *Endogone*. The genus *Endogone* (Gr. Endo=inside; gone=seed) was first described by Link in 1809 which form mycorrhizal associations with higher plants and also produce hypogeous sporocarps in soil. The history of AMF taxonomy can be grouped under different periods as suggested by Stürmer (2012).

THE DISCOVERY PERIOD (1845-1974)

In 1845, Tulasne and Tulasne published a brief description of the species *Glomus microcarpus* and *Glomus macrocarpus* in a manuscript written in Latin and later transferred the two species to the genus *Endogone* (Tulasne and Tulasne, 1851). The genus *Sclerocystis* was proposed by Berkeley and Broome (1873) to encompass species forming spores in small sporocarps. Both genera were classified in the family *Endogonaceae*, order *Mucorales*. Interestingly, *Glomus* and *Sclerocystis* were described before the term "mycorrhiza" was coined by Frank in 1885. The first monograph of the family *Endogonaceae* treated in the order *Mucorales* (*Zygomycetes*) has been prepared by Thaxter (1922). Fungi of this family were located in four genera producing spores in sporocarps: *Endogone* Link: Fries, *Glaziella* Berk., *Sclerocystis* Berk. & Br., and *Sphaerocreas* Sacc. & Ellis. In 1935, Zycha transferred the one species of *Sphaerocreas* to *Endogone*. The existing genera included both chlamydosporic and zygosporic species. Thaxter (1922) and Godfrey (1957) considered chlamydosporic species to be anamorphs of those producing zygospores, following the finding of both types of spores in sporocarps of *Glomus fasciculatum* (at that time known as *Endogone fasciculata* and *E. microcarpa*).

During this period, the widely used method of wet sieving to extract sporocarps and non-sporocarpic spores from soils was developed by Gerdemann and Nicolson (1963), which led to an increase in taxonomic activity. Collaboration between Gerdemann and Trappe resulted in the classical publication "The *Endogonaceae* in the Pacific Northwest" by Gerdemann and Trappe in 1974. The authors in this publication proposed a classification for fungal taxa including 30 species forming arbuscular mycorrhiza. They recognized *Glomus* as a valid genus distinct from *Endogone*, as previously proposed by the Tulasne brothers, and erected two new genera, *Acaulospora* and *Gigaspora*, which had also been shown to form arbuscular mycorrhizal associations. In this classification (**Table 1**), the genera *Glomus*, *Sclerocystis*, *Acaulospora*, and *Gigaspora* were placed in the family *Endogonaceae*, order *Mucorales*, phylum *Zygomycota*, together with *Endogone* and two other genera, *Glaziella* and *Modicella*, which were not known to form arbuscular mycorrhizal associations. Later, *Modicella* was transferred to the family *Mortierellaceae* by Trappe (1982) and *Glaziella* was transferred to the *Ascomycota* by Gibson *et al*. (1986). A new genus, *Complexipes*, erected by Walker (1979) and tentatively placed in *Endogonaceae* was later transferred to *Discomycetes* (Trappe, 1982). The classification by Gerdemann and Trappe (1974) was important for the taxonomy of these organisms, and it provided a sound basis for systematic knowledge during several years to follow.

THE ALPHA TAXONOMY PERIOD (1975-1989)

This period contributed to the establishment of a solid morphological basis for identification and classification of glomeromycotan fungi. The 15 years are characterized by: (1) the proposal of several new genera and families, (2) a profuse description of new species, and (3) the proposal for standardization of phenotypic characters of arbuscular mycorrhizal fungal (AMF) spores to describe new species. New taxa forming arbuscular mycorrhizal associations were proposed based on living and fossil evidence. A new genus, *Entrophospora*, was erected by Ames and Schneider (1979) based on the observation that the formation of a "saccule" prior to spore development. Walker and Sanders (1986) differentiated between species of *Gigaspora* proposed by Gerdemann and Trappe (1974) based on whether spore

germination occurred through a flexible "shield" on an inner flexible wall or directly through the spore wall, and they used the former character to define a new genus, *Scutellospora*. During this period, a large number of new species were described. Walker in the UK established an interest in this group of fungi, initially publishing with Trappe (USA), but later also establishing collaboration with several other researchers, principally Koske (USA). After initial work with Nicolson (UK), Schenck (USA) also established a group describing new species, and partly stemming from work with Schenk, Spain and Sieverding published new species from their work in Colombia. Towards the end of this period, Blaszkowski (Poland) and Morton (USA) began to publish new species based on morphological characteristics.

Only 12 years after the monograph by Gerdemann and Trappe (1974), the number of described glomeromycotan species had jumped to 77 (Trappe, 1982), and 6 years later, Schenck and Pérez (1988) listed 126 species. In parallel, different keys for AMF species identification developed, such as the synoptic key of Trappe (1982), the dichotomous key of Hall and Fish (1979), and Hall (1984), and keys for groups of species (e.g., Koske and Walker, 1985). A significant step forward for those interested on AMF taxonomy and identification at the time was publication of the "Manual for the Identification of VA Mycorrhizal Fungi" (Schenck and Pérez, 1988) which compiled all summary species descriptions. All descriptions during this time were based on morphological features of spores. Spore subcellular structures, which are diverse, largely accounted for most differences between species. Walker also proposed a "murograph" that consists of a graphic representation to depict the different wall types and groups found in a spore. Berch (1986) in her treatise on the *Endogonaceae* suggested the use of the word "wall layers" instead of "wall". The different wall layers described were laminated, evanescent, membranous, amorphous, coriaceous and germinal. Towards the end of this period, Morton (1988) critically evaluated all morphological criteria used to classify and identify AMF species and suggested some approaches to clarify taxonomy concepts.

THE CLADISTICS PERIOD (1990-2000)

This period is marked by a new classification and the entry of molecular biology into systematics of glomeromycotan fungi. It is characterized mainly by: (1) proposal of a cladistic classification for AMF based on phenotypic characters, (2) description of new taxa based on fossil records, (3) proposal of a spore development model with re-evaluation of terminology for spore subcellular characters, and (4) use of genetic characters to define taxa and elucidate evolutionary relationships. One of the important landmarks in these 11 years was the first cladistic analysis of glomeromycotan fungi and the proposal of a new classification. Morton (1990) proposed two main clades, one consisting of *Gigaspora* and *Scutellospora* species and the other harboring *Glomus*, *Sclerocystis*, *Acaulospora*, and *Entrophospora*. This cladistic analysis, together with additional information from spore ontogeny and mode of spore germination, formed the basis for a radical change in classification (Morton and Benny, 1990) (**Table 1**). Genera of AMF were removed from the order *Endogonales* and placed in the newly erected order *Glomerales* (published as *Glomales*) in the families *Glomeraceae* (*Glomus* and *Sclerocystis*), *Acaulosporaceae* (*Acaulospora* and *Entrophospora*), and *Gigasporaceae* (*Gigaspora* and *Scutellospora*). *Glomeraceae* and *Acaulosporaceae* were hypothesized to be closely related and placed in the sub-order *Glomineae* and the family *Gigasporaceae* in the sub-order *Gigasporineae*. This was the first new classification proposed since Gerdemann and Trappe (1974) had considered that AMF should remain in the phylum *Zygomycota* some 25 years earlier.

Morton (2000) proposed that the arbuscular mycorrhizal symbiosis had arisen during two distinct periods rendering the order *Glomerales* polyphyletic: *Glomineae* and *Gigasporineae* would represent two evolutionary branches. Evidence to support this hypothesis is related to the mode of spore formation (Franke and Morton, 1994), morphology of fungal mycelium (Brundrett and Kendrick, 1990), types of infective propagules (Biermann and Linderman, 1983; Jasper *et al*., 1989), and cell wall composition (Gianinazzi-Pearson et al., 1994). The classifications of Gerdemann and Trappe (1974) and Morton and Benny (1990) did not state clearly into which class the AMF species should be included: *Endogonales* in the former and *Glomerales* in the latter were left in the class *Zygomycetes*. Cavalier-Smith (1998) later proposed that fungal species establishing (vesicular) arbuscular mycorrhizas with plants could be grouped in a new class, the *Glomomycetes*, within a new phylum, *Archemycota*. The number of new species described in this "cladistics period" totaled one third of that described in the previous "alpha-taxonomy" period. One of the main events during these 11 years was the use of SSU gene sequences to elucidate evolutionary relationships among taxa within the order *Glomerales*. This period ends with the identification of two ancestral clades based on rDNA sequences (Redecker *et al*., 2000).

THE PHYLOGENETIC SYNTHESIS PERIOD (2001 TO TILL TODAY)

This ultimate period is characterized by: (1) the proposal of a new classification based solely on genetic characters (SSU rRNA gene), (2) description of new taxa based on the fossil record, and (3) the creation of new taxa and a new classification based on a combination of phenotypic and genetic characters. The most important event in this period has been the naming by Schüßler *et al*. (2001) of a new phylum within the kingdom Fungi to group all AMF species. The proposed phylum *Glomeromycota* is based on a phylogenetic analysis of SSU rRNA gene sequences. Four new orders (*Paraglomerales*, *Archaeosporales*, *Diversisporales*, and *Glomerales*) and new families were proposed (**Table 1**). The term "glomerospores" was coined by Goto and Maia (2006) to denominate spores formed by fungi in the *Glomeromycota*. After the new classification by Schüßler *et al*. (2001), the last 10 years have been characterized by descriptions and proposals of new families and genera for both ancient and extant AMF, with some of the taxa proposed still in debate among taxonomists. A more radical expansion of genera and families in the

Glomeromycota was proposed by Oehl *et al*. (2008) based on interpretation of the previous works of Walker *et al*. (2004), De Souza *et al.* (2005), Ahulu *et al*. (2006), and Redecker *et al.* (2007). In the past few years, two distinct classifications have been further proposed for the *Glomeromycota* (**Table 1**), both of which are characterized by a rearrangement of the genus *Glomus sensu lato* shown previously to be polyphyletic by Schwarzott *et al*. (2001). Schüßler and Walker (2010) performed a phylogenetic analysis of glomeromycotan fungi, based on near-full-length SSU rRNA gene sequences and proposed a new family and three new genera. They separated *Glomus* into the genera *Funneliformis*, *Sclerocystis*, and *Rhizophagus* in the family *Glomeraceae* with the remaining species of *Glomus* and *Claroideoglomus* in the family *Claroideoglomeraceae*. *Rhizophagus* was first proposed by Dangeard (1900) and synonymized with *Glomus* by Gerdemann and Trappe (1974). Inspection of the protologue of *Rhizophagus populinus* revealed that this fungus is an AMF species, and it was resurrected by Schüßler and Walker (2010) to harbour AMF species that form large numbers of spores in the roots. Schüßler and Walker (2010) recognized that their phylogeny is incomplete because no living material is available for molecular analyses of many previously described glomeromycotan species. Therefore, some species were retained in their original genus but referred to as "species of uncertain position." Oehl *et al*. (2011) proposed a rearrangement of species in the genus *Glomus sensu lato* and erected the genera *Simiglomus* and *Septoglomus* in the *Glomeraceae*, and *Viscospora* in the *Claroideoglomeraceae*. An evidence-based consensus for the classification of AMF (*Glomeromycota*) was published by Redecker *et al*. (2013). The authors point out that recent publication of numerous new taxa at all level within *Glomeromycota* has created confusion and operational difficulties for those working with AMF. The fungi being obligate symbionts pose problems not encountered for many other groups of organisms. The taxonomy of AM fungi thus has undergone intensive investigation and has experienced lots of controversy and radical transformations. However the characteristics of some of the commonly occurring AM fungi used by majority of mycorrhiza workers to identify them are given below.

Glomus: Spores formed blastically on subtending hyphae, singly, in loose aggregates or in a sporocarp. Vesicles are thin walled and ellipsoid. Intraradical hyphae rarely coiled, with cross-connecting branched hyphae. Mycorrhiza stains darkly. Arbuscules with flared or cylindrical trunks with incremental narrowing of branch hyphae. Spores with spore wall formed by a variable number of layers all originating from the subtending hyphae, no germinal walls differentiated. Germination through the lumen of the subtending hyphae or through the spore wall.

Acaulospora: Spores formed laterally from the neck of a sporiferous saccule which leaves one scar on the spore surface. Vesicles vary in shape with knobs and concavities. Intraradical hyphae straight or coiled near the entry points. Mycorrhiza stains weakly. Arbuscules with flared or cylindrical trunks with incremental narrowing of branch hyphae. Spores with spore wall formed by three layers and two inner germinal walls each with two thin layers that can be adherent. The innermost germinal wall has a beaded surface. Germination through a flexible, plate like germination orb.

Entrophospora: Spores formed within the neck of a sporiferous saccule which leaves two scars on the spore surface. Vesicles, arbuscules, intraradical hyphae and mycorrhizae staining as in *Acaulospora*. Spores with spore wall formed by two layers. Other spore subcellular structures and germination identical to that in *Acaulospora*.

Gigaspora: Spores formed terminally on a bulbous sporogenous cell; auxiliary cells finely papillate or echinulate. No vesicles produced. Intraradical hyphae frequently coiled, especially near entry points, often knobby or with projections. Arbuscules with swollen trunks with abrupt narrowing of branch hyphae. Spores with spore wall formed by two permanent layers, no inner germinal walls differentiated. At germination, a thin layer interspersed with warts differentiate and germ tube grows throughout the spore wall.

Scutellospora: Spores formed terminally on a bulbous sporogenous cell; auxiliary cells almost smooth to knobby. No vesicles produced. Arbuscules and intraradical hyphae similar in morphology to *Gigaspora*. Spores with spore wall formed by two permanent layers and 1-3 inner germinal walls, each with two layers. Germ tube grows from flexible, plate- like germination shield that differentiates on the surface of the last germinal wall.

CONCLUSIONS

Taxonomic and systematic studies of AMF can be traced back to the early works of the Tulasne brothers (1845) and the Thaxter (1922) revision of *Endogonaceae*. In the last 45 years, the classification of this group of fungi has undergone considerable transformations, from being merely descriptive and based solely on spore morphology (Gerdemann and Trappe, 1974) to being based on cladistic analysis of genetic and phenotypic characters. Morton and Benny's (1990) classification is based on the analysis of phenotypic characters (spore morphology and mycorrhizal characters), classifications of Schüßler *et al*. (2001), that of Schüßler and Walker (2010) on genetic characters (sequence variation of the SSU rDNA), and that of Oehl *et al.* (2011) on combined genetic and phenotypic characters. Up to 2001, these fungi were included in one class, one order, three families, and six genera; 10 years later, with the use of genetic characters, they are distributed into one to three classes, four to five orders, 11-14 families, and 18-29 genera depending on the classification scheme followed (**Table 1**). Though some emendations to the classification of AMF (Redecker *et al*., 2013) has come from time to time; Schüßler et al. (2001) classification has been generally accepted by mycorrhiza researchers and mycologists. This classification is valid till today as evidenced by Hibbett *et al*. (2007) in their comprehensive phylogenetic classification of the kingdom Fungi. Identification of AMF can be done by referring to the "Manual for the Identification of VAM Fungi" by Schenck and Perez (1990) and the INVAM website by Joe Morton. http://invam.caf.wvu.edu.

Table 1: Proposals of classification of glomeromycotan fungi within the kingdom Fungi

Phylum	Class	Order	Family	Genera
Gerdemann and Trappe (1974)				
Zygomycota	*Zygomycetes*	*Endogonales*	*Endogonaceae*	*Glomus* *Sclerocystis* *Acaulospora* *Gigaspora*
Morton and Benny (1990)				
Zygomycota	*Zygomycetes*	*Glomerales*	*Glomeraceae*	*Glomus* *Sclerocystis*
			Acaulosporaceae	*Acaulospora* *Entrophospora*
			Gigasporaceae	*Gigaspora* *Scutellospora*
Schüßler *et al.* (2001)				
Glomeromycota	*Glomeromycetes*	*Glomerales*	*Glomeraceae*	*Glomus*
		Diversisporales	*Gigasporaceae*	*Gigaspora* *Scutellospora*
			Acaulosporaceae	*Acaulospora* *Entrophospora*
			Diversisporaceae	*Diversispora*
		Paraglomerales	*Paraglomeraceae*	*Paraglomus*
		Archaeosporales	*Archaeosporaceae*	*Archaeospora*
			Geosiphonaceae	*Geosiphon*
Schüßler and Walker (2010)				
Glomeromycota	*Glomeromycetes*	*Glomerales*	*Glomeraceae*	*Glomus* *Funneliformis* *Sclerocystis* *Rhizophagus*
			Claroideoglomeraceae	*Claroideoglomus*
		Diversisporales	*Gigasporaceae*	*Gigaspora* *Racocetra* *Scutellospora*
			Acaulosporaceae	*Acaulospora*
			Entrophosporaceae	*Entrophospora*
			Pacisporaceae	*Pacispora*
			Diversisporaceae	*Diversispora* *Otospora* *Redeckera*
		Paraglomerales	*Paraglomeraceae*	*Paraglomus*
		Archaeosporales	*Archaeosporaceae*	*Archaeospora*
			Ambisporaceae	*Ambispora*
			Geosiphonaceae	*Geosiphon*
Oehl *et al*. (2011)				
Glomeromycota	*Glomeromycetes*	*Glomerales*	*Glomeraceae*	*Glomus* *Funneliformis* *Simiglomus* *Septoglomus*
			Claroideoglomeraceae	*Claroideoglomus* *Viscospora*
		Diversisporales	*Diversisporaceae*	*Diversispora* *Redeckera* *Otospora*
			Entrophosporaceae	*Entrophospora*
			Acaulosporaceae	*Acaulospora* *Kuklospora*
			Pacisporaceae	*Pacispora*
		Gigasporales	*Gigasporaceae*	*Gigaspora*
			Scutellosporaceae	*Scutellospora* *Orbispora*
			Racocetraceae	*Racocetra* *Cetraspora*
			Dentiscutataceae	*Dentiscutata* *Fuscutata* *Quatunica*
	Archaeosporomycetes	*Archaeosporales*	*Archaeosporaceae*	*Archaeospora* *Intraspora*
			Ambisporaceae	*Ambispora*
			Geosiphonaceae	*Geosiphon*
	Paraglomeromycetes	*Paraglomerales*	*Paraglomeraceae*	*Paraglomus*

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Fungi in cold deep seas: a hot topic*

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ABSTRACT

Deep-sea is characterised by low temperatures, elevated hydrostatic pressures, low nutrients, total darkness and constant salinity. Presently numerous workers are engaged in this topic resulting in several publications, newer insights and contributions to biodiversity and understanding the physiology of deep-sea fungi. We used several techniques to isolate and culture fungi from deep sea sediments collected at ~ 5000 m water depth in the Central Indian Basin (CIB). These were identified by conventional, morphological taxonomy as well as by molecular taxonomy. Fungi were also directly detected in formalin-preserved deep-sea sediments. We also studied culture-independent fungal diversity at several locations in the CIB. Several environmental gene libraries were constructed after amplifying the sediment DNA using universal and fungal-specific internal transcribed spacers (ITS) and universal 18S rDNA primer pairs. This approach resulted in the recovery of several fungal operational taxonomic units (OTUs). A few culturable-phylotypes and several fungal OTUs demonstrated high divergence from the existing sequences in the GenBank. Several of them were new reports for deep-sea sediments indicating hidden treasure of fungal diversity. Physiological characterization of the cultured fungi revealed that most of the fungi were mesophiles, psychrotolerants and barotolerant. Several of them produced cold-active enzymes having tolerance to high salinity. Thus it appears that deep sea sediments are repository for novel fungi. Their role in deep-sea ecosystem needs to be studied in future.

KEYWORDS : Culture dependent, culture independent, deep-sea, fungal diversity, ocean drilling program

INTRODUCTION

Microbial diversity in cold deep sea waters was a mystery a few decades ago, but it is not so anymore with advent of new sampling devices, culturing and identification tools. This was more so for the presence of fungi. Low temperatures, elevated hydrostatic pressure and low nutrient availability mark the deep sea conditions. Barghoorn and Linder (1944) demonstrated the presence of marine fungi in submerged wood. Fungi from water samples from surface to 4500 m in subtropical oceanic waters (Roth *et al.*, 1964), in shells from deep sea waters of, 4610 m depth (Höhnk, 1969) and in submerged wooden panels at 1615-5315 m depth (Kohlmeyer, 1977) were the earliest reports in deep sea. Poulicek *et al.* (1986) demonstrated mycelial fungi growing inside shells of molluscs at 4800 m depth in the Atlantic. Presence of mycelial fungi in calcareous fragments of shells collected from depths of 300-860 m in the Bay of Bengal was demonstrated (Raghukumar and Raghukumar, 1998). After demonstrating their presence and detection, came the era of culturing them under simulated deep-sea conditions and identifying them by morphological taxonomy and molecular techniques. This was followed by directly isolating DNA from deep-sea sediments and water and amplifying using fungal specific markers. Thus these rapid strides led to several reports on fungi in deep-sea sediments from various oceanic sites.

COLLECTION OF SAMPLES FROM DEEP-SEA

Sterilized Niskin bottles are used for collecting water samples from oceanic depths. Sediments are collected by box corers or multiple corer or long gravity cores (Raghukumar *et al*., 2010). Box corer are useful when the topography of the ocean floor is flat. Sub-cores are collected from the centre of such a box core with sterile PVC cylinders. Subsections of various lengths can be extruded from these sub-cores using sterilized scalpel into sterile plastic bags to avoid aerial contaminations **(Fig. 1).** Long gravity cores are also similarly used for getting subsections. Precaution to avoid contamination is of utmost importance. This can be monitored on board a research vessel by washing the samplers with a jet of hot water or steam followed by alcohol sterilization. Samples from hydrothermal vent sites are collected with titanium syringes or robotic arms on submersibles (Burgaud *et al*., 2010).

DIRECT DETECTION AND CULTURING OF DEEP-SEA FUNGI

Seeing is believing. This holds true for proof of fungi in deep-sea, be it sediment, water or calcareous substrata, coral or any other animal shells. Fungal filaments were directly detected in calcareous fragments obtained from deep sea after treating them with EDTA to dissolve calcium carbonate partially followed by staining with fluorescent brightner, Calcofluor white. They were observed under an epifluorescence microscope (Raghukumar and Raghukumar, 1998). Detection of fungi and germinating spores in deep-sea sediments by employing this technique was reported subsequently (Raghukumar *et al.*, 2004; Damare *et al.* ,2006b). Damare *et al.* (2006b) further demonstrated presence of the commonly isolated fungus, *Aspergillus terreus* in deep-sea sediments by immunofluorescence probes developed using polyclonal antibodies. Burgaud *et al.* (2010) reported presence of yeasts in animals from deep-sea hydrothermal vents by using fluorescent *in situ* hybridization (FISH) technique. These studies indicated that detection of fungi in deep-sea materials might remain undetected by conventional microscopy.

ISOLATION OF FUNGI FROM DEEP-SEA SEDIMENTS

It is recommended that fungi are isolated on board the research ship immediately after retrieval of samples from deep-sea to minimize contamination chances from land-based mycota. Strict procedures to avoid aerial contamination

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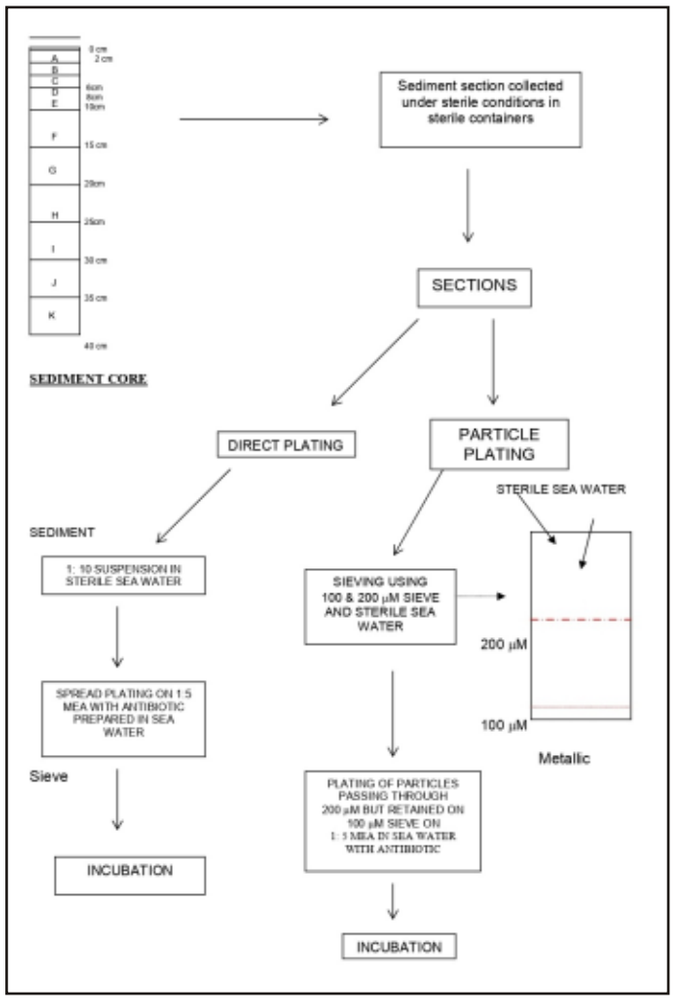

Fig. 1. Schematic diagram showing collection of sediment sample in a core, cutting sections of this core of sediment, collection in sterile bag, plating of sediments in different ways to isolate fungi. Courtesy Samir Damare.

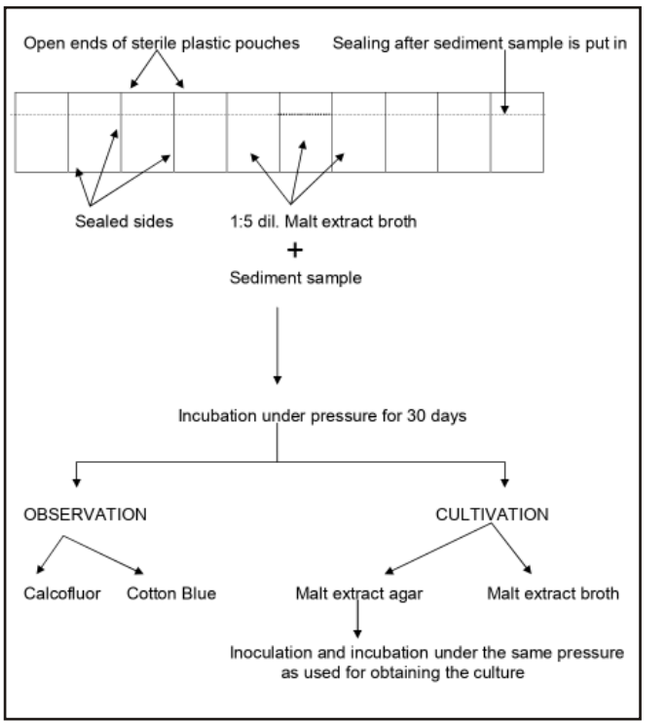

Fig. 2. Schematic diagram showing culturing of fungi in deep-sea culture vessel. Direct observation for the presence of fungi in deep-sea sediments was carried out by staining with Calcofluor or cotton blue. Courtesy Samir Damare.

during isolation on board are to be followed. The laboratory should be draft free and working benches or laminar flow hood sterilized with alcohol and all handling of sediments and culture media should be done using laboratory grade hand gloves and around spirit lamp/Bunsen burner. Petri plates with media should be exposed at all handling spaces to check for aerial contaminations. Contaminants that appear on these control plates are to be identified and such strains with identical morphology seen in sampling plates are to be eliminated.

Fungi are isolated by conventional dilution plating method wherein ~0.1 g of sediment is suspended in ambient sterile seawater, vortexed and 100 µl aliquots are spread plated on different media prepared with ambient seawater. Another method employed is particle plating method wherein 1 g of sediment slurry is passed through 200 and 100 µm mesh size screens and particles retained on 100 µm mesh are spread-plated on various media prepared with ambient seawater. For both these techniques the plates are incubated at 5-10º C and 0.1 MPa (1 bar) pressure. Pressure incubation technique involves suspending 0.5 g of sediment in 2 ml of seawater malt extract broth in sterile plastic pouches of 4x4 cm size. The open ends of pouches are sealed and suspended in water column in deep-sea culture vessel (Tsurumi &Seike Co., Japan) and pressurised to the required pressure. These are incubated at 5º C for 25-30 days. At the end of incubation period the pressure vessels are depressurized extremely slowly and 100 µl of aliquots are spread-plated in various media and incubated further for 8-10 days at 1 bar pressure, at 5 and 25º C **(Fig. 2)**. Fungal colonies appearing are isolated in pure cultures and maintained for further work. Pressure incubation technique yielded higher percentage of culturable fungi in comparison to other methods **(Table 1).** Newer techniques as discussed by Raghukumar *et al.* (2010) need to be tested for tapping this great reservoir of deep-sea for fungal diversity.

Table 1. Number of fungi isolated by different methods during three cruises in the Central Indian Basin (CIB).

Method used for isolation	Total No. of Sediments samples used	Number of fungi recovered###	Percentage Recovery
Particle plating	532	101	19
Dilution plating	260	50	19
Pressure incubation#	263	94	36
Direct incubation in MEB## at 5° C	153	3	2

#Incubated at 30 MPa pressure; ##malt extract seawater broth; ### Number of distinct morphological types obtained during 3 cruises in the CIB

GROWTH OF FUNGI UNDER SIMULATED DEEP-SEA CONDITIONS

Pressure vessels were used by Raghukumar and Raghukumar (1998) for culturing fungi from deep-sea sediments. Use of oxygen-permeable polypropylene bags for suspending cultures made oxygen available to cultures during its growth in pressure vessels. Under such conditions several filamentous fungi and yeast from deep sea sediments showed good biomass build up **(Table 2).** A few of the terrestrial fungi also showed biomass build up under hydrostatic pressure of 20 MPa at 5 as well as 30º C (Damare *et al.*, 2006b).

Table 2. Number of fungi and yeast that showed growth under various growth conditions.

Growth conditions	No. of filamentous fungi	No. of yeast
0.1 MPa/30° C	16	12
0.1 MPa/5° C	16	12
20 MPa/30° C	16	12
20 MPa/5° C	16	12
Although all the fungi and yeast showed growth under all the culture conditions, the biomass produced varied from 5 to 55 mg dry weight in 20 ml of culture medium.		

Some of the fungi showed abnormal morphology immediately after initial isolation from deep-sea sediments (Damare *et al*., 2006b). They showed extremely long conidiophores and vesicles covered by long hyphae, instead of phialides of metulae or conidia, as is typical of the genus *Aspergillus*. These features disappeared after few subculturing. Several non-sporulating fungi formed spores after a few subculturing. Some of the fungi showed distinct swellings when grown under simulated deep-sea conditions in malt extract broth but grew normally in sediment extract medium (Damare and Raghukumar, 2008). Two deep-sea fungi showed microcyclic conidiation at 10 MPa hydrostatic pressure (Raghukumar and Raghukumar, 1998) wherein germinating conidium formed further conidia without forming vegetative mycelium. This phenomenon is reported to occur under nutrient limitation and helps fungi in early completion of their life cycle.

Spores of a few deep-sea fungi germinated in sediment extracts of different dilutions at 20 MPa pressure at 30º C, but not at 5º C (Damare *et al.*, 2008). At 5º C spores failed to germinate even at 0.1 MPa after incubation period of 20 days. When initiated with mycelia inoculums, all of these fungi grew and produced biomass at elevated hydrostatic pressure and low temperature, but the spores did not germinate under these conditions. Thus low temperature and not elevated hydrostatic pressure appeared to be a limiting factor for spore germination. Thus, metabolically active fungal mycelia fragments are more tolerant to elevated hydrostatic pressure and low temperature than dormant fungal spores. It appears that mycelia fragments have better chances of survival and propagation in deep-sea conditions than spores.

It can be hypothesized that some of the wind- blown spores and mycelial fragments as well as those landing in deep sea with terrestrial runoffs remain active whereas a greater part remain dormant. These dormant propagules during their ascent to the surface undergo depressurization shock and /or availability of nutrients make them metabolically active. On this line, a 4 m long sediment core from a depth of 5900 m of the Chagos Trench in the Indian Ocean yielded several fungi down to 370 cm length of the core. *Aspergillus sydowii* isolate obtained from the depth of 360 m depth showed growth at 50 MPa pressure and 5º C temperature. Germination of spores of *A. sydowii* at 100, 300 and 500 bar pressure and 5º C temperature confirmed its barotolerance and nativity to deep-sea sediments. The sediment at this depth was estimated to be 0.43 million years old by using radiolarian index (Raghukumar *et al*., 2004). Such fungi provide an opportunity to understand cellular adaptations involved in long-term survival under extreme conditions. Discovery of such paleobes will allow us a virtual time travel to the past and pick up organisms for potential biotechnological explorations.

An orange pigmented yeast cells showed filament formation resulting in pseudomycelia under 20 MPa pressure and 5º C temperature (Damare *et al.*, 2006b). Differentially expressed genes in a psychrotolerant deep-sea yeast, *Cryptococcus* sp subjected to 50 MPa (500 bar pressure prevailing at 5000m depth in the Central Indian Basin) and low temperature of 5º C were analysed using suppression substractive hybridization technique (SSH). A total of 17 and 20 upregulated genes were identified at 50 MPa and 50 MPa/5º C, respectively (Singh *et al.*, 2012c). These differentially expressed genes showed homology to the expressed sequence tags (ESTs) listed in the NCBI database. They coded for proteins involved in arachidonic acid metabolism, amino acid transport and unsaturation of membrane fatty acids demonstrated to assist in survival of microbes under stress conditions. About 50% of the upregulated genes matched with hypothetical proteins at a percentage similarity of ≤96, suggesting their probability of being novel. Further detailed analysis of such upregulated genes under deep-sea conditions may elucidate mechanisms adapted by microorganisms for survival.

A deep-sea isolate of the psychrotolerant yeast *Cryptococcus* sp. isolated from polymetalic nodule-bearing sediments of the Central Indian Basin showed considerable growth in the presence of $ZnSO_4$, $CuSO_4$, Pb $(CH_3COO)_2$ and $CDCl_2$ at 100 mg/L concentration at 30 and 15º C (Singh *et al.*, 2013). Scanning electron microscopic images revealed altered cell surface morphology of the cells in the presence of heavy metals in comparison with the controls. Fourier transform infrared spectroscopy (FTIR) and Energy-dispersive X-ray analysis (EDAX) demonstrated adsorption of heavy metals to the cells. Atomic absorption spectrometric analysis demonstrated removal of the heavy metals in the range of 30-90% from the culture supernatant after 4 days of growth at 30º C. Being isolated from polymetallic nodule-bearing sediments, this isolate may have evolved a defensive mechanism to detoxify the environment by bioaccumulation of heavy metals and also developed tolerance to them. Thus such microorganisms need to be explored for their ecological role in deep-sea sediments.

DIVERSITY OF CULTURE-DEPENDENT FUNGI IN DEEP-SEA

Several fungi were isolated from water samples collected from surface to 4500 m depth in subtropical Atlantic Ocean (Roth *et al.*, 1964). Deep sea fungi were observed to grow in wooden panels submered at 1615-5315 m depth (Kohlmeyer, 1997). However, these were not cultured. Poulicek *et al.* (1986) documented mycelial fungi inside shells of molluscs at 4830 m depth in the Atlantic. Raghukumar *et al.* (1992) cultured fungi from surface sterilized calcareous fragments collected from 300-860 m depth in the Bay of Bengal. *Aspergillus ustus* and *Graphium* sp. were the most commonly isolated fungi from calcareous sediments from the Arabian sea (Raghukumar and Raghukumar, 1998). Takami (1999) isolated *Penicillium lagena* and *Rhodotorula mucilaginosa* from a depth of 10,500 m sediment samples of the Mariana Trench in the Pacific Ocean. *Aspergillus sydowii* and several non-sporulating fungi were isolated from a 4 m long sediment core taken from Chagos Trench in the Indian Ocean from a depth of 5900 m (Raghukumar *et al.*, 2004). Damare *et al.* (2006b) reported *Aspergillus* sp. as the most dominant form, followed by several non-sporulating cultures from the Central Indian Basin. Most of these reports used classical morphology-based taxonomy to identify culturable fungi **(Table 3).**

Table 3. Deep-sea fungi isolated from various oceanic regions identified by classical morphology-based taxonomy.

Fungi isolated & (substrates)	Oceanic sites and depths	Reference
Filamentous fungi (water samples)	Atlantic Ocean, 0-4500 m	Roth *et. al*. (1964)
Ascomycetous fungi (wooden panels)	1615-5315 m	Kohlmeyer (1997)
Mycelial fungi (molluscan shells)	Atlantic Ocean, 4830 m	Poulicek *et al.* (1986)
Filamentous fungi (calcareous fragments)	Bay of Bengal, 300-860 m	Raghukumar *et. al.* (1992)
Aspergillus ustus and *Graphium* sp (Calcareous sediments)	Arabian Sea, 800 m	Raghukumar and Raghukumar (1998)
Penicillium lagena, Rhodotorula mucilaginosa (sediments)	Marianna Trench Pacific Ocean, 10,500 m	Takami *et. al.* (1999)
Aspergillus sydowii and other non-sporulating mycelia fungi (4 m long sediment core)	Chagos Trench, Indian Ocean, 5900 m	Raghukumar *et. al.* (2004)
Several mycelia fungi (sediments)	Central Indian Basin, 5000 m	Damare *et. al.* (2006b)

With advent of molecular - based taxonomy, several more fungi were reported from deep sea. Using ITS and 18S sequences of SSU rDNA to identify cultured fungi, Singh *et al*. (2010) reported 16 filamentous fungi and 12 yeast species from the Central Indian Basin. These authors noted that most filamentous fungi belonged to *Ascomycetes*, whereas most of the yeast isolates belonged to *Basidiomycetes*. About 62 filamentous fungi, mostly belonging to ascomycetes were isolated from animals in various deep-sea hydrothermal vent sites (Burgaud *et al.*, 2009). Dupont *et al.* (2009) reported a new genus and species of ascomycete, *Alisea longicola* by analysis of 18S and 28S rDNA sequences and morphological characters. This species was found on sunken wood obtained from Pacific Ocean off Vanuatu Islands. Several yeast and yeast-like fungi were isolated from cold hydrothermal environment and basalt rock surfaces from an active deep-sea volcano, Vailulu'u Seamount, Samoa (Connell *et al.*, 2009). Burgaud *et al*. (2010) obtained 32 isolates of yeasts associated with deep-sea fauna at hydrothermal vents.

In the recent past Zhang *et al.* (2014) obtained 20 culturable phylotypes from a depth ~4000 m in the East India Ocean. Of these 30% are new reports from deep-sea sediments. Several reports on occurrence of Malassezia-like organisms in marine environment have appeared. These organisms are associated from dandruff in human beings to pathogens of marine biota. They are associated with healthy corals and sponges. They may be commensals or latent pathogens waiting for loss of immunity in their hosts or some environmental triggers to become pathogens (Amend, 2014). Rédou *et al.* (2015) investigated culturable fungal community from sediment core at a depth of 1928 meters below sea floor (mbsf) sampled from Canterbury Basin (New Zealand). About 200 filamentous fungi and yeasts, belonging to *Ascomycetes* and *Basidiomycetes* were isolated. Wei *et al* (2018) isolated 106 fungal isolates from 10 sediment samples collected at a depth range of 4545-7068 m in the East Pacific Ocean. These fungi were identified based on morphological characteristics and ITS ribosomal DNA sequencing. These belonged to 12 genera including *Aspergillus, Aureobasidium, Candida, Cladosporium, Cystobasidium, Devriesia, Knufia, Nigrospora, Penicillium, Rhodotorula, Sarocladium* and unclassified *Xylariales*.

Ocean drilling programs (ODP) started recently in collaboration with different institutions has enabled examination of microbial abundance and diversity in sediments collected below oceanic seafloor. Biddle *et al.* (2005) recovered ascomycetous fungi belonging to the genera *Cladosporium, Penicillium* and *Acremonium* spp. by direct plating and by enrichment culturing technique from sediment core collected at 200 mbsf from 252 m water depth on the outer shelf edge of the Peru Margin. They were identified by ITS sequencing **(Table 4)**.

Table 4. Deep-sea fungi isolated from various oceanic sites identified by molecular-based taxonomy. Either ITS, 18S and 28S rDNA sequences or a combination of these sequences were used for identification.

Fungi isolated and substrates	Oceanic sites and depths	Reference
16 filamentous fungi and 12 yeasts (sediments)	Central Indian Basin , 5000 m	Singh *et al*. (2010)
62 filamentous fungi (hydrothermal vent animals)	Various hydrothermal vents	Burgaud *et al.* (2009)
Alisea longicola (sunken wood)	Pacific Ocean	Dupont *et al.* (2009)
Yeast and yeast-like fungi	Cold hydrothermal environment, deep-sea volcano,Vailulu'u Seamount, Samoa	Connell *et al.* (2009)
32 yeasts (hydrothermal vent fauna)	Hydrothermal vent	Burgaud *et. al.* (2010)
20 culturable phylotypes	East India Ocean, 4000 m	Zhang *et. al*. (2014)
Malassezia-like organisms	Several marine environment	Amend (2014)
200 filamentous fungi and yeasts (sediments)	Canterbury Basin, New Zealand, 1928 mbsf	Rédou *et. al.* (2015)
106 fungi 9sediments)	East Pacific Ocean, 4545-7068 m	Wei *et. al.* (2018)
Ascomycetous fungi (sediments)	Peru Margin, 200 m below sea floor (mbsf)	Biddle *et. al.* (2005)

DIVERSITY OF CULTURE-INDEPENDENT FUNGI FROM DEEP SEA

With the advent of accurate, fast and clean techniques to isolate DNA samples from natural habitats and availability of high throughput sequencers, assessment of microbial diversity without culturing has been widely reported. Demonstration of fungi as one of the major groups among microbial eukaryotes in deep sea (Lopez-Garcia, 2001; Edgcomb *et al*. ,2002), by methods employing amplification of sediment DNA with fungal specific primers to study culture-independent fungal diversity gained popularity. Using such techniques Le Calvez *et al*. (2009) reported unsuspected diversity from hydrothermal vent samples. They reported new species in three fungal phyla namely, *Chytridiomycota*, *Ascomycota* and *Basidiomycota*. From deep-sea sediments 37 m below seafloor of the Peru Margin and Peru Trench, Edgcomb *et al*. (2011) recovered fungal sequences from DNA and RNA-based clone libraries. Fungal sequences were recovered from deep-sea methane seeps

Table 5. Diversity of culture-independent fungi from deep-sea

Fungi phylotypes reported & substrates	Oceanic site and depth	Reference
Fungal signatures among microbial eukaryotes	Deep-sea sediments	Lopez-Garcia (2001); Edgcomb *et. al.* (2002)
New species from Chytridiomycota, Ascomycota & Basidiomycota	Hydrothermal vent	Le Calvez *et. al.* (2009)
Fungal sequences from DNA & RNA-based clone libraries	37 m below sea floor from Peru Margin & Peru Trench	Edgcomb *et. al.* (2011)
Methane seeps	Sagami Bay, Japan	Takishita (2007)
Gas hydrate-bearing sediments	Ocean drilling program sites in the Pacific	Cao (2010)
New fungal sequences from Methane hydrate-bearing sediments	South China Sea	Lai *et. al.* (2007)
32 fungal taxa (8 of them new) from sediments	Central Indian Basin, ~5000 m	Singh *et. al.* (2011)
20 distinct fungal taxa belonging to Ascomycota and Basidiomycota. & 7 totally new	Central Indian Basin, ~5000 m	Singh *et. al.* (2012a)

(Takishita, 2007), gas hydrate-bearing sediments (Cao, 2010). Lai *et al.* (2007) reported several fungal sequences from methane hydrate-bearing deep-sea sediments that are not associated to any known fungi or fungal sequences in public data bases **(Table 5)**.

Culture-independent fungal diversity was intensely studied at different locations and various depths in the Central Indian Basin (CIB) using multiple primer sets. Singh *et al*. (2011) evaluated fungal diversity at 3 locations (station A, B and C) in the CIB by culture-independent approach. Community DNA isolated was amplified using 3 primer sets namely, fungal-specific ITS (primer pair a), universal ITS (primer pair b), and universal 18S rDNA primer pair (primer pair c). A total of 39 fungal operational taxonomic units (OTU), having 32 distinct fungal taxa were recovered from a total of 768 clones generated from 16 environmental clone libraries. Out of these, 8 sequences appeared to be new having less than 97% similarity with known sequences. A majority of sequences belonged to diverse phylotypes of *Ascomycota* and *Basidiomycota* **(Table 5).** Individual primer set appeared to amplify different fungal taxa occasionally. Out of the total 22 OTUs recovered from primer pair a and b, eight OTUs affiliated with sequences reported from marine environment. Out of these 8 OTUs, three were new sequence types. **(Table 5)**. Fungal taxa were unevenly distributed at different stations, some being exclusively in station A, B or C. Distribution of fungal taxa in stations A, B and C was 10, 21 and 16, respectively, indicating richness of diversity in station B.

In a slightly modified approach Singh *et al.*, (2012a) used sediment sample from one station but used four different primer sets. These were fungal-specific primer set and 3 different universal 18S rDNA primers **(Table 5)**. One environmental library was constructed with each of these 4 primer sets and 48 clones per library were sequenced. These sequences yielded 8 fungal OTUs with ITS and 19 OTUs with 18S rDNA primer pairs, respectively. These OTUs belonged to 20 distinct fungal taxa of the phyla *Ascomycota* and *Basidiomycota*. Seven sequences showed divergence by 79-97% from the known sequences in the existing data base and therefore appear to be novel. Phylogenetic affiliation of a few sequences with known environmental sequences from marine and hypersaline habitats suggest their autochthonous nature or adaptation to marine habitat. Amplification of fungal sequences with eukaryotic as well as fungal-specific primers indicates that among eukaryotes, fungi appear to be a dominant group in the sampling site.

None of the above work reported presence of sequences that clustered close to known taxa belonging to *Zygomycota* and *Chytridiomycota*. Cathrine and Raghukumar (2013) pointed out that the taxonomic position of fungal sequences that clustered close to known fungal taxa could be clearly identified. However, many of the environmental sequences clustered away from the known fungal taxa and grouped to form environmental clades. Analysis of these environmental sequences shows that a few distinct environmental clusters can be identified within *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Zygomycota*.

FUNGAL DIVERSITY BY SIMULTANEOUS CULTURE-DEPENDENT AND CULTURE-INDEPENDENT APPROACH

Singh *et al.* (2012b) further investigated fungal diversity in two deep-sea sediment cores collected at a depth of ~40 cm below sea floor of CIB by adopting culture-dependent and culture-independent approach. Partial region of 18SrDNA of pure fungal isolates was PCR amplified with NS1 and NS4 (White *et al.*, 1990). Sediment DNA samples were amplified using fungal specific ITS primer and universal 18S rDNA primer sets. A total of eight environmental clone libraries from (2 sediment cores x 2 depths each x 2 primer sets each) were constructed and 48 clones were screened from each of these libraries. They recovered 19 culturable fungi and 46 OTUs, respectively **(Table 6)**. Some of the fungi such as *Cerrena, Hortaea* and *Aspergillus* sp. were recovered by culture-dependent as well as culture-independent approaches. Eleven OTUs from environmental libraries showed high divergence (86-97%) from the existing sequences in the Genbank (NCBI database). These two approaches together detected a total of 12 distinct fungal genera and 42 OTUs, respectively from two sediment cores suggesting presence of high fungal diversity in deep-sea sediments.

Table 6. Fungal diversity in deep-sea by simultaneous culture-dependent and culture-independent approaches. Cultured fungi were identified on the basis of 18S rDNA gene sequences. Phylogenetic affiliations of the OTUs obtained using primer set NS1/NS2 (of 18S rDNA gene sequences) were compared.

Cultured fungi (closest identified relative)	Fungal operational taxonomic units (OTUs)
Nigrospora oryzae	Uncultured soil ascomycete (3)
Cladosporium sp	Uncultured fungus clone (2)
Trametes versicolor	Uncultured *Aspergillus* clone
Chaetomium elatum	*Phialosimplex caninus*
*Aspergillus versicolo*r (2)	Uncultured marine non-fungal eukaryote
Ascotricha lusitanica	*Saccharomyces* sp.
Pleaospora herbarum	Uncultured marine fungus clone
Cladosporium sp.	*Pycnosporus* sp.
Eurotium herbariorum	Uncultured *Malassezia* clone
Cerrena sp. (4)	*Sterigmatomyces halophilus*
Penicillium griseofulvum (2)	*Dothideomycete* sp
Sagenomella sp	*Cerrena unicolor*
Hortaea werneckii (2)	
Numbers within brackets denote number of isolates/OTUs obtained.	

ROLE OF FUNGI IN THE DEEP-SEA HABITAT

Besides bacteria, major role of fungi is in biotransformation of organic matter in terrestrial environment. However, the role of fungi in deep-sea is mostly hypothesized without concrete evidence. Damare and Raghukumar (2008) demonstrated that besides biodegradation fungi are important in forming aggregates in deep sea. These aggregates help in binding the soil, prevent leaching of enzymes and may serve as food to the detritus-feeding animals in deep sea. Fungi produce a large amount of exopolysaccharides which help in binding the sediment and also offer a major source of food to detritivores. Melanin and chitin, the polymers of fungal cell walls are not easily degradable and thus, fungus-mediated C storage is more persistent than that sequestered by bacteria (Bailey *et al.*, 2002). Several of the fungal sequences and Malassezia-like organisms are known to be animal parasites (Nagano *et al*., 2010) and may impact host population in deep-sea and hydrothermal vents. Some of them may be symbionts and have stimulating effects on host defence responses (Domart-Coulon *et al*., 2004). Nagano *et al*. (2010) reported 14 OTUs belonging to DSF-1 group dominating in oxygen-depleted environment like methane cold seeps. These might be anaerobic fungi or facultative anaerobes. Siderophore-producing and heavy metals tolerant yeasts in deep sea may play an active role in biomineralization processes (Connell *et al*., 2009, Singh *et al*., 2013). Methylotrophic yeasts reported in methane seeps (Lai *et al.*, 2007) may be used as biomarker for tracing methane seeps in deep-sea. Several extracellular enzymes produced by fungi may be used as indicators of nutrient cycling processes. Alkaline phosphatase activity of fungi play an important role in regeneration of inorganic phosphate through its catalysis to organic esters to inorganic P (Chróst, 1991; Raghukumar *et al.*, 2010). Damare *et al*. (2006a) demonstrated that 11% of the total of 221 fungi isolated from deep-sea sediments of the CIB produced low-temperature active alkaline protease. Miura *et al*. (2001) purified two novel endopolygalacturonases active at 0-10º C from a deep-sea yeast isolated from the Japan Trench at a depth of 4500-6500 m. Deep-sea fungi have added new dimension to the discovery of natural products too (Petit, 2011).

CONCLUSION

As is evident from this discussion, studies on deep-sea fungi from cold has become a hot topic. A vast biodiversity of fungi from cultured and culture-independent approaches is being reported from different oceanic regions. Understanding their role in ecological processes and potential biotechnological applications are pursued in great detail.

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'What are fungi?' A Revisit

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ABSTRACT

The concept of fungi based on an absorptive mode of nutrition that prevailed till the 1970s changed with discoveries brought about subsequently by molecular phylogeny studies. The term 'fungi' was thenceforth confined to the opisthokontan lineage, termed popularly as the Kingdom Fungi, while the *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* that belonged to the Kingdom *Straminipila* were relegated to 'pseudofungi' or 'fungi-like organisms'. We argue here that the term 'fungi' should be used in a broad sense based on a nutritional mode and ecological function. We support our arguments based on those of a number of other eminent mycologists. We further suggest that to avoid ambiguity, the opisthokontan lineage of fungi should be termed as belonging to the Kingdom *Mycetae*. The term 'fungi' then would constitute a polyphyletic group of 'mycetaen fungi' and 'straminipilan fungi' that are found in the Kingdom *Straminipila*.

Keywords: Opisthokont, Fungi, Kingdom, *Mycetae*, Straminipila, mycetaen, straminipilan.

Seventeen years after a similar question was discussed by Cavalier-Smith (2001) and at a time when systematists are gradually succeeding in establishing an accurate, evolutionary and phylogenetic classification of organisms, a revisit to the topic on what fungi are, is still relevant.

Fungi as understood till 1980

There have been various definitions and taxonomic organizations of fungi by earlier mycologists, such as Saccardo in his Sylloge Fungorum of 1884 and in the books of Gwynne-Vaughan and Barnes in 1926 and Gaumann and Dodge in 1928. Many of these, based on the two Kingdom classification of organisms, classified fungi under plants and included bacteria under fungi as 'Schizomycetes'. For the sake of brevity, we will not consider these and confine to definitions after an arbitrary time period after 1950, subsequent to the exclusion of bacteria, the prokaryotes, from others that are eukaryotes.

Thus, Bessey, E.A. (1950) defined fungi as 'chlorophyll-less nonvascular plants whose reproductive or vegetative structures do not permit them to be assigned to positions among recognized groups of algae or higher plants, and as excluding the Bacteria (which are typically one-celled and lack a typical nucleus) and the *Mycetozoa* (which have an animal type of structure and reproduction)'. Ainsworth (1973) considered the following features to be important for an organism to be considered a fungus: "(1) Free-living, parasitic or mutualistic symbionts, devoid of chlorophyll. (2) Cell wall composition is very variable, majority contain chitin and glucan. (3) Reserve food materials are oil, mannitol and glycogen. (4) Except some unicellular members, majority are filamentous."Alexopoulos and Mims (1979) defined fungi as achlorophyllous, saprobic or parasitic organisms with unicellular or more typically, filamentous soma (thallus), usually surrounded by cell walls that characteristically consist of chitin and other complex carbohydrates, nutrition absorptive, except in the slime molds (Division *Gymnomycota*) where it is phagotrophic, propagation typically by means of spores produced by various types of sporophores; asexual and sexual reproduction usually present. This definition is very similar to that of Alexopoulos in his earlier book published in 1962.

The concept of fungi during the period of 1950 to 1980 was as follows.

1. Eukaryotic, devoid of chlorophyll.
2. Unicellular or filamentous.
3. Heterotrophic and osmotrophic in nutrition, except for the slime molds.
4. Cell wall made of chitin or glucans.

Fungi, as defined by various authors till this period included posteriorly uniflagellate, anteriorly uniflagellate and biflagellate zoosporic fungi (chytrids, *Hyphochytriomycetes* and *Oomycetes*, respectively), the *Zygomycetes*, *Ascomycetes*, *Basidiomycetes* and the asexual fungi (**Table 1**).

Concepts OF fungi that emerged since the 1970s

Our understanding of fungi underwent a major change with the publication of Whittaker (1969) who concluded that the conventional two Kingdom classification of eukaryotes was inadequate and proposed a five kingdom classification, which elevated Fungi to the level of a Kingdom. He also created the Kingdom *Protista* that included a heterogenous assemblage of unicellular organisms. The idea was further supported by Whittaker and Lynn Margulis (1978). Whittaker's circumscription of the Kingdom Fungi included all

Table 1: Classification of fungi over time by various mycologists

Ainsworth (1973)	Alexopoulos and Mims (1979)	McLaughlin et al. (2001)	Cavalier-Smith (2001)	Alexopoulos, Mims and Blackwell, 2002	Hibbett et al. (2007)	Webster and Weber (2007)	Adl et al. (2012)
Kingdom *Mycota* -	Kingdom *Myceteae* -	*Mycota* -	Kingdom *Fungi* -	Kingdom *Fungi* -	Kingdom *Fungi*	Kingdom *Fungi* Kingdom *Straminipila* And Kingdom *Protozoa*	*Fungi*
					Phylum *Microsporidia*	-	*Microsporidia*
Division *Eumycota*			Subkingdom 1. *Eumycota*			Kingdom *Fungi*	
Subdivision *Mastigomycotina* Class *Chytridiomycetes* Class *Hyphochytrio-mycetes* Class *Plasmodiophoro-mycetes*	Division *Mastigomycota* Subdivision *Haplomastigo-mycotina* Class *Chytridiomycetes* Class *Hyphochytridio-mycetes* Class *Plasmodiophoro-mycetes*	*Eumycota*, *Chytridiomycota*	Phylum *Archemycota* Subphylum *Dictyomycotina* Class *Chytridiomycetes* Class *Enteromycetes* Subphylum *Melanomycotina* Infraphylum *Allomycotina* Class *Allomycetes*	Phylum *Chytridiomycota*	Phylum *Chytridiomycota* Phylum *Neocallimastigo-mycota* Phylum *Blastocladio-mycota*	*Chytridio Mycota*	*Neocalli-mastigaceae* *Chytridiomycota* *Blastocladiales*
			-	Kingdom *Straminipila*	-	Kingdom *Straminipila*	-
Class *Oomycetes* Class *Hypho-chytriomycetes*	Subidivision *Diplomastigomyco-tina* Class *Oomycetes* Class *Hypho-chytriomycetes*	*Pseudomycota* *Oomycota* *Hyphochytrio-mycota*	-	Phylum *Oomycota* Phylum *Hupho-chytridiomycota* Phylum *Laburin-thulomycota*	-	*Hyphochytrio-mycota* *Labyrinthulom ycota* *Oomycota*	-
-	Division *Amastigomycota*	*Eumycota*	-	Kingdom *Fungi*	-	Kingdom *Fungi*	-
Subdivision *Zygomycotina*	Subdivision *Zygomycotina*	*Zygomycota*	Infraphylum *Zygomycotina*	Phylum *Zygomycota*	Phylum *Glomeromycota* *Phylum Incertaesedis*	*Zygomycota*	*Mucoromycotina* *Mortierellaceae* *Entomophthorales* *Zoopagales* *Kickxellomyco-tina*
-	-	-	Subkingdom *Neomycota*	-	Śubkingdom *Dikarya*	-	*Dikarya*
Subdivision *Ascomycotina* Subdivision *Basidiomycotina*	Subdivision *Ascomycotina* Subdivision *Basidiomycotina*	*Dikaryomycota*	Phylum *Ascomycota* Phylum *Basidiomycota*	Phylum *Ascomycota* Phylum *Basidiomycota*	Phylum *Ascomycota* Phylum *Basidiomycota*	*Ascomycota* *Basidiomycota*	*Ascomycota* *Basidiomycota*
Classes *Acrasiomycetes* *Labyrinthula*les *Myxomycetes* *Plasmodiophoro-mycetes* [?]	Subdivision *Acrasiogymnomyc otina* Class *Acrasiomycetes* Class *Myxomycetes* Subdivision *Plasmodiogymno mycotina* Class *Protosteliomycetes*	- -	- -	Protists Phyla *Plasmodio-phoromycota* *Dictyostelio-mycota* *Acrasiomycota* *Myxomycetes*	- -	- -	- -

groups considered traditionally as fungi by mycologists, as given above and as circumscribed by Ainsworth (1973) (**Table 1**).

Advances in cell wall chemistry, biochemical pathways and electron microscopy in the 1970s had begun to show differences within fungi, as they were understood up to that time. Moore (1980) was probably the first to limit the use of 'fungi' to eukaryotes that were 'heterotrophic, not phagotrophic; often with walls and multinucleate hyphae; walls, when present, with β-glucan and usually chitin, at least in spore walls; lysine biosynthesis by aminoadipic acid (AAA) pathway; mitochondria and peroxisomes present, or secondarily lost as in Microsporidia; flattened mitochondrial cristae; plastids and tubular mastigonemes absent." This effectively excluded *Myxomycetes*, *Oomycetes* and *Hyphochytriomycetes*.

By the 1980s, it was clearly established that *Chytridiomycetes*, *Zygomycetes*, *Trichomycetes*, *Ascomycetes* and *Basidiomycetes* formed a monophyletic group related most closely to Kingdom *Animalia* (Cavalier-Smith, 1987). This lineage was called 'fungi' by the author, a terminological practice that has become the norm today. *Oomycetes*, which had hitherto been considered fungi, but which were shown to belong to the Kingdom '*Chromista*', also called the Kingdom *Straminipila*, were marginalized as 'pseudofungi'. These phylogenetic relationships, based firmly on molecular sequences are now well established and beyond dispute (Cavalier-Smith, 2001; Steencamp, *et al*., 2006; Adl *et al*., 2012; Baldauf *et al*. 2013).

Thus, organisms that were considered fungi till the period even up to 1980 are now known to belong to two distinct lineages. One of these forms a monophyletic group of the Superkingdom *Opisthokonta* that includes the Kingdom *Animalia*, and the others, namely the *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomyctes* belong to the Kingdom *Straminipila* or Kingdom *Chromista*. We will henceforth in this articlce call these the opisthokontan lineage of fungi and the straminipilan lineage of fungi, respectively, in line with our arguments that follow.

Terms used for 'Fungi'

Fungi, together as a polyphyletic assemblage, or as a monophyletic, opisthokontan lineage have been named variously in nomenclature (Table 1).

- Whittaker (1969) called them 'Kingdom *Fungi'*.
- Ainsworth (1973) used the term '*Mycota'*.
- Alexopoulos and Mims (1979) used the term 'Kingdom *Myceteae'*.

Terminologies of these authors referred to a polyphyletic assemblage, which included 'fungi' belonging to both the opisthokontan lineage and the straminipilan lineages.

The opisthokontan lineage of fungi have been termed variously as 'Kingdom *Fungi'*, 'Fungi' and '*Eumycota'* by various authors.

- 'Kingdom *Fungi'*, a term originally used by Whittaker was also the one adopted by Moore (1980), Cavalier-Smith (1987, 2001) and Hibbett *et al*. (2007) . These authors have used this term exclusively for the monophyletic, opisthokontan lineage of fungi. The formal nomenclatural term recommended by Hibbett *et al*. (2007) is 'Kingdom: *Fungi* R. T. Moore, *Bot. Mar*. 23: 371 (1980)'.
- The term 'Fungi', but not Kingdom *Fungi*, has been used by Kirk *et al*. (2008), Adl *et al*. (2012) and Baldauf *et al*. (2013) to refer to the opisthokontan lineage of fungi. Adl *et al*. (2012), in their classification, did not recognize a Kingdom level, or even any other suprageneric hierarchy. According to Baldauf *et al*. (2013), 'Fungi' belong to a larger, monophyletic clade, the *Holomycota* that comprises not only fungi with their absorptive mode of nutrition, but also many related phagotrophic, unicellular forms. These include the *Rozellida*, which are mostly known from environmental sequences, the *Nucleariida*, which are strictly amoeboid and *Fonticula alba*, which is also an amoeba that was previously classi? ed as a slime mold. The unicellular and strictly parasitic *Microsporidia* are also part of the *Holomycota*. Holomyciota share a common ancestor with *Holozoa*, the latter comprising Kingdom *Animalia* or *Metazoa*, as well choanozoans (**Fig. 1**).
- Silar *et al*. (2016), who also recognize *Holomycota*, have used the term '*Eumycota'* for the opisthokontan fungi. The term has also been used by Ainsworth (1973) and McLaughlin and McLaughlin (2001) for these fungi.. These latter two authors recognized the distinct evolutionary lineages of opisthokontan and straminipilan fungi, but brought them together under the umbrella of '*Mycota'*. According to them, the *Mycota* encompassed the '*Eumycota'* comprising the opisthokontan and the 'Pseudomycota' that comprised the straminipilan fungi.

'Kingdom *Fungi'* has been the most popular term for the opisthokontanlineage of fungi. This has led to the usage of the term 'fungi' exclusively to members of this group

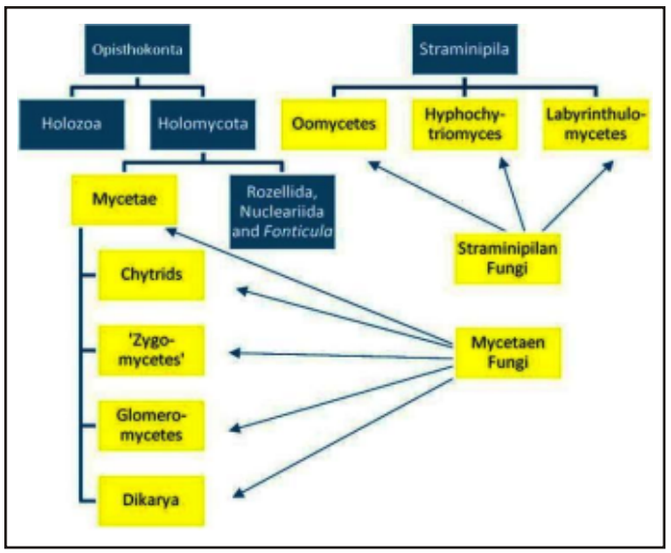

Fig. 1. Representation of Mycetaen fungi and Straminipilan Fungi.

(Cavalier-Smith, 1987, 2001; Hibbett *et al*. 2007; Kirk *et al*., 2008; Adl *et al*., 2012), or to members of one of the lineages of the larger group, the *Holomycota* (Baldauf *et al*., 2013). *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* have now been relegated to "fungal-analogues", "untrue fungi", 'pseudofungi' or 'fungal-like organisms", implying that these are not 'fungi'.

Thus, 'fungi' is mostly used at present in an evolutionary sense, as a monophyletic group.

Arguments for defining fungi as a polyphyletic group

Many mycologists have strongly recommended that the term 'fungi' should be used in a broad ecological and functional sense, instead of strictly on the basis of evolution. Several arguments have been forwarded to support this view.

- Nearly seventy years ago, Bessey (1950) wondered whether the fungi were monophyletic or arose from different ancestors, making way to a consideration of the term fungi being used in a polyphyletic sense.
- Barr (1992)expressed the view that a definitionof fungi based solely on phylogeny was inconsistent with pragmatic needs of mycologists for whom a consideration of ecological or nutritional groupings would be more suitable. He further argued that considering the role that plant pathology has played in mycology and the enormous role of oomycetes in causing plant diseases, a non-inclusion of these organisms, as well as the *Hyphochytriomycetes* and slime molds in a study of mycology could lead to a neglect in their teaching. The solution lies in recognizing a polyphyletic assemblage. According to him, the name "fungi" should be retained in the popular or colloquial, polyphyletic sense to include kingdoms, or parts of kingdoms, that make up logical groups for the benefit of the applied biologist, mycologist or phytopathologist. He suggested the term 'Union of Fungi' to include opisthokontan fungi, as well as the straminipilan ones.
- McLaughlin and McLaughlin (2001) also observed that 'the term fungi has assumed an ecological meaning for all organisms with a similar nutritional mode'. Based on this interpretation, they addressed the monophyletic opisthokontan lineage, as well as 'pseudofungi' *sensu* Cavalier-Smith, which comprised the *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* together as '*Mycota'* (**Table 1**).
- Dick (2001), in his monumental monograph of the *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* addressed these organisms as 'straminipilous fungi', thus emphasizing the need to understand fungi in a broad ecological and functional role, rather than on a strictly evolutionary relationship.
- Alexopoulos *et al*. (2002) recognized the term 'fungi' to represent a polyphyletic assemblage. They considered three groups of fungi. Fungi of the Kingdom Fungi comprised the *Chytridiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota*. Those belonging to the Kingdom *Stramenopila* consisted of the *Oomycetes*, *Hyphochytriomycetes* and the *Labyrinthulomycetes*. The protozoans, *Plasmodiophoromycetes*, dictyostelids, *Acrasiomycetes* and myxomyceteal were also treated by them as part of mycology.
- Webster and Weber (2007) also agreed with Barr and others 'who take a biological approach to the subject and regard fungi as organisms sharing all or many key ecological or physiological characteristics'. They circumscribed 'fungi' based on their lifestyle and in a manner that included both fungi belonging to the opisthokontan lineage, as well as those that had been classified under the Kingdom *Straminipila*, the latter too being considered fungal phyla.
- Kirk et al. (2008) have also followed a broad,

polyphyletic interpretation of fungi, which included all of Kingdom *Fungi* and certain members of the Kingdom *Chromista* and Kingdodm *Protozoa*.

- Beakes *et al*. (2014) also recommended that "fungi" should be considered a biological lifestyle. They further advised that it was important that the straminipilan fungi "continue to be considered an integral part of mycology and not be excluded or marginalized because of their different evolutionary origins".

There are many compelling reasons for considering fungi in a broad, polyphyletic sense. Exclusion of the *Oomycetes*, *Hypochytriomycetes* and *Labyrinthulomycetes* from 'fungi' would be a great loss to the science of mycology.

From a practical point of view, especially plant pathology and industrial mycotechnology, the straminipilan fungi deserve as much attention from mycologists, plant pathologists and biotechnologists. Downy mildew diseases caused by *Sclerospora* and *Plasmopara*, as well as *Phytophthora* that also causes soft rot diseases on diverse economic crop plants require to be studied as intensively as the rust and smut diseases caused by the "true" basidiomycetous fungi.

By ignoring straminipilan fungi, one would be excluding the study of a large part of ecosystem functioning and dynamics from mycology. In some ecosystems, these groups actually play a larger part than the opisthokontan fungi. For example, members of *Labyrinthulomycetes* appear to contribute much more in terms of biomass and energy transfer mechanisms in the marine ecosystem than the opisthokontan fungi (Raghukumar, 2017). The *Oomycetes*, which like the *Labyrinthulomycetes* appear to be of marine origin (Beakes et al., 2014) play a significant role as parasites of marine organisms. Indeed, the inclusion of these organisms under 'fungi' will make mycology much more interesting to marine microbiologists and marine biologists alike, since opisthokontan fungi, as far as the evidence points out today, seem only to be secondary invaders of the sea.

Estimation of fungal diversity based on DNA sequencing has presently skyrocketed as compared to the conservative estimate of 1.5 million proposed by Hawksworth (1991). Even in the absence of a live specimen and based exclusively on environmental nucleic acid sequence analysis, new taxa such as *Hawksworthiomyces* have been described (de Beer *et al*., 2016). In such a scenario, it is unfortunate that the unfolding rich diversity of *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes*, conventionally considered and studied as Fungi for a long time is not considered. We believe that there is an urgent need for a consensus among biologists to appreciate the extent and biodiversity dimensions in fungi that includes both the opisthokontan and straminipilanlineages of fungi.

It appears that segregation of the straminipilian fungi as "pseudofungi" is unwarranted as this will lead to mycologists neglecting such a large group of important genera and species in future. Since none other than mycologists and plant pathologists have any knowledge or expertise to make meaningful scientific contributions to this group of fungi, progress of research on these fungi will be severely hampered.

In view of the above, Raghukumar (2017) subscribed to the views of those who advocated that fungi should be considered in a broad ecological sense to include straminipilan, as well as opisthokontan lineages that fulfilled the criteria of being eukaryotic organisms with an osmotrophic or absorptive mode of nutrition.

If fungi are considered polyphyletic, those who study straminipilan fungi are mycologists, as much as those who study opisthokontan fungi. As a corollary, one could say that fungi are biological entities studied by mycologists. We stresss that mycologists should continue to spare their best efforts in studying and understanding fungi in the broadest sense.The knowledge that we have acquired through advances in biochemistry and molecular biology of these groups should be meaningfully used for a better understanding of the biodiversity in the mycological realm in its broadest sense.The loss of opportunities and scientific knowledge would indeed be serious if mycologists in future years do not wish to study all these groups together.

Choices for defining Fungi

We are confronted with two logical choices to address the two groups of fungi, depending on whether we view them as from an evolutionary point of view or that of nutritional mode and ecological function.

(1) If fungi are defined strictly based on their evolutionary lineage, they would be restricted only to members of the '*Holomycota'* (Baldauf *et al.,* 2013). *Holomycota*, which term implies 'total fungi' is a mixed bag, consisting of 'fungi' with an absorptive mode of nutrition, as well as organisms with a phagotrophic mode. In such a case, the definition of fungi needs to be modified to include organisms with two different modes of nutrition. Fungi, then will not strictly comprise organisms understood as those with an absorptive

nutrition, but as an assemblage that also includes phagotrophic organisms. This will go against the common understanding of mycologists of fungi being organisms with absorptive nutrition.

(2) Alternatively, if we adopt the term fungi only for organisms with an absorptive mode of nutrition, our concept of fungi should be expanded to make the term polyphyletic.

We reiterate what we have stated before that the second of the two choices given above, that 'fungi' should be defined in a broad sense based on lifestyle and ecological function in a manner very similar to that of 'algae'. The term 'algae' is used in a polyphyletic sense, and are understood as 'eukaryotic, nonvascular, photosynthetic organisms' and encompass straminipilan (diatoms, brown algae), viridiplantae (green algae) and the red algae. Barr (1992) indeed cited the use of the term 'algae' to support his argument.

The term 'fungi' then would embrace the opisthokontan lineage generally called as Kingdom *Fungi*, as well as *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* that belong to the Kingdom *Straminipila*.

Terminologies for the polyphyletic assemblage of fungi

A definition of fungi that includes the opisthokontan, as well as the straminipilan lineages of organisms with the same ecological functioning, will make the exclusive use of the terms Fungi or the Kingdom *Fungi* only for the opisthokontan lineage illogical and would obviously lead to confusion. This point has also been raised by Barr (1992) and Webster and Weber (2007). It would then be reasonable to apply an alternative term for the opisthokontan fungi. There appear to be two choices if a polyphyletic interpretation of fungi is accepted.

1. Eumycotan fungi and Straminipilan fungi: Barr (1992), as well as McLaughlin and McLaughlin (2001) recommended that the opisthokontan lineage of fungi be called the Kingdom *Eumycota*. Silar (2016) employed the same term. However, in our opinion this term too is ambiguous, since it also implies that only fungi belonging to *Eumycota* are 'true fungi'. In like terms, it is misleading to call the straminipilan group of fungi as 'pseudo-fungi' or 'fungal-like organisms', because it implies that these are not fungi. However, these indeed are fungi in the ecological sense that we have defined them. We suggest that these should be named 'Straminipilan fungi' in the manner discussed by Dick (2001).

2. Holomycotan and straminipilan fungi: *Holomycota* (Baldauf *et al*., 2013) is a clearly defined group. Fungi belonging to Holomycota could then be considered holomycotan fungi and the other group would be straminipilan fungi. However, the term '*Holomycota'* signifies the totality of fungi, thus automatically excluding the use of 'straminipilan fungi'. Therefore, this combination of terms is not be acceptable.

3. Mycetaen fungi and Straminipilan fungi: The opisthokonttan group of fungi have been referred to as the Kingdom *Myceteae* by Alexopoulos and Mims (1979) to include both the opisthokontan and straminipilan fungi. A simpler version, Kingdom *Mycetae* has been popular among a large number of mycologists and teachers (eg., Launchbaugh and Urness, 1992; Manoharachary *et al*., 2016). We suggest that this term is appropriate in place of Kingdom *Fungi* for the opisthokontan lineage. A similar proposal to use the term Kingdom *Mycetae* for the opisthokontan lineage and calling these mycetaen fungi, while the *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* corresponded to the straminipilan fungi has also been made by Raghukumar (2017) (**Fig. 1**).

CONCLUSION

We recommend that mycologists should consider the usage of the term 'fungi' in a polyphyletic sense to include the opisthokontan, as well as straminipilan organisms with an absorptive mode of nutrition. We further suggest that the term Kingdom *Fungi* should be replaced by Kingdom *Mycetae*.

In conclusion, fungi belong to two distinct evolutionary groups. One group belongs to the Kingdom *Mycetae*, or just *Mycetae* of *Holomycota*, which are a group of opisthokontan organisms, related to Kingdom *Animalia* or *Holozoa*. The second group comprises the groups *Oomycetes*, *Hyphochytriomycetes* and *Labyrinthulomycetes* that belong to the Kingdom *Straminipila*. The two groups are respectively called the mycetaen fungi and the straminipilan fungi (Fig. 1). This approach will lead to a positive outlook on what fungi are and facilitate a deeper and broader appreciation in understanding of fungal biodiversity in its total perspective.

Fungi would then be defined as 'Unicellular, or filamentous eukaryotic organisms that possess an osmotrophic mode of nutrition'.

The broad characteristics of fungi, based on those of Webster and Weber (2007) are as follows.

1. Nutrition: Heterotrophic (lacking photosynthesis), osmotrophic, feeding by absorption rather than ingestion.
2. Vegetative state: Non-motile, single-celled or in

the form of mycelium of hyphae showing internal protoplasmic streaming.

3. Cell wall: Typically present, usually based on chitin, cellulose or other polysaccharides.

4. Nuclear status: Eukaryotic, uni- or multinucleate, the thallus being homo- or heterokaryotic, haploid, dikaryotic or diploid.

5. Life cycle: Simple or complex.

6. Reproduction: Asexual, sexual or parasexual (i.e. involving nuclear fusion followed by gradual de-diploidization) and/or asexual (i.e. purely mitotic nuclear division).

7. Propagules: By means of non-motile or motile spores. Motile spores may be posteriorly uniflagellate, anteriorly uniflagellate or biflagellate.

8. Sporocarps: May be present in mycelial forms. Microscopic or macroscopic and showing characteristic shape.

9. Habitat: Ubiquitous in terrestrial, freshwater and marine habitats.

10. Ecology: Important ecological roles as saprotrophs, mutualistic symbionts, parasites, or hyperparasites.

11. Distribution: Cosmopolitan.

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Arbuscular Mycorrhizal (AM) diversity in some threatened North West Himalayan flora of Kinnaur

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ABSTRACT

The AM associations are integral symbiotic associations of both wild and cultivated plants. It has been widely investigated in the cultivated plants but less so in the wild plants. In this article the AM diversity in some very important and threatened wild plants of the tribal belt of Kinnaur in Himachal Pradesh is being reported for the first time. The study area has a vast wealth of medicinal, aromatic and other economically important plants. With, the upsurge in tourism and increasing developmental projects like the Hydro electric dams, etc the floral diversity and their associated mycorrhizal diversity has come under great threat. It is in this context that along with floristics, investigations of AM fungi has been undertaken on the following threatened plants, majority of which are endemic to the region. Of late, much attention has been paid to the use of AM fungi in the establishment of forests and improvement of soil fertility. The plants investigated are: *Acer caesium, Abies spectabilis, Betula utilis, Juglans regia, Rhododendron campanulatum, Quercus ilex, Hippophae tibetana, Sophora mollis, Elaeagnus umbellata, Rheum australe, Ribes alpestre, Juniperus communis, Piptanthes nepalensis, Saussurea costus* and *Fritillaria roylei.* The reported three genera are *Acaulospora, Gigaspora* and *Glomus* and in these three genera 13 species of AM fungi have been reported and illustrated. The genus *Glomus* is the most predominant with 10 species.

Key Words: AM Fungi, VAM, threatened, reforestation, floristics

INTRODUCTION

Mycorrhiza, meaning 'fungus-roots', is a symbiotic association between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth (Frank, 1885). The vast majority (95%) of plant species form one or the other type of mycorrhizae (Trappe, 1977). There are seven types of mycorrhizae, which are associated with different groups of plants. These are ecto, ectendo, endo, arbutoid, ericoid, monotropoid and orchidaceous type. Out of the seven mycorrhizal types, endomycorrhiza or Vesicular Arbuscular Mycorrhiza (VAM) or the Arbuscular Mycorrhiza (AM) is most predominant being associated with almost 90% of the plant species.

The term Vesicular Arbuscular Mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in the *Glomales*, but because a major subclass lacks the ability to form vesicles in roots; Arbuscular Mycorrhiza (AM) is now the preferred acronym. The Arbuscular Mycorrhizae (AM) form highly branched 'arbuscules' (the term literally means little trees) within root cortical cells. Other structures produced by some AM fungi include, 'vesicles'- thin-walled, lipid-filled structures that usually form in intercellular spaces, such AM fungi are called as VAM. The hyphae of AM fungi are usually recognizably distinct from other kinds of soil fungi. Their reproductive spores can be formed either in the root or more commonly in the soil. The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants. Major AM plant families include *Polygonaceae, Urticaceae, Poaceae, Fabaceae, Taxodiaceae, Taxaceae, Cupressaceae, Aceraceae, Juglandaceae, Podocarpaceae, Casuarinaceae* and the Pteridophytes. Plants with rare AM associations include *Caryophyllaceae, Brassicaceae, Chenopodiaceae* and *Cyperaceae*. Their distribution is widespread and they have been reported in plants growing in Arctic, temperate and tropical regions. Their habitat is also very diverse, they have been reported from sand dunes, coal mines and aquatic environments (Bagyaraj, 2011). They are distributed in about 1000 genera of plants in 200 families. They have at least 300,000 receptive hosts in the world flora, for about 220 species of AM fungi (Bagyaraj, 2015). The fungi that form AM are currently all classified in the order *Glomales*, which is further divided into suborders based on the presence or absence of vesicles (Morton and Benny, 1990).

Arbuscules are primary structures involved in the bi-directional transfer of nutrients between fungal symbionts and host plants (Cox and Tinker, 1976). The hyphae literally form a bridge that connects the plant root with large areas of soil and serves as a pipeline to funnel nutrients back to the plant. In return, the plant must supply the VAM fungi with carbon for its growth and energy requirements. This plant-fungal relationship is an elegant association and its development is evidently regulated by several factors. Different soil bacteria enhance the promotion of root colonization by the VAM fungi. Plant produced discharges (exudates) sent out through their roots that contain specific compounds activating the VAMF to stimulate the hyphal growth.

The symbiotic association increases the uptake of certain nutrients, particularly P, Cu and Zn, by the plant, due to exploration by the external hyphae of the soil beyond the root hair and depletion zones. VAM are known to increase tolerance to heavy metals, saline soils and drought (Michelson and Rosendahl, 1990); decrease transplant shock (Sylvia *et al*., 1993) and inhibit fungal pathogens (Garcia *et al*., 1988; Jalali and Chand, 1988; Marx, 1973); increase resistance against nematodes (Sikora and Schönback, 1975); and weeds (Jordan *et al*., 2000); increase uptake of water (Dudderidge *et al*., 1980) and drought resistance of young seedlings (Parke *et al*., 1983). They help the plants to tolerate cold (Harley and Smith, 1983) as well as high temperature (Marx and Bryan, 1971) and also provide tolerance to soil

(Barea, 1991) and to heavy metal toxicity (Henning, 1993).

AM association in plants is known to help in increased growth in several crops like: grapes, soybean, potatoes, onion, cowpea, apple, raspberry, strawbwerry, *Andropogon gerardii, Salvia officinalis, Thymus vulgaris*, cacti (Possingham and Obbink, 1971; Ross, 1971; Graham et al, 2001; Jain and Sethi, 1988; Ikombo *et al.,* 1991; Granger *et al*., 1983, Gnewkow and Marschneri, 1989; Gianinazzi *et al*., 2002; Niemi and Vestberg, 1992; Hetrick *et al*., 1986; Camprabi *et al*., 1992; Jose *et al*., 1990).

Recently much attention has been paid to the use of AM fungi in reinstatement of forest and improvement of soil fertility. They are considered as an important biological tool for balancing soil nutrients, nutrient loss and the sustainability of forest ecosystems (Chamola *et al*., 1999; Giri *et al*., 2003; Cavagnaro *et al*., 2015). They have potential use in reclamation and revegetation of wastelands due to their potential for increasing growth, survival, and biomass production under conditions of environmental stress (Giri *et al*., 2007; Kaur and Mukerji, 1999). The role of mycorrhizal fungi in the improvement of quality of planting stock is well recognised (Mukerji *et al*., 1996; Dixon *et al*., 1997; Chen *et al*., 2018) and their practical application in stressed conditions can lead to successful afforestation and restoration (Barr, 2010) programmes and eco-restoration of degraded areas (Palmer, *et al.*, 1997; Alexander *et al*., 1992; Al-Karaki, 2013; Manaut *et al*., 2015; Asmelash *et al*, 2016; Sharma and Jha, 2017).

Much work has been done on the cultivated plants of economic importance and their mycorrhizal association. But there is practically no work on mycorrhizal association of wild plants. Further, there is literally no work undertaken on the unique flora found in the North West Himalayan tribal belts. Hence, in the present study the mycorrhizal association of some important wild plants of Baspa valley of Kinnaur has been investigated to ascertain the type and extent of associations.

MATERIAL AND METHODS

Location and Scope: The area explored during the present study falls in district Kinnaur of Himachal Pradesh. Kinnaur previously formed Chini tehsil of Mahasu district and came into being as an independent tribal district during 1960. During pre-independence times, Kinnaur was a part of the erstwhile Bushahr State. The district derives its name from 'Kannaura' or 'Kinnara', the original inhabitants of the region, which have been listed as a scheduled tribe. The Kinnaur district also forms international border with Tibet and commands a special place in Himachal Pradesh because of its unique culture, history and geographical features.

The specific area of study investigated in the present study is called Baspa Valley, popularly referred to as the Sangla Valley following the name of main village. Baspa valley derives its name from the river Baspa and forms one of the major valleys in the district. The study area lies on both sides of Baspa River and extends from Karchum (1,700 m), near its confluence with river Satluj to Ranikanda (4,200 m), about 15 kms upstream of Chitkul, the last village in the valley. As far as altitudinal range of the study area is concerned, an upper altitudinal benchmark of 4,750 m above mean sea level at Rupin Pass has been maintained. The geological limits of the area lie between 31º 15' to 31º 36' North latitude and 78º 10' to 78 º 31' East longitudes. Nestled in the interior, but awesomely majestic Himalayas, this valley is related to the epic Mahabharata. So secluded is the region that, the Pandavas are believed to have spent the last years of their exile here. Today, however, the valley is better recognized as heaven for the outgoing, adventurous type people and is definitely a trekker's paradise.

Topographic Features: The Baspa River with a total length of about 72 Kms, broadly follows a south-east to north-west course in the study area and divides the valley into north-east and south-west facing slopes. In general, the topography of the study area ranges from flat valleys to gentle to precipitous slopes, scree slopes, glacial moraines and lofty peaks. The upper part of the valley is surrounded by barren ridges, whereas the lower regions are flatter with plenty of green pastures and cultivated lands. The rise in altitude is more abrupt along the right bank of the river than along the left. It is due to the reason that the high Kinner Kailash ridge-forming boundary of the valley rises from the basin of the Baspa river over a very short horizontal distance. The side valleys along left bank of Baspa River are fairly deep but cascading streams in these valleys also form gorges resulting in steepness of slopes. General elevation of the valley ranges from about 1,700 m at the confluence of river Baspa with Satluj to more than 6,000 m along the Kinner Kailash range along the right bank of the river Baspa. There are various passes that link the valley to other areas.

Climate: Due to its geographical location, the climate of the region, in general, differs from the climate in the adjacent Shimla and Lahaul and Spiti districts of the state. It has a long winter from October to May and a short summer from June to September; April to May is spring and September to October is autumn. Therefore, the short mild summers, brief light monsoons in the mid valley, extreme cold arid conditions bereft of monsoons in the upper part of the valley and prolonged winters with moderate to heavy snowfall characterize the general climate of the area.

Collection of VAM samples from the soil: Soil was collected by digging around the plant selected. In case of herbs the collection was made by digging out the entire plant and collection of the soil attached to its fine roots. In case of trees the soil attached to the root hairs was collected. About, 100 gms of soil was taken as sample for analysis in the laboratory.

Methodology for isolation of AM spores from the soil: To isolate AM spores from the soil, modified method of wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was used in the present study. In this technique about 10 g of air-dried soil sample was placed in beaker containing 500 ml of water. The soil suspension was stirred for 5 min. The coarse particles were allowed to settle down. Thereafter soil suspension was passed through stacked sieves of different mesh number (100, 200, and 300) in the increasing order. Contents of the beaker were decanted through the sieves.

Since AM fungal spores are lighter, they float on the surface of water. The spore suspension was immediately filtered through Whatman filter paper No.-1. The AM fungal spores form a distinct ring on the filter paper. The filter paper containing spores is spread on a Petri-plate for observation under binocular microscope. Spores were easily distinguished from soil particles by their characteristic hyaline to coloured subtending hyphae.

For identification the spores were transferred on a slide with the help of a needle and observed under compound microscope. Later, all slides were observed carefully under oil immersion for segregation and identification into genera and species. The standard criteria followed for identification e.g. colour, size, shape, wall characteristics, contents and surface ornamentations of the spores, nature of spore, the number and arrangement of the spores in sporocarps and the presence or absence of peridium for the sporocarps were carefully recorded. (Hall, 1984, 1987; Morton, 1988, 2002; Raman and Kumar, 1988; Schenck and Perez, 1987; Trappe, 1982; Walker, 1981).

RESULTS AND DISCUSSION

Enumeration of VAM Fungi: The plants selected for mycorrhizal association were short listed after analyzing the general flora of the valley (Jishtu, 2005). A tentative list of plants was worked out and then after repetitive consultations with the local inhabitants, a list of 15 plants was finalized which are listed in **table-1**. Care was taken to include those species that were endangered/ threatened or locally important.

The endangered/ threatened plants that were short listed are *Acer caesium, Abies spectabilis, Betula utilis, Juglans regia, Rhododendron campanulatum, Quercus ilex, Hippophae tibetana, Sophora mollis, Elaeagnus umbellata, Rheum australe, Ribes alpestre, Juniperus communis, Piptanthes nepalensis, Saussurea costus* and *Fritillaria roylei* (**Plate - 1**). Threat status has been considered as per the Conservation Assessment and Management Prioritisation (CAMP) Workshop (Shimla, 2010) and IUCN (ver. 3.1) Red List Data (Zhang *et al*., 2011; Goraya *et al*., 2013; Saha *et al.,* 2015; Rivers and Allen, 2017; Rankau *et al*., 2017; Chen *et al*., 2018).

As it emerges from the present study and the similar studies by other workers elsewhere, *G. mosseae*, is the most predominant AM fungus. That is why this species has been extensively used for mass propagation and enhanced yield of oats, barley, clovers, potatoes, alfa-alfa, onions, etc., (Muromtsev *et al*., 1990; McArthur and Knowles, 1993; IJdo *et al*., 2011). The results regarding the mycorrhizal associations depict that the genus *Glomus* has a dominant association in the plants of the valley, being associated with all the plants examined for AM. Further, among the genus *Glomus*, the species *G. mosseae* is the most prevalent in its occurrence. It is in agreement with other similar works on mycorrhizae carried out elsewhere (Pindi *et al.,* 2008; Manoharachary *et al*., 2008; Bagyaraj, 1991). Khaliel (1988) reported *G. mosseae* to be the more dominant species in the sand humus, in Riyadh.

Such diversity studies on AM fungi are required if these are to be used for nursery inoculations because studies have shown dependency of plants on mycorrhiza, though the plants greatly differ in their needs on mycorrhizal infection. Selection of efficient strain can result only from exhaustive surveys. The efficient AM strains have been shown to enhance growth of several forest tree species and medicinal plants or plantation crops (Tilak *et al.*, 2010; Bhagyaraj, 2011; Lakshmipathy *et al*., 2000; Manoharachary and Reddy, 1995). Of late the role of AM fungi is being emphasized for the conservation of endangered plants (Evelin *et al*., 2019).

In the present study, the AM spores isolated and identified from the threatened plants are, represented only by three genera, viz., *Acaulospora, Gigaspora* and *Glomus*. The salient features of these genera are as follows:

***Acaulospora*:** It has azygospore type of spores, which is formed within a lateral swelling of the sporogenous saccule. This genus is known to form vesicular arbuscular mycorrhiza produced singly in soil or in sporocarps. Presently, 28 species belong to this genus. Spores are globose, subglobose or ellipsoidae with oil contents.

***Gigaspora*:** These are azygospores borne on a tip of a bulbous hypha. They produce large spores in soil. About 8 species belong to this genus.

***Glomus*:** Presently 77 species are known in this genus. Spores are formed in compact sporocarps in loose clusters in small fascicles or as single spore in soil.

Descriptions of the Species: The AM fungi isolated from the selected plants of the valley are represented by the following species.

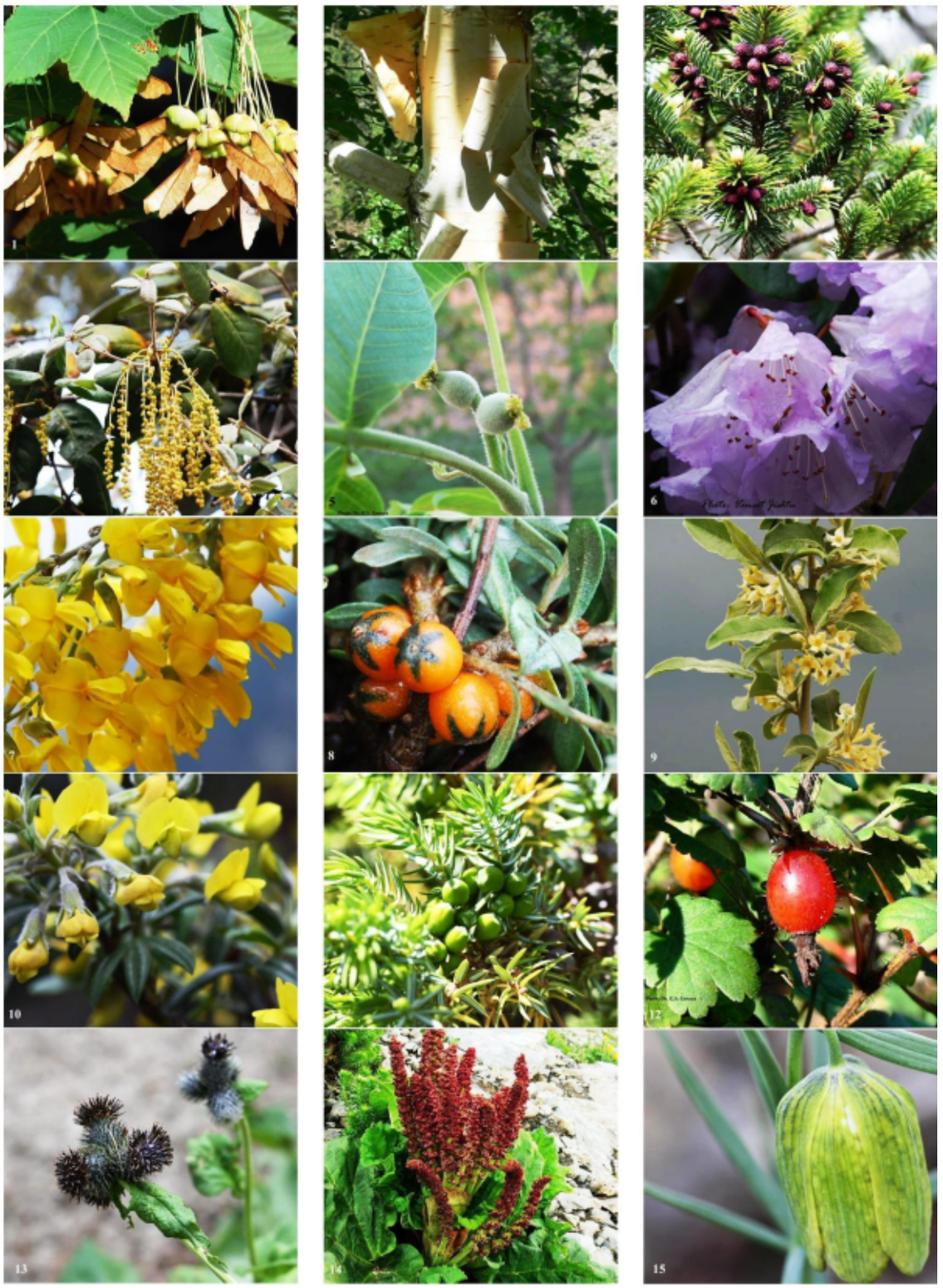
Acaulospora foveata Trappe and Janos: Chlamydospores formed singly, globose to ellipsoidal measuring 182 - 30 (-410) x 115 - 350 (-480) µm, yellow-brown to light reddish-brown, turning red-brown to brown black at maturity; surface uniformly pitted. Outer spore wall yellowish or reddish-brown with an adherent but separable hyaline inner wall; spore contents of small hyaline guttules.

Acaulospora laevis Gerdemann and Trappe: Spores formed singly, globose to sub-globose, ellipsoid or reniform, irregular, dull yellow, turning deep yellow-brown to red, 140-220 µm in diameter. Spore wall continuous, three layered with an outer rigid yellow-brown to reddish-brown with two hyaline inner membranes; spore contents globose to almost polygonal (reticulate).

Gigaspora albida Schenck and Smith: Azygospores formed singly in soil; spherical, colour dull white with light greenish yellow, 232 - 252 x 235 - 250 µm. Spore wall continuous; outer being thin. A bulbous suspensor, separating it from the spore contents is seen. Azygospore attached to a single, hyaline to yellow, bulbous suspensor, attached to septate hyphae with fine hyphal branches.

Gigaspora calospora Nicolson and Gerdemann: Azygospores formed solitary in soil with colour ranging from pale yellow to greenish yellow; spore wall thick, perforated, 2-layered with the in-between space being hyaline yellow.

Plate-I



1. *Acer caesium* Wall. ex Brandis; 2. *Betula utilis* D. Don; 3. *Abies spectabilis* (D.Don) Mirb.; 4. *Quercus ilex* L.; 5. *Juglans regia* L.; 6. *Rhododendron campanulatum* D. Don; 7. *Sophora mollis* (Royle) Baker; 8. *Hippophae tibetana* Schltdl.; 9. *Elaeagnus umbelata* Thumb.; 10. *Piptanthus nepalensis* (Hook.) D. Don; 11. *Juniperum communis* L.; 12. *Ribes alpestre* Wall. ex Decne.; 13. *Saussurea costus* (Falc.) Lipsch.; 14. *Rheum australe* D.Don.; 15. *Fritillaria roylei* Hk.

Table 1: Selected plant *taxa* with the associated AM species

S. No.	Plant Species (Family)	Threat Status	Mycorrhizal Spores
1.	*Rhododendron campanulatum* D. Don (***Ericaceae***)	VU	*Gigaspora colkospora, Glomus macrocarpum, G. constrictum, Glomus* spp.
2.	*Quercus ilex* L. (***Fagaceae***)	LC	*Glomus multisubtansum, G. macrocarpum, Gigaspora albida, Glomus entunicatum,*
3.	*Sophora mollis* (Royle) Baker (***Fabaceae***)	Endemic; populations in decline	*Acaulospora laevis, Glomus constrictum, G. macrocarpum, G. mosseae, G. macrocarpum.*
4.	*Elaeagnus umbellata* Thunb. (***Elaeagnaceae***)	Endemic; populations in decline	*Glomus fasciculatum, G. mosseae.*
5.	*Juniperus communis* L. (***Cupressaceae***)	VU	*Gigaspora albida, Glomus mosseae, G. fasciculatum*
6.	*Betula utilis* D. Don (***Betulaceae***)	EN	*Glomus aggregatum, G. mosseae, G. macrocarpum.*
7.	*Piptanthus nepalensis* (Hook.) D. Don (***Fabaceae***)	Endemic; populations in decline	*Gigaspora albida, G. constrictum, G. fasciculatum, G. intrasadies.*
8.	*Abies spectabilis* (D.Don) Mirb. (***Pinaceae***)	NT	*Glomus mosseae, G. fasciculatum, G. fecundisporum.*
9	*Acer caesium* Wall. ex Brandis; *(**Aceraceae**)*	LC	*Acaulospora laevis, Glomus constrictum, G. macrocarpum, G. mosseae, Gigaspora gigantea.*
10	*Saussurea costus* (Falc.) Lipsch. (***Asteraceae***)	CR	*Acaulospora foveolata, Glomus mosseae, Glomus* spp.
11.	*Hippophae tibetana* Schltdl. (***Elaeagnaceae***)	Endemic: restricted distribution	*Gigaspora albida, Glomus mosseae, G. fasciculatum*
12.	*Juglans regia* L. (***Juglandaceae***)	LC	*Glomus constrictum, G. fasciculatum, G. aggregatum.*
13.	*Fritillaria roylei* Hook. (***Liliaceae***)	EN	*Gigaspora albida, Glomus aggregatum, G. fasciculatum*
14.	*Ribes alpestre* Wall. ex Decne.; (***Grossulareaceae***)	Endemic to the region	*Acaulospora foveolata, Glomus mosseae, G. constrictum,*
15.	*Rheum australe* D. Don (***Polygonaceae***)	VU	*Glomus aggregatum, G. mosseae, Glomus* spp.
CR=Critically Endangered; EN= Endangered; NT=Near Threatened; VU=Vulnerable; LC=Least Concern			
Threat status as per CAMP Workshop (Shimla, 2010) and IUCN 3.1 (Zhang *et al*., 2011; Goraya *et al*., 2013; Saha *et al*., 2015; Rivers and Allen, 2017; Rankau *et al*., 2017; Chen *et al*., 2018)			

Spore contents are hyaline, vacuolated and reticulated with a suspensor like cell attached, being smooth, hyaline to light brown.

Gigaspora gigantea Nicolson and Gerdemann: Azygospores formed solitary in soil; spherical, ellipsoidal, cylindrical to irregular; colour bright yellow with greenish tinge, 183 - 500 X 291 - 812 µm. Outer spore wall thin, tightly covering a thick walled, continous endospore; a single bulbous suspensor, with slender hyphae, extending from the suspensor to the base of the spore.

Glomus aggregatum Schenck and Smith: Chlamydospores formed in loose clusters, being globose, yellow. Spore surface smooth; wall 1-2 layered; outer thick and light coloured, inner layer thin. Spore contents white to hyaline globules. Hyphal envelope absent; pore at spore wall closed by inner wall septum.

Glomus albidum Schenck and Smith: Chlamydospores globose to sub globose, white to yellow, 150 - 182 µm, in diameter. Spore surface coarse rough; wall 2 layered of equal thickness. Spore contents white to hyaline globules. Hyphal envelope absent; pore at spore wall closed by presence of septum.

Glomus constrictum Trappe: Chlamydospores globose to sub globose, yellow to brown, 142 - 180 µm, in diameter. Spore surface smooth; wall 2 layered of equal thickness. Spore contents bright - yellow globules. Sporogenous hypha one, cylindrical, contracted at the points of attachment, first straight and then recurved. Pore at spore wall closed by presence of septum. Sporocarp not observed.

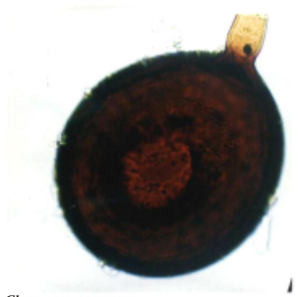
Glomus etunicatumm Becker and Gerdemann: Chlamydospores globose to sub globose, dirty yellow 118 - 120 µm in diameter. Spore surface smooth; wall 2 layered, outer thin hyaline and inner thick. Spore contents hyaline globules. Sporogenous hyphae one, cylindrical. Pore at spore wall closed by presence of septum. Sporocarp absent.

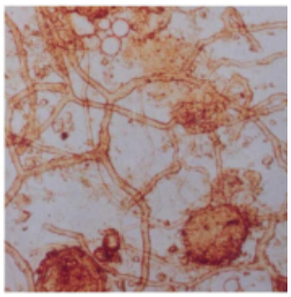
Glomus intraradices Schenck and Smith: Chlamydospores single or in clusters, globose to sub globose, yellow to brown, 140 - 162 µm, in diameter. Spore surface smooth or rough; wall 2 or 3 layered, outer hyaline yellow, inner walls darker than outer. Spore contents orange to brown globules. Sporogenous hyphae one, cylindrical. Pore at spore wall occluded by spore thickening, which forms a tubaeform juncture at attachment of hypha.

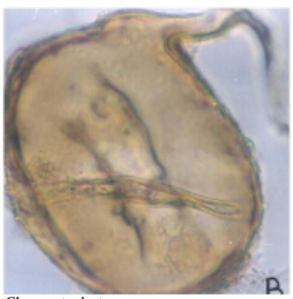
Glomus fecundisporum Schenck and Smith: Chlamydospores formed singly or in loose clusters, being globose, elongate to irregular. Spore wall yellow brown to dark brown, rough with adhering debris; inner and outer walls of approximately equal thickness. Spore contents sub hyaline to grey- white. Extra mycelial hyphae with outer surface.

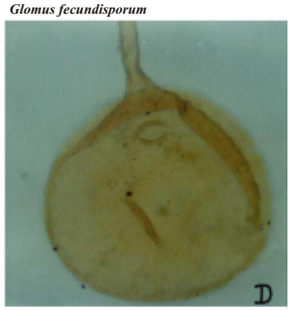
Glomus fasciculatum Thaxter, *sensu* Gerd: Chlamydospores

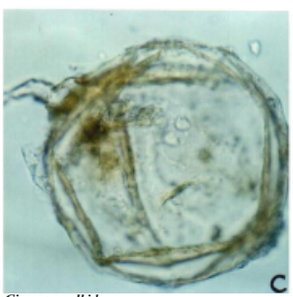
Plate 2


Glomus macrocarpum


Glomus fecundisporum


Glomus etunicatum


Glomus mossae


Gigaspora albida

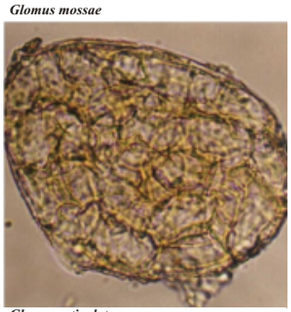
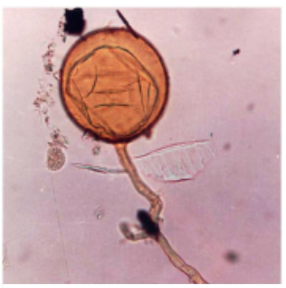
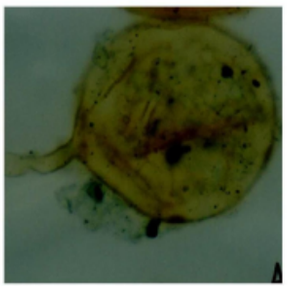

Glomus reticulatum

Plate 3

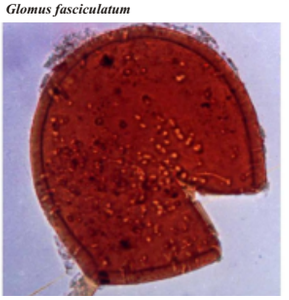

Glomus fasciculatum


Glomus spp.


Gigaspora gigantea


Glomus fasciculatum


Glomus constrictum


Acaulospora foveata

borne, irregularly globose, 86 µm, - 112.8 µm. Spore wall relatively thick, pale yellow brown, hyphae attached. Spore contents sub hyaline to grey- white. Extra mycelial hyphae with outer surface. Spores contain numerous fat globules, tending to be irregular in shape.

Glomus macrocarpum Gerdemann and Trappe: Chlamydospores single or in loose clusters, globose to sub globose, yellow to dark-brown, 140 - 190 µm, in diameter. Spore surface smooth or slightly rough; wall 2 layered. Spore contents yellow to brown hyaline globules. Sporogenous hyphae one, flared towards the point of attachment. Pore at spore wall occluded by spore thickening or plug. Sporocarp not observed.

Glomus mossae Nicolson and Gerdemann: Chlamydospores yellow to brown, 105 - 310 x 110 - 305 µm, globose to ovoid, somewhat irregular with one funnel shaped base; divided from subtendenging hyphae by a recurved septum; walls thick with white or hyaline outer membrane and a thick brownish-yellow inner layer.

Glomus multisubtensum Mukerji *et. al.*: Chlamydospores single or in loose clusters, globose to sub globose, light to dark-brown, 120 - 180 µm in diameter. Hyphal envelope absent. Spore surface smooth or slightly rough; wall with 2 inseparable layers, outer thick, brown and inner layer pale yellow. Sporogenous hyphae 2 to many, always arise from one end of the spore, hyaline, pale yellow, cylindrical. Pore at spore wall closed by septum. Sporocarp not observed.

The results regarding the mycorrhizal associations are given in **table - 1**. From this table it is clear that *Glomus mosseae* is the dominant species in the plants of the Baspa valley. The different *Glomus* species are *Glomus aggregatum, G. constrictum, G. fasciculatum, G. fecundisporum, G. macrocarpum, G. multisubtansum* and *G. entunicatum* (**Plate 2 & 3**). The other AM genera and species associated with the plants were *Gigaspora albida, G. gigantea, G. constrictum, G. colkospora, G. fasciculatum* and *G. intrasides, Acaulospora laevis* and *A. foveata*. In a recent study by Banta *et al*., (2018) on the diversity of AM spores in the rhizospheric soils of the cold desert areas of Kinnaur (HP) it was observed that *Glomus, Acaulospora* and *Gigaspora* were the predominant AM genera. Among the *Glomus* species, *G. macropoda* was the most dominant species, followed by *G. geosporum* and *G. mosseae.*

Earlier workers with different plant species have reported similar AM associations, dominant being the genus *Glomus*; and *G. mosseae*, being the most predominant AM fungus here and elsewhere which has also been extensively used for mass propagation and enhanced yield of oats, barley, clovers,

potatoes, alfa-alfa, onions, lettuce, etc., (Alexander *et al*., 1989; Muromtsev *et al*., 1990; McArthur and Knowles, 1993; Akhtar and Abdullah, 2014; Garmendia and Mangas, 2014; Vani *et al*., 2014) and other globally important food security crops (Ceballos *et al*., 2013). Of late, these AM inoculations have also been found profitable in plant production at a large agricultural scale (Chen *et al*., 2018).

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Characteristics and Applications of a Thermostable and Acidic Exochitinase of the Thermophilic Mould *Myceliophthora thermophila*

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ABSTRACT

The thermophilic mould *Myceliophthora thermophila* (Apinis) van Oorschot produces an extracellular thermo-acid-stable chitinase, which has been purified to apparent homogeneity by affinity adsorption followed by hydrophobic interaction chromatography. The pure enzyme is a monomer with a molecular mass of 43.0 kD on SDS-PAGE and pI of 4.0. Based on MALDI-ToF-MS/MS and LC-MS analysis of peptides, it was identified as glycosyl hydrolase family 18 protein. The chitinase is optimally active at pH 4.0 and 55 °C with $T_{1/2}$ values of 9 and 3 h at 55 and at 70°C, respectively. The K_m and V_{max} values (for colloidal chitin hydrolysis) are 0.396 mg ml^{-1} and 25.25 nkats mg^{-1} S^{-1}. It is strongly inhibited by Hg^{2+}, Al^{3+} and Fe^{3+}, but stimulated by Mn^{2+}, Ca^{2+} and Ba^{2+}. The chitinase is tolerant to organic solvents and detergents. It liberates N-acetylglucosamine and chitobiose from colloidal chitin degradation and has a potential to degrade powdered chitin and chitosan. The high temperature optimum and thermostability makes it a suitable candidate for the production of pharmacologically important N-acetylglucosamine and chitobiose from chitin. The cell walls of spores and hyphae are disrupted in *Fusarium oxysporum*, *Curvularia* sp. and *Stachybotrys* sp. due to the degradation of chitin in the cell walls. The enzyme arrests hatching of eggs in the nematode, *Meloidogyne incognita* and induces mortality in the insect larvae of *Aedes aegypti* as well as mealy bug (*Maconellicoccus hirsutus*).

Keywords: Exochitinase, *Myceliophthora thermophila*, colloidal chitin, N-acetylglucosamine, chitobiose

INTRODUCTION

Chitin is the most underexploited biomass resource available on the Earth. Several million tonnes of chitin residues are produced annually from sea food processing industries worldwide. Recently soluble derivatives of chitin such as N-acetylglucosamine (NAG) and chitobiose have attracted attention particularly in the field of medicine. NAG has therapeutic potential in osteoarthritis, inflammatory bowel disease and skin hyperpigmentation (Chen *et al.*, 2010). Chitooligosaccharides have been proposed as anti-microbial agents, enhancers of the immune response and anticancer agents. The monomers and oligomers are currently produced by acid hydrolysis of chitin using concentrated HCl, which is an inefficient process and also has economical, environmental and technical concerns (Sashiwa *et al*., 2003).

Enzymatic chitin hydrolysis is not only mild and environment-friendly but is also more efficient as the extent of hydrolysis and the consistency of the products can be controlled (Waghmare and Ghosh, 2010). The enzymatic hydrolysis has also been shown to produce N-acetylglucosamine in relatively higher yields than the acid hydrolysis (Pichyankura *et al*., 2002). The process, however, is slow and requires long reaction times, during which hydrolysis reactors are susceptible to contamination (Kuk *et al*., 2005). The use of thermostable enzymes at higher reaction temperatures can minimize contamination risks (Berka *et al.*, 2011). Additional advantages of hydrolysis at elevated temperatures include enhanced mass transfer, reduced substrate viscosity and the potential for enzyme recycling (Margaritis and Merchant, 1986; Unsworth *et al*., 2007).

Interest in chitinolytic enzymes in the field of biological control has arisen due to their involvement in antagonistic activity against pathogenic chitin-containing pests. These pathogenic organisms can be controlled by degrading vital structures (peritrophic membrane and cuticle of insects, fungal cell walls, and eggshells of nematodes) where chitin plays a critical role and can be considered a target for biocides (Kramer and Muthukrishnan, 1997; Patil *et al*., 2000). The absence of chitin in plants and vertebrate animals allows the consideration of safe and selective 'target' molecules for control of chitin-containing pathogens. Several microbial chitinases have shown antagonistic activities against plant pathogenic fungi, nematodes and insect larvae (Siddiqui and Mahmood, 1996; Kramer and Muthukrishnan, 1997; Patil *et al*., 2000). Chitinases can be added as a supplement to the commonly used fungicides and insecticides to make them more potent and to minimize the application of harmful chemical components present in fungicides and insecticides (Bhushan and Hoondal, 1998). Unlike chemical fungicides and bacterial agents, the enzyme-based formulations need repeated application as they would not be viable and active for long time (Neeraja *et al*., 2010). Thus attention is needed on the stability of active enzyme for extended times during storage, transport and field application (Kim and Je, 2010). Stability is also beneficial for biocatalysis as prolonged shelf life of the catalyst reduces process costs. Attempts have, therefore, been made to increase the thermal tolerance of enzymes from fungi (Kim *et al*., 2008; Kim and Je, 2010).

Thermophilic fungi have high potential for industrial applications as a source of thermostable enzymes (Johri *et al*., 1999; Singh *et al*., 2016). Biomass-degrading enzymes from thermophilic fungi consistently demonstrate higher hydrolytic capacity (Wojtczak *et al*., 1987). Thermophilic fungal strains are also capable of rapid growth with minimized viscosity at relatively elevated growth temperatures, thereby enhancing productivity in fermentors (Jensen and Boominathan, 1997). Although chitinases have been reported from a few thermophilic fungi, the application potential of these thermostable chitinases in efficient chitin

degradation remains to be explored (McCormack *et al*., 1991; Guo *et al*., 2005; Li *et al*., 2010; Kopparapu *et al*., 2012).

Myceliophthora thermophilia (Apnis) van Oorschot is a thermophilic mould that grows optimally at 45°C and has attracted attention because of its potential to produce thermostable enzymes. It is capable of producing some industrially important hydrolytic enzymes such as phytase, lipase, cellulase, xylanase and others (Satyanarayana *et al*., 1985; Singh and Satyanarayana *et al*., 2006). The recently sequenced genome of *M. thermophila* isolate ATCC42464 harbours a large number (>210) of glycosyl hydrolases, and thus, the fungus is being considered as an all-purpose decomposer (Berka *et al.*, 2011). Although a 45 kD endochitinase and a 70 kD exochitinase have been reported from this fungus based on ESI-MS/MS analysis of extracellular proteins (Visser *et al.*, 2011), a detailed investigation on biochemical characteristics and application potential of the chitinases has not yet been reported. In this investigation, a chitinase from *Myceliophthora thermophila* was purified, characterized and its applicability in generating chitooligosaccharides has been shown. The biocontrol potential of this thermostable chitinase is also being reported for the first time.

MATERIALS AND METHODS

Source of the thermophilic mould

Myceliophthora thermophila was isolated from a soil sample collected from Gujarat state of India on Emerson's YpSs agar (Emerson, 1941) supplemented with 0.5 % chitin at 45 °C. The mould has been maintained at 4 °C and as glycerol stock at -20 °C.

Molecular identification

The mycelial biomass was suspended in the extraction buffer (50 mM Tris-HCl, pH 8; 50 mM EDTA; 2% SDS; 1% Triton X 100) along with glass beads (diam. 0.5 mm) [1:1.6:0.6] and homogenized for 2 min. in a bead beater (20 sec pulses with intermittent cooling in ice). After centrifugation, genomic DNA extracted from the cell-free supernatant and purified according to Lee *et al*. (2000), has been used as a template for PCR amplification of ITS sequences.

For amplification of ITS sequence, universal primer pair ITS1 (TCCGTAGGTGAACCTGCGG) and 4 (TCCTCCG CTTATTGATATGC) was used. The resulting amplicon of 0.5 kbp was excised and purified with DNA extraction kit (Geneaid) and sequenced. The sequence was used for identifying the fungus with the help of the BLASTn program (www.ncbi.nlm.nih.gov/BLAST) and multiple-sequence alignments using Clustal W program.

Chitinase production

The mould was cultivated in 250 ml Erlenmeyer flasks containing Emerson's YpSs medium in which starch was substituted with colloidal chitin (0.5 %) and 0.1% lactose as inducers. The flasks were incubated at 45 °C and 250 rpm in an incubator shaker for 5 days, and the cultures were harvested by filtration and the cell- free filtrates were used in chitinase assays.

Chitinase assay and protein determination

The chitinase was assayed using colloidal chitin as the substrate prepared from commercial chitin according to Tanaka *et al*. (1999). The released reducing sugars were determined using dinitrosalicylic acid (DNSA) reagent (Miller, 1959) with N-acetylglucosamine (NAG) as the standard. One unit of chitinase is defined as the amount of enzyme that liberates 1 nmol of reducing sugar per second. The protein content in the enzyme samples was determined according to Lowry *et al.* (1951) using bovine serum albumin (sigma) as the standard.

Chitinase purification

The cell-free culture filtrate was concentrated by lyophilisation and re-suspended in 0.1 M acetate buffer (pH 4). The enzyme (114 nkats) was mixed with colloidal chitin (50 mg) and stirred on a magnetic stirrer at 4 °C for 4 hrs, and the colloidal chitin was then collected by centrifugation at 10,000 g for 10 min. and washed twice with ice-chilled deionised water. Desorption of chitinase was done with 0.1 M acetate buffer at 45 °C for 12 hours. Chitin was removed as pellet and the supernatant containing the enzyme was concentrated and further purified by hydrophobic interaction column (9x1.5 cm, bed volume 10 ml) chromatography using phenylsepharose matrix. The column was pre-equilibrated with 0.1 M acetate buffer (pH 4) followed by 1.0 M ammonium sulphate for binding the protein to a hydrophobic column. The proteins were eluted at 1 ml/min with a stepwise decreasing ammonium sulphate gradient from 1.0 to 0.0 M.

Determination of molecular weight and zymography

Sodium dodecylsulfatepolyacrylamide gel electrophoresis (SDSPAGE) was carried out according to Laemmli (1970) in 12 % polyacrylamide gel. The activity of the purified enzyme was demonstrated on native polyacrylamide gels. After electrophoresis, the separating gel was overlaid on the substrate gel containing 1 % colloidal chitin. Both gels were then incubated at 60 °C for 4 hours. Proteins were visualized after staining with 0.1% Coomassie brilliant blue R-250. Images of electrophoresis gels were recorded with GelDoc 2000 (Bio-Rad).

Determination of isoelectric point

Isoelectric focusing (IEF) was performed using BioRad Mini IEF Cell Model 111. The gel was cast with Bio- Lyte 3/10 ampholyte with pH operating range 3.7-9.3. The initial voltage was 100 V for 15 min followed by 200 V for 15 min. The voltage was finally increased to 450 V for 60 min. The focusing was carried out at 4 °C, and the gel was fixed in tricloroacetic acid and the bands were visualized after staining with Coomasie brilliant blue (0.04 %).

Peptide fingerprinting

The purified protein bands fractionated by SDS-PAGE were cleaved by trypsin, and the peptides were sent to The Centre for Genomic Application (TCGA), New Delhi for peptide mass spectrometric analysis by LC/MS (Agilent 1100 series 2D NanoLC MS). Mass spectrometry data were compared with data in the NCBI and Swiss Prot databases using the

Mascot search algorithm.

Characterization

Effect of pH and temperature for activity : The optimum temperature for the enzyme was determined by assaying the enzyme activity at different temperatures, while the heat stability was analyzed by measuring the residual activity after subjecting enzymes to 45 °C and 60 °C. The pH optimum for the enzyme activity was determined by conducting enzyme assay at different pH using different buffers [glycineHCl buffer (pH 3.05.0), NaH2PO4Na2HPO4 buffer (pH 6.08.0), borate buffer (pH 8.09.0), and glycine-NaOH buffer (pH 1012)] at 55 °C.

Kinetic studies : The effect of substrate concentration was studied by performing chitinase assay with different concentrations of colloidal chitin ranging between 0.1 % and 1.0 % and measuring the liberated reducing sugars by using DNSA. The kinetic constants K_m and V_{max} were determined graphically from the LineweaverBurk plot.

Effect of cations, inhibitors, solvents and detergents : The effect of various cations and modulators on the enzyme activity was assessed by incorporating them into the reaction mixtures at 1.0 and 5.0 mM. Similarly various solvents and detergents were incorporated into the reaction mixture at 10% concentration followed by chitinase activity.

Substrate specificity : The substrate specificity of the chitinase towards various natural substrates was measured by using crystalline powdery chitin, non-crystalline colloidal chitin and chitosan at 2 % concentration as substrates in the reaction mixtures. The activity against chromogenic derivative pNp-(Glc-NAc) was determined by incubating 50 µl of enzyme with 50 µl of 25 mM pNp-(Glc-NAc) in 100 mM acetate buffer (pH 4) at 55 °C for 30 min, and determining the released p-nitrophenol at 410 nm. One unit of enzyme activity is defined as the amount that liberates one µmole of p-nitrophenol per minute under the specified conditions.

Enzymatic hydrolysis of chitin : The effect of enzyme dosage on chitin hydrolysis was assessed by incorporating varied amounts of enzyme (20-140 U) in the reaction mixtures containing 100 mg swollen chitin. As the $T_{1/2}$ of chitinase at 55 °C was 9 hours, the initial enzyme concentration was replenished after 9 hours of incubation. The supernatants after centrifugation were analyzed using DNSA reagent. The yield of NAG was estimated from a calibration curve of NAG. The hydrolysis products in the supernatants were also analyzed by HPLC (Waters 600 E) using a carbohydrate column (carbohydrate column, 3.9 x 300 mm), with a mobile phase [acetonitrile : water (70:30)] at a flow rate of 1 ml/min using refractive index detector.

Determination of the biocontrol potential of the chitinase Activity against pathogenic fungi : Antifungal activity of the purified chitinase was estimated by a hyphal extension-inhibition assay as described by Kopparapu *et al*. (2012). The selected fungi were cultured in 100 x 15 mm Petri dishes containing 10 ml of potato dextrose agar. The plates were incubated at 30 °C until fungal growth was observed. Purified chitinase (35 U) was spotted on sterile paper discs (6 mm in diameter), at a distance of 0.5 cm away from the rim of the fungal colony. The plates were incubated at 30 °C for 72 h until mycelial growth envelopes disks and forms zones of inhibition around disks impregnated with chitinase.

Biocontrol against nematode eggs : Approximately equal sized egg masses of *Meloidogyne incognita* were taken in a microtitre plate (one egg mass per well) and 500 µl of purified enzyme (17.5 U) was dispensed into a well. Water and buffer (0.4 M acetate buffer, pH 4.0) were taken as control. The plate was incubated at 28 °C for 12 days and observed under microscope (16 X) after 3, 6, 9 and 12 days for hatching of eggs and appearance of juveniles. Juveniles in each well were counted.

Biocontrol against insects : The biocontrol efficacy of the enzyme samples against 3rd instar larvae of *Aedes aegypti* and grape mealy bugs was determined at National Chemical Laboratory, Pune by Dr. M.V. Deshpande and his coworkers. Five larvae of *Aedes aegypti* were kept in 1:5 dilution of enzyme in tap water and observed for 5 days. Corrected mortality was calculated by Abbott's formula (Abbott, 1925):

$$\% \text{ Mortality} = (\% \text{ Kill in treated} - \% \text{ kill in control}) / (100 - \% \text{ Kill in control}) \times 100$$

For bioassay with *Maconellicoccus hirsutus*, 20 mealy bugs after treatment with lyophilized chitinase, were transferred on sprouted potato and observed for 7 days. Mortality was calculated as above.

RESULTS

The amplification of genomic DNA using the primer pair ITS1 and ITS4 resulted in the fragments of approximately 500 kbp. This fragment consisting of the complete ITS region, including ITS1, 5.8S rRNA and ITS2, was sequenced (Genbank Accession no. KT 287076). Based on the phylogenetic analysis using BLAST, the thermophilic fungal strain was identified as *Myceliophthora thermophila*.

The chitinolytic enzyme was detected in the culture supernatant when *M. thermophila* was grown in the medium containing colloidal chitin as the substrate and N-acetylglucosamine as the inducer. More than 90 % of the activity was adsorbed on colloidal chitin. A summary of purification of chitinase is presented in **Table 1**. The purified protein was homogenous on SDS PAGE with molecular mass of 43.0 kD (**Fig. 1**). The comparison of results of native and SDS PAGE revealed that the chitinase is a monomer. Zymogram of chitinase showed a single zone of digested colloidal chitin (**Fig. 1**). The Iso-Electric-Focusing (IEF) gel revealed that it has isoelectric point of 4.0 (**Fig. 1**).

The purified protein was identified by MALDI-ToF (MS/MS) analysis by comparing the peptide fragments liberated by

Table 1. Purification of chitinase from *M. thermophila*

Purification steps	Total activity (nkats)	Total protein (mg)	Sp. activity (nkats/mg)	Yield (%)	Purification fold
Crude	113.89	62.9	1.81	100	1
Affinity adsorption	66.86	3.6	18.57	58.7	10.25
Hydrophobic interaction chromatography	16.5	0.21	78.57	14.54	43.4

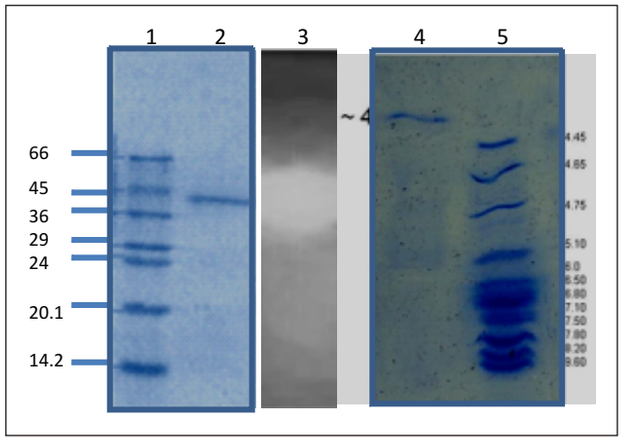

Fig. 1. Detection of molecular weight by SDS-PAGE and isoelectric focusing of the purified chitinase from *M. thermophila*. Lane 1 contains molecular mass markers, lane 2 contains the purified chitinase, lane 3 shows the zymogram, lane 4 shows the purified protein and lane 5 contains pI markers

trypsin digestion with the calculated masses of expected peptides. The spectra matched 11 tryptic peptides that could be correlated with a glycosyl hydrolase family 18 protein from *Myceliophthora thermophila* ATCC 42464 (GenBank Accession No. AE O58299) with 42 % sequence coverage and ion score of 78 in mascot search (**Table 2**). LC-MS analysis of the tryptic peptides also revealed identity of the purified chitinase to glycosyl hydrolase family 18 protein from *Myceliophthora thermophila* ATCC 42464 with 19 % sequence coverage and a score of 565.

Table 2. Peptides identified in the purified chitinase* of *M. Thermophila*

Peptide position	Mass of peptide (m/z)	Amino acid sequence of the matched peptides
38–56	2253.1027	R.QSSGYKNIVYFTNWGIYGR.N
44–56	1602.8098	K.NIVYFTNWGIYGR.N
57–79	2675.4187	R.NYQPDQLPASQLTHVLYSFANIR.S
128–151	2458.2830	K.TLLSIGGWTYSATFPAAASTAESR.A
275–298#	2476.3830	K.ALSDYVAAGVDPAKIVLGMPIYGR.S
289–298	1118.6019	K.IVLGMPIYGR.S
299–330	3470.8011	R.SFEATDGLGKPFTGVGQGSWESGVWDYKVLPR.A
331–352	2336.0971	R.AGATVQYDEEAGATYSYDPATR.E
331–364#	3713.8269	R.AGATVQYDEEAGATYSYDPATRE LISFDTVDMVK.K
374–387	1531.7051	K.GFAGSMFWEASADR.T

***** Calculated mass, 46.181 and calculated pI, 5.11

Peptides identified by LGMS/MS

LSIGGWT: Consensus sequence motif known to play a role in substrate binding

The temperature and pH optima for the activity of chitinase are 55 °C and 4.0, respectively (**Fig. 2**). The chitinase retained 100% activity when exposed to 45 °C for 12 hours. The $T_{1/2}$ values of the chitinase are 9 h at 60 °C and 3 h at 70 °C. The apparent K_m and V_{max} values of the pure chitinase (colloidal chitin) are 0.396 mg ml^{-1} and 25.25 nkats mg^{-1} s^{-1}, respectively.

The cations Mn^{2+}, Ca^{2+} and Ba^{2+} stimulated chitinase activity, while Hg^{2+}, Al^{3+} and Fe^{3+} strongly inhibited chitinase at 5mM concentration (**Table 3**). EDTA has no effect on chitinase, while β-mercaptoethanol, PMSF, Woodward's reagent K and N-bromosuccinamide inhibited chitinase activity to a varied extent (**Table 3**). In the presence of methanol, hexane, ethanol, butanol, acetone, toluene and isoamyl alcohol, the enzyme retained 63-85 % activity (**Table 3**). The chitinase is resistant to detergents and displayed 76 % residual activity in the presence of 10 % SDS (**Table 3**).

Table 3. Effect of various modulators on chitinase activity

Effect	Relative enzyme activity (%)	
Control	100	
Cations	**1mM**	**5mM**
Cu^{2+}	98.05	84.76
Fe^{2+}	106.20	90
Zn^{2+}	98.74	113.26
Al^{2+}	80.11	-
Hg^{2+}	63.45	-
Mg^{2+}	110.32	103.61
Mn^{2+}	119.59	**166.66**
Ba^{2+}	109.55	**138.37**
Co^{2+}	101.1	102.56
Ca^{2+}	113.24	**135.43**
Na^{+}	104.34	102.26
Fe^{3+}	87.97	-
Inhibitors		
Mercaptoethanol	31.28	9.83
Dithiothretol	63.91	41.64
Iodo acetic acid	96.38	54.88
N-Ethylmaleimide	86.59	65.86
N-Bromosuccinimide	84.91	27.43
Woodwards reagent K	86.32	52.11
EDTA	101.12	98.93
PMSF	59.15	50.15
Solvents	**(10%)**	
Methanol	76	
Hexane	85	
Ethanol	75	
Butanol	65	
Acetone	63	
Toulene	97	
Isoamyl alcohol	73.8	
Detergents	**(10%)**	**(20%)**
Tween 80	108.42	87.37
Tween 40	100.86	97.51
Tween 20	119.01	86.33
Triton-X-100	120.14	87.51
SDS	76.45	50.8

The purified *M. thermophila* chitinase displays maximum activity on colloidal chitin. The relative activity of the chitinase on colloidal chitin was 2.08-fold and 1.88-fold higher than on the powered (crystalline) chitin and deacetylated chitosan (**Table 4**). The activity towards synthetic substrate p-nitrophenyl N-acetyl-β-d-glucosaminide (PNP-GlcNAc) substrate was also detected inferring an associated chitobiase activity with the chitinase (**Table 4**).

The hydrolysis was maximum at enzyme concentration of 80 U /100 mg chitin. Increasing the enzyme dosage further had very little observable effect on the yield of reducing sugars. The hydrolysis products could be recovered by removing insoluble substrate by centrifugation, and the supernatant could be lyophilized for obtaining NAG and chitobiose in powder form.

The end products of colloidal chitin hydrolysis are N-acetylglucosamine and chitobiose, suggesting an exo-type

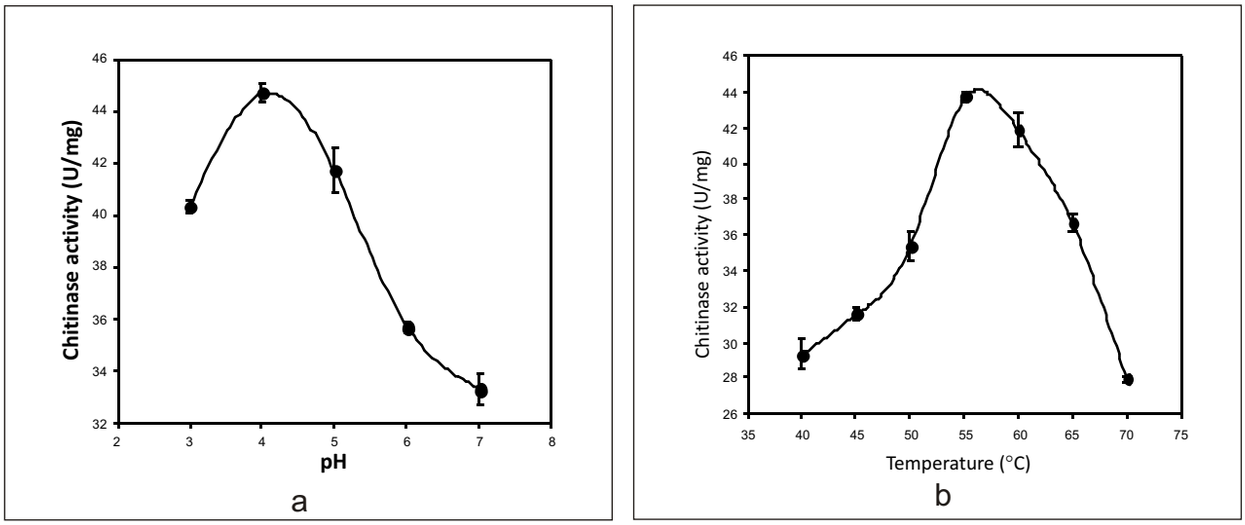

Fig. 2. Effect of (a) pH and (b) temperature on chitinase activity

Table 4. Substrate specificity of *M. thermophila* chitinase

Substrate	Specific activity (U/mg)
Colloidal chitin	9.6
Powdery chitin	4.6
Chitosan	5.1
pNp-GlcNAc[a]	7.2

Table 5. HPLC analysis of the end products liberated by the action of exochitinase of *M. thermophila* on colloidal chitin

Time (h)	NAG(%)	Chitobiose(%)
3	14	86
6	38	62
12	43	57

action of *M. thermophila* chitinase (**Table 5**). A high yield of chitobiose was attained after 3 h of the reaction and it declined with the concomitant increase in NAG. The hydrolysate consisted of 43 % NAG and 57 % chitobiose after 12 h of

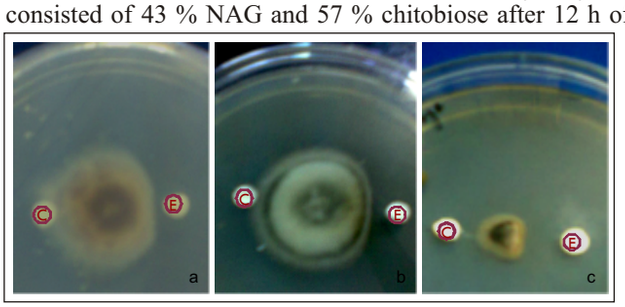

Fig. 3. Inhibition zones of chitinase against (a) *Fusarium oxysporum* (b) *Curvuleria* sp. and *Stachybotrys* sp. (c)

hydrolysis (**Table 5**).

The chitinase inhibited hyphal growth of *Fusarium oxysporum*, *Curvularia* sp. and *Stachybotrys* sp. as depicted by inhibition zones around disc impregnated with 35 U of pure chitinase (**Fig. 3**). The lysis of hyphal walls and resultant appearance of pores was evident after chitinase treatment of *Fusarium oxysporum* and *Stachybotrys* sp. mycelium. The spores of *Curvuleria* sp. appeared distorted under scanning electron microscopy (**Fig. 4**).

Both crude culture filtrate and purified chitinase effectively inhibited hatching of *Meloidogyne incognita* eggs. About 400 juveniles hatched from one egg mass in each well that served as control. However, when an egg mass was treated with chitinase, there was a complete inhibition of hatching of eggs

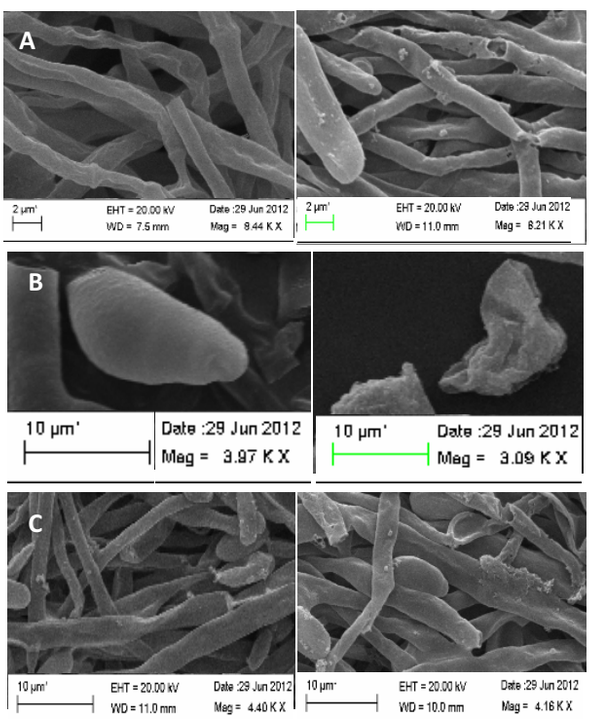

Fig. 4. Scanning electron micrographs showing control (left) and chitinase treated (right) mycelium of *Fusarium oxysporum* (A); spores of *Curvularia* sp. (B) and (C) mycelium of *Stachybotrys* sp.

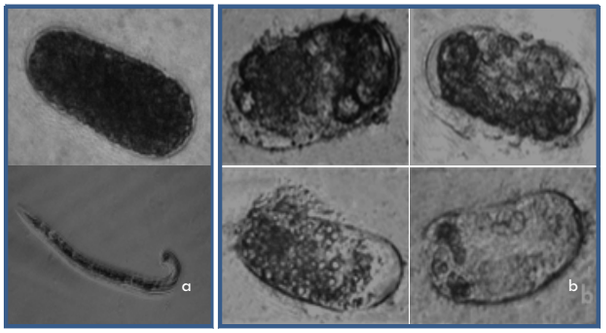

Fig. 5. Chitinase treatment of nematode eggs (a) control showing normal eggs and hatched juveniles and (b) Unhatched eggs showing clearance of egg cytoplasm after enzyme treatment.

(**Table 6**). The microscopic observation of eggs revealed appearance of vacuoles, partial degradation of egg shell and clearance of egg cytoplasm (**Fig. 5**).

It was observed that the chitinase from *M. thermophila* has larvicidal potential against the 3rd instar larvae of *Aedes aegypti*. The pure chitinase as well as crude culture filtrate of *M. thermophila* exerted 50 and 75% mortality of the larvae, respectively (**Table 7**). The crude enzyme extract of *M. thermophila* is also active against grape mealy bugs (*Maconellicoccus hirsutus*). The concentrated extract with 1 U chitinase caused 50% mortality in 7 days (**Table 8**).

DISCUSSION

The thermophilic fungus isolated in this investigation was identified as *Myceliophthora thermophila* based on morphology. The ITS sequence showed high similarity (99 %) to those of *Myceliophthora* species. It produces a chitinase extracellularly when grown in the presence of chitin as in *Thermomyces lanuginosus* (Guo *et al*., 2005) and *Talaromyces emersonii* (Hendy *et al*., 1990). The soluble sugars like glucosamine, N-acetylglucosamine and lactose induced chitinase in *M. thermophila* as reported by Tiunova *et al*. (1983) and Ulhoa and Peberdy (1991) in *Trichoderma viride* and *T. harzianum,* respectively.

Although *M. thermophila* has been reported to produce chitinase based on molecular data, this is the first report on purification and characterization of a chitinase from the fungus. The purified chitinase appeared as a single homogenous band on SDS PAGE with an apparent molecular mass of 43 kD. Usually, fungal endochitinases are monomeric proteins (Kopparapu *et al.*, 2012). The molecular mass for most of the fungal chitinases has been reported to vary from 27 to 190 kD. The reported molecular masses of thermophilic fungal chitinases, however, fall in the narrow range of 43-48 kD (Guo *et al*., 2005; Li *et al*., 2010; Kopparapu *et al*., 2012). The molecular mass of the chitinase is comparable to those reported for other thermophilic and mesophilic fungi: 43.7 kD from *Paecilomyces thermophila*, 43 kD from nematophagous fungi, *Verticillium chlamydosporium* and *V. suchlasporium* (Tikhonov *et al*. 2002), and 43-45 kD from *Metarrhizium anisopliae* (Leger *et al*., 1996). The acidic pI of the chitinase compares well with that of *Metarrhizium anisopliae* (pI 4.8) [Leger *et al*. 1996]. *Talaromyces emersonii* produced four chitinases with pI values in the range of 3.6 - 4.6 (Hendy *et al*., 1990).

Table 6. Hatching of *Meloidogyne incognita* eggs in the presence of chitinase

Days of Incubation	Number of juveniles		
	Control	Buffer	Enzyme
3	66±5	71±12	5±2
6	161±8	168±14	10±1
9	346±16	329±10	11±2
12	405±22	408±15	10±2

Table 7. Bioassay with 3rd instar larvae of *Aedes aegypti*

Treatment	Chitinase (U/ml)	Protein (mg ml^{-1})	Larval mortality (%)
Crude	0.87	1.11	75
Purified	0.7	0.393	50

Table 8. Bioassay with *Maconellicoccus hirsutus*

Treatment	Chitinase (U)	Lipase (U)	Protein (mg ml^{-1})	Mortality (%)
Concentrated enzyme extract	0.2	1	5	35
Concentrated enzyme extract	1.07	5	25	50

Generally fungal chitinases belong to the family 18 of glycosyl hydrolases (Seidl, 2005). The chitinase was identified by MALDI-ToF MS/MS and LC-MS as a glycosyl hydrolase family 18 protein from *M. thermophila* ATCC 42464. Furthermore, the calculated values for molecular mass and pI of *M. thermophila* ATCC 42464 protein are close to the experimental values being reported in this investigation. Chitinases from thermophilic fungi are composed of a single catalytic domain that is similar to other fungal chitinases (Li *et al*., 2010). Alignment of amino acid sequences of the chitinases from thermophilic fungi, *Thermoascus aurantiacus* var. *levisporus* and *Chaetomium thermophilum, Paecilomyces thermophila* and the most similar other fungal chitinases revealed that these chitinases share the LSIGGWT and DXXDXDXE motifs that are considered to be substrate-binding site and a chitin-catalyzing domain, respectively (Watanabe *et al*., 1993; Hollis *et al.*, 2000; Li *et al*., 2010). One of these two sequence motifs (LSIGGWT) is also present in the identified peptides from the chitinase of *M. thermophila*.

Fungal chitinases are active in the pH range of 4.0-7.0. The pH optimum for the activity of chitinase of *M. thermophila* is 4.0, which is very close to those of *Thermomyces lanuginosus* (4.5) [Guo *et al.*, 2005] and other thermophilic fungal chitinases (McCormack *et al.*, 1991; Li *et al*., 2010]. The acidic pH optimum will be advantageous for industrial application as chitin extraction from the shell waste involves demineralization with dilute acid. The optimum temperature for the activity of *M. thermophila* chitinase is 55 °C, which is similar to the chitinase of *Thermomyces lanuginosus* (Guo *et al*., 2005) and *Bacillus licheniformis* strain JS (Waghmare and Ghosh, 2010). The optimum temperatures for the chitinases of *Thermoascus aurantiacus* and *Chetomium thermophilum* are 50 and 60 °C, respectively (Li *et al*., 2010).

The K_m value of 0.39 mg ml^{-1} colloidal chitin is comparable with the earlier reports (Kudan and Pichyangkura, 2009; Watanabe *et al*., 2003). Low K_m values indicate high affinity of the enzyme to the substrate, which makes the enzyme significant for industrial use as the substrate to product conversion rate is high for enzymes with low K_m values (Ahmed *et al*., 2007).

High stability of chitinase is generally considered an economic advantage in industrial processes because of the reduced enzyme turnover. Most fungal chitinases have an optimum activity at 20-40 °C and are not stable at high temperatures (Li, 2006). Some mesophilic fungal chitinases are reported to have high temperature optimum but are not stable for long at their temperature optima (Binod *et al*., 2005). However, chitinases from thermophilic fungi have both high optimum temperature for activity and high thermostability (Guo *et al*., 2005; McCormack *et al*., 1991; Li *et al*., 2010). A chitinase of *Talaromyces emersonii* has maximum activity at 65 °C with a half-life of only 20 min at 70 °C (McCormack *et al*., 1991). In this investigation, the chitinase retained 100 % activity when exposed to 45 °C for 12 hours. The $T_{1/2}$ values of chitinase are 9.0 h at 60 °C and 3 h at 70 °C. This chitinase has higher thermostability than other chitinases reported from thermophilic fungi. The chitinase from *Thermomyces lanuginosus* SY-2 has a $T_{1/2}$ of only 25 min at 65 °C and less than 10 min at 70 °C (Run-fang *et al*., 2008). Chitinases from *Thermoascus aurantiacus* var. *levisporus* and *C. thermophilum* have half life values of 10 min and 50 min at 70 °C, respectively (Li *et al*., 2010). The higher thermostability of the chitinase from *M. thermophila* makes it a potent candidate for industrial chitin hydrolysis.

The cations Mn^{2+}, Ca^{2+} and Ba^{2+} stimulate chitinase activity, while Hg^{2+}, Al^{3+} and Fe^{3+} strongly inhibit chitinase. The chitinases from *Ralstonia* sp. A471 (Sutrisno *et al*., 2004) and *Bacillus* MH-1 (Sakai *et al*., 1998) are also activated by Mn^{2+} and Ca^{2+}, and the chitinase from bacterium C4 was activated by Mn^{2+} (Yong *et al*., 2005). EDTA did not inhibit the chitinase significantly, indicating that metal ions are not required for the activity. *M. thermophila* chitinase is inhibited by a carboxyl group modifier Woodward's reagent K as in *Streptomyces thermoviolaceus* OPC-520, suggesting that the carboxyl group of aspartate and/or glutamate probably plays a role in catalysis (Tsujibo *et al*., 2000). The chitinase is susceptible to N-bromosuccinamide as in the chitinases of *Streptomyces thermoviolaceus* OPC-520, *Pseudomonas* sp. YHS A-2 and *Enterobacter* sp. NRG4 indicating the importance of tryptophan residue(s) for substrate binding or catalysis (Lee *et al*., 2000). The chitinase displays tolerance to organic solvents such as toluene, hexane, methanol and others. High levels of thermal stability are correlated positively with the stability in the presence of organic solvents (Cowan, 1997).

Substrate specificity studies of different chitinases have shown that colloidal chitin is the best substrate among different forms of crystalline and amorphous chitin or chitosan (Waghmare and Ghosh, 2010; Dai *et al*., 2011). Two recombinant chitinases from *Thermoascus aurantiacus* var. *levisporus* and *Chaetomium thermophilum* showed higher activities on colloidal chitin than powered chitin and chitosan (Li *et al*., 2010) as in *M. thermophila*. Activity on synthetic substrate p-nitrophenyl N-acetyl-β-d-glucosaminide (PNP-GlcNAc) was detected inferring that a chitobiase activity is associated with the chitinase of *M. thermophila* as in *Metarhizium anisopliae* (Kang *et al*., 1999) and *Streptomyces* sp. NK1057 (Nawani and Kapadnis, 2004).

Hydrolysis of colloidal chitin with *M. thermophila* chitinase resulted in the liberation of NAG and chitobiose like that of *B. licheniformis* SK-1 (Pichyankura *et al*., 2002). *M. thermophila* chitinase produces chitobiose as the major hydrolysis product initially with small amounts of NAG. Chitobiose was the major end product of chitin hydrolysis by the chitinase of *Pyrococcus kodakarensis* KOD1 (Tanaka *et al*., 1999) and *B. licheniformis* (Waghmare and Ghosh, 2010). In the hydrosylate of *M. thermophila* chitinase, the proportion of NAG increased and that of chitobiose decreased with time. A similar observation was made with the exochitinase Chi71A from *Bacillus thuringiensis* subsp. *pakistani* (Thamthiankul *et al*., 2001). The chitin oligosaccharides are known to have biological functions such as antitumor activity and elicitor action (Kuk *et al*., 2005). Also chitobiose is important among chitooligosaccharides as it has been reported that chitobiose and chitotriose are appreciably absorbed from the gastrointestinal tract when given orally, while higher oligomers are not (Chen *et al*., 2005).

It is well established that chitinases are important components of plant pathogenesis related (PR) proteins and the disease resistance is related to their capacity to degrade cell wall of chitin containing pests such as fungi. The chitinase of *M. thermophila* inhibits the growth of *Fusarium oxysporum*, *Curvularia* sp. and *Stachybotrys* sp. Scanning electron microscopy revealed peeling off of the spore walls and pores in the mycelium. Chitinase was identified as the antifungal agent in the cell free culture filtrate of fluorescent pseudomonads that inhibited mycelial growth of *Fusarium oxysporum* f. sp. *dianthi*, the causative agent of vascular wilt of carnation (Ajit *et al*., 2006). Chitinases from *Trichoderma aureoviride* DY-59 and *Rhizopus microsporus* VS-9 inhibited microconidial germination in *Fusarium solani* (Nguyen *et al*., 2008).

Apart from fungi, nematode egg shell is composed of chitin. The egg is the most resistant stage in the nematode life-cycle and is a threat for agricultural crops as they remain in soil for long periods (Gortari and Hours, 2008). The chitinase of *M. thermophila* effectively inhibits hatching of *Meloidogyne incognita* eggs. The microscopic observation of eggs revealed clearance of egg cytoplasm. The developing eggs and juveniles of *Globodera pallida* (potato cyst nematode) become vacuolated and transparent suggesting hydrolysis of the egg and juvenile contents following treatment with chitinase and protease (Tikhonov, 2002). Incubation of *Meloidogyne incognita* eggs in the presence of chitinase from *Lecanicillium psalliotae* significantly inhibited egg hatching in *in vitro*. Approximately 38.2 % of eggs did not develop or hatch when treated with chitinase, and chitinase and protease, thus, hatching rate was reduced by 56.5 % (Gan *et al*., 2007).

The chitinase of *M. thermophila* has larvicidal potential against the 3rd instar larvae of *Aedes aegypti*. The purified chitinase and crude culture filtrate of *M. thermophila* caused 50 and 75 % mortality of the larvae, respectively. The higher mortality observed with crude preparation can be attributed to the contribution of other enzymes (protease and lipase) of the culture filtrate as reported by Mendonsa (1996).

The chitinase of *M. thermophila* is also active against grape mealy bugs (*Maconellicoccus hirsutus).* The chitinase induced mortality after 7 days. Katke and Balikai (2008) reported that *Verticillium lecanii* and *Metarhizium anisopliae* were effective in managing grape mealy bug. A chitinase from *Trichoderma harzianum* has been shown to negatively affect growth and morphogenesis of *Helicoverpa armigera*, when used in feed or topically applied (Binod *et al*., 2007).

The exochitinase of *M. thermophila* is a promising biocatalyst for the production of NAG and chitobiose from chitin residues, therefore, the gene that encodes this chitinase was cloned and heterologously expressed in *Pichia pastoris* (Dua *et al*., 2017). The high temperature optimum and the thermostability of chitinase are added advantages for its application in the recycling of chitinous residues from food industries at elevated temperatures, thereby increasing hydrolytic rates and reducing contamination risks. This chitinase has also a broad spectrum biocontrol potential against fungi, nematodes and insects.

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Role of Mycorrhizal Fungi in Forestation*

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ABSTRACT

The loss of forest cover results in ecosystem imbalance. As a result of deterioration of the biosphere, soil degradation, over exploitation of forests and population explosion accelerates the rate of deforestation and affects climate. Thus role of forestation in the sustainable development of the ecosystem holds greater promise. In this regard Arbuscular Mycorrhizal fungi as beneficial symbionts of forest plants not only help in the establishment of forest seedlings but also help in their growth, besides increasing soil fertility and stability, tolerance to biotic and abiotic stresses. A brief review on this aspect has been presented.

Keywords: AM fungi, forestation, establishment, plant growth, soil.

ECTOMYCORRHIZA

The ectomycorrhizal roots are characterized by three features namely, i) a mantle which is formed by fungal sheathing or colonization of short feeder roots, ii) the Hartig's net, which is an intercellular hyphal penetration between cortical cells, and iii) colonized feeder roots, showing a morphological differentiation through increased branching and elongation. The root system is heterorhizic with two components, long roots of unlimited growth and short stubby roots of restricted growth. All the root apices grow and may become partially or fully colonized by Mycorrhizal fungus. Root apices when fully colonized grow slowly, branch dichotomously and develop a sheath around them and partially infected root apices continue to grow actively (**Fig. 1A, 2B**).

The dichotomously branched root system along with dense mycelial connections occupy a large volume of soil and acts as a physiologically active organ of absorption. The mycorrhizal association helps in increased uptake of nutrients, water and confers tolerance to root pathogens, drought, edaphic and environmental factors. Ectomycorrhizal fungi provide host plants (macrosymbiont) with growth hormones including auxins, gibberellins, cytokinins and growth regulating "B" vitamins. Mycorrhizal fungi protect the delicate root system from attack by pathogenic soil borne fungi through, a) use of surplus carbohydrates, b) provides physical barrier, c) secretion of antibiotics e.g. diatretyne nitrite, and d) favouring beneficial rhizosphere microflora around the roots. Mycorrhizal fungi contributes to soil organic matter and nutrient cycling in forest ecosystem. Ectomycorrhizal fungi can be grown in pure cultures and therefore has a potential practical utility in regeneration and reclamation of barren lands.

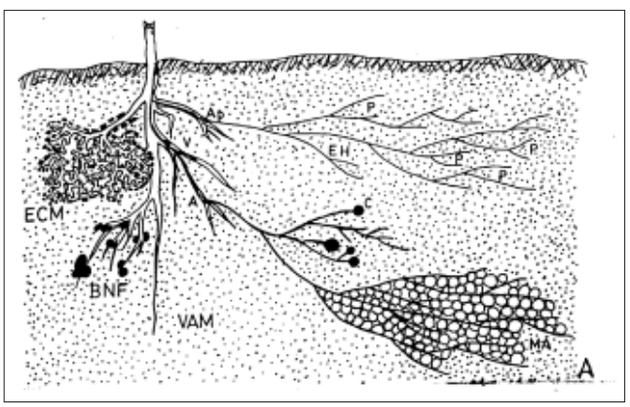

Fig. 1: A. Diagram of three major symbiotic associations with plant roots in the rhizosphere. Ectomycorrhiza (ECM); Nitrogen fixation (BNF) and Arbuscular Mycorrhizal (VAM), P - Phosphate ion; MA - Macro aggregate.

ARBUSCULAR MYCORRHIZAL FUNGI (AM)

Arbuscular Mycorrhizal fungi are characterized by three main features within the plant roots i.e. i) an internal hyphal system connected to external hyphal network through the initial entry points, ii) intracellular arbuscules, which are dichotomously branched, tree like structures enclosed by host plasmalemma and are apparent sites of nutrient exchange between the fungus and the plant, and iii) the vesicles, which are terminal and/or intercalary, thin-walled expanded structures and are not delimited by a septum and contain large quantities of lipids (**Fig. 2C**).

Arbuscular mycorrhizal fungi are obligate symbionts and can only multiply and spread in association with a host plant root. The major obstacle in exploiting the potential use of Arbuscular Mycorrhizal fungi in inoculation studies and forestation programmes are , a) the large scale production of high quality inoculum, b) standardization of inoculum dosage which is infective enough to produce desired growth response, and c) selection of AM symbiont or host fungal combination under given environmental conditions or for particular environmental conditions. The basic requirements for the production of high quality inoculum are small, bulk, high infectivity, highly effective, good shelf life and free from harmful pathogens. The ability of AM fungi to penetrate and spread in roots of the target crop under production condition is the measure of infectivity (Abbott and Robson, 1981a).

The ability of AM fungi to enhance growth or induce stress tolerance are measure of its effectiveness. Effectiveness of a mycorrhizal fungal species may depend upon extramatrical hyphal development, spore multiplication, propagule survival and root colonization. Inoculum effectivity interacts with concentration and position of the inoculum (Abbott and

*Dedicated to the memory of late Prof. B.P.R. Vittal, CAS in Botany, Madras University, Chennai.

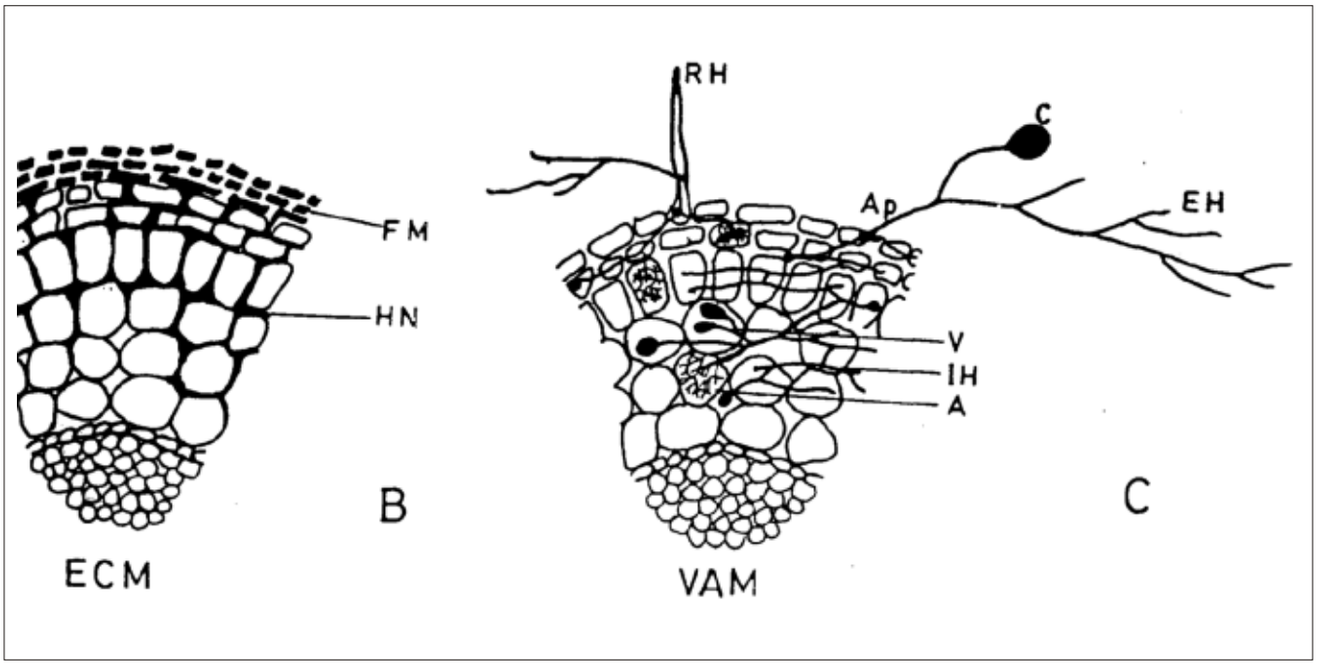

Fig. 2 **B**. & **C**. Transverse section of a root with a schematic representation of Ectomycorrhiza (B), (FM - Fungal Mantle; HN - Hartig's Net) and arbuscular mycorrhiza (C). RH-Root hair; Ap-Appressorium; V- Vesicle; EH-External hyphae; IH - Internal hyphae; C-Chlamydospore.

Robson, 1981b) as well as its infectivity (Wilson and Trinick, 1983).

The selection, establishment, multiplication and management of superior AM fungal species is a pre-requisite for the success of reforestation efforts. The AM fungi persists in soil as spores, colonized root or hyphal network and can be a source of effective inoculum. The Arbuscular mycorrhizal fungi can be established and maintained on interim cover crops such as grasses in pot cultures for routine experimentation. In pot culture AM fungi colonizes the living host plant roots and its extramatrical hyphae proliferate in the surrounding soil producing auxiliary and resting spores. Thus, the pot cultures provide soil based mixed inoculum consisting of spores, infected roots and hyphae. The pot cultures are complex system comprising of colonized host plant roots, AM fungal spores, soil microfiora and edaphic factors. The root inoculum is far easier to obtain in large quantities than spores and it is possible to harvest a large amount of inoculum in short time. The soil inoculum is considered to be more rapidly infective than spore inoculum, possibly because of a greater number of infective propagules i.e. AM spores, external hyphae, AM colonized root fragments consisting of vesicles, arbuscules and intraradical hyphae. The plant roots provide a suitable ecological niche and the associated beneficial soil microflora favours the germination of AM spores.

The root based hyphal network in soil rather than the resting spores is the primary inoculum for seedlings that become established on natural grassland. When used as inoculum, fragments of roots containing intraradical vesicles have induced more rapid colonization and a greater growth response in the host plant than the spores of same species (Abbott and Robson, 1981b; Powell, 1976; Warner and Mosse, 1980).

Mycorrhizal infection typically occurs just behind the root apex, presumably because the rate of root growth carries the apex beyond the infective AM fungal spore before the penetration takes place. The AM root colonization depends upon the host plant-fungal interactions, soil-inoculum potential, edaphic and environmental conditions.

For field sown crops the inoculum must be introduced at the time of seed sowing. The technique used is the addition of a potential soil inoculum to the furrow below the seed at the time of planting (Hayman, 1982). For successful inoculation, the introduced Arbuscular Mycorrhizal fungi would need to form substantial colonization in competition with naturally occurring indigenous AM populations and also increase plant growth.

Screening for efficient mycorrhizal fungi in promoting tree growth on adverse sites is an important step in using these fungi for forestation. The basic requirement is site specific evaluation of efficient mycorrhizal fungal species based on species diversity of the existing indigenous AM population. AM fungi individually or as mixed population have a capacity to adopt to different environments. The choice of mixed inocula ensures wider adaptation to different environmental conditions and greater consistency in benefits to the host plant the Arbuscular mycorrhizal fungi differ in their rate of promoting plant growth and their performance depends on interaction between fungus, the soil where it is growing, as well as the environmental factors. The most common method to evaluate AM species effectiveness is to test plant growth response in adverse conditions such as nutrient deficient soils. The selected host-endophyte combination is introduced in natural field conditions to improve plant growth and survival.

The selected Arbuscular Mycorrhizal symbiont should be able to enhance nutrient uptake by host plant, adaptation to soil environment and persistence in soil maintaining abilities effectivity as well as infectivity. The introduced symbiont should be able to colonize new plant roots quickly, maintain superiority over the existing indigenous populations of AM fungi and the extramatrical hyphae should proliferate extensively in soil facilitating easier absorption in nutritionally poor zone.

For plant growth to respond to inoculation with AM fungi, there must be either an absence or low inoculum potential of indigenous AM fungi and species which are less effective than the inoculant fungi in ability to stimulate nutrient uptake by plants (Abbott and Robson, 1981a and b). The external hyphae of AM fungi proliferates extensively in the soil and provide a larger absorptive and physiologically active area for nutrient and water absorption. The passage of nutrients from soil to the growing plant involves three steps, 1) mobilization of nutrients from soil to the surface of the growing plant root, 2) movement from surface to the interior of root i.e. nutrient uptake, and 3) transport of the absorbed nutrients to the shoot i.e., translocation.

The Arbuscular mycorrhizal fungi increase plant growth in the nutritionally poor soils, as in tropics and the access to poorly soluble form of phosphate is of interest. Phosphate is an essential element for plant nutrition and can be assimilated as soluble phosphate. However, in the soil a large part of the phosphorus is poorly soluble and has low mobility in the soil. Arbuscular Mycorrhizal fungi bridges the depletion zone and have a high affinity for soluble phosphate or have a high phosphorus solublizing and mobilizing potential. Mycorrhizal plants are known to use the same source of inorganic phosphate in soil as do plant roots but have greater access by growth of fungal hyphae supplementing the plant roots. This also appears to be the way in which mycorrhiza responses to poorly soluble phosphate such as rock phosphate. The AM fungi have surface phosphatases that enable them to obtain soil phosphate more rapidly than non-mycorrhizal roots. AM inoculations have beneficial effects on plant growth in low fertility soil as compared to high fertility soils. The high soil nutrient supply, particularly phosphorus suppresses mycorrhizal formation. Thus, the soil fertility is one of the important environmental parameter regulating mycorrhizae response. Mycorrhizae are significantly affected by edaphic factors such as soil pH, moisture, organic matter and temperature. AM fungi do not readily adapt to soils with a pH different from their soil of origin and that pH change restricts AM establishment. However, AM fungi adapt to edaphic conditions characterized in part by soil pH and pH is important in limiting the distribution of some AM fungi.

The arid and semi-arid regions are associated with alkaline and sandy soils with high pH and low soil moisture. The vegetation is typically xerophytic, sparse and found to be highly Arbuscular Mycorrhizal dependent. The nutrient deficient soils with low population densities of indigenous AM fungi limit the productivity of vegetation. Arbuscular mycorrhizal fungi improve water, nutritional and eco-physiological processes of the host plants and confer tolerance to such harsh conditions of water stress and nutrient deficiency. The AM colonization of seedling root systems helps in greater nutrient and water uptake and ensures better growth and survival in the fields.

The Arbuscular mycorrhizal technology is an integral component of seedling production and regeneration efforts. However, the use of AM mycorrhizae as potential tool for tree seedling production is still at nascent stages but the advances in mycorrhizal research have highlighted the scope of their use in sustainable land development and forest cover. The major constraint in drawing benefits for field application is the large scale production of AM inocula. Under such set of limitations, the possible area of successful utilization of AM mycorrhizae is nursery raised tree seedlings which requires much lower quantity of inoculum to make plants mycorrhizal. The pre-inoculation of seedlings in the nursery with selected AM symbionts results in healthy juvenile seedling growth with well developed mycorrhizal root system and provides the best opportunity for the introduction of superior, beneficial AM fungal strains at new forestation site. AM fungi confer tolerance to various biotic and abiotic stressants and reduce seedling loss and enhance primary establishment, growth and survival on outplanting in the field. Seedling quality and field performance are largely governed by processes occurring in the rhizosphere of seedlings. The mycorrhizal symbiosis improves nutrient acquisition and utilization efficiency, water relations and root absorption efficiency which are vital for survival and growth in adverse edaphic and environmental conditions.

The arid, semi-arid and wasteland soils are generally deficient in mineral nutrients, particularly phosphorus and have saline-alkaline problematic soils. Seedlings planted on such lands show poor juvenile growth and high mortality. Inoculation of the seedlings with Arbuscular Mycorrhiza in the nursery is important for forestation and rehabilitation of degraded land areas.

The conditions for utilizing AM fungi in the field (Fitter, 1985) are, i) the levels of reliance of Plants on AM fungi for nutrition and stress resistance, ii) the seasonality of association establishment, iii) host plant endophyte compatibility, and iv) nutritional or other edaphic characteristics of the host soil.

The Arbuscular Mycorrhizal symbiosis is beneficial for plant growth and survival in many ways:

Increased nutrient uptake from soil by increasing the absorptive surface area of the root system. The extramatrical AM hyphae proliferate in bulk soil and make available poorly mobile elements such as phosphorus, zinc and copper. The Vesicular-arbuscular (VA) mycorrhizal plants are well nourished as compared to non-mycorrhizal plants in nutrient deficient soils. The AM fungi confer drought tolerance to plants by increasing the water absorption capacity of the root system or by altering the host physiology. The external hyphal network contributes to the process of creating a stable soil aggregate structure, improving soil structure for better aeration and water percolation. The Arbuscular Mycorrhizal fungi increase the beneficial microbial populations in the

mycorrhizosphere with increased enzymatic activities. The tripartite relationship between host, AM fungus and *Rhizobium* results in higher nitrogen fixation and increased availability of phosphorus improving plant growth response. Mycorrhiza plays an important role in changing the ecology of a given site and is used to reclaim degraded lands. VA mycorrhiza reduces the incidence and/or severity of root diseases and provides resistance to root borne diseases as well as resistance against harmful pathogenic organisms including nematodes. Mycorrhizal associations promote mineral cycling and are key components of efficient closed nutrient cycle of natural ecosystems. The VA mycorrhiza increases plant tolerance to various biotic and abiotic stressants including alkalinity, salinity, toxicities associated with mining operations, heavy metals and mineral imbalances. Mycorrhiza confers resistance to high soil temperatures, improves soil fertility and phytobiomass production per unit area of land. Arbuscular Mycorrhizae have a potential use as biofertilizers and replace the fertilizer requirements of trees in areas of marginal fertility and reduce the need for current high levels of fertilizers. Arbuscular mycorrhizaI symbiosis confers tolerance to survive transplantation shock and increases the primary establishment, growth and survival of seedlings on outplanting. Arbuscular mycorrhizal technology is an efficient, low input biotechnology forestation tool for marginal, degraded, arid and semi-arid lands.

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Studies on Coprophilous Agaricoid Mushrooms: An Appraisal

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ABSTRACT

The present paper is an attempt to review and provide systematic information about the ecology, diversity, distribution and human relevance of wild coprophilous mushrooms the world over. The information provided herein is derived from a study carried out in the state of Punjab in India and records contained in more than 125 authentic monographic publications and research articles throughout the world. During the survey from the years 2007 to 2011in Punjab state, a total number of 172 collections have been observed, growing as saprobes on dung of various domesticated and wild herbivorous animals in pastures, open areas, zoological parks, and on dung heaps along roadsides or along village ponds, etc. High coprophilous mushrooms' diversity has been established and a number of rare and sensitive species were recorded during the study. Also while analysing the relevant references related to coprophilous mushrooms and their ecological places it was noted that dung is an important substrate which serves as a favorable niche for the growth of a variety of mushrooms throughout the world. The present paper aims to create awareness for conservation of the fascinating world of coprophilous fungi in their natural habitats. The status and taxonomic placement of each taxon in this review is updated as per MycoBank Database. The paper can serve as base line information and indicator for further mycological studies in India as well as in other countries with similar scenarios.

Keywords: *Agaricomycetes*, *Basidiomycota*, biodiversity, dung, mushrooms, distribution

INTRODUCTION

Fungi grow everywhere and in all sorts of habits and habitats. The saprotrophic fungi are the primary agents responsible for decomposition of organic matter. These fungi bring about spoilage of food and damage fabrics, paper, leather and other organic matter. They also grow on dung of all kinds of animals (Dix and Webster, 1995). In scientific terms the 'dung-loving' fungi are known as 'coprophilous' or 'fimicolous'. They represent a diverse community of morphologically and physiologically specialized mycota which provide a biological force for the decomposition and recycling of animal faeces (Richardson, 2008). The world over studies on coprophilous fungi suggests that this group plays an important role in carbon flow, ecosystem energetics and in the formation of soil (Halfter and Matthew, 1971; Angel and Wicklow, 1974, 1975; Kumar *et al.*, 1995). They possess a wide variety of adaptive characteristics that assist their survival and reproduction on dung. These characteristics include the phototrophic nature of the spore-producing structures, adhesive projectiles of their spores enabling attachment to the herbages, pigmentation in spores which provide protection against UV exposure and the resistance of their spores to digestive enzymes and acids in the animal gut (Dix and Webster, 1995; Richardson, 2008). The undigested carbohydrates, hemicelluloses and lignin, along with amino acids, vitamins, growth factors and minerals in the dung, aid their colonization and growth (Kumar *et al.,* 1995). The physical structure of dung, its pH value and high moisture contents are reported to be the major contributing factors for its suitability as a fungal substratum (Morrison 1959; Lodha, 1974).

Coprophilous fungi belong to wide range of taxonomic groups including *Acrasiales*, *Mycetozoa*, *Mucorales*, *Pezizales*, *Sordariales*, *Coprinaceae* and some other basidiomycetous fungi (Kirk *et al.*, 2008). The present review, however, has been focused on the diversity and distribution of lamellate basidiomycetous coprophilous macrofungi belonging to the order *Agaricales* (Singer, 1986; Kirk *et al.,* 2008).

Coprophilous mushrooms are reported to occur more frequently on dung of herbivores than carnivores. The dung of meat-eating animals being quite rich in proteins possesses bacteria and some insects which plays a pivotal role in decomposition (Bell, 1983; Richardson, 2001b). As compared in herbivorous dung much of the content is cellulose and lignin which are mostly decomposed by basidiomycetous fungi as bacteria cannot decompose these complex substances. Coprophilous mushrooms have seldom been documented on reptilian or amphibian dung, indicating that coprophily in fungi might have developed among the warm-blooded animals (Webster, 1970). Some of the common dung addicts as documented by Arora (1986) include *Agaricus bisporus* (J.E. Lange) Imbach, *Agrocybe pediades* (Pers.:Fr.) Fayod, *Bolbitius titubens* (Bull.)Fr., *Clitocybe nuda* (Bull.) H.E. Bigelow & A.H. Sm., *C. tarda* Peck, *Stropharia semiglobata* (Batsch) Quél, *Volvariella speciosa* (Fr.) Singer, *Conocybe* sp., *Coprinus* sp., *Panaeolus* sp., *Psilocybe* sp., etc.

Herbivorous dung has been reported to contain the macerated and undigested remains of plant food and vast quantities of bacteria and animal waste products, such as broken-down red blood cells and bile pigments, etc. (Lodha, 1974; Webster, 1970). It is reported to be rich in water-soluble vitamins, growth factors, and mineral ions, some of which are metabolic by-products of the microbes in herbivore's gut (Lambourne and Reardon, 1962). It is also reported to contain a large amount of readily available carbohydrates (Richardson, 2001b). The nature of herbivore dung has been reported to largely depend on the efficiency of the digestive tract of the animal, which, in turn, has been reported to depend on the animal's digestive anatomy and its microflora. Ruminants are reported to produce fine-textured dung as compared to horses, with a less efficient digestive system, which have been reported to produce much coarser dung (Bell, 1983; Ing, 1989; Richardson, 1998; 2003). Because of the great variation in the feeding habits, habitats, and digestive systems of herbivores, a variety of mushrooms are

documented to grow indiscriminately on any herbivore dung. Their greatest variety has been reported on cow, buffalo, horse, elephant and rabbit dung, but this is reported to be because the majority of research throughout the world has remained focused on the dung of these animals only.

THE WORLD SCENARIO

The distribution of coprophilous fungi is primarily influenced by the presence of herbivores in an area, type of vegetation, kind of dung, climatic conditions and latitudinal environmental gradients (Webster, 1970; Angel and Wicklow, 1975; Kumar *et al.* 1995; Piontelli *et al.*, 2006). The coprophilous mushrooms are common during the rainy season, especially when the relative humidity in the environment is very high. These mushrooms are quite diverse and cosmopolitan in their distribution as is evident from the information gathered from the published literature over a period of time. There are about 35 agaricoid mushroom genera spread over 10 families on which sufficient literature has been published which have been presented in tabulated form **(Table 1).**

COPROPHILOUS AGARICS FROM AUSTRALIA/ OCEANIA

The Australia and Oceania region is surrounded by the Indian, Southern and Pacific Oceans. It includes the entire Australian mainland, such big islands as New Zealand, Tasmania, New Guinea (only its eastern half), and many thousands of tiny, tropical islands of Melanesia, Micronesia and Polynesia regions, scattered throughout the South Pacific. Among Australia's herbivorous wild animals are the Kangaroo, Koala, Gliders and Wallaby. Domestic animals include Horses, Cattle, Goats, Sheep, and Donkey etc. Very little information could be gathered about the occurrence of fimicolous agarics from this region.

Watling and Taylor (1987) in their documentation on the family *Bolbitiaceae* from New Zealand reported *Pholiotina vexans* (P.D. Orton) Bon from cow dung, *Conocybe pubescens* (Gillet) Kühner from horse dung, alongwith two unnamed *Conocybe* species which were also recorded growing on dung. Peter and Buchanan (1995) reported *Psilocybe* species from New Zealand. *Psilocybe argentina* (Spegazzini) Singer was found growing on sheep dung, *P. coprophila* (Bull.: Fr.) P. Kumm. on sheep and horse dung and *P. subcoprophila* (Britzelm.) Sacc. on horse dung. Hausknecht and Krisai-Greilhuber (2003) reported *Panaeolus antillarum* (Fr.) Dennis growing on horse manure heaps from Australia.

COPROPHILOUS AGARICS FROM EUROPEAN REGION

Significant amount of literature is available on coprophilous fungi in general and agarics in particular from the European region. Singer (1977) recorded 06 species of *Agrocybe* Fayod, namely *A. coprophila* Katajev, *A. cubensis* (Murrill) Singer, *A. fimicola* (Speg.) Singer, *A. neocoprophila Singer, A. platysperma* (Peck) Singer, *A. sacchari* (Murrill) Dennis; *10 species of Bolbitius Fr., namely B. coprophilous* (Peck) Hongo, *B. demangei* (Quél.) Sacc. & D. Sacc., *B. exiguous* Singer, *B. glatfelteri* Peck, *B. gloiocyaneus* G.F. Atk., *B. lacteus* J.E. Lange, *B. mesosporus* Singer, *B. umanetucnsis* Singer, *B. variicolor* G.F. Atk., *B. vitellinus* (Pers.) Fr.; 02 species of *Conocybe* Fayod *viz., C. albipes* (G.H. Otth) Hauskn., *C. brunneidisca* (Murrill) Hauskn. and *Pluteolus glutinosus* Clem. growing on dung from this region.

Orton and Watling (1979) recorded *Parasola schroeteri* growing on cattle and horse dung from Europe. Watling (1982) in his work "British Fungus Flora- Agarics and Boleti" reported 13 species falling in 03 agaric genera growing on dung from Scotland and British Isles. These include *Agrocybe subpediades* (Murrill) Watling, *Bolbitius titubans* (Bull.) Fr., *B. variicolor* G.F. Atk., *B. vitellinus* (Pers.) Fr., *Conocybe antipus* (Lasch) Fayod, *C. coprophila* (Kühner) Kühner, *C. farinacea* Watling, *C. fuscimarginata* (Murrill) Singer, *C. intrusa* (Peck) Singer, *C. lenticulospora* Watling, *C. murinacea* Watling, *C. pubescens* (Gillet) Kühner, and *C. rickenii* (Jul. Schäff.) Kühner. These species have been documented from variety of dung types including those of cattle, rabbit, horses, etc.Moser (1984) reported *Panaeolus alcidis* Moser growing on moose dung and on roe deer and reindeer droppings from Sweden.Watling and Gregory (1987) while investigating the British fungi described 16 species of coprophilous mushrooms including 08 species of *Psilocybe,* 07 species of *Panaeolus* and 01 species of *Stropharia.*

The contributions of Uljé and Bas (1988, 1991) and Uljé and Noordeloos (1993, 1997, 1999) to the taxonomy of coprophilous macrofungi from Europe especially Netherlands belonging to coprinoid genera are outstanding. Uljé and Bas (1988) reported 03 species, namely *Parasola megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple, *P. misera* (P. Karst.) Redhead, Vilgalys & Hopple and *P. schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple from pure dung. Uljé and Bas (1991) described 10 species, namely *Coprinus stellatus* Buller, *Coprinellus bisporus* (J.E. Lange) Vilgalys, Hopple & Jacq. Johnson, *C. congregatus* (Bull.) P. Karst., *C. curtus* (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson, *C. ephemerus* (Bull.) Redhead, Vilgalys & Moncalvo, *C. heptemerus* (M. Lange and A.H.Sm.) Vilgalys, Hopple & Jacq. Johnson, *C. heterosetulosus* (Locq. ex Watling) Vilgalys, Hopple & Jacq. Johnson, *C. marculentus* (Britzelm.) Redhead, Vilgalys & Moncalvo, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo and *C. sassii* (M.Lange & A.H.Sm.) Redhead, Vilgalys & Moncalvo from various dung types of different herbivorous animals. Uljé and Noordeloos (1993) documented 06 species, namely *Coprinopsis cordispora* (T. Gibbs) Gminder, *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo, *C. poliomalla* (Romagn.) Doveri, Granito & Lunghini, *C. pseudocortinatus* (Locq. ex Cacialli, Caroti & Doveri) Doveri, *C. pseudonivea* (Bender & Uljé) Redhead, Vilgalys & Moncalvo, and *C. utrifera* (Joss. ex Watling) Redhead, Vilgalys & Moncalvo, from dung habitats. Uljé and Noordeloos (1997) reported 05 species belonging to coprinoid macrofungi, namely *Coprinopsis filamentifera* (Kühner) Redhead, Vilgalys & Moncalvo, *C. luteocephala* (Watling) Redhead, Vilgalys & Moncalvo, *C. sclerotiorum* (Horvers & de Cock) Redhead, Vilgalys & Moncalvo, *C. vermiculifera* (Joss. ex Dennis) Redhead,

Vilgalys & Moncalvo, and *C. xenobia* (P.D. Orton) Redhead, Vilgalys & and Moncalvo, from dung of herbivore animals. Uljé and Noordeloos (1999) reported 08 species, namely *Coprinopsis bicornis* (Uljé & Horvers) Redhead, Vilgalys & Moncalvo, *C. cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo, *C. krieglsteineri* (Bender) Redhead, Vilgalys & Moncalvo, *C. lagopus* (Fries) Redhead, Vilgalys, & Moncalvo, *C. macrocephala* (Berk.) Redhead, Vilgalys & Moncalvo, *C. pseudoradiata* (Kühner & Joss. ex Watling) Redhead, Vilgalys & Moncalvo, *C. radiata* (Bolton) Redhead, Vilgalys & Moncalvo and *C. scobicola* (P.D. Orton) Redhead, Vilgalys & Moncalvo from dung localities.

Samorini (1993) reported *Panaeolus cyanescens* (Berk. and Broome) Sacc. as common mushroom growing in the manure of buffalo, cow, and horse in Italy. Jordon (1995) in "The Encyclopedia of Fungi of Britain and Europe" reported 25 species belonging to 07 genera as coprophilous in habit. These include 11 species of *Coprinus*, 04 of *Panaeolus,* 04 of *Psilocybe,* 03 of *Bolbitius,* and 01 species each of *Conocybe, Stropharia* and *Lepiota*. Stamets (1996) documented *Panaeolus subbalteatus* (Berk. & Br.) Sacc. growing caespitosely or gregariously on dung or in well manured ground in autumn, spring and summer seasons and *P. acuminatus* (Schaeff.) Quél. growing scattered to gregariously in well-manured grounds or on dung from Europe.

Richardson and Watling (1997) presented four keys to the coprophilous fungi. Out of these, Keys 1 and 2 are for the coprophilous ascomycetes, Key 4 for the determination of the members of coprophilous *Zygomycota* while Key 3 for the determination of dung-inhabiting basidiomycetes. In Key 3 a total number of 66 species, including 29 species of *Coprinus,* 12 species of *Conocybe,* 08 species of *Psilocybe,* 06 species of *Panaeolus*, 03 species of *Psathyrella,* 02 of *Stropharia*, and 01 species each of *Agrocybe, Bolbitius, Clitocybe*, *Lepista*, *Leucocoprinus,* and *Volvariella* has been keyed out.Kytövuory (1999) originally described *Protostropharia alcis* (Kytöv) Redhead, Thorn & Malloch from Boreal Region of Europe, where it grows on elk dung.

Doveri (2004) published the first monograph on coprophilous fungi from Italy entitled "Fungi Fimicoli Italici". It is regarded as the starting point of a survey on fungi obligatorily or facultatively growing on any kind of dung. It included keys and descriptions covering 80 taxa of *Basidiomycota* and 214 of *Ascomycota* growing both in the natural state and in damp chamber cultures of dung of different herbivorous animals from Italy. Doveri (2010) listed 80 species of coprophilous *Agaricales* detected in the field from Italy and categorized these depending upon their dung source. These included 14 species of *Coprinopsis,* 13 species of *Conocybe,* 11 species each of *Coprinellus* and *Panaeolus,* 06 species of *Psilocybe,* 05 species each of *Agrocybe* and *Coprinus*, 04 species of *Bolbitius,* 03 species each of *Psathyrella* and *Stropharia,* 02 species of *Parasola,* and 01 species each of *Lepista, Leucocoprinus,* and *Volvariella*. Out of the total taxa documented, 54% were reported to be associated with bovine dung, 42% with equine dung, and the remaining 4% with dung of other herbivores. Out of the various agaricoid mushrooms documented about 47% represented species of *Coprinus s.l.* Doveri *et al.* (2010) re-examined the genus *Coprinellus* from its establishment, through demotion as a synonym of *Coprinus,* and up through its current reinstatement. They isolated an agaric from chamois dung and, based on morphological data, regarded it as a new species *Coprinellus mitrinodulisporus*. Doveri (2011) in "Addition to Fungi Fimicoli Italici" updated the work on coprophilous fungi reporting 43 additional species (5 *Basidiomycota* and 38 *Ascomycota*) new to Italy with the introduction of new keys, descriptions of species, and an account on their ecology.

Richardson (2004) reported a total of 81 species of coprophilous ascomycetous and basidiomycetous fungi from 32 herbivore dung samples collected from Iceland. The agaricoid members namely *Coprinopsis cordispora* (T. Gibbs) Gminder, *C. macrocephala* (Berk.) Redhead, Vilgalys and Moncalvo, *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo, *C. pseudoradiata* (Kühner & Joss. ex Watling) Redhead, Vilgalys & Moncalvo, *C. stercorea* (Fries) Redhead, Vilgalys & Moncalvo, *Coprinellus heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, *Parasola misera* (P. Karst.) Redhead, Vilgalys & Hopple, *Panaeolus antillarum* (Fr.) Dennis, *P. semiovatus* (Sowerby) S. Lundell & Nannf., and *Psilocybe subcoprophila* (Britzelm.) Sacc. were reported to be very common on dung. Richardson (2011) in 'Additions to the Coprophilous Mycota of Iceland' article recorded 11 mushrooms from animal dung, namely *Coprinellus heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & and Jacq. Johnson, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, *Coprinopsis cordisporus* (T. Gibbs) Watling & M.J. Richardson, *C. ephemeroides* (DC.: Fr.) Watling & M.J. Richardson *C. nivea* (Pers.: Fr.) Redhead, Vilgalys & Moncalvo, *C. radiata* (Bolton: Fr.) Redhead, Vilgalys & Moncalvo, *C. stercorea* (Fr.) Redhead, Vilgalys & Moncalvo, *Panaeolus papilionaceus* (Bull.: Fr.) Quél., *P. semiovatus* (Sowerby: Fr.) var. *phalaenarum* (Fr.) Ew. Gerhardt, *Parasola misera* (P. Karst.) Redhead, Vilgalys & Hopple and *Psilocybe coprophila* (Bull.: Fr.) P. Kumm.

Hausknecht *et al.* (2005) described in all 56 taxa of the genus *Conocybe* from Finland, out of which 08 species, namely *C. farinacea* Watling, *C. fimetaria* Watling, *C. fuscimarginata* (Murrill) Singer, *C. lenticulospora* Watling, *C. pubescens* (Gillet) Kühner, *C. rickenii* (Jul. Schäff.) Kühner, *C. singeriana* Hauskn. and *C. watlingii* Hauskn. have been recorded growing on herbivorous dung or manure. Hausknecht and Contu (2007) reported *Conocybe brunneidisca* (Murrill) Hauskn. from dung localities or in fertilized meadows from Italy. Hausknecht *et al.* (2010) reported *Bolbitius excoriates* Dähncke, Hauskn., Krisai, Contu & Vizzi as a new species growing gregariously on horse dung from Spain.

Larssona and Örstadiusb (2008) while working on dung inhabiting mushrooms in the Nordic countries identified 14 *Psathyrella* species. They documented *Psathyrella fimiseda* Örstadius & E. Larss. and *P. merdicola* Örstadius & E. Larss.

from cow dung, *P. romagnesi* Kits van Wav. from mixtures of straw and dung of horse, more seldom of cow, *P. scatophila* Örstadius & E. Larss. from the dung of badger, horse, cow, and elk, *P. hirta* Peck, *P. purpureobadia* Arnolds, *P. sphaerocystis* P.D. Orton, *P. stercoraria* Kühner & Joss., *P. saponacea* F.H. Møller from horse and cow dung, *P. tenuicula* (P. Karst.) Örstadius & Huhtinen from the dung of wild boar, deer and their allies, *P. conopilus* (Fr.) A. Pearson & Dennis and *P. microrhiza* (Lasch) Konrad & Maubl. from unspecified dung, *P. potteri* A.H. Sm. from mixtures of dung and straw, sometimes on raw dung, and *P. prona* (Fr.) Gillet from manured soil.

Prydiuk (2010) reported fimicolous representatives of the coprinoid taxa as a result of both the use of moist-chambers and the field research from the territory of Ukraine. In all 8 species, namely *Coprinellus bisporus* (J.E. Lange) Vilgalys, Hopple & Jacq. Johnson, *C. brevisetulosus* (Arnolds) Redhead, Vilgalys & Moncalvo, *C. curtus* (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson, *C. heterosetulosus* (Locq. ex Watling) Vilgalys, Hopple & Jacq.Johnson, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, *C. congregatus* (Bull.) P. Karst., *C. ephemerus* (Bull.) Redhead, Vilgalys & Moncalvo and *Parasola miser* (P. Karst.) Redhead, Vilgalys & Hopple were reported to be associated with dung. Prydiuk (2011) recorded 9 representatives of the coprinoid fungi collected during their investigations on coprophilous mushrooms. Out of these five species, *Coprinopsis cordispora* (T. Gibbs) Gminder, *C. foetidella* (P.D. Orton) A.Ruiz & G. Muñoz, *C. pseudonivea* (Bender & Uljé) Redhead, Vilgalys & Moncalvo, *C. pseudoradiata* (Kühner & Joss. ex Watling) Redhead, Vilgalys & Moncalvo and *C. utrifera* (Joss. ex Watling) Redhead, Vilgalys & Moncalvo] were collected in Ukraine for the first time. For *Coprinopsis ephemeroides* (DC.) G. Moreno, *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo and *C. radiata* (Bolton) Redhead, Vilgalys & Moncalvo new localities were registered. Házi *et al.* (2011) on the basis of morphological characters and species phylogeny inferred from ITS1-5.8S-ITS2 and β - tubulin gene sequences described a new coprophilous species, *Coprinellus radicellus* Házi, L. Nagy, Papp & Vágvölgyi, from Sweden.

Gierczyk *et al.* (2011) documented a list of fifty-five coprinoid fungi found in Poland. They reported *Coprinellus bisporus* (J.E. Lange) Vilgays, Hoppe & Jacq. Johnson; *C. congregatus* (Bull.) P. Karst., *C. curtus* (Kalchbr.) Vilgalys, Hoppe & Jacq. Johnson; *C. flocculosus* (DC.) Vilgays, Hoppe & Jacq. Johnson; *C. heptemerus* (M. Lange & A. H. Sm.) Vilgalys, Hopple & Jacq. Johnson; *C. heterosetulosus* (Watling) Vilgalys, Hopple & Jacq. Johnson; *C. marculentus* (Britzelm.) Redhead, Vilgalys & Moncalvo; *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo; *C. plagioporus* (Romagn.) Redhead, Vilgalys & Moncalvo; *Coprinopsis candidata* (Uljé) Noordel., *C. cordispora* (T. Gibbs) Noordel.; *C. cothurnata* (Godey) Redhead, Vilgalys & Moncalvo; *C. foetidella* (P.D. Orton) Noordel., *C. jonesii* (Peck) Redhead, Vilgalys & Moncalvo; *C. narcotica* (Batsch) Redhead, Vilgalys & Moncalvo; *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo; *C. pseudofriesii* (Pilát and Svrček) Redhead, Vilgalys and Moncalvo; *C. pseudonivea* (Bender & Uljé) Redhead, Vilgalys & Moncalvo; *C. pseudoradiata* (Watling) Redhead, Vilgalys & Moncalvo; *C. radiata* (Bolton) Redhead, Vilgalys & Moncalvo; *C. trispora* (Kemp & Watling) Redhead, Vilgalys & Moncalvo; *C. tuberosa* (Quél.) Doveri, Granito & Lunghini; *C. xenobia* (P.D. Orton) Redhead, Vilgalys & Moncalvo; *Coprinus sterquilinus* (Fr.) Fr.; *Parasola megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple; *P. misera* (P. Karst.) Redhead, Vilgalys & Hopple and *P. schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple as coprophilous species growing on herbivorous dung and dung mixed with straw. Gierczyk *et al.* (2014) described 19 coprinoid fungi, found in Poland, out of which *Coprinellus radicellus* Házi, Nagy, Vágvölgyi & Papp was recorded growing on moose dung; *Coprinopsis annulopora* (Enderle) P. Specht & H. Schubert on horse manure; *C. candidolanata* (Doveri & Uljé) Keirle, Hemmes & Desjardin on deer and sheep dung and *C. scobicola* (P.D. Orton) Redhead, Vilgalys & Moncalvo on dung compost.

Ruiz and Ruiz (2016) reported *Coprinopsis foetidella* (P. D. Orton) A. Ruiz & G. Muñoz growing on alpaka dung from Navarre in Spain. Melzer (2017) provided an exhaustive dichotomous key including most of the previously described coprinoid species from Europe, some of which have since been transferred from the genus *Psathyrella*. This key is based predominantly on microscopic characteristics and drawings. Some macroscopic and ecological features are also included for presenting an overview of more than 200 coprinoid mushrooms growing on soil, litter, wood, herbicol, open habitat, indoor, burned ground, calcareous ground along with those growing on dung and fertilized substratum.

COPROPHILOUS AGARICS FROM ANTARCTICA

Antarctica is a remote and inhospitable continent. The climate is the coldest and driest known on Earth; nevertheless it is not uniform across the continent, and different climatic regions can be distinguished (Øvstedal and Smith, 2001). The prevailing Antarctic conditions of low temperature, low water availability, frequent freezethaw cycles, low annual precipitation, strong winds, high sublimation and evaporation, high incidence of solar and especially ultraviolet radiation together constitute significant limiting factors for plant and animal life. Therefore, the biology of Antarctica, more than other continents, is dominated by microorganisms (Friedmann, 1993; Ruisi *et al.*, 2007), with a high level of adaptation and able to withstand extreme conditions.

There are about 20 species of macro-fungi (mushrooms) that have been reported to exist in the Antarctic, according to the British Antarctic Survey (BAS), which has several research stations around the peninsula region. The mushrooms belonging to the genus *Galerina* which can live on many different substrates such as wood, moss or other types of organic materials were discovered on an island off the Antarctic Peninsula (Rejcek, 2012). This proved the existence of mushrooms in continental Antarctica, although the fruiting bodies on dung have never been recorded.

COPROPHILOUS AGARICS FROM SOUTH AMERICAN REGION

Much of the work on coprophilous mushrooms in the South American region is largely from Brazil, Falkland, Venezuela, Columbia, etc. Guzmán (1978a) described *Panaeolus venezolanus* Guzmán from Venezuela. It was documented growing gregariously on cow dung or on rich soils, in meadows of the subtropical forests. Arora *(1986)* documented *P. cubensis* (Earle) Singer from the dung localities of Columboa. Stamets (1996) documented *Panaeolus subbalteatus* (Berk. & Br.) Sacc. growing caespitosely or gregariously on dung or in well manured ground in autumn, spring and summer seasons from South America.

Richardson (2001a) documented eight species of coprophilous mushrooms from the state of Matto Grosso do Sul, Brazil. These are *Cyathus stercoreus* (Schwein.) De Toni, *Coprinopsis stercorea* (Fr.) Redhead, Vilgalys & Moncalvo, *C. cordispora* (T. Gibbs) Gminder, *C. radiata* (Bolton) Redhead, Vilgalys & Moncalvo, *Coprinellus curtus* (Kalchbr.) *Vilgalys*, *Hopple & Jacq*. *Johnson,* C. *heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, along with an unidentified species similar to *Coprinopsis stercorea* (Fries) Redhead, Vilgalys & Moncalvo. Cortez and Coelho (2004) reported *Stropharia semiglobata* (Batsch) Quél. growing solitary on horse dung from Brazil. Wartchow *et al.* (2007) published three *Psilocybe* (Fr.) P. Kumm. growing on dung in Pernambuco State, Northeastern Brazil. Wartchow *et al.* (2010) reported *Panaeolus cyanescens* (Berk. & Broome) Sacc. growing on cow dung in man-made pastures from Pernambuco State, Northeast Brazil. Cortez and Silveira (2008) while conducting the survey of the genus *Stropharia* in the Brazilian State of Rio Grande do Sul recorded three dung-inhabiting species of the genus. They revealed the occurrence of *Protostropharia alcis* subsp. *austrobrasiliensis* (Cortez & R.M. Silveira) C. Hahn growing gregariously on cow dung substrate. *Protostropharia dorsipora* (Esteve-Rav. & Barassa) Redhead and *P. semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys, both were recorded growing solitary on cow dung in pastures.

Watling and Richardson (2010) recorded 28 taxa of coprophilous mushrooms from the Falkland Islands. These belong to 11 genera namely *Agrocybe, Bolbitius, Conocybe, Clitocybe, Clitopilus, Coprinellus, Coprinopsis, Parasola, Panaeolus, Psilocybe,* and *Stropharia* of 05 families namely *Bolbitiaceae*, *Entolomataceae*, *Psathyrellaceae*, *Strophariaceae*, and *Tricholomataceae* of *Basidiomycota*. Out of the species recorded, *Clitocybe amarescens* Harmaja was collected from cattle and sheep dung-enriched soil; *Coprinellus brevisetulosus* (Arnolds) Redhead, Vilgalys and Moncalvo, *Coprinellus curtus* (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson, *C. heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson, *Coprinopsis ephemeroides* (DC.) G. Moreno, *C. pachysperma* (P.D. Orton) Redhead, Vilgalys & Moncalvo, *Psilocybe coprophila* (Bull.) P. Kumm., *P. cubensis* (Earle) Singer, *P. moelleri* Guzmán and *Panaeolus papilionaceus* (Bull.) Quél. from horse dung; *Coprinellus pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, *Coprinopsis vermiculifera* (Joss, ex Dennis) Redhead, Vilgalys & Moncalvo, *Conocybe digitalina* (Velen.) Singer, and *C. magnispora* (Murrill) Singer exclusively from cattle dung; *Coprinopsis cordispora* (T. Gibbs) Gminder, *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo, *Psilocybe subcoprophila* (Britzelm.) Sacc., *Agrocybe fimicola* (Speg.) Singer, *Bolbitius vitellinus* (Pers.) Fr., *Panaeolus antillarum* (Fr.) Dennis, *P. subfirmus* P. Karst. and *P. semiovatus* (Sowerby) S. Lundell & Nannf. from cattle and horse dung; and *Coprinopsis radiata* (Bolton) Redhead, Vilgalys & Moncalvo and *Coprinopsis stercorea* (Fr.) Redhead, Vilgalys & Moncalvo from horse, sheep and rabbit dung; *Parasola misera* (P. Karst.) Redhead, Vilgalys & Hopple has been reported from horse, cattle, sheep and rabbit dung; *Conocybe pubescens* (Gillet) Kühner from horse and cow dung; *Clitopilus passeckerianus* (Pilát) Singer from sheep dung, and *Prototropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys from horse, cattle, sheep dung, and hare dung pellets, respectively.

Calaça *et al.* (2014) published a checklist of coprophilous fungi and other fungi recorded on dung from Brazil. They confirmed 18 taxa belonging to order *Agaricales* growing on herbivorous dung which are *Cyathus stercoreus* (Schwein.) De Toni; *Coprinellus curtus* (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson; *C. heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson; *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo; *Coprinopsis cordispora* (T. Gibbs) Gminder; *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo; *C. radita* (Bolton) Redhead, Vilgalys & Moncalvo; *C. stercorea* (Fr.) Redhead, Vilgalys and Moncalvo; *Parasola misera* (P. Karst.) Redhead, Vilgalys & Hopple; *Psilocybe argentina* (Speg.) Singer; *P. caeruleoannulata* Singer ex Guzmán; *P. coprophila* (Bull.) P. Kumm.; *P. cubensis* (Earle) Singer; *P. merdaria* (Fr.) Ricken; *P. pegleriana* Guzmán; *P. subcubensis* Guzmán; *Protostropharia alcis* (Kytöv.) Redhead, Thorn & Malloch and *P. semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys. Melo *et al.* (2016) reported twelve species of dung inhabiting mushrooms during a survey of coprophilous fungi in Pernambuco, northeastern Brazil. These mushrooms are *Bolbitius demangei* (Quél.) Sacc. & D. Sacc.; *Conocybe siliginea* (Fr.) Kühner; *Coprinellus angulatus* (Peck) Redhead, Vilgalys & Moncalv; *C. marculentus* (Britzelm.) Redhead, Vilgalys & Moncalvo, *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo; *C. cothurnata* (Godey) Redhead, Vilgalys & Moncalvo; *C. pseudoradiata* (Kühner & Joss. ex Watling) Redhead, Vilgalys & Moncalvo; *C. stercorea* (Fr.) Redhead, Vilgalys & Moncalvo; *C. vermiculifer* (Joss. ex Dennis) Redhead, Vilgalys & Moncalvo; *Coprinopsis foetidella* (P.D. Orton) A.Ruiz, G. Muñoz; *C. patouillardii* (Quél.) G. Moreno and *Panaeolus antillarum* (Fr.) Dennis. Seger *et al.* (2017) reported *Protostropharia alcis* ssp. *austrobrasiliensis* (Cortez & R.M. Silveira) C. Hahn and *P. dorsipora* (Esteve-Rav. & Barassa) Redhead as growing on manure of cattle and horse, inside forest and in pastures from South Brazil.

COPROPHILOUS AGARICS FROM NORTH AMERICAN REGION AND CANADA

There are reports of agarics growing on different types of dung some parts of North America and Canada. Lange and Smith (1953) while working on the coprinoid ephemerus group reported 09 coprophilous species, namely *Coprinellus bisporus* (J.E. Lange) Vilgalys, Hopple & Jacq., *C. congregatus* (Bull.) P. Karst., *C.* ephemerus (Bull.) Redhead, Vilgalys & Moncalvo, *C. heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson, *C. heterosetulosus* (Locq. ex Watling) Vilgalys, Hopple & Jacq. Johnson, *C. marculentus* (Britzelm.) Redhead, Vilgalys & Moncalvo, *C. pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo, *C. sassii* (M. Lange & A.H. Sm.) Redhead, Vilgalys & and Moncalvo and *Coprinus stellatus* Buller from North America.

Miller (1968) reported *Panaeolus solidipes* (Peck) Sacc. growing solitary on horse dung during summer season from Alaska in US and Yukon in Canada. Ghouled (1972) reported *Psilocybe cubensis* (Earle) Singer and *Panaeolus cinctulus* (Bolton) Saccardo usually located on cow manure in North America. Van de Bogart (1976) reported 04 species viz., *Coprinus comatus* var. comatus (Müll.) Gray, *C. roseistipitatus* Bogart, *C. spadiceisporus* Bogart and *C. umbrinus* Cookie & Massee, belonging to coprophilous habitats from Washington, United States. Van de Bogart (1979) documented 03 species inhabiting dung of herbivores. Out of these, *Coprinopsis radiata* (Bolton) Redhead, Vilgalys & Moncalvo was collected from dung of all kinds of herbivores, *C. cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo on horse dung, and Coprinus undulatus Bogart on compost heaps.

Stamets (1978) indicated the association of *Psilocybe cubensis* (Earle) Singer with elephant dung in the southeastern United States. Badham (1984) reported that *Psilocybe cubensis* (Earle) Singer grows commonly in the dung of cattle and horses in North America which is probably the most commonly eaten hallucinogenic mushroom in this area. One of the most common descriptions of the psychological effects of this mushroom given is that of a "dream-like" state. Moser (1984) reported *Panaeolus alcidis* Moser growing on moose dung from Saskatchewan and Canada.

According to Ammirati *et al.* (1985), *Psilocybe semilanceata* (Fr.) P. Kumm. is widespread in North America, eastern Canada and the Pacific Coast and grows scattered to gregarious in lawns, meadows, pastures and on or near dung. Arora (1986) reported *Panaeolus solidipes* (Peck) Sacc. growing scattered to gregarious on horse dung and on manure in the month of January from California and in September from Arizona and *Panaeolus cyanescens* (Berk. & Br.) Sacc. growing solitary to scattered or in groups on or near dung in the paustures of Hawaii islands. Stamets (1996) recorded *P. cyanescens* (Berk. & Br.) Sacc. growing scattered to gregariously on dung in pastures and fields from Hawaii, Louisiana and Florida in the United States, *P. subbalteatus* (Berk. & Br.) Sacc. growing caespitosely or gregariously on dung or in well manured ground in autumn, spring and summer seasons and *P. acuminatus* (Schaeff.) Quél. growing scattered to gregariously in well-manured grounds or on dung from North America. He reported *Psilocybe semilanceata* (Fr.) P. Kumm. growing scattered to gregarious in pastures, fields, lawns or rich grasslands grazed by sheep and cows from California and British Columbia.

Arora (1986) in "Mushrooms Demystified" documented 21 species growing on dung, manure, or compost piles from different parts of North America including California, Colorado, Washington, Mexixo, etc., The documented species include *Chlorophyllum rhacodes* (Vittad.) Vellinga, *Coprinus ephemeroides* (Bull.) Fr., *C. spadiceisporus* Bogart, *C. sterquilinus* (Fr.) Fr., *C. umbrinus* Cooke & Massee, *Coprinopsis radiata* (Bolton) Redhead, Vilgalys & Moncalvo, *C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo, *Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson, *C. ephemerus* (Bull.) Redhead, Vilgalys & Moncalvo, *Parasola miser*a (P. Karst.) Redhead, Vilgalys & Hopple, *Panaeolus campanulatus* (Bull.) Quél., *P. cyanescens* (Berk. & Br.) Sacc., *P. semiovatus* (Sowerby) S. Lundell & Nannf., *P. solidipes* (Peck) Sacc., *P. subbalteatus* (Berk. & Br.) Sacc., *Psilocybe coprophila* (Bull.) P. Kumm., *P. cubensis* (Earle) Singer, *Stropharia semiglobata* (Batsch) Quél., *Agrocybe pediades* (Pers.: Fr.) Fayod, *Conocybe tenera* (Schaeff.) Fayod and *Bolbitius titubans* (Bull.) Fr. Arora (1986) documented *P. cyanescens* from the dung ilocalities of Hawaiian Islands.

Keirle *et al.* (2004) documented twentynine species belonging to *Coprinus, Podaxis, Coprinopsis*, *Coprinellus*, and *Parasola* from the Hawaiian Islands. As many as 10 collections belonging to *Coprinus, Coprinopsis* and *Coprinellus* were reported to be dung inhabiting. Out of these, *Coprinus sterquilinus* (Fr.) Fr., *Coprinopsis radiate* (Bolton) Redhead, Vilgalys & Moncalvo, *Coprinopsis sclerotiorum* (Horvers & de Cock) Redhead, Vilgalys & Moncalvo and *Coprinopsis villosa* L. Nagy, Desjardin, Vágvölgyi & Papp were reported from horse dung, *Coprinopsis cordispora* (T. Gibbs) Gminder, *Coprinopsis stercorea* (Fries) Redhead, Vilgalys & Moncalvo, and *Coprinellus pellucidus* (P. Karst.) Redhead, Vilgalys & Moncalvo from horse and cow dung and rarely on goat dung. *Coprinopsis candidolanata* (Doveri & Uljé) Keirle, Hemmes & Desjardin was recorded growing on goat dung, *C. cothurnata* (Godey) Redhead, Vilgalys & Moncalvo on cow dung and *Coprinellus curtus* (Kalchbr.) *Vilgalys*, *Hopple* & *Jacq*. *Johnson* from deer dung.

COPROPHILOUS AGARICS FROM AFRICAN REGION

Not much work is available specifically on the coprophilous agarics of this region. Pegler (1977) in his monumental work on agaric flora of East Africa reported 13 mushrooms including 03 species of *Psilocybe* (Fr.) P.Kumm., 03 species of *Coprinus* Pers., 03 species of *Panaeolus* (Fries) Quél., 01 species each of *Agrocybe* Fayod, *Bolbitius* Fr., *Conocybe* Fayod, and *Stropharia* (Fries) Quél. growing in coprophilous habitats. Stamets (1996) reported dung inhabiting *Panaeolus tropicalis* Oláh from Central Africa, *P. africanus* Oláh growing on hippopotamus and elephant dung from Central and South Africa and *P. subbalteatus* (Berk. & Br.) Sacc. growing caespitosely or gregariously on dung or in well

manured ground in autumn, spring and summer seasons from many parts of the continent.

According to Reid and Eicker (1999), the species *Panaeolus antillarum* (Fr.) Dennis is able to grow on dung from a wide range of herbivorous mammals including cattle, horses, buffaloes, elephants and rhinoceros. They recorded it growing on pile of stable manure, on elephant dung, and on cattle dung in open pasture during the months of March and April from South Africa.

COPROPHILOUS MUSHROOMS FROM ASIAN REGION

In the Asian subcontinent much of the work on coprophilous mushrooms has been done in India, which has been dealt separately. However, there are scattered reports of some work on these fungi from Sri Lanka, China, Thailand Nepal, Iraq, Turkey, Combodia, etc. Pegler (1986) while working on agaric flora of Sri Lanka recorded *Bolbitius fissus* Berk. and Broome, *Coprinellus fimbriatus* (Berk. & Broome) Redhead, Vilgalys & Moncalvo*, Coprinopsis macrocephala* (Berk.) Redhead, Vilgalys & Moncalvo and *Psilocybe pseudobullacea* (Petch) Pegler from unspecified dung, *Panaeolus rubricaulis* Petch & *P. cyanescens* (Berk. & Broome) Sacc. from manured soil, and *Psilocybe rostrata* from elephant dung.

McKenna (1988) recorded *Psilocybe cubensis* often occuring in association with the manure of *Bos indicus* in Thailand. McKenna (1992) in his book 'The Archaic Revival' has created a web of understanding that he has gleaned from both his psychedelic experiences and research. The work stretches from the prehistoric veldt of Africa to the unimaginable world beyond the transcendental object at the end of history. He describes in this book that at an archeological site in the Non Nak Tha region of northern Thailand, the bones of zebu cattle were unearthed in conjunction with human remains. We know that *Psilocybe cubensis* flourishes in the manure of cattle and buffaloes in this region of northeastern Thailand. Terence McKenna has suggested that the temporal and physical relationship between the human bones and the bones of cattle gives conclusive evidence that psychoactive mushrooms were known to the people who frequented this region about 15,000 years ago.

Zhishu *et al.* (1993) have reported *Panaeolus antillarum* (Fr.) Dennis growing gregariously on cow dung and *P. cyanescens* (Berk. & Br.) Sacc. growing scattered to gregariously on dunghills or grass from China's Guangdong Province. Stamets (1996) reported dung inhabiting *Panaeolus tropicalis* Oláh from Cambodia. Guzmán and Kasuya (2004) noted *Psilocybe pseudobullacea* (Petch) Pegler and *P. subcubensis* Guzmán growing on rhinoceros manure from Nepal.

Pollock (1976) reported *Panaeolus tropicalis* Ola'h as "fruiting in the dung of cattle and wild animals" from Cambodia (Kampuchea) in Southeast Asia. Türkoğlu *et al.* (2007) reported *Coprinopsis macrocephala* (Berk.) Redhead, Vilgalys & Moncalvo growing on horse manure, in the month of May from Kayseri, Turkey. Ediriweera *et al.* (2015) described *Panaeolus sphinctrinus* (Fr) Quél. and *P. foenisecii* (Pers.) J. Schröt. on elephant dung for the first time from dry zone forest reserves of Sri Lanka. Wang and Tzean (2015) identified dung-associated four taxa, *Panaeolus antillarum* (Fr.) Dennis, *Conocybe nitrophila* (Hauskn.) Wang & Tzean*, Psilocybe angulospora* Wang & Tzean and *Protostropharia ovalispora* Wang & Tzean*,* from Qingtiangang, Yangmingshan National Park in Taiwan.

Al-Khesraji (2018) collected macrofungi specimens from Tikrit and Dujail districts of Salahadin Governorate, North Central Iraq between 2017 and 2018. *Panaeolus papilionaceus* (Bull. ex Fries) Quél. was found growing singly or gregariously on cow dung; fruiting spring and winters. Toma *et al.* (2018) found *Panaeolus papilionaceus* growing on dung of horses and cows in Erbil city of Kurdistan region of Iraq.

THE INDIAN SCENARIO

The striking variation in Indian climate plays a determinate role in growth and development of wide variety of mushrooms including coprophilous mycoflora. During the past four decades much progress has been made in the field of mushroom research in India in general. The review of literature reveals the following articles which have been published on coprophilous mushrooms and about 140 species belonging to about 30 genera are known to be growing wild on dung localities in India.

COPROPHILOUS AGARICS FROM NORTH INDIA

The earliest contribution on coprophilous mushrooms from India was by Rea (1922) who recorded 10 coprophilous species from the state of Punjab. These were *Coprinellus ephemerus* (Bull.) Redhead, Vilgalys & Moncalvo from rabbit dung; *Bolbitius tener* Berk. from donkey dung; *B. vitellinus* (Pers.) Fr. from horse dung; *Coprinus filiformis* Berk. & Broome from the dung of nilgai; *C. gibbsii* Massee & Crossl.*, C. hendersonii* (Berk.) Fr. and *C. stellaris* Quél. from dung of Zebra; *C. nycthemerus* Fr. from cow dung; *C. papillatus* (Batsch) Fr. from sambhar dung; and *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys from camel dung.

Mahju (1933) reported mushrooms on dung of herbivores collected from various zoological gardens. *Bolbitius vitellinus* (Pers.) Fr. was found growing on horse dung; *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo on unspecified animal dung and *Coprinus papillatus* (Batsch) Fr. on sambhar dung from Punjab. Ginai (1936) contributed to the study of coprophilous mushrooms by isolating 3 genera belonging to basidiomycetes from the dung of donkey, nilgai, zebra, cow and camel. *Bolbitius tener* Berk. was documented from donkey dung; *Coprinus filiformis* Berk. & Broome from dung of nilgai; *C. gibbsii* Massee & Crossl. and *C. hendersonii* (Berk.) Fr. from Zebra dung; *C. nycthemerus* Fr. from the dung of cows and *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys from the dung of camel from Punjab.

Rawla *et al.* (1982) reported *Agrocybe semiorbicularis* (Bull.) Quél. growing on dung and *Leucocoprinus cretatus* Lanzoni growing on manure heaps and heavily manured beds from

Punjab. Sarwal and Rawla (1983) documented coprophilous species of *Conocybe* growing on horse dung [*Conocybe siliginea* f. *rickenii* (Jul. Schäff.) Arnolds] from Punjab. Purkayastha and Chandra (1985) listed *Agaricus brunnescens* Peck from the manure heaps in Punjab. Kaushal and Grewal (1992) reported *Coprinus comatus* (O.F. Müll.) Pers. growing on horse dung, and *C. papillatus* (Batsch) Fr. growing on panther dung from Punjab.

Saini and Atri (1995) reviewed the exploratory work on mushrooms from Punjab and listed 94 taxa spread over 24 genera from Punjab plains, out of which 16 species are listed to be coprophilous. These include *Agaricus brunnescens* Peck from manure heaps; *Agrocybe pediades* (Fr.) Fayod from mixed dung; *Bolbitius tener* Berk. from donkey dung; *Bolbitius vitellinus* (Pers.) Fr., *Conocybe siliginea* f. *rickenii* (Jul. Schäff.) Arnolds and *Coprinus comatus* (O.F. Müll.) Pers. from horse dung; *C. filiformis* Berk. & Broome from dung of nilgai; *C. gibbsii* Massee & Crossl.; *C. hendersonii* (Berk.) Fr. and *C. stellaris* Quél.from dung of Zebra; *C. nycthemerus* Fr. from dung of cows; *C. papillatus* (Batsch) Fr. growing on sambhar and panther dung; *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo from unspecified animal dung; *Coprinellus ephemerus* (Bull.) Redhead, Vilgalys & Moncalvo from rabbit dung; *Leucocoprinus cretatus* Lanzoni from manure heaps and heavily manured beds and *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys growing on camel dung.

Atri and Kaur (2004) gave an illustrated account of 10 taxa of coprinoid macrofungi recorded from Patiala. Out of these, 03 taxa were reported from coprophilous habitats. *Coprinellus micaceus* var. *macrosporus* Atri & Kaur was collected growing in clusters on cattle dung manured soil under *Psidium guazava* tree in the month of January while *Coprinopsis patouillardii* (Quél.) G. Moreno was recorded growing on dung under *Albizzia lebbek* tree in September and *Coprinopsis radiata* (Bolton) Redhead, Vilgalys & Moncalvo was documented from cattle dung in the month of September.

Atri *et al.* (2009a) recorded and described 03 species with a coprophilous habitat from Punjab, *viz*. *Bolbitius titubans* (Bull.) Fr. growing solitary on buffalo dung in the month of September, *Conocybe brachypodii* (Velen.) Hauskn. & Svrček growing in groups on cattle dung in June and *C. crispa* (Longyear) Singer growing in caespitose cluster on cattle dung in August. Atri *et al.* (2012) made collections of *Conocybe* Fayod from various dung localities of Punjab. They described four coprophilous species of the genus, namely *Conocybe apala* (Fr.) Arnolds growing solitary or scattered on buffalo dung; *C. subxerophytica* var. *brunnea* Hauskn. growing in groups on horse dung; *C. subxerophytica* var. *subxerophytica* Singer & Hauskn. growing scattered on buffalo dung and *C. uralensis* Hauskn., Knudsen & Mukhin growing in groups on buffalo dung heap. All were recorded for the first time from India.

Amandeep *et al.* (2013a) described two new coprophilous varieties of *Panaeolus* from Punjab, India. *P. africanus* var. *diversistipus* Amandeep Kaur, NS Atri & Munruchi Kaur was found growing solitary on a cattle dung heap and *P. speciosus* var. *pilocystidiosus* Amandeep Kaur, NS Atri & Munruchi Kaur was growing scattered on cattle mixed dung. Amandeep *et al.* (2013b) reported six coprophilous species of the genus *Bolbitius* Fr., namely *B. coprophilus* (Peck) Hongo*, B. demangei* (Quél.) Sacc. & Sacc*., B. glatfelteri* Peck*, B. marginatipes* Zeller*, B. titubans* (Bull.) Fr. and *B. vitellinus* (Pers.) Fr. from a variety of herbivorous dung types. A dichotomous key to aid in the identification of these taxa was given.

Kaur *et al.* (2013a) discovered a large spored variant of *Rhodocybe popinalis (Fr.)* Singer, *R. popinalis* var. *macrosporus* Amandeep Kaur, NS Atri & Munruchi Kaur, growing on a mixed cattle and horse dung heap from Punjab. Kaur *et al.* (2013b) described a new species, *Psathyrella fimicola* NS Atri, Munruchi Kaur & Amandeep Kaur, found growing on horse dung from Patiala district of Punjab state. Kaur *et al.* (2013c) described and illustrated a new mushroom variety, *Protostropharia semiglobata* var. *punjabensis* Amandeep Kaur, NS Atri & Munruchi Kaur, growing on cow dung in Punjab. Hahn (2014) provided an overview of the taxonomy and ecology of the genus *Protostropharia* and a key of the genus including taxa not detected in Europe. He proposed a new combination for *Protostropharia semiglobata* var. *punjabensis* and regarded it as subspecies of *Protostropharia alcis* and named it as *Protostropharia alcis* subsp. *punjabensis* (Amandeep Kaur, NS Atri & Munruchi Kaur) C. Hahn.

Amandeep *et al.* (2014) discussed the diversity of *Coprinopsis* P. Karst. species from the coprophilous habitats from throughout the Punjab state. Twelve taxa, namely *C. cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo*; C. cothurnata* var. *equsterca* Atri, A. Kaur & M. Kaur*; C. foetidella* (P. D. Orton) A. Ruiz & G. Muñoz*; C. lagopides* var. *lagopides* (P. Karst.) Redhead; Vilgalys & Moncalvo*; C. lagopus* (Fr.) Redhead, Vilgalys & Moncalvo*; C. macrocephala* (Berk.) Redhead, Vilgalys & Moncalvo*; C. nivea* (Pers.) Redhead, Vilgalys & Moncalvo*; C. pseudonivea* (Bender & Uljé) Redhead, Vilgalys & Moncalvo*; C. radiata* (Bolton: Fr.) Redhead, Vilgalys & Moncalvo*; C. radiata* var. *macrocarpa* Atri, A. Kaur & M. Kaur*; C. scobicola* (P.D. Orton) Redhead, Vilgalys & Moncalvo and *C. vermiculifer* (Joss.: Dennis) Redhead, Vilgalys & Moncalvo were reported. Out of these, C. *radiata* var. *macrocarpa* and *C. cothurnata* var. *equsterca* were new mushroom varieties. In this paper, all these taxa were described, illustrated, and compared with similar species. A dichotomous key for their identification was also given.

Kaur *et al.* (2014a) reported two new coprophilous species of *Panaeolus*, namely *P. cyanoannulatus* Atri, M. Kaur & A. Kaur and *P. lepusstercus* Atri, M. Kaur & A. Kaur from Punjab. *Panaeolus cyanoannulatus* was collected on a mixed cow and horse dung heap and *P. lepusstercus* was located growing scattered on rabbit pellets. Kaur *et al.* (2014b) described two new species of *Agaricus, A. stellatus-cuticus* Atri, M. Kaur & A. Kaur and *A. flavistipus* Atri, M. Kaur & A. Kaur, collected on sheep dung and buffalo dung, respectively. Kaur *et al.* (2014c) discussed the *diversity of Panaeolus* growing on herbivorous dung from Punjab. An account of 16 species collected from a variety of coprophilous habitats were

described and discussed. Kaur *et al.* (2014d) gave an account of two *Agrocybe* species, *viz*. *A. microspora* Singer & *A. pediades* (Fr.) Fayod collected from coprophilous habitats of Punjab state. Kaur *et al.* (2014e) documented *Panaeolus sphinctrinus* var. *minor* (Fr.) Singer, *P. tropicalis* Oláh and *Psathyrella castaneifolia* (Murrill) A.H. Sm. growing on dung from Punjab state.Kaur and Kaur (2015) reported *Psilocybe uda* var. *elongata* (Pers.) Gillet and *P. coprophila* (Bull.) P. Kumm. growing scattered on animal dung from Punjab.

Amandeep *et al.* (2015a) recorded the diversity of species of the genus *Conocybe* collected on dung from Punjab. This research paper represented 22 collections belonging to 16 *Conocybe* species growing on five diverse dung types. Amandeep *et al.* (2015b) worked out the taxonomic details of eight coprophilous agarics, namely *Agaricus cupreobrunneus* (Schäffer & Steer: Møller) Pilát, *A. halophilus* Peck, *Coprinus comatus* var. *caprimammillatu* Bogart, *Lepiota epicharis* var. *occidentalis* Dennis, *L. thrombophora* (Berk. & Br.) Sacc., *L. subincarnata* J.E. Lange, *L. xanthophylla* P.D. Orton and *Leucocoprinus straminellus* (Sowerby) Pat., belonging to the family *Agaricaceae* from various dung localities of Punjab state in India. All these taxa were described along with their dung sources, illustrated with line drawings of morphological and anatomical features and compared with similar such taxa from elsewhere. Habitat photographs and a key to their determination have also been provided. Amandeep *et al.* (2015c) gave an account of five *Psathyrella* species from Punjab state along with key for their identification. The collections of the identified taxa were obtained from a variety of coprophilous habitats having different herbivorous dung types. These belong to *Psathyrella kauffmanii* var. *kauffmanii* Smith, *P. vanhermanii* Smith, *P. fimicola* N.S. Atri, Munruchi Kaur & Amandeep Kaur, *P. sphaerocystis* Orton and *P. flocculosa* (Earle) A.H. Smith. For all the taxa, dung types on which they were found growing are also mentioned.

Amandeep *et al.* (2015d) gave an account of the eco-taxonomic studies on the coprophilous mushrooms in Punjab, India. The information is primarily based on the survey to various dung localities of the state undertaken during the years 2007-2011. A total number of 172 collections of coprophilous mushrooms belonging to 95 species spread over 20 genera and 07 families of the order *Agaricales* were examined. In this paper an account of the distribution of these mushrooms in Punjab in different seasons, regions, habitats, and growing habits along with their economic utility, habitat management and conservation has been discussed. Amandeep *et al.* (2015e) published a checklist consisting of 135 coprophilous species belonging in 27 genera and 10 families of the Order *Agaricales* from India. The geographical distribution of the species covering 13 States (Assam, Bihar, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Maharashtra, Orissa, Punjab, Tamil Nadu, Uttar Pradesh and West Bengal) and 2 Union Territories (Chandigarh, New Delhi) has been discussed in this manuscript. The checklist is an attempt to provide updated information regarding the diversity of coprophilous agarics in India. Kaur *et al.* (2016) documented *Agaricus bernardii* (Quél.) Sacc. growing around the heap of dung manure from Punjab. The species is reported to be edible in literature.

Vishwakarma *et al.* (2017) published a checklist of 153 species of macrofungi belonging to 34 families primarily based on the survey of the north eastern part of Uttar Pradesh state, especially Gorakhpur. They reported *Coprinus comatus* (Müll.: Fr.) Gray; *Coprinopsis cothurnata* (Godey) Redhead, Vilgalys & Moncalvo; *C. foetidella* (P.D. Orton) A.Ruiz & G. Muñoz; *Panaeolus ater* (J.E. Lange) Kühner and Romagn.; *P. papilionaceous* (Bull.) Quél.; and *Calocybe indica* Purkayastha and A. Chandra as coprophilous, growing on animal dung. Singh *et al.* (2018) undertook a study on the taxonomy and diversity of macrofungi w.e.f. March 2014 to July 2016 in different localities of Gorakhpur district of Uttar Pradesh. Out of 14 *Coprinus* species collected and identified, they found habitat of two species coprophilous. *Coprinus comatus* (O.F. Müll.) Pers. was found growing in groups on manure and *C. radiatus* (Bolt.: Fr.) S.F. Gray growing in groups on cow dung.

COPROPHILOUS AGARICS FROM SOUTH INDIA

Natarajan and Raaman (1983, 1984) reported 14 mushrooms growing on dung from South India. Out of these, 05 species belong to the genus *Psilocybe*, 05 to *Panaeolus*, 03 to *Conocybe*, and 01 species to *Protostropharia.* From amongst the species documented *Psilocybe aztecorum* R. Heim, *P. argentina* (Speg.) Singer, and *P. gigaspora* Natarajan & Raaman were reported to be associated with cow dung; *P. coprophila* (Bull.) P. Kumm. on mixed dung and *P. cubensis* (Earle) Singer on elephant dung and manure. *Panaeolus africanus* Oláh, *P. antillarum* (Fr.) Dennis, *P. annulatus* Natarajan & Raaman, *P. subbalteatus* (Berk. & Br.) Sacc. and *P. cyanescens* (Berk. & Br.) Sacc. were reported from elephant dung; *Conocybe ambigua* Watling, *C. semiglobata* Kühner & Watling, and *Pholiotina plumbeitincta* (G.F. Atk.) Hauskn., Krisai & Voglmayr from cow dung; and *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo and Vilgalys on unspecified dung.

Bhavani Devi (1995) enlisted 134 taxa known from Kerala, a state on India's tropical Malabar Coast. Out of these 134 taxa, 13 taxa have been reported to occur in coprophilous habitats. These are *Amanita solitaria* (Bull.) Mérat from cow dung heaps; *Bolbitius vitellinus* on dung; *Conocybe antipus* (Lasch) Fayod on manure and compost heaps; *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo from cow dung; *C. radiata* (Bolton) Redhead, Vilgalys & Moncalvo from dung heaps; *Panaeolus semiovatus* (Sowerby) S. Lundell & Nannf. from elephant dung; *Panaeolus solidipes* (Peck) Sacc. from manured ground; *P. ater* (J.E. Lange) Kühner and Romagn. ex Bon from the droppings of herbivorous animals; *Leucocoprinus cepistipes* (Sowerby) Pat. from manured ground on humus rich soil; *Psilocybe coprophila* (Bull.) P. Kumm. from elephant dung; *Protostropharia semiglobata* (Batsch) Redhead; Moncalvo & Vilgalys from dung or manured soil; *Volvopluteus gloiocephalus* (DC.: Fr.) Vizzini; Contu and Justo from manured ground; *Chlorophyllum molybdites* (G. Mey.) Massee from the basins of plants where manuring is done.

Vrinda *et al.* (1999) reported *Panaeolus acuminatus* Quél. and *Parasola conopilus* (Fr.) Örstadius & E. Larss. growing scattered on elephant dung from Kerala. Thomas *et al.* (2001) documented *Bolbitius coprophilous* (Peck) Hongo, *Conocybe pseudopubescens* K. A. Thomas, Hauskn. & Manimohan and *C. volvata* K. A. Thomas, Hauskn. & Manim. growing on elephant dung and *C. zeylanica* (Petch) Boedijn on the heap of dried cow dung from Kerala. Thomas and Manimohan (2002) reported 05 coprophilous species of *Psilocybe* from Kerala state. These are *P. argentina* (Speg.) Singer from cow dung; *P. coprophila* (Bull.) P. Kumm., *P. subaeruginascens* Höhn and *P. subcubensis* Guzmán from elephant dung and *P. cubensis* (Earle) Singer from the manured soil with heavy traffic of cattle. Thomas and Manimohan (2003) documented *Agrocybe guruvayoorensis* K. A. Thomas & Manim. growing on elephant dung during the months of July-October in Kerala state. Manimohan *et al.* (2007) documented 19 species representing 12 genera and 05 agaric families associated with dung of both wild and domesticated elephants from Kerala state. These are *Agrocybe guruvayoorensis* K. A. Thomas & Manim.; *Bolbitius coprophilous* (Peck) Hongo; *Conocybe brunneoaurantiaca* K. A. Thomas, Hauskn. & Manim.; *C. pseudopubescens* K. A. Thomas, Hauskn. and Manimohan; *C. volvata* K. A. Thomas, Hauskn. & Manim.; *Crucispora rhombisperma* (Hongo) E. Horak; *Entoloma anamikum* Manim.; A. V. Joseph & Leelav.; *Macrocybe gigantea* (Massee) Pegler & Lodge; *Panaeolus antillarum* (Fr.) Dennis; *P. cyanescens* (Berk. & Br.) Sacc.; *P. rickenii* Hora; *Pholiotina indica* K. A. Thomas, Hauskn. & Manim.; *Psilocybe coprophila* (Bull.) P. Kumm.; *P. pegleriana* Guzmán; *P. subaeruginascens* Höhn; *P. subcubensis* Guzmán; *Stropharia bicolor* Pegler; *S. rugosoannulata* Farl.: Murrill and *Volvariella volvacea* (Bull.) Singer. Noordeloos *et al.* (2007) documented the occurrence of *Crucispora rhombisperma* (Hongo) E. Horak on the elephant dung from Kerala state. Arun Kumar and Manimohan (2009) recorded *Leucocoprinus pusillus* T.K.A. Kumar & Manim. growing on manure rich soil from Kerala state.

COPROPHILOUS AGARICS FROM EASTERN INDIA

Bose (1920) reported *Coprinellus fimbriatus* (Berk. & Br.) Redhead,Vilgalys & Moncalvo and *Panaeolus cyanescens* (Berk. & Br.) Sacc. growing on herbivorous dung from West Bengal in eastern India. Dhancholia and Sinha (1990) recorded two coprophilous mushrooms, *viz*. *Leucoagaricus meleagris* (Gray) Singer and *Leucocoprinus cepistipes* (Sowerby) Pat. growing on cow dung from Odisha, located in eastern India.Verma *et al.* (1995) found *Lepiota leprica* (Berk. & Br.) Sacc. growing solitary or in groups in open fields and pastures on cow dung or organic matter rich soil in North-East Hills. Andheria (2012a) reported an unnamed mushroom growing on elephant dung from Pakke Tiger Reserve, Arunachal Pradesh.

COPROPHILOUS AGARICS FROM WESTERN INDIA

Karun and Sridhar (2015) documented five species belonging to four genera of *Agaricales* growing on elephant dung in the Brahmagiri Wildlife Sanctuary of Western Ghats of Karnataka in the south western region of India. These were *Conocybe pubescens* (Gillet) Kühner, *Coprinopsis patouillardii* (Quél.) G. Moreno, *Panaeolus fimicola* (Pers.) Gillet, *Psilocybe coprophila* (Bull.) P. Kumm. and *P. fimetaria* (P.D. Orton) Watling. Andheria (2012b) reported an unnamed mushroom growing on Nilgai dung in Umred Karhandla Wildlife Sanctuary, Maharashtra. Andheria (2012c) reported an unnamed mushroom growing on elephant dung from BRT Tiger Reserve, Karnataka.

RELEVANCE OF COPROPHILOUS AGARICS

The fascination of humans for mushrooms growing on dung goes back to the earliest times. In their search for edible foods, early hunter-gatherers followed the manure trails of the large migratory herds. Being hungry and curious, early humans commonly consumed the small meaty mushrooms, some of which were psychoactive. Some such mushrooms commonly occurring on the dung of ruminants were species of various agaricoid genera including *Psilocybe, Panaeolus*, etc. Many of these mushrooms were largely valued not only as food sources, but for the expansion of consciousness and perception they induced. Over the years, substantial knowledge has accumulated about the use of mushrooms, their recipes and effects. Archeological records suggest that early humans knew about mushrooms' special effects and because of this they consumed them intentionally especially during the festive seasons. Several writers have suggested that major religious ideas were inspired by the intake of such entheogenic mushrooms and plants (Lowy and Wasson, 1969; Arthur, 2000; Allen and Arthur; 2003).

The information about their human relevance is compiled in **Table 2**. It is based on the literature and no personal observations were made in this regard.

DISCUSSION

The data given in this overview is an attempt to compile and provide updated information regarding the diversity and utility of coprophilous agarics at one place which otherwise is lying scattered in literature. It appears that, despite the effort and the information, there is still a long way to go in terms of developing a basic knowledge about the diversity of the mycota growing on animal dung. The work, perhaps, cover only a part of the actual diversity of these mushrooms the world over as most of the relevant original information is literature-based and many of the papers bear only limited information on habit, habitat and economic potential. However, the knowledge generated by the work is of immense utility as it may serve as a key revealing the diversity and ecology of mushrooms which grow on herbivorous dung. The review demonstrates that dung is a significant substrate which serves as a favorable niche for the growth of a variety of mushrooms. Geographically, coprophilous mushrooms are distributed worldwide and most of them belong to the families *Agaricaceae, Psathyrellaceae* and *Strophariaceae*.

The coprophilous agarics play a significant role in the sustenance of ecological balance on the earth. But throughout the world the natural habitats with dung deposits such as pastures, grasslands, open fields, etc. are getting destroyed because of the various developmental activities. As a result, most of the coprophilous taxa may be in danger of getting extinct. Their ecological relationships with their herbivorous

Table 1. Some reports about documentation of agaricoid coprophilous mushroom genera (Nomenclature Source MycoBank).

Family	Recorded Genera	References
Agaricaceae Chevall.	***Agaricus*** L.: Fr.	Srivastava (1978), Kannaiyan and Ramasamy (1980), Purkayastha and Chandra (1985), Saini and Atri (1995), Kaur *et al.* (2014b), Amandeep *et al.* (2015b,d)
	Chlorophyllum Massee	Manjula (1980), Natarajan and Manjula (1981), Bhavani Devi (1995), Amandeep *et al.* (2015d)
	Coprinus Pers.	Rea (1922), Mahju (1933), Ginai (1936), Van de Bogart (1976, 1979), Pegler (1977, 1986), Arora (1986), Uljé and Bas (1988, 1991), Kaushal and Grewal (1992), Uljé and Noordeloos (1993, 1997, 1999), Jordon (1995), Saini and Atri (1995), Richardson and Watling (1997), Richardson (2001a), Atri and Kaur (2004), Keirle *et al.* (2004), Richardson (2004), Doveri (2010), Prydiuk (2010), Amandeep *et al.* (2015b,d), Singh *et al.* (2018).
	Crucispora E. Horak	Manimohan *et al.* (2007), Noordeloos *et al.* (2007), Amandeep *et al.* (2015d)
	Cyathus Haller	Richardson (2001a)
	Lepiota (Pers. ex Fr.) S.F. Gray	Arora (1986), Dhancholia and Sinha (1990), Jordon (1995), Verma *et al.* (1995), Amandeep *et al.* (2015b,d)
	Leucoagaricus (Locquin) Sing.	Manjula (1980, 1983), Dhancholia and Sinha (1990), Bhavani Devi (1995), Amandeep *et al.* (2015d)
	Leucocoprinus Pat.	Patel and Kamat (1935), Rawla *et al*. (1982), Dhancholia and Sinha (1990), Bhavani Devi (1995), Saini and Atri (1995), Richardson and Watling (1997), Doveri (2010), Amandeep *et al.* (2015b,d)
	Podaxis Desv.	Keirle *et al.* (2004)
Amanitaceae R. Heim: Pouzar	***Amanita*** Pers.	Bhavani Devi (1995), Amandeep *et al.* (2015d)
Bolbitiaceae Sing.	***Bolbitius*** Fr.	Rea (1922), Mahju (1933), Singer (1977), Pegler (1977, 1986), Watling (1982), Arora (1986), Jordon (1995), Saini and Atri (1995), Richardson and Watling (1997), Thomas *et al.* (2001), Manimohan *et al.* (2007), Doveri (2010), Watling and Richardson (2010), Amandeep *et al.* (2013b, 2015d)
	Conocybe Fayod	Pegler (1977), Watling (1982), Natarajan and Raaman (1983, 1984), Sarwal and Rawla (1983), Arora (1986), Watling and Taylor (1987), Bhavani Devi (1995), Saini and Atri (1995), Richardson and Watling (1997), Thomas *et al.* (2001), Hausknecht *et al.* (2005), Hausknecht and Contu (2007), Manimohan *et al*. (2007), Atri *et al.* (2009, 2012), Doveri (2010), Watling and Richardson (2010), Karun and Sri dhar (2015), Amandeep *et al.* (2015 a,d)
	Panaeolina Maire	Noordeloos *et al.* (2007)
	Pholiotina Fayod	Natarajan and Raaman (1983, 1984), Watling and Taylor (1987), Thomas *et al.* (2001), Manimohan *et al.* (2007), Amandeep *et al.* (2015d)
	Pluteolus (Fr.) Gillet	Singer (1977)
Entolomataceae Kotlába & Pouzar	***Clitopilus*** (Fr. ex Rabenh.) P. Kumn.	Watling and Richardson (2010)
	Entoloma Fr.: Kummer	Thomas *et al.* (2001), Manimohan *et al.* (2007), Amandeep *et al.* (2015d)
	Rhodocybe Maire	Kaur *et al.* (2013a), Amandeep *et al.* (2015d)
Lyophyllaceae Jülich	***Termitomyces*** R. Heim	Amandeep *et al.* (2015d)
	Calocybe Kühner ex Donk	Vishwakarma *et al.* (2017)
Mycenaceae Overeem	***Mycena*** (Pers.) Roussel	Amandeep *et al.* (2013b, 2015d)
Pluteaceae Kotl. and Pouzar	***Volvariella*** Speg.	Richardson and Watling (1997), Manimohan *et al.* (2007), Doveri (2010), Amandeep *et al.* (2015d)
	Volvopluteus Vizzini, Contu and Justo	Bhavani Devi (1995), Dutta *et al.* (2011), Amandeep *et al.* (2015d)
Psathyrellaceae Vilgalys, Moncalvo and Redhead	***Coprinellus*** P. Karst.	Bose (1920), Rea (1922), Mahju (1933), Lange and Smith (1953), Van de Bogart (1976), Pegler (1977,1986), Manjula (1983), Arora (1986), Uljé and Bas (1988, 1991), Uljé and Noordeloos (1993, 1997, 1999), Jordon (1995),Saini and Atri (1995), Richardson and Watling (1997), Richardson (2001a), Atri and Kaur (2004), Keirle *et al.* (2004), Richardson (2004), Doveri (2010), Prydiuk (2010), Watling and Richardson (2010), Amandeep *et al.* (2015d)
	Coprinopsis P. Karst.	Lange and Smith (1953), Van de Bogart (1976), Pegler (1977, 1986), Arora (1986), Uljé and Bas (1988, 1991), Uljé and Noordeloos (1993, 1997, 1999), Bhavani Devi (1995), Jordon (1995), Richardson and Watling (1997), Richardson (2001a), Atri and Kaur (2004), Keirle *et al.* (2004), Richardson (2004), Doveri (2010), Prydiuk (2010), Watling and Richardson (2010), Amandeep *et al.* (2014, 2015b,d), Karun and Sridhar (2015)
	Panaeolus (Fr.) Quél.	Bose (1920), Pegler (1977), Natarajan and Raaman (1983, 1984), Watling and Taylor (1987), Watling and Gregory (1987), Bhavani Devi (1995), Jordon (1995), Stamets (1996), Richardson and Watling (1997), Vrinda *et al.* (1999), Manimohan *et al.* (2007), Doveri (2010), Watling and Richardson (2010), Amandeep *et al.* (2013a, 2015d), Kaur *et al.* (2014a,c), Karun and Sridhar (2015), Al-Khesraji, (2018); Toma *et al.,* (2018).
	Parasola Redhead, Vilgalys & Hopple	Pegler (1977), Watling and Taylor (1987),Vrinda *et al.* (1999), Keirle *et al.* (2004), Doveri (2010), Prydiuk (2010), Watling and Richardson (2010), Amandeep *et al.* (2015d)
	Psathyrella Fr.: Quél.	Richardson and Watling (1997), Larsson a and Örstadius b (2008), Doveri (2010), Kaur *et al.* (2013b, 2014c), Amandeep *et al.* (2015d)
Strophariaceae Singer & Smith	***Agrocybe*** Fayod	Pegler (1977), Singer (1977), Rawla *et al.* (1982), Watling (1982), Arora (1986), Saini and Atri (1995), Richardson and Watling (1997), Thomas and Manimohan (2003), Manimohan *et al.* (2007), Doveri (2010), Watling and Richardson (2010), Kaur *et al.* (2014d), Amandeep *et al.* (2015d)
	Protostropharia Redhead, Moncalvo & Vilgalys	Rea (1922), Ginai (1936), Pegler (1977), Natarajan and Raaman (1983), Arora (1986), Watling and Taylor (1987), Bhavani Devi (1995), Jordon (1995), Saini and Atri (1995), Richardson and Watling (1997), Watling and Richardson (2010), Doveri (2010), Kaur *et al.* (2013c), Amandeep *et al.* (2015d)
	Psilocybe (Fr.) P. Kumm.	Massee (1901), Pegler (1977, 1986), Natarajan and Raaman (1983, 1984), Arora (1986), Watling and Taylor (1987), Watling and Gregory (1987), Bhavani Devi (1995), Jordon (1995), Stamets (1996), Richardson and Watling (1997), Thomas and Manimohan (2002), Richardson (2004), Manimohan *et al.* (2007), Doveri (2010), Watling and Richardson (2010), Karun and Sridhar (2015), Amandeep *et al.* (2015d)
	Stropharia (Fr.) Quél.	Pegler (1977), Arora (1986), Watling and Taylor (1987), Watling and Gregory (1987), Jordon (1995), Richardson and Watling (1997), Manimohan *et al.* (2007), Doveri (2010), Watling and Richardson (2010), Amandeep *et al.* (2015d)
Tricholomataceae R. Heim: Pouzar	***Clitocybe*** (Fr.)	Richardson and Watling (1997), Watling and Richardson (2010)
	Lepista (Fr.) W.G. Sm.	Richardson and Watling (1997), Doveri (2010)
	Macrocybe Pegler & Lodge	Manimohan *et al.* (2007), Amandeep *et al.* (2015d)

Table 2. Relevance of Coprophilous Mushrooms

Characteristic	Name of the coprophilous taxa	References
Mushrooms with edibility potential	*Agaricus bernardii, A. campestris, A. placomyces, A. cupreobrunneus, A. halophilus, Coprinus comatus, Coprinus sterquilinus, Coprinopsis cinerea, Coprinellus micaceus, Chlorophyllum rhacodes, Leucoagaricus naucinus, Panaeolus acuminatus, P. rickenii, P. semiovatus , P. solidipes Protostropharia semiglobata, Volvopluteus gloiocephala,* and *Termitomyces radicatus.*	Kauffman (1918), Murrill (1922), Bose and Bose (1940), Atkinson 1961, Kaul and Kachroo (1974), Pegler (1977, 1983), Pegler and Piearce (1980), Purkayastha and Chandra (1985), Arora (1986), Singer (1986), Stamets (1996), Atri *et al.* (2007, 2009b), Amandeep *et al.* (2015c), Kaur *et al.* (2016)
	Coprinopsis atramentaria is particularly interesting since it is edible unless consumed with alcohol.	Bresinsky and Besl (1990)
	Coprinellus micaceus is considered ideal for omelettes, and as a flavor for sauces.	http://en.wikipedia.org/wiki/*Coprinellus_micaceus*
	Panaeolus acuminatus It has been listed to make a good strawberry milkshake.	Singer (1986)
Inedible and poisonous mushrooms	*Agaricus xanthodermus, Conocybe albipes, Chlorophyllum molybdites, Leucocoprinus cepistipes, Lepiota xanthophylla, L. subincarnata* are not worth consideration for human consumption. They are reported to be inedible and poisonous in literature.	Arora (1986), Singer (1986), Kerrigan (1986), Hall (2003), Vellinga (2001, 2003), Hallen *et al. (*2003)
	Symptoms of *Chlorophyllum molybdites* poisoning are reported to occur about 1-3 hours after the meal and persist for up to six hours or even longer.	Vellinga (2003), Kuo (2005)
	Lepiota subincarnata has been reported to be very toxic because of the presence of amanitins and amatoxins and its consumption is reported to be potentially lethal.	Vellinga (2001), Hall (2003)
Mushrooms with potential medicinal utility	*Coprinus comatus*, *Coprinopsis radiata, C. lagopus* and *Coprinellus micaceus* are reported to possess antibiotic properties against bacteria and fungi.	Ohtsuka*et al.* (1973), Botton and Siehr (1975), Efremenkova*et al.* (2001), Zenkova*et al.* (2003)
	Coprinus comatus has antidiabetic, antifungal and antibacterial properties.	Efremenkova *et al.* (2001), Zenkova *et al.* (2003)
	Coprophilus macrofungi (*Coprinus comatus*, *C. plicatilis*, and *C. cinereus*) are the producers of several bioactive metabolities (tri-sesquiterpenes, quinones, glucans, proteins, etc.) and enzymes (protease, phenoloxidases, etc.) with immune modulating, antifungal, antioxidant, thrombolytic, hypoglycemic and anti-protozoal effects and these metabolites can be extracted and identified. Polysaccharides extracted from the mycelial culture of *C. cinereus* have been shown to contain anti tumour effects. The polysaccharides obtained from *C. comatus*, tested in mice, revealed hypolipidemic effects and antioxidant properties suggesting that their antioxidant activity could be directly or indirectly responsible for its hypoglycemic and hypolipidemic properties.	Manoharachary *et al.* (2014)
	Tanzanian *C. cinereus* grown on dried grasses supplemented with cow dung manure exhibits activity against *Escherichia coli*, *Aspergillus niger* and *Candida albicans*.	Mwita *et al.* (2010)
	Coprinopsis radiata is reported to have antimicrobial and anticancer action.	Anisova *et al.* (1987)
	Coprinopsis quadrifidus has been reported to produce an antibiotic called quadrifidin.	Singer (1986)
	Coprinellus micaceus has been reported to possess antibacterial and antibiotic properties. The species also possesses a unique chemical sterol 'Micaceol' with potential for use in cancer chemotherapy.	Zahid *et al.* (2 006)
	Chlorophyllum molybdites, although produces ill effects in many individuals, has been reported to contain 08 steroidal derivatives, two of which are reported to be important in the treatment of human gastric cancer, besides possessing antitumor and antiviral components.	Chang and Hayes (1978), Didukh *et al.* (2004)
	Agaricus campestris is reported to be used for the treatment of ulcers, and bed sores.	http://en.wikipedia.org/wiki/*Agaricus_campestris*
	Beside these taxa, *Lepiota* and *Leucocoprinus* are the other commonly found genera in the coprophilous habitats which are reported to possess bioactive compounds with scope for utilization in human welfare.	Didukh *et al.* (2004)
Psychoactive/ Hallucinogenic properties	The most famous hallucinogenic mushrooms belong to *Psilocybe* and *Panaeolus.*	Stamets (1996), Allen and Gartz (1997), Guzmán *et al.* (2000), Trappe (2005)
	Coprophilous *Psilocybe aztecorum, P. cubensis, P. coprophila , P. mexicana, P. natalensis, P. semilanceata,* are known to have hallucinogenic properties. Some hallucinogenic species of *Panaeolus,* namely *P. africanus, P. acuminatus, P. antillarum, P. ater, P. castaneifolius, P. cyanescens, P. pa pilionaceus* var. *parvisporus, P. sphinctrinus, P. subbalteatus,* and *P. tropicalis* are reported to grow throughout the world on dung and well manured grounds.	Guzmán (1978), Margot and Watling (1981), Singer (1986), Stamets (1996) Allen and Gartz (1997)
Ecological aspect	Coprophilous Mushrooms play a significant role in the decomposition of the fecal materials, carbon flow and ecosystem energetics.	Angel and Wicklow (1974, 1975)
	Responsible for recycling the nutrients in animal faeces and in the formation of soil.	Kumar *et al.* (1995)
	An important source of nutrients for coprophagous and mycophagous arthropods.	Halfter and Matthew (1971)
Industrial use	*C. atramentaria* has been reported to be the main source of *Coprinus* ink which had utility for retouching work in photography and for specific effects in writing and drawing.	Singer (1986)

hosts must be conserved and attention be given to the study of these fungi which otherwise play a major role as natures recyclers and replenishers. More consideration should be given to rarely investigated habitats, as zoological reserves, studs, pastures or compost piles where many ink-cap species new to science or uncommonly recorded can found out.

CONCLUSION

As is apparent from the above account, the field of coprophilous mushrooms remains largely underexplored. There are many areas which are still unexplored from taxonomic and sociobiological point of view. So as to update and strengthen the knowledge about their occurrence, distribution and importance, a co-rdination amongst the mushroom mycologists on a wider scale is needed. The mycologists from throughout the world need to come together so as to undertake investigations for inventorization of these mushrooms by surveying the various dung localities and evaluate these for their nutritional and nutraceutical constituents. So as to identify edible and medicinally important mushrooms, sociobiological aspects of these mushrooms need to be explored by coordinating with the local inhabitants who are regularly using these mushrooms in their day to day life.

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Arbuscular Mycorrhizal (AM) Biotechnology and its Applications

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ABSTRACT

Arbuscular mycorrhizae (AM) are one of the widely distributed types of fungi forming symbiotic associations with almost all land plants. The beneficial association of these Glomalean fungi from the phylum *Glomeromycota* with plant roots dates back to about 460 million years ago, making it the most ancient type of symbiosis. These fungi constitute a crucial functional group of the soil micro-biome by determining the efficacy of agro-ecosystem through formation of a close interface between soil and plant roots. The extra-radical mycelial network associates with plant roots to take up resources from nutrient depletion zones (especially P) in the soil and in turn receive carbohydrates from the host plant, thus influencing plant productivity, diversity and ecosystem sustainability. The symbiosis plays a key role in nutrient cycling (C, N, P), plant tolerance to abiotic and biotic stresses, formation of stable soil aggregates, and various other ecosystem functions, making it important in restoration and conservation of disturbed lands. Advances in the scientific understanding on AM symbiosis have enhanced the potential for implementation of AM biotechnological approaches in different ecosystem processes. However, it is important to support further developments for production of efficient AM inocula and its application in biofertilization of crops to guide sustainable efforts. In this chapter, various strategies for AM inoculum production including pot cultures (soil based) and *in vitro* culture and its application in production of fruit, vegetable and plantation crops and floriculture are discussed.

Keywords: Arbuscular mycorrhizal fungi, agro-ecosystem, inoculum, phosphorus, extra-radical hyphae, biofertilizer

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi represent a group of efficient soil micro biota that can greatly contribute to crop development and productivity, and ecosystem sustainability. AM fungi are able to establish a mutual symbiosis with the root organs of 80% of plant families, they directly influence the plant growth through increased uptake of available soil phosphorus (P) and other essential non-labile soil mineral nutrients, and also have indirect benefits in stabilizing soil aggregates leading to soil formation, in preventing erosion, and in alleviating plant stress caused by biotic and abiotic factors leading to improved agricultural productivity (Smith and Read, 2008; Gianinazzi *et al*., 2010). Moreover, AM fungal diversity can have a direct effect on the ecosystem by driving the structure of plant communities (van der Heijden *et al*., 1998a, 1998b), ameliorating the quality of soil by improving its aggregation and organic carbon content, and finally a positive impact on ecosystem productivity (Oehl *et al*., 2003) thus making them essential for the functioning of terrestrial ecosystems (Bedini *et al*., 2009). The beneficial effects of AM fungi on plant performance and soil health are of interest for the reclamation and re-vegetation of degraded lands and thus of importance for the sustainable management of agricultural ecosystems (Oehl *et al*., 2003; Barrios, 2007).

In the sustainable agricultural systems, the two important components: crop management and soil management play a crucial role in proper functioning of the agro-ecosystem. Crop management is usually essential to sustain the soil fertility with low agricultural inputs. This involves crop rotation and intercropping (Harinikumar *et al*., 1990) which also helps to manage effective and infective AM populations. It is also observed that prior cropping with AM fungal host plant crop can increase the fertility of a soil so that yield benefits are achieved in subsequent crops (Dodd *et al*., 1990). Integrated alternatives for different cropping systems using AM fungi are necessary to maintain the nutrient balance. The profuse use of P fertilizers and chemicals causes pollution problems and health hazards. So the use of AM fungi is being encouraged in agriculture (Dessai, 2013).

The use of AM fungi in agriculture could lead to a considerable decrease in the amount of chemical pollutants in soil and water (Giovannetti, 2001). This clearly indicates the potential of AM fungi for promoting a low chemical input agriculture (Atkinson *et al*., 2002). The recent development of molecular probes able to differentiate AM fungi within roots and soils (Jacquot *et al*., 2000; Jacquot-Plumey *et al*., 2001) opens new biotechnological perspectives for defining their population biology and therefore employ management strategies for their use in agriculture. Modern intensive agricultural practices such as chemical fertilization, pest control, continuous monoculture and tillage affect plant-AM fungal interaction and association. Describing the community of AM fungal diversity at a site is an important step in determining the effects of agricultural treatments on AM fungi and for the formulation of management strategies for these fungi. The beneficial effects of AM fungi on plant growth have led to the development of AM fungi as bioinoculants for agriculture, horticulture and forestry (Mohammad *et al*., 1995). They present valuable opportunities for current agricultural practices with regard to various biotic and abiotic stress conditions making effective utilization of these symbiotic soil fungi indispensable for sustainable agriculture (Sensoy *et al*., 2007).

The use of AM fungi as 'biofertilizers' in agriculture is becoming a worldwide phenomenon and has been successfully used in places like Taiwan, South Africa and United States (Juang, 2007). Their potential as a biofertilizer lies in their mycorrhizal benefits and plant-soil interactions, hence, their selection as inoculum for management of crops in the field are widely studied (Atkinson *et al*., 2002). The exact definition of biofertilizers remain unclear, however, they are commonly referred to as the use of beneficial soil microorganisms to improve availability and uptake of mineral nutrients required for plant growth (Vessey, 2003). In order to exploit these microbes as biofertilizers, the ecological complexity of these microbes in the mycorrhizosphere needs to be taken into consideration (Khan, 2006).

AM SYMBIOSIS AND AGRICULTURAL ECOSYSTEM SERVICES

Crop nutrition: Enhanced uptake of P is the main benefit obtained by host plants through AM association, and plant P status is often the main controlling factor in the formation of plant-fungal relationship (Graham, 2000). AM fungi can play a significant role in crop P nutrition, increasing the total uptake and in some cases P use efficiency (Koide *et al*., 2000) which is directly associated with increased growth and yield (Gosling *et al*., 2006). When AM fungal colonization is disrupted, uptake of P, plant growth and in some cases yield can be significantly reduced (Gosling *et al*., 2006).

When high concentration of available soil P is present, many crops fail to respond to colonization by native AM fungi (Ryan *et al*., 2002; Gosling *et al*., 2006). Under such conditions, the colonization of roots by AM is often suppressed (Kahiluoto *et al*., 2001). Though P uptake usually dominates the consideration of AM association, it has become increasingly apparent that AM can be important in the uptake of other nutrients by the host plant. Zinc (Zn) nutrition is most commonly reported as being influenced by AM association, though uptake of Cu, Fe, N, K, Ca and Mg have been reported as being enhanced (Smith and Read, 1997; Clark and Zeto, 2000). In some cases, it is the availability of these other nutrients, which control the formation or initiation of AM symbiosis (Ryan and Angus, 2003). AM fungi may also enhance plant uptake of N from organic sources (Hodge *et al*., 2001). AM fungi also interfere with the phytohormone balance of host plants which influences plant development (Rouphael *et al*., 2015).

Crop protection against pathogens: AM fungi also play a role in the suppression of crop pests and diseases, particularly soil-borne fungal diseases (Paulitz and Linderman, 1991; Borowicz, 2001). When plant root cell is colonized by AM fungi, the pathogen is excluded from that cell (Gosling *et al*., 2006). AM fungi also suppress pathogenic nematodes (Talavera *et al*., 2001), above ground fungal diseases (Feldmann and Boyle, 1998) and herbivores (Gange *et al*., 2002). Though the mechanisms involved are complex, changes in nutritional status, resulting in changes to leaf defensive chemicals, are likely to be involved in above ground interactions with herbivores (West, 1995). As with soil fungal pathogens, the most effective control is achieved when colonization by AM fungi takes place before pathogen attack (Sylvia *et al*., 2001).

Crop water relations: AM fungi are able to increase the host plants tolerance to water stress (Augé, 2004) and high salinity (Mohammad *et al*., 2003). Several mechanisms have been proposed to explain the effect, including increased root hydraulic conductivity, improved stomatal regulation, osmotic adjustment of the host and improved contact with soil particles through the binding effect of hyphae, enabling water to be extracted from smaller pores (Augé, 2001, 2004). Often both water and mineral nutrient uptake are higher in drought stressed mycorrhizal plants than in non-mycorrhizal plants (Srivastava *et al*., 2002).

Heavy metal tolerance: AM fungal association helps in alleviation of heavy metal induced stress (Hall, 2002) and the extent of alleviation can vary depending on the heavy metal involved, its concentration in the soil, the fungal symbiosis partner and the conditions of plant growth (Turnau and Mesjasz-Przybylowicz, 2003). AM colonization can have a significant impact on the expression of several plant genes coding for proteins involved in heavy metal tolerance and detoxification (Hildebrandt *et al*., 2007). Enhanced tolerance to specific heavy metals by fungi isolated from soils contaminated with Pb, Zn, Cd or Cu has been observed by González-Guerrero *et al*. (2005). AM fungi tolerant to increased heavy metals application readily colonize host roots despite low spore counts (Jacquot-Plumey *et al*., 2001). Secretion of glycoprotein glomalin by AM fungal hyphae also helps in heavy metal binding to soil (González- Chávez *et al*., 2004). The large surface area explored by AM fungal extra-radical hyphae creates an important sink for soil heavy metal binding since hyphae of heavy metal tolerant AM fungi display a higher affinity to heavy metals than plant cells (Joner *et al*., 2000).

Soil stability (soil structure and aggregation): AM fungi have a direct effect on soil structure, which is important in an agricultural context, where cultivations, trafficking and low levels of soil organic matter all tend to result in damaged soil structure (Leifheit *et al*., 2014). The host plant transfers as much as 20% of all fixed C to the fungal partner (Jakobsen and Rosendahl, 1990) and in agricultural soils AM fungi can produce significant biomass (Rillig *et al*., 1999), this in turn has an impact on soil structure. AM fungi bind soil micro-aggregates into larger macro-aggregates through the enmeshing effects of their hyphae (Tisdall, 1991). In addition, AM fungal hyphae secrete glomalin, an extra-cellular fungal glycoprotein. Glomalin accumulation in soil (Rillig *et al*., 2001) exerts a strong influence on soil aggregate stability and soil porosity (Rillig, 2004) there by promoting aeration and water movement, essential for better root growth and development and microbial activity (Amaranthus, 1999). General hyphal exudation and rapid hyphal turnover (Staddon *et al*., 2003) provide C to other soil microorganisms indirectly promoting aggregate stability (Jastrow *et al*., 1998). The overall effect of hyphal enmeshment and C inputs can result in significant enhancement of soil structure and stability (Bethlenfalvay *et al*., 1999).

NUTRITIONAL BENEFITS THROUGH AM FUNGAL ASSOCIATION

Phosphorus: Phosphorus is one of the essential mineral nutrients for plants. The preferred form of P absorbed by plants is ortho-phosphate (Vance, 2003). Due to the fact that P is generally non-labile, narrow depletion zones are formed around P-absorbing roots leading to restricted plant growth (Hinsinger *et al*., 2005). Therefore, plants have developed mechanisms such as beneficial associations with soil biota, to increase their access to soil P. One of the most important symbiotic associations is the formation of mycorrhizae, mutualistic symbiotic relationship between plant roots and specific soil fungi. Arbuscular mycorrhizal association usually increases the growth of the plants by enhancing the uptake of mineral nutrients especially P (Tinker, 1978). It has

been reported that the rate of uptake of nutrients by mycorrhizal plants is faster than that by non-mycorrhizal plants (Son and Smith, 1988).

Effective P absorption by the external or extra-radical hyphae is related to a) formation of polyphosphates in the hyphae and thus, maintaining low internal phosphate (Pi) concentrations b) the small hyphal diameter leading to a relatively larger volume delivering P per unit surface area compared to the root surface area (Jungk and Claassen, 1989) and correspondingly 2-6 times higher P influx rate per unit length of hyphae (Jakobsen *et al*., 1992) and production of extracellular acid phosphatases which catalyze the release of P from organic complexes in soil.

Phosphatases represent a broad range of intracellular as well as soil accumulated activities that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Speir and Ross, 1978). Phosphatase enzymes are also directly involved in the acquisition of phosphorus by plants. Their importance, however, is not always obvious. The proposition that plants with lower activities of root phosphatases may gain and use phosphorus more readily than plants with higher ones has been put forward by McLachlan (1980) who found that acid phosphatase activity was lower in plants more efficient in P-uptake than grown under P-deficient conditions. Mycorrhizal colonization had been shown to influence root phosphatase activity. Acid phosphatase may be associated with the growth and development of the fungus within the host tissue (Gianinazzi *et al*., 1979) as well as with phosphorus acquisition in the rhizosphere. Alkaline phosphatase activity specific to AM fungi have been reported (Bertheau, 1977). This enzyme activity is closely linked to both the mycorrhizal growth stimulation and the arbuscular phase of the colonization and there is strong evidence that it is of fungal origin (Gianinazzi-Pearson and Gianinazzi, 1978).

Carbon: The major fluxes in the AM symbiosis appear to be of C from plant to fungus and of P, and possibly N, from fungus to plant. Reverse C movement from fungus to plant appears only to occur in special cases where the plant has an unusually restricted C supply, most notably in achlorophyllous plants (Bidartondo *et al*., 2002). Carbon supplied from the host to the fungal symbiont is derived from plant sugars and is thought to be transported by passive efflux (Willis *et al*., 2013). The intra-radical hyphae and/or arbuscules take up hexose, a substantial amount of which is used in lipid, trehalose and glycogen synthesis before translocation to extra-radical mycelia (Bago *et al*., 2003). Up to 20% of total photosynthate, always of recent assimilate partitioned to roots, may be supplied to the fungus. Movement of lipid bodies from intra-radical to extra-radical hyphae has been imaged by real-time immunofluorescence technique (Bago *et al*., 2002a). Other work has also shown movement in the opposite direction (Bago *et al*., 2002b). Much of this C is utilized in fungal maintenance and growth and there is evidence that the AM mycelial web releases C into the mycorrhizosphere (Toljander *et al*., 2007), just as roots exude into the rhizosphere soil matrix, influencing biota populations (Jones *et al*., 2009).

Nitrogen: AM fungi also assist in the transfer of N from Organic Matter (OM) and leaf litter to host plants (Leigh *et al*., 2008). As in case with P uptake, AM fungi do not enhance the acquisition of N when present at low levels in soil (Reynolds *et al*., 2005) but can make a significant contribution to plant N requirement (Hodge and Fitter, 2010), particularly in dry soils where mobility to the direct pathway via roots is restricted (Tobar *et al*., 1994). The hyphal pathway converts inorganic N taken up from the labile pool into amino acids, and translocates it principally as arginine from extra-radical to intra-radical hyphae (Govindarajulu *et al*., 2005). Here the N is converted to inorganic N compounds (urea) before passing to the host as NH_4^+ with the resulting C skeletons from arginine breakdown being re-incorporated into the fungal C pools (Govindarajulu *et al*., 2005). AM fungal extra-radical hyphae are thought to contribute indirectly to leguminous plant N status but only in reduced P conditions, supplying essential P and micronutrients to nitrogen-fixing organisms (Smith and Read, 2008).

Uptake of other elements: The symbiosis also contributes in the uptake of other micronutrients by the host plant. Uptake of nutrients such as Na, K, Mg, Ca, B, Fe, Mn, Cu, and Zn is influenced by AM colonization (Cardoso and Kuyper, 2006; Meding and Zasoski, 2008). Mycorrhizal plants may contain higher total quantities of some elements than non-mycorrhizal plants because of their greater biomass. In general nutrient uptake is likely to be affected by P deficiency or whatever is limiting plant growth (Yano-Melo *et al*., 1999).

AM FUNGAL INOCULUM PRODUCTION AND MULTIPLICATION

Inoculum Production: AM fungal inoculum has been utilized in agriculture, horticulture, landscape restoration, and site remediation for almost two decades (Hamel, 1996). In the early 1990s, researchers described multiple ways in which AM species management would be useful for sustainable systems, including agro-systems and restoration (Bethlenfalvay and Linderman, 1992; Pfleger and Linderman, 1994). In a long-term study comparing organic and conventional agriculture, Maeder *et al*. (2002) found that AM were stimulated in organic treatments, which was correlated to enhanced system health (faunal diversity, soil stability, and microbial activity) and to increased crop efficiency.

Sources of AM inoculum: Current production systems rely on soil-based systems (plots or pots), which are not sterile and are often contaminated with other AM species, and other microbes, including pathogens (Gianninazzi and Bosatka, 2004). Non-soil based approaches include *in vitro* systems involving the use of Ri T-DNA transformed plant root organs (genetically modified with *Agrobacterium rhizogens*) to grow on media under sterile conditions. These are much cleaner, but have a limited production capacity (Declerk *et al*., 2005).

Soil based systems or pot cultures: Soil from the root zone of a plant hosting AM can be used as inoculum. Such inoculum is composed of dried root fragments or colonized root fragments, AM spores, sporocarps, and fragments of hyphae. Soil may not be a reliable inoculum unless one has some idea of the abundance, diversity, and activity of the

indigenous AM species. Spores can be extracted from the soil and used as inoculum but such spores tend to have very low viability or may be dead or parasitized. In such a case, soil sample can be taken to set up a 'trap culture' using a suitable host plant to boost the number of viable spore propagules for isolation, further multiplication and also to produce pure or monospecific cultures.

Pure cultures or monospecific cultures are obtained after a known isolate of AM and a suitable host are grown together in a medium (sterilized soil/sand) optimized for development of AM association and spore formation. It consists of spores, colonized root fragments, and AM hyphae.

Host plant species: The plant grown to host AM fungi in the inoculum production medium should be carefully selected. It should grow fast, be adapted to the prevailing growing conditions, be readily colonized by AM, and produce a large quantity of roots within a relatively short time (45-60 days). It should be resistant to any pests and diseases common in the inocula production environment.

Gilmore (1968) recommended strawberry (*Fragaria* sp.) for open pot culture propagation of AM fungi. The range of plant species used since then are too numerous to list. Some common temperate host plants included *Zea mays* (corn), *Allium cepa* (onion), and *Arachis hypogaea* (peanut). Widely-used tropical hosts included *Stylosanthes* spp., *Paspalum notatum* (bahia grass) and *Pueraria phaseoloides* (kudzu) (http://invam.wvu.edu/methods/cultures/host-plant-choices).

The host plant should also be fertilized by periodic additions of a nutrient solution such as Hoagland's solution (especially - P) so as to manage the chemical composition of the medium and to regulate the formation of AM association. To ensure that most of the spores in the inoculum are mature, it is essential to grow the host plant for 12-14 weeks. The medium is then allowed to dry slowly by reducing the frequency of watering over a week and then withdrawing water completely. The inoculum can then be further multiplied.

***In vitro* systems or root organ cultures:** Ri-plasmid transformed root cultures were pioneered by Mugnier and Mosse (1987). A natural genetic transformation of plants by the ubiquitous soil bacterium *Agrobacterium rhizogenes* Conn. (Riker *et al*., 1930) produces a condition known as hairy roots. This stable transformation (Tepfer, 1989) produces Ri T-DNA transformed plant tissues that are morphogenetically programmed to develop as roots. Their modified hormonal balance makes them particularly vigorous and allows profuse growth on artificial media (Tepfer, 1989). *Daucus carota* L. (carrot) and *Convolvulus sepium* L. (bindweed) were among the earliest species to be transformed using *A. rhizogenes* Conn. (Tepfer and Tempé, 1981). For *in vitro* culture of AM fungi using Ri T-DNA roots, the disinfected AM fungal propagules (spores and colonized root fragments) are plated on to Modified Strullu Romand (MSR) media for germination after which the germinated propagules are associated with actively growing Ri T-DNA transformed roots for establishment of AM symbiosis (Bécard and Fortin, 1988).

APPLICATIONS

AM fungi and fruit crops: Many fruit tree species are dependent on AM colonization for survival and growth (Schubert and Cammarata, 1986). In *Musa* species, the beneficial effects of AM under *in vitro* conditions (Declerck *et al*., 1995) and field conditions (Sukhada, 1994) are well documented. Mycorrhizal fungi have been shown to increase growth in *Malus* seedlings, both in field and glasshouse conditions (Plenchette *et al*., 1983; Reich, 1988). Improvement of growth and mineral uptake in *Citrus* species is well documented (Menge *et al*., 1978). The response of AM fungi in strawberry (*Fragaria* x *ananassa*) has also been tested (Vestberg *et al*., 2000). Khade and Rodrigues (2008a, 2008b) recorded 18 AM fungal species belonging to four genera *viz., Acaulospora, Glomus, Gigaspora* and *Scutellospora* in mono-culture plantation of *Carica papaya*. Wang *et al*. (2013) identified 18 AM fungal species belonging to 3 different orders, *Archaeosporales* (1 species), *Diversisporales* (7 species) and *Glomerales* (10 species) from rhizosphere soils of *Citrus reticulata* Blanco (red tangerine) rootstock in hillside *Citrus* orchards. In field conditions, grapevine roots normally are colonized by AM fungi (Balestrini *et al*., 2010). Presence and identification of AM fungi has also been detected in different isolated avocado orchards in Michoacan, Mexico (Bárcenas *et al*., 2006). Soares *et al*. (2005) identified 9 native AM species *viz., Rhizophagus clarus, Glomus spurcum, Scutellospora fulgida, G. macrocarpum, G. invermaium, Acaulospora colombiana, S. pellucida, A. appendiculata* and *S. heterogama* from a passion fruit plantation in Brazil with *R. clarus* and *G. spurcum* being the most predominant species. Singh and Prasad (2006) observed maximum colonization and spore population in *Litchi* orchards from Uttar Pradesh and reported colonization by AM species belonging to the genera *viz., Glomus*, *Gigaspora*, *Rhizophagus* and *Acaulospora*. Kachkouch *et al*. (2012) evaluated mycorrhizal status in *Olea europaea* and also conducted the survey of AM species in the olive grove soils of Morocco. They recorded spores belonging to *Glomus, Entrophospora, Gigaspora, Acaulospora* and *Scutellospora*, with *Glomus* being the dominant genus. Sarwade *et al*. (2011) reported the AM association in *Annona squamosa* from Maharashtra, India. They reported the association of *Glomus* and *Acaulospora* with *A. squamosa*. Sukhada (2012) studied the diversity of AM fungi in seven root stocks of mango and found *Glomus* and *Acaulospora* to be the major genera in the rhizosphere with *Rhizophagus fasciculatus* and *Funneliformis mosseae* as the predominant AM species.

AM fungi and vegetable crop plants: Diversity of AM association in different crops is currently of great interest due to the important role played in different crops. AM fungal distribution and diversity in different plant species of a particular agro-ecological zone are important in order to evaluate the natural status of AM fungi in that region (Dessai, 2013). Many researchers reported the abundance of AM spores in rhizospheres of different crops (Friberg, 2001; Sinegani and Sharifi, 2007; Mathimaran *et al*., 2007). Franke-Snyder *et al*. (2001) compared the composition and structure of the AM fungal communities associated with *Zea mays* and

Glycine max in a conventional and two low-input farming systems for better understanding of the relationship among AM fungi present in different agricultural systems. Akond *et al*. (2008) investigated fifteen vegetable crop plant species in Bangladesh to measure their affinity in harbouring mycorrhizal fungi. Hindumathi and Reddy (2011) reported the occurrence and distribution of AM fungi and microbial flora in the rhizosphere soils of *Vigna radiata* and *Glycine max*. Sinegani and Sharifi (2007) reported that the number of AM spores was significantly higher in the rhizosphere of *Solanum lycopersicum* and *Allium cepa*. Grigera *et al*. (2007) reported that AM fungi are active during the reproductive growth stages of maize and may benefit high productivity of maize crops by facilitating P uptake. Zhao *et al*. (2010) reported that the AM fungal inoculation can reduce watermelon replant problems through effectively modifying the soil microbe population and community structure, and increasing the soil enzyme activities. Manjunath *et al*. (1981) studied the effects of AM inoculum on growth of onion. AM inoculation increased yield of chillies, tomato, capsicum and other vegetables (Mamatha and Bagyaraj, 2000). Douds *et al*. (2007) reported an increase in potato yield in a high P soil due to AM inoculation. Sheng *et al*. (2012) studied the inoculation of AM fungi and reported that intercropping with pepper improves soil quality and watermelon crop performance in a system previously managed by monoculture. AM fungus *Rhizophagus fasciculatus* enhanced fruit growth and quality of *Capsicum annuum* plants exposed to drought (Mena-Violante *et al*., 2006). Eight different pepper genotypes inoculated by two AM fungi (*R. intraradices* and *Gi. margarita*) showed greater dry weights compared to non-inoculated plants (Sensoy *et al*., 2007). Castillo *et al*. (2009) studied the effect of AM fungi on an ecological crop of *Capsicum annuum* and explained that the inoculation with native fungi decreased transplantation stress thus accelerating the maturation stage of plants and resulting in higher and better yield quality. El-Shaikh and Mohammed (2009) reported enhancement of yield of okra through AM inoculation. Lingua *et al*. (2002) studied the mycorrhizal-induced differential response to a yellow disease in tomato where symptoms induced by the phytoplasma were less severe when the plant was colonized by AM fungi which showed improved morphological parameters and reduced nuclear senescence. Mycorrhizal tomato plants had significantly less infection by *Alternaria solani* than non-mycorrhizal plants (Fritz *et al*., 2006). Inoculation of *R. fasciculatus* significantly reduced nematode population, number of galls and root knot index besides increasing the growth, plant biomass, P uptake and yield of tomato plant (Shreenivasa *et al*., 2007). Akhtar and Siddiqui (2010) studied the effect of AM fungi on the plant growth and root-rot disease of chickpea. Application of AM fungi mostly resulted in significant suppression of nematode multiplication and root galling damage in tomato and carrot over two crop cycles indicating that the AM fungi persist and remain protective against root-knot nematodes (Liu *et al*., 2012). AM fungi increase the plant growth and nutrient uptake, decrease yield losses of tomato under saline conditions and improve salt tolerance of tomato (Al-Karaki *et al*., 2001; Hajiboland *et al*., 2010). Dessai and Rodrigues (2012) conducted a survey of different vegetable crop plants cultivated in Goa to assess the associated AM fungal diversity and recorded a high spore density in *Zea mays* (95.33 spores $100g^{-1}$ soil) with *Acaulospora scrobiculata* being the dominant AM species. Dessai (2013) evaluated AM fungal diversity in vegetable crop plants of Goa, India and reported a rich diversity of AM fungal species associated with vegetable crops. Fifty one AM fungal species belonging to eleven genera *viz., Acaulospora, Glomus, Gigaspora, Funneliformis, Dentiscutata, Rhizophagus, Claroideoglomus, Racocetra, Simiglomus, Ambispora* and *Scutellospora* were recovered from the rhizosphere soil. The study also revealed a significant increase in growth and nutrient uptake in *C. annuum* and *Abelmoschus esculentus* upon inoculation with dominant indigenous AM fungal species in all the treatments compared to un-inoculated control plants.

AM fungi and plantation crops: AM fungi are widespread in agricultural systems. Numerous studies have reported the natural diversity of AM fungi in the soils of coffee orchards, as well as the presence of AM colonization in coffee roots (Theodoro *et al*., 2003; Muleta *et al*., 2007). Lopes *et al*. (1983) identified 22 AM species in coffee rhizosphere soil from a Brazilian coffee production region; with *Acaulospora* and *Glomus* as the most frequently occurring genera. These were also the predominant genera found in other coffee-cultivated soils in Venezuela, Colombia and Mexico (Riess and Sanvito, 1985; Toro-Garcia, 1987; Cruz, 1989). Other AM genera, such as *Scutellospora, Gigaspora* and *Sclerocystis*, have also been described in different coffee orchard soils (Colozzi-Filho and Cardoso, 2000). Recently, in a native forest in Ethiopia, where coffee coexists with other trees in its original ecosystem, high AM species richness has been reported, with representatives of five genera of AM. *Glomus* was the dominant genera, followed by *Gigaspora, Acaulospora, Entrophospora* and *Scutellospora* (Muleta *et al*., 2007). Sanchez *et al*. (2005) found a positive effect of AM inoculation on coffee P concentrations, which resulted in higher growth when compared with non-inoculated plants. Arbuscular mycorrhizal associations have been well reported in palms, such as *Cocos nucifera* (Thomas *et al*., 1993), arecanut (Bopaiah, 1991), and, more recently in some tropical palms (John, 1988) under natural conditions. In India, a number of fungi belonging to four genera viz., *Glomus, Gigaspora, Sclerocystis*, and *Acaulospora* have been found to form mycorrhizal associations with coconut (Harikumar and Thomas, 1991). The occurrence of a mixed population of AM has been commonly recorded from the coconut rhizosphere soils. Research on genotypic dependency of AM in coconut revealed higher colonization rate in tall varieties compared to dwarf ones (Thomas and Ghai, 1987). The quantitative and qualitative distribution of AM also varied in response to a single crop or a combination of intercrops (Ramesh, 1984). Ananthakrishnan *et al*. (2004) studied mycorrhizal association in cashew (*Anacardium occidentale*) grown in different plantation areas of south India and recorded AM species belonging to the genera *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* in the rhizosphere soils. The species of *Glomus*, *G. aggregatum*, *G. fasciculatum* and *G. mosseae* were the most abundant in the majority of the

experimental sites and hence were selected and used for experimental studies. Of the three AM species *G. fasciculatum* significantly increased shoot length, internode number and length, leaf number, stem diameter, root length and number. Karthikeyan *et al*. (2005) studied the response of tea (*Camellia sinensis*) to inoculation with six species of indigenous AM fungi, *Acaulospora scrobiculata*, *Glomus aggregatum*, *G. fasciculatum*, *G. geosporum*, *G. intraradices* and *Scutellospora calospora* under plantation nursery conditions. AM inoculated tea seedlings showed an increased growth and nutritional status over un-inoculated seedlings. The extent of growth and nutritional status enhanced by AM fungi and the mycorrhizal dependency of the host varied with the species of AM fungi. However, AM association decreased nutrient use efficiencies. Seedlings inoculated with *S. calospora* had greater biomass and seedling quality than other mycorrhizal seedlings. Given the unusual morphology of the oil palm root systems and the results from experimental studies it appears that oil palms are strongly mycorrhizal dependent (Phosri *et al*., 2010). Well-established field-grown oil palm roots are naturally heavily colonized by AM fungi (Blal and Gianinazzi-Pearson, 1990). Both the African and American oil palms (*Elaeis oleifera*) produce thick cylindrical adventitious roots and do not produce root hairs (Corner, 1966). Root hairs are used by most plants for efficient water and nutrient uptake and, therefore, oil palms are probably functionally dependent on AM fungi to obtain their nutrition (Corley and Tinker, 2003).

AM fungi and Floriculture: There are fewer studies on the association and diversity of AM fungi in ornamental flowering plants. Ranganayaki and Manoharachary (2001) studied AM colonization in *Tagetes erecta* plants under natural field conditions and found the rhizosphere soil harbouring *Acaulospora foveata, Entrophosphora* sp*., Funneliformis constrictum, R. fasciculatus, G. heterosporum, G. hoi, Sclerocystis pakistanika* and *Scutellospora nigra* among which *R. fasciculatus* was predominant. Muthukumar *et al*. (2006) studied mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Radhika and Rodrigues (2010) found *Glomus maculosum, G. glomerulatum* and *Acaulospora scrobiculata* associated with *Hibiscus rosa-sinensis* while carrying out survey of AM fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. Yang *et al*. (2011) reported AM colonization in *Magnolia cylindrica*. Johnson *et al*. (1982) studied the effect of flower bud development in *Chrysanthemum* on AM formation and showed that carbon and nutrient allocation patterns between mycorrhizal and non-mycorrhizal plants can influence flowering. Gaur *et al*. (2000) evaluated effects of mixed AM inocula and chemical fertilizers in a soil with low P fertility on growth and flowering in *Petunia hybrida, Callistephus chinensis* and *Impateins balsamina*. An increase in P and K concentration in shoots of AM inoculated plants along with an improvement in both flower number and vegetative phase of plants was reported. Sohn *et al*. (2003) evaluated the effect of different timing of AM inoculation on rooting rate, colonization percentage and early plantlet growth at transplanting stage and successive plant growth, nutrient uptake and flower quality of *Chrysanthemum morifolium.* A significant difference in plant growth, nutrient uptake and flower quality was observed in AM inoculated plants compared to non-inoculated plants. Soil pasteurization and inoculation with AM fungi can alter plant characteristics that affect the quality and composition of corms and cut flower production in *Brodiaea laxa* (Scagel, 2004). Gange and Smith (2005) evaluated the effect of AM inoculation in three species of annual plants viz., *Centaurea cyanus, Tagetes erecta* and *T. patula* and reported that AM inoculation influenced visitation rates of pollinating insects to these plants due to increase in total plant size, flower number and size and, amount of pollen produced. Long *et al*. (2010) evaluated effects of *Gigaspora* and *Glomus* on *Zinnia elegans* and showed that mixed inoculum is not much effective in promoting growth as compared to inoculation with *Glomus* alone. Asrar and Elhindi (2011) studied the effect of *F. constrictum* on growth, pigments and P content of *Tagetes erecta* plant grown under different levels of drought stress and observed that AM inoculation positively stimulated all growth parameters. Vaingankar and Rodrigues (2012) conducted a study to screen the most efficient AM fungal bioinoculant to evaluate its possible effects on growth, yield and flower fresh weight loss in two commercially ornamental plant species *viz., Chrysanthemum morifolium* Ramat. and *Tagetes erecta* L. The study revealed that *Glomus intraradices* was the most efficient AM fungal bioinoculant that increased flower number in both the plant species. This was attributed to its ability to colonize and multiply at a faster rate than the other AM fungal species used in the study.

CONCLUSION

The search for an effective microorganism having role in seed germination, nutrient acquisition, vegetative growth, productivity and tolerance to environmental stress factors has led the researchers to explore possibilities of using AM fungi in agro-ecosystems making these myco-symbionts a pivotal factor for improvement and management of the plant production systems. Mycorrhizal fungi also play an important role in reducing the fertilizer usage. The benefits provided by AM fungi can vary depending on the AM species or strains used for inoculation and in turn can affect the crop productivity. Screening of AM fungal diversity in the field or natural environment where a native community is present is necessary so as to obtain maximum benefits through AM association.

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Characterization of physico-chemical properties of chitin extracted from *Coprinopsis cinerea*, a coprophilous fungus

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ABSTRACT

Chitin has great applicability in biomedical and biotechnological fields because of its non-toxic, biodegradable and biocompatible nature, and having antimicrobial and antioxidant properties. The most common source of chitin is the crustaceous shell; however, mushrooms are an alternative source for isolating these biopolymers because their cell wall has a high content of chitin. The main objective of this study was to isolate chitin from the coprophilous mushroom *Coprinopsis cinerea* and to determine its physico-chemical properties. The material characterization was done using X-ray diffraction (XRD), Fourier transform infrared (FTIR)spectroscopy, Scanning electron microscopy (SEM) and Thermogravimetric analysis (TGA). We extracted 42.8 grams of chitin per 100 grams of mycelial biomass of *C. cinerea*. The extracted chitin had a significant similarity with commercial chitin, including diffractogram peaks, characteristic infrared analysis bands. The crystallinity index (CrI) value of chitin was calculated as 57.48%. The scanning electron micrograph (SEM) indicated the presence of nanofibre on the surface of the chitin. The maximum degradation temperature of *C. cinerea* chitin was found to be 378°C by Thermogravimetric analysis (TGA). This is the first report of extraction of chitin from *C. cinerea*, a coprophilous fungus and determination of its physico-chemical properties. This data gives us basic information on the possibility of introducing this biomaterial in the field of biomedicine.

Keywords : Coprophilous mushroom, *Coprinopsis cinerea* ,Chitin, XRD, FTIR, TGA

INTRODUCTION

Chitin is a structural amino polysaccharide found in the cell wall of yeast, fungi, protists and diatoms as well which also forms the exoskeletons of broad variety of invertebrates including sponges, worms, mollusks and, especially, arthropods species. It has a wide range of uses in fields such as cosmetics, pharmacy, medicine, bioengineering, biological material science, agriculture, textiles and environmental engineering based upon its nontoxic, ecofriendly, biocompatibility and biodegradability characteristics (Ospina *et al*., 2014; Kaya *et al.,* 2015; Ospina *et al*., 2015; Hoque *et al.,* 2018). Commercially, chitin is obtained by processing the outer skeleton of crustaceans such as shrimp, crab, prawn and crayfish after they have been consumed as food. However, the availability of crustacean shell waste is limited and is subject to season and supply. In recent years, chitin extracted from fungal mycelia is gaining importance. Fungal mycelia can be cultivated throughout the year and can be performed in bioreactor with all automated and controlled conditions, therefore, mycelium biomass produced in each batch is homogeneous in quality and quantity (Yen and Mau, 2007; Abdou *et al.,* 2008).

Coprinopsis cinerea is a coprophilous fungus, which in nature grows on herbivores dung (Buller, 1931; Uljé and Noordeloos, 1999). It is commonly called as inky cap mushroom and serves as a model oraganism for fruit body development in basidiomycetes (Kues and Liu, 2000). Till date there is no report on the extraction of chitin from *C. cinerea*. This study describes the extraction of chitin from *C. cinerea* cultivated under submerged culture conditions and analysis of its physico-chemical properties by using various advanced analytical techniques.

MATERIAL AND METHODS

Chemicals: The chemicals and reagents (analytical grade) used in the media and reagent preparation were purchased from Sisco Research Laboratory (SRL) and Himedia, Mumbai, India.

Fungal culture: The pure culture of *C. cinerea* (KX468975) is a lab isolate, isolated by us from horse dung. The culture used for the present study was maintained in 2% wheat flour agar (WFA) medium at 4°C (Mohankumar and Savitha, 2017).

Growth of mushroom mycelium (*C. cinerea*): Submerged fermentation was carried out to cultivate *C. cinerea* (KX468975) in 2% wheat flour broth (WFB) medium incubated for 7 days at 30°C and pH 6 under dark (Mohankumar and Savitha, 2017). After growth, the mycelia was separated from culture filtrate and used for the extraction of chitin.

Chitin extraction: The extraction of chitin from the mycelium of *C. cinerea* was carried out according to the method described by Ospina *et al.,* (2015). Forty grams dried mycelia biomass of *C. cinerea* was homogenized in 100 ml of deionized water. The suspension obtained was centrifuged at 7000 rpm for 15 min, and the precipitate was submitted to deproteinization with NaOH (4 M) at a ratio of 1:20 (p/v) by constantly stirring at 100°C for 2 hrs. Later, it was washed repeatedly with deionized water, centrifuged and supernatant was discarded. The deproteinization treatment was conducted twice and the pellet was dried at 50°C until it reached a constant weight.

X-ray diffraction (XRD) studies of chitin: X-ray diffraction analysis was performed on extracted chitin (in triplicate) using X´Pert PRO MPD equipment with a 1.8 kW (40 mA y45 kV) ceramic Cu tube, K-alpha radiation at 1.5406 A°. The crystallinity index (CrI) was calculated according to the following formula described by Erdogan *et al.* (2017).

$$CrI = [(I_o - Iam)/I_o] \times 100$$

Where, I_o = Maximum intensity at 2θ 20°

Iam = Intensity of amorphous diffraction at 2θ 16°

Fourier transform infrared spectroscopy (FTIR) of chitin: Fourier transform infrared spectroscopy (FTIR)

analysis for chitin was done using an Agilant spectrophotometer that had aDTGS PerkinElmerdetector. The samples were dispersed in KBr under anhydrous condition to form pellets. The analysis was conducted at a temperature of 24°C, with 4000450 cm^{-1}wavenumber range and 4 cm^{-1}resolution (Ospina *et al.,* 2014).

Scanning electron microscopy of chitin: Scanning electron microscopy was used to examine the surface morphology of the chitin extracted from *C. cinerea*. Examination was carried out on samples coated with gold using a TESCAN VEGA 3 scanning electron microscope.

Thermogravimetric analysis (TGA) of chitin: A thermogravimetric analysis was performed for chitin according to the method described by Ospina *et al.* (2015). Test was performed in an air atmosphere with a TGA Q500 device at a warming speed of about 10°C/min. The extracted sample was heated up at a temperature ranging from 0 to 900°C to check the thermostability.

RESULTS AND DISCUSSION

Yield of chitin: The chitin flakes extracted from *C. cinerea* by the method given by Ospina *et al*. (2015) was small in size, more homogeneous and light brown in color (**Fig. 1**). The color is possibly due to the fact that there is a presence of lignin. The dry weight of chitin in *C. cinerea* was found to be 42.8%. Ospina *et al.,* (2015) reported that the dry weight of chitin in crustaceans varied between 13% and 42% and in some insect species it varied between 2.59% and 36%. Our results clearly indicate that, the chitin content of *C. cinerea* is higher than that of crustaceans and insects. Vetter (2007) reported the chitin contents in the pileus and stipe of some cultivated mushroom species such as *Agaricus bisporus* (pileus-6.67%, stipe-3.71%), *Pleurotus ostreatus* (pileus-3.78%, stipe-2.8%) and *Lentinula edodes* (pileus-8.07%, stipe-6.5%). Similarly, Kaya *et al.* (2015) reported the dry weight of chitin in *Fomitopsis pinicola* to be 30.11%. As it is seen above, there are significant differences even between mushroom species in terms of their chitin content.

Scanning electron microscopy (SEM): The surface morphology of the chitin of *C. cinerea* was examined with scanning electron microscopy (SEM). The results indicated that there are nanofibres on the surface of the chitin of *C. Cinerea* (**Fig. 2**). This is in agreement with the results documented by Ifuku *et al.* (2011) who reported the presence of uniformity in structure of nanofibrils present on the surface of chitin in five mushroom species , namely *Pleurotus eryngii*, *Agaricus bisporus*, *Lentinula edodes*, *Grifola frondosa* and *Hypsizygus marmorcus*. On the contrary, Erdogan *et al.* (2017) reported that the chitins from *Lactarius vellereus* and *Phyllophora ribis* did not possess any nanofibres on their surface. A similar study reported that chitin of *Xanthoria parietina*, a lichen species, also did not show any nanofibres on the surface of the chitin (Kaya *et al.,* 2015). Surface morphology is reported to be one of the important factors in the characterization of chitin which accounts for their application in various biomedical fields. In this regard Muzarelli (2011) documented that nanofibre chitin of fungal origin can be used in anti-tumor applications and immune-modulating activity.

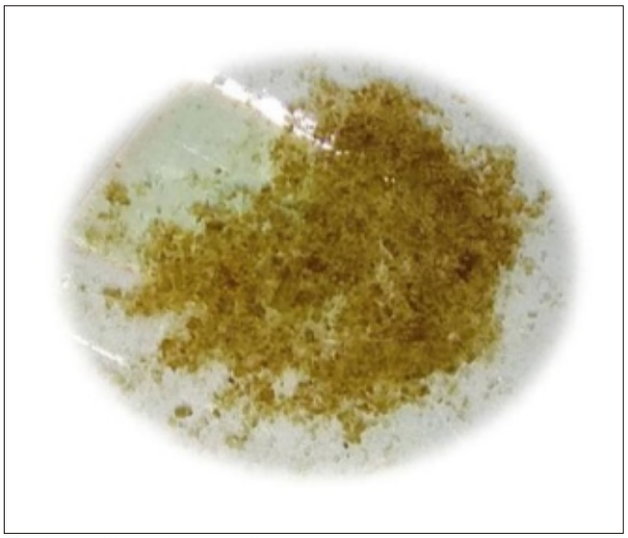

Fig 1: Chitin flakes of *Coprinopsis cinerea*

X-ray diffraction (XRD): Crystallinity of chitin extracted from *Coprinopsis cinerea* was determined by X- ray diffraction analysis. Diffractogram shows three sharp crystalline peaks at 2θ= 6.02°, 20° and 29.5°. These peaks are similar to the ones reported by Ospina *et al.* (2014), in case of

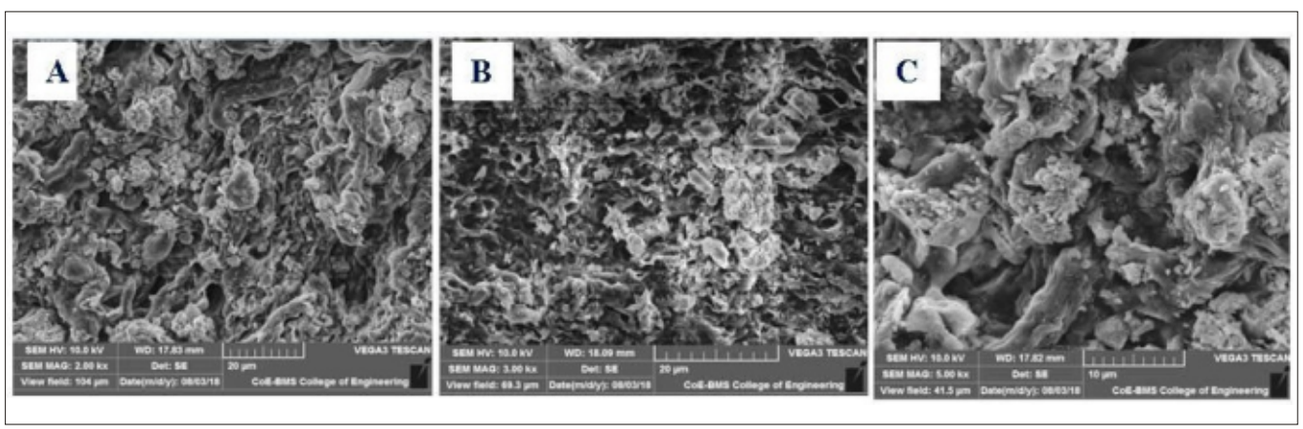

Fig 2: Scanning electron micrographs of chitin of *Coprinopsis cinerea*: A) 2000X, B) 3000X and C) 5000X.

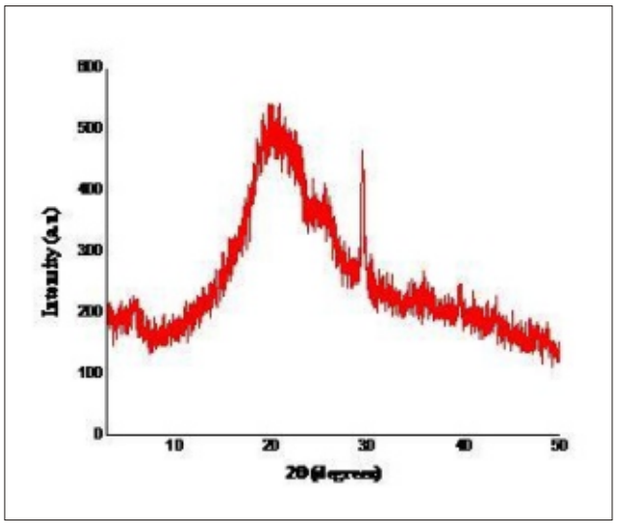

Fig 3: X-ray diffractogram of chitin of *C. cinerea*

chitin from *Ganoderma lucidum* which is reported to give crystalline peaks at 5.6°, 21.7° and 30.1°. On the contrary, crustacean chitin is reported to have two sharp peaks around 9° and 19° (Wang *et al.,* 2013). Similarly, Erdogan *et al* . (2017) reported sharp crystalline peaks at 9.20° and 19.64° in case of chitin extracted from *Lactarius vellereus* and in case of *Phyllophora ribis* at 9.38° and 19.60°.

Crystallinity index (CrI) of the chitin extracted from *Coprinopsis cinerea* was calculated as 57.48%. In literature, the CrI values reported are around 67.8% and 64.1% for crab and shrimp chitins (Hajji *et al.,* 2014). In case of chitin from *Lactarius vellereus* and *Phyllophora ribis*, Erdogan *et al.* (2017) reported CrI values of 64% and 49%, respectively. Ifuku *et al.* (2011) documented that CrI values of five mushroom species varied between 47.6% and 88.5%. Crystallinity index (CrI) values are reported to be the indicators of the purity of chitin (Ifuku *et al.,* 2011). Higher the crystallinity index higher the purity.

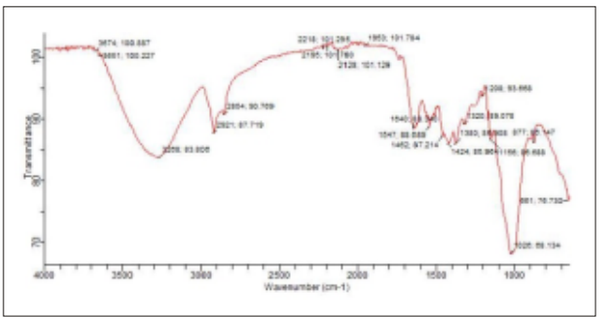

Fig 4: FTIR spectrum of chitin of *C. cinerea*

Fourier transform infrared spectroscopy (FTIR): FTIR analysis of chitin extracted from *C. cinerea* is shown in **Fig. 4**. The FTIR shows chitin representing bands at 3674, 3268, 2854, 1640, 1547, 1380, 1320, 1156 and 1020 cm^{-1} (**Table 1**). This is in agreement with the FTIR analysis of chitin extracted from *Ganoderma lucidum* (Ospina *et al*., 2014). The band at 3268 cm^{-1} represents the N-H stretching and the band at 2854 cm^{-1} represents the C-H stretching. Teng *et al.* (2001) reported that bands near to 2900 cm^{-1} are representative bands for chitin. The band at 1640 cm^{-1} corresponds to amide I stretching C=O, while the band at 1547 cm^{-1} to the stretching or N-H deformation of amide II.

Thermogravimetric analysis (TGA): The result of the TGA thermal details of chitin extracted from *Coprinopsis cinerea* is shown in **Fig. 5** and **Table 2**. The curve in the **Fig. 5** shows an evidence of a loss of weight in three stages. The first stage of 11.87% weight loss occurs in the range between 24 and 103° C which could be a result of the loss of absorbed water. This process is reported to be an endothermic reaction, which resulted from the existence of adsorbed and bound water in the samples (Yang *et al.,* 2015). The second stage starts at approximately 103° C and continues to 378° C and during this interval there is 55.06% weight loss. This weight loss is attributed to saccharide degradation in the molecular structure (Ospina *et al*., 2015). In the third stage there is 13%

Table 1: FTIR bands of chitin samples isolated from *Coprinopsis cinerea* and commercial chitin

Functional group and vibration modes	Classification	Wavenumber (cm^{-1}) frequency	
		***C. cinerea* chitin**	**Commercial chitin**
O-H stretching	-	3674	3437
N-H stretching	-	3268	3261-3103
CH_3 sym.stretch and CH_2 asym. stretch	Aliphatic compounds	2218, 2195	2392
CH_3 sym.stretch	Aliphatic compound	2854	2862
C=O secondary amide stretch	Amide I	1640	1665
C=O secondary amide stretch	Amide I	-	1621
N-H bend, C-N stretch	Amide III	1547	1553
CH_2 ending and CH_3 deformation	-	1424,1468	1428
CH bend, CH_3 sym. deformation	-	1380	1375
CH_2 wagging	Amide III, components of protein	1320	1311
Asymmetric bridge oxygen stretching	-	1156	1154
Asymmetric in-phase ring stretching mode	-	-	1115
C-O-C asym. stretch in phase ring	Saccharide rings	-	1069
C-O aym. stretch in phase ring	-	1020	1020
CH_3 wagging	Along chain	-	951
CH ring stretching	Saccharide rings	-	897

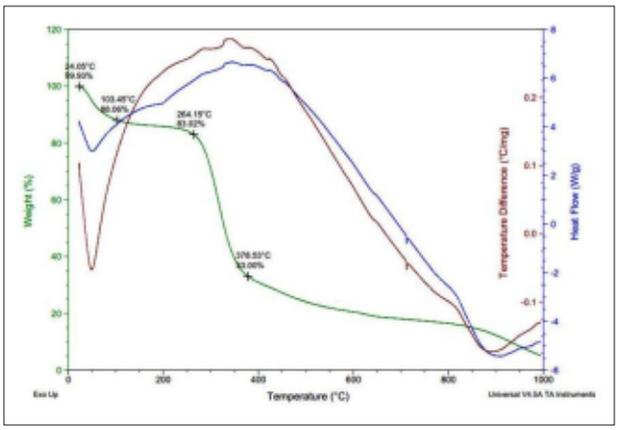

Fig 5: Thermogram of chitin of *C. cinerea*

weight loss in a temperature range of 378-800° C, which is reported to a thermal decomposition of glucosamine (Ramya *et al.,* 2012). It is noted that, chitin start degrading at 264.15° C, so it is only safe to work with this material only up to this temperature.

The maximum degradation temperature of chitin obtained from *C. cinerea* was measured at 378°C. This is in agreement with the results of Jang *et al.,* (2004) who reported 300-400°C, the degradation temperature values for α-chitin. Erdogan *et al.* (2017) reported the degradation temperature values of chitin from *Lactarius vellereus* and *Phyllophora ribis*were to be 354 and 275°C. In crustaceans and insects the maximum degradation temperature values of chitin was found to be 350° and 380° C. Considering these results, it is

Table 2: TGA thermal analysisof chitin of *Coprinopsis cinerea*

Percentage decomposition (%)	Decomposition temperature (°C)
10	80
20	280
30	300
40	320
50	330
60	350
70	420
80	700
90	980

seen that the thermal stability of the chitin obtained from *Coprinopsis cinerea* was higher than the chitin of crustaceans and few mushrooms.

CONCLUSION

Chitin is an important polysaccharide found as supporting material in the cell wall of mushrooms. Due to its high biocompatibility, it has generated an attractive interest in various fields such as biomedical, pharmaceutical, food and environmental industries for various applications. In the present study, chitin was extracted from the mycelia of *C. cinerea* and evaluated its physico-chemical properties using SEM, XRD, FTIR and TGA studies. We extracted 42.8% of chitin from mycelia of *C. cinerea*, which is the highest amount among the coprophilous mushrooms. The high thermal stability, good crystallinity index value and the nanofibres on the surface of the chitin extracted by us makes the usage of this material in the food packaging industries and as a drug delivery channel. In future, the chitin obtained from *C. cinerea* can be exploited for the synthesis of chitosan and other chitin derivatives.

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New species and new records of *Melanommataceae* (*Pleosporales*) from Andaman Islands

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ABSTRACT

Andaman Islands are less investigated for fungal diversity and very meagre information is available on fungi. In our investigations on diversity of ascomycetous fungi from Andaman Islands we encountered one new species and three new records belonging to the family *Melanommataceae*. The new species, namely *Bertiella striatispora* sp. nov., is different from other species of the genus in having striations in the ascospores. The new records are three species belonging to the genus *Byssosphaeria* including *Byssosphaeria jamaicana, B. keithii* and, *B. schiedermayeriana.* These taxa are described and illustrated in this paper. A dichotomous key and a table are also provided to delineate the new species *B. striatispora* from other species of the genus.

KEY WORDS: Fungal diversity, *Pleosporales*, new ascomycetes, *Byssosphaeria* spp.

INTRODUCTION

Although Andaman and Nicobar Islands are rich in plant diversity, the fungal diversity has been poorly investigated. This is mainly due to the distance from the mainland of the Indian subcontinent. In a recent checklist of fungi from Andaman and Nicobar Islands Niranjan and Sarma (2018a) listed 446 fungi. Further, new species and new records are being added by our research team and other workers (Niranjan and Sarma, 2018 b, c, d, e, f; Rajamani *et al.,* 2018). *Pleosporales* is one of the largest orders in *Dothideomycetes* (Mugambi and Huhndorf, 2009; Tian *et al.,* 2015). The family *Melanommataceae* belonging to this order (Wijayawardene *et al.,* 2017; 2018) has members that are mostly saprobic and are distributed throughout the world by colonization of decaying twigs and woody litter in terrestrial, marine and freshwater environments (Tian *et al.,* 2015). The morphological characters of the *Melanommataceae* have been updated by different authors (Barr, 1990; Sivanesan, 1984; Zhang *et al.,* 2012; Hyde *et al.,* 2013; Tian *et al.,* 2015). The family *Melanommataceae* is mainly characterized by globose or depressed perithecial ascomata, trabeculate pseudoparaphyses, bitunicate and fissitunicate asci, pigmented and phragmosporous ascospores (Tian *et al.,* 2015). Many new species have been added to this family recently (Mugambi and Huhndorf, 2009; ChacónZapata and TapiaPadilla, 2013; Tian *et al.,* 2015; Li *et al.,* 2016; Almeida *et al.,* 2017), including several additions and exclusions of genera (Lumbsch and Huhndorf, 2007; Kirk *et al.,*2008; Wijayawardene *et al.,*2014; 2018 ; Tian *et al.,* 2015). Currently this family has 24 accepted genera (Wijayawardene *et al.,* 2018).

Bertiella (Sacc.) Sacc. (1899) is a genus belonging to *Melanommataceae* and has species that are mostly saprobic on lignicolous substrates and are predominantly known from the north temperate zone (Almeida *et al.,* 2017). This genus is morphologically characterised by superficial, subglobose ascomata and bitunicate, cylindroclavate or clavate, short stalked asci containing eight, fusiform, septate, hyaline to brown ascospores. Though only two species have been included by Wijayawardene *et al.* (2018), the current number has increased to 6 species including the present new taxon proposed in this paper.

Byssosphaeria Cooke (1879), another genus in *Melanommataceae*, has morphological differences from the existing genera in possessing the ostiole with reddish-orange or greenish tinge at pore. Currently, 25 species are accepted in this genus (Wijayawardene *et al.,* 2017). Most of them produce hyaline to brown fusiform ascospores with 3 septa or rarely 5 septate (Tennakoon *et al.,* 2018). *Byssosphaeria* was previously synonymized with *Herpotrichia*, but Barr (1984) felt that *Herpotrichia* was heterogeneous and restored *Byssosphaeria* in addition to other genera included in the synonymy.

MATERIAL METHODS

Dead and decomposing twig samples fallen on the forest floor in the Andaman Islands, India were collected in large polythene bags. They were rinsed with running tap water to remove the debris, dried overnight, and packed into new plastic bags for shipment to the laboratory for further processing. Before undertaking the microscopic examination, the twigs were placed individually into plastic bread boxes, lined with sterile tissue paper, rehydrated by sprinkling sterile distilled water and incubated for one week to 3 months. The samples were then examined under a Stereo Zoom microscope (Optika SZMLED, Italy) to locate the fungal fruit structures. Hand sections were taken wherever necessary. The fruit bodies were cut with a razor/blade and the sections were transferred to a microslide, mounted (in lactophenol or lactophenol cotton blue). Slides were then examined under the compound microscope (Olympus CH20i, Japan) for morphological characteristics. Nikon ECLIPSE TiU upright microscope with DIC objectives fitted with Nikon DSFi2 digital camera, Japan to fetch photomicrographs. Measurements were carried out with Nikon NIS Elements Imaging Software version 4.4 program. Photoplates were prepared with the help of Microsoft power point, and Adobe Photoshop version 7.0. Morphological identification was carried out by referring the recent literature (Tian *et al.,* 2015; Almeida *et al.,* 2017 and Wijayawardene *et al.,* 2018). The specimens and descriptions were deposited at the Ajrekar Mycological Herbarium (AMH), Pune and the Department of Biotechnology, Pondicherry University.

RESULTS AND DISCUSSION

Taxonomy:

Bertiella striatispora M. Niranjan & V.V. Sarma sp.nov.

Figs.1: a-q

Mycobank: MB 830985 ; "Facesoffungi: FOF 06134".

Diagnosis: *Bertiella striatispora* is distinct from existing species of the genus in having brown ascospores with longitudinal striations on the surface rather than hyaline and smooth surface.

Etymology: ***The species name has been based on the presence of striations on ascospores.***

Classification- *Melanommataceae*, *Pleosporales*, *Dothideomycetes*.

Saprobic on an unidentified twig. **Teleomorph:** *Ascomata* 185-233 (-434) × 225-276 (-345) µm, perithecial, globose to sub globose, soft, superficial, solitary to gregarious, surrounded by dark brown septate setae of 6-6.4 µm dia. Peridium 33-40 µm wide. *Hamathecium*: *Pseudoparaphyses* 2.2 µm wide, septate, branched, even, longer than asci. *Asci* 82.5-112.5 × 12.5-17.5 µm ($\bar{X}$= 94.2 × 14.6, n=25), bitunicate, 8-spored, clavate, apex rounded with an apical chamber when immature, at maturity it disappears, narrow towards base, short pedicellate. *Ascospores* 26.2-37.5 × 7.5-10 µm ($\bar{X}$= 30 × 8.2, n=25), biseriate, fusiform, 1 septate, often constricted at the septum and hyaline when young, pale brown or gray, collapsing, oblong with linear striations at maturity. **Anamorph:** Undetermined.

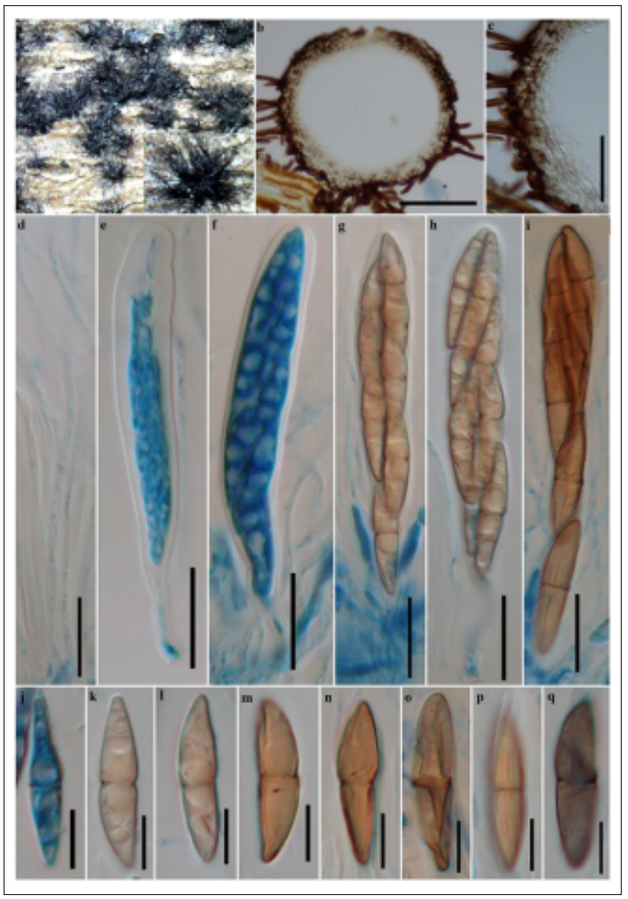
Distribution: India.

Material examined: INDIA, Andaman and Nicobar Islands, Middle Andaman, Bharatpur (12˚29'58"N 92˚52'53"E). Recorded on unidentified twig, February 3, 2016. M. Niranjan and V.V.Sarma (PUFNI 330).

Remarks: The family *Melanommataceae* has been revised and a key has been provided for both sexual and asexual morphs by Tian *et al.*(2015). *Bertiella* is similar to *Byssosphaeria* in having the superficial ascomata with long setae around the ascomata, but is distinct in having ostioles without a coloured tint unlike *Byssosphaeria* that has reddish-orange or greenish tint at pores. The genus *Bertiella* consists of five species (Almeida *et al.,*2017). Among the different species of *Bertiella*, the new taxon resembles *B. ellipsoidea* in having the long setae around the ascomata. The ascomata and ascospores of *B.striatispora* are larger, while asci are smaller in length and width when compared to *B. ellipsoidea*. Further, *Bertiella striatispora* also differs in possessing dense setae around the ascomata, fusiform ascospores with longitudinal striations that are brown at maturity. Hence a new species *B. striatispora* is introduced in the genus *Bertiella* based on the above mentioned morphological characteristics and differences (**Table1**).

Byssosphaeria jamaicana (Sivan.) M.E. Barr, *Mycotaxon* **20** (1): 30 (1984)**.** **Fig: 2. a-m**

Classification **-** *Melanommataceae*, *Pleosporales*, *Dothideomycetes*.



Figs: 1. ***Bertiella striatispora*** (PUFNI 330 Holotype)- **a.** Ascomata, **b.**Vertical section, **c.** Peridium, **d.** Pseudoparaphyses, **e-i.** Asci, **j-q.** Ascospores. Scale bars: **b**=100µm,**c=**50 µm, **d-i**= 20 µm, **j-q**= 10 µm.

Saprobic on unknown twig. **Teleomorph:** *Ascomata* 542-564 × 570-598 µm, perithecial, clusters on surface, superficial, sub globose, brown, coriaceous, ostiolated canal surrounded by a rinse of orange tissue, setae 5.1 µm, covered lateral side of ascomata. *Peridium* 80 µm wide, comprising thin brown textura prismatica cell layers, middle thick yellow and inner thin hyaline textura subglobosa and textura angularis cells, respectively. Hamathecium: *Pseudoparaphyses* filamentous, septate, unbranched, guttulated. *Asci* 100-110 × 8.2-9.0 µm, bitunicate, 8 spored, overlapping uniseriate at below, clavate, smooth, persistent, short pedicel. *Ascospores* 20.8-26.7 ×4.2-5.9 µm, ($\bar{X}$= 24.2 × 50, n=25), biseriate, with 1 median septum having a strong constriction, pale brown, apical cell wider than the basal cell, smooth-walled, straight to slightly curved with obtuse ends. **Anamorph:** Undetermined.

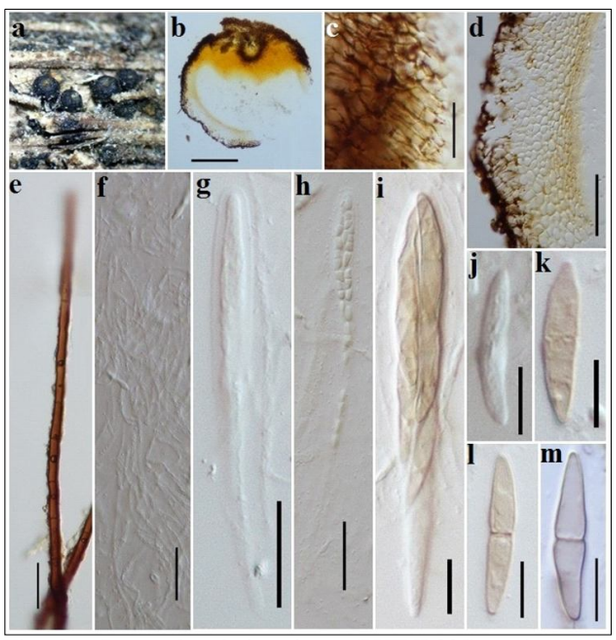
Distribution: Costa Rica. India, Panama and USA.

Material examined: INDIA, Andaman and Nicobar Islands, Middle Andaman, Nimbudera (12˚43'40"N 92˚53'1.9"E). Recorded on unidentified twig, February 3, 2016, M. Niranjan and V.V. Sarma **(**PUFNI 295).

Remarks: *Byssosphaeria* is a genus established by Cooke

Table: 1. Morphological characters of *Bertiella* species*.

	Ascomata		Asci		Ascospores				
Species	**Shape**	**Size** (µm)	**Shape**	**Size** (µm)	**Shape and colour**	**Septa**	**Sheath**	**Size** (µm)	**Reference**
B.striatispora	Subglobose	185–233 (–434) × 225–276 (345.3)	Clavate	82.5–112.5 × 12.5–17.5 µm	Ellipsoid, Brown	1	Absent	26.2–37.5 × 7.5–10	Present study
B. botryosa Morgan	Subglobose	200	Cylindric–clavate	100–110 × 11–14	Fusiform, Hyaline	1–3	Absent	20–30 × 5–6	Morgan (1904)
B. ellipsoidea Ekanayaka, Zhao & K.D. Hyde	Subglobose	200–250	Cylindric–clavate	112–160 × 10–12	Ellipsoid,Hyaline	1	Absent	14–18× 7-9.6	Hyde *et al.* (2016)
B. gelatinosa D.A.C. Almeida, Gusmão & A.N. Mill	Discoid to sub globose	200–550	Clavate	124–188 × 14–22	Fusiform, Hyaline	1	Present	42–55 × 6.5–9	Tian *et al.*(2015)
B. macrospora (Sacc.) Sacc . & Traverso	Subglobose	———	Cylindric–clavate	130–150 × 22	Fusiform, Hyaline	1	Absent	22–43 × 5–9	Eriksson and Yue (1986); Mugambi and Huhndorf (2009)
B. rhodospila (Berk. & M.A. Curtis) M.E. Barr.	Pyriform	190–440	Clavate	50–85 × 5 – 11	Fusiform, Hyaline	3	Absent	16–25 × 3–5	Barr*et al.* (1986)
* This table is modified from Tian *et al.*(2015) to include the present new taxon									



Figs: 2. ***Byssosphaeria jamaicana*** (PUFNI 295)**- a.** Ascomata on decaying host, **b.** Vertical section of ascomata, **c.**Textura prismatica, **d.**Textura subglobosa, **e.** Setae, **f.** Pseudoparaphyses, **g.-i.** Asci, **j.-m.** Ascospores. Scale bar: **b**= 200 µm, **d**= 50 µm, **e-h**=20 µm, **i-m**=10 µm.

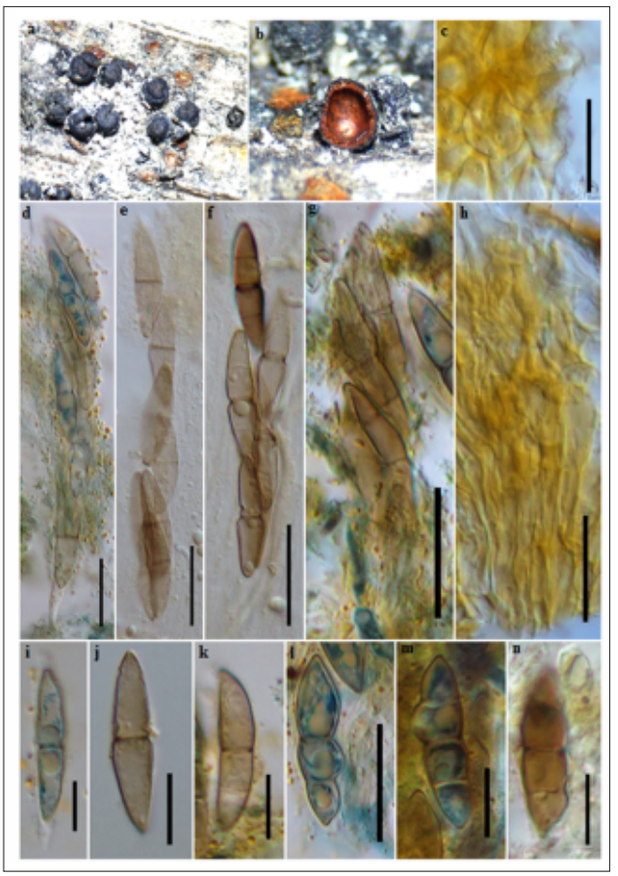
(1879). There are currently 25 species included in this genus (Wijayawardane *et al.,* 2017). This genus is characterized by superficial to semi-immersed, separate or gregarious ascomata, with rounded ostioles (Barr, 1990). The pore area may be pale to bright yellow, orange or red, and the surface may be irregular or slightly rough, with dependent hyphal appendages fusing with the subiculum below. The asci are cylindrical to claviform and the hamathecium is composed of trabeculate pseudoparaphyses. The ascospores are ellipsoidal to fusoid, symmetrical, hyaline to pale reddish or light brown, with one to several septa often with a mucilaginous sheath or appendages. The 'type' collection of *B. jamaicana* has larger asci and wider ascospores than our collection. This is the first report of this fungus from the Andaman Islands and India.

Byssosphaeria keithii (Berk. & Broome) Cooke, *Grevillea* (1879). **Figs: 3. a-l**

Classification *-Melanommataceae*, *Pleosporales*, *Dothideomycetes*.

Saprobic on unidentified twig. Teleomorph: *Ascomata* 448-517 × 418-535 µm ($\bar{X}$=484.3 x 473.8, n=5), perithecioid, superficial, mostly scattered, coriaceous, subiculated, subglobose to oblong or circular, brown, hyphal setae connected to lateral peridium of ascomata, flat at the base and apex, central ostiole collapsing. *Peridium* 77 µm wide, composed of outer black acellular coat, inner hyaline to pale yellow cells of textura subglobosa. Hamathecium: *Pseudoparaphyses* 1.8 µm wide, septate, unbranched. *Asci* 120-173.3 × 10.1-12.4 µm, ($\bar{X}$=143 × 10.9, n=25), bitunicate, 8-spored, clavate, having small rounded end with apical chamber, long pedicels, persistent. *Ascospores* 24.1-30.4 × 5.1-7.2 µm ($\bar{X}$=26.2 × 6.1, n=25), overlapping, triseriate, fusiform, initially hyaline becoming pale brown, 1septate with constriction, straight to

Figs: 3. ***Byssosphaeria keithii*** **(**PUFNI 17512**)- a.** Ascomata, **b.** Vertical section, **c.** Peridium, **d.** Pseudoparaphyses, **e.- f.** Asci, **h.** Hyphae and **g., i-l.** Ascospores. Scale bars: **b.=**100µm, **c-h.=**50µm, **d-f.** =20 µm and **g., I-l.=**10 µm.



Figs: 4. ***Byssosphaeria schiedermayeriana*** (PUFNI 17477 Holotype) **-a.** Ascomata, **b.**Vertical section, **c.** Textura angularis, **d-g.** asci, **h.** Pseudoparaphyses, **i-n.** ascospores. Scale bars: **c-h.,l.**= 20 µm, **i-k.,m.,**

slightly curved, fusiform, becoming obtuse, brown, 3septate with slight constrictions at maturity, with mucilaginous sheaths and appendages 2.2 × 2.5 µm in length. **Anamorph:** Undetermined.

Distribution: India and Sweden.

Material examined: INDIA, Andaman and Nicobar Islands, South Andaman, Wright mayo, (11˚47'36.0"N 92˚42'35.1"E). Recorded on unidentified twig, March 28, 2017, M. Niranjan and V.V. Sarma (PUFNI 17512).

Remarks: *Byssosphaeria* species possess 3 or 5 septate ascospores. The ascospores of *B. keithii* are similar in length to *B. xestothele*, but are 3 septate rather than one septate found in the latter. When compared to the type of *B. Keithii,* our record of *B. keithii* has smaller ascomata, larger asci and slightly smaller ascospores. The present report of *B. keithii* is the first from Andaman and Nicobar Islands, India.

Byssosphaeria schiedermayeriana (Fuckel) M.E. Barr, *Mycotaxon* **20** (1): 34 (1984). **Figs. 4 a-n**

Classification **-***Melanommataceae*, *Pleosporales*, *Dothideomycetes*.

Saprobic on an unidentified twig. **Teleomorph:** *Ascomata* superficial, single, sub globose with wide, cap-like structure closing the ascomata, subiculated on wood, coriaceous, outer thin carbonaceous, middle thick orange and inner hyaline layer of textura angularis cells. *Hamathecium*: *Pseudoparaphyses* septate, smaller than asci, uneven in width broad to narrow towards the end, 2.5-4.2 µm. *Asci* 75-100 × 12.5-15 µm ($\bar{X}$=99.16 × 13.75, n=10), unitunicate, 8-spored, overlapping uniseriate, clavate to cylindrical, short pedicel, deliquescent, with obtuse apical ends. *Ascospores* 27.5-37.5× 7.5-10.5 µm ($\bar{X}$=30.8 × 8.6, n=25), hyaline to pale brown, fusiform, usually with 1central septum with a strong constriction and rarely 3-septate with slight constrictions, acute ends, straight to slightly curved, smooth walled. **Anamorph:** Undetermined.

Distribution: Kenya, India, New Zealand, Germany and USA.

Material examined: INDIA, Andaman and Nicobar Islands, North Andaman, Diglipur, Kalighat (13˚13'43"N 92˚56'35"E). Recorded on unidentified twig, January 6, 2017, M. Niranjan and V.V. Sarma (PUFNI 17477).

Remarks: *Byssosphaeria* is characterised with superficial subiculated ascomata that are turbinate with a rounded pore

and apical area that is usually light coloured (Barr, 1984).Currently this genus has 25 species (Wijayawardene *et al.,* 2017). Our taxon closely resembled *Byssosphaeria schiedermayeriana* in asci and ascospore measurements with a slight variation in ascospore colour,which become dark brown at maturity. Earlier reports on this fungus show that the matured ascospores are pale-brown, have acute ends and mucilaginous sheaths and appendages. Our collection of *B. schiedermayeriana* has smaller ascomata and ascospores compared to the type of *B. schiedermayeriana* (Chen and Hsieh, 2004).

KEY TO *BERTIELLA* SPECIES

1. Ascomata covered with outwardly projecting, long setae.....2
1' Ascomata without setae and presence of hyaline ascospores…….....3
2. Ascospores brown with longitudinal striations ….....……***B.striatispora***
2' Ascospores hyaline ….....*B. ellipsoidea*
3. Ascospores surrounded by a gelatinous sheath.....*B. gelatinosa*
3' Ascospores lacking a sheath 4
4. Ascomata wall cephalothecioid.....*B. macrospora*
4' Ascomata wall not cephalothecioid.....5
5. Ascomata red at the apex ..... *B. rhodospila*
5' Ascomata black at the apex.....*B. botryosa*

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Noteworthy species of genus *Melanoleuca* (*Trichlomataceae, Agaricales*) from India

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ABSTRACT

Three species of genus *Melanoleuca* Pat. viz. *M. exscissa* (Fr.) Singer, *M. paedida* (Fr.) Kühner & Maire and *M. subalpina* (Britzelm.) Bresinsky & Stangl. were collected from Jammu and Kashmir, India and are taxonomically described. An identification key based upon the morphoanatomical characters is also provided. All the three species included in this paper are reported for the first time from India.

Key Words: *Melanoleuca*, amyloid, gelatinized pileus cuticle.

INTRODUCTION

Melanoleuca Pat. is a well defined genus of family *Trichlomataceae* falling under order *Agaricales* (Kirk *et al*., 2008) and suborder *Pluteineae* (Dentinger *et al.,* 2015). This genus is characterized in possessing a collybioid to tricholomatoid habit, color of the pileus varying in the shades of grey and brown: lamellae are mostly white to pale yellowish in color: adnexed, sinuate, adnate to subdeccurent in their attachment. Microscopically, the basidiospores are mostly ellipsoid, amyloid with prominent ornamentations: cheilocystidia and pleurocystidia are mostly lageniform to fusoid ventricose, sometimes with apical encrustrations and clamp connections are lacking in this genus (Pegler and Young, 1973; Singer, 1986; Boekhout, 1988, 1999; Bon, 1991; Vesterholt, 2008). Kirk *et al*. (2008) documented fifty species of this genus while the latest MycoBank record (http://www.mycobank.org/2018) lists 338 validly published species of *Melanoleuca*. From India this genus is represented by only four species (Upadhyay *et al*., 2017). Out of these, *M. melaleuca* (Pers.) Murrill was documented by Dhancholia *et al.* (1991) from Uttrakhand, *M. subrimosa* Murrill by Mohanan (2011) from Kerala and *M. subpulverulenta* (Pers.) Singer and *M. roseobrunnea* Murrill by Watling and Gregory (1980) from Jammu and Kashmir.

MATERIAL AND METHODS

The fungal forays were undertaken from time to time to various places in Kashmir during spring and summer for the collection of gilled mushrooms. These collections were worked out as per the standard methodology given by Atri *et al.,* (2005, 2017). The concepts of the classification and generic names followed are as given by Kirk *et al.,* (2008) and MycoBank (2018). The color terminology of Kornerup and Wanscher (1978) has been followed to describe the color of the various carpophores parts. Following Singer (1986) the basidiospore quotient (Q) was calculated by ratio of mean length divided by mean breadth of numerous basidiospores. The specimens were dried and deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (Punjab) India, under the Accession No. PUN.

TAXONOMIC OBSERVATIONS

The taxonomic descriptions of the presently investigated species of genus *Melanoleuca*, namely *M. exscissa* (Fr.) Singer, *M. paedida* (Fr.) Kühner & Maire and *M. subalpina* (Britzelm.) Bresinsky & Stangl are given in the ongoing account as per the sequence of their segregation in the identification key given below.

Key to the investigated species of the genus *Melanoleuca*

1 Stipe twisted rope like; basidia with siderophilous granules; gill edges sterile.....***M. exscissa***

1′ Stipe not twisted; basidia without siderophilous granules; gill edges heteromorphous.....**2**

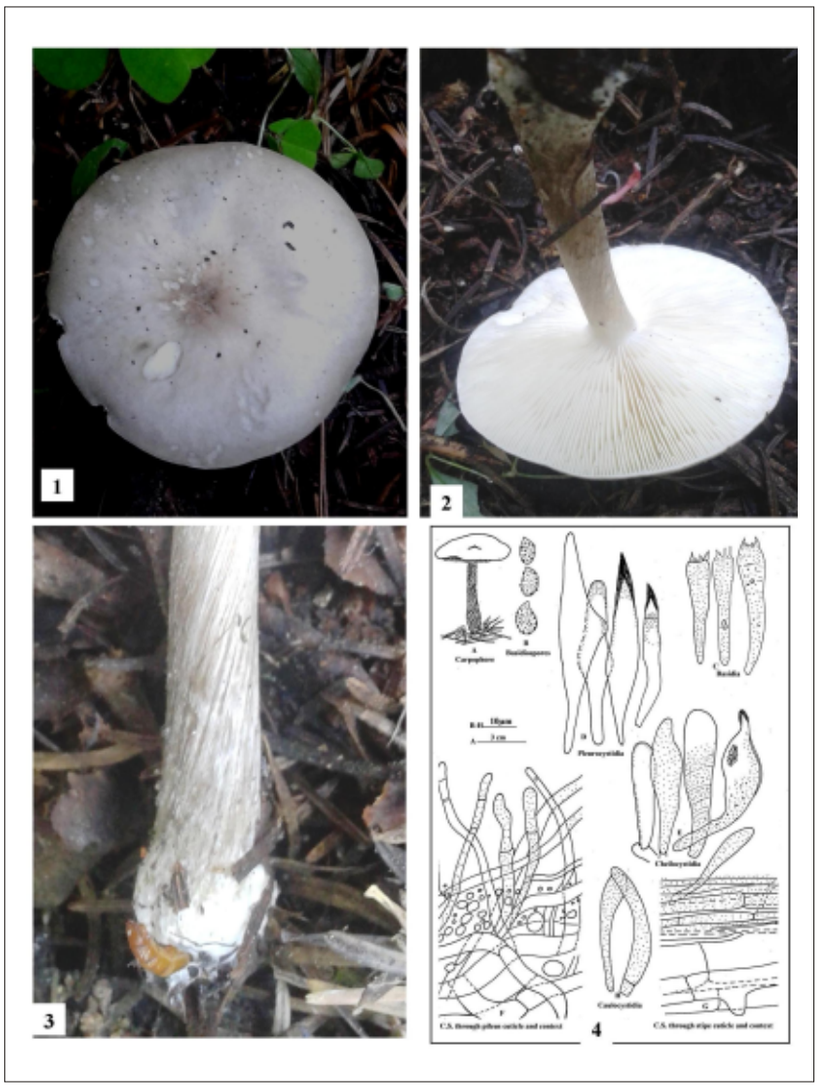
2 Sporophores growing in groups or scattered on *Picea* cones; cap applanate; cuticle fully peeling; pleurocystidia and cheilocystidia stinging hair like.....***M. paedida***

2′ Sporophores growing solitary or scattered on needles of *Pinus*; cap infundibuliform; cuticle half peeling; pleurocystidia and cheilocystidia lageniform to fusoid ventricose.....***M. subalpina***

Melanoleuca exscissa (Fr.) Singer, *Cavanillesia* **7**: 125, 1935. **Figs. 1-4**

Sporophores 4.8-8.0 cm in height. Pileus 3.0-5.0 cm broad, applanate, umbonate, umbo acute; surface grey ($20B_1$), reddish grey ($7B_2$) at the centre, dry; margin regular; cuticle fully peeling; flesh up to 0.2 cm broad, white, unchanging; taste and odor mild. Lamellae up to 0.5 cm broad, adnate to sub decurrent, crowded, unequal, white ($2A_1$), unchanging, lamellulae present in 1-4 lengths. Gill edges smooth. Stipe central, 5.0-7.0 cm long, 0.5-1.0 cm broad, tapering upwards with a bulbous base, twisted, rope like, white ($2A_1$) to grey ($7B_2$), unchanging, scaly, scales appressed fibrillose, white, hollow, exannulate.

Basidiospores 6.4-8.0 × 4.8-5.6 (6.4) µm (excluding ornamentation), Q = 1.3, broadly ellipsoid, rough walled, granular, ornamented, ornamentation verrucose 0.8-1.6 µm high; amyloid; apiculate, apiculus 0.83 - 1.6 µm long. Basidia 33.0-41.5 × 5.0-8.3 µm, clavate, granular, siderophilous granules present, tetrasterigmate; sterigmata 1.6-3.32 µm long. Pleurocystidia 49.0-75.0 × 8.3-13.28 µm, fusoid, ventricose to lageni form with pointed to broad inflated tips, apically incrusted, secondary septa present, abundant; cheilocystidia 33.2-50.0 × 5.81-11.6 µm, clavate to lageniform with rare apical incrustations, rarely clamped, thickly granular, not crowded. Hymenophoral trama regular. Gill edges sterile. Pileus cuticle hyphal, ixocutis made up of



Figs.1-4 ***M. exscissa*: 1.** Sporophore growing solitary on leaf litter of conifers, **2.** Underview of cap bearing white and crowded lamellae and twisted rope like stipe**. 3.** Twisted stipe with bulbous base **4.(A-H): A.** Sporophore, **B.** Basidiospores, **C.** Basidia, **D.** Pleurocystidia, **E.** Cheilocystidia, **F.** C.S. through pileus cuticle and context, **G.** C.S. through stipe cuticle and context, **H.** Caulocystidia**.**

horizontally tangled gelatinized septate hyphae giving rise to a regular turf of closely septate granular to hyaline projecting 3.3-5.6 µm broad hyphae with apical ends somewhat spathulate; context hyphal, made up of 6.4-12.8 µm broad, gelatinized, septate, irregularly placed, hyaline, inflated hyphae intermixed with 8.3-21.58 µm broad, hyaline cells. Stipe cuticle hyphal, ixocutis, made up of longitudinally tangled hyphae, giving rise to sparcely placed caulocystidia; caulocystidia 33.2-41.5 × 4.15-5.81 µm broad, clavate to cylindrical, granular; context hyphal made up of 8.3-25.0 µm broad, longitudinally placed, hyaline, septate hyphae.

Collection Examined: Jammu and Kashmir; Kulgam, Cherenbal Kounsernag (2600 m), $33^0$35.627′N $074^0$50.771E, growing solitary on leaf litter of conifers in coniferous forest, July 28, 2014, Nazir Ahmad Malik, PUN 9076.

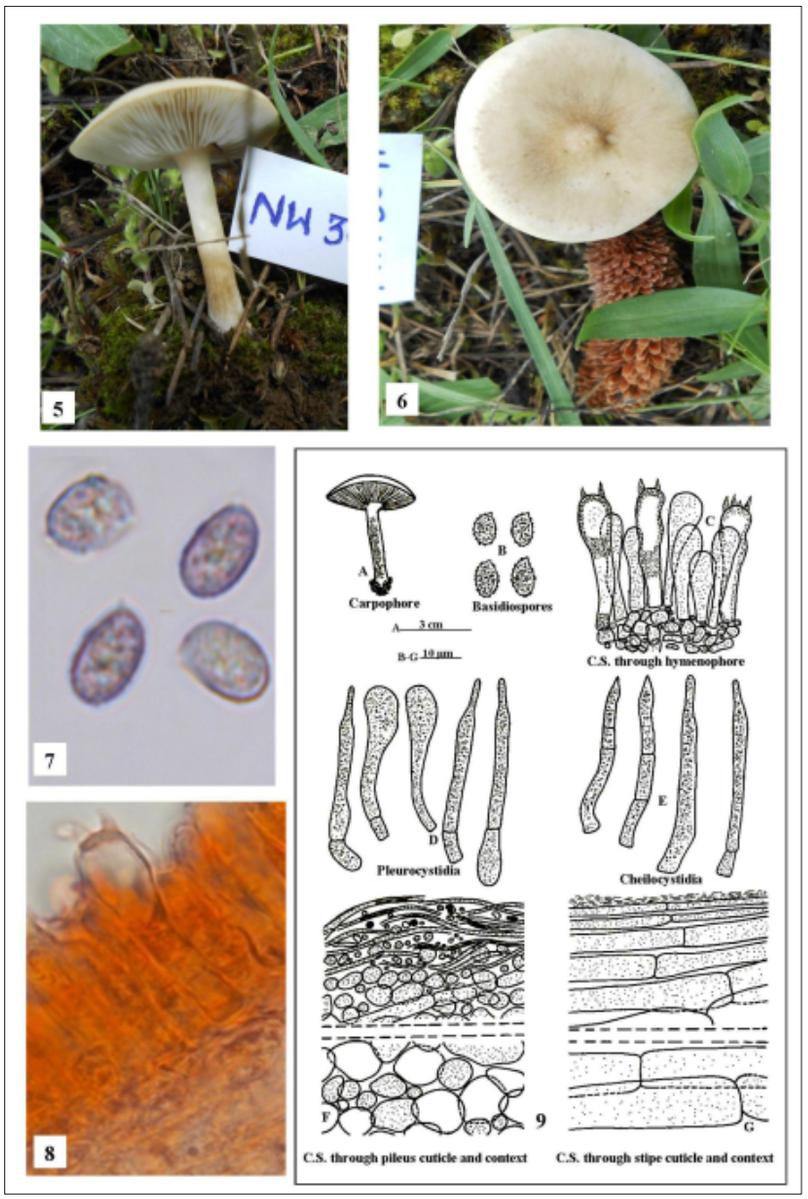
Distribution and Ecology: According to Breitenbach and Kränzlin (1991) *M. exscissa* is a rare species, distributed in Europe, commonly occurring during spring in pastures or along forest edges. Antonin *et al.* (2017) reported this species as widely distributed in Europe growing under *Pinus* and *Quercus* on sandy soil and on montane meadow or pastures in grass up to subalpine vegetation zone. During the present study this species was found growing solitary on leaf litter of conifers in coniferous forest of Kashmir Himalayas at 2600 m in the rainy season.

Remarks: All the morphanatomical characters of the presently examined collection match well with the description of *M. exscissa* as given by Breitenbach and Kränzlin (1991) and Antonin *et al.* (2017). This species is characterized in possessing grey ($20B_1$) to reddish grey ($7B_2$) cap with an acute umbo and hollow, twisted rope like stipe. The basidiospores are verrucose, amyloid and the pleurocystidia and cheilocystidia are fusoid ventricose, lageniform with apical encrustation and secondary septa. All the key features of this collection fits into the details provided by Breitenbach and Kränzlin (1991) for *M. exscissa*. This species is not earlier known from India thus is a new record.

Melanoleuca paedida (Fr.) Kühner & Maire, *Bulletin de la Société Mycologique de France* **50:** 18 (1934). **Figs. 5- 9**

Sporophore up to 4.5 cm in height. Pileus up to 3.8 cm broad, applanate, umbonate, umbo acute; margin irregular, non striate, splitting at maturity; surface greyish orange ($5B_3$) with brownish orange ($5C_4$) centre, moist; cuticle fully peeling; flesh up to 0.1 cm thick, creamy white, unchanging; odor mild. Pileal veil absent. Lamellae adnexed to notched, subdistant, forked towards the margins, unequal, not in series, broad (up to 0.2 cm), white ($10A_1$) to pale yellow ($4A_3$), unchanging; gill edges smooth, white; lamellulae present. Stipe central, up to 3.5 cm long, up to 0.5 cm broad above and 0.6 cm broad at the base, almost equal in diameter throughout; surface white to pale orange ($5A_3$), unchanging, hollow, scaly, scales fibrillose to floccose, exannulate.

Basidiospores 7.47 - 9.96 × 5.81- 6.64 µm Q = 1.3; ellipsoid, verrucose, warty, amyloid; apiculate, apiculus up to 0.83 µm long. Basidia 23.24 - 34.86 x 7.47 - 9.13 µm, clavate, granular, bisterigmate to tetrasterigmate; sterigmata 3.32-5.0 µm long, granular. Pleurocystidia 28.22-53.12 x 5.81-10.0 µm, claviform to stinging hair like with tubular apex, septate, thickly granular. Cheilocystidia 33.2-51.46 x 5.0-8.3 µm, stinging hair like with tubular apex, secondary septa present, thickly granular, not abundant, gill edges heteromorphous.



Figs.5-9 ***M. paedida***: ***5.*** Sporophore growing solitary in natural habitat, **6.** Carpophore with prominent umbo on the pileus surface, **7.** Microphotograph showing amyloid ornamented basidiospores, **8** Basidium, **9 (A-G) A.** Sporophore, **B.** Basidiospores, **C.** Basidia, **D.** Pleurocystidia, **E.** Cheilocystidia, **F.** C.S. through pileus cuticle and context, **G.** C.S. through stipe cuticle and context.

Hymenophoral trama regular. Pileus cuticle gelatinized, made up of sub-radially tangled, granular, 1.66 - 2.49 µm broad hyphae; pilocystidia absent; context made up of 4.98 - 14.94 µm broad, hyaline to granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged, 2.5 - 5.8 µm broad, septate hyphae; context hyphal, made up of, 6.64 - 15.0 µm broad, septate, hyphae. Clamp connections absent throughout.

Collection Examined: Jammu and Kashmir, Baramulla, Tangmarg (1850 m) 34^0 10.911′ N $074^0$21.955′ E growing in groups on *Picea* cones in the open coniferous forest, Naseema Aqbar Wani, PUN 9295, May 4, 2013.

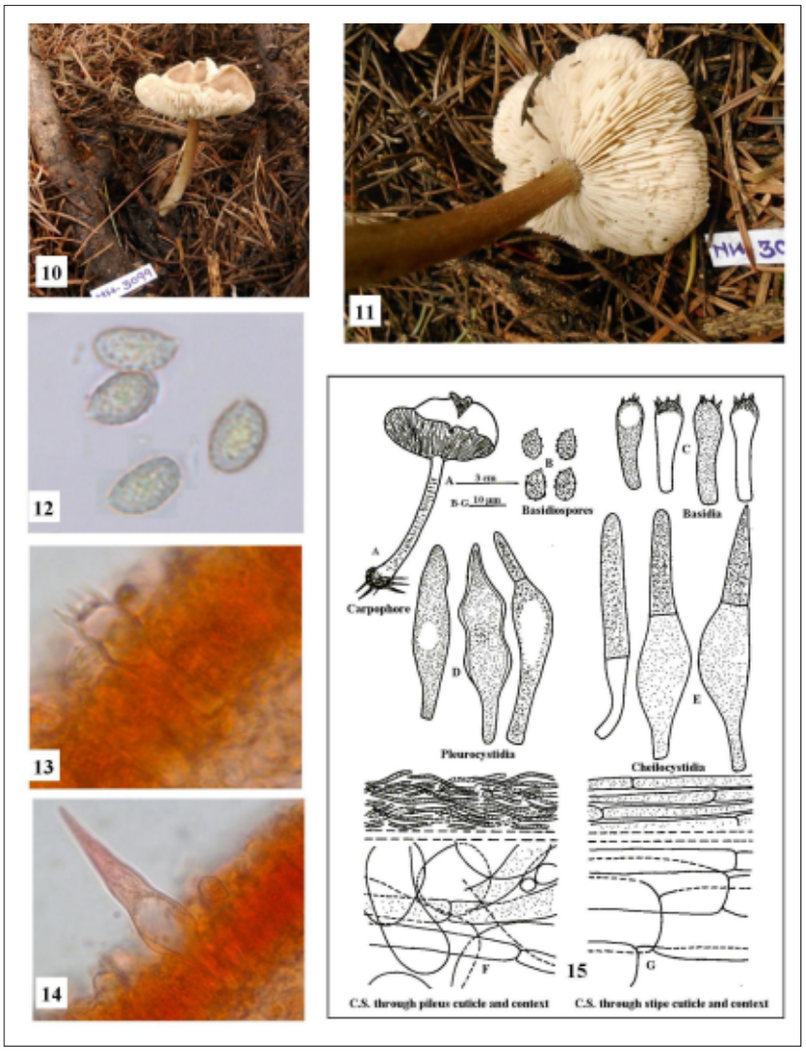
Distribution and Ecology: Breitenbach and Kränzlin (1991) collected *M. paedida* from Switzerland at an altitude of 550 m growing in forest meadows and cow pastures and from under *Picea* near the forest edge in the month of April during spring. The present collection has been found growing in groups on *Picea* cones scattered in open grasses near the forest edge in the month of May from Jammu and Kashmir.

Remarks: The details of the present collection match well with the description provided for *Melanoleuca paedida* by Breitenbach and Kränzlin (1991) and Kalmer *et al.* (2018). Spore size, carpophore morphology, secondary septa in cheilocystidia and pleurocystidia are in conformity but the encrustations as described for *M. paedida* by Breitenbach and Kränzlin (1991) are not very clear in the presently examined collection although these are densely granular. The present collection was documented growing scattered on cones of *Picea* in the open area. The only difference being in the gill edge color which in *M. paedida* is yellowish brown while this character is absent in our collection. Presently, this species has been recorded for the first time from India.

Melanoleuca subalpina (Britzelm.) Bresinsky & Stangl., *Beihefte zur Zeitschrift für Pilzkunde* **1**: 46 (1976). **Figs. 10-15**

Sporophore up to 8.5 cm in height. Pileus up to 5.5 cm broad, infundibuliform, undulating, umbonate, umbo obtuse, splitting near the umbo; margin irregular, non striate; splitting at maturity; surface pale orange ($5A_3$) to brownish orange ($5C_4$) with a darker greyish orange ($5B_4$) centre, shiny; cuticle half peeling; flesh up to 0.4 cm thick, white, unchanging; odor mild. Pileal veil absent. Lamellae adnexed to subdecurrent to somewhat notched, close, rarely forked towards the margins, unequal, not in series, broad (up to 0.5 cm), white ($10A_1$) to brownish orange ($5C_4$), unchanging, gill edges smooth, lamellulae present. Stipe central, up to 7.2 cm long, up to 0.5 cm broad above, up to 0.5 cm broad in the middle and up to 0.7 cm broad at the base, with a slightly bulbous base, surface light brown ($7D_5$), unchanging, hollow; scaly, scales fibrillose to floccose, white mycelial mat present at the base of the stipe, exannulate.

Basidiospores 7.47 - 9.13 × 4.15 - 5.81µm Q = 1.8, ellipsoid, verrucose, rough, with blunt tipped isolated warts, amyloid; apiculate, apiculus up to 0.83 µm long. Basidia 25.0-33.2 x 7.5-8.3 µm, clavate, granular, without basal clamps, tetrasterigmate; sterigmata 2.5-5.0 µm long, granular.



Figs. 10-15 ***M. subalpina*** **10.** Sporophore growing solitary to scattered on needles of *Pinus,* 11. Underview of cap showing adnexed to somewhat notched lamellae, 12. Microphotograph showing amyloid ornamented basidiospores, 13. Basidium, 14. Pleurocystidium fusoid ventricose with long tubular apex. 15(A-G) **A.** Carpophore, **B.** Basidiospores, **C.** Basidia, **D.** Pleurocystidia, **E.** Cheilocystidia, **F.** C.S. through pileus cuticle and context, **G.** C.S. through stipe cuticle and context.

Pleurocystidia 50.0 - 96.3 x 8.3-15.0 µm, lageniform to fusoid ventricose with long tubular apex, double walled, secondary septa observed in few, thickly granular in the upper portion, tips non encrustrated, abundant. Cheilocystidia 56.44 - 89.64 x 6.64-9.13 µm, similar to pleurocystidia; gill edges heteromorphous. Hymenophoral trama regular. Pileus cuticle gelatinized, made up of sub-radially tangled, granular, 1.66 - 2.49 µm broad hyphae; pilocystidia absent; context made up of 6.64-15.0 µm broad, septate, granular, hyphae intermingled with 4.15-35.0 µm broad hyaline to granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged, 3.32-5.81 µm broad, septate hyphae; context hyphal, made up of 6.64-3.24 µm broad, septate, hyphae. Clamp connections absent throughout.

Collection Examined: Jammu and Kashmir, Kupwara, Bangus Valley (2500 m) 34^0 16.550' N 074^{0}12.892 E, growing solitary to scattered on needles of *Pinus*, in coniferous forest, Naseema Aqbar Wani, PUN 9296, August 1, 2014.

Distribution and Ecology: *M. subalpina* was reported growing in an alpine pasture among grasses in the month of June from Pontresina by Breitenbach and Kränzlin (1991). The present Indian collection has been found growing solitary to scattered on needles of Pinus in coniferous forest in the month of August.

Remarks: The present collection matches well with the description provided for *M. subalpina* by Breitenbach and Kränzlin (1991). It is characterized by the presence of silky shining cap, with a broad dark umbo in the centre and undulate margin of the cap, visibly broader stipe base, notched forking gills especially near the margin; densely granular apex of cheilocystidia and pleurocystidia. But apical cap of crystals as described by Breitenbach and Kränzlin (1991) could not be seen. Further, the caulocystidia could not be located in the presently worked out collection may be due to the fact that the caulocystidia are reported to be present only in the apex of the stipe by Breitenbach and Kränzlin (1991). *M. subalpina* is recorded for the first time from India.

CONCLUSION

With the addition of three species of *Melanoleuca* documented in this manuscript, the total number of species reported from India has gone to seven.

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Biochemical basis of systemic acquired resistance in potato induced by different SAR elicitors in response to challenge inoculation of late blight pathogen

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ABSTRACT

The *Omycetes*, also known as "water molds", are a group of several hundred organisms that include some of the most devastating plant pathogens. Among oomycete pathogen; *Phytophthora infestans* causing late blight of potato is most important foliar pathogen, causing significant yield losses. The present study was conducted to reduce fungicide load and work out alternate method for control of this disease. Different SAR compounds were tested and foliar sprays of different conc. of Salicylic acid, Jasmonic acid and Bion (Benzothiadiazole-BTH) and β- amino butyric acid were given for inducing resistance in potato against late blight pathogen. Concentration of Salicylic acid, Jasmonic acid and Bion @ 500 µM, and β- amino butyric acid @ 50 mM gave best control of disease among all tested concentrations. Protein content of treated potato plants varied from 6.4 to 7.7 mg/g fresh weight compared to 4.0 mg/g fresh weight in control. Induction of proteins and defense enzymes was systemic in nature in response to all the four elicitors. The SAR compounds tested also stimulated the activities of pathogenesis related proteins (Pr- proteins) i.e. β-1,3 glucanase, Peroxidase (POD), and defense related proteins i.e. Polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL) from 26 to 99 % indicating induced resistance in treated potato plants as compared to control. Electrophoretic protein profiling of treated potato plants also confirmed the induction of pathogenesis-related proteins ranging from 15- 75 kDa along with some other proteins. Total chlorophyll and carotenoids also showed spike of 1% to 100 % in response to elicitors. Salicylic acid gave best results showing 77.7 % disease control followed by Jasmonic acid showing 75.1% while Bion and Beta amino butyric acid gave 69 % disease control as compared to control plants. Thus integration of disease tolerance and salicylic acid spray schedule resulted in effective, eco-friendly as well as economical control of late blight of potato.

KEYWORDS: Potato, systemic acquired resistance, salicylic acid, Jasmonic acid, β-amino butyric acids (BABA), Benzothiadiazole (BTH).

INTRODUCTION

Potato (*Solanum tuberosum* L.*)* is fourth most important food crop of the world after wheat, rice and corn. Late blight caused by the *Phytophthora infestans* (Mont.) de Bary, is the major decimator of potato cultivation costing over 12 billion USD losses worldwide (Haverkort *et al.,* 2008). Late blight has tremendous potential to cause up to 80% reduction in the yield in susceptible varieties of potato. All the commercial varieties of potato cultivated in Punjab state of India are moderate to highly susceptible to late blight. Moreover, host resistance to *P. infestans* is not generally stable due to development of new races of the pathogen. Therefore, fungicides play an important role in management of this disease. However, a strain of *P. infestans* resistant to fungicide metalaxyl was reported in Europe in 1981, in Canada and the US in the 1990s and thereafter worldwide; this resulted in the loss of effective late blight disease control, especially against tuber blight (Dowley and O'sullivan, 1991). Similarly, in India, Metalaxyl resistant strains of *P. infestans* were also reported (Kaur *et al.*, 2010). To date, Mancozeb and Ridomil gold fungicides are commonly used to control potato late blight in India.

Application of signaling molecules i.e. Jasmonic acid (JA), Salicylic acid (SA) and β-Amino butyric acid (BABA), etc. is a new promising way of disease management. These molecules are reported to induce systemic acquired resistance (SAR) against pathogens in many crops by activating genes encoding PR-proteins like β -1,3-glucanase (PR-2), chitinase (PR-3), peroxidase (PR-9) and several other stress related proteins (Enkerli *et al.*, 1993). According to Durrant and Dong (2004) Systemic Acquired Resistance is a mechanism of induced defense that vest in long lasting protection against broad spectrum of pathogens. Walters and Fountaine (2009) and Pieterse *et al* (2009) asserted that, at least three types of systemic acquired resistance is known, which is shown to be effective against biotrophic fungi and oomycetes: systemic acquired resistance (SAR), induced systemic resistance (ISR) and β-amino butyric acid induced resistance (BABA-IR). SAR can be induced by treatment with various agents, such as acibenzolar-S-methyl (ASM), a photostable functional analogue of salicylic acid (SA) that is associated with the accumulation of SA and pathogenesis related (PR) proteins, and is dependent on the regulatory protein NPR1 (non-expressor of PR-gene 1) (Durrant and Dong, 2004). Exogenous application of SA, or of its functional analogues 2, 6-dichloroisonicotinic acid (INA) and ASM, can activate PR gene expression and resistance in plants without pathogen inoculation (Van Loon *et al.*, 2006). Many biochemical, cytological and molecular changes are associated with SAR in plants that are systemically protected against pathogens. Among these, cell-wall strengthening, the production of phytoalexins and PR proteins, the hypersensitive reaction (HR), and callose deposition is associated with BABA-induced resistance to *Helminthosporium parasitica* in *Arabidopsis thaliana* (Zimmerli *et al.*, 2000). In view of these observations, the present study was conducted to test application of SAR elicitors Jasmonic acid (JA), Salicylic acid (SA), Benzothiadiazole (BTH) and β-Amino butyric acid (BABA) to enhance plant defense to control late blight disease and reduce fungicide load on potato crop. .

MATERIAL AND METHODS

Potato cultivars/hybrids: Three varieties of potato i.e. Kufri Badshah (moderately tolerant), Kufri Jyoti (tolerant) and Kufri Pukhraj (susceptible) were procured from department of vegetables, Punjab Agricultural University, Ludhiana and used for the study.

Sowing of crop and testing of different doses of elicitors: The selected varieties were raised in rows and replicated thrice using standard package of practices in month of October with plot size of 2m X 3m. Standardization of concentration of Jasmonic acid (JA), Salicylic acid (SA), Benzothiadiazole (BTH) and β- amino butyric acids (BABA) for the induction and over expression of proteins in 30 plants (10 X 3 replication) of each variety i.e. tolerant (Kufri Jyoti) and susceptible (Kufri Pukhraj) cultivars of potato was done. Different concentrations of each elicitor tried as spray (prepared in 500 ml double distilled water) are as follows:

1. Jasmonic acid of 50µM, 250µM, 500µM, 1000 µM,
2. Salicylic acid of 50 µM, 250 µM, 500 µM,1000 µM,
3. Bion (Benzothiadiazole) of 50 µM, 250 µM, 500 µM,1000 µM, and
4. β-amino butyric acid of 20 mM, 30mM, 50 mM, 100mM

Three-week-old sprouts were sprayed using an atomizer. Water sprayed plants of corresponding varieties were kept as untreated control.

Collection of plant tissue samples: Leaf sampling was done after 24, 48, 72, 96, 120, 144 hrs and at weekly intervals after elicitors spray. Samples were brought to laboratory in ice packs and stored at -80°C to prevent denaturation of proteins.

Estimation of total soluble proteins: Leaf blade tissue excluding veins (0.5 g) was weighed and homogenized in 25 mM Tris HCl buffer (pH 8.0) in a precooled pestle and mortar on the ice bath and centrifuged at 10,000 rpm for 25 minutes at 4^0C. Supernatant was used for protein estimation (Lowry *et al.*, 1951). Bovine serum albumin (BSA) standards (20-100 g) were run along with the test samples and the concentration of protein was calculated from the standard curve of BSA and expressed as mg/g fresh weight of tissue. Tissue was sampled from at least three leaves.

Protein profiling: Protein profiling by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Walker, 1996) was done for leaves of all the three varieties sprayed with 500 µM of SA, JA, BTH and 50 mM of BABA. Standard protein ladder with molecular weights ranging from 6-180 kDa was also run. Gels were stained with Comassie brilliant blue for visualizing changes in bands in the range of 6-50 kDa and compared with their respective control.

Extraction and estimation of enzymes: One hundred mg of leaf tissue from each plant was crushed in a pre chilled pestle and mortar with 2 mL 0.1M sodium phosphate buffer (pH 7.5) containing 10 mM 2-mercaptoethanol in the presence of 1% polyvinyl-pyrrolidone (PVP). The homogenate was centrifuged at 13,000 g at 4^0C for 30 min (Eppendorf 5804R) and clear supernatant was used for estimating peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity. Standard procedures of Claiborne and Fridovich (1979), Burrell and Rees (1974), and Zauberman *et al* (1991) were employed for the estimation of Peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, respectively. For estimation of -1, 3 glucanase, Dinitrosalicylic acid (DNSA) was used as reagent. Reddish brown colour developed was measured at 575 nm. The enzyme activity has been expressed as µg of glucose released/min/g FW (Kauffmann *et al.,* 1987)

Estimation of chlorophylls and carotenoids: For estimation of chlorophylls, 0.2 g of leaf sample was taken and to this added 1 mL of Dimethyl Sulfoxide (DMSO). This solution was kept overnight for colour development. The optical density was measured at 649 nm and 665 nm (Barnes *et al.,* 1992). The amount of carotenoids was estimated by the method of Kirk and Allen (1965).

Preparation of sporangial suspension for challenge inoculation: Fresh sporulations of *P. infestans* sporangial solution at conc. of approx. 4.0 x 10^4 sporangia per mL were prepared by dislodging sporangia from sporulating leaves in double distilled water and used for challenge inoculations in potato experiments.

Determination of disease severity: Elicitors were sprayed after 21 days of sowing. After 2 days of elicitor spray pathogen inoculations with *P. infestans* sporangial solution at conc. of 4.0 x 10^4 sporangia per mL was done using an atomizer to create disease. High relative humidity was maintained for next 72 hrs by spraying water. Observations on disease severity were recorded using the scale given by Thind and Mohan (1998). Per cent Disease Index was calculated using following expression:

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of numerical rating}}{\text{Total no of samples x Maximum of rating scale}} \times 100$$

Per cent disease incidence and disease severity was observed from 7 to 14 days post inoculation. The leaves were collected for seven consecutive days after inoculation and analyzed for various biochemical estimations. The statistically significant difference (CD 5%) in biochemical parameters in response to foliar spray of SA, JA, BABA and BTH on three varieties was calculated using CRD factorial analysis.

RESULTS AND DISCUSSION

Total Protein: The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to selected elicitors and pathogen inoculation revealed statistically significant difference. JA, SA and BTH i.e., at 500 µM and BABA at 50 mM was sprayed after 21days of planting followed by challenge inoculation of pathogen. These elicitors were applied on three different varieties of potato, namely Kufri Badshah, Kufri Jyoti, Kufri Pukhraj **(Table 1)**. Mean maximum protein induction was observed at 500 µM of SA in Kufri Jyoti i.e. 7.7 mg/g FW followed by 7.3 mg/g FW in Kufri Badshah and 6.9 mg/g FW in Kufri Pukhraj. JA at 500 µM proved to be second best treatment with mean maximum protein induction observed in Kufri Jyoti i.e. 7.1 mg/g FW followed by 7.0 mg/g FW in Kufri Badshah and by 7 mg/g FW in Kufri Pukhraj. BTH at 500 µM gave mean maximum protein induction of 6.5 and 6.7 mg/g FW in Kufri Badshah, and Kufri Jyoti, whereas 6.4 mg/g FW in Kufri Pukhraj. BABA gave mean maximum protein induction of 6.8 mg/g FW at 50mM of concentration in Kufri Jyoti and 6.7 and 6.5 mg/g FW in Kufri Badshah, Kufri Pukhraj, respectively.

B-1, 3 glucanase activity: The data pertaining to changes in β-

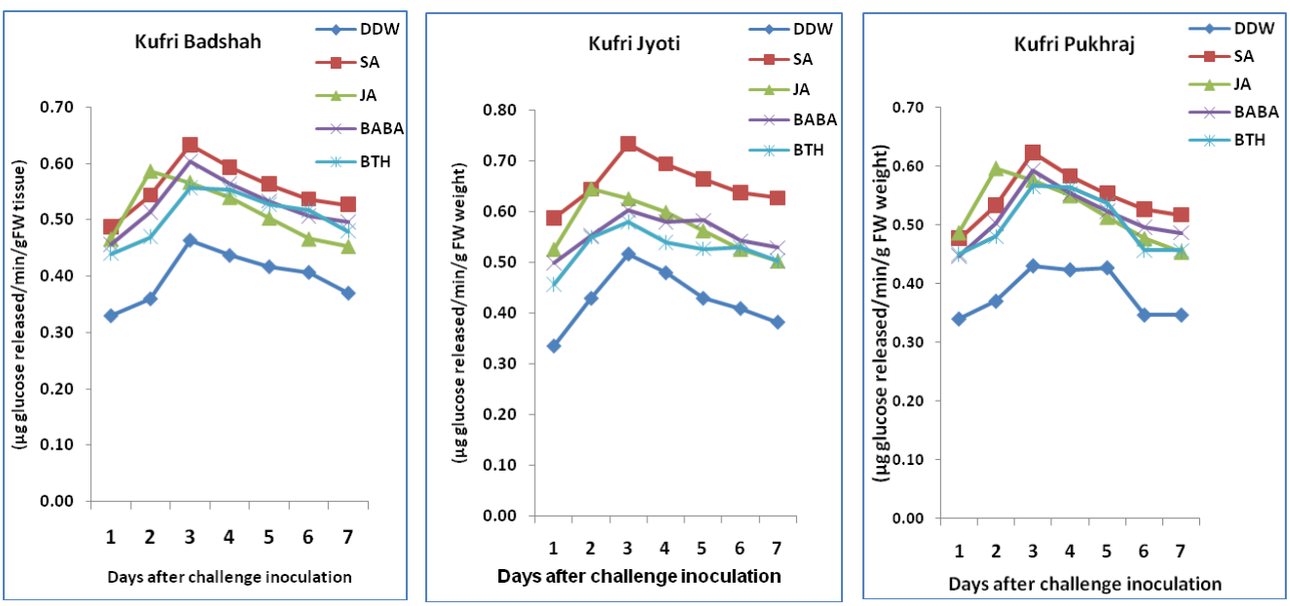
Table 1: Leaf protein (mg/gFW) induction studies in response to elicitors in potato varieties challenge inoculated with *P. infestans*.

Total protein content (mg /g FW)									
		Days after challenge inoculation							
Variety	Treatment	1	2	3	4	5	6	7	Mean
Kufri Badshah	Control	3.4	3.8	5.2	4.4	4.4	3.7	3.6	4.1
	SA (500µM)	6.4	6.8	8.4	8.2	7.8	7.2	6.6	7.3
	JA(500 µM)	6.4	6.8	7.8	7.4	7.2	6.8	6.5	7.0
	BABA(50mM)	6.0	6.5	7.5	7.1	6.8	6.5	6.3	6.7
	BTH (500µM)	6.0	6.4	7.5	6.9	6.6	6.3	6.1	6.5
Kufri Jyoti	Control	3.7	4.1	5.5	4.8	4.4	4.2	3.9	4.4
	SA (500µM)	6.6	7.3	8.7	8.5	8.1	7.8	6.8	7.7
	JA(500 µM)	6.4	6.8	8.1	7.6	7.2	6.8	6.5	7.1
	BABA(50mM)	6.2	6.7	7.7	7.3	7.0	6.6	6.5	6.8
	BTH (500µM)	6.1	6.5	7.6	7.1	6.8	6.5	6.3	6.7
Kufri Pukhraj	Control	3.5	3.9	4.8	4.3	4.1	3.9	3.8	4.0
	SA (500µM)	6.0	6.7	7.8	7.5	7.2	6.9	6.5	6.9
	JA(500 µM)	6.2	7.3	7.7	7.5	7.0	6.6	6.4	7.0
	BABA(50mM)	5.9	6.3	7.3	6.9	6.7	6.4	6.3	6.5
	BTH (500µM)	5.8	6.2	7.3	6.8	6.6	6.2	6.0	6.4
CD (5%)	Variety (A)-0.022; Elicitor (B)- 0.029; Time interval (C)-0.035 ; AB- 0.051; AC-0.060; BC-0.078; ABC- 0.135								

1, 3 glucanase activity recorded at periodical interval in combined response to elicitors and challenge inoculations of pathogen revealed statistically significant difference. SA caused 37 % increase in β-1, 3 glucanase activity in leaves, whereas JA and BTH resulted in 27%, BABA gave 30% increase in β-1, 3 glucanase activity w.r.t control in Kufri Badshah. Similarly, in Kufri Jyoti SA caused 53 % increase in β-1, 3 glucanase activity in leaves, whereas JA and BABA resulted in almost 32%, and BTH gave 23% increase in β-1, 3 glucanase activity w.r.t control. In Kufri Pukhraj SA caused 42% increase in β-1, 3 glucanase activity in leaves, whereas JA resulted in 37%, BABA gave 34% and BTH gave 31% increase in β-1, 3 glucanase activity w.r.t control indicating that SA is better inducer of β-1, 3 glucanase activity among all the four elicitors with maximum β-1, 3 glucanase activity in Kufri Jyoti (**Fig. 1**). The total β-1, 3 glucanase activity increased up to 3rd day in all three elicitors except JA where peak value was observed on second day of spray and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM SA resulted in higher mean β-1, 3 glucanase activity followed by JA followed by BABA and then BTH. Kufri Jyoti showed better β-1, 3-glucanase activity followed by Kufri Badshah and Kufri Pukhraj. BABA and BTH treatments were at par w.r.t. β-1, 3 glucanase activity in all the three varieties.

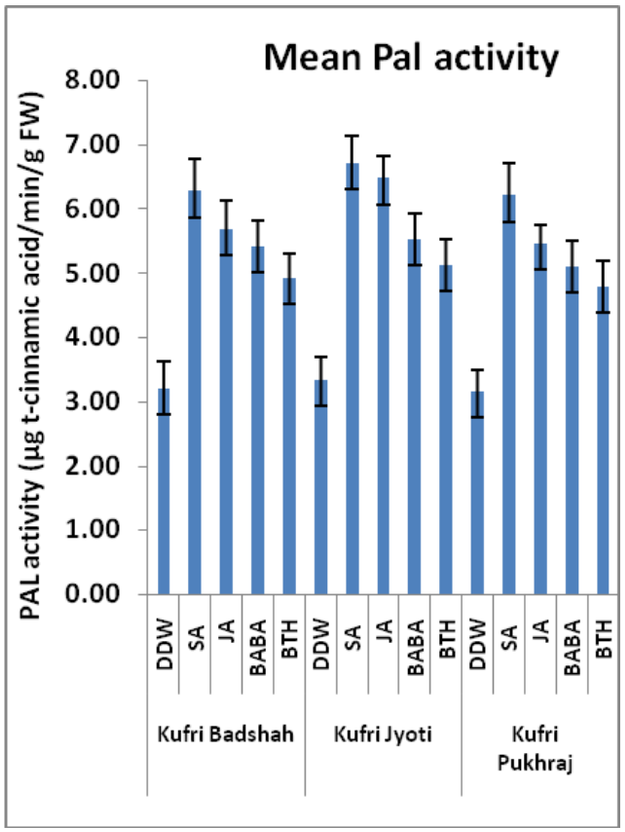
Phenylalanine ammonia lyase (PAL): The data pertaining to changes in Phenylalanine ammonia lyase (PAL) activity recorded at periodical interval of 24 hrs till a week in response to selected best doses revealed statistically significant difference. SA showed the spike of 96 % in PAL activity in leaves, whereas JA resulted in 78%, BABA gave 69% and BTH gave 54% increase in PAL activity w.r.t control in Kufri Badshah. Similarly, in Kufri Jyoti SA caused 102% increase in PAL activity in leaves, whereas JA resulted in 94%, BABA gave 65% and BTH gave 54% increase in PAL activity w.r.t control. In Kufri Pukhraj SA caused 102% increase in PAL activity in leaves, whereas JA resulted in 78 %, BABA gave 67% and BTH gave 56% increase PAL activity w.r.t control indicating that SA is better inducer of PAL activity among all the four elicitors with maximum PAL activity in Kufri Jyoti. The total PAL activity increased up to 4th day in all four elicitors and thereafter registered decline irrespective of variety and elicitor treatment (**Fig. 2**).

Peroxidase (POD): The data pertaining to changes in peroxidase (POD) activity revealed that in Kufri Badshah; SA caused 57 % increase in peroxidase activity in leaves, whereas JA resulted in 42%, BABA gave 34% and BTH gave 32% increase in peroxidase activity w.r.t control. Similarly, in Kufri Jyoti SA caused 107% increase in peroxidase activity in



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 1 Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on -1, 3-glucanases activity (g glucose released/min/ gFW weight) in leaves of different potato varieties challenge inoculated with *P. infestans*.



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 2 : Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PAL activity (µg t-cinnamic acid/min/g FW) in leaves of different potato varieties challenge inoculated with *P. infestans*

leaves, whereas JA resulted in 81%, BABA gave 65% and BTH gave 53% increase in peroxidase activity w.r.t control. In Kufri Pukhraj SA caused 75% increase in peroxidase activity in leaves, whereas JA resulted in 69%, BABA gave 59% and BTH gave 52% increase in peroxidase activity w.r.t control indicating that SA is better inducer of peroxidase activity among all the four elicitors with maximum peroxidase activity in Kufri Jyoti (**Table 2**).

Table 2: Effect of foliar spray of SA, JA, BABA and BTH on peroxidase activity (ΔA /min/g FW) in leaves of potato varieties challenge inoculated with *P. infestans*

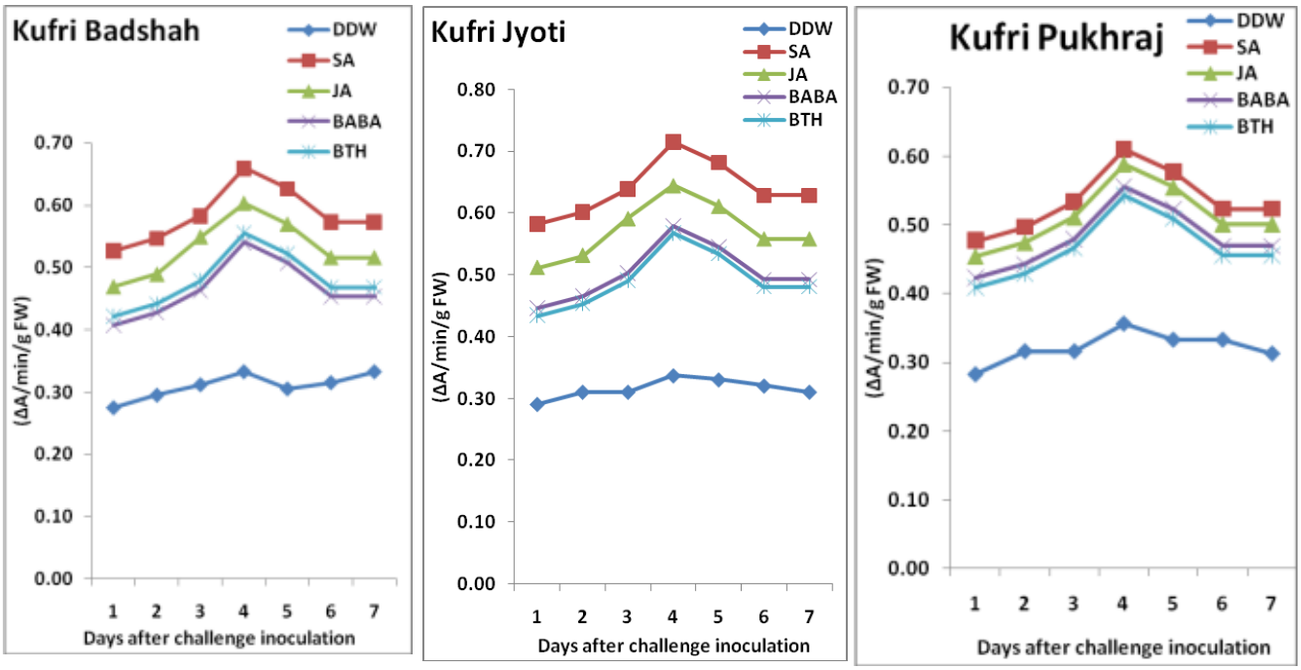
Peroxidase activity (ΔA /min/g FW)									
Variety	Treatment	Days after challenge inoculation							Mean
		1	2	3	4	5	6	7	
Kufri Badshah	Control	21.40	24.93	37.76	34.27	31.90	29.16	28.27	29.67
	SA(500µM)	37.70	42.74	54.94	52.74	49.20	45.54	44.97	46.83
	JA(500µM)	34.20	38.27	49.10	46.30	44.14	42.47	41.14	42.23
	BABA(50m)	32.50	36.37	47.90	43.77	40.87	40.14	37.94	39.93
	BTH (500µM)	32.67	35.97	46.77	43.77	40.94	37.94	37.87	39.42
Kufri Jyoti	Control	21.67	22.37	31.80	29.54	27.40	27.01	24.97	26.39
	SA (500µM)	45.49	50.53	62.73	60.53	56.99	53.33	52.76	54.62
	JA(500 µM)	39.00	43.07	53.90	51.10	48.94	47.27	45.94	47.03
	BABA(50m)	35.39	39.26	50.79	46.66	43.76	43.03	40.83	42.82
	BTH (500µM)	33.27	36.64	47.44	44.44	41.61	38.61	38.54	40.08
Kufri Pukhraj	Control	22.54	23.40	23.90	25.37	26.27	27.10	27.97	25.22
	SA (500µM)	34.80	39.84	52.88	50.48	46.71	42.64	42.07	44.20
	JA(500 µM)	34.70	38.77	49.60	46.80	44.64	42.97	41.64	42.73
	BABA(50m)	32.40	36.-27	47.80	43.67	40.77	40.71	38.35	40.00
	BTH (500µM)	31.52	34.82	45.62	42.62	39.79	37.01	37.31	38.38
CD (5%)	Variety (A)-0.16; Elicitor (B)- 0.21; Time interval (C)-0.25 ; AB- 0.37; AC-0.44; BC-0.57; ABC- 1.01								

Polyphenol oxidase (PPO): The data pertaining to changes in Polyphenol oxidase (PPO) activity revealed that amongst the different elicitors treatment in Kufri Badshah; SA caused 74 % increase in PPO activity in leaves, whereas JA resulted in 77%, BABA gave 53% and BTH gave 60% increase in PPO activity w.r.t control. Similarly, in Kufri Jyoti SA caused 100% increase in PPO activity in leaves, whereas JA resulted in 78%, BABA gave 56% and BTH gave 53% increase in PPO activity w.r.t control (**Fig 3**). Similar pattern was observed in Kufri Pukhraj as well. Statistically significant difference for all four elicitors w.r.t. time interval was observed. SA was found to induce maximum PPO activity 0.72 ΔA /min/g FW at 4TH day interval, respectively followed by JA showing maximum PPO activity 0.65 ΔA /min/g FW at 4TH day interval. BABA and BTH showed maximum PPO activity 0.58 and 0.57 ΔA /min/g FW at 4TH day interval in Kufri Jyoti. Whereas minimum PPO activity varied from 0.29 to 0.34 ΔA /min/g FW for control treatment between all the respective time intervals with mean value of 0.32 ΔA /min/g FW.

Raut and Borkar (2014) from their studies also reported that application of SA via seed dip treatment and seedling dip treatment elicited increase in chitinase and β-1, 3-glucanase activity in susceptible tomato cultivars and induced resistance to *Alternaria* leaf blight. Rajab *et al* (2009) showed prominent increase in the PAL activity when calli of *Sesamum prostratum* were challenged with *Fusarium oxysporum* f. *sesame* crude toxin metabolite of varying concentrations. The activated PAL activity leads to production of phenolics, which are further converted into more reactive species by phenol oxidases and peroxidases (Heath, 2000). ASM significantly induced β-1,3-glucanase activity which increased with time in inoculated seedlings, as confirmed by the presence of PR-2. Analysis of three other acidic (PR-1C, PR-5S, PR-8) and one basic (PR-6) PR proteins in the ASM-treated seedlings showed that only PR-1 and PR-5 were slightly and slowly induced (4-5 days after treatment), but this induction was more pronounced after inoculation with *P. parasitica* (Ziadi *et al.,* 2001).

The maximum increases in chitinase, β-1, 3-glucanase, PO and PPO activity occurred in root and shoot tissue from Bion® seed treated material inoculated with fungus (Whan *et al.,* 2008).

Kim and Hwang (2014) also observed that pepper plants showing high activity of PAL enzyme were resistant to the infection of *Xanthomonas campestris* pv. *vesicatoria.* The expression of genes encoding hydrolytic enzymes such as chitinases and β-1,3-glucanases that degrade the cell wall of microbes and may



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 3 Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PPO activity (ΔA/min/g FW) in leaves of different potato varieties challenge inoculated with *P. infestans*

be involved in the release of elicitor molecules and the synthesis of pathogenesis related proteins (PR proteins). These responses might trigger in the whole plant a long-lasting systemic acquired resistance (SAR) which is effective against a large spectrum of pathogens (Ricci, 1997).

Present study reports that potato plants showed increase in the activities of defense related enzymes after treatment with different elicitors as compared to control. Additional enhancement in the activities was observed upon inoculation with pathogen. The biochemical defence response was better in response to combined exposure of elicitors followed by pathogen, as plant gets primed with elicitor spray. SAR plant defense does not always get directly activated upon first exposure to stimulus instead may need priming and is often associated with a faster and stronger induction of the plant defence on subsequent exposure to abiotic and/or biotic stress (Conrath, 2011). Elicitors alone without challenge inoculation of *P. infestans* primed the plants but biochemical activity showed higher response with combined effect of elicitor plus pathogen (**Table 3**). Of the various elicitors used, SA followed by JA, BABA and BTH were found to be more effective in eliciting the level of enzymes which play an important role in defense; in potato against late blight pathogen.

Total chlorophyll: The data pertaining to changes in leaf total chlorophyll in response to combined effect of elicitor followed by challenge inoculation with pathogen revealed statistically significant difference amongst the various elicitors applied on three different varieties of potato (**Table 4**). Amongst the different elicitors treatment in Kufri Badshah; SA caused 13% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3.5% increase, BABA gave 11% and BTH gave 10% increase in total chlorophyll (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA caused 17% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3% increase, BABA gave 16% and BTH gave 12% increase in total chlorophyll (mg/ g FW) w.r.t control. In Kufri Pukhraj SA caused 16% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in increase of only 2%, BABA gave 14% and BTH gave 13% increase in total chlorophyll (mg/ g FW) w.r.t control indicating that SA is better inducer of total chlorophyll among all the four elicitors with

Table 3. Comparison of Biochemical activities in response to SA, JA, BABA and BTH in leaves of potato varieties; with and without challenge inoculation of pathogen.

Potato cultivar	Elicitor	Total proteins (mg /g FW)		β-1, 3 glucanase (µg glucose released/min/g FW)		PAL (µg t-cinnamic acid/min/g FW)		Peroxidase activity (ΔA /min/gFW)		Polyphenol oxidase (ΔA/min/g FW)	
		Data is mean of seven readings taken at 24 hours interval upto a week post challenge inoculation									
		Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)
Kufri jyoti (tolerant)	Control	3.7	4.4	0.35	0.43	2.54	3.34	18.50	26.39	0.25	0.32
	SA	7.3	7.7	0.55	0.66	5.64	6.70	41.96	54.62	0.46	0.64
	JA	7.0	7.1	0.47	0.57	4.75	6.47	36.26	47.03	0.47	0.57
	BABA	6.0	6.8	0.44	0.56	4.72	5.51	33.26	42.82	0.43	0.50
	BTH	5.2	6.7	0.42	0.53	4.33	5.12	32.10	40.08	0.42	0.49
Kufri Pukhraj (susceptible)	Control	3.4	4.0	0.30	0.38	2.27	3.16	17.45	25.22	0.25	0.32
	SA	6.4	6.9	0.47	0.54	5.41	6.20	36.16	44.20	0.44	0.53
	JA	6.0	7.0	0.44	0.52	4.66	5.45	34.96	42.73	0.42	0.51
	BABA	4.9	6.5	0.43	0.51	4.31	5.10	32.06	40.00	0.41	0.48
	BTH	4.6	6.4	0.44	0.50	4.00	4.79	30.50	38.38	0.40	0.47

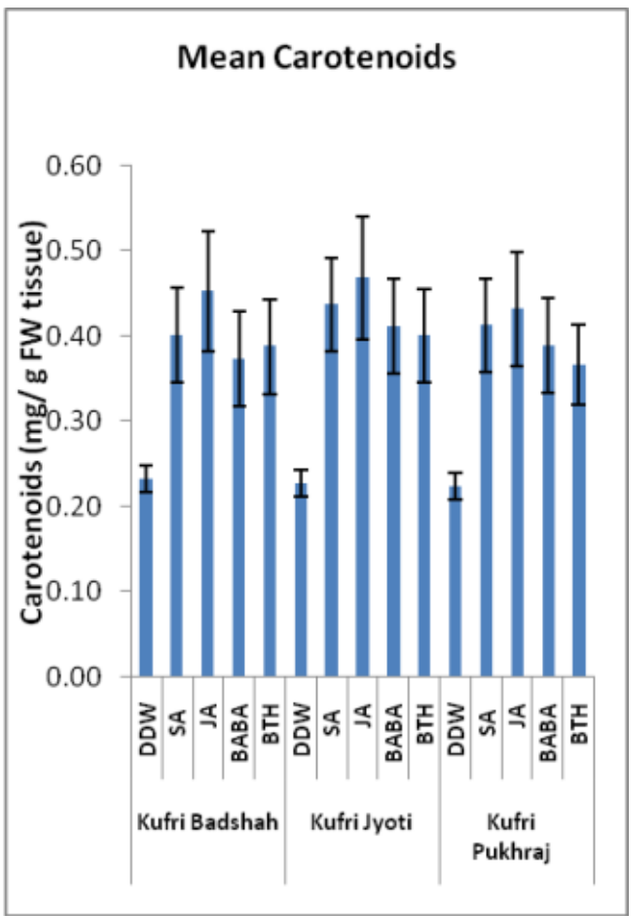
DDW- Double distilled water after 2 days of elicitor spray;
PI- *P. infestans* with sporangial solution at conc. of 4.0 x 10^4 sporangia per mL after 2 days of elicitor spray

Table 4: Effect of foliar spray of SA, JA, BABA and BTH on total chlorophyll (mg/gFW) in leaves of different potato varieties challenge inoculated with *P. infestans*

Total chlorophyll (mg/gFW)									
Variety	Treatment	Days after challenge inoculation							Mean
		1	2	3	4	5	6	7	
Kufri Badshah	Control	1.46	1.46	1.42	1.40	1.38	1.37	1.36	1.41
	SA (500µM)	1.55	1.57	1.58	1.59	1.61	1.62	1.64	1.60
	JA(500 µM)	1.41	1.43	1.44	1.45	1.47	1.48	1.50	1.46
	BABA(50mM)	1.53	1.55	1.56	1.57	1.59	1.60	1.61	1.57
	BTH (500µM)	1.51	1.53	1.54	1.55	1.57	1.58	1.59	1.55
Kufri Jyoti	control	1.48	1.50	1.48	1.46	1.44	1.42	1.41	1.46
	SA (500µM)	1.66	1.68	1.71	1.79	1.76	1.70	1.70	1.71
	JA(500 µM)	1.44	1.46	1.52	1.54	1.54	1.53	1.52	1.51
	BABA(50mM)	1.63	1.65	1.69	1.77	1.73	1.68	1.68	1.69
	BTH (500µM)	1.59	1.61	1.64	1.72	1.69	1.63	1.63	1.64
Kufri Pukhraj	Control	1.48	1.50	1.49	1.46	1.43	1.42	1.40	1.46
	SA (500µM)	1.63	1.65	1.69	1.77	1.73	1.68	1.68	1.69
	JA(500 µM)	1.45	1.44	1.55	1.52	1.51	1.49	1.48	1.49
	BABA(50mM)	1.61	1.63	1.67	1.74	1.71	1.66	1.66	1.67
	BTH (500µM)	1.60	1.62	1.65	1.73	1.70	1.64	1.64	1.65
CD (5%)	Variety (A)-0.0022; Elicitor (B)- 0.0028; Time interval (C)-0.0033 ; AB- 0.0049; AC-0.0058; BC-0.0076; ABC- 0.0131								

Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

maximum content in Kufri Jyoti. It was observed that the chlorophyll content increased with JA treatment in foliar application, even though control values were at par with 500 µM JA treated plants.



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 4: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on carotenoids (mg/ g FW) in leaves of different potato

Carotenoids: The data pertaining to combined effect on leaf carotenoids (mg/ g FW) in response to various elicitors followed by challenge inoculation; revealed statistically significant difference amongst the various elicitors applied on potato **(Fig. 4).** After 21 days of planting, the plants were sprayed with elicitors, and were further, challenge inoculated with pathogen after 2 days. Amongst the different elicitors treatment in Kufri Badshah; SA caused 74% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 94% increase, BABA gave 61% and BTH gave 69% increase in carotenoids (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA treatment resulted in 91% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 100% increase, BABA gave 78% and BTH gave 74% increase in carotenoids (mg/ g FW) w.r.t control. In Kufri Pukhraj SA treatment resulted in 86% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in increase of 91%, BABA in 77% and BTH in 73% increase in carotenoids (mg/ g FW) w.r.t control indicating that JA is better inducer of carotenoids among all the four elicitors with maximum content in Kufri Jyoti followed by Kufri Badshah and Kufri Pukhraj. It was observed that the carotenoids content showed peak on 4th day of treatment in all the tested elicitors.

Electrophoretic study (SDS-PAGE) of protein extract of different potato cultivars: Acidic extracellular forms of these PR proteins built up at the commencement of plant resistance, indicating that they have a value as molecular markers for the expression of SAR. PR proteins have a low molecular weight (5-75 kDa), and they are thermostable, highly resistant to proteases, extractable, and stable at low pH (<3). These PR proteins are known to provide resistance against various pathogens. PR proteins are categorised into structurally homologous families. Some of these PR-protein families have direct antimicrobial activities. Electrophoretic study of protein pattern for potato varieties in response to selected best doses of JA, SA and BTH i.e, at 500 µM and BABA at 50 mM, resolved in the molecular weights ranging from 6-180 kDa with respect to standard protein markers. Specific bands falling in the range of 6-75 kDa were reported in treated samples as compared to their respective control (**Plate 1**). It is known that PR proteins fall under the range of 15.5 KDa to 75 KDa (Van Loon *et al.,* 2006), Which, signified that application of SA, JA, BABA and BTH in potato resulted in PR protein induction along with some other proteins.

DISEASE DATA

Disease severity was observed from 14 days post challenge inoculation. SA treatment gave per cent disease control of 75.43, 77.07, and 77.29 in Kufri Badshah, Kufri Jyoti and Kufri Pukhraj. Minimum per cent disease control of 61.22 was observed in BTH treated Kufri Jyoti cultivar. Therefore, single spray of elicitors gave 61 to 77 % disease control of late blight disease **(Table 5)**.

Katoch (2007) showed that treatment with SA or

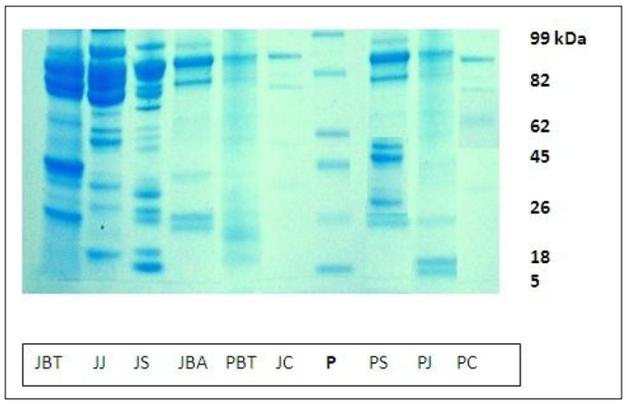


Plate 1: SDSPAGE of leaf proteins of different potato varieties at 500 M of SA, 500 M of JA, 500 M of BTH and 50 mM for BABA. JBT Kufri Jyoti treated with BTH, *JJ* Kufri Jyoti treated with JA, *JS* Kufri Jyoti treated with SA, JBA Kufri Jyoti treated with BABA , PBT Kufri Pukhraj treated with BTH, *JC* Kufri Jyoti-control, ***P***-protein ladder; PS Kufri Pukhraj treated with SA, *PJ* Kufri Pukhraj treated with JA; PC - Kufri Pukhraj control.

Inoculation with *Erysiphe polygoni* resulted in synthesis of new protein, whereas treatment with ABA did not show any variation in the protein profile as compared to the control. It may be that 5mM SA and inoculation with *E. polygoni* shares a mechanism for induction of synthesis of a new protein. Ziadi *et al* (2001) reported that foliar application of BTH induces systemic resistance in many crops and higher activity of β-1, 3-glucanases. Andreu *et al (*2006) showed that when BABA was applied to the foliage at early stages of crop development, a protective effect against late blight of potato was observed, and increase was observed in level of β-1, 3-glucanase and aspartyl protease (StAP1) in post harvest tuber samples. Sorokan *et al* (2014) reported that application of salicylic acid in potato against *P. infestans* showed increased peroxidase activity compared to control plants. Moharekar *et al* (2003) stated that salicylic acid increased the synthesis of many pigments like carotenoid, xanthophylls but it also decreases the amount of chlorophyll pigments in wheat and mung and chlorophyll *a/b* ratio in wheat plantlets. This stimulatory response of ascorbic acid, salicylic acid and other antioxidants might be due to the phenomenon of antioxidant scavenging to provide protection to chloroplast and chlorophyll against degradation caused by reactive oxygen species. Foliar application of SA (1.4×10^{-4} M) to *Brassica napus* was found to enhance chlorophyll concentration (Hayat *et al* 2005).

Table 5: Efficacy of elicitors in controlling late blight of potato 14 days after challenge inoculation with *P. Infestans*

% Disease severity after 14 days of challenge inoculation										
Sr. No.	**Variety**	**DDW/ control**	**SA**	**% Disease control**	**JA**	**% Disease control**	**BABA**	**% Disease control**	**BTH**	**% Disease control**
1	K Pukhraj	55.04	12.50	77.29	13.73	75.06	16.00	70.93	16.53	69.97
2	K Badshah	31.86	7.83	75.43	8.48	73.39	9.25	70.97	10.15	68.13
3	Kufri Jyoti	22.33	5.12	77.07	8.78	60.69	8.13	63.58	8.66	61.22
CD (5%)	Varieties (A)- 0.87 ; Elicitors (B)- 0.45; AB: 1.3									
DDW- Double distilled water										

CONCLUSION

In conclusion, it may be possible to, in certain cases, replace conventional chemical fungicides with any of the four elicitors especially SA, provided, spray is done before the onset of disease to prime the plants. It could also be concluded that primed plants with salicylic acid act better for suppressing the severity of late blight disease of potato, by stimulating their own inbuilt resistance under field conditions. Moreover, it is considered as safe, cost-effective and easily applied for such diseases. Blanket spray of salicylic acid can be done on vegetable crops before onset of diseases to enhance plant defense against oomycete pathogens. Only 8-10 grams of SA is required (Dissolved in 100 litres of water) for spraying crop and it approximately cost only Rs 15/ Acre per spray, making it very economical and safe.

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Taxonomic characterization and diversity of wood inhabiting Polypores from Chennai

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ABSTRACT

Eleven polypore species, *Ganoderma philippii*, *G. resinaceum*, *Lenzites elegans*, *Microporus xanthopus*, *Polyporus grammocephalus*, *P. alveolaris*, *Pycnoporus sanguineus*, *Rigidoporus lineatus*, *Trametes lactinea*., *T. pubescens* and *T. trogii* belonging to three families of order *Polyporales* are described on the basis of specimens collected from three localities of Chennai (Tamil Nadu, India). Of these, eight species namely *Ganoderma philippii*, *Microporus xanthopus*, *Polyporus alveolaris*, *Pycnoporus sanguineus*, *Rigidoporus lineatus*, *Trametes lactinea*, *T. pubescens* and *T. trogii* are described from the study area for the first time.

Keywords: *Polyporales*, polypores, white rot, taxonomy, diversity,

INTRODUCTION

Polypores play a vital role in preserving the earth's biosphere because of their ability to decay wood that brings about the subsequent interaction between all life (Johansson *et al*., 2007). These fungi have been classified under different groups from time to time based on morphotaxonomic and molecular characters (Ryvarden and Gilbertson, 1993; 1994; Binder *et al*., 2005; Kirk *et al*., 2001; 2008).

Polyporales, the major order of polypores, was proposed by Gäumann (1926) with ten families grouped together on the basis of morphological features. Binder *et al.* (2013) and Justo *et al*. (2017) recognised 41 families within order *Polyporales*. However, as per Mycobank (2019) there are 77 families listed under this order. As per Kirk *et al.* (2008) about 1800 species have been described under *Polyporales*. The members of this order are peculiar in having resupinate to effused reflexed to pileate basidipcarps (Binder *et al.,* 2005). The hyphal anatomy, ranges from monomitic to dimitic to trimitic (Gilbertson and Ryvarden, 1986).

The taxonomic studies on polypores from Tamil Nadu were initiated by Sundaramani and Madurajan (1925). It was followed by the significant contributions by Natarajan and Kolandavelu (1985), Selvam *et al*., (2012), Malarvizhi (2014) and Priyamvada *et al*., (2017). The review of literature motivated the authors to explore the diversity of *Polyporales* in and around Chennai. The present paper is an attempt for the morpho-taxonomic characterization of polypores of order *Polyporales* in the metropolitan city, Chennai.

Several collection trips were carried out for the collection of polypore basidiomata in three localities of Chennai, namely Guindy, Adyar and Tambarum during the month of November to January in 2015- 2017. These basidiomata were worked out following the standard techniques and the dried specimens were deposited in Herbarium, Centre for Advanced studies in Botany, University of Madras, Chennai, Tamil Nadu, India. The colour standards followed were as per Kornerup and Wanscher (1978).

RESULTS AND DISCUSSIONS

A total of 24 polypore specimens collected from the study area were worked out and have been grouped into 11 species spread over 6 genera belonging to 3 families namely *Ganodermataceae* (*Ganoderma*), *Meripilaceae* (*Rigidoporus*) and *Polyporaceae* (*Lenzites, Microporus, Polyporus, Pycnoporus* and *Trametes*) of order *Polyporales*.

Morphotaxonomic characterization

Ganoderma philippii (Bres. & Henn. ex Sacc.) Bres. Iconogr. *Mycol*. **21**: tab. 1014, 1932. - *Fomes philippii* Bres. & Henn. ex Sacc., *Sylloge Fungorum* **9**: 180, 1891. **Fig. 1**

Basidiomata annual, pileate, solitary, applanate, non-laccate, broadly attached, corky to woody when fresh, hardening on drying; pilei up to 12 × 17 × 5 cm (length × breadth × thickness). **Pilear surface** zonate, brownish grey (5F2) to light brown (6D8) to brown (6E4) to dark brown (7F4, 7F6) to brownish grey (5D2) when fresh, somewhat darkening on drying. **Pore surface** grey (7E1) to brownish orange (5C5) to pale orange (5A3) to light brown (7D5); pores round to angular, 3-5 per mm. **Context** homogenous, brown (6E4) to dark brown (7F67), up to 1.5 cm in thickness. **Pore tubes** dark brown (7F67), more than 1 mm in depth. **Margins** acute, orange white (5A2) to dark brown (7F4), up to 2 mm in thickness. **Hyphal system** trimitic; generative hyphae hyaline, thick-walled, clamped, branched, up to 5.2 µm in width; skeletal hyphae hyaline, unbranched, thick-walled, rarely encrustated, up to 3.9 µm in width; binding hyphae branched, thick-walled, up to 2.9 µm in width. **Pilocystidia** clavate, 18-32 × 6-12 µm. **Basidia** clavate, 2 - 4 sterigmate, with a basal clamp, 11.5 - 25.3 × 2.3 - 4.6 µm. **Basidiospores**

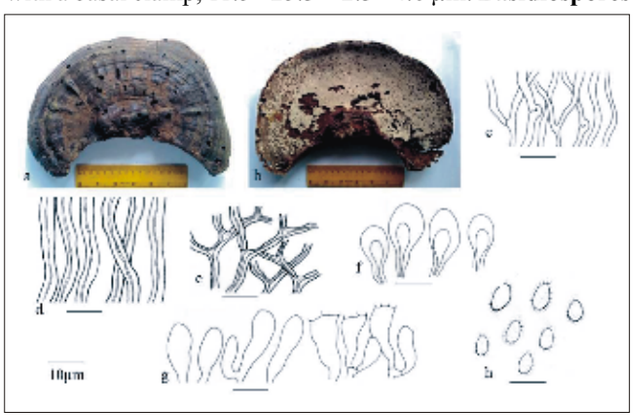

Fig. 1 ***Ganoderma philippii*****:** a. Basidiomata, b. Pore Surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Pilocystidia, g. Basidioles and Basidia, h. Basidiospores.

broadly-ellipsoid, brown, thick-walled, truncate at the apex, (4.6-) 4.8 - 9.2 (-10.4) × (4.1-) 4.6 - 6.4 (-6.9) µm, Q=1.2; exospore thin, subhyaline, smooth; endospore thick, brown, echinulate; cyanophilous **(**CN^+**)**, inamyloid (IKI^-).

Specimen examined: India, Tamil Nadu, Chennai, Guindy (13°00'34"E, 80°14'15"N), MLCASB277,12.09.2016, Kezhocuyi Kezo; University of Madras, 13°00'35"E, 80°14'16"N, MLCASB279, 12.09.2016, Kezhocuyi Kezo; Guindy, 13°00'04"N, 80°14'25"E, MLCASB347, 13.10.2017, Lakleiphi Tallanao.

Remarks: *Ganoderma philippii* is characterized by applanate basiodiomata with non-laccate abhymenial surface, homogenous context and broadly-ellipsoid basidiospores. The species has been earlier reported from Maharashtra (Bhosle *et al*., 2010), West Bengal (Sharma, 2012), Uttarakhand (Singh *et al.,* 2014). It is the first report from the study area.

Ganoderma resinaceum Boud., *Bulletin de la Société Mycologique de France* **5**: 72, 1889. **Fig. 2**

Basidiomata annual, pileate, sub-stipitate, broadly-attached, corky when fresh, becoming hard on drying; pilei up to 10 × 6 × 2 cm. **Pilear surface** azonate, violet brown (10F4, 11F8) when fresh, dark brown (9F8) on drying. **Pore** surface greyish orange (5B3) to orange white (5A2) to brown (7E7) to reddish brown (8E8); pores round to angular, somewhat irregular, 2-3 per mm. **Context** duplex, light brown near abhymenial surface, brown near tube layer, up to 9 mm in thickness, dark brown in KOH. **Pore tubes** light brown (5D7), up to 1 cm in depth. **Margins** acute, wavy, abhymenial margins reddish brown (8E8), hymenial margins pale orange (5A3) to brownish orange (5C5), up to 3 mm in thickness. **Hyphal system** trimitic; generative hyphae hyaline, thick-walled, branched, clamped, up to 5.2 µm in width; skeletal hyphae unbranched, thick-walled, rarely septate, up to 5.2 µm in width; binding hyphae irregularly branched, up to 4.4 µm in width. **Pilocystidia** clavate, apically encrusted, 25 - 42.5 × 7.5 - 12.5 µm. **Basidia** clavate, 4-sterigmate, with a basal clamp, 11.5 - 25.3 × 2.3 - 4.6 µm. **Basidiospores** ellipsoid, brown, thick-walled, truncate at the apex, (6.9-) 7.4 - 9.2 (-9.4) × (4.6-) 5.1- 6.9 (-9.2) µm, Q=1.4; exospore thin, subhyaline, smooth; endospore thick, brown, echinulate; CN^+, IKI^-

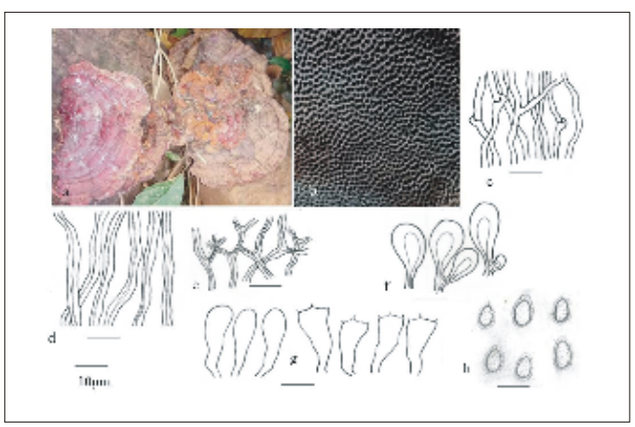

Fig. 2 ***Ganoderma resinaceum*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. skeletal hyphae, e. binding hyphae, f. Pilocystidia, g. Basidioles & Basidia, h. Basidiospores.

Specimen examined: India, Tamil Nadu, Chennai, Tambarum**,** 12°55'11"N, 80°07'14"E; 12°55'12"N, 80°07'14"E; 12°55'11"N, 80°07'15"E, MLCASB161, MLCASB168, MLCASB192, 13.10.2015, Tenzing Sangmo; Guindy, 13°00'35"E, 80°14'16"N, MLCASB278, 12.09.2016, Kezhocuyi Kezo.

Remarks: The presence of annual, pileate, solitary, basidiomata with pilocystidia and palisade end cells on the pilear surface and brown thick-walled, ellipsoid basidiospores are the key characters of *Ganoderma resinaceum.* This species is also known from Himachal Pradesh (Dhanda 1977; Kaur 2013), Jammu and Kashmir (Dhanda 1977), Maharashtra (Foroutan and Vaidya 2007; Ranadive *et al*., 2011; Ranadive 2013), Uttarakhand (Sharma 2000; 2012, Singh 2016), West Bengal (Sharma 2000; 2012), Punjab and Chandigarh (Kaur *et al*., 2017 and Brar *et al*., 2018) and Tamil Nadu (Malarvizhi, 2014).

Lenzites elegans (Spreng.) Pat., *Essai taxonomique sur les familles et les genres des Hyménomycètes*: 89*,* 1900. *-Daedalea elegans* Spreng., *Kongliga Svenska Vetenskaps akademiens Handlinger Ser*. 3, **8**: 51, 1820. **Fig. 3**

Basidiomata perennial, sessile, laterally attached, flabelliform or circular, corky and flexible when fresh, rigid on drying; pilei up to 8 cm × 10 cm × 3 cm. **Pilear surface** zonate, finely tomentose, slightly uneven**,** white (4A1) to grey (4B1) when fresh, fairly unchanged on drying. **Pores** round to angular to lamellate, 1- 2 per mm, radially split, up to 2 mm wide, lamellate portion up to 20-25 per cm when measured tangentially. **Context** homogenous, white (4A1), up to 8 mm thick near the base, leathery when dry. **Pore tubes** orange red (8B8), up to 20 mm in depth. **Margins** acute, even or lobed, white (4A1) on both pilear and pore surface, up to 3 mm in thickness. **Hyphal system** trimitic; generative hyphae hyaline, thin-walled, clamped, up to 4 µm in width; skeletal hyphae dominating, light-yellowish, thick-walled, up to 6 µm in width; binding hyphae hyaline, thick-walled, irregularly branched, up to 3.5 µm in width, **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-stergimate, with a basal

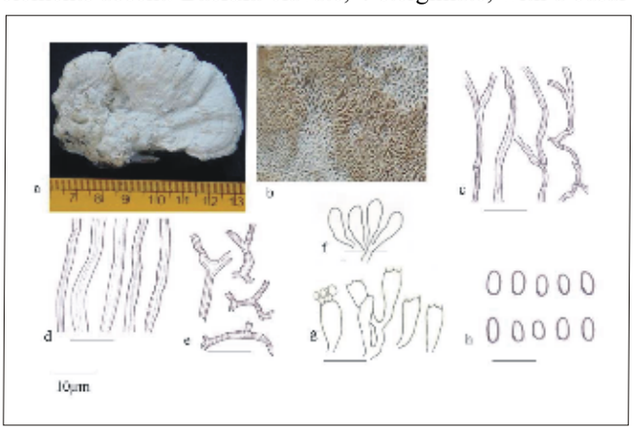

Fig. 3. ***Lenzites elegans:*** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores.

clamp, 17-22 × 5-8 µm. **Basidiospores** ellipsoid, hyaline, smooth, thin-walled, (4-) 4.2-5.8 (-6) × 2-4 µm, Q = 1.52, CN^+, Amyloid (IK^+).

Specimen examined: India, Tamil Nadu, Chennai, Guindy, 13º00'05''N 80º14'26''E, MLCASB008, 31.10.2015, Kezhocuyi Kezo; Guindy, 13º00'37''N 80º14'17''E, MLCASB358, 31.10.2015, Kezhocuyi Kezo; Guindy, 13º00'05''N 80º14'26''E, MLCASB347, 13.10.2017, Lakleiphi Tallanao.

Remarks: *Lenzites elegans* is characterized by their poroid to lamellate hymenophore and has been earlier reported from Himachal Pradesh (Sharma, 2000; 2012; Kaur, 2013), Kerala (Mohanan, 2011), Uttarakhand (Bagchee *et al*., 1954; Thind *et al.,* 1957), West Bengal (Bakshi, 1971), Tamil Nadu (Priyamvada *et al.,* 2017).

Microporus xanthopus (Fr.) Kuntze, *Revisio generum plantarum* **3** (2): 494, 1898. **-** *Polyporus xanthopus* Fr., *Observationes mycologicae* **2**: 255, 1818. **Fig. 4**

Basidiomata annual, pileate, solitary, centrally stipitate, infundibuliform, sometimes two or more basidiocarps grow together; pilei up to 6 × 5 × 0.3 cm. **Pilear surface** glabrous, glossy, concentrically zonate with alternating shades of brown (6C4, 6D6 and 7D6), thickening towards the centre.. **Pore surface** white (4A1) when fresh, grey (4B1) on drying; pores angular to circular, minute, almost invisible to the naked eye, 7-9 per mm. **Context** homogenous, white, up to 1 mm in thickness, tissues turning dark black in KOH. **Pore tubes** white (4A1), up to 2 mm in depth. **Margins** wavy to lobed, up to 2 mm in thickness. **Stipe** somewhat round, brown (7D6), glabrous, covered with a thin light cuticle, up to 6 cm × 0.6 cm, expanding to a disk-like base which is up to 1 cm in width. **Hyphal system** trimitic; generative hyphae branched, thin-walled, clamped, up to 3 µm in width; skeletal hyphae dominant in context as well as trama, thick-walled, up to 6 µm in width; binding hyphae torturous, thick-walled, frequently branched, up to 3 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-sterigmate, with a basal

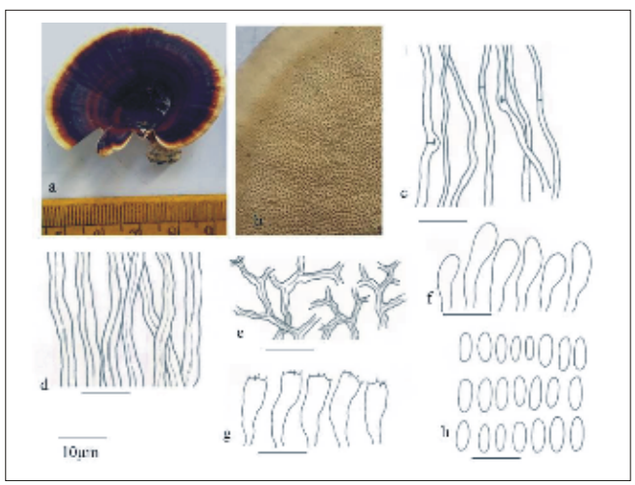

Fig. 4. ***Microporus xanthopus*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores.

clamp, 12 - 18 × 4.5 - 5.5 µm. **Basidiospores** cylindrical, hyaline, (5-) 5.2 - 7.4 (-7.6) × (2.1-) 2.4 - 3.1 (-3.3) µm, Q= 2.2, CN^-, IK^-.

Specimen examined: India, Tamil Nadu, Chennai, Guindy 12º59'50''N 80º14'27''E, MLCASB247, 17.12.2015, Kezhocuyi Kezo.

Remarks: *Microporus xanthopus* is recognized by its infundibuliform basidiocarps with a glossy and concentrically zonate pileus; brown, glabrous stipe with disk like base and minute pores. It has been earlier reported from Andhra Pradesh (Sharma, 2012), Assam (Ashwani *et al*., 2013; Bhattacharjee *et al*., 2015*),* Kerala (Leelavathy and Ganesh, 2000; Mohanan 2011) and Meghalaya (Sharma, 2012; Bhattacharjee *et al*., 2015*).* It is the first report from the study area.

Polyporus grammocephalus Berk., *London Journal of Botany* **1**(3): 148, 1842. **Fig. 5**

Basidiomata annual, solitary, pileate, dimidiate, flabelliform, laterally attached with a stipe like contracted base; pilei up to 8 cm × 5.5 cm ×0.6 cm. **Pilear surface** glabrous, with numerous fine radial lines, light orange (5A4) when fresh**,** turning brownish orange (5A3) on drying. **Pore surface** brownish orange (5A3) both when fresh and on drying; pores round to angular, 3-5 per mm. **Context** homogenous, brownish orange (5A3), up to 3 mm in thickness. **Pore tubes** brownish orange (5A3), up to 2 mm in depth. **Margins** acute, light orange (5A4) both on pilear and pore surface, up to 1 mm thick. **Stipe** usually absent, but a short stipe like base present which is up to 6 mm in diameter. **Hyphal system** dimitic; generative hyphae hyaline, clamped, thin-walled, up to 4.8 µm in width; binding hyphae thick-walled, abundant, up to 6 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-sterigmate, with a basal clamp, 15 - 22.7 × 5.5 - 8.2 µm. **Basidiospores** ellipsoid, smooth, thin-walled, (4.8-) 5 - 6.5 (-6.9) × (2.2) 2.6 - 3.1 (-3.4) µm, CN^+, IK^-, Q = 1.9.

Specimen examined: India, Tamil Nadu, Chennai, Guindy 13º00'37''N 80º14'17''E, MLCASB190, 31.10.2015, Tenzing Sangmo; Guindy, 13º00'05''N 80º14'26''E, MLCASB221, MLCASB239, 31.10.2015, Kezhocuyi Kezo; Guindy (13°00'33''E, 80°14'17''N), 12.09.2016, Kezhocuyi Kezo;

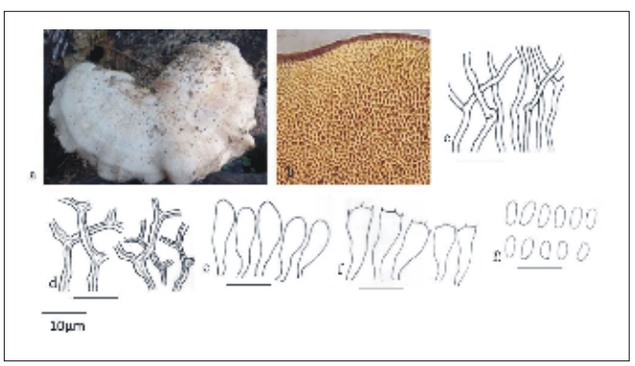

Fig. 5. ***Polyporus grammocephalus*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Binding hyphae, e. Basidioles, f. Basidia, g. Basidiospores.

MLCASB341, India, Tamil Nadu, Chennai, Guindy, 13°00'33"E, 80°14'26"N, MLCASB261, 12.09.2016, A. Arockia Mahimai Jayaseelan; Guindy, 13º00'05"N 80º14'26"E, MLCASB347, 13.10.2017, Lakleiphi Tallanao.

Remarks: *Polyporus grammocephalus* is unique in having solitary, flabelliform pilei with a narrower constricting attachment, comparatively small-sized pores and dimitic hyphal system. It has been earlier reported from Himachal Pradesh (Sharma, 2000; 2012; Kaur, 2013), Kerala (Leelavathy and Ganesh, 2000), Madhya Pradesh (Roy and De, 1996), Maharashtra (Roy and De, 1996; Ranadive *et al*., 2011), Punjab (Dargan *et al*., 2006), Uttarakhand (Dhanda, 1977; Roy and De, 1996; Sharma, 2000, 2012), West Bangal (Roy and De, 1996), Tamil Nadu (Kumar and Kumar, 2017).

Polyporus alveolaris (DC.) Bondartsev & Singer, *Annales Mycologici* **39** (1): 58, 1941. **-** *Merulius alveolaris* DC., *Flore française* **6**: 43, 1815. **Fig. 6**

Basidiomata annual, pileate, sessile to stipitate, circular to dimidiate; pilei up to 7 cm × 6 cm × 0.3 cm. **Pilear surface** smooth, azonate, yellowish white (4A2) when fresh**,** turning light yellow (4A4) on drying. **Pore surface** greyish orange (5B3) when fresh, almost unchanged on drying; pores regular, polygonal, usually 1 per mm. **Context** homogenous, light yellow (4A4), up to 1mm in thickness. **Pore tubes** greyish orange (5B3), up to 2 mm in depth. **Margins** thin, acute, yellow (4A4) on the pilea**r** surface**,** yellowish white (4A2) on the pore surface. **Hyphal system** dimitic; generative hyphae hyaline**,** thin-walled, clamped, up to 4.8 µm in width; binding hyphae thick-walled, aseptate, much branched, up to 5.2 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-sterigmate, with a basal clamp, 19.5 - 29 × 7 - 9.2 µm. **Basidiospores** cylindrical, hyaline, smooth, (9.2-) 9.5 - 13.8 (-14.2) × (4-) 4.5 - 5.1 (-5.4) µm, CN^+, IK^-, Q = 2.7.

Specimen examined: India, Tamil Nadu, Chennai district, Guindy, 13º00'04"N 80º14'26"E, MLCASB220, 31.10.2015, Kezhocuyi Kezo.

Remarks: *Polyporus alveolaris* is easily identifiable by its circular to dimidiate basidiocarp with large polygonal pores and is earlier reported from Arunachal Pradesh (Sharma, 2000; 2012), Himachal Pradesh (Kaur, 2013), Meghalaya (Sharma, 2000; 2012) West Bengal (Roy and De, 1996). It is the first report from the study area.

Pycnoporus sanguineus (L.) Murrill, *Bulletin of the Torrey Botanical Club* **31**(8): 421, 1904. **-** *Boletus sanguineus* L., *Species Plantarum*: 1646, 1763. **Fig. 7**

Basidiomata annual, pileate, sessile, broadly attached, attenuate at the base, flabelliform, imbricate, rarely overlapping, leathery when fresh, rigid on drying; pilei up to 8 cm × 5 cm × 4 mm. **Pilear surface** coraceous, broadly zonate, reddish orange (7B8) to brownish orange (7C8) when fresh, vivid red (10A8) to brownish red (8C8) on drying. **Pore surface** flame scarlet (7A8) when young, becoming copper red (7C8) to orange red (8B8) with age; pores angular to circular, 4-7 per mm. **Context** homogenous, reddish orange (7B8), hard near attachment, leathery near margins, up to 3 mm in thickness, dark black in KOH. **Pore tubes** orange red (8B8), up to 1 mm in depth. **Margins** acute, thin, brownish red (8C8) on both pilear and pore surface. **Hyphal system** trimitic; generative hyphae thin- to thick-walled, clamped, branched, up to 5 µm in width; skeletal hyphae dominating, thick-walled, up to 6 µm in width; binding hyphae thick-walled, frequently branched, up to 5 µm in width. **Basidia** clavate, 4-sterigmate, with basal clamp, 12 - 15 × 4 - 5 µm. **Basidiospores** cylindrical, hyaline, (4.7-) 5.1-6.4 (-7.3) × (-2.2) 2.4 - 2.8 (-3) µm, weakly CN^+, IK^-, Q = 2.

Specimen examined: India, Tamil Nadu, Chennai, Guindy, 12º59'50"N 80º14'27"E, MLCASB233, 31.10.2015, Kezhocuyi Kezo; Adyar, 13º00'37"N 80º15'48"E, MLCASB022, 31.10.2015, Kezhocuyi Kezo.

Remarks: *Pycnoporus sanguineus* has been characterized by reddish orange to brownish orange basidiocarp and is differentiated from *P. cinnabarinus* by the presence of thinner basidiocarp and larger pore size (Sharma, 2012). The earlier reports of this species are from Kerala (Leelavathy and Ganesh, 2000; Mohanan 2011), Meghalaya, Mizoram (Bhattacharjee *et al*., 2015), Uttarakhand (Sharma, 2012), Tamil Nadu (Selvam *et al*., 2012). It is the first report from the study area.

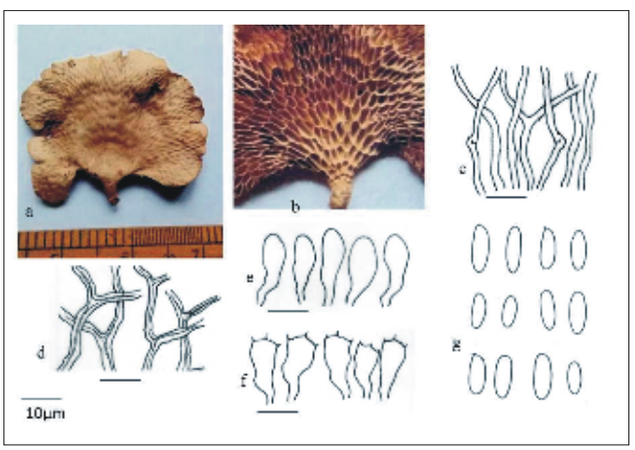

Fig. 6. ***Polyporus alveolaris*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Binding hyphae, e. Basidioles, f. Basidia, g. Basidiospores.

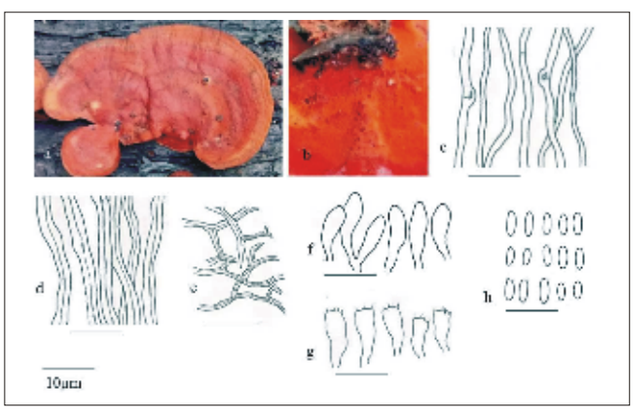

Fig. 7 ***Pycnoporus sanguineus*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores.

Rigidoporus lineatus (Pers.) Ryvarden, *Norwegian Journal of Botany* **19**: 236, 1972. **-** *Polyporus lineatus* Pers., *Botanique* (*Nagpur*) **5**: 174, 1827. **Fig. 8**

Basidiomata annual, pileate, sessile, solitary to imbricate, dimidiate, brittle and hard when dry; pilei up to 3 cm × 2 cm × 10 mm. **Pilear surface** concentrically zonate, pale orange (5A3) when fresh, turning to greyish orange (5B5) on drying. **Pore surface** pale orange (5A3) when fresh, turning to brownish orange (5C5) on drying; pores round to angular, 6 - 7 per mm. **Context** homogenous, pale yellow (4A3), up to 2 mm in thickness. **Pore tubes** yellowish white (4A2), radially fibrous, up to 3 mm in depth. **Margins** acute, greyish orange (5B5) on pilear surface**,** pale orange (5A3) on pore surface, up to 5 mm in thickness. **Hyphal system** monomitic; generative hyphae hyaline, thin- to thick-walled, simple-septate, moderately branched, up to 8 µm in width. **Cystidia** rare, club-shaped, thick-walled, coarsely encrusted, 35.4 - 42.8 × 7.8 - 12.6 µm. **Basidia** clavate, 4-sterigmate, without basal clamp, 11.5 - 12.6 × 4.1 - 4.6 µm. **Basidiospores** cylindrical, hyaline, smooth, thin-walled, (4.1-) 4.3 - 4.6 (-5) × (2.3-) 2.5 - 2.9 (-3.4) µm, CN^+, IK^-.

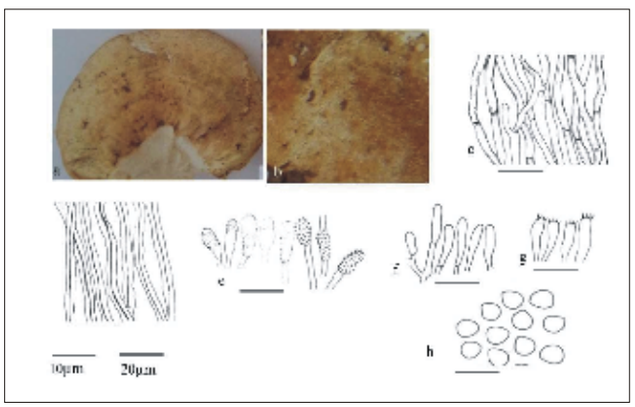

Fig. 8 ***Rigidoporus lineatus*****:** a. Basidiomata, b. Pore surface, c. Thin walled generative hyphae, d. Thick walled generative hyphae, e. Encrusted cystidia, f. Basidioles, g. Basidia, h. Basidiospores.

Specimen examined: India, Tamil Nadu, Chennai, Guindy, 13°00'04''N 80°14'26''E, MLCASB349,13.10.2017, Lakleiphi Tallanao.

Remarks: The dimidiate basidiocarps that become hard and rigid on drying and larger encrusted cystidia are the key characters of *Rigidoporus lineatus*. This species has been earlier reported from Kerala (Leelavathy and Ganesh, 2000), Uttarakhand and Meghalaya (Sharma, 2012). It is the first report from Chennai.

Trametes lactinea (Berk.) Sacc., *Sylloge Fungorum* **6**: 343, 1888. **-** *Polyporus lactineus* Berk., *Annals and Magazine of Natural History* **10**: 373, 1843. **Fig. 9**

Basidiomata annual, pileate, broadly attached, solitary, applanate, dimidiate, semicircular, corky when fresh, woody on drying; pilei up to 16 cm × 12 cm × 3.3 cm. **Pilear surface** azonate, becoming warted with age, white (4A1) when fresh,

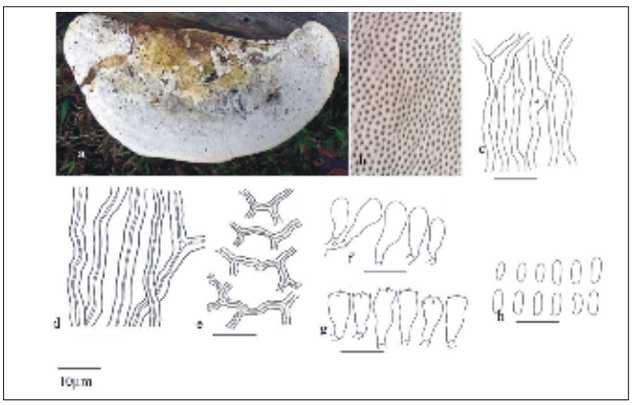

Fig. 9 ***Trametes lactinea:*** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores**.**

greyish white (4A2) on drying. **Pore surface** white (4A1) when fresh, changing to greyish white (4A2) on drying; pores round to angular, 2-3 per mm. **Context** homogenous, white (4A1), up to 13 mm in thickness, weakly darkening in KOH. **Pore tubes** white (4A1), up to 2 cm in depth. **Margins** acute, white (4A1), up to 2 mm in thickness. **Hyphal system** trimitic; generative hyphae hyaline, clamped, thin-walled, up to 4 µm in width; skeletal hyphae dominating, pale-yellow, thick-walled, up to 6 µm in width; binding hyphae rather abundant, thick-walled, coralloid branching, up to 4 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-sterigmate, with a basal clamp, 14 - 16 × 5.2 - 5.8 µm. **Basidiospores** cylindrical, thin-walled, (5-) 5.5 - 7.2 (-7.5) × (2.1-) 2.3 - 2.6 (-2.8) µm, CN^+, IK^-, Q = 2.3.

Specimen examined: India, Tamil Nadu, Chennai, Guindy, 13°00'05''N 80°14'26''E, MLCASB351, 31.10.2015, Kezhocuyi Kezo.

Remarks: *Trametes lactinea* is characterized by white applanate, larger basidiocarps with somewhat warted pilear surface. The species was earlier reported from Himachal Pradesh (Dhanda, 1977), Kerala (Leelavathy and Ganesh, 2000), Maharashtra (Ranadive, 2013), Uttarakhand (Roy and De, 1996, Sharma, 2012), West Bengal (Banerjee, 1947; Roy and De, 1996). It is the first report from the study area.

Trametes pubescens (Schumach.) Pilát, *Atlas Champ. Eur., Polypor., B*: 268, 1939. - *Boletus pubescens* Schumach., *Enumeratio Plantarum, in Partibus Sællandiae Septentrionalis et Orientalis Crescentium* **2**: 384, 1803 **Fig. 10**

Basidiomata annual, pileate, broadly attached, solitary; pilei up to 8 cm × 7 cm × 8 mm. **Pilear surface** faintly zonate, sulcate, tomentose, becoming hispid with age, brownish orange (5C5) to light brownish (6D6) when fresh**,** brownish orange (5C5) to dark brown (6F6) on drying. **Pore surface** brownish orange (5C5) when fresh**,** oak brown (5D6) on drying; pores round to angular, 4 - 5 per mm. **Context duplex**, brownish orange (5C5), dense towards the pore surface and brown (6F6) loose towards the pilear surface, without black line below the tomentum, up to 2 mm in thickness. **Pore tubes** oak brown (5D6), up to 5 mm in depth. **Margins** acute, golden

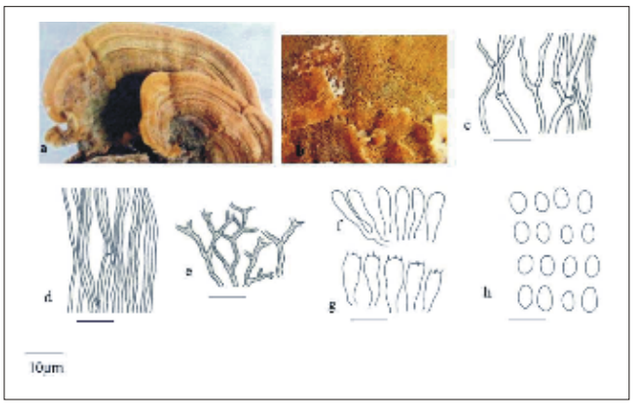

Fig. 10 ***Trametes pubescens*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores**.**

brown (5D7) on both pilear and pore surface, up to 2 mm in thickness. **Hyphal system** trimitic; generative hyphae thin-walled, clamped, up to 5 µm in width; skeletal hyphae thick-walled, abundant, up to 8 µm in width; binding hyphae thick-walled, much-branched, up to 4 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4-sterigmate, with a basal clamp, 10 - 15 × 4.6 - 5.7µm. **Basidiospores** cylindrical, hyaline, (4.6-) 5.7 - 6.9 (-7.2) × (1.8-) 2.1 - 2.3 (-2.5) µm, CN^+, IK^-, Q = 2.3

Specimen examined: India, Tamil Nadu, Chennai, Guindy, 13º00'07" N 80º14'24"E, MLCASB344, 13.10.2017, Lakleiphi.

Remarks: *Trametes pubescens* is identified in the field on the basis of faintly zonate, tomentose pilear surface, duplex context without distinct black line and smaller pores. The species is described earlier from Andhra Pradsh (Sharma, 2012), Kerala (Mohanan 2011), Uttarakhand (Sharma, 2012). It is the first report from the study area.

Trametes trogii Berk. in *Trog. Verz. Schweiz. Schw. Suppl.* **2**: 52, 1850. **Fig. 11**

Basidiomata annual, pileate, broadly attached, solitary, semicircular, applanate to convex; pilei up to 8 cm × 3 cm × 15 mm. **Pilear surface** tomentose, azonate, yellowish white (4A2) when fresh**,** brownish orange (5C5) on drying. **Pore surface** brownish yellow (5C8) both when fresh and on drying; pores round to angular, 1-2 per mm. **Context** duplex, brownish orange towards pilear surface, yellowish white (4A2) towards the pore surface, without distinct black line, up to 3 cm in thickness. **Pore tubes** brownish yellow (5C8), up to 1 cm in depth. **Margins** acute, brownish orange (5C5), up to 6 mm in thickness. **Hyphal system** trimitic; generative hyphae hyaline, thin-walled, clamped, up to 2.5 µm in width; skeletal hyphae dominating, thick-walled, up to 5.2 µm in width; binding hyphae abundant, tortuous, intertwined, up to 4.5 µm in width. **Cystidia** and other sterile elements absent. **Basidia** clavate, 4- sterigmate, with a basal clamp, 14 - 17.5 × 5.5-6 µm . **Basidiospores** cylindrical, thin-walled, hyaline, (7-) 7.5 - 8.7 (-9.1) × (2.5-) 3 - 3.5 (-3.7) µm, CN^+, IK^-, Q = 2.5.

Specimen examined: India, Tamil Nadu, Chennai, Guindy**,** 13°00'33"E, 80°14'16"N, MLCASB341, 12.09.2016, Kezhocuyi Kezo.

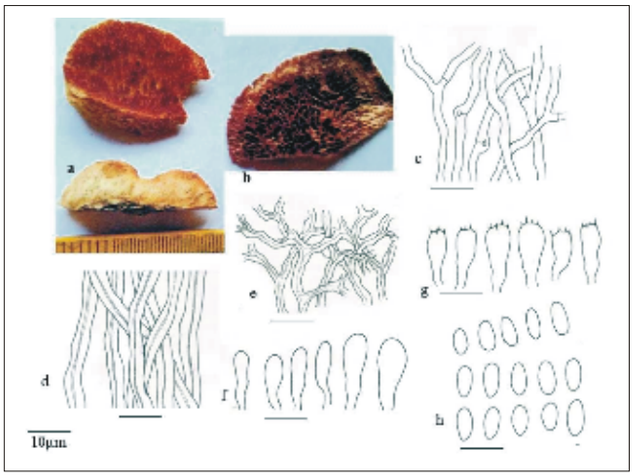

Fig. 11 ***Trametes trogii*****:** a. Basidiomata, b. Pore surface, c. Generative hyphae, d. Skeletal hyphae, e. Binding hyphae, f. Basidioles, g. Basidia, h. Basidiospores.

Remarks: *Trametes trogii* has comparatively thicker pilei with yellowish white to brownish orange, tomentose pilear surface, duplex context which is without black line and larger pores. It has been earlier reported from Himachal Pradesh (Sharma, 2012; Kaur, 2013), Uttarakhand (Sharma, 2012), Maharashtra (Ranadive, 2013). It the first report from the study area.

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The discussion should be logical, precise and clearly relate to the results obtained. Avoid verbosity and lack of clarity. Results and Discussion should be presented separate sections for articles exceeding 3 printed pages. Articles less than 3 printed pages should combine Results and Discussion.

CONCLUSIONS

Brief concluding remarks from the study must be given following discussion, which should be different than the abstract.

ACKNOWLEDGMENTS

Must acknowledge the Institution for the facilities where work has been carried out and the financial support received.

REFERENCES

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Hawksworth, D.L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol. Res.* **105**: 1422-1432.

Samson, R.A. and Varga, J. 2010. Molecular systematic of *Aspergillus* and its teleomorphs. In: *Aspergillus: Molecular Biology and Genomics*. (Eds.: Machida, M. and Gomi, K.). Caister Academic Press, 19-40.

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