Tulasi Satyanarayana
Sunil Kumar Deshmukh
Mukund V. Deshpande *Editors*

Progress in Mycology

An Indian Perspective

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Preface

Mycology is the branch of biology that deals with the systematic study of fungi, including their biology, genetic and biochemical features, taxonomy and phylogeny, and their uses to humans as a source of medicine, food, and psychotropic substances, and their role in poisoning or infection. They are essential for nutrient cycling because of their ability to degrade cellulose, hemicellulose, pectin and lignin, which are major components of plant organic matter. On the other hand, they cause serious human, animal and plant diseases and thus have numerous negative impacts on human life. Fungi are, however, also relatively understudied, but are an essential, fascinating and biotechnologically useful group of organisms with an incredible biotechnological potential for industrial exploitation.

Fungi are a unique group of organisms, different from all others in their behaviour and cellular organisation. The uniqueness of fungi is reflected in the fact that they have the status of a kingdom, equivalent to the plant and animal kingdoms. Extensive efforts have, therefore, been made to estimate the number of fungal species. According to Hawksworth and Lucking (2017), Blackwell (2019) and Wu et al. (2019), 3.8, 5.1 and 12.0 million species, respectively, are present on Earth. Among them, approximately 140,000 have so far been characterised and named [Ann. Rev. Microbiol. 74: 291–313(2020)]. It may take long time to describe all the extant fungal species. Moreover, big chunk of this fungal diversity is non-culturable which can be detected from the sequences of 18S rRNA genes and internal transcribed spacers (ITS). Wu et al. (2019) suggested physical type based on specimens and gDNA type (genome DNA or digital type). Culture-independent methods for species discovery have emerged in the recent years, which provide new insights into fungal diversity. Developments in the next-generation sequencing technologies and bioinformatics permit detection of fungal species from metagenomes/microbiomes. Currently, phylogenetic classification divides the kingdom Fungi into eight phyla/divisions: Ascomycota, Basidiomycota, Mucoromycota, Entorrhizomycota, Zoopagomycota, Blastocladiomycota, Chytridiomycota and ophisto-sporidia [Ann. Rev. Microbiol. 74:291–313(2020)].

The exploitation of filamentous fungi will contribute significantly to the realisation of a secure, sustainable and bio-based future. To achieve this, the following key questions and challenges may need to be answered:

1. How do we exploit filamentous fungi in more efficient and sustainable ways in biotechnological, pharmaceutical and agrochemical industries?
2. What is the best way to develop new antifungals and sustainable strategies to fight plant and human pathogens?
3. How can synthetic biology contribute to the design of optimised fungal genomes?
4. What are the optimal approaches to analyse and provide access to fungal-omics data to realise their full potential?
5. What are fungal model organisms good for?
6. Which technologies need to become developed and integrated to better understand fungal growth and development?
7. Which training programs are key for the next generation of fungal scientists?

Attempts are being made globally to understand the diversity of culturable and non-culturable fungi in a great variety of environments. Fungi were cultured from samples collected from 174 m deep Soudan iron ore mine (Minnesota, USA). The ITS region was sequenced for identification and phylogenetic analysis. Ascomycota are the dominant fungi followed by Basidiomycota and Mucoromycota. Out of 164 identified taxa, 108 belong to the Ascomycota and 26 and 31 to Basidiomycota and Mucoromycota, respectively. There are also 46 taxa that do not match (<97% BLAST GenBank identity) the sequenced fungal species. The mine environment is a relatively extreme environment for fungi, with the presence of high levels of heavy metals, complete darkness and poor nutrient availability [PLoS One (2020) https://doi.org/10.1371/journal.pone.0234208].

From the rock samples of Antarctica Island, 386 fungal isolates belonging to 20 genera have been recently isolated. The predominant fungal genera were *Cladophialophora*, *Cladosporium*, *Cyphellophora*, *Eichleriella*, *Paracladophialophora*, and *Penicillium.* The Antarctic Peninsula region appears to be under the effects of global climate changes, which may expose and accelerate the rock weathering processes [Extremophiles (2019) 23:327–336].

There is a possibility of developing novel applications of fungi. For instance, mycelium of the mushroom *Lentinula edodes* was developed with enhanced selenium (Se) content that was found to be safe to calves based on cytotoxicity tests [PLoS ONE 15 (5):e0233456 (2020)].

The calves fed with selenium-enriched *L. edodes* mycelium had higher body weight gains than those of the control. The administration of *L. edodes* enriched with selenium had a beneficial effect on the health of calves.

A Canadian startup called Anahit Therapeutics is constructing a large psilocybin cultivation farm in Jamaica, where it hopes to grow psychedelic mushrooms to use in new pharmaceuticals. It was planned to build modular cultivation facilities that not only grow the psychedelic mushrooms but also extract their active ingredient, psilocybin. Doctors have investigated psilocybin's ability to treat myriad conditions ranging from depression to eating disorders, and this new facility is a major bid to bring those treatments to reality. According to a recent press release, high-tech mushroom-growing facilities with tightly controlled environments that start by implanting spores onto a sterile substrate yield harvestable mushrooms within 4–9

weeks. Each facility can generate just over 34 pounds of psilocybin per year; Anahit plans to build five more facilities once this first one is done, bringing the total yield to about 172 pounds.

There are immense possibilities in producing useful products such as drugs by synthetic biology. For instance, Srinivasan and Smolke recently developed a strain of *Saccharomyces cerevisiae* by genetic engineering that converts simple sugars and amino acids into two tropane alkaloids, hyoscyamine and scopolamine; these block the action of the neurotransmitter molecule acetylcholine and are thus useful in the treatment of nausea, gastrointestinal problems, excessive bodily secretions and neuromuscular disorders like Parkinson's disease [Nature 585:614–619 (2020)].

Investing in basic research may seem, at first sight, a costly affair. There are, however, numerous examples of fungal diversity in the past demonstrating as to why investing in basic research pays off in the long run, and even more reasons, why it is today more important than ever to renew an interest in basic research on fungi. With the new trend to a more sustainable, health-oriented living, and constant reports of hazardous chemicals found in food and cosmetics, the demand for more ecological, more 'natural' alternatives is high. This is again where fungi can step in.

In India, mycologists like C.V. Subramanian, K.G. Mukherji, K.S. Thind, S.B. Saxena, M.N. Kamat, M.J. Thirumalachar and several others and their students have made significant and praiseworthy contributions in understanding the various aspects of fungi, including diversity and role in different ecosystems, and their beneficial and detrimental activities.

In the present book, an attempt has been made to discuss developments in mycology with a special emphasis on the progress made in mycology in India. The book is divided into three parts. Part I includes five chapters on historical developments in mycology, marine mycology, fossil fungi, plant pathology and culture collections in India; V.V. Sarma, B.D. Borse and Sarma, R.N. Kharwar and coworkers, A.K.M. Tripathi and S.K. Singh have made herculean efforts in describing history and developments on different aspects of fungi, respectively. Part II includes ten chapters contributed by distinguished mycologists on symbiotic and pathogenic fungi. Part III includes six chapters on different groups of fungi, such as soil fungi, aquatic hyphomycetes, Myxomycetes, yeasts, mushrooms and thermophilic fungi. We sincerely wish that the book will be useful to students, scholars, teachers and scientists working in broad areas of botany, microbiology, biotechnology and life sciences.

We are grateful to all contributors for readily accepting our invitation and contributing well-written chapters within the stipulated time in their areas of expertise and specialisation. And thanks are also due to Springer Nature for publishing the book.

New Delhi, Delhi, India — Tulasi Satyanarayana
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Editors and Contributors

About the Editors

Tulasi Satyanarayana has been Professor Emeritus at the Division of Biological Sciences & Engineering, Netaji Subhas University of Technology, New Delhi after superannuating from the University of Delhi in June 2016. He has over 280 scientific papers and reviews, 11 books and 3 patents to his credit. He is a fellow of the National Academy of Agricultural Sciences (NAAS), Academy of Microbiological Sciences (AMSc), Biotech Research Society (I) [BRSI], Mycological Society of India (MSI) and Telengana Academy of Sciences. He is a recipient of Dr. Manjrekar award of AMI, Dr. Agnihotrudu Memorial award of MSI and Malaviya Memorial award of BRSI for his significant contributions. He mentored 30 scholars for Ph.D., and was president of AMI and MSI. He successfully completed 17 major research projects sanctioned by various Govt. agencies. His research efforts have been focused on microbial diversity and enzymes, metagenomics, carbon sequestration and bioethanol production from lignocellulosics.

Sunil Kumar Deshmukh received his Ph.D. in Mycology from Dr. H.S. Gour University, Sagar (M.P.) in 1983. A veteran industrial mycologist who spent a substantial part of his career at Hoechst Marion Roussel Limited [now Sanofi India Ltd.], Mumbai and Piramal Enterprises Limited, Mumbai in drug discovery. He has to his credit 8 patents, 125 publications and12 books on various aspects of Fungi and natural products of microbial origin. He was the president of the Mycological Society of India (MSI). He is a fellow of MSI, the Association of Biotechnology and Pharmacy, the Society for Applied Biotechnology and the Maharashtra Academy of Sciences. He was the Fellow at Nano-Biotechnology Centre, TERI, New Delhi, and Adjunct Associate Professor in Deakin University, Australia till Jan. 2019 who had been working on the development of natural food colours, antioxidants and biostimulants through nanotechnology intervention.

Mukund V. Deshpande obtained his Ph.D. in 1982 in Biochemistry and D.Sc. in Microbiology of the University of Pune in 1994. He has been working extensively on the use of fungi and fungal products in Biotechnology. Dr. Deshpande successfully completed more than 35 research projects funded by national and international funding agencies like Indo-Swiss Collaboration in Biotechnology (ISCB) for

development of mycoinsecticide, Indo-Belarus programme of DBT on biopesticides, Indo-Mexico programme on fungal dimorphism, to name a few. Dr. Deshpande is an elected fellow of Maharashtra Academy of Sciences (FMASc, 1994) and the Society for Biocontrol Advancement (FSBA, 2010). He has to his credit more than 150 research papers, reviews and chapters, 8 patents, 8 books and a number of popular articles. He has his own start-up Greenvention Biotech located in Urli-Kanchan, Pune for the translational activities in Agricultural Biotechnology.

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Part I

History and Developments in Indian Mycology

Historical Developments in Indian Mycology

1

V. Venkateswara Sarma

Abstract

Mycological developments in India, like other colonial states, had great influence from British mycologists. A great deal of contribution has come from Edwin J. Butler before independence. In fact, it has been conveniently segregated as pre-Butler and post-Butler period to trace the development of mycology in India. Several active centres of mycology were then established. Early part of the nineteenth century was dominated by descriptive taxonomic research followed by fungal plant pathology. This was followed by physiological aspects and recently the biotechnological applications. Recent advances in molecular biology have complemented the biodiversity studies in addition to various other branches of mycology. These aspects are discussed in this chapter.

Keywords

Fungi · E.J. Butler · Taxonomic studies · Applied mycology · Fungal diseases

1.1 Introduction

The mycological research in India could be traced back to the colonial rule of British raj where the initial records of fungi had been reported. The first compilation of mycological surveys came from Butler and Bisby (1931). These initial records from India were basically collections made by others and taken to the UK for further examination. The first fungus to be reported from India was that of *Podaxon pistillaris* (= *Scleroderma pistillare*) collected by Koenig (1767–1785) from Tharangambadi, Thanjavur district, Tamil Nadu, India (Fries 1829; Butler and

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Bisby 1931). When a corvette 'Galathea' berthed at Nicobar Islands for 2 months during its voyage (1845–1847), Didrick Ferdinand collected several specimens and passed them on to Fries who examined and reported in his publication (Fries 1855). At the beginning of the nineteenth century Wight collected a number of fungi in India to be examined later on by Klotzschin 1832, 1833 and Berkeley in 1839 (Berkeley 1839). Montagne (1846) examined specimens sent by Belanger (1825–1829) and by Peroottet from Nilgiri hills, Tamil Nadu (1840), and reported fungi from the collections of India. J. Helfer collected a few specimens which were examined by Corda in 1854. Before 1850 there were only around a hundred fungal species reported from India (Corda 1854). Later Currey (1874) reported some fungi from Myanmar and West Bengal of India. Cooke, who was working at Kew, described a significant number of Indian fungi based on the collections of specimens sent by Robenhorst, Brefeld, Distel and Patouillard (Butler and Bisby 1931). Around the early twentieth century, P. Hennings examined the specimens sent by Gollan in the United Provinces (Hennings 1900, 1901). When Massee published the series 'Fungi Exotici' in the Kew Bulletin, the treatise included several fungi from India also. Theissen reported fungi from Bombay based on the collections sent by his colleague Father E. Blatter (Theissen 1909). Some of the fungi reported by Saccardo also included fungi from India for which he had access. During the first two and half decades of the twentieth century, the father and son duo of P. Sydow and H. Sydow examined hundreds of fungi from India sent from Pusa and Coimbatore, India. C.G. Lloyd collected the larger fungi from different parts of India and deposited in some of the larger herbaria (Butler and Bisby 1931). While the above account mainly deals with collections of specimens from India and their examination and identification in England, some work also was simultaneously carried out in India itself. For example, two medical officers of the Indian army contributed a pioneering work. Of the two, D.D. Cunningham published fungi belonging to Mucoraceae and Uredinales from Calcutta and other parts of eastern India. A. Barclay worked extensively on rusts in Simla and worked out the life histories of several Uredinales of Himalayas. After Barclay's death, Sir George Watt made observations on fungal diseases of tea and other crops. Butler, who arrived in Calcutta in 1901, was transferred to Dehra Dun in 1902 and Pusa in 1905 and officially attached to Pusa Institute up to 1920 with mycology, particularly the parasitic fungi. He was assisted by W. McRae, F.J.F. Shaw, J.F. Dastur and others. Later on the agricultural departments in various provinces of India included studies of plant diseases, and hence mycology also flourished to some extent in dealing with fungal diseases. The compilation by Butler and Bisby (1931) shows that there were 2351 fungi recorded from India, including 384 rusts, 69 cup fungi (Discomycetes), 394 Pyrenomycetes, 13 Hemiascomycetes, 100 Ustilaginales (smuts), 318 Polyporalean members, 462 other Hymenomycetes, 75 Gasteromycetes, 196 Hyphomycetes and Mycelia sterilia, 265 Melanconiales and Sphaeropsidales (coelomycetes), 12 Archimycetes, 4 Chytridiales, 45 Oomycetes and 14 Zygomycetes. For the above account, the author mostly referred the compilation of Butler and Bisby (1931), and liberally used the information provided therein. Other than the above, Butler's monograph on *the* genus *Pythium* (Butler 1907) and the book on Fungi and Disease in Plants (Butler

1918) were classic contributions. Subramanian (1986) mentioned that 'the development of mycology in India owes a great deal to E.J. Butler' and highlighted 'Butler's contributions to mycology and applied mycology were not only basic and fundamental but also relevant to the needs of a country where a balanced development of the twin disciplines of mycology and plant pathology was of utmost importance'. The mycological developments in India are discussed broadly as pre-Butler and post-Butler periods.

Though Butler left India in 1921, much of the work immediately followed was stimulated by him. Some of the examples include studies on *Phytophthora* by J.F. Dastur, *Cerebella* by L.S. Subramaniam, and fungal diseases of agricultural and plantation crops such as coconut, arecanut, coffee and tea by L.C. Coleman, S. Sundararaman, W. McRae, M.J. Narasimhan and A.C. Tunstall (Subramanian 1986). The other important works were on polypores by S.R. Bose, smuts by B.B. Mundkur, wheat rust epidemiology by K.C. Mehta, tree rusts by K.D. Bagchee, Gasteromycetes particularly phalloids by C.G. Lloyd, graminicolous *Helminthosporium* spp. by M. Mitra, downy mildews particularly *Sclerospora* by B.N. Uppal, and *Fusarium* by G.W. Padwick.

1.2 Post-Colonial Period (Post-Butler Period)

Subramanian (1986) mentioned that the period 1950–1975 was one of the intense mycological explorations. Major contributions on rusts and smuts came from Mundkur and M.J. Thirumalachar, additions of fungi to Madras from T.S. Ramakrishnan and K. Ramakrishna, ascomycetes from M.N. Kamat and his students, and larger fungi including the Clavariaceae, the agarics and the Discomycetes from K.S. Thind and his associates. Further group explorations included those on Cercosporae and Meliolinae by Thirumalachar, C.G. Hansford; *Synchytria* by B.T. Lingappa; *Pythium* by M.S. Balakrishnan; *Phytophthora* by K.M. Thomas, T.S. Ramakrishnan; the Mucorales, the Aspergilli and Penicillia by B.S. Mehrotra; Fusaria by C.V. Subramanian; The Hyphomycetes by C.V. Subramanian and his associates; the Coelomycetes by K. Ramakrishnan, C.V. Subramanian and K.R.C. Reddy; the lichens by D.D. Awasthi; and the Myxomycetes by V. Agnihotrudu, K.S. Thind, Indira Kalyanasundaram and T.N. Lakhanpal.

This section is divided into major groups like Basidiomycetes, Ascomycetes, Lower fungi, Plant pathology and mycology and Miscellaneous groups. Several mycologists have summarized in their reviews. Several fungal genera or groups have been monographed; thanks to the support that came from the Indian Council of Agricultural Research due almost entirely to the keen interest and liberal backing of its Vice-President M S Randhawa. These include The Clavariaceae of India by Thind (1961), Indian Cercosporae by R S Vasudeva (1963), The Mucorales of India by R N Tandon (1968), Indian Polyporaceae by Bakshi (1971), Hyphomycetes by C V Subramanian (1971) and The Myxomycetes of India by K S Thind (1977). The publication of these monographs on fungi (and others on algae), which Randhawa

vigorously supported with great foresight, imagination and dedication, is undoubtedly one of the most significant turning points in the development of mycology (and algology) in India. In addition, mention should be made of the following monographs: Ustilaginales of India by Mundkur and Thirumalachar (1952), The Chytrids of India with a supplement of other zoosporic fungi by J S Karling (1966, Sydowia, Beih.6), Indian Myxomycetes by T N Lakhanpal and K G Mukerji (Lakhanpal and Mukerji 1981, Cramer), The Resupinate Aphyllophorales of the Northwest Himalayas by S S Rattan (1977, Cramer) and South Indian Agaricales by K Natarajan and N Raman (1983, Cramer). The contributions on various groups of fungi are elaborated in the following pages.

1.3 Basidiomycota

1.3.1 Agaricales

The position on Indian Agaricales was reviewed by **Sathe** and Rahalkar (1975). Later **Manjula** (1983) provided an exhaustive list of Agaricoid and Boletoid fungi from India and Nepal. From India, the following centres and researchers have contributed mainly on mushroom diversity: (a) Sathe and co-workers from South West India (Sathe and Rahalkar 1975), (b) **Kapoor** and associates in and around Delhi, Rawla and his students, (c) **Saini** and **Atri** and their students in North India and **Lakhanpal** and his co-workers, (d) **Kaul** and his associates in Himalayan region and (e) **K. Natarajan** and co-workers from South India. In the north-western Himalayas a lot of work has been done (Lakhanpal 2004). Several contributions have come from this region. These include Watling and Gregory who have provided a comprehensive list of taxa from Jammu and Kashmir (vide Lakhanpal 2004). Abraham (1991) provided a list of agarics along with ecological notes on 250 species from Kashmir; Lakhanpal (1995) concluded that the diversity is less when compared to the vastness and diversity in the mountainous range. A checklist of Indian Russulaceae was provided by Atri et al. (1994). A list of 94 taxa belonging to 24 genera from Punjab was recorded by Saini and Atri (1985). Bhavani Devi (1995) reviewed the work on mushrooms from Kerala. A list of 212 species of agarics belonging to 63 genera was provided by Patil et al. (1995) from Maharashtra. Similarly, a list of 95 species of mushrooms was reported by **Verma** et al. (1995). **NKS Harsh** of the Forest Research Institute, Dehradun worked on mushroom diversity from Dehra Dun. He and his associates are developing a Red List of India's fungi. He has worked on ethnomycology to improve tribal economy in Madhya Pradesh and Jharkhand (Harsh et al. 1993; Harsh and Tiwari 1996). **BRM Vyas** studied the physiological and biotechnological aspects of polyporalean members (Vyas and Molitoris 1995).

K. Natarajan, who was with the CAS in Botany as Professor at the Department of Botany, University of Madras, Chennai, established an active group to work extensively on mushroom diversity of South India which was hitherto a neglected region (Natarajan 1975). Natarajan (1995) listed 457 species of agarics belonging to

76 genera from South India excluding Kerala. He along with his co-workers published several new genera and new species: *Hemimycena indica*, *Pulveroboletus parvulus*, *Agricochaete indica*, *Hebelomina maderaspatana* and *Marasmius nilgiriensis*, *Gymnopilus giganteus, Gymnopilus minutosporaus, Kuehneromyces terrestris, Melanotus macrosporus, Phaeomarasmium globisporus, Panaeolus microsperma, P. annulotus, P. setulosus, Pholiota truncate, Psathyrella cordispora, Psathyrella kodaikanalensis, Psilocybe gigaspora* and *P. guzmanii*, *Eutoloma nilgiriensis* and *E. indica*. **V. Kaviyarasan**, who did a Ph.D. under K. Natarajan from CAS in Botany, University of Madras, studied the mushroom diversity from South India and reported several interesting species (Kumar and Kaviyarasan 2011). From the same institution, **Malarvizhi Kaliyaperumal** is continuing the diversity of mushrooms by using molecular sequence analyses too. She has published her work in several journals (Kaliyaperumal 2013; Kaliyaperumal et al. 2018).

S.S. Saini worked as a Professor in the Department of Botany, Punjabi University. **N.S. Atri** carried out his Ph.D. under S. S. Saini (Saini and Atri 1984). Together they have published several papers on mushrooms of North West India. N.S. Atri has taken up a faculty position in the Department of Botany, Punjabi University, Patiala, Punjab, and worked until his retirement as Professor in 2019. He has published several monographs and papers on mushroom diversity, more particularly on Russulaceae (Atri 2020). During his long years of research, he and his co-workers investigated the families of Agaricaceae, Amanitaceae, Bolbitiaceae, Tricholomataceae, Polyporaceae, Lyophyllaceae, Coprinaceae, Pluteaceae, Entolomataceae, Inocybaceae, Marasimaceae, Mycenaceae, Pleurotaceae, Psathyrellaceae, Strophariaceae and Russulaceae. These studies resulted in 1700 collections belonging to 700 taxa, of which 250 were described for the first time and 34 were new to science including *Macrocystidia indica*, *Russula minutalava. rrobusta, Russula natarajanii, Lactarius annulocystidiatus, Chlorolepiota indica* and *Chlorolepiota brunneotincta* (Atri et al. 2016), to mention a few. His lab also extensively studied on the exosporial ornamentation at SEM level on taxa belonging to *Russula, Lactaria* and *Lactifluus* (vide Atri 2020). **Munruchi Kaur,** who did Ph. D. under N.S. Atri on mushroom diversity from North West India, is continuing her studies from this region (Kaur et al. 2011). **G.S. Dhingra** has been investigating on the biodiversity of mushroom fungi working from the Department of Botany, Punjabi University (Dhingra and Singh 2008). Along with his co-worker **Avneetpal Singh** of the same university, he had published several papers (Singh et al. 2010).

K.B. Vrinda of Tropical Botanic Garden & Research Institute, Kerala, along with her co-worker **C.K. Pradeep** has been working on mushroom diversity of Kerala; they had published several papers (Vrinda et al. 2005); this group published new species in the genera *Plutues* and *Volvariella*. They had also surveyed the ectomycorrhizal fungi in three different forest types of Western Ghats forests of India (Pradeep and Vrinda 2010). **Yashpal Sharma** from the University of Jammu has been working on both diversity and ethnomedicinal aspects of mushroom fungi from Jammu and Kashmir and published several papers (Kumar et al. 2015). **Krishnendu Acharya** in the Department of Botany, University of Calcutta, has been working on both morphological and molecular diversity of mushroom fungi in

addition to the bioactive compounds produced by different fungi (Acharya et al. 2012). **Kanad Das** affiliated to Botanical Survey of India surveyed mushroom diversity, particularly the Russulales in the Sikkim hills. His studies also included the ectomycorrhizal fungi. He, along with his co-workers, had published several new species, particularly of the genus *Russula* (Das et al. 2008, 2010).

1.3.2 Polyporales

A Comprehensive Treatise on Indian Polyporaceae was provided by **S.R. Bose** through a series of papers from Bengal published in 11 parts and 143 supplements from 1919 to 1946 (Bose 1934, 1944, 1946, see Ranadive 2013). His studies on the effects of radiation of some polypores and nature of pigments in coloured polypores were one of the pioneering works on Polyporales (Aneja and Mehrotra 2011). Other important contributions of Aphyllophorales came from several mycologists. **Bagchee** (1961) worked as a mycologist at the Forest Research Institute, Dehradun; he worked on many aspects of mycology including biology and pathology of rust fungi on conifers, soil borne diseases and their control, and timber decay and their control (Bagchee and Bakshi 1950). **B.K. Bakshi** along with associates worked on Aphyllophoralean fungi. He was working in Forest Research Institute and College, Dehradun. He also published a monograph on Indian Polyporaceae on trees and timber (Bakshi 1971) and a book titled 'Forest Pathology: Principles and Practice in Forestry' (Bakshi 1976). **K.S. Thind** at Punjab University, Chandigarh, initiated research on the systematics of Basidiomycota including Aphyllophorales and other groups (Thind 1980). Thind and his students studied extensively the polyporalean diversity from India (Thind 1975) from Mussoorie hills and North Western Himalayas. Though polyporoid fungi have very hard basidiomata due to the presence of skeletal and binding hyphae, he worked on those rare members of Aphyllophorales that have monomitic hyphal system with inflated generative hyphae that impart fleshy texture (Thind 1976). Hyphal pegs occur as clusters of sterile and erect hyphae projecting out from the hymenial surface of some polypores supposedly originating from the tramal or subhymenial regions. The hyphal pegs vary in size, shape and arrangement and are important at specific level in several genera. Thind and Anand (1956) reported fascicles of sterile hyphae originating from the context and protruding beyond the hymenium in *Clavulina hispidulosa.* **K. Natarajan** and his students (Natarajan and Kolandavelu 1985) from CAS in Botany, University of Madras, studied the Aphyllophorales. **J.G. Vaidya** and his students made ecological observations of polypores in addition to looking into their medicinal value (Vaidya 1987). He also published a book on 'Biology of Fungi' (Vaidya 1995). **J.R. Sharma** of Botanical Survey of India surveyed Hymenochaetaceae of India and also published genera of Indian Polypores (Sharma 1995, 2000). In addition to the above, several monographs and books have also been published: 'Polypores of Kerala' was published by **Leelavathy** and Ganesh (2000). **Hakimi** et al. (2013) recently published a book on 'Resupinate Aphyllophorales of India'. Prasher (2015) published a book titled 'Wood-Rotting Non-Gilled

Agaricomycetes of Himalayas'. A checklist of the 256 species of aphyllophoraceous fungi from Maharashtra State was recorded by **Ranade** et al. (2012).

M.J. Thirumalachar and **B.B. Mundkur** published the first monographic account on rusts (Thirumalachar and Mundkur 1949). Thirumalachar studied the spore taxonomy and spore morphology in rust fungi (Thirumalachar 1969) in addition to the cytological studies (Thirumalachar and Govindu 1954) and he also published a new Uredinalean genus *Elateraceium* (Thirumalachar and Patil 1966). **P. Ramachar**, working at Mycology and Plant Pathology Research Laboratory, Department of Botany, Osmania University, Hyderabad (Telangana), investigated on the diversity of rusts in South India, particularly from Hyderabad (Ramachar and Salam 1954; Salam and Ramachar 1955; Ramachar et al. 1978; Ramachar 1990).

1.4 Ascomycota

In addition to work on the anamorphic fungi, particularly the hyphomycetes, **C.V. Subramanian** also initiated work on ascomycetes at the Centre for Advanced Studies in Botany, University of Madras. He and his associates including **B.C. Lodha** and **K.V. Chandrasekhara** started off with coprophilous fungi and fungal succession on dung. Several interesting ascomycetous fungi were described from the studies on dung and excreta of animals (Subramanian and Lodha 1968; Subramanian and Chandrasekhara 1976). In the mid-1970s, an interest had been developed on teleomorph (sexual state) and anamorph (asexual state) connections as part of the whole fungus (holomorph) studies (Subramanian 1979, 1980). Developmental morphology of anamorph and teleomorphs of a number of taxa in the Erisiphales, especially the Aspergilli, were studied by Rajendran (Subramanian and Rajendran 1981). These authors found that there is a whole range of forms including naked clusters of asci (*Edyuillia*) to forms with typical cleistothecia (*Eurotium*, *Chaetosartorya*, *Neosartorya*) to stromatic forms. **D.J. Bhat**, who did Ph.D. (1979) under C.V. Subramanian, studied the developmental morphology of Hypocreales. Several of the taxa they studied showed typical *Nectria* type centrum and ascomatal development with conidial states being moniliaceous and characterized by the *Trichoderma* type of phialoconidiogenesis. **D. Ananthapadmanabhan** studied the Diaporthales and Diatrypales from South India and published new genera and new species (Ananthapadmanabhan 1988). He joined the Institute of Microbial Technology (IMTECH) and worked in the Microbial Type Culture Collection Centre (MTCC); he has just retired after long years of service. **G. Sekar** studied another interesting group Coronophorales, where several anamorphs were linked to their teleomorphs. Also, they could establish such links to species in the genera *Helminthosporum, Diplococcium, Mammaria, Trichocladium* and *Moorella*.

M.N. Kamat, working at the University of Poona, and later from Agarkhar Research Institute, Pune, contributed in the area of plant pathology a great deal on ascomycetous pathogens. Kamat et al. (1978) monographed the genus *Phyllachora.* He has published two books on plant pathology entitled 'Practical Plant Pathology'

(Kamat 1953) and 'Introductory Plant Pathology' (Kamat 1956) and two volumes on Handbook of Mycology Vol. I and II (Kamat 1959, 1961). **K.G. Mukherji**, working at the University of Delhi, worked on several aspects of fungi. He published several new species along with his associates. For example, Mukerji et al. (1969) studied the Indian species of *Xylaria* and allied genera from Delhi. The genera *Xylaria,* and *Hypoxylon* were studied by Thind and Waraitch (1969) in West India from Himalayan region; taxonomic characterization of 193 osmophilic and osmotolerant yeasts was undertaken by **Kumbhojkar** (1969, 1972) that resulted in the isolation of *Saccharomyces rouxii, S. bisporus, Schizosaccharomyces octosporus, S. sloofffiae* and *Torulopsis etchellsii*. Major contributions on the family Clavicipitaceae came from Govindu and Thirumalachar (1963), Srinivasan (1963), Kulkarni (1963), Thirumalachar (1969) and Ullasa (1971). Several new genera belonging to Ascomycetes and other groups have been described from India. A few examples include *Achaetomium* (Rai et al. 1964), *Tripterosporella* (Subramanian and Lodha 1968), *Muelleromyces* (Anahosur 1968/1969) in Sphaeriales (= Sordariomycetes).

J.S. Dargan, who was a Professor in the Department of Botany, Punjabi University, Patiala, Punjab, extensively worked on the family Xylariaceae from Northern India. Dargan (2006) reviewed the status and progress of family Xylariaceae in India. He along with his co-workers published the diversity of Xylarialean fungi mainly from Northwest Indian regions. They studied the genera *Daldinia, Rosellinia, Hypoxylon, Xylaria, Kretzschmaria* and *Helicogermslitia* and reported more than 100 taxa belonging to these genera (vide Dargan 2006). Agharkar Research Institute, Pune, has become one of the important centres from where contributions had been made on xylariales, including Anahosur (1968/1969), **Alaka Pande** (Pande 1984) and Pande and Rao (1995). These authors, in a series of papers, published on the Xylariaceae of Western India including eight new species belonging to *Anthostoma*, *Anthostomella*, *Rosellinia*, *Hypoxylon* and *Xylaria*. Alka Pande published a book on 'Ascomycetes of Peninsular India' (Pande 2008) and included brief details of more than 1500 taxa from Peninsular India.

V.B. Hosagoudar, who was working at Tropical Botanical Research Institute, Peechi, Kerala, surveyed extensively two groups of leaf colonizing ascomycetes, viz. Asterinales and Meliolales, and published several new genera and new species in these two groups (Hosagoudar 2010, 2012; Hosagoudar 2013). He not only worked on the mainland but also surveyed Meliolales from Andaman Islands (Hosagoudar 2013). Also, he worked on Asterinales in India (Hosagoudar 2012).

V. Venkateswara Sarma, who is working in the Department of Biotechnology, Pondicherry University, has initiated morphological and molecular studies of fungi from marine and terrestrial habitats focusing on Ascomycetes group. Several new genera and new species have been described from his lab (Devadatha et al. 2018, 2019; Phookamsak et al. 2019; Hongsanan et al. 2020; Niranjan and Sarma 2018).

Praveen Gehlot, who is working as a Professor at CAS in Botany, Jai Narayan Vyas University, Jodhpur, Rajasthan, has surveyed microfungi from Rajasthan and published several new species of Ascomycetes. He has also worked on the anamorphic fungi. He along with his group published a book on 'Pyrenomycetes Fungi' (Kaur et al. 2010).

1.5 Anamorphic Fungi

The asexual morphs of ascomycetes and basidiomycetes can thrive for several generations without resorting to the sexual mode. This has made the early mycologists to include a provision in the ICBN code to have separate names for asexual states. Now this provision has been removed with 'one fungus, one name' provision brought into the Melbourne code wherein irrespective of the states in which a fungus has been recorded, it will be recognized with only one name. However, in the past, due to the then prevailing situation, a separate classification has been advanced for the benefit of mycologists. C.V. Subramanian has been one of the pioneers to propose a classification for anamorphic fungi particularly the hyphomycetes (asexual or mitosporic fungi that are not enclosed within the fruit bodies).

C.V. Subramanian got his Ph.D. working under the supervision of T.S. Sadasivan in 1948 for his thesis on 'Soil Conditions and Wilt Diseases in Plants with Special Reference to *Fusarium vasinfectum* Atk. on Cotton'. Later on, in 1957, he received the D.Sc. from Madras University for his published work on 'Floristic and taxonomic studies on fungi Imperfecti'. Professionally he held different positions at IARI, New Delhi, and Department of Botany, University of Rajasthan, before finally moved to the University of Madras as Professor of Botany and later became Director of Centre of Advanced Study in Botany (1964–1985). After his post-doctoral work at Kew under Mason, he started exploration of hyphomycetes and other groups in India and South East Asian nations. This pioneering work resulted in the publication of several new genera and new species of hyphomycetes that were finally compiled into a monograph on 'Hyphomycetes' published by the Indian Council of Agricultural Research in 1971. In fact, this masterpiece laid a foundation for his subsequent explorations along with his students and co-workers in India on hyphomycetes that also made a cementing effect and lasting connectivity with the second- and third-generation mycologists in India (Bhat and Vittal 2014). He established the Mycological society of India (MSI) in 1973 during the International Symposium on Taxonomy of Fungi held at the CAS in Botany, University of Madras. Also, he founded the journal KAVAKA, which means a fungus in Sanskrit, being the transactions of the MSI and served as its editor until 1998. He supervised 24 Ph.D. students on various aspects of mycology. He is fond of giving Sanskrit names to genera and species of fungi. Although many are there a few are presented here: *Angulimaya, Ashtaanga, Bahusaganda, Bahusandhika, Bahusakala, Bahusutrabeeja, Drumopama, Dwayabeeja, Dwayaloma, Koorchaloma, Kutilakesa, Lomachasaka, Nalanthamala, Paathramaya, Prathiigada, Prathoda, Tharoopama* and *Vakrabeeja.* Subramanian discussed on the concepts of characters for classification of hyphomycetes and proposed a hierarchical system of classification in 1962 for hyphomycetes by recognizing six basic spore types, viz., blastospore, gangliospore, porospore, phialospore, arthrospore and meristem arthrospore, and raising six families correspondingly: Torulaceae, Bactridiaceae, Helminthosporaceae, Tuberculariaceae, Geotrichaceae and Coniosporiaceae (Subramanian 1962). He opined that based on conidium ontogeny, most of the

genera of Hyphomycetes could be accommodated in the particular families (Subramanian (1971)). He revised the hierarchical system of classification of hyphomycetes in 1983 by bringing out another volume titled 'Hyphomycetes: Taxonomy and Biology' (Subramanian 1983). His group also pursued developmental morphological studies of different taxa including those belonging to Hypocreales and Eurotiales. Also, it was around that time itself it was felt that there should be attempts to bring in unitary naming and classification of fungi with more importance given to holomorphs based on connecting both anamorph (asexual state) and teleomorph (sexual state) based on cultural studies. Hence, he and his co-workers were one of the pioneers to attempt to connect the sexual and asexual states through cultural studies. However, since only a few fungi sporulate in cultures and that too in majority of the cases only asexual states, it became difficult to provide such connections during that time to all fungi (Subramanian and Bhat 1977a, b, 1978, 1983). Now, we know that with the advent of molecular sequence analyses, the sexual and asexual states could be connected. Subramanian also wanted to develop mycology in a large way in India and Tropical countries, and he expressed his views through a status report on biodiversity of fungi published in a couple of articles (Subramanian 1986, 1992a, 2007). He initiated revisions of important fungal genera and major groups. For example, he revised the genus *Sporidesmium* and raised several new genera: *Ellisembia, Stanjehughesia, Reptophragmia, Penzigomyces, Acarocybellina, Gangliophora* and *Hemicorynesporella* (Subramanian 1992b). Also, Subramanian and Sekar (1990) surveyed the ascomycete order Coronophorales along with its asexual states in India and reported 14 genera in the family with a monographic account.

B.P.R. Vittal obtained Ph.D. under C.V. Subramanian on leaf litter fungi (Subramanian and Vittal 1979, 1980). He took up a faculty position at CAS in Botany and pursued research on the diversity of fungi colonizing decomposing leaf litter in terrestrial ecosystems and marine fungi colonizing woody litter in mangroves. Apart from studies on taxonomy and ecology of litter fungi, BPRV initiated aerobiological studies for the first time and developed an active school of aerobiology. Along with several of his students, he carried out work on fungal diversity colonizing leaf litter of different plants and published several papers on new fungi in addition to the ecological observations. With **M. Dorai** he published several new species and new genera including *Civisubramaniania eucaliptii*, *Cercospermalongispora, Dactylaria eucalypti, Minimidochium indicum, Kellermania intermedia* and *Stachybotrys ramose* collected from *Eucalyptus* litter (Dorai and Vittal 1988; Vittal and Dorai 1991). Later on, along with several of his students, he surveyed the biodiversity of leaf litter fungi from South India, particularly the Eastern Ghats of Tamil Nadu (Shanthi and Vittal 2010; Shanthi and Vittal 2012). Apart from his studies on litter fungi from terrestrial habitats, BPRV also initiated studies on diversity and ecology of marine fungi in mangroves from east of India including Cauvery delta, Tamil Nadu (Ravikumar and Vittal 1996), and Godavari and Krishna deltas, east coast of India (Sarma and Vittal 2001, 2004).

D.J. Bhat did his doctoral studies under C.V. Subramanian (1976) at CAS in Botany, University of Madras, and a project under him that resulted in several

publications (Subramanian and Bhat 1978, 1981, 1983). After holding an academic position in Ethiopia, he took up a faculty position in the Department of Botany, University of Goa, Goa, and retired as Professor and Head of the department in 2010. During this time, he and his co-workers explored the wet evergreen forests of Western Ghats in Silent Valley in the Kerala State for fungi and other places in south India (Bhat and Kendrick 1993; Bhat 2008). These and other publications of his work not only resulted in the establishment of several new genera and more than 70 new fungal species but also reported several new records from India on hyphomycetes which are now consummated into Ascomycota. Bhat had established a fungal culture repository in the Goa University and added different fungal cultures of different fungal groups to the inventory.

J. Muthumary did her Ph.D. on Coelomycetes of South India under C.V. Subramanian. She continued her studies concentrating on coelomycetes and published a few monographs and books including a 'Monograph of *Septoria* species in India' (Muthumary 1999) and 'Indian Coelomycetes' (Muthumary 2013).

Kamal, who is with the Department of Botany, Deen Dayal Upadhya Gorakhpur University, Gorakhpur, U.P., India, has been working on anamorphic fungi. He published a book on Cercosporoid fungi (Kamal 2010). He along with his co-workers published several new taxa belonging to the group hyphomycetes (Kamal et al. 1980a, b; 1981; Braun and Crous 2003; Kumar et al. 2007; 2012; Meenu 1998; Sharma et al. 2003; Singh and Kamal 2012).

M.K. Rai has retired as the Professor and Head of the Department of Biotechnology, Amaravati University, Maharashtra. He conducted research extensively on the coelomycetous fungi particularly the *Phoma* spp. (Rai 1989) and has published a treatise on *Phoma* (Rai 1998) in addition to publishing other books (Deshmukh and Rai 2005).

Presently biodiversity studies on leaf litter fungi are carried out at ARI, Pune, and at a few other centres. **S.K. Singh, P.N. Singh** and **K.C. Rajesh Kumar** are working in this field at ARI, Pune (Singh and Singh 2016; Singh et al. 2017; Rajeshkumar and Sharma 2013; Rajeshkumar et al. 2016). **Rashmi Dubey** of Botanical Survey of India, Pune, India, has been working on anamorphic fungi and published several papers in this area (Dubey 2016; Dubey and Pandey 2019). **Shambhu Kumar** is another mycologist presently working on anamorphic fungi at the Kerala Forest Research Institute, India. He had described several new species along with his co-workers (Singh et al. 2013).

D.J. Bhat also studied the aquatic hyphomycetes from Western Ghats, South India (Subramanian and Bhat 1981). **K.R. Sridhar** in the Department of Biosciences, Mangalore University, Mangalore, Karnataka, studied elaborately the diversity, ecology and physiology of aquatic hyphomycetes from Western Ghats streams (Sridhar and Kaveriappa 1992). **Suresh Chandra Sati** of the Department of Botany, Kumaun University, Nainital, U.P., worked extensively on aquatic hyphomycetes of Kumaun region and published several new records and interesting fungi (Sati and Tiwari 1990).

1.6 Lower Fungi

It was once again E.J. Butler who contributed an article entitled 'An Account of the Genus *Pythium* and Some Chytridiaceae' published in 1907, while working at the Imperial Agricultural Department in Dehra Dun (U.P.) and Pusa (Bihar), as mentioned by Butler and Bisby (1931). He also established a new genus and species, *Allomyces arbuscula,* in the order Blastocladiales in 1911 (Butler and Bisby 1931). Another significant contribution on water moulds came from **Chaudhuri** (Chaudhuri 1931; Chaudhuri et al. 1947) in the order Saprolegniales. Several other early workers also contributed on zoosporic fungi. **Iyengar** (1935) reported two new fungi in the genus *Coelomomyces* that are parasitic on larvae of *Anopheles*. **Lacey** (1949, 1955) worked on aquatic phycomycetes and reported species belonging to the genera *Chytridium, Olpidium* and *Rhizophydium*. **Thirumalachar** (1942) reported *Olpidium uredines* to be parasitic within the uredospores of *Hemileia canthii*. **Sachindra Nath Dasgupta** did his Ph.D. at London University and on return to India joined the Lucknow University and retired as a Professor. At Lucknow University, he developed an active team to work on different aspects of fungi including aquatic fungi (appreciated by Sparrow), tip necrosis of mango, economical control of the diseases including usage of Borax spray. Dasgupta and John (1953) investigated into the aquatic fungi of Lucknow. **B.S. Mehrotra**, who retired as Professor from the Department of Botany, University of Allahabad, Allahabad, India, worked extensively on both Zoosporic fungi and the Zygomycetes particularly the Mucorales. He initially focused more on the physiology of Saprolegniales (Mehrotra 1949, 1951, 1952). Then his contributions were more on Mucorales. He published several new taxa in this group (Mehrotra and Mehrotra 1962, 1969). He published a book titled 'The Fungi: An Introduction' published by Oxford and IBH (Mehrotra 1967). **Patil** (1960) reported *Olpidium uredinis* as parasitic within the uredospores of *Uromyces leptodermus.* Ghosh and Dutta (1962) reported the soil fungi occurring in the paddy fields of Orissa that included lower fungi. Bhargava (1944) reported fertilization in *Isoachlya anisospora* in Bihar. **Bhargava** and **Srivastava** (1966) introduced a new species in the genus *Isoachlya*, viz., *Isoachlya luxurians*. **Pavgi** and **Singh** (1975) reported the occurrence of *Olpidium brassicae* in India. Dayal and Thakurji (1968) conducted taxonomic studies on aquatic fungi from Varanasi. **Rai** and **Misra** (1977) reported a new variety of *Achlya stellata*. **Ram Kumar Saksena** served as a Professor at the University of Allahabad. RK Saksena worked on the physiology, taxonomy and cytology of Peronosporales and Saprolegniales in addition to Mucorales. He made a notable contribution also on the extra nuclear cytology of fungi, especially of mitochondria. He carried out detailed studies on soil and aquatic fungi. **Shyam Bahadur Saksena** who did a Ph.D. under R.K. Saksena named a new genus after his mentor as *Saksenaea vasiformis* a soil fungus belonging to Zygomycota. This fungus has certain unique features and resembles zoospores producing fungus Nowakowskiella of Chytridiomycetes. Based on this fact, he proposed that Mucorales could have directly evolved from *Nowakowskiella*-like fungus. Later on, Hesseltine in 1973 raised a new family Saksenaeaceae to accommodate *Saksenaea* along with

Echnisporangium. S.B. Saksena, working at the University of Saugor, Sagar, Madhya Pradesh, developed a strong school of Mycology and Plant Pathology. After working in S.D. Garrett's lab in Cambridge, he initiated laboratory studies at Sagar on root-infecting fungi of the soil including the control of *Phytophthora* blight of Piper betle. He had also worked on the effect of fumigation of soils and its effect on *Trichoderma viride*, which resisted and survived. **Ram Dayal** did a Ph.D. under R.K. Saxena and contributed a lot to our understanding of aquatic fungi particularly the chytrids. He along with his co-workers also studied the nematophagous fungi and biological control and also brought out a book titled 'Zoosporic Fungi of India' published by Usha Kiran (Dayal and Kiran 1988). **R.S. Mehrotra** studied the Phytophthora diseases of various crops and fungal soil borne diseases and bio-control of plant pathogens. He published a monograph on Phytophthora diseases in India (Mehrotra and Agarwal 2001) and various other books including Plant Pathology (Mehrotra 1980, Mehrotra and Aneja 1990). **A.K. Sarbhoy** who initially worked in the Department of Botany, Allahabad University, shifted later to Indian Agricultural Research Institute, New Delhi, and retired as Professor. He worked on soil fungi and published a monograph on Mucorales. Also, he published five supplements of 'Fungi of India' by CBI. **K.G. Mukherji** from the Department of Botany, University of Delhi, and **T.N. Lakhanpal** from the Department of Biosciences, H.P. University, Simla, Himachal Pradesh, have worked extensively on the diversity of myxomycetes of North India and have published a book on Indian Myxomycetes (Lakhanpal and Mukerji 1981). **C. Manoharachary** (1973) working in the Department of Botany, Osmania University, Hyderabad, India, surveyed aquatic phycomycetes from Hyderabad (India). Chowdhry and **Agarwal** (1980) studied the distribution of some aquatic fungi. Kiran and Dayal (1980) and Sarkar and Dayal (1983) reported new records of freshwater chytrids from Varanasi including biological forms of *Rhizidium varians.* **R.D. Khulbe** worked in the Department of Botany, Kumaun University, Nainital, U.P., and he had extensively worked on aquatic fungi. He published a 'Manual of Aquatic Fungi' (Khulbe 2001). Khulbe et al. (1983) reported *Pythium torulosum* for the first time from India. **Hasija** and Khan (1982) also reported chytrids from India. Manoharachary had worked on the taxonomy and ecology of freshwater fungi and published several reviews (Manoharachary 1981, 1991). **V. Agnihotrudu** surveyed Myxomycetes from several places in South India (Agnihotrudu 1952) and North East India (Agnihotrudu 1959) and published a series of papers which culminated in bringing out a list of the Indian Myxomycetes up to 1961 (Agnihotrudu 1961). **Indira Kalyanasundaram** working at CAS in Botany worked on the taxonomy, ecology and physiology of Myxomycetes (Indira 1975; Venkataramani and Kalyanasundaram 1986). **C. Manoharachary** surveyed myxomycetes from Telangana state along with his co-workers (Manoharachary et al. 2012; Manoharachary and Rajithasri 2015). **VD Ranade** worked extensively on myxomycetes and recently brought out a checklist of myxomycetes in India along with **Kiran Ramachandra Ranadive** and others (Ranade et al. 2012).

The main groups of lower fungi that occur in marine environments are Thraustochytrids, Labyrinthulids and Halophytophthoras. These were extensively

studied from Indian waters by **Seshagiri Raghukumar** who was working at the National Institute of Oceanography, Goa, India (Raghukumar 1988a, b, 2002a, b). He has worked on taxonomy, biodiversity and ecology and biotechnology of thraustochytrids. The lower fungal groups mentioned above have very low diversity with few genera known. From Indian waters he was involved in the description of two new species including *Schizochytrium mangrovei* (Raghukumar 1988a) and *Thraustochytrium gaertnerium* (Bongiorni et al. 2005). He has enumerated thraustochytrids from the Arabian Sea (Raghukumar 1985). Also, he has initiated usage of immunofluorescence techniques for detection of thraustochytrids and found *Ulkeniavisurgensis* in a hydroid (Raghukumar 1988b) in addition to the occurrence of thraustochytrid fungi in corals and coral mucus (Raghukumar and Balasubramanian 1991). Raghukumar (2002a, b) studied ecology of marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). Raghukumar et al. (1996a) provided an account of morphology, taxonomy and ecology of Thraustochytrids and Labyrinthuloids. Raghukumar (2002a, b) studied ecology of marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). His group isolated *Aplanochytrium yorkensis* from the equatorial Indian Ocean and investigated its morphology and physiology (Damare et al. 2006). He has published on the biotechnology of thraustochytrids (Raghukumar 2008). His group published ecological observations on thraustochytrids and fungal components of marine detritus based on laboratory and field studies on decomposition of brown alga *Sargassum cinereum* leaves of the mangrove *Rhizophora apiculata* Blume (Raghukumar et al. 1994a, b, c; Sharma et al. 1994). He has published a volume on Fungi in Coastal and Oceanic Marine Ecosystems covering different aspects of lower and higher marine fungi in marine ecosystems (Raghukumar 2017).

Chandralatha Raghukumar also working from the National Institute of Oceanography, Goa has published on diseases caused by lower fungi on marine algae and animals. For example, an account of fungal parasites of marine green algae, e.g., *Cladophora* and *Rhizoclonium* by *Sirolpidium bryopsidis* of Oomycota, *Olpidium rostriferum* of Chytridiomycota and *Coenomyces* sp., and *Labyrinthula* sp. from the beaches of Goa and Lakshadweep islands were reported by her (Raghukumar 1986a). Also, she has recorded *Chytridium polysiphoniae* (Chytridiomycota), a fungal parasite, on the red alga *Centroceras clavulatum* from Goa (Raghukumar 1986b). She gave an account of fungal parasites of marine algae from Mandapam (South India) and reported *Pontisma lagenidioides* (Oomycota) parasite of *Chaetomorpha media* and *Chytridium polysiphoniae* on *Sphacelaria* sp. (Raghukumar 1987a) in addition to fungal parasites of marine algae *Cladophora* and *Rhizoclonium* (Raghukumar 1987b). A survey of the Indian coast for the presence of parasitic fungi in marine algae was conducted by Raghukumar and Raghukumar (1994). Their study on 35 filamentous algae resulted in the finding of 15 fungal parasites with percentage of algal cells infected in nature varying from $<5\%$ to 60% for different species. They found that seven fungal genera belonging to Mastigomycotina, Labyrinthulales and Thraustochytriales were commonly found as parasites on various algae. Eight zoosporic fungal parasites of marine biota have been described from India, including *Ulkenia amoeboidea* (Raghukumar 1996a).

Some of the above groups of fungi are not considered as true fungi and have been separated from Fungi. However, since traditionally they are studied under mycology, they are included here in our discussion.

1.7 Plant Pathology

Most of the plant pathological studies involved fungal pathogens, and hence mycology and plant pathology were flourishing together.

T.S. Sadasivan was one of the pioneering plant pathologists who worked on the host–pathogen interactions. He did a Ph.D. under S.D. Garret in soil microbiology and also worked at Rothamsted Experiment Station. After holding different positions, he became the Director of the Botany Laboratory, University of Madras, Chennai, in 1944 at the retirement of M.O.P. Iyengar, the first Director of University Botany Laboratory. His group studied soil-borne diseases affecting cash crops such as cotton, pigeon pea and rice which made a better understanding of the soil-borne pathogens in the early part of the century. He also introduced the concepts of competitive saprophytic ability and stressed the importance of effects of Rhizosphere in the host–pathogen interactions. He has served as the Editor of Journal of Phytopathology and was also the President of Indian Phytopathological Society (1964) and Mycological Society of India (1975).

K.C. Mehta is one of the pioneering workers on wheat rust problem, who presented a comprehensive work on 'annual recurrence of rusts of wheat and barley in India' wherein he explained the death of rust spores in the summer due to heat in the plains and that the rusts can survive in the hills in the form of urediniospores (uredospores) due to their long distance dispersal. His main contribution has been on the investigations on the life cycle of cereal rusts in India.

B.B. Mundkur

B.B. Mundkur (1896–1952) worked at the Indian Agricultural Research Institute from 1931 to 1947, and later on as the first Deputy Director at the Directorate of Plant Protection, Quarantine, and Storage, New Delhi. He was instrumental in instituting the Indian Phytopathological Society in India in 1948 in addition to starting a new journal 'Indian Phytopathology'. He has published a book titled 'Fungi and Plant Diseases' (Mundkur 1949). He had pioneered the work on control of cotton wilt through varietal resistance. He has also contributed for the identification and classification of a large number of Indian smut fungi.

V. Agnihotrudu though worked on Myxomycetes also contributed in the area of plant pathology. The status of fungicidal research in India was traced by him up to 1985 (Agnihotrudu 1985).

Ram Narayan Tandon did a Ph.D. from University of London and worked as a Professor at University of Allahabad. He has studied the fungal diseases of crop plants and fruit trees in addition to the post-harvest diseases that cause diseases and spoilage during storage and suggested a number of control measures against different diseases. He has also concentrated on the physiology of fungi and the biochemical changes that take place in hosts due to post-harvest diseases of fruits and

vegetables. His notable contributions are 'Mucorales of India', 'Physiological Studies on Some Phytopathogenic Fungi' and 'A Supplement to the List of Indian Fungi'. **G.P. Agarwal** who did a Ph.D. under R.N. Tandon became a Professor in the Rani Durgavati University, Jabalpur, M.P., and he has worked on fungi causing plant diseases at Jabalpur and surrounding areas and published a series of papers. He and his students contributed considerably on different nutritional aspects of the fungi, biological control in addition to fungal infections of humans and aquatic fungi.

R.S. Mehrotra was a Professor in the Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. He has contributed a great deal in the area of plant pathology both in teaching and in research. He has published several textbooks for college and researchers besides publishing several regular research articles. In addition to plant pathological aspects, he had interests in biocontrol and delivered a presidential address 'on certain aspects of Trichoderma taxonomy, etiology, biology and biocontrol' (Mehrotra 1997).

1.8 Miscellaneous Groups of Fungi

1.8.1 Marine Fungi

The first report on typical marine fungi from mangroves came from **Raghukumar** (1973a, b). The following centres were/are involved in the research on marine fungi. These are (a) **B.P.R. Vittal** and his students from C.A.S. in Botany, University of Madras, Chennai, (b) National Institute of Oceanography, Goa by S. Raghukumar and C. Raghukumar, (c) **K.R. Sridhar** and his students from Department of Biosciences, University of Mangalore, (d) **K. Raveendran**, from the Department of Botany, Sir Syed College, Kannur, Kerala from West coast of India, (e) and presently **V. Venkateswara Sarma** from the Department of Biotechnology, Pondicherry University. Higher marine fungi occurring on mangroves of Godavari and Krishna deltas in Andhra Pradesh and Pichavaram and Muthupet mangroves in Tamil Nadu and Pondicherry mangroves have been investigated (Ravikumar and Vittal 1996; Sarma and Vittal 2000, 2004; Vittal and Sarma 2005, 2006; Sarma 2016; Devadatha et al. 2018, 2019; Devadatha and Sarma 2018). Similarly, survey-type studies have been conducted from the West coast (**Borse** 1988; Borse et al. 2013; Borse and Pawar 2005; Ananda and Sridhar 2001; Manimohan et al. 2011; Maria and Sridhar 2002; Sridhar and Maria 2006; Nambiar and Raveendran 2007; Patil and Borse 1985; Prasannarai and Sridhar 2001) and other places (Chinnaraj 1992, 1993; Chinnaraj and Untawale 1992). Studies on biotechnological applications of these marine fungi, however, are very few (Raghukumar 1996b). A few studies have been reported on the extracellular enzymes and their industrial applications (Raghukumar 2000a, b; Raghukumar et al. 1996a, b). More details are presented in a separate chapter on history of marine fungi. **M. Kalaiselvam** at CAS in Marine Biology, Parangipettai, near Chidambaram, Tamil Nadu, is working on the marine fungi and their applications (Kalaiselvam 2015).

1.8.2 Thermophilic Fungi

Approximately 60 fungal species are capable of growth in the temperature range of 40–60 °C (Johri et al. 1999; Salar 2018). B.N. Johri, who retired as Professor and Head of the Department of Microbiology, G.B. Pant University of Agriculture and Technology, Pant Nagar (U.P.), and several other groups from a number of Indian Universities/institutes have extensively isolated thermophilic fungi from soil, compost and several other environmental samples and investigated into their diversity, ecological, physiological and biotechnological aspects (Johri et al. 1999; Singh et al. 2016; Salar 2018).

1.8.3 Deep-Sea Fungi

Very little was known about the deep-sea fungi until the last two decades. The research requires special probes and cruise ships. Such facilities are available only with premier institutes. Further, deep-sea conditions pose harsh environmental conditions including high hydrostatic pressure. To get a better picture about marine fungal diversity, nowadays researchers resort to both culture-dependent and culture-independent approaches. Exploiting the facilities available, **Chandralatha Raghukumar** and her group working at the National Institute of Oceanography, Goa have explored the Arabian Sea and Indian Ocean region and reported the deep-sea fungi. These studies have resulted in very interesting observations about deep-sea fungi and published in several journals (Damare and Raghukumar 2008; Raghukumar and Damare 2008; Raghukumar et al. 2010; Singh and Kamal 2012). Raghukumar (2011) has summarized the diversity of culturable and non-culturable fungi in deep-sea habitats, their physiology, ecological roles and biotechnological potentials in an excellent review through her presidential address to the MSI in 2011. This subject is a hot topic, nowadays, and, as she mentioned, deep-sea offers least explored habitat for prospecting fungi with novel molecules and enzymes. She opined that newer methods of culturing and isolation and studying adaptation mechanisms of fungi in deep-sea realm pose future challenges.

1.8.4 Fossil Fungi

R.K. Saksena and **S.K.M. Tripathi** affiliated to Birbal Sahni Institute of Paleobotany, Lucknow, India, have worked extensively on fossil fungi and contributed a monographic account on Indian fossil fungi (Saxena and Tripathi 2011).

1.8.5 Medical Mycology

R.S. Randhawa had worked on dermatophytes and surveyed soil inhabiting dermatophytes and related keratinophilic fungi of India (Randhawa and Sandhu

1965). He described a new genus *Keratinophyton terreum* from soil in India (Randhawa and Sandhu 1964). **L.N. Mohapatra** worked in the Department of Microbiology at the All India Institute of Medical Sciences, New Delhi. He reviewed the available information on medical mycology in India the past, present and future up to 1985 (Mohapatra 1985). **Jagdish Chander**, who worked as Professor in the Department of Microbiology, Government Medical College and Hospital, Chandigarh, published a book titled 'Textbook of Medical Mycology' (Chander 2018). **H.C. Gugnani** working as Professor in the Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, worked on the occurrence of pathogenic fungi in soils in India and Nepal (Gugnani et al. 2007). Anuradha Chowdhary, who is working as Professor in the same department, focused attention on multidrug-resistant human fungal pathogens and clinical significance of non-sporulating fungi especially basidiomycetes in the patients of respiratory diseases (Choudhary et al. 2018; de Groot et al. 2019). **C. Rajendran** was with National Institute of Communicable Diseases, Delhi, where he studied different skin diseases, dermatophytosis and utility of antifungal agents in controlling them (Rajendran 1987; Rajendran et al. 1990).

1.8.6 Keratinophilic Fungi

G.R. Ghosh was with Indian Veterinary Research Institute, Izatnagar, Bareily, UP, India. He along with his co-workers carried out investigations on the Keratinophilic fungi from feathers of wild birds and domestic fowls in addition to soil samples from five districts of Odisha (Sur and Ghosh 1980). **S.C. Agarwal** of the Department of Applied Microbiology and Biotechnology, Dr. H.S. Gour Vishwavidyalaya, Sagar, investigated the keratinophilic fungi and related dermatophytes from different soils (Kushwaha and Agarwal 1976; Deshmukh and Agarwal 2003). **R.K.S. Kushwaha** worked as Professor at the Department of Botany, Christ Church College, Kanpur. He and his co-workers studied keratinophilic fungi on Indian birds and isolated from feathers of most common Indian birds including domestic chicken, domestic pigeon, house sparrow, house crow, duck and rose-ringed parakeet (Dixit and Kushwaha 1991). **P.C. Jain** of the Department of Botany, University of Sagar, Sagar, MP, India, continued on keratinophilic fungi from soils and reported some additions to Indian *Malbranchea* (Jain and Agrawal 1979). **S.K. Deshmukh** of Piramal Enterprises Limited, Mumbai, made surveys on the occurrence of keratinophilic fungi in selected soils of different parts of India including Madhya Pradesh and the Ladakh (Deshmukh and Agarwal 1983; Deshmukh et al. 2010).

1.8.7 Lichenized Fungi

S.R. Kashyap initiated the lichenological studies in India. He and his students collected specimens and identified them with the help of A.L. Smith and published

in the book by G.L. Chopra in 1935 that included 75 taxa of lichens from the Himalayas (Awasthi 1983).

D.D. Awasthi established an active centre on lichenized fungal research at the Department of Botany, Lucknow University, and has contributed his services for around 6 decades until his sudden demise on 21.8.2011. A detailed historical information of the developments of lichenology were presented in the book titled 'Catalogue of Lichens from India, Nepal, Pakistan and Ceylon' by Awasthi (1965).

Later on, **Singh** (1980) published 'Lichenology in Indian Subcontinent' (1966–1977), where he provided a detailed information on lichenological research in different centres of India. He mentioned that the research contribution had mainly come from four centres including National Botanical Research Institute, Lucknow; Botanical Survey of India; Maharashtra Association for the Cultivation of Science, Pune (presently Agarkhar Research Institute), and Department of Botany, Lucknow University. From the last institution, several generic revisions were published and these included *Rhizopcarpon* (Awasthi and Singh 1977), additions to the lichens of Nepal (Awasthi and Sharma 1978) and Silent Valley lichens (Singh 1982).

P.G. Patwardhan and his students extensively surveyed lichens from the Western Ghats of India. Some of their work is included in the following publications: on new Parmelia (Patwardhan and Prabhu 1977), terricolous lichens (Patwardhan et al. 1977), Theletremataceae of India (Patwardhan and Nagarkar 1980) and Pyrenocarpus lichens of Eastern India (Nagarkar and Patwardhan 1981).

1.8.8 Aerobiology

S.T. Tilak from Aghakar Research Institute, Pune, extensively carried out investigations on aerobiology in India. He published a book titled 'Aerobiology' (Tilak 1998). Further, he worked on the epidemiology of the fungal diseases on plants through aerobiology (Tilak and Babu 1984). **BPR Vittal** in addition to biodiversity and ecology of litter fungi from terrestrial and marine environments also initiated aerobiological studies at the Centre for Advanced studies in Botany, University of Madras. These included surveys of air mycoflora of farm environments using Burkard Volumetric spore trap, enumeration of airborne fungi from extramural, intramural and occupational environments, and clinical studies with aeroallergens, which resulted in several publications (Bhuvaneswari and Vittal 2005; Pugalmaran and Vittal 1999; Udaya Prakash et al. 2011; Vittal 2005). **A.B. Singh** who was with CSIR-Institute of Genomics and Integrative Biology, Delhi University Campus, Delhi, worked on aerobiology of pollen and fungal spores. He has worked on indoor airborne fungi in poultry farms and examined them as occupational sensitizers in poultry workers (Singh and Singh 1996). Also, he studied the common environmental allergens causing respiratory allergy in India (Singh and Kumar 2002; Singh 2017).

1.8.9 Endophytic Fungi

T.S. Suryanarayanan, Director of VINSTROM (Vivekananda Instittue of Tropical Mycology), Mylapore, Chennai, initiated studies on endophytic fungi (Suryanarayanan et al. 1998, 2003, 2009, 2012). He examined different aspects of endophytic fungi including their diversity, ecology, physiology and secondary metabolites and the collections included from mangroves, shola forests and Andaman Islands (Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000; Kumaresan and Suryanarayanan 2001). **J. Muthumary** worked on the bioactive compounds from endophytic fungi particularly on taxol (Gangadevi and Muthumary 2007, 2008). **Ravindra Kharwar** and his associates have studied the anticancer compounds produced by fungal endophytes (Kharwar et al. 2011). In addition to the endophytic fungal diversity (Gond et al. 2007), their team is focusing on chemical and functional diversity of natural products from plant-associated endophytic fungi (Verma et al. 2009). S.K. Deshmukh of Nicholas Piramal Enterprises Limited, Mumbai, have also studied bioactive metabolites produced by endophytic fungi from Indian subcontinent (Deshmukh et al. 2015; Deshmukh 2018).

1.8.10 Entomopathogenic Fungi

M.V. Deshpande associated with National Chemical Laboratory (NCL), Pune, carried out investigations on entomopathogenic fungi for over three decades. His team worked on fungi, fungal enzymes and other compounds that serve as novel biopesticides and in other applications (Deshpande 1998; Kapoor and Deshpande 2013; Chaudhary et al. 2013; Ghormade et al. 2011; Yadav and Deshpande 2010).

1.8.11 Trichomycetes

J.K. Mishra, who retired as Professor from the Department of Botany, Sri. N.P.G. College Lucknow, had extensively worked on Trichomycetes colonizing different groups of insects and published several books, review articles and research articles on this group (Mishra 1998; Lichtwardt et al. 1999). The class Trichomycetes covers the obligate symbiotic fungi that commonly thrive in the guts of arthropods. They are considered as commensals with little effect on the hosts, although in stressful environments they may confer an advantage to colonized hosts and occasionally act as pathogens. The books JK Mishra published include 'Trichomycetes and Other Fungal Groups' (Mishra and Horn 2001), 'Illustrated Genera of Trichomycetes and Fungal Symbionts of Insects and Other Arthropods' (Mishra and Lichtwardt 2000) in addition to various edited volumes on general fungi. Also, he worked on zoosporic fungi and published a monographic account on *Pythium* from India (Mishra 1996).

1.8.12 Vesicular and Arbuscular Mycorrhizal Fungi

D.J. Bagyaraj retired as Professor from the Department of Agricultural Microbiology, University of Agricultural Science, GKVK campus, Bangalore. He has extensively studied on VA mycorrhizal fungi on various aspects including their biodiversity and use in practical agriculture (Bagyaraj 1984, 1992; Bagyaraj and Balakrishna 1998–1999, Harinikumar and Bagyaraj 1995). **K.G. Mukherji** conducted studies on Vesicular-Arbuscular Mycorrhizal fungi on *Kalanchoe spicata* and *Eclipta alba* (Mukerji and Ardery 1985). **B.L. Jalali** was working at CCS Haryana Agricultural University, Hisar, Haryana. He had studied the effect of VAM fungi in controlling plant diseases and effect of pesticides on VAM fungi (Jalali and Jalali 1991; Jalali and Chhabra 1991). **N. Raman** studied the diversity and proposed techniques to study mycorrhizae and investigated their efficacy in mine spoilt soils (Raman and Mohan Kumar 1988; Raman et al. 1993). **Bernard F. Rodrigues** is a Professor in the Department of Botany, University of Goa, Goa; he is working on different aspects of Arbuscular Mycorrhizal fungi. These include their taxonomy (Rodrigues 2008) and their occurrence in medicinal plants (Radhika and Rodrigues 2010; Radhika et al. 2012), in vegetable crop plants of Goa (Dessai and Rodrigues 2012), aquatic and marshy plant species (Radhika and Rodrigues 2007), pteridophytes (Khade and Rodrigues 2002), and coastal sand dune vegetation of Goa (Rodrigues and Jaiswal 2001). **Alok Adholeya**, who is with The Energy and Resources Institute (TERI), New Delhi, has been working extensively on different applied aspects of VAM fungi including phytoremediation prospects in heavy metal contaminated soils (Gaur and Adholeya 2004). A modified method for estimation of VAM fungal spores in soil has been suggested by Gaur and Adholeya (1994), while the effects of the particle size of soil-less substrates upon Arbuscular mycorrhizal fungal inoculum production had been investigated by Gaur and Adholeya (2000). His lab is also associated with large-scale inoculum production of arbuscular mycorrhizal fungi (Adholeya et al. 2005).

1.8.13 Yeasts

G.S. Prasad has served as Director of Microbial Type Culture Collection Centre (MTCC) of IMTECH, Chandigarh, for many years and presently is with Central University of Hyderabad as Director-Research. His group had worked on diversity and molecular phylogeny of yeasts from different ecological niches of India. His group studied the diversity of yeasts from two high temperature regions of India, viz., coal bed regions of Andhra Pradesh and Rajasthan, and described new species *Debaryomyces singareniensis* and *Cryptococcus rajasthanensis,* respectively (Saluja and Prasad 2007a, b). Also, he and his group have described new species and new records from different environments including the oil refinery in Digboi, Assam (Prasad et al. 2005). **S. Shivaji**, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, had carried out investigations on yeast diversity from Antarctic sea (Shivaji and Prasad 2009). He had also worked in the field of

Table 1.1 List of mycologists active in service, their affiliation, areas of research and new genera and new species published by them

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
1	**J. Savitha**	Department of Microbiology and Biotechnology Bangalore University, Jnanabharathi Campus, Bangalore-560 056, Karnataka	Enzymes as well as primary and secondary metabolites that include bioactive compounds, lipids and organic acids have been discovered from soil and coprophilous fungi leading to a productive growth of bio-commercialization	Working on physiology and biotechnology of fungi
2	**V. Mohan**	Institute of Forest Genetics and Tree Breeding Coimbatore-641 002, Tamil Nadu	Arbuscular Mycorrhizal (AM) fungi of different forest tree species, medicinal plants, horticulture and agriculture crops in varied ecosystems	Reported several new records of AM and ectomycorrhizal fungi in India
3	**N. Mathivanan**	Centre for Advanced Studies in Botany University of Madras, Guindy Campus Chennai-600 025, Tamil Nadu	Biological control of plant pathogens; plant and microbial metabolites, marine microbiology and biotechnology and extremophile	Working on fungal plant pathogens and biotechnology of fungi
4	**M. Sudhakara Reddy**	Dept. of Biotechnology Thapar University Patiala-147 004, Punjab	Molecular diversity of fungi and bacteria, bioactive compounds from endophytic fungi, bioremediation, sustainable agriculture	Working on biotechnology of fungi
5	**Munruchi Kaur**	Department of Botany Punjabi University Patiala-147 002, Punjab	Mycology, plant pathology, mushroom systematic and culturing	*Agaricus sgtellatus-cuticus, A. flavistipus, Psathyrella fimicola, Panaeolus cyanoannulatus, P. lepus-stercus, Chlorolepiota indica, Russula himalayensis, Lactarious annulocystidiatus*
6	**Rupam Kapoor**	Department of Botany University of Delhi Delhi-110 007	Interaction of plants with pathogenic and symbiotic fungi	Working on host and pathogen interactions of pathogenic and symbiotic fungi

7	**M. Krishna Mohan**	Dept. of Biotechnology and Bioinformatics Birla Institute of Scientific Research Statue Circle, Jaipur-302 001, Rajasthan	Microbial diversity analysis of drinking water, salt lakes and desert ecosystems, microbial metagenomics, molecular and functional profiling, and characterization of industrially important enzymes	Working on biotechnology of fungi
8	**R.N. Kharwar**	Mycopathology and Microbial Tech. Lab Department of Botany Banaras Hindu University Varanasi-221 005, Uttar Pradesh	Microbial endophytes, bioactive compounds, fungal nanocomposites	*Pseudocercospora biophytiicola, P. clematicola, Cladosporium bauhiniana, Stenella cassinigena, Zasmidium cassines, Z. fabaceicola, Mycovellosiella adinae-cofdifoli, M. desmodigena, M. neri*
9.	**S. Shishupala**	Department of Microbiology Davangere University Shivagangotri Campus Davangere-577002, Karnataka	Mycology, phytopathology, immunotechnology	Working on fungal pathogens and biotechnology of fungi
10	**M. Kalaiselvam**	CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu	Marine microbiology with special reference to marine mycology	Working on marine fungi and their applications
11	**K.B. Vrinda**	Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode Thiruvananthapuram-695 562, Kerala	Mushroom taxonomy and diversity	*Entoloma brunneopapillatum, Entoloma brunneosquamulosum, Entoloma griseolimosum, Entoloma brunneocarnosum, Hygrocybe rubida, Hygroaster fucatus, Entoloma crassum, Entoloma suaveolens, Entoloma aurantioquadratum, Auritella foveata, Pluteus brunneosquamulosus, Pluteus velutinus, Pluteus silentvalliyanus, Pluteus luteostipitatus, Tubaria virescens, Pluteus delicatulus, Lactarius ignifluus, Hygrocybe parvispora, Russula leelavathyi, Inocybe purpureoflavida, Inocybe virosa*

(continued)

Table 1.1 (continued)

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
12	**Krishnendu Acharya**	Department of Botany University of Calcutta 35, Ballygunge Circular Road Kolkata-700 019 West Bengal	Macrofungal diversity and medicinal prospects; innate immunity in plants and myco-nanotechnology	*Marasmius vladimirii, Marasmius midnapurensis, Chlorophyllum pseudoglobossum, Russula kanadii, Marasmiellus foliiphilus, Marasmius indopurpureostriatus, Russula hookarii, Ramaria subalpina, Russula buyckii, Russula intervenosa, Russula arunii, Trogia benghalensis, Clitocybula albida, Russula darjeelingensis, Agaricus duplocingulatoides, Lactarius benghalensis, Hygrocybe lucida, Marasmius indojasminodorus, Lactifluus midnapurensis, Lactarius brunneocinnamomeus, Chlorophyllum squamulosum, Russula benghalensis*
13	**S.K. Singh**	National Fungal Culture Collection of India, B4#36, Biodiversity and Palaeobiology Group, Agharkar Research Institute, Pune-411004, Maharashtra	Biodiversity, systematics, conservation and bioprospecting of fungi	*Ellisembia karadkensis, Arthrinium rasikravindrii, Manoharachariella indica,* and many others
14	**Yashpal Sharma**	Department of Botany, Mycology and Plant Pathology Lab., University of Jammu Jammu-180 006	Diversity, ethnomycology and biochemical characterization of wild mushrooms of North-west Himalayan region of Jammu & Kashmir and Ladakh	Several new records to India were reported from their studies
15	**Dilip Hande**	Botany Department Shri Shivaji Science College, Morshi Road, Amravati-444 603, Maharashtra	Fungal diversity and biotechnology	*Eupropolella indica, Helicospoium melghatianum, Ajrekarella asetosa, Acanthophiobolus indicus, Ophiobolus melghatibus*

16	**Naveen Kango**	Department of Microbiology Dr. Harisingh Gour Vishwavidyalaya Sagar-470003, Madhya Pradesh	Production and characterization of cellulases, xylanases, mannanases, fructosyltransferases from fungi; prebiotic oligosaccharides using fungal enzymes; valorization of biomass using fungal enzymes: bioethanol generation	Working on physiology and biotechnology of fungi
17	**V. Venkateswara Sarma**	Dept. of Biotechnology, School of life Sciences, Pondicherry University Pondicherry–605014	Morphological and molecular diversity of marine fungi and ascomycetes from terrestrial habitats. Isolation, screening for enzymes and bioactive compounds for antiquorum sensing and other activities	One new family: Paralophiostomataceae 9 New genera: *Vismaya, Curvatispora, Vittaliana, Raghukumaria, Thyridariella, Murinectria, Halocryptosphaeria, Pseudoastrosphaeriellopsis, Paralophiostoma* 52 New species *Deniquelata vittalii, Thyridariella mangrovei, T. mahakoshae, Vaginatispora microarmatispora, Morosphaeria muthupetensis, Pontoporeia mangrovei, Vittaliana mangrovei, Raghukumaria keshaphalae, Fusicolla bharathavarshae, Pseudoastrosphaeriellopsis kaveriana, Zopfiella indica, Amphisphaeria mangrovei, Hypoxylon teeravasati, Peroneutypa mangrovei, P. indica, P. polysporae, Nigrograna samueliana, Phaeoseptum carolshearum, P. manglicola, Verruconis mangrovei, Lanspora cylindrospora, Halocryptosphaeria avicenniae, Neodevrisiea manglicola, Biatriospora borsei, Cytospora fusispora,*

(continued)

Table 1.1 (continued)

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
				Kamalomyces polyseptatus, Fissuroma kavachabeejae, F. microsporum, Neoastrosphaeriella alankrithabeejae, Astrosphaeriella uniseptata, Pithomyces hyalosproa, Bertiella striatispora, Allocryptovalsa truncata, Murinectria murispora, Paralophiostoma hysterioides, Botryobambusa apiculiformisproa, Brunneiapiospora appendiculata, Cllioplea macrospora, Cryptoascoma shodasabeejae, Diatrypella macroasca, Leptosphaeria sadvibhajanabeejae, L. verruculosa, Montagnula varkabeejae, Ostreichnion beejakoshae, Rizalia falcata, Rosselinia attentuata, R. tetraspora, Ascobolus gomayapriya
18	**Paras Nath Singh**	National Fungal Culture Collection of India, Biodiversity and Paleobiology Group, MACS, Agharkar Research Institute, G.G. Agarkar Road Pune-411 004, Maharashtra	Fungal diversity, systematics of micromycetes, conservation and biopotential of potential of fungi	*Neoacladium indicum* gen. et sp. nov., *Hyweljonesia indica* sp. nov., *Clinoconidium lauracearum* sp. nov.
19	**Sashirekha Suresh Kumar**	Dept. of Botany, Mithibai College Vile Parle (W), Mumbai-400 056, Maharashtra	Fungal biotechnology including protein profiling of fungi and dye degradation by fungi	Working on fungal biotechnology
20	**C.K. Pradeep**	Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode Thiruvananthapuram-695 562, Kerala	Mushroom taxonomy and diversity	*Entoloma brunneopapillatum, Entoloma brunneosquamulosum, Entoloma griseolimosum, Entoloma*

				brunneocarnosum, Hygrocybe rubida, Hygroaster fucatus, Entoloma crassum, Entoloma suaveolens, Entoloma aurantioquadratum, Auritella foveata, Pluteus brunneosquamulosus, Pluteus velutinus, Pluteus silentvalliyanus, Pluteus luteostipitatus, Tubaria virescens, Pluteus delicatulus, Lactarius ignifluus, Hygrocybe parvispora, Russula leelavathyi, Inocybe purpureoflavida, Inocybe virosa
21	**Pratyoosh Shukla**	Department of Microbiology Maharshi Dayanand University Rohtak-124001, Haryana	Bioprospecting of fungi, metabolic engineering for enzymes, metagenomics	Working on fungal biotechnology
22	**Rashmi Dubey**	Botanical Survey of India	Diversity of anamorphic fungi and plant pathogenic fungi	New genera: *Sawantomyces indicus, Sheathnema indicum, Beltramono costei* New species: *Goosiomyces bambusicola, Zygosporium cocos, Z. dilleniae, Sporidesmium bilgiriense, Kamalomyces mahabaleshwarensis, Pseudoacrodictys steviae, Tharoopama livistonae, Bispora aeglei, Elotespora indica, Thirumalacharia thanensis, Periconia chandoliensis, Stachybotrys citri, Volutella rauvolfii, Cercosporidium ziziphus, Curvularia ocimi*
23	**Sanjai Saxena**	Department of Biotechnology and Environmental Sciences Thapar University Patiala-147 004, Punjab	Plant-microbe/animal-microbe interaction, natural product-based drug discovery, mycoherbicides	*Muscodor tigerii, Muscodor indica*

(continued)

Table 1.1 (continued)

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
24	**Kanad Das**	Botanical Survey of India Cryptogamic Unit P.O. Indian Botanic Garden Howrah-711 103, West Bengal	Mushroom taxonomy and diversity	2 New genera and 130 new species New genera: *Mycorrhaphoides, Indoporus* New species: *Hericium bharengense, H. yunthangense, Mycoleptodonoides sharmae, Lactarius abbotanus, L. byssaceus, L. capitatus, L. sanjappae, L. verbekenae, L. dafianus, L. dhakurianus, L. dwaliensis, L. elaioviscidus, L. ermineus, L. maitlyensis, L. mayawatianus, L. montoyae, L. mukteswaricus, L. crenulatus, L. croceigalus, L. indochrysorrheus, L. olivaceoglutinus, L. pyriodorus, L. yumthangensis, L. vesterholtii, Lactifluus dissitus, L. leptomerus, L. versiformis, Russula mayawatiana, R. appendiculate, R. tsokae, R. khanchanjungae, R. mukteshwarica, R. natarajanii, R. vaurasiana, R. koeleggiensis, R. netrabaricus, R. sharmae, R. dubdiana, R. sikkimensis, R. shingbaensis, R. thindii, Nidula shingbaensis, Inonotus ryvardenii, Austroboletus olivaceoglutinosus, Boletus sharmae, B. lakhanpalii, B. recapitulates*. Only 50 are shown here and 80 not shown

25	**N.K. Udayprakash**	Department of Biotechnology Vels University, Velan Nagar, Pallavaram Chennai-600117, Tamil Nadu nkudayaprakash@gmail.com	Aeromycology, air spora, fungal allergens in air	Working on airobiology and fungal allergens
26	**Praveen Gehlot**	Mycology Laboratory Department of Botany, Jai Narain Vyas University Jodhpur-342001, Rajasthan	Fungal taxonomy, diversity, anamorphic fungi, ascomycetes, macrofungi, DNA barcoding	New genus—*Rosellinopsis* New species—*Anthostoma macrocarpa, Endoxylon aravilla, Valsaria microspora, Coniochaeta abuensis, Podospora rajasthansis, P. variabalata, Zignoella rajasthansis, Diatrypis gaumukhi, Eutypella panwariella, Peroneutypa rajasthansis, Pseudomassaria indica, Anthostomella caudata, Kretzchmaria mangiferae, K. pyriformis, K. ornamantalis, K. rajasthansis, Hypoxylon rajasthansis, Sphaerulina indica, Didymella agaves, D. mucosa, Didymosphaeria heteroasca, D. abuensis, Trematosphaeria macrospora, Leptosphaeria tori, Asterosphaeriella macrospora, Pleomassaria lantanae, Pleospora camarensis, Malanomma indicum*
27	**V. Kumaresan**	Tagore Arts College Puducherry-605003	Diversity of fungal endophytes and their bioactive potential, and mushroom diversity	*Helioephala natarajanii, Hygrocybe manadukaensis, Hygrocybe natarajanii, Entoloma vittalii*
28	**K. Malarvizhi**	Centre for Advanced Studies in Botany, University of Madras Guindy Campus, Chennai-600025, Tamil Nadu	Morphological and molecular diversity of basidiomycetes. Fungal pigments and volatile compounds	Working on molecular diversity of basidiomycetes and biotechnology of fungi

(continued)

Table 1.1 (continued)

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
29	**T.S. Murali**	Division of Biotechnology School of Life Sciences, Manipal University, Manipal-576 104, Karnataka	Fungal endophytes and bioprospecting. Molecular diversity analysis of fungi	Fungal endophytes and biotechnology of fungi
30	**Samir Damare**	Marine Biotechnology Laboratory National Institute of Oceanography Dona Paula, Goa-403 004	Marine fungi, deep-sea fungi, proteomics of fungi	Deep-sea fungi
31	**D. Nagaraju**	Government Degree College Eturnagaram, Warangal-506165 Andhra Pradesh	Soil fungi, VAM fungi, anamorphic fungi	New genera: *Bhadradriella hyalina, Hyalocephalotrichum indica* New species: *Custingophora lignicola, Chaetopsina indica, Ulocladium gpagarwalii, Ulocladium lignicola*
32	**Damodar Shenoy Belle**	National Institute of Oceanography, Visakhapatnam	Diversity and taxonomy of marine fungi and bacteria	*Oxydothis bambusicola*, *Pseudohalonectria miscanthicola*, *Colletotrichum communis*
33	**K.C. Rajeshkumar**	National Fungal Culture Collection of India B4#36, Biodiversity and Palaeobiology Group Agharkar Research Institute Pune-411004, Maharashtra	Fungal taxonomy, nomenclature and phylogeny; fungal culture collection; plant pathology and quarantine	*Pilidiella crousii, Chaetospermum setosum, Ellisembia karadkensis, Pseudocercospora kamalii, Tamhinispora srinivasanii, Talaromyces amyrossmaniae, Hemibeltrania cinnamomi , Taiwanascus samuelsii, Tubeufia sahyadriensis* and many others
34	**Baghela**	National Fungal Culture Collection of India B4#36, Biodiversity and Palaeobiology Group Agharkar Research Institute Pune-411004, Maharashtra	Phylogenetics, DNA barcoding, and genetic engineering of fungi	*Apophysomuyces elegans, Cantherellus sikimensis, Boletus lakhanpalii, Russula butyroindica,Boletus recapitulatus, Blastobotrys bombycis Vittaliana mangrovei, Mycorrhaphoides stalpersii, Exosporium gymnemae, Wickerhamiella shivajii, Suhomyces drosophiliae* and many others

35	**Rahul Sharma**	National Centre for Cell Sciences, NCCS Complex, SP Pune University Complex, Ganeshkhind, Pune-411 007, Maharashtra	Keratinophilic fungi, systematics and molecular phylogeny of onygenalean fungi, dermatophytes-related fungi	**New genera (5)** *Auxarthronopsis, Tamhinispora, Matsushimamyces, Currahmyces, Canomyces* **New species (12)** *Auxarthronopsis bandhavgarhensis, Gymnoascus verrucosus, Pseudocercospora kamali, Volvariella sathei, Matsushimamyces bohaniensis, Arthrinium rasikravindri, Tamhinispora indica, 8. Curvularia lonarensis, Keratinophyton turgidum, Ctenomyces indicus, Canomyces reticulatus, Currahmyces indicus*
36	**Avneet Pal Singh**	Department of Botany Punjabi University Patiala-147 002, Punjab	Mycology and plant pathology with interest in taxonomy of wood rotting corticioid fungi belonging to class—Agaricomycetes, Subphylum—Agaricomycotina (Phylum—Basidiomycota)	New genera: *Repetobasidiopsis* New species: *Athelopsis parvispora, Clavulicum hallenbergii, Vararia longicystidiata, Inonotus tramisetifer, Flavophlebia sphaerospora, Aleurodiscus himalaicus, Radulodon acacia, Hyphoderma hallenbergii*
37	**Shambhu Kumar**	Kerala Forest Research Institute (KFRI), Peechi, Kerala, India	Diversity of anamorphic fungi	*Passalora musicola, P. caesalpiniicola, Cercospora apii, C. prosopidicola, Cladosporium cycadacearum, Taeniolella sapinndii, Phyllactinia braunii, Corynespora celastri, C. ficigena, Zasmidium smilacis-proliferae, Pseudocercospora bischofigena, Corynespora clerodendrigena, C. crotonicola, C. glochidicola, C. titarpaniensis, Curvularia martyniicola, Alternaria polypodiicola*

(continued)

Table 1.1 (continued)

S. No.	Name of the mycologist	Affiliation	Area of research	Association in the publication of New genera or new species
38	**J. Cathrine Sumathi Manohar**	Biological Oceanography Division National Institute of Oceanography Dona Paula, Goa-403 004	Studies on fungi in the oxygen-deficient marine habitats, physiological adaptations to live in the absence of oxygen and their nitrate reduction capacity. Ecological and biotechnological importance of fungi from mangrove habitats. The relative importance of fungi in comparison with bacteria, archaea and viruses in the marine habitats.	*Tritirachium candoliense*
39	**M. Kumar**	Department of Plant Biology and Plant Biotechnology Madras Christian College (Autonomous) Tambaram, Chennai-600 059, Tamil Nadu	Mushroom and other macrofungal biodiversity especially in the Eastern Ghats and tropical dry evergreen forests of South India, cultivation of wild edible mushrooms, bioprospecting of wild mushrooms	*Volvariella minuta*

extremophiles (Shivaji and Ray 1995) and also worked on psychrophilic yeasts from Antarctica (Satyanarayana et al. 2005). **T. Satyanarayana** and his co-workers from the Department of Microbiology, University of Delhi South Campus, New Delhi, isolated a yeast strain from flower buds of *Woodfordia fruticosa* which was identified as *Pichia anomala*; this yeast was reported to be a good source of phytase (Vohra and Satyanarayna 2001). Jyoti Prakash Tamang, who is with the Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, India, is blending morphological and molecular phylogenetic analyses in studying yeasts. He along with his co-workers studied yeast diversity in fermented foods and beverages using both culture-dependent and culture-independent approaches (Jeyaram et al. 2011; Tamang and Fleet 2009; Sha et al. 2016, 2018) A table is provided about the active researchers of mycology in India and new taxa published by them (Table 1.1).

1.9 Conclusions

Several active centres of mycology that were traditionally involved in biodiversity research have shifted focus to other branches of science. Taxonomists have become endangered species. This has to reverse as the diversity of fungi is huge and we have a few workers to focus on this area. The molecular approach is complementing the morphological methods. This combination would continue in future harmoniously to describe new species. Hopefully the golden era of biodiversity research would come back as to what is expected.

In this book chapter, major historical developments have been captured and important laboratories/departments involved have been briefly covered. If any of areas is missing, it is inadvertent and due to limitation of space and time. Several workers who have contributed to the area of physiology and biotechnological aspects of fungi are dealt with separately in this and a sequel to this book.

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History of Marine Mycology in India

2

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Abstract

About 225 marine fungi and fungus-like organisms have been listed in India, including Labyrinthulomycetes (14), Chytridiomycetes (4), Oomycetes (4), Ascomycetes (149), Basidiomycetes (3), Hyphomycetes (39) and Coelomycetes (12). All these species were reported from intertidal wood, driftwood, intertidal mangrove wood, salt marsh plants, water and sediment samples, algae and animals, and propagules (ascospores/conidia) of marine fungi in foam samples. The most speciose genera are from Ascomycetes including *Aigialus* (5 spp.), *Aniptodera* (7 spp.), *Arenariomyces* (4 spp.), *Bathyascus* (5 spp.), *Ceriporiopsis* (3 spp.), *Corollospora* (14 spp.), *Halosarpheia* (6 spp.), *Lulworthia* (5 spp.), *Nimbospora* (3 spp.), *Saagaromyces* (3 spp.), *Dyfrolomyces* (5 spp.), *Savoryella* (5 spp.) and *Trichocladium* (6 spp.). A few species colonize both the marine and freshwater habitats (*Aniptodera chesapeakensis*, *A. lignatilis, Natantispora retorquens*, *Savoryella lignicola* and *Zopfiella vlatipes*). Seven genera and 44 new species have been described from India. Developments in marine fungal research in India are discussed.

Keywords

Marine fungi · Chytridiomycota · Ascomycota · Basidiomycota · Fungal diversity · Mitosporic fungi · Marine ecology

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2.1 Introduction

Till late 1930s, marine mycology was not thought as a discrete branch of science. The investigations of Sparrow Jr (1936) and Barghoorn and Linder (1944), however, provided the impetus to the study of marine fungi all over the world. Professor Jones (2011) rightly stated that 'some may regard the study of marine fungi as somewhat esoteric but they do play a vital role in ecology of marine ecosystems and in the food web of the oceans'. So far only 22 species of zoosporic (lower marine fungi) fungi have been recorded from marine waters of India (Borse et al. 2017). Goldstein (1973) stated in his review on marine zoosporic fungi that 'these organisms are swimming in an ocean of benign neglect'. Though a few initial reports on marine fungi from India came from mycologists from other countries who visited India (late 1950s through 1960s), sustained efforts came from a few institutes such as C.A.S. in Botany, University of Madras, Chennai (1970s onwards), S.P. University, Pune (1980s onwards), National Institute of Oceanography, Goa (1985 onwards), and Department of Biological Sciences, Mangalore University, Mangalore, Karnataka (1997 onwards), among others.

In the ensuing pages, we have traced different research works and publications, and presented the account in a chronological order. Since there are numerous publications, it is beyond the scope of this chapter to discuss about the individual papers and their work in detail. However, the list of publications provided in the Reference section would be of use to the researchers in order to find further details.

2.2 History of Marine Mycology in India

2.2.1 Diversity of Lower Marine Fungi

Chakravarty (1979) reported *Schizochytrium aggregatum* (Labyrinthulomycota, Thraustochytrids) from Tamil Nadu. This was probably the first record of this phylum in India. Further, these fungi were studied by Dr. Seshagiri Raghukumar and Dr. Chandralata Raghukumar (National Institute of Oceanography, Goa) and their students, since 1986. Raghukumar (1986a) presented an account of fungal parasites of marine green algae, *Cladophora* and *Rhizoclonium* and reported *Sirolpidium bryopsidis* (Oomycota; this was probably the first record of this phylum in India), *Olpidium rostriferum* (Chytridiomycota; this was probably the first record of this phylum in India), *Coenomyces* sp. and *Labyrinthula* sp. from the beaches of Goa and Lakshadweep islands.

Raghukumar (1986b) recorded *Chytridium polysiphoniae* (Chytridiomycota), a fungal parasite, on the red alga *Centroceras clavulatum* from Goa, as a new report from India. Raghukumar (1987a) gave an account of fungal parasites of marine algae from Mandapam (South India) and reported *Pontisma lagenidioides* (Oomycota) parasite of *Chaetomorpha media* and *Chytridium polysiphoniae* on *Sphacelaria* sp. Raghukumar (1987b) recorded fungal parasites of marine algae *Cladophora* and *Rhizoclonium*.

Raghukumar (1988a) described *Schizochytrium mangrovei*, a thraustochytrid from mangroves in India. Raghukumar (1988b) detected the thraustochytrid protist *Ulkenia visurgensis* in a hydroid, using immunofluorescence. Raghukumar and Lande (1988) reported shell disease of rock oyster *Crassostrea cucullata* caused by *Ostracoblabe implexa* (Oomycota, *incertaesedis*). Raghukumar and Balasubramanian (1991) reported occurrence of thraustochytrid fungi in corals and coral mucus. Raghukumar et al. (1992a) studied the association of thraustochytrids and fungi living with marine algae. Eight zoosporic fungal parasites of marine biota have been described from India, including *Ulkenia amoebiodea* (Raghukumar 1996a). An account of morphology, taxonomy and ecology of Thraustochytrids and Labyrinthuloids was further provided by Raghukumar (1996b).

Raghukumar and Raghukumar (1994) conducted a survey of the Indian coast to explore the presence of parasitic fungi in marine algae. They examined 35 filamentous algae and found that 15 harboured fungal parasites. The percentage of algal cells infected in nature varied from <5% to 60% for different species. A total of seven fungal genera belonging to Mastigomycotina, Labyrinthulales and Thraustochytriales were found regularly as parasites on various algae. Ramasamy et al. (1996) reported *Lagenidium callinectes* (Oomycota) infection and its control in cultured larval Indian tiger prawn *Penaeus monodon* Fabricus from Madras (Chennai now), Tamil Nadu.

Raghukumar (2002) studied ecology of marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). Bongiorni et al. (2005) described a new species, namely, *Thraustochytrium gaertnerium* from mangroves of Goa. Damare and Raghukumar (2006) isolated *Aplanochytrium yorkensis* from the equatorial Indian Ocean and investigated its morphology and physiology.

2.2.2 Diversity of Higher Marine Fungi

Before 1973, research on higher marine fungi (sexual and asexual morphs of Ascomycota and Basidiomycota) were based on collection and transfer of intertidal wood and submerged wood test blocks for further studies in foreign countries. These included Becker and Kohlmeyer (1958) who have recorded the presence of soft rot fungi on small fishing crafts in India. The only species reported was *Halosphaeria quadricornuta* (Now *Antennospora quadricornuta*). This was probably the first record of a marine Ascomycete from India. Kohlmeyer (1959) described the new genus *Paraliomyces* with *P. lentiferus* as its type species on intertidal wood collected from Madras (now Chennai) coast. Kohlmeyer et al. (1967) described a new species of *Corollospora* (Ascomycota), *C. pulchella* with its imperfect state *Clavariopsis bulbosa* (now *Clavatospora bulbosa*) from Kerala coast. *Clavatospora bulbosa* was probably the first record of marine mitosporic fungus in India. Jones (1968) reported five higher marine fungi from test blocks of wood submerged at Bombay and Cochin coasts.

N. B. Nair was the first Indian researcher who explored higher marine fungi on intertidal wood from Kerala coast (Nair 1970). Seshagiri Raghukumar was the first

Indian researcher who has investigated the higher marine fungi on intertidal decaying parts of mangroves from Tamil Nadu coast and reported 12 species of Ascomycota and six mitosporic fungi from Madras coast (Raghukumar 1973). Later, S.D. Patil (S. P. Pune University) and his co-workers (Borse, Ramesh, Shrivastava) surveyed marine fungi from Maharashtra coast. B.P.R. Vittal (Madras University, Chennai) and co-workers (Ravikumar and Sarma) explored marine fungi from the mangroves of the coast of Andhra Pradesh and Tamil Nadu.

K.R. Sridhar (Mangalore University) and co-workers (Ananda, Prasannarai, Maria, Ghate) investigated marine fungi from Karnataka and other coastal states of the country. A.G. Untawale (NIO, Goa) and his co-worker, Chinnaraj, recorded marine fungi from Andaman-Nicobar and Lakshadweep Islands. P. Manimohan (University of Calicut) and co-workers (Raveendran, Nambiar and Khan) surveyed marine fungi from Kerala and other parts of India. B.D. Borse (SSVPS's Dr. P.-R. Ghogarey Science College, Dhule) and co-workers (K.N. Borse, N.S. Pawar, N.B. Pawar, Tuwar, Kamble and Gosavi) reported marine fungi from West Bengal (Sundarbans), Orissa, Gujarat, Goa, Diu-Daman and Union territory of Pondicherry. V.V. Sarma and his co-worker B.D. Devadatha studied marine fungi from Union Territory of Pondicherry and Muthupet mangroves of Tiruvarur district (Tamil Nadu).

Patil and Borse (1982, 1983a, b, 1985, 1986) published a series of papers dealing exclusively with higher marine fungi from the coast of Maharashtra. Patil and Borse (1983b) reported *Halocyphina villosa* on intertidal roots of *Avicennia alba* from Maharashtra coast. This was probably the first record of a marine Basidiomycete in India. They also described two new marine species of Ascomycota (*Didymella avicenniae* and *Halosarpheia ratnagiriensis* (now *Saagaromyces ratnagiriensis*). Kohlmeyer and Vittal (1986) described *Lophiostoma mangrovei* (now *Rimora mangrovei*), a new marine ascomycete from India.

Borse (1984, 1985, 1987a, b, c, d, e); Borse, 1988, 2000a, b; Gosavi and Borse, 2017, 2018 published a series of papers dealing with higher marine fungi from Maharashtra coast and described three new species of Ascomycota [*Aigialus mangrovei, A. rhizophorae* and *Pleospora avicenniae* (now *Halojulella avicenniae*)] from mangroves of Maharashtra. Venkatesan and Natarajan (1985) reported the occurrence of *Cirrenalia pygmea* along with several marine-derived fungi from Pichavaram mangroves. Hyde and Borse (1986a) described a new genus *Biatriospora* (Ascomycota) with *B. marina* as its type species. Hyde and Borse (1986b) described a new species *Massarina velataspora* (now *Morosphaeria velataspora*) from Maharashtra coast and Seychelles (Arabian Sea).

Ravikumar and Vittal (1987) surveyed marine fungi from Tamil Nadu coast and reported ten species of Ascomycota occurring on intertidal wood of various mangroves. Raghukumar (1988c) reported four lignicolous marine fungi from the coast of Goa and Lakshadweep Islands. Raghukumar et al. (1988a) described a new species of mitosporic fungi, namely, *Cirrenalia basiminuta* on materials collected from Goa and Kuwait coast. Borse et al. (1988) investigated the frequency of occurrence of higher marine fungi from Maharashtra coast and observed that *Massarina velatospora* was the most dominant species in mangrove habitats,

while *Antennospora quadricornuta* was the most dominant species from beach habitats.

Borse et al. (1988) reported some marine fungi from Maharashtra coast. Borse and Shrivastava (1988) reported *Halosarpheia abonnis* and *Zopfiella latipes* as additions to the fungi of India. Ravikumar and Purushothaman (1988a, b) reported *Cirrenalia tropicalis* and *Corollospora intermedia* from Tamil Nadu coast. Sridhar and Kaveriappa (1988) studied the occurrence and survival of aquatic hyphomycetes in brackish and seawater samples. Shrivastava Alka (1989) reported seven species of Ascomycota from Bombay coast. Borse and Hyde (1989) described a new genus of Ascomycota (*Acrocordiopsis* with *A. patilii* as its type species), occurring on mangrove wood from Maharashtra and Seychelles (Island, Arabian Sea). Ramesh and Borse (1989) added some higher marine fungi to the list from Maharashtra coast.

Ravikumar and Vittal (1991a) described a new species of *Bathyascus* (*B. mangrovei*) from Pichavaram estuary, Tamil Nadu. Ravikumar and Vittal (1991b) reported two species of *Corollospora* (*C. angusta* and *C. filiformis*) from Tamil Nadu. Sridhar and Kaveriappa (1991) reported five Ascomycetes and four mitosporic fungi from Mangalore coast. Hyde et al. (1992) described a new species of Ascomycota (*Massarina armatispora*) occurring on intertidal mangrove wood from Andaman Islands, Goa and Karnataka coasts, while Chinnaraj and Untawale (1992) reported nine species of Ascomycota from these three mangrove belts.

Chinnaraj (1992) recorded 32 species of higher marine fungi from Lakshadweep Islands. He had also recorded 63 species of higher marine fungi from six mangrove tree species from Andaman and Nicobar Islands (Chinnaraj 1993). Chinnaraj (1994) compiled higher marine fungi from mangroves. Borse and Shrivastava (1994) reported *Ascocratera manglicola* and *Massarina thalassiae* (now *Halomassarina thalassiae*) from Maharashtra coast. Nandan et al. (1993) recorded 14 higher marine fungi from Goa coast with *Aniptodera mangrovei* and *Ocostaspora apilongissima* as new reports to the fungi of India.

Sridhar and Prasannarai (1993) reported four ascomycetes from Mangalore coast, in which *Halosarphie aviscosa* (now *Panorbis viscosus*) and *Trematospaeria mangrovei* were new reports for Indian marine fungi. Shrivastava Alka (1994, 1995) recorded 10 species of mitosporic fungi and 14 marine ascomycetes from the coast of Bombay (Maharashtra). Ravikumar and Vittal (1996) surveyed fungal diversity on *Rhizophora mucronata* and reported 48 marine fungi from Pichavaram mangroves of Tamil Nadu. Of these, *Rimora mangrovei, Verruculina enalia, Halocyphina villosa* and *Monodictys pelagica* were more prevalent. Raghukumar (1996c) gave an account of fungi in the marine realm highlighting the status, challenges and prospects.

Prasannarai and Sridhar (1997) studied the effect of incubation on the occurrence of marine fungi from west coast of India. Ananda et al. (1998) studied the occurrence of higher marine fungi on marine animal substrates from Mangalore coast. Raghukumar (1998) recorded three Ascomycetes (*Abysomyces hydrozoicus*, *Bathyascus vermisporus* and *Oceanitis scuticella*) and two mitosporic fungi (*Allescheriella bathygena* and *Periconia abyssa*) for the first time from deep-sea sediments of Indian marine waters (Table 2.1).

Table 2.1 New genera and species of marine fungi described from India

S. no.	Taxon	Substrate	Location	References
	Labyrinthulomycota			
1	*Schizochytrium mangrovei* S. Raghukumar	*R. mucronata*, *A. officinalis*	Goa	Raghukumar et al. (1988a)
2	*Thraustochytrium gaertnerium* Bong et al.	Mangroves	Goa	Bongiorni et al. (2005)
	Ascomycotina			
3	**Acrocardiopsis patilii* Borse and K.D. Hyde	*A. alba*, *R. mucronata*	Maharashtra	Borse and Hyde (1989)
4	*Aigialus mangrove* Borse	*R. mucronata*	Maharashtra	Borse (1987e)
5	*A. rhizophorae* Borse	*R. mucronata*	Maharashtra	Borse (1987e)
6	*Bathyascus mangrovei* Ravikumar and Vittal	*R. apiculata*	Tamil Nadu	Ravikumar and Vittal (1991a)
7	**Biatriospora marina* K.D. Hyde and Borse	*A. marina*	Maharashtra	Hyde and Borse (1986a)
8	*Corollospora indica* Prasannarai et al.	Calcareous substrates	Karnataka	Prasannarai et al. (2000)
9	*C. pulchella* Kohlm et al.	Intertidal wood	Kerala	Kohlmeyer et al. (1967)
10	*Didymella avicenniae* S.D. Patil and Borse	*A. alba*	Maharashtra	Patil and Borse (1985)
11	*Halosarpheia ratnagirinsis* (now *Saagaromyces*) S.D. Patil and Borse	Mangrove wood	Maharashtra	Patil and Borse (1982)
12	*Julella avicenniae*(Borse) K.D. Hyde	*A. alba*	Maharashtra	Borse (1987b)
13	*Lophiostoma* (now *Rimora*) *mangrovei* Kohlm and Vittal	*R. mucronata*	Tamil Nadu and Maharashtra	Kohlmeyer and Vittal (1986)
14	*Massarina armatispora* K.D. Hyde et al.	Intertidal mangrove wood	And. Islands, Karn. and Goa	Hyde et al. (1992)
15	*Massarina* (now *Morosphaeria*) *velataspora* K.D. Hyde and Borse	*R. mucronata*	Maharashtra	Hyde and Borse (1986b)
16	**Paraliomyces lentiferus* Kohlm.	Intertidal wood	Tamil Nadu	Kohlmeyer (1959)

17	*Tirispora mandoviana* V.V. Sarma and K.D. Hyde	*R. mucronata*	Goa	Sarma and Hyde (2000)
18	*Passeriniella mangrovei* Maria and K.R. Sridhar	*R. mucronata*	Karnataka	Maria and Sridhar (2002a)
19	*Cirrenalia basiminuta* Raghukumar and Zainal	Mangrove wood	Karnataka	Raghukumar et al. (1988b)
20	*Vaginatispora microarmatispora* Devadatha, V.V. Sarma and E.B.G. Jones	Mangrove wood	Tamil Nadu	Devadatha et al. (2017)
21	*Thyridariella mangrovei* *Devadatha, V.V. Sarma, K.D. Hyde, Wanas and E.B.G. Jones	*Avicennia marina* Mangrove wood	Tamil Nadu	Devadatha et al. (2018a)
22	*Thyridariella mahakoshae* Devadatha, V.V. Sarma, K.D. Hyde, Wanas and E.B.G. Jones	*Avicennia marina*	Tamil Nadu	Devadatha et al. (2018a)
23	*Deniquelata vittali* Devadatha, V.V. Sarma and E.B.G. Jones	*Suaeda monoica*	Tamil Nadu	Devadatha et al. (2018b)
24	*Morosphaeria muthupetensis* Devadatha, V.V. Sarma and E.B.G. Jones	*Rhizophora mucronata*	Tamil Nadu	Devadatha et al. (2018c)
25	*Pontoporeia mangrovei* Devadatha and V.V. Sarma	*Suaeda monoica*	Tamil Nadu	Devadatha and Sarma (2018)
26	*Vittaliana mangrovei* *Devadatha, Nikita, A. Baghela and V.V. Sarma	*Avicennia marina*	Tamil Nadu	Devadatha et al. (2019)
27	*Raghukumaria keshaphalae** Devadatha, V.V. Sarma and E.B.G. Jones	*Aegiceras corniculatum*	Tamil Nadu	Jones et al. (2019)
28	*Fusicolla bharatavarshae* Devadatha, V.V. Sarma and E.B.G. Jones	*Avicennia marina*	Tamil Nadu	Jones et al. (2019)
29	*Pseudoastrosphaeriellopsis*kaveriana* *Devadatha, Wanas. Jeewon and V.V. Sarma	*Avicennia marina* *Suaeda monoica*	Tamil Nadu	Phookamsak et al. (2019)
30	*Zopfiella indica* Devadatha, Jeewon and V.V. Sarma	Mangrove wood	Tamil Nadu	Phookamsak et al. (2019)
31	*Amphisphaeria mangrovei* Devadatha and V.V. Sarma	*Suaeda monoica*	Tamil Nadu	Phookamsak et al. (2019)

(continued)

Table 2.1 (continued)

S. no.	Taxon	Substrate	Location	References
32	*Hypoxylon teeravasati* Devadatha, V.V. Sarma and E.B.G. Jones	*Avicennia marina* *Suaeda monoica*	Tamil Nadu	Phookamsak et al. (2019)
33	*Peroneutypa mangrovei* Devadatha and V.V. Sarma	*Avicennia marina*	Pondicherry	Phookamsak et al. (2019)
34	*Nigrograna samueliana* Devadatha, V.V. Sarma and E.B.G. Jones	*Avicennia marina*	Tamil Nadu	Dayarathne et al. (2019a)
35	*Phaeoseptum carolshearianum* Devadatha, V.V. Sarma and E.B.G. Jones	*Avicennia marina*	Tamil Nadu	Dayarathne et al. (2019a)
36	*Phaeoseptum manglicola* Devadatha, V.V. Sarma and E.B.G. Jones	*Avicennia marina*	Tamil Nadu	Dayarathne et al. (2019a)
37	*Peroneutypa indica* Devadatha, V.V. Sarma and E.B.G. Jones	*Suaeda monoica*	Tamil Nadu	Dayarathne et al. (2019a)
38	*Peroneutypa polysporae* Devadatha, V.V. Sarma and E.B.G. Jones	*Suaeda monoica*	Tamil Nadu	Dayarathne et al. (2019b)
39	*Verruconis mangrovei* Devadatha, V.V. Sarma and E.B.G. Jones	*Excoecaria agallocha*	Tamil Nadu	Hyde et al. (2020)
40	*Lanspora cylindrospora* Devadatha, V.V. Sarma and E.B.G. Jones	*Suaeda monoica*	Tamil Nadu	Hyde et al. (2020)
41	*Halocryptosphaeria avicenniae* Devadatha and V.V. Sarma	*Avicennia marina*	Tamil Nadu	Dayarathne et al. (2020)
42	*Neodevrisea manglicola* Devadatha, V.V. Sarma and E.B.G. Jones	*Rhizophora mucronata*	Tamil Nadu	Yuan et al. (2020)
43	*Biatriospora borsei* Devadatha and V.V. Sarma	*Avicennia marina*	Tamil Nadu	Hongsanan et al. (2020)
44	*Halocryptosphaeria* bathurstensis* (Dayarathne and K.D. Hyde) Dayarathne, V.V. Sarma, Devadatha and K.D. Hyde	*Avicennia marina*	Tamil Nadu	Dayarathne et al. (2020)

Borse et al. (1999a) recorded 15 marine ascomycetes from Goa coast and 8 marine fungi from Diu Island with *Cryptovalsa halosarceicola* as a new record to India (Borse et al. 1999b). Prasannarai et al. (1999) reported seasonal occurrence and colonization of marine fungi from Mangalore harbour with *Didymosphaeria lignomaris* and *Nimbospora effuse* as new records for the Indian coast. Sarma and Vittal (1998–1999) confirmed the seasonal occurrence of manglicolous fungi from Godavari and Krishna deltas, east coast of India. They found a higher diversity during wet season (July to November) than dry season (January to May).

Borse et al. (2000a) examined the frequency of occurrence of marine fungi from Pirotan Island (Gujarat) and collected 29 species of higher marine fungi on mangroves. Borse et al. (2000b) reported ten Ascomycetes and three Dueteromycetous (anamorphic) fungi along with a high frequency of occurrence from Daman coast. Borse et al. (2000a) reported three species of *Savoryella* from Maharashtra, Orissa and West Bengal with *Savoryella appendiculata* as a new record to India. Borse et al. (2000b) published a list of 83 species (62 Ascomycota, 3 Basidiomycota, and 18 Mitosporic fungi) from the coast of Maharashtra. Prasannarai et al. (2000) described a new species of *Corollospora* (*C. indica*) from west coast of India.

Sarma and Vittal (2000) surveyed the biodiversity of mangrove fungi on individual substrata of *Rhizophora apiculata* (prop roots, seedlings and wood) and *Avicennia* spp. (pneumatophores, roots and wood) from Godavari and Krishna deltas and found that fungi have preference to a particular part/substrate of the plant. For example, the prop roots of *R. apiculata* and wood of *Avicennnia* spp. have shown a higher percentage occurrence than other substrata/parts of these plants. Sarma and Hyde (2000) described a new species of *Tirispora* (*T. mandoviana*) from Goa on *Rhizophora mucronata*. Prasannarai and Sridhar (2000–2001) recorded 11 species of higher marine fungi as new records for fungi of India. Sarma et al. (2000) prepared an interactive CD-ROM for mangrove fungi covering 85 fungi from Indian and Hong Kong mangroves.

Sarma and Vittal (2001) collected 88 species of manglicolous fungi colonizing nine host plants from Godavari and Krishna deltas (Andhra Pradesh, India) of which *Rhizophora apiculata* and *Avicennia* spp. supported a large number of fungi when compared to other hosts (Figs. 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, and 2.9). Sarma et al. (2001a) studied frequency of occurrence of mangrove fungi from the east coast of India and found *Verruculina enalia* to be the very frequently occurring fungus from mangroves of Andhra Pradesh state. Patil and Borse (2001) reported 50 species of higher marine fungi along with their frequency of occurrence from Gujarat coast. Borse and Borse (2001) reported *Remispora* as a new generic record and *Trematosphaeria lineolatispora* a new record for India. Borse and Pawar (2001) reported *Carbosphaerella* and *Dryosphaera* as new generic records to the fungi of India from West Bengal.

Ananda and Sridhar (2001a) described *Aniptodera indica* (Synonym of *Tirispora unicaudata* Jones and Vrijmoed) from west coast of India. Ananda and Sridhar (2001b) reported mycoflora on dead animal substrates in mangrove habitats of Karnataka coast. Vishwakiran et al. (2001) recorded 33 species of marine fungi

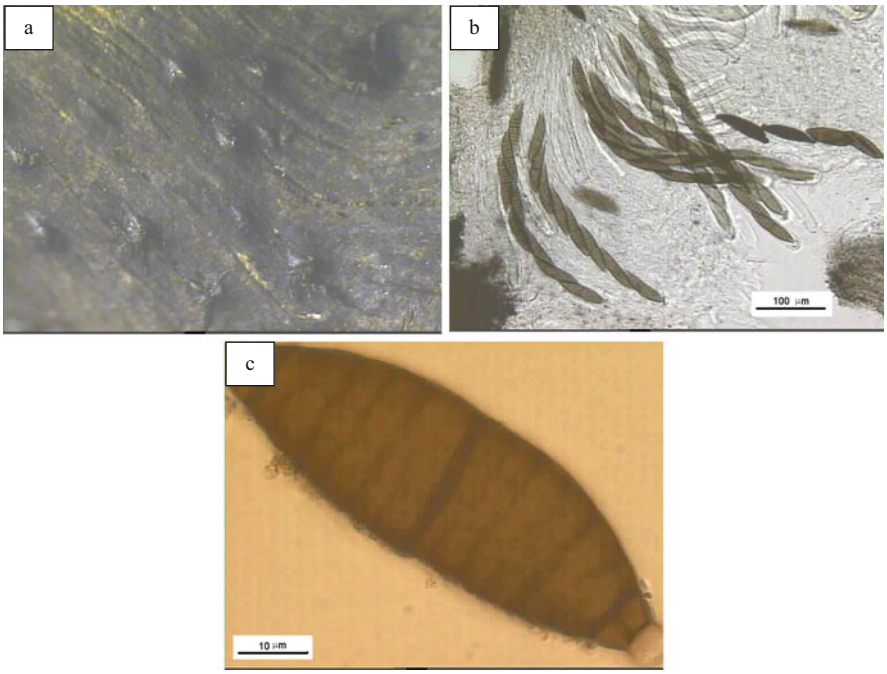

Fig. 2.1 *Aigialus mangrovei*: (**a**) Ascomata (fruit bodies), (**b**) Asci, (**c**) Muriform ascospore with end cells hyaline

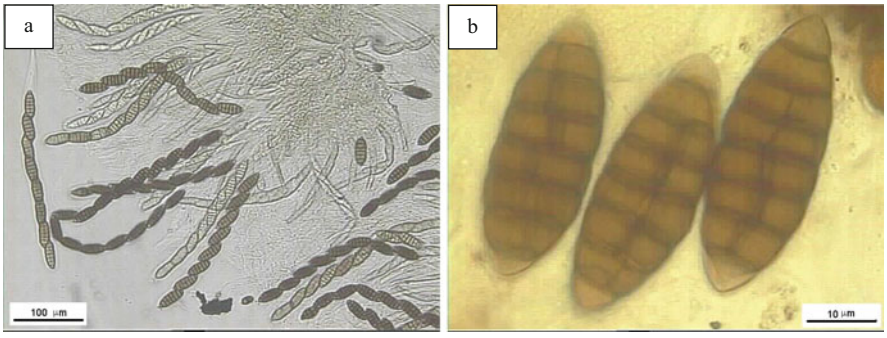

Fig. 2.2 *Aigialus mangrovei*: (**a**) Asci, (**b**) Ascospores

(20 Ascomycota, 1 Basidiomycota and 12 Mitosporic fungi) on mango panels submerged in the Mondovi and Zhuari estuarine systems of Goa.

Prasannarai and Sridhar (2001) investigated the diversity and abundance of higher marine fungi from west coast of India and reported 88 species belonging to 48 genera including 22 species reported as new records for the Indian Peninsula. Sridhar and Prasannarai (2001) reviewed biogeography and biodiversity of higher marine fungi in tropics. Borse et al. (2000b) reported propagules of seven

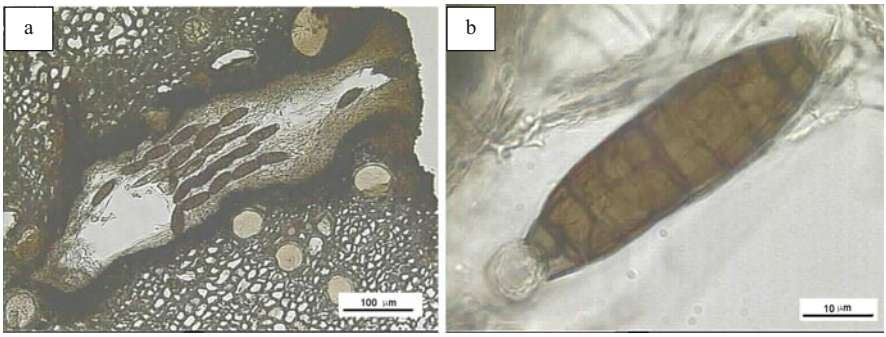

Fig. 2.3 *Aigialus parvus*: (**a**) Sagittal section through ascoma showing asci, (**b**) Ascospore

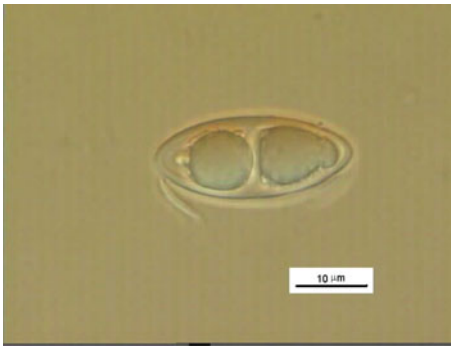

Fig. 2.4 Unipolar appendage ascospore of *Aniptoderachesapeakensis*

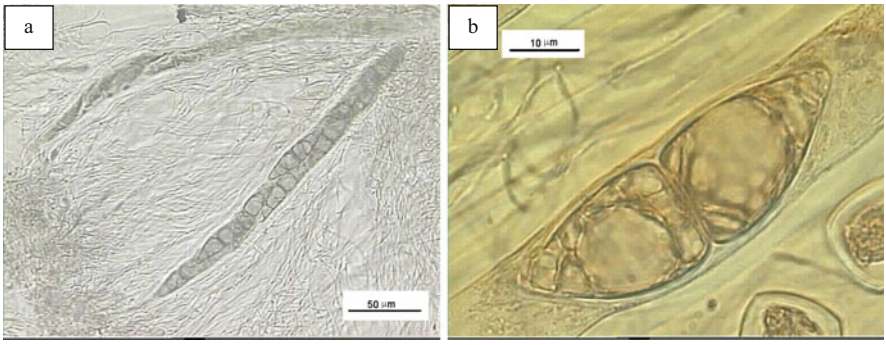

Fig. 2.5 *Ascocratera manglicola*. (**a**) Asci. (**b**) Ascospore

Ascomycetes and two mitosporic fungi in foam samples from sandy beaches of Orissa and propagules of seven Ascomycetes and one mitosporic fungus from foam samples of Sundarbans (Borse et al. 2000b). Sarma et al. (2001b) studied the frequency of occurrence of mangrove fungi from the east coast of India covering

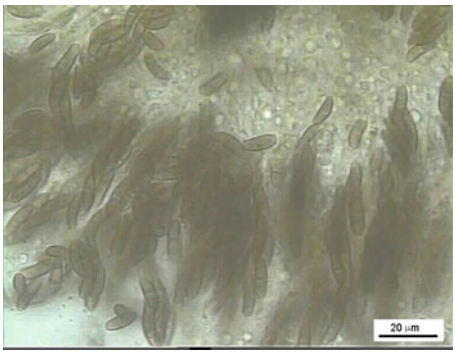

Fig. 2.6 Asci and allantoids ascospores of *Cryptosphaeria mangrovei*

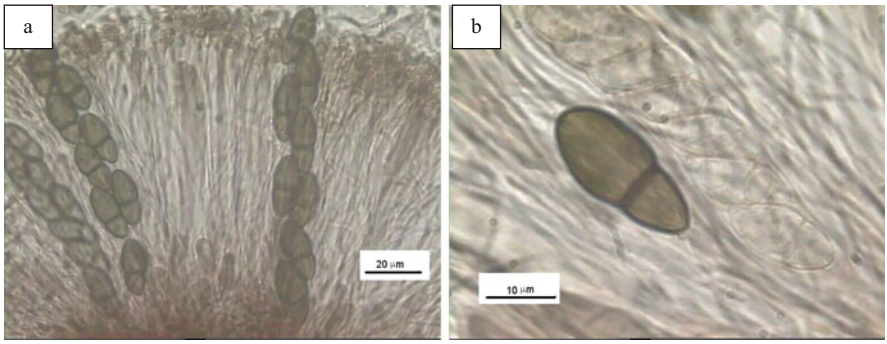

Fig. 2.7 *Dactylospora haliotrepha*: (**a**) Asci, (**b**) Ascospore with longitudinal striations

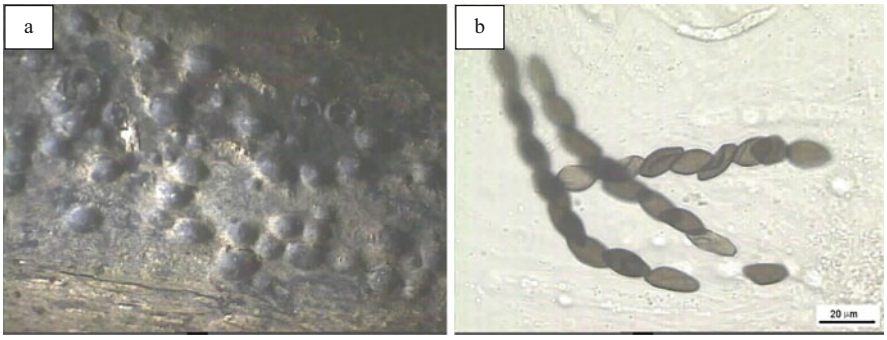

Fig. 2.8 *Halorosellinia oceanica.* (**a**) Ascomata that are superficial, (**b**) Asci showing ascospores within that have germ slits

Godavari and Krishna deltaic mangroves. They found that *Verruculina enalia, Halocryptosphaeria bathurstensis, Rimora mangrovei, Hydea pygmea, Rhizophila marina* and *Cryptospheria mangrovei* were recorded with higher frequency in that order from Godavari delta. At Krishna delta, *V. enalia, Sclerococcum haliotrephum,*

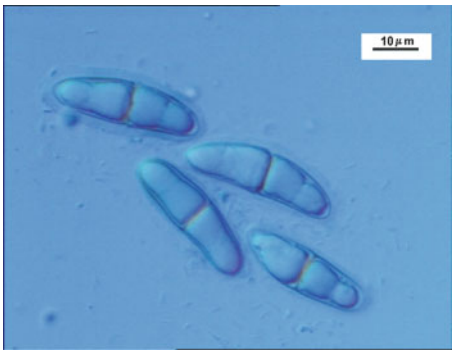

Fig. 2.9 Ascospores of *Halomassarinathalassiae* with mucilaginous sheaths

Halocryptosphaeria bathurstensis, Lulworthia sp., *Halosarpheia abonnis* and *Halocyphina villosa* showed a higher frequency of occurrence in that order. Though these deltaic mangroves have only 150 km distance and similar host plants, some differences were found in the frequently occurring fungi recorded.

Borse et al. (2002a) reported nine species of *Corollospora* from the coast of Orissa. Borse et al. (2002b) reported three species of *Arenariomyces* from Sunderban region. Shindikar and Borse (2002) reported 21 species of higher marine fungi (14 Ascomycetes, 1 Basidiomycetes and 6 mitosporic fungi) from mangrove swamps of Vikhroli (Mumbai). Maria and Sridhar (2002a) reported a total of 78 species belonging to 45 genera comprising 46 Ascomycetes, 1 Basidiomycete and 31 mitosporic fungi on woody litter of mangroves along the west coast of India.

Maria and Sridhar (2002b) described a new species of Ascomycota (*Passeriniella mangrovei*) from the mangrove forest of India. Ananda and Sridhar (2002) explored the diversity of endophytic fungi in the roots of mangrove species on the west coast of India. An investigation on the vertical distribution of manglicolous fungi on prop roots of *Rhizophora apiculata* at Krishna delta by Sarma and Vittal (2002) revealed that certain fungi occur in a vertically zonated pattern in terms of a high percentage occurrence at submerged region or intertidal region. Maria and Sridhar (2003a) surveyed the diversity of filamentous fungi on woody litter of five mangrove plant species from the southwest coast of India. Maria and Sridhar (2003b) examined endophytic fungal assemblage of two halophytes from west coast mangrove habitats. Ananda and Sridhar (2003) investigated filamentous fungal assemblage of mangrove fungi from Coconut Island and Minicoy Island of the Arabian Sea.

Ananda and Sridhar (2004) analysed the diversity of filamentous fungi on decomposing leaf and woody litter of mangrove forest in the southwest coast of India. Maria and Sridhar (2004) observed the pattern of fungal colonization of immersed wood in mangroves of the southwest coast of India. Sarma and Vittal (2004) proposed a dichotomous key to identify the 88 mangrove fungi that they have recorded earlier (Sarma and Vittal 2001), including 65 Ascomycetes, 1 Basidiomycete and 22 Mitosporic fungi from mangroves of Godavari and Krishna deltas of east coast of India.

Kumaran et al. (2004a) reported fossil records of marine manglicolous fungi from Malvan (Maharashtra). Kumaran et al. (2004b) observed spores of the fungus

Cirrenalia indica on the lignite beds of Malvan (Maharashtra) and indicated that these lignites are autochthonous and deposited in a near-shore environment. Pawar and Borse (2004) reported 12 species of higher marine fungi collected on samples of mangroves and salt marsh plants from the coast of Sundarbans.

Borse et al. (2005a) reviewed the diversity and taxonomy of marine fungi from Maharashtra. Borse and Borse (2005) recorded 18 mitosporic fungi from the coast of Orissa. Pawar and Borse (2005a, b) reported marine fungi from Sundarbans including 15 mitosporic species. Borse et al. (2005b) recorded five Ascomycetes for the first time from Sundarbans, east coast of India, while Pawar et al. (2005) reported five Ascomycetes from east coast of India. Maria et al. (2005) investigated the antimicrobial and enzyme activities of mangrove endophytic fungi of southwest coast of India. Vittal and Sarma (2005) compiled the fungal diversity associated with decaying substrates of mangrove plants in Godavari and Krishna deltas (Andhra Pradesh) on the east coast of India. Borse and Pawar (2005) reported 13 species of marine fungi (12 Ascomycetes and 1 mitosporic fungus) in the seawater foam samples collected from Mahe (Pondicherry).

Borse and Tuwar (2006) reported eight species of marine fungi (seven Ascomycetes and one mitosporic fungus) in foam samples from Goa coast. Vittal and Sarma (2006) reviewed diversity and ecology of fungi on mangroves of Bay of Bengal region and listed a total number of 131 species belonging to 77 genera. Sridhar and Maria (2006) reported 66 fungal species on mangrove woody litter of *Rhizophora mucronata*. Nambiar and Raveendran (2006) reported a comparative account of mangrove associated marine fungi of Valapattanam estuary in Kannur district (Kerala). Nambiar et al. (2006) studied marine fungi from north Malabar region of Kerala coastal waters and recorded 31 marine fungi from Valapattanam estuary, Kannur District (Kerala).

Sarma and Vittal (2007) sampled soils of Coringa mangroves of Godavari and Kothapalem mangroves of Krishna delta, east coast of India, for fungi over a 2-year period. At Coringa mangroves, 34 filamentous soil fungi were recorded wherein *Aspergillus niger, A. terrus, A. japonicas* and *Trichoderma* sp. formed the core group fungi, while 41 fungi were recorded at Kothapalem mangroves with *A. terreus, A. niger, Penicillium funiculosum, Trichoderma* sp. and *P. citrinum* forming the core group fungi.

Raveendran and Manimohan (2007) published a book entitled 'Marine Fungi of Kerala'. They described 80 species of marine fungi from Kerala Coast that included 61 Ascomycetes, 1 Basidiomycete and 18 mitosporic fungi. Nambiar and Raveendran (2007) reported 52 marine fungi from three estuaries of North Malabar region of Kerala (35 Ascomycetes, 2 Basidiomycetes and 15 mitosporic fungi). Kamble et al. (2008) recorded five species of marine ascomycetes from Kutch (Gujarat).

Nambiar and Raveendran (2008a) recorded 22 marine fungi from Kerala. These included 16 Ascomycetes, 1 Basidiomycete and 6 mitosporic fungi. Nambiar and Raveendran (2008b) reported 61 marine fungi from coastal wetlands of Kerala. These included 42 Ascomycetes, 2 Basidiomycetes and 17 mitosporic fungi. Nambiar and Raveendran (2008c) recorded 18 marine fungi from Pondicherry and

Mahe and 23 marine fungi from estuaries of North Malabar region of Kerala (Nambiar and Raveendran 2008d). Nambiar and Raveendran (2008e) studied biodiversity of marine mangrove fungi of Valapattanam and Pichavaram mangrove forests (South India).

Nambiar et al. (2008a) recorded 30 marine fungi from backwaters and brackish waters of North Malabar region of Kerala. Nambiar et al. (2008b) reported 51 marine fungi from four localities of Tamil Nadu (Tuthukudi, Chennai, Kanyakumari and Pichavaram). Sridhar (2009a) reviewed marine fungi on mangroves of the Indian Peninsula. Sridhar (2009b) studied the fungal diversity of Pichavaram mangroves.

Nambiar and Raveendran (2009a, b) studied influence of coir retting on mangrove ecosystem and recorded 19 marine fungi from mangroves of Kerala and in another study reported lignicolous marine fungi in selected wetlands of north Malabar (Kerala). Nambiar and Raveendran (2009c) observed the frequency of arenicolous marine fungi on sand buried wood substrates along Kerala beaches. Nambiar and Raveendran (2009d, e, f, g, h, i) recorded manglicolous marine fungi on *Avicennia* and *Rhizophora* along the Kerala coast.

Nambiar and Raveendran (2009j) investigated marine mycoflora of Andhra Pradesh (India). Nambiar and Raveendran (2009k) gave an account of marine mycoflora of south India with special emphasis on lignicolous marine fungi. Nambiar and Raveendran (2009l) examined the eco-biodiversity of marine mycoflora of the Gulf of Mannar biosphere reserve. Nambiar and Raveendran (2009m) provided a detailed account of manglicolous marine fungi of Kerala.

Shini et al. (2009–2010) studied assemblage and diversity of higher marine fungi from Thalassery (Kerala) and Bakkhali (West Bengal), the underexplored mangroves. Sridhar et al. (2010) studied fungal colonization and breakdown of sedge (*Cyperus malaccensis*) in a mangal located along the coast of Karnataka. Nambiar and Raveendran (2010a) enumerated the frequency and abundance of arenicolous marine fungi along South Indian Beaches. Nambiar and Raveendran (2010b) studied the impact of hospital waste on mangrove fungi of Kerala. Nambiar and Raveendran (2010c) observed the frequency and abundance of marine fungi of South India. Nambiar and Raveendran (2010d) examined the arenicolous marine fungi of Kerala. Nambiar and Raveendran (2010e) observed the frequency and abundance of algicolous marine fungi along Indian coasts.

Kamble et al. (2011) recorded six species of marine ascomycetes from Gujarat coast. Nambiar and Raveendran (2011a) reported *Arenariomyces triseptatus* and *Halospheria appendiculata* as new records of marine fungi from India. Nambiar and Raveendran (2011b) reported *Anthostomella nypensis* and *Biconiosporella corniculata* as additions to the marine mycoflora of India. Khan and Manimohan (2011) reported *Etheirophora bijubata*, *Remispora quadriremis* and *Robillarda rhizophorae* as new records for India.

Borse et al. (2013) provided a checklist of marine fungi of India. Sarma and Raghukumar (2013a) made observations on manglicolous marine fungi of Choraomangroves (Goa). They found *Aigialus grandis, Trichocladium achrasporum, Morosphaeria ramunculicola, Halorosellinia oceanica, Rimora mangrovei, Scleorococcum haliotrephum* and *Rhizophila marina* as the core group

fungi at this site. Sarma and Raghukumar (2013b) found three ecologically distinct groups of mangrove fungi on *Rhizophora apiculate* at Choraomangroves, Goa, west coast of India, including commensals (e.g. *Aigialus grandis*), mutualists (e.g. *Trichocladium achrasporum* and *Verruculina enalia*) and antogonists (e.g. *Rimora mangrovei*). Borse and Gosavi (2013) recorded two mitosporic fungi as new to India. Sivakumar (2013, 2016) reviewed diversity of marine mangrove fungi. Manohar et al. (2014) isolated *Tritirachium candoliense* sp. nov., a Basidiomycetous fungus from the Arabian sea.

Nambiar and Raveendran (2014) made an investigation on the diversity of marine fungi occurring on *Avicennia* sp. and *Kandelia candel* along the Kerala coast. Nambiar and Raveendran (2015) observed frequency of marine fungi on animal substrates along the west coast of India. Ghate and Sridhar (2015) contributed to the knowledge on macrofungi in mangroves of the Southwest India. Sarma (2015) provided a key for identification of the genera of marine fungi recorded on *Rhizophora* spp. Sohal and Negi (2015) investigated into the diversity of marine mangrove fungi from Mumbai coast (Maharashtra).

Tuwar et al. (2016) recorded *Corollospora besarispora* and *C. fusca* from Goa coast. Borse et al. (2016) recorded *Savoryella melanospora* form Maharashtra. Devadatha et al. (2017) introduced a new species, *Vaginatispora microarmatispora* (Ascomycota) from Muthupet mangroves, Tamil Nadu coast, South India. Gosavi and Borse et al. (2017, 2018) reported marine fungi from Maharashtra. Gosavi et al. (2018) reported marine mitosporic fungi from Maharashtra. Pawar et al. (2018a, b) studied marine fungi from Sundarbans (West Bengal). Borse et al. (2018) reviewed marine and freshwater fungi of India. Devadatha et al. (2018a) reported a new genus *Thyridariella* with two new species in this genus including *T. mangrovei* and *T. mahakoshae* from Muthupet mangroves of Tamil Nadu, east coast of India. *Deniquelata vittalii*, a novel Indian saprobic marine fungus on *Suaeda monoica,* and two new records of marine fungi from Muthupet mangroves, east coast of India, were reported by Devadatha et al. (2018b). *Morosphaeria muthupetensis,* a new species in Morosphaeriaceae, was reported from Muthupet mangroves, India, based on morphological characterization and multigene phylogenetic inference (Devadatha et al. 2018c). Devadatha and Sarma (2018) reported *Pontoporeia mangrovei,* a new marine fungus, from Muthupet mangroves, east coast of India, along with a new geographical and host record for *Falciformispora lignatilis*. A new genus has been described in honour of Prof. B.P.R. Vittal as *Vittaliana mangrovei* gen. et sp. nov. in Phaeosphaeriaceae from Pondicherry mangroves in India, based on morphology and multigene phylogeny (Devadatha et al. 2020). Similarly, another new genus has been raised in honour of Seshagiri Raghukumar and Chandralatha Raghukumar as *Raghukumaria. Raghukumaria keshaphalae* was recorded on *Aegiceras corniculatum* along with a new species *Fusicolla bharatavarshae* on *Avicennia marina* from Muthupet mangroves by Jones et al. (2019). Also five new marine fungal species were added from Indian mangroves in a new compilation. These include a new genus, *Pseudoastrosphaeriellopsis* and its new species *Pseudoastrosphaeriellopsis kaveriana* on *Avicennia marina* and *Suaeda monoica*; *Zopfiella indica* on intertidal mangrove wood; *Amphisphaeria mangrovei* on *Suaeda*

monoica; *Hypoxylon teeravasati* on *Avicennia marina* and *Suaeda monoica*, all from Muthupet mangroves and *Peroneutypa mangrovei* on *Avicennia marina* from Thengaithittu mangroves, Pondicherry, on the east coast of India (Phookamsak et al. 2019). Recently, five more new marine fungal species were reported from India including *Nigrograna samueliana* on *Avicennia marina* from Parangipettai, Tamil Nadu; *Phaeoseptum carolshearianum* and *Phaeoseptum manglicola* on *Avicennia marina* wood and *Peroneutypa indica* and *Peroneutypa polysporae* on *Suaeda monoica* from Muthupet mangroves, Tamil Nadu, east coast of India (Dayarathne et al. 2019a). Phylogenetic revision of Savoryellaceae and evidence for its ranking as a subsclass was published recently (Dayarathne et al. 2019b). The other new marine fungal species reported are *Verruconis mangrovei* on *Excoecaria agallocha*, and *Lanspora cylindrospora* on *Suaeda monoica* from Muthupet mangroves, Tamil Nadu, east coast of India (Hyde et al. 2020). *Halocryptosphaeria* has been introduced as a new genus to accommodate *Halocryptosphaeria bathurstensis* by a transfer of *Eutypa bathurstensis* (Dayarathne et al. 2020). Also, a new species has been introduced in this genus, viz., *Halocryptosphaeria avicenniae* (Dayarathne et al. 2020). Recently *Neodevrisea manglicola* on *Rhizophora mucronata* from Parangipettai mangroves, Tamil Nadu, India (Yuan et al. 2020) and *Biatriospora borsei* on *Avicennia marina* from Muthupet mangroves, Tamil Nadu, east coast of India (Hongsanan et al. 2020), were reported as novel species.

2.3 Role of Marine Fungi in Ecology and Their Potential Biotechnologies

Durve and Bal (1960) reported a shell disease of *Crassostrea gryphoides* Schlotheim from Bombay, the aetiology is not yet understood (Ramaiah 2006). Raghukumar (1985) enumerated thraustochytrids from the Arabian Sea. Salique et al. (1985) made observations on fungal populations in the Vellar estuary and Pichavaram mangroves. Raghukumar et al. (1988b) monitored the colonization of marine fungi on seven different kinds of wood panels pretreated with wood preservatives in Mandovi estuary (Goa).

Raghukumar et al. (1990) noticed the abundance of thraustochytrid fungi in the Arabian Sea. Raghukumar (1990) made speculations on niches occupied by fungi in the sea in relation to bacteria. The fungal invasion of massive corals was investigated by Raghukumar and Raghukumar (1991). Raghukumar et al. (1992b) isolated the endolithic fungi from deep-sea calcareous substrata and conducted laboratory studies. Sathe-Pathak et al. (1993) and Sharma et al. (1994) studied thraustochytrid and fungal component of marine detritus of the brown alga, *Sargassum cinereum*.

Raghukumar et al. (1994a) reported production of laccase and other lignocellulose-modifying enzymes by marine fungi. Raghukumar et al. (1994b, 1995) demonstrated the role of thraustochytrids and fungi in the decomposition of leaves of the mangrove *Rhizophora apiculata* in the laboratory as well as in the field. Raghukumar et al. (1996) tested the efficacy of marine fungi on the degradation of

lignin and decolourization of paper mill bleach plant effluents. Ravishankar and Suryanarayanam (1998) reported that the increasing salinity elevated the activities of polyol dehydrogenase (NADP+), D-mannitol dehydrogenase and peroxide of the mangrove fungus *Cirrenalia pygmea*. The association of arbuscular mycorrhizal fungi with *Launaea sarmentosa* was observed on maritime sand dunes (Beena et al. 1997).

The barotolerence of fungi isolated from deep-sea sediments had been reported by Raghukumar and Raghukumar (1998). Raghukumar and Raghukumar (1999) reported thraustochytrid fungoid protists in faecal pellets of the tunicate *Pegea confoederata* and their tolerance to deep-sea conditions and implications in degradation processes. Raghukumar et al. (1999) investigated into the lignin modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment.

Raghukumar (2000) proved decolourization of beach plant effluents and synthetic dyes by fungi from marine habitats. Suryanarayanan and Kumaresan (2000) reported endophytic fungi of some halophytes from an estuarine mangrove forest from Tamil Nadu. Beena et al. (2000a) reported fungal endophytes of three sand dune plant species of west coast of India. Kumaresen and Suryanarayanan (2001) studied the occurrence and distribution of endophytic fungi in a mangrove community. Kumaresen and Suryanarayanan (2002) studied endophytic fungi in young, mature and senescent leaves of *Rhizophora apiculata* and provided evidence for the role of endophytes in mangrove litter degradation. Devarajan et al. (2002) studied endophytic fungi associated with the tropical seagrass *Halophila ovalis* (Hydrocharitaceae). Beena et al. (2000b) studied the diversity of arbuscular mycorrhizal fungi on the coastal sand dunes of the west coast of India. Raghukumar et al. (2001a) studied the dynamics of thraustochytrid protists in the water column of the Arabian Sea. Raghukumar et al. (2001b) observed thraustochytrid protists as a component of marine microbial films.

Oil pollution in marine environments occur mainly due to coastal oil refinery effluents, regular shipping operations, industrial and municipal waste disposal and occasional oil-spills caused by oil tanker accidents and any blow-outs from off-shore oil-well platforms (Clark 1996). Different groups of microorganisms including bacteria, unicellular yeasts and filamentous fungi have been implicated to play an important role in hydrocarbon degradation in the marine environment. The filamentous fungi with such proven capabilities reported are species belonging to *Aspergillus, Aureobasidium, Candida, Cladosporium, Geotrichum, Penicillium, Trichosporon* and *Pichia*; these were earlier considered as marine-derived fungi as they were usually reported from the terrestrial environments (Ahearn et al. 1971; Bhosle and Mavinkurve 1984; vide Zinjarde et al. 1998). In addition to the filamentous fungi, some yeasts also have been shown to be involved in oil degradation; *Yarrowia lipolytica* had been reported to be one such yeast that could degrade aliphatic fractions of crude oil (Zinjarde et al. 1998). M.V. Deshpande at the National Chemical Institute, Pune, Maharashtra, has been working along with his students on oil degradation by yeasts since 1990s. They have found that *Y. lipolytica* strains to have capability to undergo morphological changes from yeast to mycelium

in response to the prevailing environmental and nutritional conditions, e.g. presence of casein plus olive oil or *N*-acetylglucosamine (GlcNAc) or olive oil in their growth medium (Rodriguez and Dominguez 1984). Zinjarde et al. (1998) isolated fungal cultures from oil-polluted seawater near Mumbai, India; *Y. lipolytica* NCIM 3589 was shown to be dimorphic in behaviour and degrades oil pollutants. Interestingly this yeast degrades aliphatic fractions of crude oil in yeast form in addition to pure alkanes in pure form within 20–48 h under aerobic conditions. Further, these authors reported that under slight anaerobic conditions (low oxygen tensions or when oxygen was replaced by nitrogen or carbon dioxide), this organism switches over to mycelial form. Based on their results, they also suggested that mutants with monomorphic yeast form may be useful in enhanced alkane degradation. A similar observation was reported by Palande et al. (2014) with *Y. lipolytica* var. *indica* wherein the new variety not only degraded the hydrocarbons but also used them as carbon source. These investigators reported that the terrestrial strains of *Y. lipolytica* are more efficient in hydrocarbon degradation in mycelial form than the yeast form. Campos-Góngora et al. (2018) developed an ornithine decarboxylase minus (*odc*-) mutant of *Y. lipolytica var. indica* and showed that the mutant does not turn into mycelial state even at the concentration of 10 mM of putrescine in the medium that produces polyamines and thus they confirmed that the mutant yeast is constitutively a monomorphic one under aerobic conditions. However, this mutant formed mycelial cells under partial anaerobic conditions. Interestingly this mutant showed higher hydrocarbon degradation proportional to higher concentration of putrescine. This mutant also has been reported to be showing a higher emulsification activity than its parental strain which could have advantage in possible technological applications of hydrocarbon degradation (Campos-Góngora et al. 2018).

Ravindran et al. (2001) studied fungi in *Porites lutea*, associated with healthy and diseased corals. Raikar et al. (2001) carried out an investigation on the degradation of hydrocarbons by thraustochytrid protists. Raghukumar (2002) reported bioremediation of coloured pollutants by terrestrial versus facultative marine fungi. Raghukumar (2002) reviewed the ecology of the marine protists, the Labyrinthulomycetes (thraustochytrids and labyrinthulids).

Jain et al. (2004) investigated enhancement in the production of polyunsaturated fatty acids, docosahexaenoic acid in thraustochytrid protists. Raghukumar (2004a, b) reviewed the role of fungi in marine detrital processes and reviewed enzymes of marine fungi in decolourization of coloured effluents. Raghukumar et al. (2004a) studied xylanases of marine fungi for their potential use for biobleaching of paper pulp, while the diversity of culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean, was studied by Raghukumar et al. (2004b). Jain et al. (2005) worked on extra-cellular polysaccharide production by thraustochytrid protists. Karunsagar et al. (2004) reviewed microbial diseases in shrimp aquaculture.

Raghukumar (2006) studied the algal–fungal interactions (symbiosis to parasitism) in the marine ecosystem. D'Souza et al. (2006) reported enhanced production of laccase by a marine fungus during treatment of coloured effluents and synthetic dyes. Damare et al. (2006a) studied fungi in deep-sea sediments of the Central Indian Basin and investigated deep-sea fungi as a source of alkaline and cold-tolerant

proteases (Damare et al. 2006b). Raghukumar (2006) reviewed marine eukaryotic diversity with particular reference to fungi. The fungal diseases of algae, marine fishes, shrimps and corals had been reviewed by Ramaiah (2006). Ravishanker et al. (2006) investigated strategies for osmoregulations in the marine fungus *Cirrenalia pygmea* Kohlm.

Raghukumar (2008a) reviewed marine fungal biotechnology from an ecological perspective. The production of PUFAs by thraustochytrids was reviewed by Raghukumar (2008b). Kamat et al. (2008) reported marine-derived fungi to be a good source of proteases. Purvetkar et al. (2009) screened marine-derived fungus *Aspergillus terreus* for Aspernoilides A and B and Butenolides. Jebaraj et al. (2010) surveyed fungal diversity in oxygen-depleted regions of the Arabian Sea. Suryanarayana et al. (2010) studied the diversity and biotechnological potential of internal mycobiota of marine macroalgae from Tamil Nadu coast. Raghukumar et al. (2010) reviewed the deep-sea fungi. Singh et al. (2010) provided phylogenetic diversity of culturable fungi from the deep-sea sediments of the Central Indian Basin and their growth characteristics. Singh et al. (2011) undertook fungal community analysis in the deep-sea sediments of the Central Indian Basin by culture-independent approach. Raghukumar and Damare (2011) provided increasing evidence for the important role of Labyrinthulomycetes in marine ecosystems. Sridhar (2012) reviewed in detail the decomposition of materials in the sea. Sarma (2012) studied the diversity and distribution of marine fungi on *Rhizophora* spp. in mangroves. Damare et al. (2012) reviewed biotechnology of marine fungi. Raghukumar (2012) published an edited book, namely, '*Biology of Marine Fungi*'. Suryanarayanan (2012a) investigated the fungal endosymbionts of seaweeds, while the diversity and importance of fungi associated with marine sponges was discussed by Suryanarayanan (2012b). Thirunavukkarasu et al. (2012) surveyed the fungal symbionts of marine sponges from Rameswaram, southern India. Venkatachalam et al. (2015) isolated endophytic fungi of marine algae and sea grasses, while their distribution and diversity were investigated by Venkatachalam et al. (2016). Raghukumar (2017) published a book, namely, 'Fungi in Coastal and Oceanic Ecosystem'. Sridhar (2017) reviewed the diversity, distribution and bioprospecting of marine filamentous fungi. The biodeterioration potential of obligate marine fungi and the role of marine fungi in sustainable development were reviewed by Sarma (2018a, b). The present status and future perspectives of marine fungal diversity were discussed by Sarma (2019) and the marine fungal ecology in the molecular era by Sarma and Jeewon (2019) in different book chapters.

2.4 Future Perspectives

Though in the past four decades the marine fungal research has been active, very few centres are now involved in the research on marine fungi. Few examples include National Institute of Oceanography, Goa, India, and Department of Biotechnology, Pondicherry University. However, it requires more centres, more researchers and

sustained efforts for marine fungal research. India has a vast coastline and marine fungi form one of the important components of bioresources.

The marine fungi and lichens of India are not fully explored. There are sporadic reports on marine fungi of mangroves from Mahanadi delta, Indian Sundarbans, Gulf of Kutch, Andaman and Nicobar Islands, etc. Similarly, marine algae, marine animals, salt marsh plants and sea grasses are unexplored substrates for marine fungi. There are only sporadic reports on lower marine fungi from various coastal areas of India. Hence these missing gaps should be taken up for future research.

There is a paradigm shift in the drug research with new environments that have extremophiles, and other such fungi are being explored. More research is required on the biotechnological research of marine fungi in India including secondary metabolites, enzymes, different applications in environmental clean-up, healthcare and industry. There is a requirement for a national facility for marine fungi in India. Nowadays molecular sequence data is incorporated in the taxonomic research and multigene sequence analysis has become a routine practice while describing new genera and new species. Hence more centres and laboratories should tune in with the latest trends of molecular diversity analyses for characterizing marine fungal taxa. Also there is a need to increase studies on metagenomics of marine fungi.

2.5 Concluding Remarks

Around 225 marine fungi and fungal-like organisms were listed by Borse et al. (2012) from India. Most of them belong to higher fungi group (Dikarya) and the remaining belongs to lower fungal groups. A recent update on marine fungi in mangroves alone from India shows that the number has risen to 339 (Sarma and Devadatha 2020, Devadatha et al. 2021). Most of the species are reported as the new additions to the fungi of India, while 7 genera and 44 species were described as new to science by various researchers based on the type material collected along the coasts of India. Not much is known about marine lichens of India (Borse et al. 2012, 2017). The mangroves of Maharashtra, Karnataka and Kerala on the west coast of India and Tamil Nadu and Andhra Pradesh of east coast of India have been more intensively investigated for the marine fungi. From other mangrove formations along west coast (Gujarat and Lakshadweep Islands) and east coast (Mahanadi delta, Sundarbans and Andaman & Nicobar Islands), only stray collections and examinations have been undertaken. Since elaborate biodiversity studies on marine fungi of the latter mangrove sites have not been conducted, there is a scope to undertake such studies from these mangrove formations. Any type of research depends on the expertise of the researcher in any particular group and his or her sustained efforts. The research on lower marine fungal groups is dismally low excepting one group, i.e. thraustochytrids.

Though India has a vast coastline of more than 6000 km, only a few centres are involved in the marine fungal research of east and west coasts. During last six decades, the marine fungal studies in India were directed more towards diversity and ecology and only one institute (NIO, Goa) involved intensively on physiological

and biotechnological aspects of marine fungi. There is a need for more centres and researchers to involve in the marine fungal research to harness the benefits from the marine fungi in various spheres such as medicine, industry and environment. While several deep-sea fungal explorations have taken place from Arabian Sea, such efforts are wanting from the east coast (Bay of Bengal region).

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History and Developments of Plant Pathology in India: Fungal Aspects

3

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Abstract

The chapter makes a modest attempt to highlight the major achievements of fungal plant pathology in India which had set a milestone in history of fungal plant pathology. The chapter summarizes the historical achievements made in the area of plant pathology with respect to diseases caused by fungal pathogens, disease biology research, epidemiology, fungicide research, disease control strategies and the introduction of plant pathology as a discipline in Indian Universities. It also highlights the present status of plant pathology in India giving an overview of the developments made in the past over years. Though the chapter by no way is a complete account of the vast ocean of information available on various aspects of the subject, it is anticipated that the historical events covered in this tells the story of Indian plant pathology beginning from the Vedic era to the younger generation and motivate for the greater challenges of the pathological problems ahead.

Keywords

Fungal diseases · Indian history · Plant pathology · Disease management

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3.1 Concept of Plant Pathology

The term "Pathology", like numerous others, in science derives from the two Greek words: pathos-suffering and Logos-to speak or discourse (Horsfall and Dimond 1959). Plant pathology is considered an integral part of plant protection and is mostly crop oriented. Plant pathology is not only concerned with the biology of the pathogens and pests and their mode of action but also with the control measures, new pesticides trials and their mechanism of action, toxicological aspects of crop protection, economics of control, etc.

Although plant pathology as a science attempts to increase our knowledge of the cause and the development of plant diseases, it is also a science with a more practical goal to control all plant diseases. Plant pathology has revealed profound and useful truths. It was among the pioneers in revealing the vast and variable world of microorganism and in identifying man's friends and foes among them. According to Stakman (1967), "It has not only helped man to solve many of his basic problems, but it has helped to illuminate his intellect and expand his intellectual horizons; it has helped man to comprehend infinity in minuteness as it had begun to comprehend infinite magnitude".

3.2 Ancient Indian Literature on Plant Pathology

With cultivation of crops started the understanding of numerous diseases of the crops in several parts of the world including the Indus civilization. The ancient testament on plant pathology was recorded in the Homer 1000 BC, and 750 BC. The well-known Greek philosopher "Democritus" in 470 BC has also talked about the blights. Theophrastus, who is also the "father of the botany", has described plant diseases in a systematic form (Agrios George 1972). His description of plants and theory of plant diseases were founded on the beliefs and theories which was nearly 2300 years old (Sharma 2018). Although the control over epidemics in that era was not possible due to lack of proper scientific knowledge, some reports show Homer employed sulphur and Theophrastus olive oil to control the plant diseases (Burkholder 1948; Martinelli et al. 2014).

India with its civilization nearly 5000 years old, the agriculture was started 4000 years ago and plant diseases have been mentioned much earlier than Theophrastus. As regards Sanskrit Literature, it is quite evident that whether it be Vedic literature or classical Sanskrit literature, all have given due importance to the plants. Though description of plants has been found in all the Sanskrit books, the present search is based on the sources with Atharvaveda (2500 BC) (Dube 2006; Shastri Acharya 2003), Kautilya's Arthaśāstra (third century BC) (Shamasastry 2005), Agni Purāna (fourth century AD) (Vyāsaśisya 1980), Brahatsamhitā (sixth century AD) (Ramakrishna 1995), Śukranīti (eighth century AD) (Miśra 1999), Krsiparāśara (sixth–tenth century AD) (Prasad 2003), Vrakshā-Ayurveda (tenth century AD) (Jugnu 2004) and Upavanavinoda (thirteenth century AD). Going by the Sanskrit Literature, it comes to light that the plant diseases and pests were well

recognized by ancient Indian seers. They developed eco-friendly ways of crop cultivation and protection utilizing organic agents as pesticides. Destruction of corns by various kinds of reptiles and vermins has been referred to in Atharvaveda. It hints of various diseases by which plants get infected. Some of these are difficult to prevent, and some were treated using specific herbs. The Sukranīti makes it clear that grains get spoilt by poisons, fire or snows or eaten by worms and insects (Dwivedi 2014). It becomes evident from such ancient literature that organized agriculture was being practised in India nearly 4000 years ago and that attention was paid to plant diseases and pest in crop production system referring to blights, root rots and wilt types of symptoms. Rig-Veda mentions the classification of plant diseases and also the germ theory of diseases. In Vedic era men were aware of the fact that diseases were caused by microorganisms. The classic book Vrakshāyurveda, written by Surapal, appears to be the first book in which details of plant diseases are included. Two groups of plant diseases were recognized: internal diseases (which now are known as physiological disorders), and external diseases, caused by microorganisms and insect pests (Dwivedi 2014), and it quotes

śārīrāngantubhedenadvihprakārāhsamāsatah ।
śarvabhū r u h a jā t ī n ām-ān t a k a h parikīrtitah ॥(Vrksāyurveda, 8.165)

Discussing these diseases the text says:

tatravātātkaphātpittāccharīrānāmsamudbhavāh ।
āgamtūnāmsamutpattihkītaśītādibhirbhavet॥ (Vrksāyurveda, 8.166),

The above text means that the internal diseases are caused by vāta, pitta and kapha, and the external ones by insects, cold weather, etc. All methods of therapeutic control over plant diseases were followed in the same manner as for humans. As chemical methods, use of milk, ghee, honey, barley flour and herbal pastes of plant extracts was practised. Oil cakes of mahua, mustard, shesham and caster were used to control root diseases which are even followed today. We find a proficient text with beautiful mantras and verse on illness on different plant organs caused by different external and internal factors. The science of treating human diseases in ancient Indian literature later took its way in the Indian system of medicine as "Ayurveda" but the science of plant pathology was somewhere lost in the ages. Symptoms of plant diseases are also mentioned in Hindu mythology, Jataka of Buddhism, and Raghuvansh of Kalidas.

3.3 Plant Pathology in India During Colonial and Post-Colonial Periods

The global history of plant pathology as a science is not old and so was the case in modern India. Possibly, a few hundred years ago, there was no science of plant pathology. Actually, the foundation of plant pathology as a science was laid in the

early part of the nineteenth century particularly when the classic work of French scientist, Prevost (1807) proved that diseases were caused by the microorganisms and he discovered the life cycle of the bunt fungus (Singh 1984). The idea of microorganisms was an impetus which attracted the smart minds that made legends later. Mycological studies in India started with the passion of Europeans (Koenig, Jacquemontt, Hooker, Wight, SulpisKurz) to collect fungal samples and send them to the UK and Europe for its identification (Berkekeyan and Currey in England, Leveille and Montagne in France). Sulpis Kurz was the Curator at Royal Botanical Garden, Calcutta (Subramanian 1986). Later on identifying fungi in India was initiated by two British scientists A.D. Cunningham and A. Barcley during the period 1850–1875 (Sharma 2018) who studied the rust and smut fungi as these were mainly affecting the wheat crop which was the staple food of India. Cunningham unveiled many details of rusts and smuts. A.D. Cunningham also identified the causal organism of red rust of tea in Assam as *Cephaleuros virescens*. Cunningham pioneered work on aero-mycology and aerobiology and made important contributions in understanding Uredinales, Mucorales and Ustilaginales. K.R. Kirtikar (1885) was the first ambitious Indian scientist to make remarkable contributions by collecting and identifying the fungi in this country.

It was after that in the first decade of the twentieth century that the first planned and organized institute was established by the British government in Samastipur district of Bihar as Imperial Agricultural Research Institute. The financial support was extended by an American philanthropist Dr. Henry Phips, after whom the name was kept PUSA, where P is for Phips and USA for the county to which he belonged. The institute was later shifted to Delhi after an earthquake in 1934, and after India's independence it was renamed as Indian Agricultural Research Institute (IARI), PUSA, New Delhi. Sir Edwin J. Butler was appointed as its first Imperial Mycologist. He stayed at the IARI, Pusa (Bihar), for 16 years (1905–1921) and established a strong school of mycology and plant pathology in India and is honoured as "Father of Indian Plant Pathology" (Raychaudhuri et al. 1972). He discovered two important diseases of sugarcane; the first one was the red rot caused by *Colletotrichum falcatum,* on which he worked with Khan (Butler and Khan 1913) at Pusa in 1913, and the second one was smut disease caused by *Ustilago sacchari.* They showed that the infection of red rot caused by *Colletotrichum falcatum* occurred through seed, soil and irrigation water (Chona 1943, 1950) and suggested simple practice for its control (Chona 1947). Butler (1918) also first described the mode of infection (Dastur 1920), life history and method of perpetuation of the pathogen *Ustilago sacchari* causing the smut disease in sugar cane. E.J. Butler was the first plant pathologist credited to undertake detailed studies on fungi *Phytophthora infestans*, causing disease in potato and castor. A monograph on potato diseases was written by Butler (1903) in 1903. His best contribution on plant pathology in India still exists in the form of the classic book "*Fungi and Disease in Plants'* published in 1918. His book became the major source of literature in understanding the critical details of plant pathology and proved to be a great inspiration to the budding plant pathologists in India. J.F. Dastur (1886–1971), who was an Indian colleague of Butler, studied the genus *Phytophthora* and infections caused by it in potato and castor. Dastur

(1915) reported that potato blight due to *Phytophthora infestans* was uncommon in the Indian plains, but favoured infections on hills causing severe damage to crops grown at an elevation of 6000 ft. and above. Late blight was later found to occur in the plains also (Lal 1948; Vasudeva and Azad 1949). Dastur defined two novel diseases of potato: tuber rot caused by a new species, *P. himalayensis,* and leaf rot caused by a species resembling *P. parasitica* (Dastur 1948).

With losses from diseases going enormous, there was an increasing importance of plant pathology being realized that led more scientists to take a keen interest in this field. It was then the cereal rusts that received attention in India in early 1907 when Milligan testified a heavy wheat rust outburst in Punjab. Studies on the epidemiology of rusts and methods of their control gave a holistic idea of the work plan to be followed in future studies and was an important contribution to plant pathology carried out in India. Dr. K.C. Mehta (Father of Indian Rust) during the first half of the twentieth century at Agra College studied the life cycle of cereal rusts in India, and it was in 1929 that he deciphered the disease cycle of cereal rusts in India specially the stem/black rusts, rejecting the theory that Barbery played a crucial role in annual recurrence of the disease (Mehta 1929). Dr. Mehta wrote a monograph on "Further Studies on Cereal Rust in India" in 1948. Raghubir Prasada (1907–1992), trained by K.C. Mehta, contributed in understanding the cereal rusts by identifying their physiological races. He also described the life cycle of flax rusts, *Melapspora lini* (Raychaudhuri et al. 1972). Dr. Prasada also contributed significantly on *Alternaria* blight of linseed and cucurbits, powdery mildews of wheat and pea and blight of pearl millet. His monumental work on linseed rust decorated him with D.Sc. degree from Agra University in 1943. Since 1935, Dr. Prasada was associated with the breeding of rust-resistant varieties of wheat. It was then after a long gap that "Indian Stem Rust Rules" for *Puccinia graminis tritici* was framed by S. Nagarajan and H. Singh (1975). By this time scientists started implementing the emerging technologies in understanding the triad of plant, pathogen and environmental interactions. Dr. S. Nagarajan and Dr. L.M. Joshi (1978) used the climatic and weather-based information to identify *Puccinia* path in India. Going back to the early twentieth century, we find that the passion for rust kept growing and CD Mayee contributed to the understanding of the groundnut rust and sunflower downy mildew and documented his work in the book "Phytopathometery". Mitra (Mitra 1931) recorded *Tilletia indica*, a new bunt (Karnal bunt) on wheat that was thought, erroneously, to be soil borne (Mitra 1931, 1937a, b, c) but later proved to be air borne, with the infections being non-systemic (Mundkur 1943a, b).

Working at Allahabad University in 1937, Prof. R.N. Tandon went to the Imperial College, London to work on the physiology of fungi under the guidance of Prof. W. Brown, FRS, and earned a Ph.D. degree from the London University in 1939. On his return to Allahabad University, he continued not only with his interest on physiology of fungi but also studied fungal taxonomy and fungal flora of different regions of the country, and prepared an exhaustive list of fungi found in the plains and hilly regions of Uttar Pradesh and neighbouring states. He studied the physiology and nutrition of different strains and species of a large number of pathogenic fungi causing storage diseases of fruits and vegetables and leaf spot diseases. These

studies not only led to a better understanding of the life processes of the organisms but also established that the classification of fungi based on nutritional requirement is not correct as even closely related species show marked differences in their nutritional requirements. He also developed strategies for the management of a large number of economically important plant diseases.

Few other diseases which were worked out in the colonial period was the green-ear disease of bajra (*Pennisetum typhoides*) which for the first time was shown by Chaudhuri in 1931 to be propagated through the oospores of the pathogen *Sclerospora graminicola* present in the soil (Chaudhuri 1928, 1932a, b). However, it was found that the artificial infection of the host could be accomplished only under very humid conditions (Uppal and Kamat 1928).

While most of the fungal pathology was focused on crop, exploration in forest pathology was instigated by Bagchee in the Himalayas while he was at the Forest Research Institute, Dehra Dun. He wrote several reviews on the canker pathogens, root and stem-rotting fungi, coniferous rusts, timber diseases, nursery diseases and the habits and ecology of forest fungi (Bagchee 1939). He also enlisted polypores and rusts infecting forest trees. The primary diseases in Indian oak were also studied by him (Bakshi and Bagchee 1950).

After the colonial period, investigations into fungal pathology were regaining prominence with increasing understanding of their biological significance, origin, function and structural diversity. S.L. Ajrekar worked on wilt of cotton, sugarcane smut and ergot of jowar and on their control measures (Raychaudhuri et al. 1972). G.S. Kulkarni printed extensive data on downy mildew and smuts of pearl millet and sugarcane. T.S. Ramakrishan studied and contributed in understanding the genera *Pythium, Phytophthora, Colletotrichum* and the rusts. Few newer diseases of wheat that came into prominence due to new agricultural practices, such as extensive use of fertilizer, intensive irrigation, and use of the new high-yielding varieties (Raychaudhuri 1968), was leaf blight of wheat caused by *Alternaria triticina*.

B.B. Mundkur identified and classified a large number of Indian smut fungi. Mundkur initiated research on varietal resistance for controlling cotton wilt and was also recognized for the systematics of a large number of Indian smut fungi. After Mundkur, it was M.J. Thirumalachar who carried forward the work on rust and smuts of India. Likewise malformation was a challenging disease of mango (*Mangifera indica*) at a time as different workers have attributed it to different causes like nutritional imbalance, virus, eriophyid mites, etc. Affected shoots from mango trees were later on shown to have *Fusarium monilijorme* (*Gibberella fujikuroi*) presence which inoculated the healthy seedlings grown in the glasshouse (Summanwar et al. 1966). The disease was later shown to be systemic in branches (Varma et al. 1969). Numerous studies attempted to understand the malformation disease in mango since its recognition in 1891, which was worked out by J. Kumar from G.B. Pant University of Agriculture and Technology revealing its aetiology and control.

The period 1950–1975 was of intense mycological studies emphasizing disease interpretation and pathogen identification. Plant pathology gained impetus by the inputs that came from mycology and closely related disciplines. These include work

Table 3.1 Monographs on Indian fungal flora

Contributor	Monograph	Year
E. J. Butler	Potato diseases of India	1903
E. J. Butler	The fungi of India	1907
E. J. Butler	Fungi and diseases in plants	1918
K.C Mehta	Further studies on cereal rusts in India	1948
Mundkur and Thirumalachar	Ustilaginales of India	1952
T. S. Ramakrishan	Diseases of millets	—
G. Rangaswamy	Pythiaceous fungi	1958
K. S. Thind	The Clavariaceae of India	1961
R. S. Vasudeva	Indian Cercosporae	1963
J. S. Karling	The Chytrids of India	1966
R. N. Tandon	The Mucorales of India	1968
B. K. Bakshi	Indian Polyporaceae	1971
C. V. Subramanian	Hyphomycetes	1971
S. S. Rattan	The Resupinate Aphyllophorales of the Northwest Himalayas	1971
K. S. Thind	The Myxomycetes of India	1977
A. P. Misra	Helminthosporium	1977
T. N. Lakhanpal and K. G. Mukerji	Indian Myxomycetes	1981
K. Natarajan and N. Raman	South Indian Agaricales	1983
Manjula	Taxonomic study of Indian Agaricales	1983
Vidhyasekaran P.	Fungal pathogenesis in plants and crops	1997
R. S. Mehrotra	Phytophthora Diseases in India	2001
R. S. Upadhyay	Wilt of pigeon-pea	2001
Kamal	Cercosporoid fungi of India	2010
B. Borse, D. J. Bhat et al.	Marine fungi of India	2012
R. S. Singh	Introduction to principles of plant pathology	2018

on *Cerebella* (L.S. Subramaniam), polypores (S.R. Bose), Gasteromycetes (C.G. Llyod), graminicolous *Helimenthosporium* species (M Mitra) and work on various fungal diseases of agricultural crops like downy mildews, especially *Sclerospora* (B.N. Uppal), *Fusarium* (G.W. Padwick) and plantation crops like areca nut, coconut, tea and coffee. K.S. Thind and his group provided inputs for the Clavariaceae, the Agarics and the Discomycetes. Other significant inputs from mycology were Polypores (B.K. Bakshi and Thind), the Cercosporae and the Meliolineae (Thirumalachar and C.G. Hansford), Synchytria (B.T. Lingappa), *Pythium* (M.S. Balakrishnan), Mucorales, the Aspergilli and Penicillia (B.S. Mehratra), Hyphomycetes (C.V. Subramanian), lichens (D.D. Awasthi) and the Myxomycetes (V. Agnihothrudu, K.S. Thind, Indira Kalyanasundaram, T.N. Lakanpal) (Subramanian 1986). Several fungal genera or groups were monographed (few supported by ICAR) which are listed in Table 3.1.

3.4 Historical Expansions in Disease Control

With the unravelling of the plant pathogens and their disease cycle, interest in controlling them ran parallel as evident from the early reports. In 1885 Ozanne (Nene 1971) first used a fungicide in India for the control of a crop disease when he used copper sulphate against sorghum smut. Later on Lawrence (Nene 1971) used Bordeaux mixture for the first time in 1904 against *Cercospora* leaf spot of groundnut. Coleman (Coleman 1915) claimed control in 1915 on *Phytophthora omnivora* var. *arecae* with Bordeaux mixture plus resin. Cotton anthracnose was controlled (Dastur 1934) by seed dressing with an organomercurial fungicide after delinking the seed with sulphuric acid (Nene 1971). Narasimhan suggested in 1930 the use of linseed oil in Bordeaux mixture for improving coverage and tenacity (Nene 1971). R.S. Vasudeva (1932) investigated root rot of cotton, a soil-borne disease, which had baffled many eminent scientists. He is credited not only for finding the true cause of disease but also for working out simple measures for its control, which were novel. These included recommendations as growing of "moth" (*Phaseolus aconitifolius*) as intercrop with cotton, which reduced soil temperatures because of its spreading nature and thus inhibited the growth of the causal fungi. This work later earned him the degree of D.Sc. of London University (Misra et al. 2016). A heat treatment technique was established by Luthra (1953) for the control of loose smut of wheat which was an altogether new approach in this area. Yeshwant Laxman Nene reported "Khaira" disease of rice at Pantnagar due to zinc deficiency (1965 AD) and authored the book "Fungicides in Plant Disease Control". M.J. Thirumalachar is well known for introducing antibiotics in plant disease control (Singh 1984). Wide use of antibiotics for the control of fungal diseases of crop plants was fairly new in India. Hindustan Antibiotics took a leading role in the commercial manufacture of antibiotics, and one of their products that has been used extensively as a seed dressing (Dharam Vir and Raychaudhuri 1969), a spray on standing crops (Pavgi and Mandokhot 1969), and a post-harvest dip was aureofungin (DharamVir et al. 1968; Swamp and Raghava 1970). He was credited with having identified more than one hundred antibiotics and in their development at commercial level. Dr. Thirumalachar was also responsible for the development of Systemic Fungicide, KT19827 for the control of Dutch Elm disease and Oak Wilt (Misra et al. 2016).

3.5 Historic Famines that Triggered Systematic Research

The historic famine which had an impact on the country and accelerated the research and sudden growth of literature in this field was the great Irish famine in the middle of the nineteenth century, which may be regarded as the dawn of modern era of plant pathology. In 1845 and 1846, Ireland faced a too tragic famine in which Ireland quickly lost two million of its eight million people from starvation, consequent disease, and immigration (Stakman 1967). When the late blight of potato was fast spreading in England, Ireland, and continental Europe, there was no clear concept among the scientists about the disease–fungus relationship, and they had no

Fig. 3.1 Irish famine caused by *Phytophthora infestans*

experimental evidence to prove it. The single event (disease outbreak) tragically forced man to realize the importance of plant diseases and the need for its control. In the middle of the nineteenth century, scientific investigations were taken up and these were traced to be due to severe outbreak of late blight of potato caused by *Phytophthora infestans* identified by the German mycologist Anton de Barry (Mehrotra 1980). De Bary studied the pathogen *Phytophthora infestans* (formerly *Peronospora infestans*) to elucidate its life cycle. During those days the origin of plant diseases was not known, and the credit for the foundation of modern experimental plant pathology goes to German scientist Anton De Bary (1831–1888), respected as "The father of modern plant pathology" (Stakman and Harrar 1957), who confirmed the findings of Prevost made in 1853. He is also credited with the discovery of heteroecious nature of rust-fungi and their parasitism. Brefeld, a colleague of de Bary, discovered the method of artificial culture of microorganism during the period 1875–1912. The Irish famine (shown in Fig. 3.1) although occurred in Europe had a great impact all over the world and so was with India.

Another instance of a serious loss in the white sugar belt of northern India particularly in the states of Bihar, Punjab and Uttar Pradesh by a fungal disease was the red rot of sugarcane reaching its peak in 1938–1939. It badly affected not

only the farmers of the sugarcane-growing areas but even the mills could make only 33% of the normal production quality.

India faced a similar famine later in 1943 in Bengal when about two millions of starvation deaths were reported. Brown spot of rice caused by *Helminthosporium oryzae* was considered to be the chief element of Bengal famine (Mehrotra 1980). Crop diseases shattered the economy of many countries. Wheat rust epidemic in 1946–1947 brought the food shortage and seeds paucity in Madhya Pradesh (India). Wheat rust appeared in epiphytic form from time to time in many countries and this disease forced the farmers in many parts of the world to change their cropping pattern. The aforesaid instances of plant disease epidemics are worth mentioning because they left their adverse effects not only on the country concerned but also on other countries. These plant disease epidemics from 1938 to 1947 stimulated greatly the growth of plant pathology in India.

3.6 Establishing Plant Pathology as a Subject

Understanding the significance of plant pathology it was realized to establish it as a separate discipline, which was although quit late in India being compared to North America and European countries, and for which colonialism was said to be an important reason. Looking at the year of establishment of different Indian Universities, teaching plant pathology as a major discipline started a bit late in Indian Universities, and it was during 1930s that at the University of Madras (estb. in 1857) under the leadership of T.S. Sadasivsan, at Allahabad (estb. in 1887) under the influence of R.N. Tandon, at Lucknow (estb. in 1927) under the guidance of S.N. Dasgutpa, and at Banaras Hindu University (estab. in 1916) under the efforts of A Lal plant pathology was recognized as a discipline. The course at these universities before 1930s was focused on structure and taxonomy of fungi but later on plant pathology was introduced as a strong school emphasizing the biochemistry of host parasite interaction. Dasgupta studied the role of enzymes in pathogenicity; a general high metabolic rate and higher level of several enzymes were shown in virulent strains (Dasgupta 1969; Verma 1964, 1971). At Lucknow University, Dr. Dasgupta established one of the finest schools of mycology and plant pathology, which attracted students from the country and abroad. His early work on "Saltation of Fungi" (published as a booklet in 1936) earned him a great repute by earning the citations in international books and journals. Sadasivan's school developed the concept of vivotoxins and worked out the mechanism of cotton wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum*. The production of fusaric acid by *F. vasinfectum* as a vivotoxin was demonstrated (Kalyanasundaram 1955). At Calcutta University (estb. in 1857), teaching plant pathology started at the Department of Botany. Although A Lal initiated the study of Plant Pathology in Botany Department of Banaras Hindu University in 1928, its real foundation was laid down by R.Y. Roy, who had received the training from William Brown at Imperial College, London. His principal area of research was taxonomy and ecology of

rhizospheric fungi with special reference to root pathology. Dr. Roy devoted almost four decades (1931–1969) in establishing a strong school of mycology and plant pathology at BHU. Dr. R.S. Dwivedi, who was the student of Dr. Roy, took the legacy ahead and continued to work and extend the research areas under a wide umbrella which included ecology and taxonomy of soil fungi, rhizosphere and root pathology, seed pathology, phyllosphere and leaf litter fungi and their biological control. The outstanding works were recognized by UGC who declared" Mycology and Plant Pathology" as a major thrust area of research under Center of Advanced Study in Botany, Banaras Hindu University in 1995.

Raychaudhuri (1967) dealt in 1967 with the development of plant pathological research, education and extension work in India. Dr. R.S. Singh engaged in teaching and research in plant pathology from 1948 till 1989 at Pantnagar is much admired for his book "Plant Diseases" which is known as "Bible of Plant Pathology" in the fungal world as it remarkably not only addresses the challenges in the area but also reflects the great interest and experience of the author gained over the years.

B.B. Mundkur, well known for his work on Indian smut fungi, is much remembered for his significant contribution to plant pathology in India through the foundation of "Indian Phytopathological Society" (IPS) in 1947, through which he started a journal in 1948 as the "*Indian Phytopathology*". Also his text book "Fungi and Plant Diseases" published by Macmillan and Co Ltd. in 1949 (Mundkur 1949) has been considered as the second book of its type after the classic work of Butler, which was later revised by Chattopadhyay in 1967. The Indian Phytopathological Society was established for promoting the cause of science of Phytopathology. The society focuses in the field of mycology, plant pathology, bacteriology, virology, phytoplasmology and nematology. During the journey of last seven decades, the society progressed with time due to the visionary sincere effort of its presidents and members, who gave a sound footing to the society, the contributions of whom are enlisted in Table 3.2.

Presently there are many research institutes under the Indian Council of Agricultural Research throughout the country which are engaged in research into various aspects of plant diseases. Few main institutes are Central Potato Research Institute, Shimla; Central Rice Research Institute, Cuttak; Central Tobacco Research Institute, Rajahmundry; Sugarcane Breeding Research Institute; Coimbatore, etc. As part of Agricultural Sciences teaching plant pathology began at Indian Agricultural Research Institutes (IARI) and they started degrees in postgraduate and doctoral level. Plant pathology expanded in its various areas of specialization like mycology, bacteriology, virology and nematology after the establishment of State Agricultural Universities during 1960s. The event "International Symposium on Taxonomy of Fungi" organized at Madras in 1973 came up with a consensus of the mycologists of India to form the Mycological Society of India with an official journal, Kavaka. Mycology of India soon found recognition in the International Mycological Association in 1977 by getting a prestigious position in its framework.

Table 3.2 Contributions of IPS presidents in pathology

S. No.	Presidents of IPS	Area of contribution	Year
1.	S. R. Bose	Medical mycology	1947 and 1949
2.	J. F. Dastur	Disease of castor oil by *Phytophthora parasitica*	1948
3.	B. B. Mundkur	Ustilaginales of India	1950 and 1952
4.	M. K. Patel	Phytopathogenic bacteria	1951
5.	R. S. Vasudeva	Root rot of cotton	1953 and 1958
6.	S. N. Dasgupta	Complex aetiology of tip necrosis of mango	1954
7.	K. D. Bagchee	Rust fungi on conifers	1955
8.	M. J. Thirumalachar	Antibiotics in plant disease control	1956 and 1965
9.	B. L. Chona	Sugarcane mosaic	1957
10.	R. Prasada	Cereal rust, *Alternaria* blight, powdery mildews	1959
11.	T. S. Ramakrishnan	Rusts and *Pythium, Phytophthora, Colletotrichum* genera	1960
12.	R. P. Asthana	Plant disease control	1961
13.	P. R. Mehta	Pesticides	1962
14.	T. S. Sadasivan	Physiology of host pathogen relationship	1963
15.	S. Sinha	Storage Diseases of Mango	1964
16.	R. N. Tandon	Fungal disease in storage fruits and vegetables	1966
17.	S. P. Raychaudhuri	Viral diseases and tissue culture.	1967
18.	R. K. Saksena	Extranuclear cytology in fungi	1968
19.	M. J. Narasimhan	Sexuality of *Phytophthora arecae*	1969
20.	H. K. Saksena	Taxonomy and cytology of *Rhizoctonia,* gram rust	1970
21.	D. N. Srivastava	Diseases of cereals and vegetables	1971
22.	Kartar Singh Thind	Physiology and taxonomy of fungi	1972
23.	S. Y. Padmanabhan	Red rot disease of sugarcane	1973
24.	K. S. Bhargava	Plant Virology	1974
25.	L. M. Joshi	Cereal Rust	1975
26.	S. B. Saksena	Fungal taxonomy	1976
27.	J. S. Chohan	Microbial spoilage of food grains	1977
28.	A. P. Misra	Epidemiology of wheat rust	1978
29.	G. Rangaswamy	Relations between soil, plant and microbes	1979
30.	R. S. Singh	Principles of Plant Pathology	1980
31.	R. L. Munjal	Karnal bunt and mushroom cultivation	1981
32.	B. B. Nagaich	Virology	1982
33.	V. V. Chenulu	Viral and phytoplasmal diseases	1983
34.	M. M. Payak	Cereal rusts	1984
35.	R. K. Grover	Fungitoxicants in disease control	1985
36.	Y. L. Nene	Viral diseases of pulses and khaira disease	1986

(continued)

Table 3.2 (continued)

S. No.	Presidents of IPS	Area of contribution	Year
37.	J. S. Grewal	Diseases of pulse crops	1987
38.	J. N. Chand	Plant bacteriology	1988
39.	Gopal Swarup	Wheat rust	1989
40.	R. S. Mehrotra	*Phytophthora* diseases	1990
41.	Harnek Singh Sohi	Mushroom cultivation	1991
42.	M. S. Chatrath	Nuclear techniques in plant pathology	1992
43.	A. K. Sarbhoy	Mycology and plant pathology	1993
44.	J. P. Verma	Cotton bacteriology	1994
45.	V. P. Agnihotri	Taxoecology and physiology of *Pythium*	1995
46.	A. N. Mukhopadhyay	Biological control of plant disease	1996
47.	C. D. Mayee	Epidemiology and management	1997
48.	Anupam Varma	Plant virology	1998
49.	S. Nagarajan	Epidemiology of the cereal rusts	1999
50.	B. L. Jalali	Mycorrhizal and host pathogen interactions	2000
51.	G. S. Shekhawat	Seed production and potato research	2001
52.	C. Manoharachary	Biodiversity, taxonomy and biotechnology of fungi	2002
53.	D. V. Singh	Aetiology, epidemiology and management of wheat diseases	2003
54.	S. M. Paul Khurana	Screening resistance against potato viruses by ELISA, ISEM	2004
55.	Y. S. Ahlawat	Citrus badna virus and ringspot virus	2005
56.	L. V. Gangawane	Fungicide resistance and biopesticides	2006
57.	Amerika Singh	Integrated pest management	2007
58.	S. S. Chahal	Diseases of cereals and sunflower	2008-2009
59.	R. K. Jain	Bacterial blight of rice	2010
60.	T. S. Thind	fungicides in plant disease control	2011
61.	R. K. Khetarpal	Seed transmitted viruses, plant quarantine, biosafety	2012
62.	Uma Shankar Singh	Eco-friendly management of plant diseases	2013
63.	M. Anandaraj	biological control of *Phytophthora*	2014
64.	A. K. Misra	Characterization and management of subtropical fruit diseases	2015
65.	P. Chowdappa	Molecular plant pathology	2016
66.	B. N. Chakraborty	Molecular plant pathology and fungal biotechnology	2017
67.	R. N. Pandey	Molecular detection of Plant Pathogens and biocontrol	2018
68.	M.P. Thakur	Mushroom cultivation	2019
69.	Dr. P. K. Chakrabarty	Molecular diagnostic tools and transgenic diploid cotton	2020

3.7 Current Developments and Future Directions

In the twenty-first century, the subject "Plant Pathology" as a science has made a marvellous progress during the past 10 years in many facets. Breakthrough in this field came when molecular biology studies started after Flor in 1955 gave the gene-to-gene hypothesis of disease resistance and susceptibility (Singh 1984). Today this eukaryotic system has therefore proved to be an excellent model system to answer the fundamental biological questions. New techniques and discoveries have followed one another attracting a large chunk of research workers to this new and prestigious area of research. Dr. M.S. Chatrath in 1971, placed in the Nuclear Research Laboratory, IARI, was a pioneer in the field of nuclear techniques in plant pathology especially in use of ionizing radiations for control of pathogens and use of radioisotopes for studying translocation of systemic fungicides. Plant pathologists are contributing more time in understanding the mechanism of pathogenesis, physiological and molecular plant pathology, biocontrol, post-harvest disease control and disease forecasting. Dr. Chohan has done pioneering research work on aflatoxin. His extensive studies have helped in managing microbial spoilage of food grains and in understanding diseases affecting fruit crops like grape, pear and peach and he has devised suitable methods for their effective management (Misra et al. 2016). Dr. N.K. Dubey's Laboratory from BHU, Varanasi, is doing pioneering work on protecting the post-harvest loss of cereals, dry fruits and spices caused by storage fungi, through "nanoencapsulation" technique, for efficient and effective control (Dwivedy et al. 2018). The research on disease management was focused by and large on chemical-based control measures during 1970–1990. In the later part of the 1990s there was a paradigm shift in focus, on disease management by including botanicals, biocontrol agents (BCA) and arbuscular mycorrhiza, etc., which had additional benefits of plant growth promotion. Many research laboratories then looked for effective antagonists as disease suppressants resulting in the emergence of research on *Trichoderma, Gliocladium, Aspergillus, Penicillium, Neurospora, Chaetomium, Dactylella, Arthrobotrys, Glomus, Bacillus subtilis, Streptomyces Rhizobium, Bacillus, Azotobacter* and *Pseudomonas fluorescence* as seed dressers and as soil augmenters in soil health (Sharma et al. 2011). Since 1990 many BCA have been identified and commercialized as they add value in meeting the sustainable development goal. These BCA inhibit plant pathogens through nutrient competition, hyperparasitism and antibiosis. Such interactions are highly regulated cascades of metabolic events, often combining different modes of action.

The emergence of enzymes and nucleic acid-based technologies in disease diagnostics like enzyme linked Immunosorbent Assay (ELISA), Lateral Flow Devices (LFD), DNA microarrays and DNA barcoding during last 25–30 years has revolutionized the discipline of plant pathology. Proteomics has emerged as an indispensable tool for understanding the cellular mechanisms of fungal pathogenicity that occur during plant pathogen interaction in all kinds of pathogenic fungi such as biotrophs, hemibiotrophs and necrotrophs. The information obtained through various proteome level studies of the interaction between hosts and fungi has led to more effective disease management strategies. The pathogenesis-related

(PR) proteins which are encoded by the host plant but induced only in pathological or related situations are also involved in hypersensitive response (HR) or systemic acquired resistance (SAR) against infections. PR proteins are an indispensable component of innate immune responses in plants and they show strong antifungal and other antimicrobial activity. Some of them inhibit spore release and germination, whereas others are associated with strengthening of the host cell wall and its outgrowths and papillae. The signal compounds responsible for the induction of PR proteins like jasmonic acid, ethylene, xylanase, salicylic acid, the polypeptide systemin, and probably many others get transported systemically to other parts of the plant and reduce disease initiation and intensity for several days or even weeks (Chakraborty 2011). Prof. H. S. Shetty has done remarkable work in the field of molecular plant pathology, contributing on the induction of immunity triggered by host recognition of pathogen-associated molecular patterns in pearl millet (Veena et al. 2016). Prof. J Kumar has not only done pioneering research on mango malformation but also got a patent on designing PCR-based detection and diagnostic assay for *Magnaporthe oryzae*, directly from infected seeds of rice, using PWL2 gene-specific primer.

A greater understanding of the cell biology of these versatile eukaryotes has underpinned efforts to engineer certain fungal species to provide novel cell factories for the production of life biomolecules. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological and biomedical applications. The discipline of plant pathology is now being studied in its different aspects like fungal phylogenetics, genomics, proteomics and molecular enzymology. Some of the frontier areas of modern science like bioinformatics which is an amalgam of genomics, proteomics, and metabolomics are playing a decisive role in understanding pathogenic organisms at a systems level.

The fungal kingdom is diverse and the hosts and habitats of these eukaryotic microorganisms are also equally diverse with the fungi being present in every ecosystem on Earth. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere and needs to be deciphered. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. Fungal plant pathology has an integral role to play in the global food security issues particularly addressing the post-harvest losses. Hence, with the changing agricultural scenario, the plant pathologist will have to play a significant role in matching the production technology with protection technology. The modern era of the plant pathology has no doubt opened many vistas in life sciences.

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Study of Indian Fossil Fungi: An Odyssey

4

S. K. M. Tripathi

Abstract

Progress of studies on Indian fossil fungi is synthesized. Diagnostic characters and occurrences of the selected fossil fungi reported from India are provided. Most of these forms have been reported from Cretaceous to Miocene sediments of India. Due to space constraints some genera established from other countries but reported to occur in Indian sediments have been omitted. Fungal remains included in the chapter belong to fossil fungal spores, chlamydospores, ascocarps, parasitic fungi, mycorrhizal fungi and Ingoldian aquatic fungi. Stratigraphic and palaeoclimatic implications of fossil fungal remains have been discussed.

Keywords

Fossil fungi · Conidia · Palynology · Fruiting bodies · Ascomycota · Basidiomycota · Paleoclimate

4.1 Introduction

Fossil fungal remains are commonly observed in the palynological preparations made from samples belonging to Cretaceous to Miocene sediments. Most of these are represented by spores, ascocarps, mycelia, and mycorrhizal fungi. Studies in fossil fungi received more attention since 1950s. Fungal diversity is linked with diversification of Angiosperms which took place during Tertiary Period. Majority of dispersed fungal spores are produced by the Ascomycetes. This group became well

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established during Cretaceous and diversified morphologically in the Tertiary Period (Jain 1974; Jain and Kar 1979; Ramanujam 1982; Tripathi 2009; Saxena and Tripathi 2011). Several types of asexual spores, the conidiospores or conidia are also very common in palynological preparations. Ascocarps (fruiting bodies) have definite shape and may occur above ground or may remain subterraneous. These are constituted by radiating rows of mycelia and contain ascospores. Fruiting bodies or ascocarps of epiphyllous fungi profusely occur in palynological preparations. These can be easily compared with extant forms and are more reliable for palaeoclimatic interpretations. Studies related with host–fungus relationship are of great significance in attempting the palaeoenvironmental conditions. Fossil fungal remains described during the early period were significant but many of the described taxa were either not validly published or their diagnoses and status were not properly defined. Kalgutkar and Jansonius (2000) tried to streamline the taxonomic status of many fossil fungal remains. Saxena and Tripathi (2011) documented the validly published species of Indian fossil fungi and provided their descriptions along with the sketches and occurrences.

Classification of fossil fungi: Fossil fungi are fragmentary in nature and hence lack characteristic features that are diagnostic of extant taxa. Except for a few Tertiary forms, most of the fossil fungal remains can seldom be compared with modern taxa. Most of the workers opine that assigning fossil forms to extant taxa will create taxonomic confusion. Therefore, fossil fungal remains are generally described under *Artificial System of classification* which is based on morphological characters only. Following this system, fossil fungal remains are described under two categories—the **Fungal Spores** and the **Ascocarps**.

4.1.1 Fungal Spores

As stated earlier, majority of fossil fungal spores belong to Ascomycetes. Palynological assemblages are also generally rich in different varieties of conidia which are produced by Fungi Imperfecti and the holomorphic Ascomycetes. These are of varied shapes and may be one- to multicelled. Spores of some fungi, especially conidia and ascospores, possess distinctive features leading to their identification and categorization with the extant forms. Fossil spores can be generally assigned to a natural class system of Phycomycetes, Ascomycetes or Basidiomycetes if the diagnostic morphographic features are observable. Some fossil materials are assigned to the class Fungi Imperfecti where spores or isolated structures (conidia, pycnidia or other sporangia, or isolated mycelia) are of exclusive morphology. Numerous types of fossil fungal spores are reported from Late Cretaceous to Cenozoic sediments. These are described under ‘dispersed spores’ which include detached spores, microscopic sporangia, hyphae or fragmented mycelia.

In a classification system proposed by van der Hammen (1956), fossil fungal spores were grouped under various morphologic categories having the suffix ‘Sporites’. Clarke (1965) proposed the suffix ‘Sporonites’ for naming the fossil fungal spores. Considering the characters like shape, size and symmetry of spores, absence/presence and number of apertures, septa characters and the wall features,

Elsik (1976) proposed a classification system for the fossil spores. He proposed artificial supra-generic categories which were primarily based on the cell number and presence or absence of apertures. Pirozynski and Weresub (1979) suggested a system named 'Saccardoan System' for classifying the fungal spores that are not referable to extant families. This scheme is based on shape and number of cells and the fungal spores are recognized as Amerospores, Didymospores, Phragmospores, Dictyospores, Scolecospores, Helicospores or Staurospores (star-like). Characteristic features of each of these groups are as follows:

Amerosporae: Spores unicellate, aperturate or inaperturate; with one pore or hilum, two or more pores, or variable apertures.

Didymosporae: Spores dicellate, inaperturate or aperturate; aperturate spores with one pore or hilum at the proximal end, or two pores, one each at or near the proximal and distal end.

Phragmosporae: Spores tri- or pluricellate, only transversely septate, aperturate or inaperturate.

Dictyosporae: Spores muriform, divided by intersecting longitudinal, transverse or diagonal septa; shapes variable, inaperturate or with a more or less distinct hilum.

Helicosporae: Spores uni- or pluricellate; body spirally coiled.

Staurosporae: Spores pluricellate; with more than one axis, or star shaped.

Scolecosporae: Spores long, pluricellate, filamentous, transversely septate, provided with one or two pore(s) or hilum at proximal or/and distal end.

4.1.2 Fruiting Bodies or Ascocarps

Ranging in size from 80 to 170 μm, the ascocarps commonly occur as parasites on epidermis of leaves, stem and flowers of higher plants. These belong to Ascomycetes and are placed in the family Microthyriaceae. The fruiting bodies are provided with radiating rows of mycelial cells giving an appearance of tissues arranged in radial fashion. Ascocarps contain asci that are surrounded by or enclosed within protective tissues. These may be globose, flask-shaped or saucer-shaped open bodies and may or may not possess an opening, the *ostiole*. Fossil fungal fruiting bodies are also classified under the artificial system. Several workers attempted to classify and formally describe the fossil ascocarps (Edwards 1922; Rosendahl 1943; Cookson 1947; Dilcher 1965; Rao 1958; Venkatachala and Kar 1969; Jain and Gupta 1970; Elsik 1978; Pirozynski 1978). Fruiting bodies are classified on the basis of presence/absence of an ostiole, shape and margin of the fruiting body, presence or absence of pore in individual cells and nature of the central part of the fruiting body. Widely accepted classification scheme to describe the dispersed ascocarps is summarized in Table 4.1. This system is primarily based on porate or aporate individual cells of multicellular fruiting body. Forms with porate individual cells are kept under the genera *Callimothallus* and *Ratnagiriathyrites*, whereas those without pores are divided into non-radiate and radiate forms. The non-radiate forms may be ostiolate or non-ostiolate. The radiate forms are further divided into genera having smooth, fimbriate or spinose margins. The radiate forms with smooth to fimbriate margins are further divided on the basis of presence, absence or nature of ostiole.

Table 4.1 Classification of fossil ascocarps

Ascocarp cells aporate, body radiate, margin smooth or irregular		Ascocarp cells porate, body radiate *(Callimothallus)* Ascocarp cells porate, body non-radiate *(Ratnagiriathyrites)*	
Ostiole distinct 1. Body made up of intertwined thin hyphae; ostiole margin thickened *(Plochmopeltinites)* 2. Ostiole bordered with single/double walled cells *(Trichothyrites)*	Ostiole indistinct *(Microthyriacites)*	Non-ostiolate 1. Central cells modified, provided with star-shaped opening *(Asterothyrites)* 2. Central cells unmodified *(Phragmothyrites)*	Body radiate, margin with projecting spines *(Parmathyrites)*
Ascocarp cells aporate, body non-radiate, ostiole irregular, body fan shaped *(Brefeldiellites)*		Elongated dehiscence, body multi fan shaped *(Euthythyrites)*	Body non-radiate, non-ostiolate *(Trichopeltinites)*

Some members of the epiphyllous fungi also produce morphologically similar fructifications (Kalgutkar and Jansonius 2000). The family Asterinaceae shows the presence of thyriothecium resembling those of Microthyriaceae. Fructifications of this family open by irregular crumbling, cracking or gelatinization of the central area forming an irregular wide opening or a stellate crack (Pirozynski 1978). Fruiting bodies of Trichothyriaceae resemble those of Asterinaceae but are lenticular rather than scutelliform. The ostiole in these forms is often protruding and may be bordered by darkly pigmented cells which sometimes bear spine-like setae. This family is represented in fossil records by *Trichothyrites*. Thalli of the family Trichopeltinaceae are irregularly branched, membranous and are composed of regular cells in radiating or parallel fashion. Fossil representatives of these common tropical epiphytes are described under the genus *Trichopeltinites*. Fructifications of the family Micropeltaceae are shield shaped and centrally ostiolate. Walls in these fruiting bodies are composed of haphazardly arranged indistinct hyphae forming a delicate hyphal reticulum at the margins. Members of this family are epiphytes growing on tropical evergreen plants. *Plochmopeltinites* are the fossil members of the family. Fruiting bodies of the epiphyllous Ascomycetes of the family Parmulariaceae superficially resemble those described earlier but are thicker and less distinctly cellular. Fossil representatives of these forms are *Callimothallus* and *Microthallites*.

4.1.3 Work on Indian Fossil Fungi

Fungal remains have been reported from the Permian (Bajpai and Maheshwari 1988) and Early Cretaceous sediments of India (Banerjee and Misra 1968; Pant et al. 1983; Bose and Banerji 1984; Tiwari and Tripathi 1995; Tripathi 2001). Diverse types of

fungal remains from Indian Tertiary sedimentary sequences have been described by Potonié and Sah (1960), Venkatachala and Kar (1969), Jain and Gupta (1970), Chitaley and Sheikh (1971), Chitaley and Patil (1972), Kar et al. (1972), Kar and Saxena (1976), Rao and Ramanujam (1976), Chitaley (1978), Chitaley and Yavale (1978), Ramanujam and Rao (1973, 1978), Patil and Ramanujam (1988), Tripathi (1989), Kumar (1990), Rao (1995), Kar et al. (2003, 2004a, b, 2005, 2006) and Srivastava (2008). Some of the forms deserve separate discussion, although these have been dealt with in the later part of the text.

Tiwari and Tripathi (1995) and Tripathi (2001) described fungal assemblages from the Intertrappean beds (Early Cretaceous) of the Rajmahal Basin, Jharkhand. These assemblages show presence of many microthyriaceous fruiting bodies. Kar et al. (2003) reported a fruiting body assignable to Polyporaceae (Basidiomycetes) from the Lameta Formation (Maastrichtian) exposed in Madhya Pradesh. This fossil, called *Lithopolyporales zeerabadensis*, resembles the modern genus *Fomes* which are found as saprophytes on dead wood of various trees. Kar et al. (2004a) described a fossil fungus showing affinity to *Colletotrichum* ascribed to the family Melanconiaceae (Deuteromycetes) from Intertrappean beds (Maastrichtian) exposed at Mohgaon Kalan Village, Chhindwara District, Madhya Pradesh. Modern species of this genus causes red rot in the economically important plants. The fossil of this fungus, named as *Protocolletotrichum deccanensis*, was found to be preserved on a leaf cuticle and shows setae on the margins of the acervuli. Kar et al. (2004b) described fossil parasitic fungi and epiphyllous fruiting bodies from the coprolite of dinosaurs. The coprolite was collected from Lameta Formation (Maastrichtian) of Central India.

Kar et al. (1972) described a unique fungal fruiting body *Cucurbitariaceites* from early Tertiary sediments of Assam. The fruiting bodies are circular to subcircular in shape and its outer region is dark in colour. The asci are cylindrical, up to 20 in number and generally develop from the inner region of the fruit body. A rupture is observed in some specimens in the central polygonal area bordered by basal parts of the asci. *Cucurbitariaceites* is distinguished from all other fossil genera of Microthyriales by its shape, darker outer layer, in the absence of true paraphyses and the presence of cylindrical asci. Kalgutkar and Jansonius (2000), while commenting on this genus, stated that it shows affinity with the extant family Cucurbitariaceae of Pseudosphaeriales. Most of the members of this order are confined to tropical areas. Potonié and Sah (1960) described forms *Lirasporis intergranifer* from the Miocene lignites of Cannanore, Kerala. The spores are oval, possess notches at the ends and have parallel longitudinal ribs over the body. Jain and Kar (1979) emended the diagnosis of this taxon and considered it as a fungal body made up of long septate mycelia which are more or less parallel to each other. Kalgutkar and Jansonius (2000) commented that this form may have some stratigraphic significance. Rao (2003) described a new fungal fruiting body *Kalviwadithyrites* from Sindhudurg Formation (Miocene), Kalviwadi, Sindhudurg, Maharashtra. Body of this cleistothecium is circular to subcircular in shape and is made up of two sets of aporate cells. Kar et al. (2005) described mycorrhizal fungi of the family Glomaceae from Miocene sediments of Mizoram. Two types of fossil

Ingoldian aquatic fungi were reported from Miocene sediments of Mizoram (Kar et al. 2006). One of these shows resemblance with the extant genus *Tetrachaetum* while the other one shows similarity with the extant genus *Ceratosporella*.

Significant Indian fossil fungal remains are discussed below in alphabetical order.

4.1.3.1 Systematic Description

(A)

Genus: *Alleppeysporonites* Ramanujam and Rao 1978.

Generic Description: Spores gently curved, branched, multicellular, nonaperturate, transversely septate, one/two branches per spore. Basal and terminal cells with a conspicuous appendage and spore wall psilate to scabrate.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Alleppeysporonites scabratus* Ramanujam and Rao 1978 (Fig. 4.1a).

Description: Spores branched, branches one to two per spore, 2–5 transverse septa per branch, cells rectangular, 6–11 × 4–6 μm in size. Basal and terminal cells have a prominent appendage which is simple and 15–22 μm long. Spore wall thin, scabrate.

Occurrence: Miocene, Warkalli Beds, Alleppey, Kerala.

Genus: *Appendicisporonites* Saxena and Khare 1992.

Generic Description: Spores inaperturate, subcircular, multicellular, each cell possessing a long process which may be septate or nonseptate, with pointed or blunt tips, wall psilate.

Classification: Ascomycetes, Microthyriales.

Species: *Appendicisporonites typicus* Saxena and Khare 1992 (Fig. 4.1b).

Description: Spores subcircular, excluding appendages 44–47 × 36–39 μm in size, multicellular, inaperturate, and each cell has a long nonseptate process, processes pointed, 43–45 μm long and 5–6 μm wide, wall psilate.

Occurrence: Eocene, Neyveli Formation, Tamil Nadu.

Genus: *Ascochytites* Barlinge and Paradkar 1982.

Description: Saprophytic sphaeropsidaceous fungus, with ostiolate pycnidia; hyphae septate, branched; conidiospores small.

Classification: Fungi Imperfecti, Sphaeropsidales.

Species: *Ascochytites intertrappeus* Barlinge and Paradkar 1982 (Fig. 4.1c).

Description: Saprophytic sphaeropsidaceous fungus with ostiolate pycnidia; pycnidia 80–100 × 60–65 μm; mycelium 1.5–2 μm broad, branched; conidia two celled, elongate, hyaline, 3.5 × 2–1.5 μm.

Occurrence: Late Cretaceous, Deccan Intertrappean, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Asterothyrites* Cookson 1947 emend. Kalgutkar and Jansonius 2000.

Emended description: Ascomata circular with radially arranged hyphae which are laterally interconnected to form a pseudoparenchymatous tissue. Cells isodiametric, squarish or elongate rectangular. Ascomata are ostiolate. Ostiole is simple, small or large, irregular or rounded.

Classification: Ascomycetes, Microthyriales.

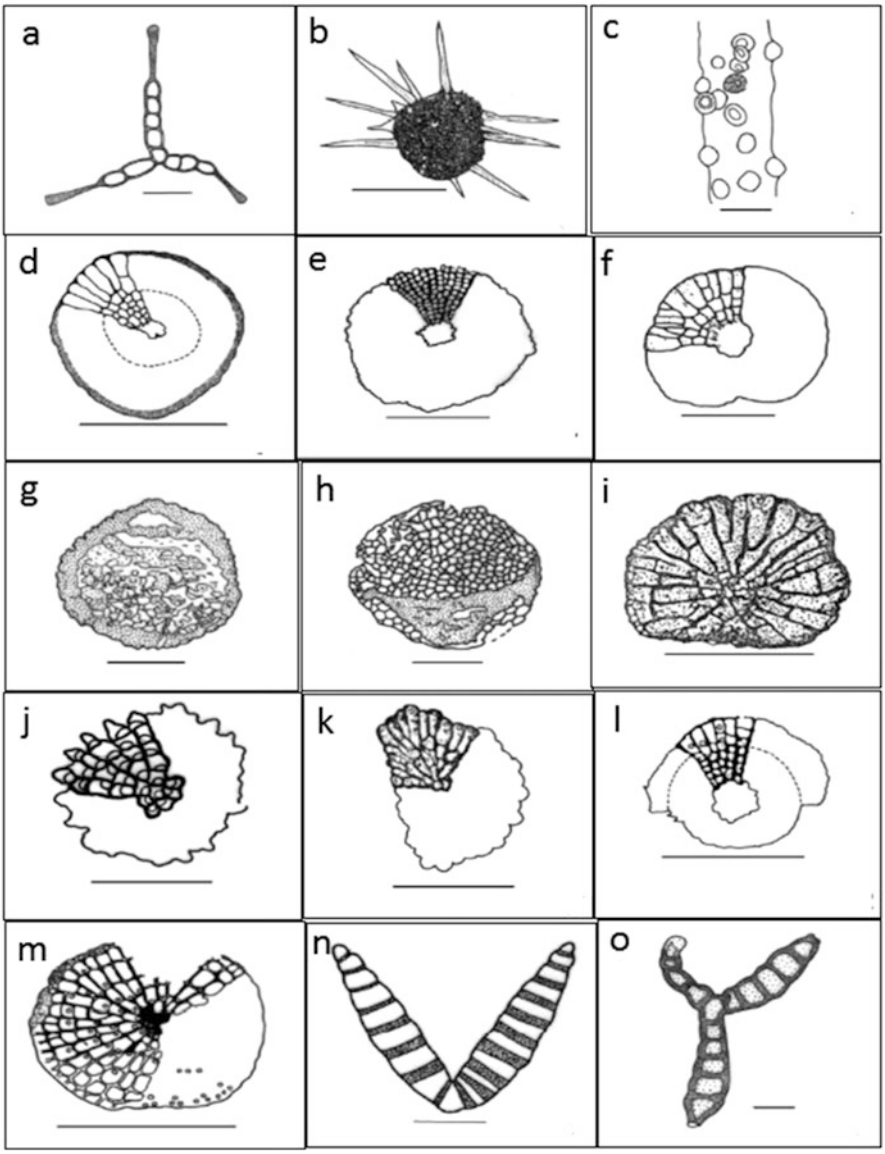

Fig. 4.1 (**a**) *Alleppeysporonites scabratus* Ramanujam and Rao 1978; Bar = 20μm. (**b**) *Appendicisporonites typicus* Saxena and Khare 1992; Bar = 50μm. (**c**) *Ascochytites intertrappeus* Barlinge and Paradkar 1982; Bar = 200μm. (**d**) *Asterothyrites edvensis* (Rao and Ramanujam 1976) Kalgutkar and Jansonius 2000; Bar = 50μm. (**e**) *Asterothyrites konkanensis* (Saxena and Misra 1990) Kalgutkar and Jansonius 2000; Bar = 50μm. (**f**) *Asterothyrites menonii* (Jain and Gupta 1970) Kalgutkar and Jansonius 2000; Bar = 25μm. (**g**) *Bireticulasporis communis* Potonié and Sah 1960; Bar = 30μm. (**h**) *Bireticulasporis indicus* Potonié and Sah 1960; Bar = 30μm. (**i**) *Callimothallus assamicus* Kar et al. 1972; Bar = 50μm. (**j**) *Callimothallus dilcheri* Rao and Ramanujam 1976; Bar = 50μm. (**k**) *Callimothallus quilonensis* Jain and Gupta 1970; Bar = 50μm. (**l**) *Callimothallus raoi* Ramanujam and Rao 1973; Bar = 50μm. (**m**) *Callimothallus senii* (Venkatachala and Kar 1969) Kalgutkar and Jansonius 2000; Bar = 100μm. (**n**) *Ceratohirudispora miocenica* Kar et al. 2010; Bar = 10 μm (**o**) *Ceratohirudispora triradiata* Kar et al. 2010; Bar = 10 μm

Species: *Asterothyrites edvensis* (Rao and Ramanujam 1976) Kalgutkar and Jansonius 2000 (Fig. 4.1d).

Earlier: *Paramicrothallites edvensis* Rao and Ramanujam 1976.

Description: Ascomata flattened, semicircular, margin firm and even, 50–85 μm in diameter; ostiolate, ostiole simple, rounded or slightly irregular, 7.5–9 μm in diameter; cells of ascomata radially arranged, 3–4.5 μm wide, squarish to rectangular near central region, elongated, 6–9 μm long and thin walled near margin, margin mostly crenate with local thickenings.

Occurrence: Miocene, Quilon Beds, Edvai, Kerala.

Species: *Asterothyrites konkanensis* (Saxena and Misra 1990) Kalgutkar and Jansonius 2000 (Fig. 4.1e).

Earlier: *Paramicrothallites konkanensis* Saxena and Misra 1990.

Description: Ascostromata subcircular, size range 94–103 × 90–98 μm, ostiolate, ostiole subcircular, ca. 7–9 μm in diameter, unthickened, hyphae radiating, forming aporate pseudoparenchymatous cells, central cells squarish, marginal cells rectangular, margin uneven.

Occurrence: Neogene, Sindhudurg Formation, Maharashtra.

Species: *Asterothyrites menonii* (Jain and Gupta 1970) Kalgutkar and Jansonius 2000 (Fig. 4.1f).

Earlier: *Paramicrothallites menonii* Jain and Gupta 1970.

Description: Ascomata circular, ostiolate, 40–60 μm in diameter, margin entire; hyphae radially arranged, interconnected to form pseudoparenchymatous cells. Central cells squarish, marginal cells rectangular, walls thin, ostiole well defined, central, 8–10 μm in diameter, simple and margin lobed.

Occurrence: Miocene, Quilon Beds, Padappakkara, Kollam, Kerala.

(B)

Genus: *Bireticulasporis* Potonié and Sah 1960.

Generic Description: Size 77.5–129 μm, circular to irregular, outline undulated to irregularly dentate, sometimes straight; the outline may be accompanied by a more or less narrow darker band which is due to folding of the peripheral wall. Wall showing two types of ornamentations, a finer reticulation in the higher focus and a coarser reticulation in the deeper focus; the lumina of the deeper reticulation larger. In the last reticulation the lumina are more or less rounded, coarser reticulation with polygonal lumina.

Classification: Fungi, Incertae sedis.

Species: *Bireticulasporis communis* Potonié and Sah 1960 (Fig. 4.1g).

Description: Size ranges from 36 to 129 μm; outline irregular circular to roundly polygonal and dentate to faintly sinuate, the *extrema lineamenta* inside may be followed by a dark ± narrow band; this band is formed by very narrow and dense tangential folds; coarser reticulum not always perfect perhaps as a result of bad preservation; the higher reticulation sometimes seen as sharp white points but in the highest focus as dark points.

Occurrence: Late Miocene, Cannanore lignite, Kannur, Kerala.

Species: *Bireticulasporis indicus* Potonié and Sah 1960 (Fig. 4.1h).

Description: Size 77.5–129 μm with irregularly circular outline, darker band along the periphery absent, muri of the coarser reticulum sometimes sinuous, lumina more or less polygonal, often having more than ten times the breadth of the muri, fine reticulation of the higher focus often clear, sometimes faint.

Occurrence: Late Miocene, Cannanore lignite, Kannur, Kerala.

Genus: *Callimothallus* Dilcher 1965.

Generic Description: Stroma round, radiate, astomate, no central dehiscence, individual cells may possess single pore, spores undetermined, size range 50–250 μm.

Classification: Ascomycetes, Microthyriales.

Species: *Callimothallus assamicus* Kar et al. 1972 (Figs. 4.1i).

Description: Ascomata subcircular to circular, non-ostiolate, 50–80 μm. Central cells triangular to polygonal from which radiating rows of cells extend outwards. Central cells porate, pores single, outer cells radially elongated.

Occurrences: Recorded from many Tertiary localities of India.

Species: *Callimothallus dilcheri* Rao and Ramanujam 1976 (Figs. 4.1J).

Description: No free mycelium, more or less circular, margin irregular, 75–120 μm in diameter, non-ostiolate, cells forming the ascomata radiating from angular central cell, cells near central region 5–6 angled and those towards periphery rectangular, 4–5 μm wide and 4–7.5 μm long, marginal cells bottle shaped with distinctly constricted neck, one pore per cell, located terminally, 1.5–2.5 μm wide, radial and tangential walls of cells thickened.

Occurrence: Miocene, Quilon Formation, Edvai, Kollam, Kerala.

Species: *Callimothallus quilonensis* Jain and Gupta 1970 (Fig. 4.1k).

Description: Ascomata, subcircular to circular, non-ostiolate, 35–65 μm in diameter, solitary, margin entire to crenate. Centre cell triangular from which radiating rows of cells extend outward, cells more elongated towards the periphery, peripheral cells porate, pore single, slightly elevated, 1–3 μm wide, placed apically, Cell wall thick.

Occurrence: Early Miocene, Padappakkara, Kollam, Kerala.

Species: *Callimothallus raoi* Ramanujam and Rao 1973 (Fig. 4.1l).

Description: Ascomata discoid, rounded, margin entire, 55–75 μm in diameter. Central part consists of irregular cavity with ragged margin, surrounded with 5 or 6 layers of dark smaller and thick-walled cells. Peripheral cells in radiating pattern, light-coloured, thinner, slightly larger than central ones rectangular, 2.5–6 × 2–4 μm. Only few cells in central and peripheral regions have single pore, 1–1.5 μm, usually distal in peripheral cells, central to proximal in central thick-walled cells.

Occurrence: Late Miocene, Warkalli lignite, Kerala.

Species: *Callimothallus senii* (Venkatachala and Kar 1969) Kalgutkar and Jansonius 2000 (Fig. 4.1m).

Earlier: *Pseudosphaerialites senii* Venkatachala and Kar 1969.

Description: Perithecium subcircular, 100–140 μm, central part darker than neighbouring regions; in the latter each stromatal cavity possesses one hypha. Hyphae radially arranged, pseudoparenchymatous; outer layer thickened and minutely setose.

Occurrence: Early Eocene, Matanomadh, Kutch, Gujarat.

Genus: *Ceratohirudispora* Kar et al. 2010.

Generic Description: Hyphomycetaceous fungi, conidiophore small, growth terminated by production of apical conidium; conidium enlarges laterally in opposite direction to produce two–three arms. Conidia 5–10 celled, septa up to 2 μm thick, with broad base and narrow tip.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Ceratohirudispora miocenica* Kar et al. 2010 (Fig. 4.1n).

Description: Conidia two armed, V-shaped, 24–38 × 5–9 μm, conidia septate, septa 6–8 in number, 2 μm thick, conidia broader in middle, narrow at base and apex, wall about 1 μm thick, laevigate.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Species: *Ceratohirudispora triradiata* Kar et al. 2010 (Fig. 4.1o).

Description: Hyphomycetaceous conidia three armed providing a triradiate appearance, arms unequal in length and size, septate, septa more or less 1 μm thick, 4–8 celled, conidia wall 1 μm thick, laevigate.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Cervichlamydospora* Kar et al. 2010.

Generic Description: Chlamydospores subcircular, dark brown-black, originate from neck of hyphae, solitary, 14–24 × 12–22 μm in size. Many hyphae adhere together at base, branch out laterally at tip; hyphae wall laevigate-granulose, grana sparsely placed, up to 1 μm thick.

Classification: Fungi Imperfecti, Amerosporae.

Species: *Cervichlamydospora nigra* Kar et al. 2010 (Fig. 4.2a).

Description: Chlamydospore subcircular, solitary, dark brown-black with a constriction at margin, margin like a shield, smooth, 13–23 × 12–21 μm; arise from neck of hyphal strand, strand composed of 8–13 hyphae, closely adhered at base and region of chlamydospore attachment, separate and loose in rest part. Central hypha strongly built, nonseptate or rarely septate; hypha wall about 1 μm thick, generally laevigate, sometimes weakly granulose.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Chlamydosporites* Paradkar 1975.

Generic Description: Mycelium of septate branched hyphae with haustoria, chlamydospores thick walled.

Classification: Basidiomycetes, Ustilaginales.

Species: *Chlamydosporites gramineus* Paradkar 1975 (Fig. 4.2b).

Description: Mycelium of profusely branched hyphae, septate, 4–6 μm broad, with haustoria, chlamydospores 10–12 μm thick with reticulate exine and thin intine.

Occurrence: Late Cretaceous, Deccan Intertrappean Series, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Circinoconites* Kar et al. 2010.

Generic Description: Conidia acrogenous, strongly spiralled, spirals 30–39 × 25–31 μm in size; solitary, coiled, 8–14 septate, fist shaped, constricted at septa regions, cells increase in diameter from base to apex, dissimilar.

Classification: Fungi Imperfecti, Phragmosporae.

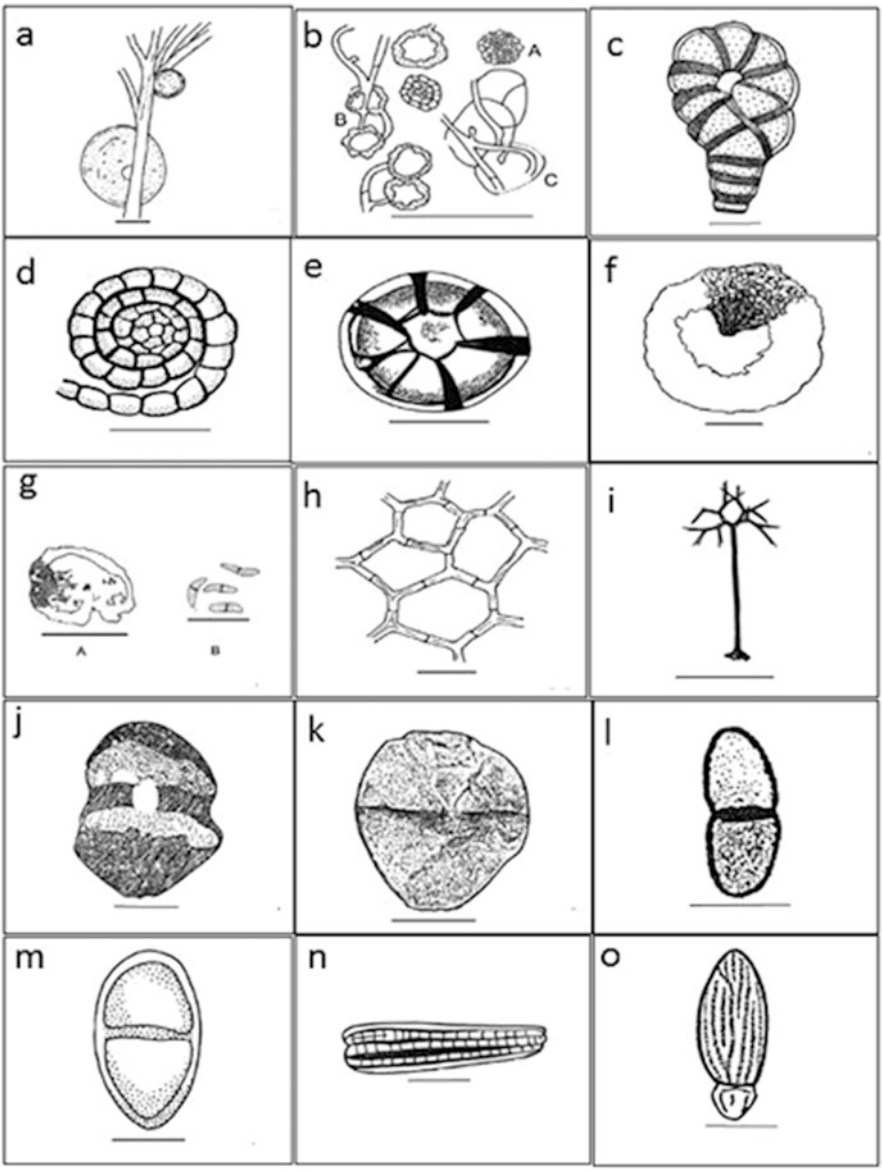

Fig. 4.2 (**a**) *Cervichlamydospora nigra* Kar et al. 2010; Bar = 10μm. (**b**) *Chlamydosporites gramineus* Paradkar 1975; Bar = 50μm. (**c**) *Circinoconites arthrus* Kar et al. 2010; Bar = 10μm. (**d**) *Colligerites kutchensis* (Kar and Saxena 1976) Jain and Kar 1979; Bar = 50μm. (**e**) *Cucurbitariaceites bellus* Kar et al. 1972; Bar = 50μm. (**f**) *Cucurbitariaceites keralensis* Varma and Patil 1985; Bar = 50μm. (**g**) *Deccanodia eocena* Singhai 1974; Bars = 250μm & 50μm respectively. (**h**) *Dendromyceliates rajmahalensis* Tripathi 2001; Bar = 10μm. (**i**) *Dendromyceliates splendus* Jain and Kar 1979; Bar = 50μm. (**j**) *Dicellaesporites constrictus* Sah and Kar 1974; Bar = 20μm. (**k**) *Dicellaesporites ellipticus* Jain and Kar 1979; Bar = 20μm. (**l**) *Dicellaesporites elongatus* Ramanujam and Rao 1978; Bar = 10μm. (**m**) *Dicellaesporites minutus* Kar and Saxena 1976; Bar = 10μm (**n**) *Dictyomykus ellipticus* Kar et al. 2010; Bar = 10 μm (**o**) *Diploneurospora tewarii* Jain & Gupta 1970; Bar = 20 μm

Species: *Circinoconites arthrus* Kar et al. 2010 (Fig. 4.2c).

Description: Conidia arise from tip of conidiophores, helicoid, 42–30 × 23–27 μm in size, made up of 10–16 cells, cells increase in size from base to top, basal cell rectangular, 3–4 in number, 6–8 × 4–6 μm in size. Lower cells straight, rest cells coiled, septate, septa up to 2 μm thick, constriction more marked in middle region, individual cells rectangular to wedge shaped, terminal cell oval-subcircular, 11–18 × 12–16 μm; spore wall 1 μm thick, mostly laevigate, sometimes weakly intrastructured.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Colligerites* Jain and Kar 1979.

Generic Description: Spores multicellular, coiled, cells generally smaller, rounded in central region and bigger, rectangular in outer region. Spore wall mostly laevigate, sometimes granulose. Pore may be present or absent in each cell.

Classification: Fungi Imperfecti, Helicosporae.

Species: *Colligerites kutchensis* (Kar and Saxena 1976) Jain and Kar 1979 (Fig. 4.2d).

Earlier: *Involutisporonites kutchensis* Kar and Saxena 1976.

Description: Coiled, laevigate, generally monoporate spores, central cells dark, hick, rhomboid to squarish, outer cells thinner longer and rectangular.

Occurrence: Palaeocene, Matanomadh Formation, Kutch, Gujarat.

Genus: *Cucurbitariaceites* Kar et al. 1972.

Generic Description: Pseudoperithecia subcircular to circular, 40–120 μm, outer region darker than inner, laevigate, Asci 1–20 μm, equal or unequal in size, cylindrical to somewhat bulging at tips in mature stage. No true paraphysis observed, but in some specimens basal part of asci may join together to form a broad irregular mesh-like structure.

Classification: Ascomycetes, Dothideales.

Species: *Cucurbitariaceites bellus* Kar et al. 1972 (Fig. 4.2e).

Description: Pseudoperithecia subcircular to circular, 40–120 μm. Peripheral part dark brown, central part translucent, characterized by presence of a polygonal area formed by the interconnection of basal parts of asci. Asci ± cylindrical, sometimes swollen tipped, always originate from upper surface of stroma.

Occurrences: Palaeocene, Tura Formation, Garo Hills, Meghalaya and also from many Indian Tertiary localities.

Species: *Cucurbitariaceites keralensis* Varma and Patil 1985 (Fig. 4.2f).

Description: Pseudoperithecia subcircular to circular, dark-brown, 86–90 μm in diameter, outer region light brown, wide (about 28–32 μm), uneven, with microreticulate ornamentation, imparting a mesh-like appearance to the peripheral zone. Central part conspicuously small, circular in outline, about 20–26 μm in diameter, with radiating lines of ad pressed asci running from the radius to the periphery of the central part. Central part darker than the peripheral region.

Occurrence: Miocene, Tonakkal, Trivandrum, Kerala.

(D)

Genus: *Deccanodia* Singhai 1974.

Generic Description: Pycnidium brown, more or less globose, non-ostiolate, thick walled; conidia many, faintly to dark brown in a mass, unequally two celled, oblong or ellipsoid.

Classification: Fungi Imperfecti, Sphaeropsidales.

Species: *Deccanodia eocena* Singhai 1974 (Fig. 4.2g).

Description: Pycnidium brown, more or less globose, 345 × 364 μm in size; conidia faintly brown, unequally two celled, oblong or ellipsoid, with their ends pointed or rounded, or one end pointed and the other round, measuring 12–24 × 2–8 μm.

Occurrence: Late Cretaceous-Maastrichtian, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Dendromyceliates* Jain and Kar 1979.

Generic Description: Hyphae thick walled, septate, cylindrical, base swollen, hyphae length divided into several cells by septa, cells with or without pores, generally uniporate. Tip of hyphae dichotomously branched 3–4 times, acutely pointed.

Classification: Fungi Imperfecti, Mycelia sterilia.

Species: *Dendromyceliates rajmahalensis* Tripathi 2001 (Fig. 4.2h).

Description: Fungal hyphae 3–3.5 μm wide, less than 1 μm thick, smooth, dichotomously branched, tips rounded, septate; septa thin; cells aporate. Free ends of hyphae with globular head, conidia separated by thin septa at places hyphae bear small baculate projections.

Occurrence: Early Cretaceous, Rajmahal Formation, Bihar.

Species: *Dendromyceliates splendus* Jain and Kar 1979 (Fig. 4.2i).

Description: Fungal hyphae thick walled, 52–165 μm long, dark brown, septate. Hyphae 3–4 times dichotomously branched, tips pointed, cells larger at unbranched region, smaller in upper part, with or without pores.

Occurrence: Miocene, Chanakkodi, Kollam, Kerala.

Genus: *Dicellaesporites* Elsik 1968.

Generic Description: Inaperturate, psilate fungal spores or algal bodies. Uniseptate, two celled, shape variable.

Classification: Fungi Imperfecti, Didymosporae.

Species: *Dicellaesporites constrictus* Sah and Kar 1974 (Fig. 4.2j).

Description: Two celled, psilate, inaperturate, 89–120 × 40–101 μm, constricted in middle, uniseptate, individual cells subcircular-oval.

Occurrence: Early Eocene, Palana lignite, Bikaner, Rajasthan.

Species: *Dicellaesporites ellipticus* Jain and Kar 1979 (Fig. 4.2k).

Description: Spores two celled, elliptical, 45–70 × 30–60 μm in size, inaperturate, septa distinct, straight, cells equal. Spore wall 1–2 μm thick, granulose-microverrucose, sculptural elements less than 1 μm high.

Occurrence: Miocene, Papanasam, Varkala, Kerala.

Species: *Dicellaesporites elongatus* Ramanujam and Rao 1978 (Fig. 4.2l).

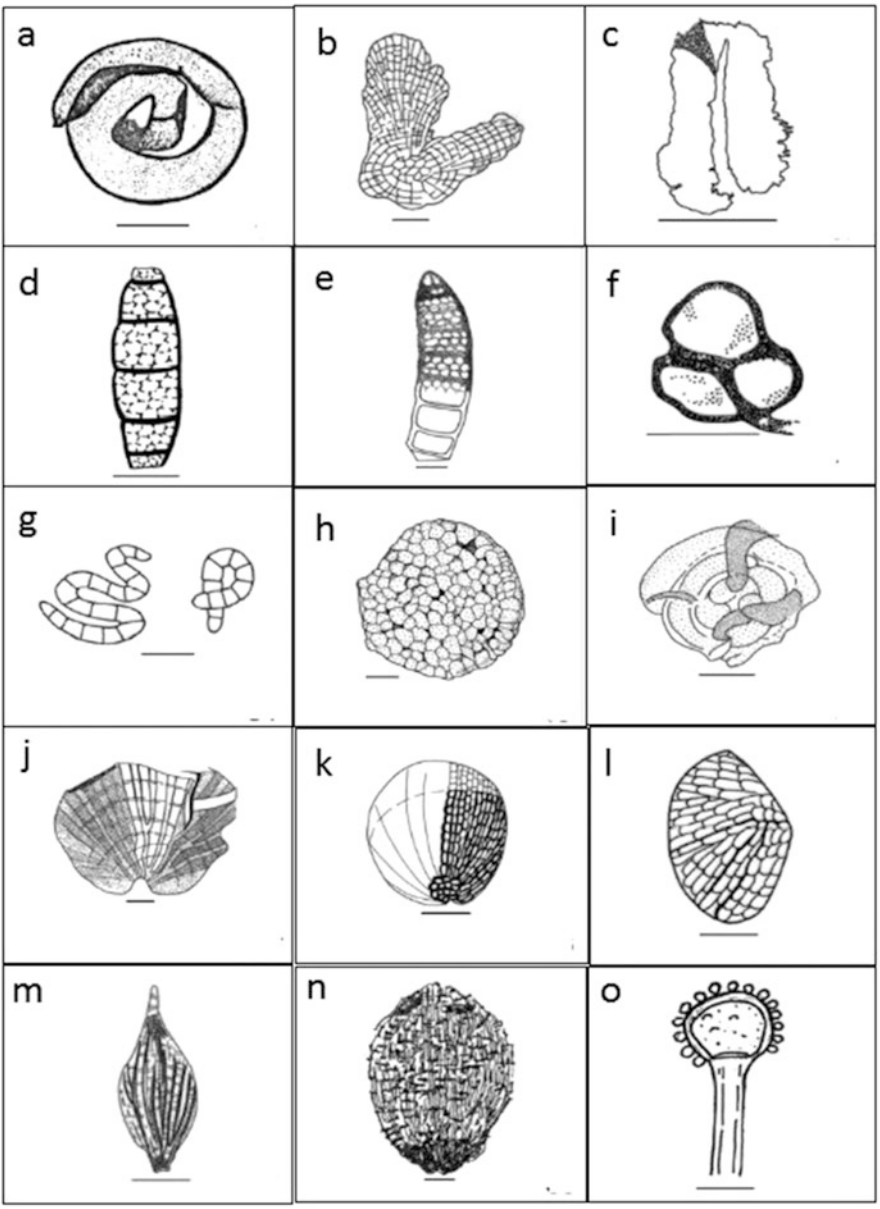

Fig. 4.3 (**a**) *Elsikisporonites tubulatus* Kumar 1990; Bar = 10μm. (**b**) *Euthythyrites bifidus* Kar et al. 2010; Bar = 10. (**c**) *Euthythyrites keralensis* Ramanujam and Rao 1973; Bar = 100. (**d**) *Foveoletisporonites indicus* Ramanujam and Srisailam 1980; Bar = 20μm. (**e**) *Foveoletisporonites miocenicus* Ramanujam and Rao 1978; Bar = 10μm. (**f**) *Hapalophragmites cumminsii* Ramanujam and Ramachar 1980; Bar = 20μm. (**g**) *Helicominites salvinites* Barlinge and Paradkar 1982; Bar = 10μm. (**h**) *Kalviwadithyrites saxenae* Rao 2003; Bar = 25μm. (**i**) *Koshalia enigmata* Sarkar and Prasad 2003; Bar = 20μm. (**j**) *Kutchiathyrites eccentricus*Kar 1979; Bar = 10μm. (**k**) *Kutchiathyrites mehrotrae* Saxena and Tripathi 2011; Bar = 40μm. (**l**) *Kutchiathyrites perfectus* (Kar et al. 2010) Saxena and Tripathi 2011; Bar = 10μm (**m**) *Lirasporis elongatus* Kar 1990; Bar = 35 μm (**n**) *Lirasporis intergranifer* Potonié & Sah 1960; Bar = 10 μm (**o**) *Lithomucorites miocenicus* Kar et al. 2010; Bar = 10 μm

Description: Spores inaperturate, dicellate, melanin coloured, ellipsoidal to almost oblong, 21–26 × 6–8 μm. Individual cells considerably elongated, spore wall 1 μm thick, transverse septum considerably thicker, wall psilate.

Occurrence: Miocene, Quilon and Warkalli beds, Kerala.

Species: *Dicellaesporites minutus* Kar and Saxena 1976 (Figs. 4.2m).

Description: Spores bicellate, septa distinct, oval, 23–33 × 7–12 μm; inaperturate, individual cells nearly same in size and shape. Spore wall up to 1.5 μm thick, laevigate.

Occurrence: Palaeocene, Matanomadh Formation, Kutch, Gujarat and also from many other Indian Tertiary localities.

Genus: *Dictyomykus* Kar et al. 2010.

Generic Description: Conidiophores small, closely placed to form a sporodochium-like cluster; conidia elliptical, basal part narrowed than terminal, 30–48 × 7–12 μm, branches parallel, 4–8 in number, develop from a basal cell, laterally fused, multiseptate, curved at tips, spore wall laevigate.

Classification: Fungi, Incertae sedis.

Species: *Dictyomykus ellipticus* Kar et al. 2010 (Fig. 4.2n).

Description: Conidiophores inconspicuous, 4–6 celled, closely adhered to each other; conidia borne singly, elliptical in shape, basal part generally narrower than terminal end, may also be equally broad at two ends, 30–48 × 9–14 μm in size. Branches 5–8, equal in length, fused laterally except at tip, transversely septate, septa 10–18, distinct, parallel to each other, no appreciable constriction at margin, terminal cell markedly curved, spore wall about 1 μm thick, laevigate.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Diploneurospora* Jain and Gupta 1970.

Generic Description: Ascospore two celled, uniseriate, elliptical, margin uneven, upper cell prominent, dark brown in colour, thick walled, wall sculptured with longitudinal ribs, lower cell hyaline, appendage-like, small in size, rib sculpture faint.

Classification: Ascomycetes, Sphaeriales.

Species: *Diploneurospora tewarii* Jain and Gupta 1970 (Fig. 4.2o).

Description: Ascospores two celled, uniseriate, cells unequal in size, length ratio nearly 3:1, both ends acute, two cells attached at base. Larger cell dark brown in colour, elliptical, 50 × 16 μm in size, exine 0.7 μm thick, sculptured, ribs prominent on one side, extending up to margins on the other side, leaving central portion free, ribs longitudinal, dichotomous, 8–10 in number, branched. Smaller cell hyaline, tail-like, 10 × 15 μm in size. Exine thin, ribs very faint, 4–5 in number.

Occurrence: Early Miocene, Padappakkara, Kollam, Kerala.

(E)

Genus: *Elsikisporonites* Kumar 1990.

Generic Description: Spores monoporate, nonseptate, tubular and coiled. Pore at outer end, nozzle-like. Spore wall smooth and hyaline.

Classification: Fungi Imperfecti, Helicosporae.

Species: *Elsikisporonites tubulatus* Kumar 1990 (Fig. 4.3a).

Description: Spores monoporate, nonseptate, tubular in shape and coiled, cell broad in the middle region, 10–12 μm wide, gradually tapering towards the ends. Pore at outer end, small, nozzle-like, 1.5 μm wide. Spore wall 1 μm thick, smooth, slightly folded, hyaline.

Occurrence: Early-Middle Miocene, Quilon Beds, Padappakkara, Kollam, Kerala.

Genus: *Euthythyrites* Cookson 1947.

Generic Description: Mycelium superficial; ascomata linear, radiate spore characters unknown.

Classification: Ascomycetes, Microthyriales.

Species: *Euthythyrites bifidus* Kar et al. 2010 (Fig. 4.3b).

Description: Ascostromate, 51–60 × 30–45 μm, dimidiate, non-ostiolate, dark brown, generally one-celled thick, darker in central region, hyphae radially arranged, anastomose to form square-rectangular pseudoparenchymatous cells, branches generally divided into two, sometimes an incipient third also visible.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Species: *Euthythyrites keralensis* Ramanujam and Rao 1973 (Fig. 4.3c).

Description: Mycelium superficial, ascomata linear, elliptical to oblong, ends rounded or flattened. Ascomata 125–350 × 60–100 μm in size, lateral margins uneven, dehiscing by a longitudinal slit, slit 7–13 μm broad, cells radiating from mid-vertical line, square to rectangular, 2.5–8 × 2.5–3.75 μm, thick walled, brownish to dark-brown. Mycelial hyphae radiating mostly from lateral marginal cells, usually flexuous, 2.5–3.75 μm thick, hyphopodiate, hyphopodia small, peg-like.

Occurrence: Upper Miocene, Warkalli lignite, Varkala, Kerala.

(F)

Genus: *Foveoletisporonites* Ramanujam and Rao 1978.

Generic Description: Spores simple, light to dark brown, multicellate, inaperturate. Spore wall two layered, inner layer forming transverse septa, surface conspicuously foveolate.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Foveoletisporonites indicus* Ramanujam and Srisailam 1980 (Fig. 4.3d).

Description: Spores elongated, light to dark brown, 5 septate, 68–74.8 × 15–20 μm, slightly constricted at central part, central two cells large, elongated, 20.4 × 17 μm, end cells relatively small, 10.2 × 5.1 μm, septa thick, conspicuous, spore wall about 1 μm thick, no vertical septa in terminal cells, surface conspicuously foveolate, foveolae irregularly aligned, 2.5 μm in diameter in central cells, slightly smaller in the end cells.

Occurrence: Miocene, Warkalli Beds, Kannur, Kerala.

Species: *Foveoletisporonites miocenicus* Ramanujam and Rao 1978 (Fig. 4.3e).

Description: Spores multiseptate, 100–120 μm long, 18–25 μm broad, transverse septa up to ten, apical cell deltoid with one or two vertical septa. Spore wall conspicuously foveolate, foveolae 1.5–3.5 μm in diameter, aligned in two or three horizontal rows in each cell.

Occurrence: Miocene, Quilon and Warkalli Beds, Varkala, Kerala, India.

(H)

Genus: *Hapalophragmites* Ramanujam and Ramachar 1980.

Generic Description: Teliospores triquetrously three celled, pedicellate, odd cell terminal, the two basal cells borne on a common stalk; wall cinnamon-brown; one germ pore in each cell.

Classification: Basidiomycetes, Uredinales.

Species: *Hapalophragmites cumminsii* Ramanujam and Ramachar 1980 (Fig. 4.3f).

Description: Teliospores triquetrously three celled, pedicellate, more or less rounded triangular to rounded, 30–42 × 30–39 μm, cinnamon-brown, wall up to 3 μm thick, smooth; pedicel up to 8 μm long; one germ pore per cell, faint, up to 2 μm in diameter.

Occurrence: Miocene, Neyveli lignite, South Arcot, Tamil Nadu.

Genus: *Helicominites* Barlinge and Paradkar 1982.

Generic Description: Saprophytic myceliate fungus; mycelium septate, branched, hyphae faint in colour; pycnidium and acervulus absent; conidia coiled in loose spirals and narrow at both ends.

Classification: Fungi Imperfecti, Helicosporae.

Species: *Helicominites salvinites* Barlinge and Paradkar 1982 (Fig. 4.3g).

Description: Saprophytic fungus found inside *Salvinia intertrappea* megaspores in space usually occupied by female gametophyte; mycelium 5–6 μm wide; conidia loosely, spirally coiled, 21–32 × 20–30 μm, narrow at both ends.

Occurrence: Cretaceous to Maastrichtian, Deccan Intertrappean, Mohgaon Kalan, Chhindwara, M.P.

(K)

Genus: *Kalviwadithyrites* Rao 2003.

Generic Description: Cleistothecium subcircular to circular, dimidiate, non-ostiolate. Two types of cells present, pores and hyphae absent. Marginal cells rectangular to polygonal in shape, larger in size, covers outer part; central cells two or three layered, squarish and isodiametric.

Classification: Ascomycetes, Microthyriales.

Species: *Kalviwadithyrites saxenae* Rao 2003 (Fig. 4.3h).

Description: Cleistothecium circular-subcircular, size range 105–115 × 95–110 μm. Dimidiate, non-ostiolate, No free hyphae. Fruiting body made up of two sets of cells, pores absent. Marginal cells rectangular to polygonal in shape, 9–12 × 10–17 μm in diameter, light brown in colour central cells of 2/3 layers, squarish and isodiametric, 4–10 μm in diameter, darker in colour.

Occurrence: Miocene, Sindhudurg Formation, Kalviwadi, Maharashtra.

Genus: *Koshalia* Sarkar and Prasad 2003.

Generic Description: Thyriothecia subspherical, multilayered, 90–150 μm in diameter, 9–10 cells arranged in compact rings around an ostiole, marginal cells extremely large, size 35–45 × 65–85 μm, inner cells small, subcircular, size 8–15 × 10–20 μm.

Classification: Ascomycetes, Microthyriales.

Species: *Koshalia enigmata* Sarkar and Prasad 2003 (Fig. 4.3i).

Description: Thyriothecia subspherical, 90–150 μm in diameter, multicellular, multilayered, 9–10 cells arranged in compact rings, 3–4 cells in each layer, ostiolate, marginal cells extremely large.

Occurrence: Late Ypresian, Subathu Formation, Himachal Pradesh.

Genus: *Kutchiathyrites* Kar 1979.

Generic Description: Ascostromata eccentric, dimidiate, non-ostiolate, radially arranged hyphae thick, dark, diverging from one another, transverse hyphae comparatively thinner, interconnecting radial ones to form squarish, pseudoparenchymatous cells.

Classification: Fungi Imperfecti, Dictyosporae.

Species: *Kutchiathyrites eccentricus* Kar 1979 (Fig. 4.3j).

Description (Kar 1979, p. 32): Ascostromata eccentric, 64–110 × 41–73 μm. Stromata dimidiate, non-ostiolate; radial hyphae diverging, dark, better developed than transverse ones; hyphae interconnecting each other to form squarish, nonporate, pseudoparenchymatous cells.

Occurrences: Oligocene, Meniyera Fort Formation, Kutch, Gujarat and also from many other Tertiary localities of India.

Species: *Kutchiathyrites mehrotrae* Saxena and Tripathi 2011 (Fig. 4.3k).

Earlier: *Kutchiathyrites* sp. Singh et al. 1986.

Description: Ascomata semicircular, some specimens look like fish scales, eccentric, size range 88–110 × 67–75 μm, non-ostiolate, dimidiate. Hyphae radially arranged thick, dark, diverging from one another; transverse hyphae comparatively thinner, interconnecting radial ones forming squarish, pseudoparenchymatous cells without having any pore. Some specimens possess spines in marginal cells.

Occurrence: Early Miocene, Bhuban Formation, Meghalaya and Assam.

Species: *Kutchiathyrites perfectus* (Kar et al. 2010) Saxena and Tripathi 2011 (Fig. 4.3l).

Earlier: *Dictyostromata perfecta* Kar et al. 2010.

Description: Stromata with two lateral sides divergent from each other, outer margin convex, slightly undulated due to pseudoreticulation, 35–42 × 23–37 μm; haustorium present or absent, 4–7 × 2–3 μm, hyaline, stromata generally conical at attachment zone; radial hyphae stronger than transverse hyphae, anastomose to form pseudoreticulation, meshes square to rectangular; faint at basal region.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Lirasporis* Potonié and Sah 1960.

Generic Description: Oval, 69 × 103 to 116 × 134 μm; longitudinal ends broadly rounded or somewhat tapering, sometimes showing irregular protuberances which form a jumbled mass; wall smooth, longitudinal ends notched; following the longer axis exist perhaps 20–30 parallel but narrow ribs showing between them spaced grana.

Classification: Fungi Imperfecti, Dictyosporae.

Species: *Lirasporis elongatus* Kar 1990 (Fig. 4.3m).

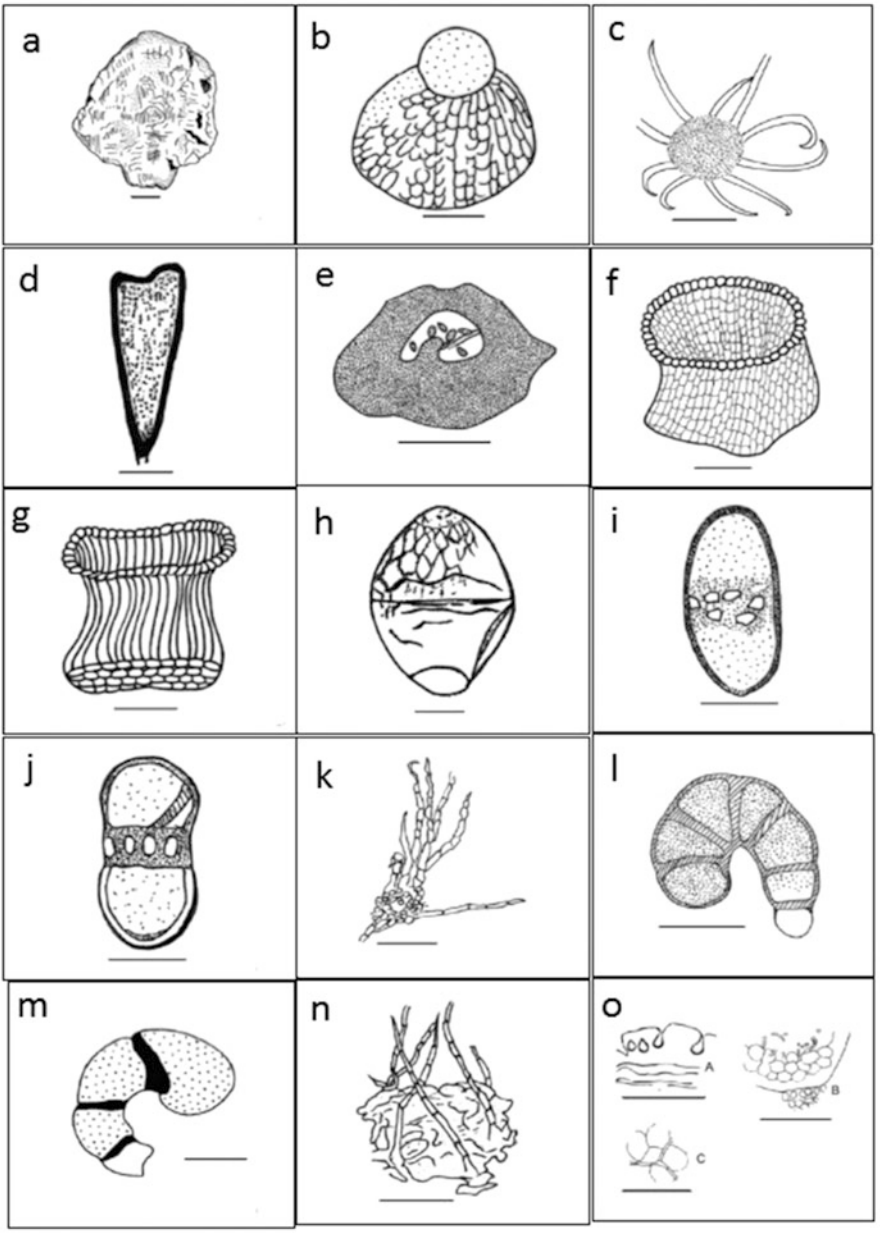

Fig. 4.4 (**a**) *Lithopolyporales zeerabadensis* Kar et al. 2003; Bar = 25μm. (**b**) *Lithosporocarpia cephala* Kar et al. 2010; Bar = 10μm. (**c**) *Lithouncinula lametaensis* Sharma et al. 2005; Bar = 20μm. (**d**) *Milesites irregularis* Ramanujam and Ramachar 1980; Bar = 10μm. (**e**) *Mohgaonidium deccani* Singhai 1974; Bar = 50μm. (**f**) *Netothyrites palaeocenicus* Misra et al. 1996; Bar = 20μm. (**g**) *Netothyrites vertistriatus* Misra et al. 1996; Bar = 20μm. (**h**) *Ornasporonites inaequalis* Ramanujam and Rao 1978; Bar = 10μm. (**i**) *Palaeoamphisphaerella*

Description Fungal body oval with elongated ends, 135 × 60 μm, broader in the middle and tapering at lateral sides. Mycelia longitudinally and transversally septate, spore wall laevigate.

Occurrence: Miocene, Rokhia borehole, Tripura.

Species: *Lirasporis intergranifer* Potonié and Sah 1960 (Fig. 4.3n).

Description: Ribs narrower than canals between them; sparse grana distributed in canals, about 10–20 grana in each canal along the longer axis. Perhaps 20 ribs on the exposed surface; 16–20 grana in an entire canal; chiefly at one of the longitudinal ends the exine is jumbled to form irregular rounded protuberances.

Occurrences: Late Miocene to Pliocene, Kannur, Kerala and also from many other Tertiary localities of India.

Genus: *Lithomucorites* Kar et al. 2010.

Generic Description: Fungal sporangia apophysate, sometimes with sporangiophore, circular-subcircular in shape, size range 25–52 × 22–49 μm. Wall about 1 μm thick, ornamented with bacula, pila and verrucae, ornamentation 2–5 μm in length, closely placed on both sides to provide negative reticulum on surface view; no slit on sporangia observed.

Classification: Zygomycetes.

Species: *Lithomucorites miocenicus* Kar et al. 2010 (Fig. 4.3o).

Description: Sporangia subcircular with serrated margin due to heavy ornamentation, 32–28 × 26–40 μm in size, sporangia wall about 1 μm thick, ornamented with bacula, pila, and verrucae, pila-bacula of 3–5 μm height, closely placed to form pseudoreticulate structure.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Lithopolyporales* Kar et al. 2003.

Generic Description: Basidiocarp macroscopic, conspicuous, shelf-like and effused refluxed. Texture tough and leathery, fruiting bodies or 'conks' perennial as evidenced by the presence of zonation in the section. They were sessile. In the section, minute hyphal strands forming the network could be seen; scattered between were found tiny dot-like spores—presumably the basidiospores.

Classification: Aphyllophorales, Polyporaceae.

Species: *Lithopolyporales zeerabadensis* Kar et al. 2003 (Fig. 4.4a).

Description (Kar et al. 2003, pp. 37–38): As for the genus.

Occurrence: Maastrichtian, Lameta Formation, Zeerabad, M.P.

Genus: *Lithosporocarpia* Kar et al. 2010.

Fig. 4.4 (continued) keralensis Ramanujam and Srisailam 1980; Bar = 10μm. (**j**) *Palaeoamphisphaerella pirozynskii* Ramanujam and Srisailam 1980; Bar = 10μm. (**k**) *Palaeocercospora siwalikensis* Mitra et al. 2000; Bar = 20μm. (**l**) *Palaeocirrenalia elegans* Ramanujam and Srisailam 1980; Bar = 20μm (**m**) *Palaeocirrenalia oligoseptata* Ramanujam & Srisailam 1980; Bar = 20 μm (**n**) *Palaeocolletotrichum graminioides* Mitra & Banerjee 2000; Bar = 25 μm (**o**) *Palaeocytosphaera intertrappeana* Singh & Patil 1980; A-Bar = 800 μm; B-Bar = 50 μm; C-Bar = 50 μm

Generic Description: Sporocarps subcircular-circular, 22–48 × 20–45 μm, often with chlamydospores, chlamydospores stalked, subcircular; sporocarp wall up to 2 μm thick, hyphae forming reticulation on both sides.

Classification: Fungi, Incertae sedis.

Species: *Lithosporocarpia cephala* Kar et al. 2010 (Fig. 4.4b).

Description: Sporocarps subcircular, margin uneven due to projection of hyphae, 28–30 × 26–32 μm in size, hyphae forming regular reticulate pattern on both surfaces, meshes mostly square in shape, sometimes rectangular. Chlamydospore present, one chlamydospore found on each sporocarp, chlamydospore subcircular, 10–22 × 8–20 μm, dark brown, laevigate, with a small stalk and globular head.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Lithouncinula* Sharma et al. 2005.

Generic Description: Cleistothecium subcircular-circular in shape, size range 51–87 μm, reticulate, provided with appendages of various sizes, appendages invariably circinate, rarely septate.

Species: *Lithouncinula lametaensis* Sharma et al. 2005 (Fig. 4.4c).

Description: C1eistotheca subcircular, appendiculate, wall psilate. Appendages of different sizes, 15–20 in number, conspicuous, circinoid, generally not septate.

Occurrence: Maastrichtian, Lameta Formation, Pisdura, Maharashtra.

(M)

Genus: *Milesites* Ramanujam and Ramachar 1980.

Generic Description: Urediniospores pedicellate, obovoid, lanceolate or irregular in shape; wall thin, hyaline or very light in colour, smooth or finely sculptured, germ pores few, faint.

Classification: Basidiomycetes, Uredinales.

Species: *Milesites irregularis* Ramanujam and Ramachar 1980 (Fig. 4.4d).

Description: Urediniospores lanceolate, or irregularly shaped, 30–45 × 12–20 μm; wall up to 1.5 μm thick, smooth or finely flecked, almost hyaline; germ pores few, indistinct.

Occurrence: Miocene, Neyveli lignite, Tamil Nadu.

Genus: *Mohgaonidium* Singhai 1974.

Generic Description: Pycnidia small, brown, thick walled, ostiolate, oval or more or less globose; conidia faintly brown, one celled, ovoid or ellipsoidal; conidiophores short and simple.

Classification: Fungi Imperfecti, Sphaeropsidales.

Species: *Mohgaonidium deccani* Singhai 1974 (Fig. 4.4e).

Description: Pycnidia ostiolate, small, oval or globose, brown, 56–96 × 52–72 μm in size. Conidiophores 5–8 μm in size, simple; conidia small, faintly brown, one celled, ovoid or ellipsoid, 3–4 × 5–6 μm in size, thin walled, smooth.

Occurrence: Late Cretaceous-Maastrichtian, Mohgaon Kalan, Chhindwara, M. P.

(N)

Genus: *Netothyrites* Misra et al. 1996.

Generic Description: Fungal fruit body pitcher shaped with distinct collar, hollow neck and main body with closed reticulated bottom, fly catcher's net-like in appearance. Proximal opening (? ostiole) distinct, bordered with dark, multicellular cells, form a distinct collar (rim) around subcircular to oval proximal opening. Main body hangs down from the collar, with a distinct neck in between. Sidewalls of the neck and main body bear a number of longitudinal ribs which run down parallel or anastomose to form reticulum, bottom of the main body densely reticulate and closed.

Classification: Fungi, Incertae sedis.

Species: *Netothyrites palaeocenicus* Misra et al. 1996 (Fig. 4.4f).

Description Fruit bodies flask shaped, longitudinally oval, 40–61 μm long, 22–54 μm in transverse axis; proximal opening (? ostiole) big, subcircular to oval measuring 22–51 μm at transverse axis, bordered with uniserial multicellular peripheral rim/collar, cells of peripheral rim rectangular, with thick surface, neck in between the proximal opening and main body distinct, broad, of almost same diameter as main body. Sidewalls of the main body bear a number of ribs/striations which emerge out from peripheral cells and run down; striation often dichotomise and anastomose to form reticulate net along sidewalls as well as at the bottom of main body, size of the meshes up to 2.4 × 3.6 μm; reticulation at the bottom very closely placed with thickened and narrow meshes.

Occurrence: Palaeocene, Subsurface, Krishna-Godavari Basin, Andhra Pradesh.

Species: *Netothyrites vertistriatus* Misra et al. 1996 (Fig. 4.4g).

Description: Fungal fruit bodies fly catcher's net shape, longitudinally oval to elongate, longitudinal axis 36–64 μm, transverse axis 24–62 μm. Proximal opening (? ostiole) distinct, big, transversely subcircular to oval, approximately 28–65 μm at transverse diameter, opening bordered by a distinct, relatively dark uni- or biserial multicellular peripheral rim. Cells of peripheral rim hard, rectangular, 2–3 × 3–6 μm in size: neck in between the main body and proximal opening narrow. Main body hang down from the peripheral rim, sidewalls bear number of unbranched, parallel running longitudinal ribs; ribs emerge out from the junctions of peripheral cells. Longitudinal ribs 2.5–4 μm away from each other, joined by thin membranous film. The bottom of the main body wall closely reticulated with thick mesh.

Occurrence: Palaeocene, subsurface, Andaman Basin.

(O)

Genus: *Ornasporonites* Ramanujam and Rao 1978.

Generic Description: Spores four celled, fusiform, diporate, cells unequal in size, basal and apical cells much smaller than two central cells; transverse septa three, central septum straight, other two septa curved. One simple pore in basal and apical cells, spore wall rugulate-reticuloid.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Ornasporonites inaequalis* Ramanujam and Rao 1978 (Fig. 4.4h).

Description: Spores fusiform to barrel shaped, tetracellate, 45–63 × 35–42 μm, cells unequal, basal and apical cells much smaller, one simple pore in basal and apical cells. Spore wall less than 1 μm thick, surface rugulate-reticuloid, muri flat, meshes irregular, often incomplete, lumina irregular, smooth.

Occurrence: Miocene, Quilon and Warkalli beds, Alleppey, Kerala.

(P)

Genus: *Palaeoamphisphaerella* Ramanujam and Srisailam 1980.

Generic Description: Spores aseptate, elliptical, oblong somewhat rhomboidal, with more or less rounded ends; with equatorial pores, surface psilate to scabrate.

Classification: Fungi Imperfecti, Amerosporae.

Species: *Palaeoamphisphaerella keralensis* Ramanujam and Srisailam 1980 (Fig. 4.4i).

Description: Spores dark brown, aseptate, elliptical to somewhat rhomboidal, 25.5–30.6 × 8.5–15.3 μm, multiporate, 3–6 equatorial pores, not always equidistant, often showing zigzag alignment, rounded to slightly ovoid or even transversely elongated, 3 μm in diameter, pore margin prominently thickened (2.2 μm), spore wall 1.7 μm thick, surface scabrate, locally coarsely so.

Occurrence: Miocene, Palayangadi and Cheruvattur, Kannur, Kerala.

Species: *Palaeoamphisphaerella pirozynskii* Ramanujam and Srisailam 1980 (Fig. 4.4j).

Description: Spores aseptate, oblong with more or less rounded ends, 28.9–34 × 10.2–15.3 μm in size, multiporate, pores 8–10, equatorial, equidistant, oval, 3.4–5.1 μm in diameter, pore margin prominently thickened (2.2 μm), spore wall 1.7 μm thick, surface psilate.

Locality: Miocene, Palayangadi and Cheruvattur, Kannur, Kerala.

Genus: *Palaeocercospora* Mitra and Banerjee 2000.

Generic Description: Stroma compact, with groups of well-developed hyphal cells, conidiophores occur singly or in fascicles of 2–7, elongated, divergent, septate; conidial scar present at the point of geniculation.

Classification: Dematiaceous fungi, Hyphomycetes.

Species: *Palaeocercospora siwalikensis* Mitra and Banerjee 2000 (Fig. 4.4k).

Description: Stroma distinct, circular with undulated outline; fascicles of conidiophores 7–20 in number, emerge from peripheral zone of stroma. Conidiophores simple, thick walled, smooth, pluriseptate with conidial scar on each cell.

Occurrence: Middle Miocene, Darjeeling Foothills, Eastern Himalaya.

Genus: *Palaeocirrenalia* Ramanujam and Srisailam 1980.

Generic Description: Spores light brown to reddish brown, inaperturate, helicoid, 1 to 1/4 times loosely coiled, multicellular, 2–6 septate, septa transverse, prominent, as thick and dark bands, cells of unequal size, terminal cell dome shaped and broader, basal cell usually cuneate, pale coloured, surface psilate.

Classification: Fungi Imperfecti, Helicosporae.

Species: *Palaeocirrenalia elegans* Ramanujam and Srisailam 1980(Fig. 4.4l).

Description: Spores light brown to reddish brown, inaperturate, helicoid, 1 to 1 1/4 times loosely coiled, 4 to 6 septate, maximum width 34–49.3 μm, basal cell cuneate to elongated-cuneate, 10 × 8.5 μm, pale coloured to almost hyaline, terminal cell dome shaped, 15.3 × 17 μm in diameter, septa prominent to form thick dark bands up to 5 μm thick, spore wall up to 1.7 μm thick, surface psilate.

Occurrence: Miocene, Warkalli Beds, Kannur, Kerala.

Species: *Palaeocirrenalia oligoseptata* Ramanujam and Srisailam 1980 (Fig. 4.4m).

Description: Spores inaperturate, helicoid or partially curved, 2/3 septate, maximum width 23.8 × 68 μm, elongated, cuneate, central cells larger than others, 34 × 23.8 μm, septa as prominent dark bands, up to 6.1 μm thick; spore wall about 1 μm thick, surface psilate.

Occurrence: Miocene, Palayangadi and Cheruvattur, Kannur, Kerala.

Genus: *Palaeocolletotrichum* Mitra and Banerjee 2000.

Generic Description: Subdermal acervuli, indistinct, long narrow stiff, pointed setae emerging from the acervulus surface.

Classification: Coelomycetes, Melanconiacea.

Species: *Palaeocolletotrichum graminioides* Mitra and Banerjee 2000 (Fig. 4.4n).

Description: The coelomycetous epiphyllous fungal remains occurring on cuticular layers show numerous setae scattered singly or in groups of 3–6 both on veins and intervenal regions, faint outline of hyphal mass of acervulus structure observed in the sub-epidermal position. Individual setae 84–126 μm long, stiff, pointed, moderately thick (4–5.5 μm), dark brown in colour, projected out from the leaf surfaces. The projected setae with a bulbous base, base 7–8.4 μm in diameter, septate, septa 5–8 in number.

Occurrence: Middle Miocene, Geabdat Sandstone, Eastern Himalaya.

Genus: *Palaeocytosphaera* Singh and Patil 1980.

Generic Description: Pycnidia in row, ostiolate, pseudoparenchymatous, black, spherical to oval; conidia single or in chains, oval to spherical, thin walled.

Classification: Fungi Imperfecti, Sphaeropsidales.

Species: *Palaeocytosphaera intertrappeana* Singh and Patil 1980 (Fig. 4.4o).

Description: Pycnidia 5–7 in row, black, ostiolate, spherical-oval, 110–165 × 70–90 μm in size; wall pseudoparenchymatous, 3–4 cells thick; conidiophores unbranched, 4 μm long; conidia oval-spherical, 2–3 μm in size, one celled, single or in chains of 2–3; mycelium branched, septate, intercellular. Host: dicotyledonous wood.

Occurrence: Late Cretaceous, Intertrappean beds, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Palaeoleptosphaeria* Barlinge and Paradkar 1982.

Generic Description: Saprophytic Fungus; mycelium branched, septate; asci in pycnidia forming acervulus; ascospores cylindrical, thick walled, elongate, and slightly curved.

Classification: Ascomycetes, Dothideales.

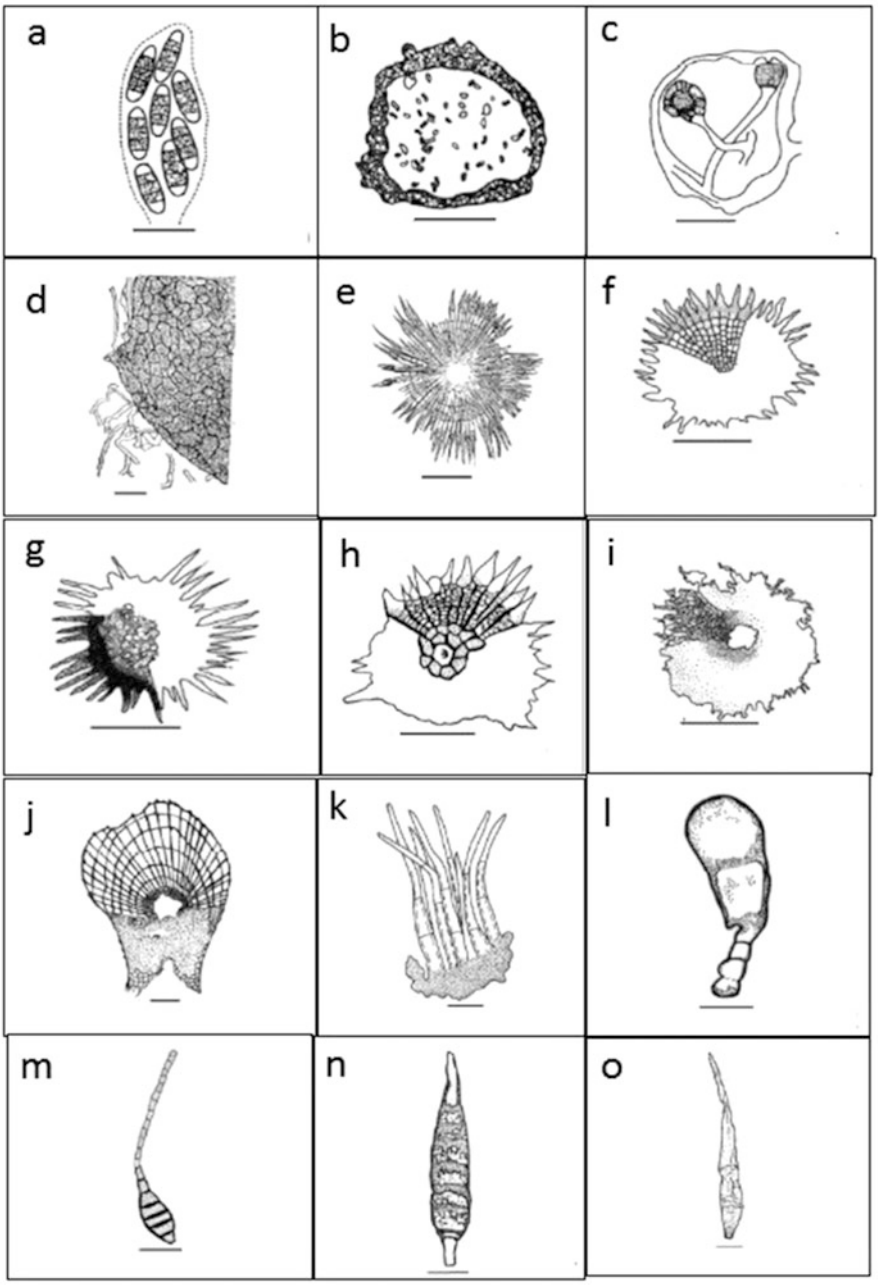

Fig. 4.5 (**a**) *Palaeoleptosphaeria intertrappeana* Barlinge and Paradkar 1982; Bar = 25μm. (**b**) *Palaeophoma intertrappea* Singhai 1974; Bar = 100μm. (**c**) *Palaeophthora mohgaonensis* Singhai 1978; Bar = 25μm. (**d**) *Palaeosordaria lagena* Sahni and Rao 1943; Bar = 20μm. (**e**) *Parmathyrites indicus* Jain and Gupta 1970; Bar = 50μm. (**f**) *Parmathyrites ramanujamii* Singh

Species: *Palaeoleptosphaeria intertrappeana* Barlinge and Paradkar 1982 (Fig. 4.5a).

Description: Asci 110–114 × 22 μm; ascospores 8, cylindrical, thick walled, brown, elongated, slightly curved, 25–35 × 9.5–11.5 μm, end cells pale, central cells enlarged. Host: decaying plant remains of *Salvinia intertrappea*.

Occurrence: Late Cretaceous, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Palaeophoma* Singhai 1974.

Generic Description: Pycnidium brown, more or less spherical; conidia one celled, hyaline, curved or spherical, thick walled; pycnidium pseudoparenchymatous.

Classification: Fungi Imperfecti, Sphaeropsidales.

Species: *Palaeophoma intertrappea* Singhai 1974 (Fig. 4.5b).

Description: Pycnidium brown and spherical, 224 × 200 μm in size, thick walled (12–32 μm) and pseudoparenchymatous; ostiole not seen. Conidia one celled, hyaline, curved or spherical, thin walled and smooth, measuring 5–8 × 2–4 μm.

Occurrence: Late Cretaceous-Maastrichtian, Mohgaon Kalan, Chhindwara, M. P.

Genus: *Palaeophthora* Singhai 1978.

Generic Description: Mycelium intracellular, branched, aseptate, sporangia either isolate or in organic connection at the tip of the mycelium, rounded or elongated, columella not present; sexual reproduction heterogamous, represented by rounded oogonia and narrow tube-like antheridia; oogonium having sac-like bodies on its wall; oospore (zygospore) with thick and rough outer wall.

Classification: Phycomycetes, Peronosporales.

Species: *Palaeophthora mohgaonensis* Singhai 1978 (Fig. 4.5c).

Description: Mycelium intracellular, 2.5–5 μm broad, aseptate, branched; detached sporangia 12–25 μm long and 5–10 μm broad, sporangia in organic connection at the tip of the mycelium, 7.6 μm in diameter, columella absent, spores present only in the detached sporangia; narrow elongated antheridia 14 × 5 μm; oogonium spherical, 12 × 11 μm; oospore (zygospore) rounded, with rough and thick outer wall, 11 μm in diameter.

Occurrence: Late Cretaceous-Maastrichtian, Mohgaon Kalan, Chhindwara, M. P.

Genus: *Palaeosordaria* Sahni and Rao 1943.

Fig. 4.5 (continued) et al. 1986; Bar = 50μm. (**g**) *Parmathyrites robustus* Jain and Kar 1979; Bar = 50μm. (**h**) *Parmathyrites turaensis* Kar et al. 1972; Bar = 50μm. (**i**) *Plochmopeltinites cooksoniae* Ramanujam and Rao 1973; Bar = 50μm. (**j**) Polyhyphaethyrites giganticus Srivastava and Kar 2004; Bar = 500μm. (**k**) *Protocolletotrichum deccanensis* Kar et al. 2004a, b; Bar = 10μm. (**l**) *Pucciniasporonites arcotensis* Ramanujam and Ramachar 1980; Bar = 10μm. (**m**) *Quilonia alleppeyensis* (Ramanujam & Rao 1978) Kalgutkar & Jansonius 2000; Bar = 20 μm. (**n**) *Quilonia attenuata* (Ramanujam & Srisailam 1980) Kalgutkar & Jansonius 2000; Bar = 50 μm. (**o**) *Quilonia miocenica* (Singh et al. 1986) Kalgutkar & Jansonius 2000; Bar = 10 μm

Generic Description: Fossil fungi referable to the Sordariaceae. Perithecia free, flask shaped, attached to a septate mycelium.

Classification: Pyrenomycetes, Sphaeriales, Sordariaceae.

Species: *Palaeosordaria lagena* Sahni and Rao 1943 (Fig. 4.5d).

Description: Perithecia black, flask shaped; body smooth and spherical, about 140 μm in diameter, external surface reticulate; neck tapering, about 180 μm long, with traces of short hairs round the tip, wall composed of one layer of cells, hypha septate.

Occurrence: Early Tertiary, Deccan Intertrappean Series, Sausar, Chhindwara, M.P.

Genus: *Parmathyrites* Jain and Gupta 1970.

Generic Description: Ascomata ostiolate or non-ostiolate, generally circular-subcircular, flat, one cell layer thick; hyphae radially arranged, interconnected, forming pseudoparenchyma; spines peripheral, spine sheath present or absent, ascospores unknown.

Classification: Ascomycetes, Microthyriales.

Species: *Parmathyrites indicus* Jain and Gupta 1970 (Fig. 4.5e).

Description: Ascomata flattened, circular, non-ostiolate, 180–190 μm in diameter, solitary, one-layer thick, radiating hyphae connected throughout whole length, central portion not well preserved. Central cells squarish, marginal cells rectangular. Cell walls thin, each peripheral cell developed into a long spine-like process. Spines about 70 in number around the periphery, unequal in size, 20–50 μm long, pointed at the apex, broader at base, walls thick, fused radially at the base, free on the upper side.

Occurrences: Early Miocene, Padappakkara, Kollam, Western Ghats and also from many other Indian Tertiary localities.

Species: *Parmathyrites ramanujamii* Singh et al. 1986 (Fig. 4.5f).

Description: Ascomata circular to subcircular, non-ostiolate, 80–90 μm in diameter. Hyphae radially arranged, interconnected, forming pseudoparenchymatous non-porate cells. Central cells squarish and marginal cells rectangular. Outer peripheral cells prominent with thickened radial walls, each peripheral cell develop into a spine-like process; spines unequal, 5–15 μm long, pointed at the apex and broader at the base, about 40 in number; wall thick, radially fused at the base forming a continuous peripheral sheath around ascomata; ascospores unknown.

Occurrence: Early Miocene, Sonapur-Badarpur Road section, Meghalaya and Assam.

Species: *Parmathyrites robustus* Jain and Kar 1979 (Fig. 4.5g).

Description: Ascostromata dimidiate, 60–110 μm; central pseudoparenchymatous cells thickened, sometimes porate, non-ostiolate. Marginal cells spinose, spines robust, closely placed, radiate, tips pointed.

Occurrence: Miocene, Chillakur Village, Varkala, Kerala.

Species: *Parmathyrites turaensis* Kar et al. 1972 (Fig. 4.5h).

Description: Ascomata circular to subcircular, ostiolate, one layered, 40–80 μm. Hyphae in the central part form a pseudoparenchymatous structure which is distinct from peripheral tissue. Development of radially elongated cells from central

polygonal ones is gradual. Outer peripheral cells generally more thickened and provided with spine-like projections; spines very well developed, up to 6 μm long with bulbous base and pointed tip. In some specimens, a few stromata are found together. Polygonal to rounded cells in central region are bigger than the rest and may form an ostiolate structure.

Occurrence: Palaeocene, Tura Formation, Garo Hills, Meghalaya.

Genus: *Plochmopeltinites* Cookson 1947.

Generic Description: **A**scomata dimidiate, formed with ascomal membranes of sinuous plectenchyma, ascospore characters unknown.

Classification: Ascomycetes, Microthyriales.

Species: *Plochmopeltinites cooksoniae* Ramanujam and Rao 1973 (Fig. 4.5i).

Description: Ascomata superficial, discoid, rounded, brown to reddish brown, 65–166 μm in diameter, ostiolate; ostiole 10–18.5 μm in diameter, irregular in shape, more or less centric, border dense, slightly raised, of dark brown colour, made up of thick-walled irregular cells; covering membrane of ascomata plectenchymatous, consisting of extremely sinuous, irregularly branched hyphae; hyphal cells 4–18 μm long, considerably thick walled (3.5–6 μm), excepting cells of peripheral layer, margin of fruit body not entire, wavy, formed of thin-walled membranous peripheral cells, free hyphae at times extending from marginal cells of ascomata.

Occurrence: Late Miocene, Warkalli lignite, Kerala.

Genus: *Polyhyphaethyrites* Srivastava and Kar (2004).

Generic Description: Ascostromatas dimidiate, generally subcircular in shape with wavy margin. Size 3.5 to 4.0 mm, no opening in the middle, but in section the middle part is ruptured occasionally giving an ostiolate appearance, 8–25 hyphae closely placed side by side to form radial and transverse strands, which are slightly twisted and rope-like. The strands are interconnected with each other to form a net-like structure. The cellular structures seen are often branched at the margin. The margin is generally setose and the cells are thicker with smaller meshes.

Classification: Ascomycetes, Microthyriales.

Species: *Polyhyphaethyrites giganticus* Srivastava and Kar 2004 (Fig. 4.5j).

Description: Same as that of genus.

Occurrence: Palaeocene, Deccan Intertrappean Beds, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Protocolletotrichum* Kar et al. 2004b.

Generic Description: Acervuli subcircular to circular in shape, 0.03–0.05 mm in diameter, sparsely distributed on cuticle; margins slightly raised, setose, setae arise around the margins, stout, 0.05–1.2 mm long and less than 0.01 mm broad, dark brown in colour, slightly swollen at base, pointed at tip, unbranched, smooth, 1–2 septate, very slightly constricted at septae.

Classification: Deuteromycetes, Melanconiaceae.

Species: *Protocolletotrichum deccanensis* Kar et al. 2004b (Fig. 4.5k).

Description: Around 30 haphazardly placed acervuli are preserved on a cuticle fragment. Setae that originate from the margins of each acervulus are unbranched and slightly divergent, with broad bases and pointed tips; in some cases their tips are broken. The cell walls of the setae are ca. 2 μm thick and more or less psilate.

Occurrence: Palaeocene, Deccan Intertrappean Beds, Mohgaon Kalan, Chhindwara, M.P.

Genus: *Pucciniasporonites* Ramanujam and Ramachar 1980.

Generic Description: Teliospores borne singly on pedicels, two celled by prominent horizontal septum; wall thick, pigmented; one germ pore in each cell, more or less terminal in upper cell, and lateral in lower cell.

Classification: Basidiomycetes, Uredinales.

Species: *Pucciniasporonites arcotensis* Ramanujam and Ramachar 1980 (Fig. 4.5l).

Description: Teliospores two celled by horizontal septum, pedicellate, obovoid to elliptical, 25–35 × 10–12 μm excluding stalk, not constricted at septum, individual cells up to 13 μm long and 12 μm broad; wall chestnut-brown, often darkly so, smooth, up to 3 μm thick, pedicel light coloured, up to 8 μm long; one germ pore in each cell, faint, up to 2 μm in diameter.

Occurrence: Miocene, Neyveli lignite, Tamil Nadu.

(Q)

Genus: *Quilonia* Jain and Gupta 1970 emend. Kalgutkar and Jansonius 2000.

Generic Description: Pluricellate hilate fungal spores, with an oval to elongate obpyriform pigmented central section, the greatest width of which tends to be near the proximal end; spore distally extended into an elongated multiseptate narrow stalk that terminates in a closed cell, although the tip of the stalk is commonly lacking; proximally, there is a short tapering stalk with a hilate scar. Both stalks tend to be thin walled or hyaline.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Quilonia alleppeyensis* (Ramanujam and Rao 1978) Kalgutkar and Jansonius 2000 (Fig. 4.5m).

Earlier: *Pluricellaesporites alleppeyensis* Ramanujam and Rao 1978.

Description: Spores straight to slightly curved, inaperturate, uniseriate, multicellate, 80–165 μm long. Septa 8–16, lower part of spore broader, apical part narrower, broader part confined to first five cells, 30 × 13 μm, narrower, part 3 μm broad, basal cell conicotruncate. Spore wall 1–1.5 μm thick, septa in the lower, broad portion thicker than spore wall, surface scabrate to finely granular in the lower part, psilate in apical part.

Occurrence: Miocene, Quilon and Warkalli beds, Alleppey, Kerala.

Species: *Quilonia attenuata* (Ramanujam and Srisailam 1980) Kalgutkar and Jansonius 2000 (Fig. 4.5n).

Earlier: *Diporicellaesporites attenuatus* Ramanujam and Srisailam 1980.

Description: Spores elongate, 30–59.5 × 10–13.6 μm, transverse septa seven, end cells prominently attenuating, paler or almost hyaline, a prominent pore in each end cell; septa conspicuous, 3 μm thick, two layered, spore wall up to 2.2 μm thick, slightly constricted at septa, surface psilate.

Occurrence: Miocene, Warkalli Beds, Palayangadi and Kannur, Kerala.

Species: *Quilonia miocenica* (Singh et al. 1986) Kalgutkar and Jansonius 2000 (Fig. 4.5o).

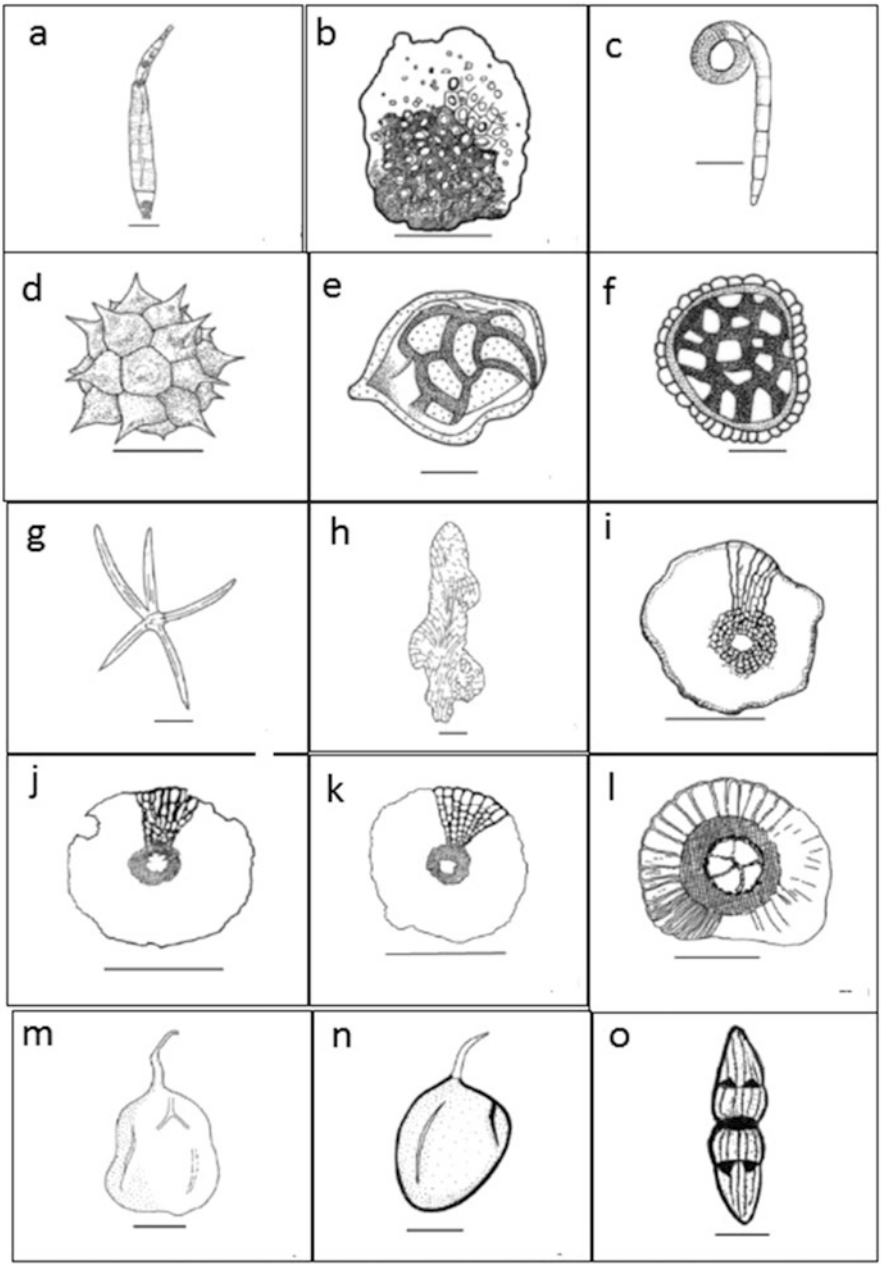

Fig. 4.6 (**a**) *Quilonia typical* Jain and Gupta 1970; Bar = 20 μm. (**b**) *Ratnagiriathyrites hexagonalis* Saxena & Misra 1990; Bar = 50 μm (**c**) *Retihelicosporonites elsikii* Ramanujam & Rao 1978; Bar = 10 μm. (**d**) *Spinosporonites indicus* Saxena & Khare 1992; Bar = 20 μm. (**e**) *Teliosporites globatus* Kar et al. 2010 Bar = 10 μm. (**f**) *Teliosporites hirsutus* Kar et al. 2010; Bar = 10 μm. (**g**) *Tetradigita stellata* Kar et al. 2010; Bar = 10μm. (**h**) *Trichopeltinites folius*

Earlier: *Inapertisporites miocenicus* Singh et al. 1986.

Description: Fungal spores elongate, 112–218 × 21–24 μm, unicellate, aseptate, inaperturate. Spores pointed at one end, blunt at the other. Spore wall hyaline, laevigate and irregularly folded.

Occurrence: Early Miocene, Bhuban Formation, Jacinta Hills, Meghalaya.

Species: *Quilonia typica* Jain and Gupta 1970 (Fig. 4.6a).

Description: Body multicellular, filamentous, 175–215 × 10–25 μm. Basal stalk distinct, with one or two rectangular cells, 8–10 μm with unevenly thickened walls. Apical portion curved, central part broad, elongate, exine 1.5–2.5 μm thick, furrow prominent, 40–67.5 × 2–2.5 μm. Some small, circular (8–10 μm) bodies occur throughout the filament.

Occurrence: Miocene, Padappakkara, Kollam, Western Ghats, South India.

(R)

Genus: *Ratnagiriathyrites* Saxena and Misra 1990.

Generic Description: Ascostromata subcircular or irregular in shape, non-ostiolate, cells not radially arranged, porate, pores generally distributed throughout stromata, cells hexagonal, bigger towards periphery than in the central region; margin thick, wavy.

Classification: Ascomycetes, Microthyriales.

Species: *Ratnagiriathyrites hexagonalis* Saxena and Misra 1990 (Fig. 4.6b).

Description: Ascostromata subcircular in shape, non-ostiolate, size range 66–114 × 55.5–90 μm, cells not radial, porate, central cells porate, marginal cells aporate, cells hexagonal, sometimes pentagonal, cells increasing in size towards periphery, margin thick, wavy.

Occurrence: Neogene, Ratnagiri beds, Amberiwidi section, Sindhudurg, Maharashtra.

Genus: *Retihelicosporonites* Ramanujam and Rao 1978.

Generic Description: Spores simple, uniseriate, multicellular, inaperturate, basal cell cuneate, other cells rectangular; apical part of spore helical, spore wall reticulate.

Classification: Fungi Imperfecti, Helicosporae.

Species: *Retihelicosporonites elsikii* Ramanujam and Rao 1978 (Fig. 4.6c).

Description: Spores multicellular with 3–8 transverse septa, 110–130 μm long, apical part helical; septa very faint or almost absent in the helical region, cells

Fig. 4.6 (continued) Kar et al. 2010; Bar = 10. (**i**) *Trichothyrites amorphus* (Kar and Saxena 1976) Saxena and Misra 1990; Bar = 50μm. (**j**) *Trichothyrites denticulatus* (Ramanujam and Rao 1973) Kalgutkar and Jansonius 2000; Bar = 50μm. (**k**) *Trichothyrites keralensis* (Rao and Ramanujam 1976) Kalgutkar and Jansonius 2000; Bar = 50μm. (**l**) *Trichothyrites ovatus* (Venkatachala and Kar 1969) Kalgutkar and Jansonius 2000; Bar = 50μm. (**m**) *Udaria saxenae* Gupta 1996; Bar = 30μm. (**n**) *Udaria singhii* Gupta 1996; Bar = 30μm. (**o**) *Varmasporites tonakkalensis* (Varma and Patil 1985) Kalgutkar and Jansonius 2000; Bar = 10μm

10–13.5 × 5–7.5 μm, wall two layered, reticulate, coarsely so in the helical region, meshes hexagonal, lumina smooth.

Occurrence: Miocene, Quilon and Warkalli beds, Kollam and Warkalli, Kerala.

Genus: *Spinosporonites* Saxena and Khare 1992.

Generic Description: Spores circular to subcircular, inaperturate; multicellular, each cell giving rise to a robustly built spine.

Classification: Ascomycetes, Microthyriales.

Species: *Spinosporonites indicus* Saxena and Khare 1992 (Fig. 4.6d).

Description: Spores subcircular, size 42–46 × 38–40 μm (excluding spines); inaperturate; multicellular, each cell giving rise to a robustly built spine; spines 7–9 μm long, up to 3 μm wide at the base and pointed at the tip.

Occurrence: Late Palaeocene-Middle Eocene, Neyveli, Tiruchirapalli, Tamil Nadu.

(T)

Genus: *Teliosporites* Kar et al. (2010).

Generic Description: Teliospores, always in mass, 21–32 × 19–28 μm, generally surrounded by gelatinous translucent sheath, up to 2 μm thick, spores laevigate; sterile cells may be associated with fertile ones, pseudobaculate appearance on surface view.

Classification: Basidiomycetes, Ustilaginales.

Species: *Teliosporites globatus* Kar et al. 2010 (Fig. 4.6e).

Description: Spore mass subcircular-oval, 21–28 × 19–27 μm, margin undulated due to the presence of gelatinous covering; 12–18 spores joined together, individual spore subcircular, 8–11 × 6–9 μm, spore wall up to 2 μm thick, laevigate, light brown; about 2 μm thick, translucent, weakly granulose, grana less than 1 μm high, sparsely placed.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Species: *Teliosporites hirsutus* Kar et al. 2010 (Fig. 4.6f).

Description: Teliospores always occur in mass, 12–24 spores joined together to form subcircular shape, 18–31 × 16–29 μm. Cells of two kinds—fertile and sterile, fertile cells bigger 12–18 × 10–16 μm, sterile cells 4–8 × 3–7 μm; spore wall about 1 μm thick, laevigate; fertile cells 12–28 in number, sterile cells numerous, covering fertile cells, closely placed to form retibaculate pattern.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Tetradigita* Kar et al. 2010.

Generic Description: Conidia blastic in development, conidium long, tubular, bent in middle to produce generally two lateral arms, giving a stellate appearance, arms equal or unequal in length (5–30 × 2–4 μm), sometimes with further branching, spore wall 1 μm thick, laevigate, translucent-light brown, mostly not septate.

Classification: Fungi, Incertae sedis.

Species: *Tetradigita stellata* Kar et al. 2010 (Fig. 4.6g).

Description: Conidiogenous cells like ordinary hyphae, conidia develop before septation of conidiogenous cells, filamentous, straight in young stage, slightly curved at maturity to bear two arms laterally at the same time; arms 14–28 × 2–4

μm, tubular, generally without septa, arms gradually tapering at terminal end, generally 4–5 armed, arm may be branched laterally or dichotomously at base; wall 1 μm thick, laevigate.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Trichopeltinites* Cookson 1947.

Generic Description: Thallus that of the Trichopeltineae, ascomata developed as thickened areas of the thallus and dehiscing by an irregular ostiole.

Classification: Ascomycetes, Microthyriales.

Species: *Trichopeltinites folius* Kar et al. 2010 (Fig. 4.6h).

Description: Ascostromata leaf-like, lobed, 90–110 × 20–25 μm, margin undulated, hyphae radially arranged, more or less parallel to each other, raised, often branched, transverse septa few, anastomose to form pseudoparenchymatous cells particularly on fruiting bodies, fruiting bodies subcircular, 8–10 μm, alternately placed, internal structure not visible due to hyphae.

Occurrence: Miocene, Bhuban Formation, Tlangsam, Mizoram.

Genus: *Trichothyrites* Rosendahl 1943.

Generic Description: Mycelium consisting of yellowish or brownish, branching, septate hyphae with occasional anastomoses, 5–6.7 μm in diameter, individual cells 28–33 μm long. Perithecia dark brown to nearly black, circular, disk shaped because of slightly upturned margin, 70–95 μm in diameter, with upper and lower membranes composed of radially arranged cells, upper membrane with a central papilla having a distinct pore or ostiole, marginal cells of membrane 4–5 μm wide, 6–8 μm long, cells of papilla more nearly quadrangular and thick walled, many marginal cells of pore prolonged into finger-like processes, cells of lower membrane all thin walled and radiating from a circular central cell, asci and spores lacking.

Classification: Ascomycetes, Microthyriales.

Species: *Trichothyrites amorphus* (Kar and Saxena 1976) Saxena and Misra 1990 (Fig. 4.6i).

Earlier: *Notothyrites amorphus* Kar and Saxena 1976.

Description: Ascostromata mostly subcircular, 45–105 × 40–98 μm, dimidiate, ostiolate; ostiole surrounded by a wall of few cells thick. Hyphae radially arranged but do not anastomose to form distinct pseudoparenchymatous cells.

Occurrence: Palaeocene, Matanomadh Formation, Kutch, Gujarat and also from many other Indian Tertiary localities.

Species: *Trichothyrites denticulatus* (Ramanujam and Rao 1973) Kalgutkar and Jansonius 2000 (Fig. 4.6j).

Earlier: *Notothyrites denticulatus* Ramanujam and Rao 1973.

Description: Ascomata discoid, dimidiate, rounded, margin smooth, 69–81 μm in diameter. Ostiolate, ostiole centric, 10–15 μm in diameter, elevated on slightly raised border. Ostiole border 3–4 layered, cells dark brown, thick walled, rounded to flattened, lumina narrow. Marginal cells of ascomata 5–16 × 3.8–6.5 μm, tangential walls thickened. Cells between ostiole border and ascomata periphery squarish to rectangular, with thickened tangential walls; 4–7 conical, teeth-like (denticular) processes protruding into ostiole cavity from inner layer of border. Denticular

processes 3–5 μm long, 4–5 μm broad basally, tip blunt or subacute, often slightly refluxed.

Occurrence: Late Miocene, Warkalli, Kerala.

Species: *Trichothyrites keralensis* (Rao and Ramanujam 1976) Kalgutkar and Jansonius (2000) (Fig. 4.6k).

Earlier: *Asterothyrites keralensis* Rao and Ramanujam 1976.

Description: Ascomata rounded, dimidiate, margin even to crenate, firm, 58–85 μm in diameter. Ostiolate, ostiole centric, round, 7–9 μm in diameter, with a prominent border of 2–3 layers of thick-walled dark brown cells. Hyphopodiate free mycelial shreds near ostiole border, rest of ascomata with strictly radially arranged, squarish to rectangular 2–4 μm wide cells; outer walls of marginal cells thickened.

Occurrence: Late Miocene, Warkalli, Kerala.

Species: *Trichothyrites ovatus* (Venkatachala and Kar 1969) Kalgutkar and Jansonius 2000 (Fig. 4.6l).

Earlier: *Sphaerialites ovatus* Venkatachala and Kar 1969.

Description: Perithecium dark brown, subcircular-circular, size range 60–150 μm. Central part of perithecium lighter, comprising 4–5 hexagonal cells, surrounded by a thick, at least two-layered, dark, plate-like, well-defined, rounded area consisting of square-hexagonal cells. Remaining part of perithecium one layered, made up of square/hexagonal pseudoparenchymatous cells of unequal length and width produced by the radiating interconnected hyphae, outer margin slightly undulating, unthickened.

Occurrence: Eocene, Bore-hole No. 14, Matanomadh, Kutch, Gujarat.

(U)

Genus: *Udaria* Gupta 1996.

Generic Description: Subcircular-ellipsoidal-oval, with germ slit (furrow) and a tube-like appendage, generally irregularly folded, psilate, size 39 × 33–116 × 81.5 μm (excluding appendage), appendage 3–8 μm broad. Position of slit and appendage variable; these two occur at opposite ends, slit elongate, small-large, running around nearly up to whole surface; situated irregularly, somewhat obliquely, often obliquely horizontal, nearly vertically in relation to appendage; one, rarely appears more. Number of folds in individual specimen varies, ranging from rare to copious, folds irregularly distributed, occasionally parallel to margin. Body shape variable due to haphazard folding and wide opening of wall at the slit.

Classification: Zygomycetes, Endogonales.

Remarks: Gupta (1996) ascribed this genus to acritarchs but this form has affinity with VAM fungi (Saxena and Tripathi 2011).

Species: *Udaria saxenae* Gupta 1996 (Fig. 4.6m).

Description: Light brown, subcircular-ellipsoidal, with slit and a tube-like appendage, 65 × 46–116 × 81.5 μm (excluding appendage), wall up to 7 μm thick, surface irregularly folded, psilate, appendage 3–8 μm broad.

Occurrence: Early Tertiary, Subathu Formation, Sirmaur, Himachal Pradesh.

Species: *Udaria singhii* Gupta 1996 (Fig. 4.6n).

Description: Deep brown, subcircular-oval with slit and a tube-like appendage, 39 × 33–75.5 × 67 μm (excluding appendage), wall ca. 2–2.6 μm thick, surface irregularly folded, psilate, appendage 4–8 μm broad.

Occurrence: Early Tertiary, Dagshai Formation, Sirmaur, Himachal Pradesh.

(V)

Genus: *Varmasporites* Kalgutkar and Jansonius 2000.

Generic Description: Fusiform, four celled, inaperturate fungal spores, with a pronounced constriction at the thick median septum, and with a distinct ribbed or striate sculpture parallel to the long axis. The two centrifugal septa may be less strongly developed.

Classification: Fungi Imperfecti, Phragmosporae.

Species: *Varmasporites tonakkalensis* (Varma and Patil 1985) Kalgutkar and Jansonius 2000 (Fig. 4.6o).

Earlier: *Fusiformisporites tonakkalensis* Varma and Patil 1985.

Description: Tetracellate, inaperturate, striate, spindle-shaped, brownish fungal spores; 48–52 × 8–10 μm. Heteroseptate, central septum 2.5 μm thick, 8 μm long, constricted, imparting a girdle shape to the spore and dividing the spore into two equal halves, each half with conical outline, pointed ends; septate, septum porate (1 μm wide), with two septal folds, 2.5 μm wide. About 5–6 longitudinal striae are seen on each exposed facet of the spore; striae 1.5 μm wide, not continuous, ending up in the median septum; spore wall 2 μm thick.

Locality: Miocene, Tonakkal, Thiruvananthapuram, Kerala.

4.1.4 Fungal Spore Stratigraphy

Although most of the fungal spores are long ranging and do not bear any stratigraphical significance but some are morphologically distinct and have restricted range in geological time. Graham (1962) suggested the possibility of using fungal spores for age determinations in palynological studies. Applicability of fungal spores in stratigraphy has increased with the record of some characteristic spores (Kalgutkar and Jansonius 2000). Numerous varieties of fungal spores are recorded from Mesozoic strata world over but their morphological complexity and frequency increases in Cenozoic (Elsik 1970). This worker noted that *Fusiformisporites* and similar longitudinally ribbed forms appear to be restricted to the Cenozoic. Elsik (*op. cit.*) further observed that fossil fungal spores described as *Exesisporites* resembling with extant *Hypoxylon* type are more frequently found in Neogene sediments. Ramanujam (1982) opined that overall diversity in morphology of fungal spore was attained by late Cretaceous and Early Tertiary. While evaluating the stratigraphic potential of fungal remains in Indian sequences, he further observed that spores with relatively simpler morphology were recorded from early Mesozoic strata but in younger sediments ornamented spores with complex morphology were recorded.

Table 4.2 Stratigraphic distribution of some fossil ascocarp genera in Indian Tertiary sediments

Taxa	Palaeocene	Eocene	Oligocene	Miocene	Pliocene
Calimothallus Dilcher	———	———	———	———	———
Cucurbitariaceites Kar et al.	———	———	———	———	———
Phragmothyrites Edwards	———	———	———	———	
Microthyriacites Cookson		———	———	———	
Kutchiathyrites Kar		———	———	———	
Kalviwadithyrites Rao				———	
Parmathyrites Jain and Gupta				———	
Plochmopeltinites Cookson				———	
*Ratnagiriathyrites*Saxena &Misra				———	
Trichopeltinites Cookson				———	
Trichothyrites Rosendahl				———	
Asterothyrites Cookson				———	
Euthythyrites Cookson				———	

4.1.5 Fossil Ascocarp Stratigraphy

Stratigraphic record of fossil Microthyriaceous fungi shows that these occur in major parts of the Cenozoic. An attempt has been made to summarize the stratigraphic distribution (at generic level only) of different fossil fruiting bodies recorded from Indian Tertiary sequences (Table 4.2). Taxa assigned to *Callimothallus* and *Cucurbitariaceites* are long ranging. Different species of *Phragmothyrites* mark their presence in Palaeocene to Miocene, *Microthyriacites* in Eocene to Miocene and *Kutchiathyrites* in Oligocene to Miocene. Forms restricted to Miocene sequences only are *Asterothyrites, Euthythyrites, Parmathyrites, Plochmopeltinites, Ratnagiriathyrites, Trichopeltinites* and *Trichothyrites.*

4.2 Palaeoclimatic Interpretations

Fungi are found in close association with specific plants and animals and if found in a fossil state are indicative of similar kind of situations during the geological past. Fossil fungi therefore, may provide useful information about the palaeoecology, past habitats and their hosts. In this regard fossil epiphyllous fungi can be more reliable

and advantageous for palaeoclimatic interpretations. Occurrence of these fossils reflects moist and humid climate of tropical to subtropical belts. Fossil peltate fungi are generally identified to the extant Microthyriaceae which are ectoparasites on leaves of higher plants of tropical to subtropical zones growing particularly in areas with high humidity. Edwards (1922) reported the occurrence of this group on conifer needles. Microthyriaceous fungi grow best in rain forests, its margins and along creek banks (Ramanujam 1982). Hence their presence is generally indicative of a wet tropical climate with heavy precipitation. The palaeohabitat interpretations based on fossil epiphyllous microthyriaceous fungi and their germ lings is well established through the studies on their modern equivalents growing on leaf litter from various Australian regions. These studies have shown the occurrence of microthyriaceous germ lings in greater number on the plants growing in moist tropical habitats. Such studies have great potential in interpreting the palaeoclimate and should be undertaken for other geographical areas. However, the ecological interpretations based on epiphyllous fungi should be made with caution because some of these occur in wider latitudinal ranges (Dilcher 1965; Selkirk 1975). It is therefore, advisable to take into consideration the complete palynological assemblage for palaeoenvironmental interpretations. In most of the cases, coordinated studies of mega fossils in association with palynological assemblages may provide more accurate information about the palaeoenvironmental conditions. Dilcher (1965) published an account of epiphyllous fungi thriving on leaves of different plants of Eocene age. Such studies bear great potential for determining the regional Palaeoclimate by comparing the fossils with extant taxa of known habitats. Environmental interpretations based on the presence of microthyriaceae may, however, sometimes be hampered due to the incorrect identification of the material. Their presence in dispersed fossil assemblage should, therefore, be ascertained before deciphering the past climate. The red alga *Caloglossa leprieurii*, generally found on grasses of brackish water marshes, may be confused with *Trichopeltinites* due to morphological resemblance. Similarly, marine green alga *Ulvella lens* also resembles the fructifications of Microthyriaceae.

Studies particularly focusing on host fungus relationship are also of great significance in attempting the palaeoenvironmental interpretations. Chitaley (1978) and Chitaley and Yavale (1978) provided valuable palaeoecological information based on the presence of fossil fungal spores in petrified plant materials from the Deccan Intertrappean beds of India. Similar kinds of interpretations were published by Kar et al. (2004a, b, 2005, 2006). These studies emphasize the importance of some fungal spores in the evaluation of palaeoenvironment. Ramanujam and Srisailam (1980) noticed the prevalence of *Paleocirrenalia*, the hilicoid spore, in Neogene sediments of Kerala, South India, and interpreted brackish to marine conditions by comparing them with modern fungi. Similarly, based on the presence of some other spores in the same strata a tropical climate has been interpreted by Ramanujam and Rao (1978) and Ramanujam and Srisailam (1980). A warm and humid environment has been interpreted by Kalgutkar and McIntyre (1991) in the Canadian Arctic owing to the presence of helicosporous fungal types. Studies of fossil fungal remains in coordination with micro- and mega fossils of other groups have sometimes been

used to infer the palaeoenvironment (Dilcher 1973; Pirozynski 1976; Ramanujam 1982). These assessments are based on the assumption that the palaeoclimatic sensitivity of fossil taxa was similar to that of the comparable modern counterparts. In this regard special stress was laid to explore the possibility of relating fossil fungal spores with those of modern fungi so as to realize their full potential in determining the ancient environment. However, only those types that could be related to the modern forms with certainty should be taken into account for this specific purpose.

4.2.1 Scope of Future Studies

Diverse fossil fungal remains have been described from Indian sediments but their affinity with modern counterparts is still less known. Such endeavour will help in elucidating the palaeoecology and evolutionary trends within this group. Stratigraphic significance of fossil fungal taxa should also be explored. Host–pathogen interaction in fossil state is another aspect which needs to be given more attention. Interaction of fungi with higher plants in light of palaeobotanical and chemical evidences are required to be worked out.

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Microbial Culture Collections in India: Historical Perspectives and Future Prospects

5

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Abstract

Culture collections maintaining microbial strains in pure forms are considered as important resource for research and development. The strains are maintained either in active condition or abiotically, a condition that assures little alterations/morphogenetic changes over decades. The first culture collection, the Kral collection, was established in Prague. Since then many culture collections have been developed in the world. Preamble to the Convention on Biological Diversity (CBD) aptly emphasizes that the countries have sovereign rights over all types of biological resources, and are responsible for conserving and using in sustainable manner for the benefit of present and future generations. Microbial culture collections having essential expertise function as repository preserve, maintain and manage microbial genetic resources and associated information. Use of various methodologies facilitate ex situ conservation which is an integral part of culture collection activities. These efforts would make the availability of authentic and high-quality strains to research community on sustained basis. Geographical enormity of India with biodiversity hotspots is the testimony of requirement of culture collections with modern preservation facilities, appropriate expertise and best strategies can cater to the need of country's R&D programmes as well as can attract biotech industries for depositing their microbial strains in culture collections. However, diminishing interest in the fundamentals of microbial taxonomy and systematics, especially among young researchers and students, is a matter of great concern. The conservation of rich microbial diversity and its judicious, sustainable use for the benefit of various sectors like agriculture,

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health care and biotech industry is yet another topic of importance requiring immediate attention. Taking a cue from activities of several international collections, this article advocates the need for more coordinated efforts by Indian culture collections with the ultimate goal of ensuring quality services, and building capacity for sustainable utilization of microbial resources of India under existing regulatory framework.

Keywords

Bioresource centres · Culture collections · Microbial gene bank · Repository · Ex situ conservation

5.1 Introduction

Diversity of living organisms, their genes and biomes are the result of evolutions that took place in millions of years (Koh et al. 2004). The unique traits or characteristic features acquired in due course of time by microbes make them valuable for various applications.

As microbial resources are the integral part of biodiversity, it comes under the sovereign rights of country and becomes essential to conserve them on sustained basis. Microorganisms and its derivatives provide raw materials to the development of microbe-based research and technology. However, the lack of comprehensive data poses immense difficulty in the evaluation of vast microbial diversity of tropical region. The main objective of any culture collection centre is to preserve and maintain pure cultures of biological materials as well as their supply to the researchers across the country or globally (Floyd et al. 2005), which is being carried out as per the guidelines of World Federation for Culture Collections (WFCC). Therefore, any culture collection or repository has crucial importance in the advancement of microbial research and development of the country.

In the new era of biotechnology, the role of microbial culture collections is increasingly important and crucial especially in the changing scenario when the government of India is enthusiastic in supporting the biotechnology industry. Many collections maintain fungi not only for teaching and pure research, fungal germplasm is immensely useful in fermentation, brewing, agriculture and medicine. The importance of such collections increased multifold after the discovery of penicillin in the 1930s, and several pharmaceutical industries continue to work on fungi as a rich source of novel compounds. Further spectacular demand for fungal cultures was witnessed after the discovery of cyclosporine which has increased interest in biotechnological processes (Smith 1981; Hawksworth 1985). It is pertinent to point out here that Biotechnology Industry Research Assistance Council (BIRAC), a flagship programme of the Department of Biotechnology, Govt. of India, is playing an important role in enhancing the strategic research and innovation capabilities of the Indian biotech industry. It has been estimated that out of about 1000 biotechnology start-ups in India, more than half of the start-ups work on diagnostics, drugs and

medical devices, about 14% focus on agricultural biotechnology, 3% in bioindustry, 1% in bioinformatics and 18% in biotechnology services (The Spotlight: Nature 564, S53–S55; 2019).

As such microbial collections also serve as genetic resource for numerous applications. Authentic or reference cultures required for regulatory compliance to health and trade are supplied by these culture collections (Sly 2010). Convention on Biological Diversity (CBD) provides sovereign rights to the states on biological materials and advocates for the conservation of biodiversity for the next generation. These culture collections maintain living microbial materials as well as their replicable parts such as genomes, plasmids, viruses and cDNAs for longer periods following standard guidelines (OECD 2001, 2007). Also, they ensure their sustainable use and management of related molecular and physiological data. In broader perspectives, traditional knowledge is also associated with bioresources, and the loss of these resources leads to social and ecological concerns. Since India is one of the most diverse and populous countries in the world, it needs greater awareness on the impact of loss of microbial wealth.

Culture collections are also recognized as biological resource centres (BRCs), which play an important role in ex situ germplasm conservation and maintenance (Singh and Baghela 2017). Basically, these collections perform academic, public service, government, private, and commercial activities such as the supply of authenticated and characterized cultures as 'seed' stocks for the industry, as 'the reference strains' for systematics and taxonomical studies, biological assays and publishing comparative scientific results.

In addition to storing live strains, culture collections also maintain the information on geographical origins, characteristic property and related ecological data of individual cultures either in the form of catalogue or in database (Singh and Baghela 2017). Moreover, they offer different knowledge-based services such as identification, storage, distribution, consultancy, and training programmes. In addition, several collections in the world provide patent deposit service also. First collection of microorganism was established in 1890 at the German University of Prague, Czech Republic (Hawksworth 1985). Collections are developed with certain objectives based on the available expertise in the field and among which long-term preservation and maintenance of microbial strains under controlled culture conditions is the primary objective of the collection. Pure cultures are very much important for biotechnological innovations in different areas such as agriculture, medicine, and industrial biotechnology. Systematic exploration as well as ex situ conservation of tropical microbial diversity is the prerequisite for any advanced research in science. For future scientific and industrial research, there is a greater need to emphasize our knowledge of microbial biodiversity as well as their availability for R&D purposes.

Huge microbial resources of India accommodate world's one of the largest biodiversity gene pools. The conservation of microbial diversity, their cells or replicable parts like genomes, plasmids, viruses and cDNAs in the environment has been realized by understanding the application of rRNA gene barcoding and the use of operational taxonomic units (OTUs) with the help of next-generation sequencing (NGS) (Arora et al. 2005; Sharma et al. 2016).

As per the WFCC-WDCM database, in India, 32 culture collections are currently existing which hold a variety of organisms like bacteria, archaea, fungi, yeasts, actinobacteria, cyanobacteria, algae, viruses, insects and plasmids, among which some are actively contributing by preserving and supplying microbial strains for basic and applied research.

5.2 Scenario of Microbial Culture Collections in the World

Microbial culture collections in the world focus on different activities, primarily to fulfil the requirement of microbe-based R&D programme. World Federation for Culture Collections (WFCC) was established in 1966 with the aim to promote the activities of the world culture collections of microorganisms and cell lines (Mallik 1992). In 1960, Professor V.B.D. Skerman, University of Queensland, Australia, along with his colleagues pioneered development of international database on cultures worldwide and later it was established as WFCC-MIRCEN World Data Centre for Microorganisms (WDCM). Both International Union of Microbiological Societies (IUMS) and the International Union of Biological Sciences (IUBS) accepted the formation of WFCC that serves as a forum and provides guidelines to Culture Collections. Besides, International conferences are also held every 4 years (see WFCC n.d.).

WDCM provides a common platform for communication for culture collections in the world and databases related to microbes and bioinformatics tools. World Directory of Collections of Cultures of Microorganisms (the World Directory) was published in 1972 for the first time. Culture collection information (CCINFO) database comprises data on the organization, services, management and scientific interests of the culture collections. Each record is further linked to other records of STRAIN database (list of holdings of culture collections: algae, cyanobacteria, bacteria, fungi, yeasts, lichens, protozoa, tissue cultures and viruses) (Jong and Birmingham 1985; Sugawara et al. 1993; Sugawara and Ma 1995; Vanderlei 1999). WFCC World Data Centre for Microorganisms (WDCM) was relocated to the Institute of Physical and Chemical Research (RIKEN), Japan, and then to National Institute of Genetics (NIG), Japan. After winning the bid for hosting WDCM in 2010, Institute of Microbiology, Chinese Academy of Sciences (IMCAS) has launched the WFCC (World Federation for Culture Collection) -MIRCEN (Microbial Resources Centres) World Data Centre for Microorganisms (WDCM) in 2011.

There are total 799 culture collections registered with WDCM from 78 countries and regions. Among 32,67,674 preserved microbes, 14,29,366 are bacteria, 867,934 are fungi, 39491 are viruses and 33,020 are cell lines (http://www.wfcc.info/#).

5.3 Culture Collections as Patent Depositories and Their Requirements

World Intellectual Property Rights Organization (WIPO), under an international treaty, i.e. Budapest Treaty, grants permission for collection to deposit microbial cultures for the purpose of patents. As per the patent law, the description of an invention should be adequate and reproducible during the life of the patent. The depositor needs to provide all the taxonomic details and also need to deposit living organism or materials in the designated depositories under Budapest Treaty (like DSMZ or MTCC). Sometimes, misidentified patent materials lead to rejection, and hence need to be checked and corrected. Novelty is an important requirement for the patent. In living organism-related patents, patent will be considered novel either by its use for which it is employed or by organism itself that has not been employed earlier for the same purpose. Therefore, legitimate identification of the organisms is essential for the legal standing of the patent.

5.4 Scenario of Microbial Culture Collections in India

Since the end of last century, work of scientific collections, identification and description of fungi was started in India. Similarly, work on other microbial systematics was initiated in different academic and research institutions at the same time. Initially, at a few places in India, ex situ conservation and characterization of microbes were started for backing microbes-based R&D programmes. Indian Type Culture Collection (ITCC) was established in 1936 at IARI, New Delhi. Upon observing the logistic difficulty, faced by academia, research institutes and industries in the country, the need of more specialized repositories in various regions of India has been realized. Due to the lack of inadequate facilities, procurement and deposit of microbes from overseas repositories were inevitable for the R&D programmes of the country.

Certain repositories in the world, such as American Type Culture Collection (ATCC), Centraalbureau voor Schimmelcultures-CBS (Current name: The Westerdijk Fungal Biodiversity Institute), Utrechtt, Netherlands, Deutsche Sammulung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ), CABI, Bioscience, Egham, UK (IMI), the Japan Collection of Microorganisms RIKEN, Tokyo (JCM), NRRL, USDA-ARS (USA), etc. have been considered reputed service providers at global level and many developing countries like India sought their services including deposition of biological materials in these international repositories. As a consequence, these valuable biological materials of our country remained in the foreign repositories due to current changes in international policy. However, on demand these materials can be repatriated by paying high cost levied by individual collection/repository. In India, the need of additional repositories at regional level has been realized to support the requirement of different sectors such as agriculture, healthcare and industry (Bhat 2000; Sarma 2003). Therefore, several microbial culture collections were established in India in

due course of time and with different objectives. Currently, there are 32 culture collections existing in India possessing different organisms, like Bacteria, Cyanobacteria, Fungi, Yeast, Actinobacteria, Algae, Archaea, Viruses, Insects, Plasmids, etc. (Table 5.1). Among these, three culture collections (MTCC, MCC and NAIMCC) have been upgraded and now recognized as Patent Depositories under IDA.

The details of a few active culture collections of India and their activities are described below:

HCIO-ITCC: The Herbarium Cryptogamae Indiae Orientalis (HCIO) is a national herbarium established by Sir. Edwin John Butler at Pusa, Bihar, in 1905 and later on shifted to Indian Agricultural Research Institute, New Delhi, in 1934. The role of mycological herbarium is highly important to preserve the fungal diversity in the form of specimens. Soon after the transfer of HCIO, Indian Type Culture Collection (ITCC) was established in 1936. The main objectives of HCIO and ITCC are to act as repositories for mycological specimens and fungal cultures, respectively. The overall activities include (a) preservation and maintenance of fungal cultures and specimens, (b) identification and supply services of fungal cultures, (c) deposition of authentic fungal cultures and diseased specimens, (d) taxonomic investigations and (e) documentation of the fungi. More than 50,000 fungal disease specimens and 4000 strains/isolates are maintained at HCIO and ITCC, respectively.

MTCC: In 1986, with the joint financial support from the Department of Biotechnology (DBT) and the CSIR, Govt. of India, Microbial Type Culture Collection and Gene Bank (MTCC) was established as national facility at the Institute of Microbial Technology (IMTECH), Chandigarh. Later on, MTCC became first International Depository Authority (IDA) of India under the Budapest Treaty recognized by the World Intellectual Property Organization (WIPO), Geneva, Switzerland, on 4th October 2002. The main work of this collection is to deposit and supply authentic microbial cultures and also provide related services to the universities, research institutes and industries. Currently, it holds over nine thousand cultures of Bacteria, Fungi, Yeasts, Actinobacteria and Plasmids (Source: https://mtccindia.res.in/).

NFCCI: MACS' Agharkar Research Institute has a great legacy for mycological research undertaken at its mycology and plant pathology group since its establishment (estd. 1946).

ARI established first mycological herbarium in western India in 1969. This repository was named as Ajrekar Mycological Herbarium (AMH) in the honour of pioneer mycologist and plant pathologist of British India, late Prof. S.L. Ajrekar, who served as the first honorary Head of the Mycology & Plant Pathology Dept. of MACS-ARI. The AMH has been in support of researchers by providing fungal and lichen specimens of Indian and foreign origins for fundamental studies on comparative systematics, taxonomic research and authentication. Besides exchanged authentic materials with many other international herbaria such as HCIO, New Delhi, CMI & Royale Botanic Gardens, England, Padova (Italy), Sweden, Argentina, etc. As such AMH serves as archive for related reference materials with passport

Table 5.1 Details of microbial culture collections registered from India

S. no.	Name of collection	State	Registered with WFCC	Holdings
1.	National Collection of Industrial Microorganisms (NCIM-WDCM 3) **Postal Address:** National Chemical Laboratory (CSIR), Dr. Homi Bhabha Road, Pashan, Pune, Maharashtra 411 008 **Telephone 1:** (91) 20-25902670 **Telephone 2:** (91) 20-25902454 **Fax:** (91) 20-25902671 **E-mail:** ncim@ncl.res.in	Maharashtra	1981	Algae (20) Bacteria (1400) Fungi (950) Yeasts (600)
2.	Culture Collection, Microbiology and Cell Biology Laboratory (NTCCI-WDCM 107) **Postal Address:** Indian Institute of Science, Bangalore 560012, Karnataka	Karnataka	1981	Bacteria (214) Fungi (78) Yeasts (43) Cell lines: animal (10) Viruses: animal (03) Viruses: bacteria (11)
3.	Culture Collection, Department of Microbiology (CCDMBI-WDCM 119) **Postal Address:** Bose Institute, 93/1 Acharya Prafulla Chandra, Calcutta 700009, West Bengal	West Bengal	1981	Bacteria (50) Fungi (40) Yeasts (12)
4.	DMSRDE Culture Collection (DMSRDE-WDCM 166) **Postal Address:** G.T. Road Post Box No.320, Kanpur 208013, Uttar Pradesh	Uttar Pradesh	1981	Bacteria (10) Fungi (167)
5.	Division of Standardisation (DBV-WDCM 173) **Postal Address:** Indian Veterinary Research Institute, Izatnagar, Bareilly 243122, Uttar Pradesh	Uttar Pradesh	1981	Bacteria (100) Fungi (01) Yeasts (02) Protozoa (03) Viruses: Plants (47)
6.	Indian Type Culture Collection (ITCC-WDCM 430) **Postal Address:** Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012 **Telephone:** (91)-9871980304 **E-mail:** prameelancha@yahoo.co.in	New Delhi	1981	Bacteria (20) Fungi (3800)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
7.	Biological Nitrogen Fixation Project, College of Agriculture (MPKV-WDCM 448) **Postal Address:** Mahatma Phule Agricultural University, Pune 411005, Maharashtra	Maharashtra	1981	Algae (03) Bacteria (10) Fungi (13)
8.	Collection of Insect Pathogens, Department of Entomology (CIPDE-WDCM 462) **Postal Address:** Marathwada Agricultural University, Parbhani 431402, Maharashtra	Maharashtra	1981	Bacteria (04) Fungi (03) Protozoa (05) Viruses: Plants (20)
9.	Fungal Culture Collection (VPCI-WDCM 497) **Postal Address:** Vallabhbhai Patel Chest Institute, University of Delhi, Delhi 110 007	Delhi	1981	–
10.	MACS Collection of Microorganisms (MCM-WDCM 561) **Postal Address:** MACS-Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004 **Telephone:** (91) 20-25325096 **Fax:** (91) 20-25651542 **E-mail:** director@aripune.org	Maharashtra	1982	Bacteria (256) Fungi (08)
11.	Food and Fermentation Technology Division, University of Mumbai (UMFFTD-WDCM 562) **Postal Address:** Dept. of Chemical Technology, Nathalal Parekh Marg, Mumbai,Maharashtra 400 019 Telephone: (91) 22-4145616 Fax: (91) 22-4145614 E-mail: prk@fft.udct.ernet.in	Maharashtra	1982	Bacteria (35) Fungi (20) Yeasts (05) Algae (03)
12.	Delhi University Mycological Herbarium (DUM-WDCM 40) **Postal Address:** Department of Botany, University of Delhi, 110007	Delhi	1984	Fungi (200)
13.	Microbial Type Culture Collection & Gene Bank (MTCC-WDCM 773) **Postal Address:** IMTECH, Sector 39-A, Chandigarh 160036, U.T. **Telephone 1:** (91) 172-690562 **Telephone 2:** (91) 172-690004 **Fax 1:** (91) 172-690632 **Fax 2:** (91) 172-690585	Chandigarh, U.T.	1998	Bacteria (1124) Fungi (1245) Yeasts (575) Plasmids (85)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
14.	National Collection of Dairy Cultures (NCDC-WDCM 775) **Postal Address:** D. M. Division, National Dairy Research Institute, Karnal 132001, Haryana **Telephone 1:** (91) 184-2259008 **Telephone 2:** (91) 184-2259198 **Fax 1:** (91) 184-2250042 **E-mail 1:** rsndri@gmail.com **E-mail 2:** registrar.ndri@gmail.com	Haryana	1998	Bacteria (400) Fungi (15) Yeasts (20) Dairy Starter Cultures (25)
15.	Anaerobic Bacterial Resource Centre (BRC-WDCM 912) **Postal Address:** Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, Andhra Pradesh **Tel Phone:** (91) 40-23134502 **E-mail 1:** chvrsl@uohyd.ernet.in **E-mail 2:** r449@sify.com	Andhra Pradesh	2007	Bacteria (210)
16.	Microbial Culture Collection (MCC-WDCM 930) **Postal Address:** National Centre for Cell Science (NCCS) Second floor, Central tower, Sai Trinity building Pashan, Pune 411021, Maharashtra-411021, **Telephone:** (91) 20-2025329000 **Fax:** (91) 20-25692259 **E-mail:** mcc@nccs.res.in	Maharashtra	2008	Bacteria (149314) Fungi (15338)
17.	Visva-Bharati Culture Collection of Algae (VBCCA-WDCM 931) **Postal Address:** Department of Botany,Visva-Bharati University, Santiniketan 731235, West Bengal **Telephone:** (91) 947-4766362 **E-mail 1:** vbcca@visva-bharati.ac.in **E-mail 2:**jrath@visva-bharati.ac.in	West Bengal	2008	Algae (50)
18.	National Fungal Culture Collection of India (NFCCI-WDCM 932) **Postal Address:** MACS' Agharkar Research Institute, G.G. Agarakar Road, Pune 411004, Maharashtra **Telephone:** (91) 20-25325103 **Fax:** (91) 20-25651542 **E-mail 1:** sksingh@aripune.org **E-mail 2:** nfcci.ari@gmail.com	Maharashtra	2008	Fungi & Yeast (5000)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
19.	Whylabs Resource Centre for Microorganisms (AYL-WDCM 934) **Postal Address:** Hyderabad 500001, Andhra Pradesh **E-mail:**whyllabs@gmail.com **E-mail 2:** whyllabs@in.com	Andhra Pradesh	2008	–
20.	Goa University Fungus Culture Collection and Research Unit (GFCC-WDCM 946) **Postal Address:** Department of Botany, Goa University, Taleigao 403206, Goa **Telephone 1:** (91) 0832-6519349 **Telephone 2:** (91) 0832-9423889629 **Fax:** (91) 0832-2451184 **E-mail 1:** nandkamat@gmail.com **E-mail 2:** nkamat@unigoa.ac.in	Goa	2009	Bacteria (180) Fungi (400) Yeasts (200) Archaea (30)
21	NII Microbial Culture Collection (NIICC-WDCM 961) **Postal Address:** NIICC National Institute for Interdisciplinary Science and Technology (CSIR), Industrial Estate, Pappanamcode, Trivandrum 695019, Kerala **Telephone 1:** (91) 471-2515276 **Telephone 2:** (91) 471-2515279 **Fax 1:** (91) 471-2491712 **Fax 2:** (91) 471-2495949 **E-mail 1:** ashokpandey56@yahoo.co.in **E-mail 2:** binodkannur@yahoo.com	Kerala	2010	Bacteria (294) Fungi (78) Yeasts (07)
22.	North Maharashtra Microbial Culture Collection Centre (NMCC-WDCM 972) **Postal Address:** North Maharashtra University, PB. 80, Umavinagar, Jalgaon 425001, Maharashtra **Telephone:** (91) 257-2257421 **Fax:** (91) 257-2258403 **E-mail 1:** drsatishnmcc@gmail.com **E-mail 2:**satish.patil7@gmail.com	Maharashtra	2010	–*
23.	National Facility for Marine Cyanobacteria (BDU-WDCM 976) **Postal Address:** Bharathidasan University, PalkalaiPerur,	Tamil Nadu	2010	Marine Cyanobacteria (290)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
	Tiruchirappalli 620024, Tamil Nadu **Telephone:** (91) 431-2407084 **E-mail:** lumaprabakar@yahoo.com **E-mail:** dharmarpraba@yahoo.com			
24.	Entomopathogen (EntoPatho-WDCM 1013) **Postal Address:** Department of Microbiology and Biotechnology, M.S. University of Baroda, Vadodara 390002, Gujarat **Telephone:** (91) - 265-2794396 **E-mail:** ingle05@yahoo.co.in	Gujarat	2012	Bacteria (150)
25.	*Chroococcus minor* (CM-WDCM 1033) **Postal Address:** Department of Biotechnology, JJT University, Ward No. -19, Behind road ways depot, Sardar Shahar, Churu 331403, Rajasthan **Telephone 1:** (91) - 9950709933 **Telephone 2:** (91)-9413536100 **E-mail 1:** omprakashchahar@gmail.com **E-mail 2:** opchahar@yahoo.co.in	Rajasthan	2013	Cyanobacteria (09)
26.	*Bacillus thuringiensis* (BT-WDCM 1036) **Postal Address:** M.S. Swaminathan Research Foundation, third Cross Street, Taramani, Chennai 600113, Tamil Nadu **Telephone:** (91) - 9941 63 9941 **E-mail:** spshanthakumar@gmail.com	Tamil Nadu	2013	Bacteria (01)
27.	Bank A Bug (BAB-WDCM 1058) **Postal Address:** Gujarat Biodiversity Gene Bank, 9/11, Udhyog Bhavan, Sector 11, Gandhinagar 382011, Gujarat Telephone: (91) - 7923252165 **E-mail:** ssabtm@gujarat.gov.in **E-mail:** snehalbagatharia@hotmail.com	Gujarat	2014	Bacteria (4696) Fungi (1764) Archaea (47)
28.	National Agriculturally Important Microbial Culture Collection (NAIMCC-WDCM 1060)	Uttar Pradesh	2014	Bacteria (2423) Fungi (3828)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
	Postal Address: Director, ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Mau 275101, Uttar Pradesh **Telephone 1:** (91) - 547 2530158 **Telephone 2:** (91) - 547 2530080 **Fax 1:** (91) - 547 2530358 **Fax 2:** (91) - 547 2530381 **E-mail:** director.nbaim@icar.gov.in **E-mail:**nbaimicar@gmail.com			Cyanobacteria (246)
29.	Col. Sir R.N. Chopra, Microbial Resource Center Jammu (MRCJ-WDCM 1117) **Postal Address:** CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, Jammu & Kashmir **Telephone:** 91-191-2585006 **E-mail:** sundeepjaglan@iiim.ac.in	Jammu & Kashmir	2015	Bacteria (52) Fungi (723)
30.	National Culture Collection of Pathogenic Fungi (NCCPF-WDCM 1118) **Postal Address:** Postgraduate Institute of Medical Education and Research (PGIMER), Medical Microbiology, Research Block-A, Sector-12, Chandigarh 160012 **Telephone 1:**(91) - 1722755155 **Telephone 2:**(91) - 1722755173 **Fax 1:**(91) - 172244401 **Fax 2:**2757173 **E-mail:**arunaloke@hotmail.com **E-mail:**anupkg3@gmail.com	Chandigarh, U.T.	2015	Fungi (4341)
31.	Global Collection of Cyanobacteria (GCC-WDCM 1165) **Postal Address:** Varanasi 221005, Uttar Pradesh **Telephone:** (91) - 7755830009 **E-mail:** sps.bhu@gmail.com	Uttar Pradesh	2017	Cyanobacteria (20)
32.	M.S. Swaminathan Research Foundation Culture Collection (MSSRFCC-WDCM 1220) **Postal Address:** M.S. Swaminathan Research Foundation, third Cross Road, Taramani Institutional area, Chennai 600113, Tamil Nadu **Telephone 1:** (91) - 04422541229	Tamil Nadu	2019	Bacteria (20500)

(continued)

Table 5.1 (continued)

S. no.	Name of collection	State	Registered with WFCC	Holdings
	Telephone 2: (91) -9840253789 **E-mail 1:** prabavathyvr@mssrf.res.in **E-mail 2:** jegan.sekar@mssrf.res.in			

Source: WFCC (n.d.) (http://www.wfcc.info/#)
*data not available
Note: Holdings/number of organisms in most of the culture collections are not updated at WFCC website, and hence number provided in table may vary from actual numbers. Similarly, date of actual establishment of collection may differ from date of registration at WFCC-WDCM

information and support teaching, research and making live specimens available for experiencing/viewing by students and teachers. AMH now houses >40,000 fungi and lichen specimens.

Later, in recognition of the authoritative work on mycology at MACS-Agharkar Research Institute, Pune, an unique National Facility for Culture Collection of Fungi was established in 2008 with the financial support by DST, Govt. of India. NFCCI principally acts as a service collection and performs basic functions as acquisition, verification, preservation and maintenance, deposit and accession, and distribution of authentic fungal strains. This well-equipped facility offers various knowledge-based services to academia, research institutions and industry. National Fungal Culture Collection of India is an exclusive repository of fungi. This repository was established having affiliation with World Federation for Culture Collections (WFCC), registered with World Data Centre of Microorganisms (WDCM 932) holding over 5000 fungal strains of different groups of indigenous fungi, which can be searched in online catalogue.

Staff at NFCCI is well acquainted with recent developments in systematics and identification of fungi. Mycology has seen dynamic changes and fungal taxonomy is in a state of flux due to recent changes in fungal code (e.g. one fungus = one name) as it is being interpreted differently. Similarly advances in sequence-based analysis of fungi are getting global prominence. In order to become competitive, NFCCI offers quality services to academia, research institutions and industry in morpho-based and molecular identification of fungi. Authenticated and well-characterized fungal strains/isolates are also supplied to evergrowing demands by the industry especially mycopesticides, biofertilizer, pharmaceutical, food and other industries and to agriculture. Capacity building training in thrust area of mycology is one of the most important mandates of the NFCCI. In the last one-decade, NFCCI created significant awareness about ex situ conservation strategies to be undertaken and trained manpower for undertaking tasks of conservation and sustainable utilization

of mycological heritage of India. NFCCI regularly organizes courses since 2011 like national workshops, certificate courses and summer courses. In the capacity building exercise, the focus is on learning the basics, viz. selective isolation, identification, taxonomy and conservation and applications of fungi of diverse taxonomic groups. Initially this type of course was introduced for the benefit of postgraduate students who aspire to pursue a career in fungal systematics, plant pathology and quarantine, and industrial mycology, microbiology, biotechnology, etc. Besides, individual training has been well recognized for faculty, post-docs, research and PG students. This training programme is open round the year. NFCCI also supply authentic fungal strains and support R&D programme of various industries. More than 700 various academic and research institutions and more than 100 industries across 26 states and 6 Union Territories (UT) in India were benefited. Besides, about 250 research personnel have been trained from India and Nepal as part of human resource development.

(Source: http://nfcci.aripune.org/).

NAIMCC-NBAIM: This collection was established by the Indian Council of Agricultural Research (ICAR) in 2001. Initially, the bureau started functioning at the Old NBPGR Building, New Delhi, and then shifted to Kusmaur, Mau Nath Bhanjan, Uttar Pradesh, in 2004. The NAIMCC is holding more than 5000 microbial accessions isolated from different parts of the country.

The basic goal of the bureau is to promote and coordinate systematic and scientific research in the area of agriculturally important microorganisms (AIMs) in order to improve the agricultural productivity. It functions as (a) registration authority for elite microbial germplasm, (b) national microbial genomic resource repository, (c) ISO 9001:2008 certified institute and (d) partner of National Agricultural Bioinformatics Grid (NABG). Overall activities of this culture collection include:

(a) Exploration and collection of agriculturally important microorganisms (AIMs).
(b) Identification, characterization and documentation of AIMs.
(c) Conservation, maintenance and utilization of AIMs.
(d) Surveillance of indigenous/exotic AIMs.
(e) Microbial biodiversity and systematics.
(f) Human resource development (HRD).

Source: http://nbaim.org.in/pages/services-culture-collectionnaimccculture-collectionnaimcc

NCIM: Established as national facility for deposit of microbial culture and associated services in 1951 with a focus on following services related to industrially important microbial strains: (a) The NCIM is dedicated to isolation, preservation and distribution of authentic industrially important microbial strains, (b) All biological materials accepted in the NCIM collection are subject to extensive quality control, phenotypic and molecular characterization, (c) It provides an extensive documentation and detailed identification information about biological materials supplied, (d) Only non-pathogenic cultures are maintained in the collection and (e) It has

online catalogue of microbial strains. Collection currently is holding about 5000 accessions including Bacteria, Fungi, Actinobacteria, Yeasts and Algae.

Source: https://www.ncl-india.org/files/NCIM/Default.aspx

NCMR (MCC): The National Centre for Microbial Resource (NCMR) is a national facility funded by the Department of Biotechnology (DBT), Government of India. NCMR is the part of National Centre for Cell Science, Pune, India, and is recognized by the World Intellectual Property Organization (WIPO), Geneva, Switzerland, as an International Depository Authority (IDA) on 9th April 2011. NCMR's overall activities include: (a) to act as a national depository, (b) supply of authentic microbial cultures, (c) provide related services to the scientific community, (d) deposit of microorganisms under the Budapest Treaty for patent purposes and (e) actively work on microbial and environmental aspects. This collection is currently holding about 149,314 bacteria and 15,338 fungi. For details of functions and activities Sharma and Shouche (2014) may be consulted.

5.5 Conclusions

Microbes comprise important domains of life like bacteria, archaea and eukarya as well as the viruses. Despite their importance, <0.1% of the extant microbial species only have been characterized, preserved and utilized for various purposes (Alain and Querellou 2009). Besides industrial applications, microbes are widely used in taxonomic studies as standard/reference strains, for production of metabolic products, for diagnostic purposes, or in biological transformation (Martin 1964). Currently almost every branch of science utilizes the processes and products of microbes. Now it is well understood that *ex situ* conservation is important for ready availability of source of living cells for scientific scrutiny of basic and applied nature. Since microbes isolated from environmental samples cannot always be recovered, they need protection from various factors like climate change, habitat destruction, etc. (Sharma et al. 2018). However, declining interest in taxonomy and systematics is the matter of great concern. As culture collection activity involves handling of the microbial cultures isolated from diverse environments, there is a great concern regarding risks and biohazards caused, mainly due to human factors and by microbes, which are categorized into 4 risk/hazard classes (H1, H2, H3 and H4) (Gams et al. 1998). By following essential Code of Practice (CoP) along with Good Laboratory Practices, we can minimize and overcome the genuine problems. Indian culture collections must re-frame the strategic plan and build up capabilities by enhancing expertise and modern infrastructure to attract the bioindustry for utilizing the services of culture collections. It will also help the collections to generate revenue for the survival and sustenance.

5.6 Future Perspectives

Since fundamental research are considered backbone, 'Taxonomy' as a science has seen dynamic change in the recent past and significantly supported the studies on biodiversity, proper authentication of the microbial strain is the primary requirement for academic interest, industrial application and also for protecting Intellectual Property Rights (IPR) on the processes and products developed through research and development. Applications of various methods play very important roles in preserving and maintaining different groups of microbes in culture collections (Onions and Smith 1984; Kirshop and Doyale 1991; Salafsky et al. 2002; Gams 2002; Deshmukh 2003).

Availability of pure/axenic culture, modern laboratory infrastructure and expertise in the field and trained manpower are the strategic requirement to fulfil the scientific requirement and increasing demands of the bioindustry in order to develop and commercialize bio-products using traditional knowledge. It is very essential for the culture collections to accept and use the OECD best practice guidelines for the safe handling of microbes. Besides protection of bioresources from variable climatic conditions (climate changes), habitat destruction, pollution etc., the other challenging tasks must be addressed.

Due to geographic enormity, research fraternity of India demands more repositories at regional level including patent depositories, which will play a key role in the long-term maintenance of patented microbial strains as industries need these for a variety of purposes. Despite 32 registered collections in India, currently only 5-6 collections are active and catering the demand. However, networking and coordination of microbial culture collections like MTCC, NFCCI, ITCC, NBAIM, NCMR (MCC) and NCIM having specific expertise are essential for providing active support to evergrowing demand of industry and academia in country. Compilation of microbial red data book of India that can reflect the status of endangered/threatened/extinct microbial taxa is important task that needs to be undertaken for their protection.

A clear-cut policy for microbial resource management in the country is essential. Suitable policy under the existing framework of Indian Biodiversity Rules (see NBA n.d.) and flagship programme of CBD would be helpful to the survival of culture collections in India. It would be easy for these collections to develop working model to generate bio-economy to self-sustenance and provide support to developing intellectual property rights of processes and products. The role of culture collections in the era of industrial biotechnology is highly significant in supplying authenticated microbial strains for comparative research and product development. However, microbial centres can also partially contribute to some of the Millennium Development Goals of United Nations by re-defining their activities. Bio-safety, ethical access and use of microorganisms are also crucial to Indian culture collections/bioresource centres in changing scenario at global level.

In a nutshell, the role of culture collections can be re-defined in new era as 'back-up of bioresources' for any industry which works on microbes or microbial products. Detailed knowledge of cultures including physiology, genomics and proteomics will

be useful for characterization of cultures/strains for different applications. As such collection can serve as reservoir of novel/interesting strains producing novel biochemicals and other bioactive metabolites which can be used for potential application in different sectors like agriculture, health and industries. Besides, collections also need to develop business models for their own survival and providing R&D support on a sustained basis.

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Part II

Symbiotic and Pathogenic Fungi

History and Development of Lichen Research in India

6

Sanjeeva Nayaka and Dalip Kumar Upreti

Abstract

The lichens of India were initially studied by European lichenologists starting from the era of Carl Linnaeus. Although Indians started exploring lichens during the late twenties of last century, they were identified by Europeans. It is now (about seven decades) that D.D. Awasthi successfully established school of lichenology in India and laid a strong foundation for the subject in the country. Later, researchers at CSIR-National Botanical Research Institute (NBRI), Lucknow, played a crucial role in introducing various aspects of lichenology such as biomonitoring, biodeterioration and bioprospecting together with both classical and modern taxonomy. CSIR-NBRI, in close association with Indian Lichenological Society, is instrumental in popularizing lichenology in the country and today over 200 researchers are practising the subject all over the country. Agarkar Research Institute, Pune, and Botanical Survey of India are the other two major organizations that contributed significantly for exploring the lichen wealth of the country and critically revising several important taxa. At present, India is represented by approx. 2900 species; north-east India (including eastern Himalaya), Western Himalaya, Western Ghats and Andaman Nicobar Islands are being considered as hotspots of lichen diversity. The lichens are used in air pollution and climate change studies; for biological activities such as antimicrobials and antioxidants to lifestyle diseases; for biodeterioration and in forestry as indicators. In the recent years Indian lichens and endolichenic fungi are widely studied for their medicinal potential and in developing antimicrobial nanoparticles. Indian researchers are publishing more than 75 research papers

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every year, and lichenology is no more a neglected branch of science in the country.

Keywords

Lichens · Fungi · Hotspots · Mycobiont · Phycobiont · Lichenologist · Bioactivities · Biomonitoring

6.1 Introduction

The lichens are unique group of organisms. Although they are made of two organisms (fungus, alga or cyanobacteria), they behave as single organism by undergoing dramatic morphological and physiological changes. From the day the dual nature was discovered, the lichens are defined variously and at least ten definitions are available so far (Hawksworth 1988; Hawksworth and Honegger 1994). Currently lichens are defined as 'a self-sustaining ecosystem formed by the interaction of an exhabitant fungus and an extracellular arrangement of one or more photosynthetic partners and an indeterminate number of other microscopic organisms' (Hawksworth and Grube 2020). Over the years, there are different estimates of total number of lichens occurring in the world. Zahlbruckner (1922–1940) estimated it to be 22,000 species, Hawksworth (1995)—13,500 species, Sipman and Aptroot (2001)—20,000 species, Feuerer and Hawksworth (2007)—17,322 species and lately according to Lücking et al. (2017) 19,409 species under 1002 Genera, 119 Families, and 40 Orders. In conclusion, it can be said that total number of lichens known so far in the world is around 20,000 species. The tropical rain forests in the world have the highest species richness of lichens in the world and 500–600 or more species can be found just within a km^2 (Lücking et al. 2009). There are more lichen species than tree or bird species in any given area of tropical jungle. Unfortunately, 50% of the tropical forests are unexplored for lichens (Aptroot and Sipman 1997).

6.1.1 Brief History of Development of Lichenology in the World

The term 'lichen' (*lie, ken*) was coined by Theophrastus (300 BC), the Greek Father of Botany (Fig. 6.1a). He used the term to indicate some outgrowths on the bark of olive trees and it is uncertain what exactly he meant by lichens. The Ancient Greek authors used the term to refer several organisms including lichens, mosses, liverworts, fungi, seaweeds and even corals. The first person to distinguish lichens by the name 'lichen' was French botanist Joseph Pitton de Tournefort (1656–1708), but he too included some thalloid liverworts and excluded many lichens. He treated lichens as separate taxonomic group under plants. Johnn Jacob Dillenius (1687–1747) reorganized the lichens as lichens as accepted in the modern concept. Carl Linnaeus (1707–1778) (Fig. 6.1b), who is considered as 'Father of Modern

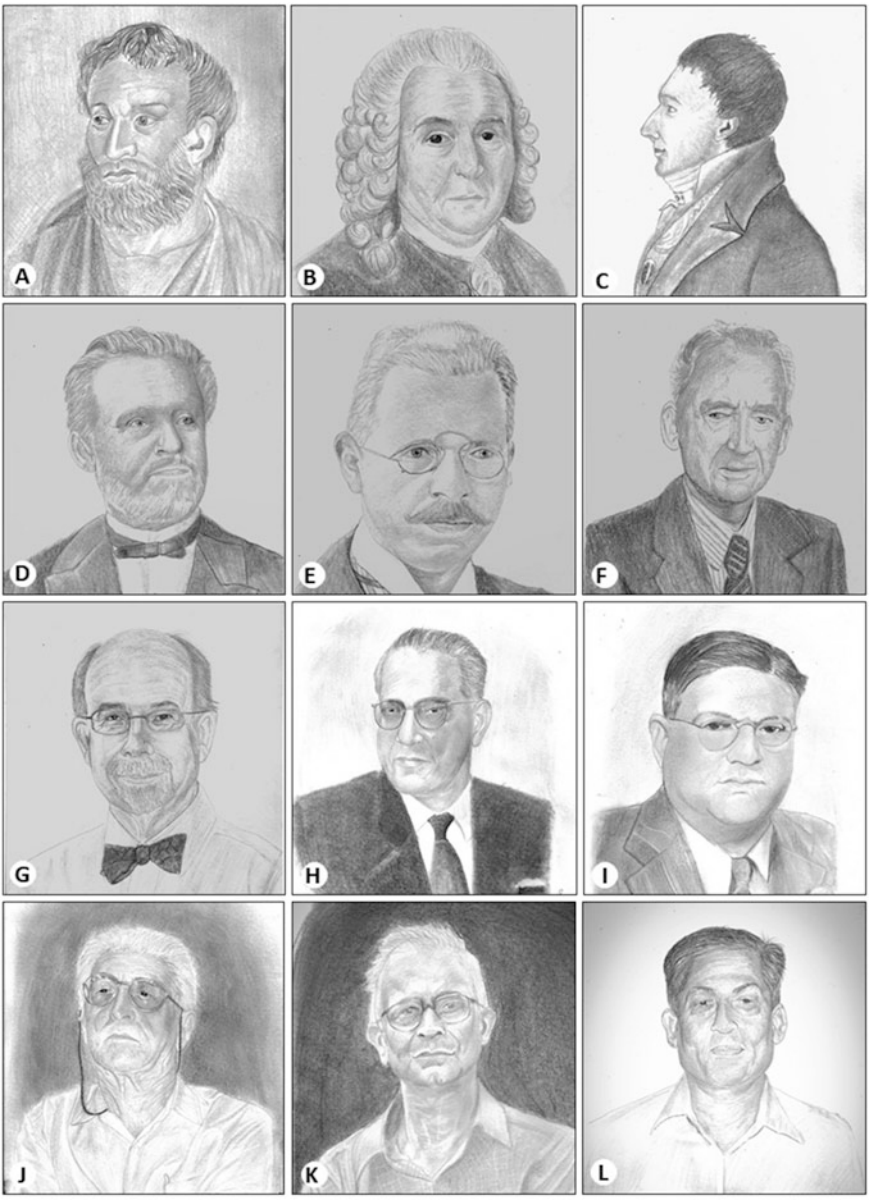

Fig. 6.1 Some important personalities who contributed to the development of lichenology in the world and in India. (**a**) Theophrastus, (**b**) Carl Linnaeus, (**c**) Eric Acharius, (**d**) Simon Schwendener, (**e**) Alexander Zahlbruckner, (**f**) Rolf Santesson, (**g**) David L. Hawksworth, (**h**) T.R. Sheshadri, (**i**) K. Biswas, (**j**) D.D. Awasthi, (**k**) Ajay Singh and (**l**) P.G. Patwardhan

Taxonomy', described 80 species under the single genus *Lichen* within 24th Class Cryptogamie in his book 'Species Plantarum'. His student Eric Acharius (1758–1819) (Fig. 6.1c), a Swedish Botanist, classified over 3300 species of lichens under 40 different genera based on morphology of the thallus and fruiting bodies. Acharius also coined several new terminologies referring to special characters present in lichens, and he is honoured as the 'Father of Lichenology'. As the usage of microscope increased in botany, the presence of algae (blue-green algae, *Nostoc*) was observed in gelatinous lichens by H. Anton de Bary (1831–1888) and his assistant C.W. Naegeli (1817–1891). However, it is Swiss botanist Simon Schwendener (1829–1919) (Fig. 6.1d) who developed the theory of dual nature of lichens and presented at Swiss Naturforscher Gesellschaft in Rheinfelden in 1867. Thereafter the debate arose on two issues: (1) What is the relation between fungi and algae? and (2) Where to place lichens? Under fungi, algae or to treat them as separate a taxonomic unit? In lichens the relation between fungus and the alga can be mutualistic, endosaprophytism, helotism or controlled parasitism. Therefore, it is ideal to call the relationship as just symbiosis (living together).

Even before the discovery of dual nature of lichens by Schwendener, there was a strong feeling among some researchers that lichens are related to fungi. However, they prefer to treat lichens as separate taxonomic group and maintain their autonomous identity. Scottish botanist Robert Morrison (1620–1683) from Aberdeen was first to call lichens as 'Muscofungi'. French botanist Michel Adanson (1727–1806) placed lichens close to fungi. Acharius, although worked extensively on lichens, treated them as a separate taxonomic group. He had strange ideas as to where lichens belonged in the system; he compared them with sponges which could dry out completely to be revived when wetted. The dual nature of lichens proposed by Schwendener was rejected by most of the leading lichenologists of the time especially by influential Finnish William Nylander (1822–1899). Based on Schwendener, another Finish lichenologist Edvard Vainio (1853–1929) constructed a classification where the lichens are regarded as parallel taxon to fungi, which was heavily criticized. Austrian lichenologist Alexander Zahlbruckner (1860–1938) (Fig. 6.1e) who considered lichens as unrelated to fungi proposed a classification system for lichens in Engler and Prantl's great work 'Das Pflanzenreich' (1900–1953). He also published a series of index to all lichen names from 1753 to 1940 which are popularly known as *Catalogus Lichenum Universalis* (1922–1940). A decade after Zahlbruckner era, Swedish lichenologist Santesson (1952) (Fig. 6.1f) successfully arranged foliicolous taxa that he studied within classification of fungi. It was followed or improvised by several lichenologists including Poelt (1973) and Henssen and Jahns (1974). Later, with the advent of better techniques such as scanning electron microscopy and DNA-based analysis, a clearer understanding of the lichens emerged, and considered as part of the fungi. O.E. Eriksson, J. Hafellner, D.L. Hawksworth (Fig. 6.1g), T.H. Lumbsch, F. Lutzoni, G. Rambold, A. Tehler and D. Triebel are some of the important contributors to modern systematics of lichens. The development of lichenology as a whole in the world is well documented in the 100th volume of Bibliotheca Lichenologica (Kärnefelt 2009), and it is beyond the scope of the present communication. The recent developments in lichenology

certainly declare that lichenology is not a narrow scientific field any more as thought by non-lichenologists.

6.2 Studies on Indian Lichens During Pre-independence Period

The studies on Indian lichens can be clearly categorized into pre- and post-independence era. Detailed contributions of the various workers to Indian lichenology during pre-independence period are already mentioned by Awasthi (1965, 2000) and Singh (1964), and it will be repetitive to mention here. However, for new readers, some of the historical events are mentioned here. Like any other group of organisms in India, lichens were also studied by Europeans during the British rule. Linnaeus (1753) was the first person to record lichens from India. He mentioned *Lichen fusiformis* L. (= *Roccella fusciformis* (L.) DC) from India in his book 'Species Plantarum'. According to Awasthi (1965), this species does not occur in India and correct identity should be *Roccella montagnei* Bél. Acharius (1810, 1814) described a total of four species (*Alectoria arabum* Ach. (= *Ramalina arabum* (Dill. ex Ach.) Meyen &Flot.), *Collema rottleri* Ach. (probably a Pannariaceae, type material is small with biotorine apothecia), *Isidium bassiae* Ach. (= *Oxneriopsis bassiae* (Willd. ex Ach.) S.Y. Kondr.), *Porina subcutanea* Ach. (actually *Pertusaria leioplaca* (Ach.) DC) from India in his classical works 'Lichenographa Universalis' and 'Synopsis Methodica Lichenum'. It is not clear the place of collection and who passed on the lichen samples to Lennaeus and Acharius from India. But it can be noted that during colonial period, lichen collectors were mostly non-lichenologists, but botanists (N. Wallich, J.D. Hooker, S. Kurz), naturalists, army personnel, doctors (D.D. Cunningham) and Jesuit missionaries who served British government. These specimens were identified by European lichenologists such as Babington, Leighton, Nylander, Stirton and Taylor. First record of lichen collection from India comes from Bélanger (1838) who reported 40 taxa from Pondicherry and Coramandel coast. Peroltet was the first person to collect lichens from Western Ghats (Nilgiri Hills) which are enumerated by Montagne (1842). A large number of Indian lichen specimens preserved at European herbaria were identified by Swiss lichenologist J. Müller Argoviensis (1874–1891). Chopra, Chaudhuri, Kashyup and Quraishi are the pioneer Indians to study the lichens of the country in the late twenties (1928) and early thirties (1931–1934) of the twentieth century. They collected lichens from Himalaya (Mussoorie, Darjeeling, Sikkim) which were identified by expert lichenologist such as A.L. Smith and Zahlbruckner.

When it comes to contribution of Indian to country's lichenology, contributions of T.R. Sheshadri (Fig. 6.1h) and K. Biswas (Fig. 6.1i) are usually forgotten. Sheshadri was a chemist rather than a lichen taxonomist, but did not restrict his studies only to lichens but also included medicinal plants for providing their chemical nature. In 1930, Sheshadri returned to India after completing his doctorate from University of Manchester and served as faculty in various organizations before settling as professor at the University of Delhi (Krishnaswamy 2004). During this

period, himself and his students studied the chemicals of several lichens including *Parmelia abessinica* Ny, *Parmelia tinctorum* Despr. (=*Parmotrema tinctorum* (Despr.) Hale) and *Usnea japonica* Vain. (Seshadri and Subramanian 1949a, b). Among them, Awasthi (1965) included few lichens of taxonomic significance in the 'Catalogue of the lichens from India'. Biswas was superintendent at Royal Botanic Garden, Calcutta, during the year 1945 (now known as Acharya Jagadish Chandra Bose Indian Botanic Garden, situated in Howrah, and governed by Botanical Survey of India). For the first time, Biswas presented an overview of Indian lichen flora and mentioned their commercial utilization (Biswas 1948). Later, along with D.D. Awasthi, he published two abstracts which discussed the distribution of over 700 lichen species in India and 3100 lichen specimens housed at Royal Botanic Garden, Calcutta (Biswas and Awasthi 1948a, b).

6.3 Studies on Indian Lichens During Post-independence Period

6.3.1 Establishment of School of Lichenology at Lucknow University

During the year 1947, D.D. Awasthi (Fig. 6.1j) while working as trainee at Botanical Survey of India, his association with Biswas inspired him to take up lichen studies. His earlier collections from western Himalaya were identified by V. Räsänen of Finland. A fully functional Lichenology Laboratory started in India only after Awasthi became a faculty member at the Department of Botany, Lucknow University, in the year 1952. Awasthi was active in lichenology for seven long decades till his death. His visit to University of Colorado, USA, as Fulbright Scholar during his early career came as a boon to Indian Lichenology. He was trained by well-known lichenologist of that time William A. Weber. During his foreign trip, Awasthi also visited most of the European herbaria for studying Indian specimens and collected exsiccate specimens and valuable literature. He established lichen herbarium at Lucknow University, travelled throughout India for collection of lichens, published about 90 research papers and seven books, described more than 75 species and guided eight students for Ph.D. He was honoured with Fellowship of the Indian Academy of Sciences (1978), Indian National Science Academy (1984), Professor P. Maheshwari Lecture Award of INSA (1991) and Acharius Medal (1992). He was well known in the world as Father of Indian Lichenology (Upreti 2012). After superannuation of Awasthi from Lucknow University in 1982, the lichen herbarium of LWU and his personal collections (AWAS) were transferred to LWG (CSIR-NBRI, Lucknow) on permanent loan basis. At present, no one is perusing lichen research at Lucknow University.

6.4 Emergence of Lichen Research at CSIR-National Botanical Research Institute, Lucknow

Ajay Singh (Fig. 6.1k) initiated lichen research at CSIR-NBRI (then called National Botanical Garden) during the 1960s. He joined this institute in 1958 as Junior Scientific Assistant and initially worked on higher plants but later switched over to the field of lichenology. To create a base for lichenological research, he gathered literature on Indian lichens scattered all over the world and complied the information in a book 'Lichens of India' (Singh 1964) with a list of approximately 1300 species. He established lichen herbarium at LWG and also excellent library of lichen literature. He mostly worked on pyrenocarpous lichens of India, discovered 42 new species and two new varieties, and published three books and about 50 research papers. It is notable that Singh completed his Ph.D. under the guidance of Awasthi. After his superannuation from CSIR-NBRI in 1982, Singh joined National Research Laboratory for Conservation of Cultural Property (NRLC), Lucknow, and continued studies on lichens biodeteriorating monuments and historical buildings of the country (Nayaka et al. 2019).

D.K. Upreti is associated with CSIR-NBRI since 1981 after completion of his Ph. D. from Lucknow University under the supervision of Awasthi. He joined as scientist at this institute in the year 1988 and served in various capacities till his superannuation in 2018. He has made outstanding contribution in the field of lichen systematics, biomonitoring, biodeterioration and bioprospecting. He discovered about hundred taxa of lichens as new to science and added 250 species as new records to the Indian lichen biota. He published about 350 research papers and 10 books and guided 30 students for their Ph.D. He extensively travelled all over the country for the exploration of lichens and also participated in 11th Indian Antarctic Expedition. Upreti was honoured with several awards and important among them are Fellowship of Indian National Academy of Science (FNA), National Academy of Science (FNASc), Prof. E.K. Janaki Amal National Award for 2015 by Ministry of Environment, Forest and Climate Change, New Delhi, and Excellence in Science and Technology Award for 2019 from the state of Uttarakhand. Currently, Upreti is working as CSIR Emeritus Scientist at CSIR-NBRI.

The Lichenology Laboratory of CSIR-NBRI is currently headed by S. Nayaka who joined the institute as Research Scholar in 2000 and as Scientist in 2001. Nayaka completed his Ph.D. under the supervision of Upreti. Although his primary interest is taxonomy, he contributed to various aspects of lichenology. The popularization of lichen research in the country is one the focus areas of Nayaka. So far, he has published about 180 research papers and three books, described 25 new species and added 80 species as new to India.

6.5 Lichen Research at Agarkar Research Institute, Pune

The lichen research at Agarkar Research Institute (ARI), Pune, was initiated by P.G. Patwardhan (Fig. 6.1l) during the early 1970s (then called Maharashtra Association for the Cultivation of Science). Patwardhan did his Ph.D. in mycology under the supervision of M.N. Kamat at ARI. He was benefitted by his association with M.E. Hale Jr. under a U.S. Project. Himself and his students extensively surveyed Western Ghats and Andaman-Nicobar Islands for lichens. Along with larger lichens, the focus of Patwardhan's group was mostly on microlichens. He established a large collection of lichens in the Agharkar Mycological Herbarium (AMH). During his career, he discovered 170 new species and published 60 research articles. After his superannuation in 1995, Patwardhan could not contribute much to lichenology as he lost his vision due to brain tumour (Makhija 2006).

The lichen research at ARI was continued with the appointment of Urmila Makhija as scientist. She was a student of Patwardhan and served the area of lichenology for more than 30 years till her superannuation in 2010. She carried forward the studies on microlichens especially belonging to Arthoniales, Graphidaceous and pyrenocarpous group. She explored Andaman-Nicobar, Lakshadweep Islands and tracts of Western Ghats with special emphasis on Maharashtra. She has described around 200 species as new to science and many more new additions to the country. She has published over hundred research papers and three catalogues.

Bharti Sharma, one of the students of Makhija, continued the tradition of studying microlichens at ARI since 1988. She extensively studied graphidaceaous lichens with special emphasis on South India. She authored about 45 research papers and one book. At present, she is continuing her studies on lichens, but her more focus is on non-lichen-forming fungi.

The lichenological research at ARI was diversified by B.C Behera who joined this institute in 1997. With his expertise in biochemistry and physiology, he established mycobiont and lichen culture for the first time in India. He is successful in bioprospecting lichens for antimicrobial, antioxidant, probiotic, cardiovascular protective and other biological activities. He has about 25 publications to his credit on the applied aspects of lichenology. Lately in 2006, Subhash B Gaikwad joined the mycology group of ARI as Technical Assistant. Although he authored a few papers on lichens, his significant contribution to lichenology is yet to come.

6.6 Initiation of Lichen Research at Botanical Survey of India

It should be mentioned here that Botanical Survey of India (BSI) is spread all over India with 16 regional centres and units and its headquarters is located at Kolkata. After initial work by Biswas and Awasthi from BSI, lichen research was restarted by Dharne and Roychowdhury (1967). However, there were only a few publications by these two researchers. Later, K.P. Singh, senior-most student of Awasthi, joined this institute at its Eastern Regional Centre, Shillong, in 1975 and extensively studied the

lichens of north-east India. He established one of the richest lichen herbaria at BSI, Shillong, comprising over 21,000 specimens and also at Central Regional Center, Allahabad, with 10,500 specimens. Singh discovered two new genera, over 45 new species of lichens new to science and several new records of lichens. He has contributed more than 150 research papers and five books on lichens, besides guiding a few students for their Ph.D. He was honoured with several awards and medals, important among them are Prof. E. A. Janaki Ammal National Award in Plant Taxonomy (2016) by Ministry of Environment, Forest and Climate Change, New Delhi, and Lifetime Achievement award by Indian Lichenological Society, Lucknow.

Another important contributor from BSI is G.P. Sinha who started his career in lichenology during 1984 as Junior Research Fellow in Flora of India project at Eastern Regional Centre, Shillong. He did his Ph.D. under the supervision of K.P. Singh on lichen flora of Nagaland. He served as scientist at BSI's Sikkim Himalaya Regional Centre, Gangtok, and currently Head of Office at Central Regional Centre, Allahabad. During his career Sinha has undertaken extensive exploration in north-east and other parts of India. He has discovered 40 new species and reported about 100 new records to India. He also contributed about 160 research papers and four books. Sinha is now at the verge of his superannuation.

At BSI's Andaman and Nicobar Regional Centre, Portblair, T.A.M. Jagadeesh Ram is undertaking lichenological research. He started his research career at BSI's Sikkim Himalayan Regional Centre, Gangtok, under All India Coordinated Project on Taxonomy. He did his Ph.D. under the supervision of K.P. Singh. After studying the lichens of Sundarbans Biosphere Reserve and Darjeeling Himalaya, at present he is focusing his studies on lichens of Andaman-Nicobar Islands.

6.7 Other Centres of Lichen Research in India

Meanwhile a few research organizations in India initiated lichen research; however, they either did not continue or changed the focus of the study. One of the students of Madhav Gadgil at the Centre for Ecological Sciences, Indian Institute of Sciences (CES, IISc), Bangalore, H.R. Negi carried out ecological studies on lichens in 1996. He also incorporated about 500 lichen specimens to herbarium JCB housed at CES, IISc. The lichen research at CES, IISc, stopped after completion of Negi's Ph.D. Nayaka was inspired by Negi to carry out lichenological research during his association with Gadgil. Negi could have continued working on lichens after his appointment as scientist at CSIR—Institute of Himalayan Bioresource Technology, Palampur, but he died very early.

Muktesh Kumar established lichen research laboratory at Kerala Forest Research Institute (KFRI), Peechi, during 1996 and guided S. Sequiera for his Ph.D. The herbarium at KFRI has the lichen specimens collected from Kerala region. Although lichen research at KFRI was discontinued after the superannuation of Kumar, his student is still pursuing it at Maharaja's College, Kochi. Almost at the same time, G.N. Hariharan joined MS Swaminathan Research Foundation, Chennai, and

established lichen research centre. Hariharan had completed his Ph.D. on lichens under the supervision of K.V. Krishnamurthy of Bharathidasan University, Tiruchirappalli, and he was actually trained by Upreti. At present, the focus of research of Hariharan includes bioprospecting lichens for novel molecules.

6.8 Role of CSIR-NBRI and Indian Lichenological Society (ILS) in Popularizing Lichen Research in India

CSIR-NBRI played a major role in popularizing lichenology in the country since the period of Upreti. He guided 30 candidates for their Ph.D. in lichens, of them at least three (P.K. Divakar, S. Nayaka, Y. Joshi) are currently well-established lichenologists while a few changed their area of research due to unavoidable circumstances and a few are yet to find suitable placement. More than 45 research projects have been handled at CSIR-NBRI sponsored by all major funding agencies of Government of India. Apart from national collaboration, institute has international collaboration with organization such as Field Museum, Chicago; M. H. Kholodny Institute of Botany, Ukraine; and Universidad Complutense de Madrid, Spain. Upreti along with Nayaka from CSIR-NBRI trained over 100 researchers from all over the country for identifying lichens. They established collaboration and also rendered technical assistance in lichen study to over 60 academic organizations (Table 6.1). Several of these organizations are still continuing to work on lichens and producing next-generation lichenologists. Both have written popular articles in Hindi, Kannada and Urdu; delivered more than 100 talks on lichens; organized about 15 workshops and served as resource persons in several events. Meanwhile scientists and research scholars of CSIR-NBRI established Indian Lichenological Society (ILS) and gave a common platform to all lichen researchers of the country. During the span of 6 years, the society has conducted two national conferences and four workshops. The society has about 200 life members from India and abroad. The ILS not only encourages lichen researchers of the country but also others working on cryptogams. ILS publishes a biannual journal Cryptogam Biodiversity and Assessment.

6.9 Trends in Indian Lichen Research

Indian lichen research primarily focused on taxonomic allied aspects including floristic and revisionary studies. Much diversification in lichen research appeared only after 1990s leading to studies on lichens growing on monuments and historical buildings (biodeterioration), ethnic usage of lichens (ethnolichenology), air pollution and climate change (biomonitoring) and utilization of lichens for biological activities (bioprospecting).

Table 6.1 Technical assistance provided by CSIR-NBRI in their effort to popularize lichenology in India

S. No.	States Name/Organization	Contact person	Current status
Andhra Pradesh			
1.	Yogi Vemana University, Kadapa	Dr. A. M. Reddy	Active
Arunachal Pradesh			
2.	North Eastern Regional Institute of Science and Technology (NERIST), Nirjuli	Dr. Ashish Paul	Active
Assam			
3.	Assam University, Silchar	Prof. Jayashree Rout	Active
4.	Nowgong College, Nagaon	Dr. Farishta Yasmin	Active
5.	Tezpur University, Tezpur	Dr. Raza Hoque	Inactive
Chandigarh			
6.	Post Graduate Government College for Girls, Chandigarh	Dr. Kiran Rana	Inactive
7.	Panjab University, Chandigarh	Prof. M.L. Sharma	Inactive
Chhattisgarh			
8.	Guru Ghasidas Vishwavidyalaya, Bilaspur	Dr. Sushil K Shahi	Active
Delhi			
9.	Ambedkar University, New Delhi	Dr. Pulak Das	Active
10.	TERI School of Advanced Studies, New Delhi	Dr. Sudipto Chatterjee	Active
Goa			
11.	Carmel College for Women, Nuvem	Dr. Sulabha Pathak	Inactive
12.	Goa University, Taleigao	Dr. M.K. Janarthanam	Active
13.	National Centre for Polar and Ocean Research (NCPOR), Vasco da Gama	Dr. S.M. Singh	Inactive
Gujarat			
14.	Gujarat Institute of Desert Ecology (GUIDE), Bhuj	Dr. Logesh A.R.	Inactive
15.	Smt. S.M. Panchal Science College, Talod	Dr. Bhaskar Punjani	Active
16.	Space Applications Centre (SAC-ISRO), Ahmedabad	Dr. C.P. Singh	Active
Himachal Pradesh			
17.	Career Point University, Hamirpur	Dr. Hem Chander	Active
18.	CSIR-Institute of Himalayan Bioresource Technology, Palmpur	Dr. Amit Kumar	Active
Jammu and Kashmir			
19.	University of Jammu, Jammu	Prof. Namrata Joshi; Prof. Yashpal Sharma	Active
20.	University of Kashmir, Srinagar	Prof. A. Reshi Zafar; Dr. A.A. Khuroo; Dr. Manzoor Ul Haq	Active

(continued)

Table 6.1 (continued)

S. No.	States Name/Organization	Contact person	Current status
Karnataka			
21.	Davangere University, Davangere	Dr. Gayatri Nagaraj	Active
22.	Jain College and University, Bangalore	Dr. B.E. Ravishankar	Inactive
23.	Kuvempu University, Shankargatta	Dr. Y.L. Krishnamurthy	Active
24.	Sri Venkataramana Swamy College, Bantwala	Dr. Vinayaka, K.S.	Active
25.	University College, Mangaluru, Mangalore	Dr. Shobha D.	Active
26.	University of Mysore, Mysore	Dr. Rajkumar H. Garampalli	Inactive
27.	Yuvaraja's College (Autonomous), Mysore	Dr. Mahesh MK	Inactive
Kerala			
28.	Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE—JNTBGRI), Palode	Dr. Biju Haridas	Active
29.	Kerala Forest Research Institute, Peechi	Dr. Muktesh Kumar	Inactive
30.	Maharaja's College, Kochi	Dr. Stephen Sequiera	Active
31.	St Berchmans College, Chengacherry	Dr. Scaria K. Verghese	Active
32.	St Thomas college, Kozhencherry	Dr. Jasy Thomas	Active
Ladakh			
33.	Defence Institute of High-Altitude Research (DIHAR), Ladakh	Dr. O.P.Chaurasia	Inactive
Madhya Pradesh			
34.	Govt. MLB College, Bhopal	Dr. Suman Trivedi	Inactive
Maharashtra			
35.	Kishinchand Chellaram College, Mumbai	Dr Hemlata Bagla	Inactive
Mizoram			
36.	Advanced Research Centre for Bamboo and Rattan (ICFRE-ARCBR), Aizwl	Dr. Sandeep Yadav	Active
37.	Mizoram University, Aizawl	Dr. Awadesh Kumar	Active
Odisha			
38.	Centurion University of Technology and Management, Bhubaneswar	Dr. K.B. Satapathy	Inactive
39.	Utkal University, Bhubaneshwar	Dr. K.B. Satapathy	Inactive
Punjab			
40.	Central University of Punjab, Batinda	Dr. Felix Bast	Active

(continued)

Table 6.1 (continued)

S. No.	States Name/Organization	Contact person	Current status
Rajasthan			
41.	Rajasthan University, Jaipur	Prof. Kailash Agarwal; Dr. Yogesh Joshi	Active
Sikkim			
42.	Sikkim State Council of Science and Technology, Gangtok	Dr. N.P. Sharma	Active
Tamil Nadu			
43.	Annamalai University, Chidambaram	Prof. K. Kandasamy	Inactive
44.	Bharathidasan University, Tiruchirappalli	Prof. N. Thajuddin	Active
45.	Bharathiar University, Coimbatore	Dr. P. Ponmurugan	Active
46.	Kamaraj College of Engineering and Technology, Madurai	Dr. Shyam Kumar	Active
47.	KS Rangaswamy College of Technology, Tiruchengode	Dr. G. Ayyappadasan	Active
48.	M S Swaminathan Research Foundation, Chennai	Dr. G.N. Hariharan	Active
Uttar Pradesh			
49.	Banaras Hindu University, Varanasi	Dr. Rajan Gupta	Active
50.	University of Allahabad, Allahabad	Prof. Anupam Dikshit	Active
Uttarakhand			
51.	G.B. Pant National Institute of Himalayan Environment (NIHE), Almora	Dr. Subrat Sharma	Active
52.	Hemvati Nandan Bahuguna Garhwal University, Srinagar	Prof. M.C. Nautiyal	Active
53.	Kumaun University, Almora	Dr. Balwant Singh	Active
West Bengal			
54.	Bose Institute, Kolkata	Prof. N. Mandal	Inactive
55.	Indian Institute of Science Education and Research- Kolkata	Dr. Anuradha Bhat	Inactive
56.	University of Calcutta, Kolkata	Dr. Santanu Paul	Active

6.9.1 Floristic and Revisionary Studies

Singh (1964) and Awasthi (1965) compiled over 1300 species of lichens reported from Indian subcontinent till that time, of which 960 are reported from India. Singh (1980a) further updated the lichen research carried out till 1978. Awasthi (1988, 1991) published keys for identification of macro- and microlichens of Indian subcontinent, which are most useful literature till date. This provided a strong foundation for further exploration and taxonomic revision of several taxa in India. The macrolichen key was updated later (Awasthi 2007) but microlichen key needs

Table 6.2 The checklist of lichens published for various states of India

	States	References
1.	Andhra Pradesh	Reddy et al. (2011)
2.	Assam	Gupta and Sinha (2018)
3.	Chhattisgarh	Bajpai et al. (2018a)
4.	Goa	Randive et al. (2017)
5.	Gujarat	Nayaka et al. (2013)
6.	Jammu and Kashmir	Sheikh et al. (2006)
7.	Karnataka	Singh (2020)
8.	Kerala	Kumar (2000)
9.	Maharashtra	Makhija et al. (2014)
10.	Manipur	Singh (1981a, b)
11.	Nagaland	Singh and Sinha (1994)
12.	Rajasthan	Sinha et al. (2015)
13.	Sikkim	Sinha and Singh (2005)
14.	Uttar Pradesh	Nayaka and Upreti (2013)

revision. Singh and Sinha (2010) published annotated checklist of Indian lichens with 2300 species of lichens. The checklist was helpful in knowing the diversity of lichens in India and in identifying under explored regions. Apart from these valuable publications over the seven decades, lichen researchers have explored almost entire country and published checklists or flora for 14 states (Table 6.2). There are numerous floristic accounts available at local to regional levels. The emphasis was also given to exploring protected areas, and a total of 34 biosphere reserves, national parks, wildlife sanctuaries and several reserve forests have been explored (Table 6.3). More than 60 taxa are critically revised (Table 6.4) mostly at Lucknow University, BSI, ARI and CSIR-NBRI.

At present, India is represented by about 2907 species of lichens under 406 genera and 79 families. It is about 14.8% of the world-known lichens. About 18% of Indian lichen biota is endemic to the country (Anonymous 2020). The lichen floristic and revisionary studies resulted in an exponential growth by adding a number of novel taxa to Indian lichen biota (Fig. 6.2). From the year 2010 to 2019, a total of 610 species are added and 90 new species are discovered from India. Several unexplored and ecologically interesting areas surveyed resulted in an increase in the number of species at the state levels (Fig. 6.3). Among different regions Western Himalaya, north-eastern India (including eastern Himalaya), Western Ghats and Andaman Nicobar Island can be considered as hotspots for lichen biodiversity with approximately 1200, 1580, 1475 and 525 species, respectively.

6.9.2 Lichen Study in Antarctica

Antarctica being a cold desert supports mostly cryptogams, and lichens are the prominent vegetation in the continent. The scientists of CSIR-NBRI had the privilege of participating in four Indian Antarctic Expeditions exclusively for exploring

Table 6.3 Protected areas surveyed by Indian lichen researchers (excluding reserve forests)

	Tittle	References
1.	Achanakmar-Amarkantak Biosphere Reserve, Madhya Pradesh and Chhattisgarh	Singh et al. (2010), Satya and Upreti (2011), Shukla and Singh (2012), Prajapati et al. (2013)
2.	Bhadra Wildlife Sanctuary, Karnataka	Vinayaka and Krishnamurthy (2010), Vinayaka et al. (2010)
3.	Bhagwan Mahavir Wildlife Sanctuary, Goa	Nayaka et al. (2004)
4.	Bhimashankar Wildlife Sanctuary, Maharashtra	Mishra et al. (2017)
5.	Binsar Wildlife Sanctuary, Uttarakhand	Bisht et al. (2014)
6.	Bondla Wildlife Sanctuary, Goa	Nayaka et al. (2004)
7.	Chail Wildlife Sanctuary, Himachal Pradesh	Nayaka et al. (2002)
8.	Chambal National Wildlife Sanctuary, Uttar Pradesh	Nayaka and Upreti (2013)
9.	Corbet Tiger Reserve and National Park, Uttarakhand	Upreti and Chatterjee (1999), Upreti and Divakar (2003)
10.	Cotigao Wildlife Sanctuary, Goa	Phatak et al. (2004), Randive et al. (2017, 2018)
11.	Dhauladhar Wildlife Sanctuary, Himachal Pradesh	Chander and Chandel (2019)
12.	Govind Wildlife Sanctuary, Uttarakhand	Bajpai et al. (2014a, b), Karakoti et al. (2014), Mishra and Upreti (2015a, b)
13.	Gundy National Park, Tamil Nadu	Balaji and Hariharan (2004)
14.	Hastinapur Wildlife Sanctuary, Uttar Pradesh	Nayaka and Upreti (2013)
15.	Hemis National Park, Ladhak	Negi and Upreti (2000)
16.	Kalakad Mundanthurai Tiger Reserve, Tamil Nadu	Vinayaka et al. (2016)
17.	Kanchendzonga Biosphere Reserve, Sikkim	Sinha (2004)
18.	Katarniaghat Wildlife Sanctuary, Uttar Pradesh.	Nayaka et al. (2011)
19.	Lothian Islands Wildlife Sanctuary, West Bengal	Jagadeesh Ram et al. (2006)
20.	Marine National Park and Wildlife Sanctuary, Gujarat	Ingle et al. (2014)
21.	Meghamalai Wildlife sanctuary, Tamil Nadu	Nayaka et al. (2001)
22.	Mehao Wildlife Sanctuary, Arunachal Pradesh	Singh et al. (2004)
23.	Mudumalai Wildlife Sanctuary, Tamil Nadu	Ingle et al. (2016)
24.	Murlen National Park, Mizoram	Thangjam et al. (2019)
25.	Nanda Devi Biosphere Reserve, Uttarakhand	Upreti and Negi (1995), Negi and Gadgil (1996), Rawat et al. (2014)

(continued)

Table 6.3 (continued)

	Tittle	References
26.	Neora Valley National Park, West Bengal	Jagadeesh Ram and Sinha (2018)
27.	Seshachalam Biosphere Reserve, Andhra Pradesh.	Anjali et al. (2017)
28.	Shettihalli Wildlife Sanctuary, Karnataka	Vinayaka (2016)
29.	Shilly Wildlife Sanctuary, Himachal Pradesh	Nayaka et al. (2002)
30.	Similipal Biosphere Reserve, Odisha	Singh and Kumar (2012)
31.	Sohelwa Wildlife Sanctuary, Uttar Pradesh	Gupta and Sinha (2017)
32.	Soor Sarovar Wildlife Sanctuary, Uttar Pradesh	Nayaka and Upreti (2013)
33.	Sundarbans Biosphere Reserve, West Bengal	Jagadeesh Ram et al. (2012)
34.	Valley of Flowers National Park, Uttarakhand	Rawat et al. (2010)

lichens of Schirmacher Oasis where Indian research station Maitri is located. Upreti and Nayaka (2011) have surveyed the whole Oasis and documented a total of 69 lichen species. The lichens are used for monitoring the air quality of Schirmacher Oasis on regular basis by estimating the heavy metals accumulated in them (Upreti and Pandey 1999). The physiology of Antarctic lichens was studied as regard to water relations and found that *Rhizoplaca melanophthalma* (DC.) Leuckert & Poelt is a good desiccation-tolerant species (Nayaka and Upreti 2019). Apart from Schirmacher Oasis, the lichens in Larsemann Hills in East Antarctica are also studied and 25 species are reported (Singh and Nayaka 2017). The third Indian research station 'Bharti' is located here in Larsemann Hills.

6.9.3 Air Pollution and Climate Change Studies

It was Nylander in the year 1866 who first correlated the absence of lichens in Paris city to air pollution. Since then more than 3000 research papers have been published on the topic worldwide and lichens are successfully utilized for air pollution studies. The lichens have proved to be reliable bioindicators in the monitoring of ecosystem changes as litmus test for ecosystem health (Hawksworth 1971). From India, Das et al. (1986) were the first to study lichens in relation to air pollution caused by vehicular activity in Kolkata city. They reported the occurrence of lichen *Parmelia caperata* (L.) Ach.(=*Flavoparmelia caperata* (L.) Hale), which is now locally extinct in the city. After the study by Das et al. (1986), much of the air pollution related studies has been carried out at CSIR-NBRI. The lichens are utilized in three different ways such as—documenting all the lichens or select species occurring

Table 6.4 Various lichen taxa critically revised by Indian lichen researchers

	Taxa	References
1.	*Alyxoria*	Joseph et al. (2016a)
2.	*Aspicilia*	Upreti and Chatterjee (2002a)
3.	*Asterothyrium*	Singh (1979)
4.	*Bacidia*	Awasthi and Mathur (1987)
5.	*Badimia*	Awasthi and Mathur (1987)
6.	*Baeomyces*	Upreti (1985a)
7.	*Brigantiaea*	Awasthi and Srivastava (1989)
8.	*Buellia*	Singh and Awasthi (1981)
9.	Caliciales	Pant and Awasthi (1989a)
10.	*Catillaria*	Pant and Awasthi (1989b)
11.	*Cetraria*	Awasthi (1982a, b)
12.	*Cetrelia*	Mishra and Upreti (2015a, b)
13.	*Cladia*	Upreti (1985b)
14.	*Cladonia*	Upreti (1987)
15.	*Coccocarpia*	Awasthi (1985)
16.	*Collema*	Akhtar and Awasthi (1980)
17.	*Cryptothecia*	Makhija and Patwardhan (1994), Jagadeesh Ram and Sinha (2016)
18.	*Dermatocarpon*	Awasthi and Upreti (1985)
19.	*Diploschistes*	Pant and Upreti (1993)
20.	*Diplotomma*	Singh and Awasthi (1990)
21.	*Dirinaria*	Awasthi (1975)
22.	*Echinoplaca*	Singh (1978)
23.	*Endocarpon*	Singh and Upreti (1984)
24.	*Evernia*	Awasthi (1982a, b)
25.	*Fellhanera*	Awasthi and Mathur (1987)
26.	Graphidaceous and Thelotremataceous lichens	Makhija et al. (2005), Chitale et al. (2009), Joshi et al. (2012), Joshi et al. (2018)
27.	*Heiomasia*	Bajpai et al. (2018b)
28.	*Heppia*	Upreti and Büdel (1990)
29.	*Herpothallon*	Bajpai et al. (2018b)
30.	*Hypogymnia*	Awasthi (1984)
31.	*Lecanora*	Upreti (1997a, b), Upreti and Chatterjee (1997, 1998)
32.	*Lecidea*	Upreti et al. (2006)
33.	*Lempholemma*	Akhtar (1981)
34.	*Lepraria*	Bajpai et al. (2018c)
35.	*Leprocaulon*	Bajpai et al. (2018c)
36.	*Leptogium*	Awasthi and Akhtar (1977, 1979)
37.	*Letrouitia*	Awasthi and Srivastava (1989)
38.	*Lithographa*	Joseph et al. (2016b)
39.	*Maronea*	Singh (1980b)
40.	*Menegazzia*	Awasthi (1984)
41.	*Mycobilimbia*	Awasthi and Mathur (1987)

(continued)

Table 6.4 (continued)

	Taxa	References
42.	*Ochrolechia*	Awasthi and Tewari (1987)
43.	*Opegrapha*	Joseph et al. (2018)
44.	Pannariaceous	Upreti et al. (2005b)
45.	*Parmeliella*	Makhija and Adawadkar (1999)
46.	Parmelioid lichens	Divakar and Upreti (2005)
47.	*Peltigera*	Awasthi and Joshi (1982)
48.	*Peltula*	Upreti and Büdel (1990)
49.	*Phlyctis*	Joshi et al. (2010, 2012)
50.	*Phyllopsora*	Upreti et al. (2002), Mishra et al. (2011)
51.	*Porpidia*	Upreti and Chatterjee (2002b)
52.	Pyrenocarpous lichens	Makhija and Patwardhan (1988, 1993, 1995), Makhija et al. (1994), Upreti (1998), Singh and Upreti (1999), Ingle et al. (2017)
53.	*Ramalina*	Sharma and Awasthi (1981), Pant and Awasthi (2003)
54.	*Rhizocarpon*	Awasthi and Singh (1977)
55.	*Sclerophyton*	Makhija and Adawadkar (2002)
56.	*Stereocaulon*	Pant and Upreti (1999)
57.	Stictaceae	Joshi and Awasthi (1982)
58.	*Stirtonia*	Makhija and Patwardhan (1998)
59.	*Synarthonia*	Joseph and Sinha (2015)
60.	Teloschistaceae	Joshi and Upreti (2008, 2011)
61.	*Tephromela*	Upreti and Chatterjee (2002c)
62.	*Usnea*	Awasthi (1986)

around the source of pollution (city centre or pollution emitting factory, industry, power-plant), either qualitatively or quantitatively; estimating the heavy metals and pollutants accumulated in the naturally growing lichens; and transplanting the lichens from healthier area to polluted sites and estimating pollutants accumulated in such lichens. Along with heavy metals, the accumulation of polycyclic aromatic hydrocarbons (PAH), persistent organic pollutants (POP), arsenic, fluorides, and physiological parameters such as chlorophyll contents, fluorescence, degradation, protein and carotenoids are analysed. Using lichen distribution data, areas are categorized into various zones of pollution, which is called 'lichen zone mapping'. Further, air purity index (IAP) was calculated using quantitative data. The lichens are perennial organisms and metal accumulation is a continuous process in them. Therefore, pollution monitoring with lichens would complement the studies carried out using machines and will help in generating baseline data. So far, only a few cities in the country such as Ayodhya, Barak Valley, Bhadravati, Bengaluru, Dehradun, Guwahati, Haridwar, Howrah, Kanpur, Katni, Kolkata, Lucknow, Mahabaleshwar, Mandav, Pauri, Pune and Rewa have been studied for pollution using lichens. India being large country with automobile-based pollution, there is a need for more studies on this aspect. Bajpai et al. (2009, 2010, 2013) worked extensively on the heavy metal accumulation and physiological changes in lichens while Shukla and Upreti

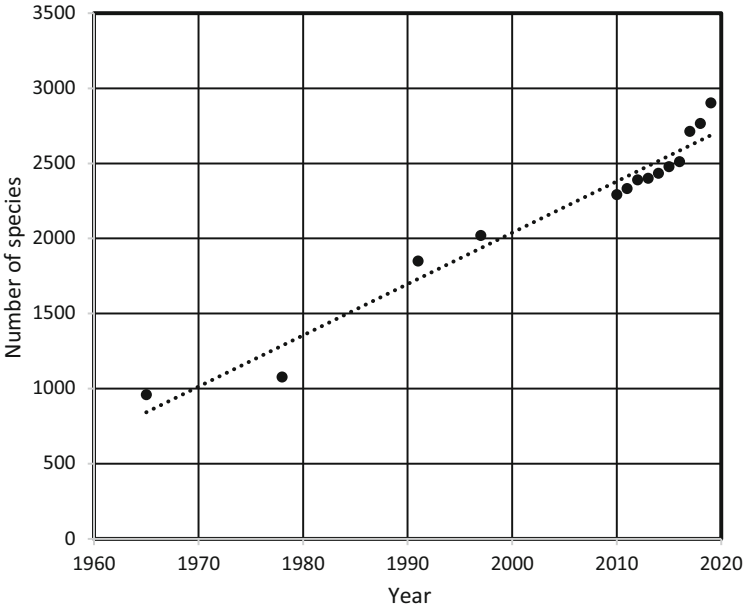

Fig. 6.2 Addition of species to Indian lichen biota since 1965 onwards

(2009, 2012) studied the PAH in lichens in north India. The studies related to biomonitoring are summarized in the book '*Lichens to Boimonitor the Ecosystem*' by Shukla et al. (2014). The pollution accumulation data combined with geostatistical (GIS) tool was helpful in marking the gradient of pollution in Uttarakhand (Bajpai et al. 2014a, b). The studies have concluded that widely distributed foliose lichens *Phaeophyscia hispidula* (Flörke) H. Mayrhofer & Poelt and *Pyxine cocoes* (Sw.) Nyl. can be utilized as model organism for air pollution monitoring studies. However, there are no detailed studies related to radionucleotide accumulation in lichens and genotoxic effect due to the pollutant accumulation.

The lichens are sensitive indicators of global warming. The spread, range expansion, increased frequency and abundance of several thermophilous epiphytic species in Europe and other countries have been attributed to global warming in the late twentieth century (Hauck 2009). The change in the lichen diversity in relation to climate change has been well worked out elsewhere (Hauck 2009; Aptroot and van Herk 2007), but such studies are rare in India. Joshi and Upreti (2010) compared their study on lichens of Pindari region in Himalayas with three decades earlier data and found that the green algae-containing lichens exhibited an increase in number than cyanolichen. Among green algal lichens, *Trentepohlia*-containing lichens were more in diversity. Further, soil- and rock-inhabiting lichens reduced in their diversity while epiphytic ones increased. The climate change studies with lichens using open top chambers, quantitative assessment of UV protective compounds are still in infancy. It is now well known that the effects of climate change can be best

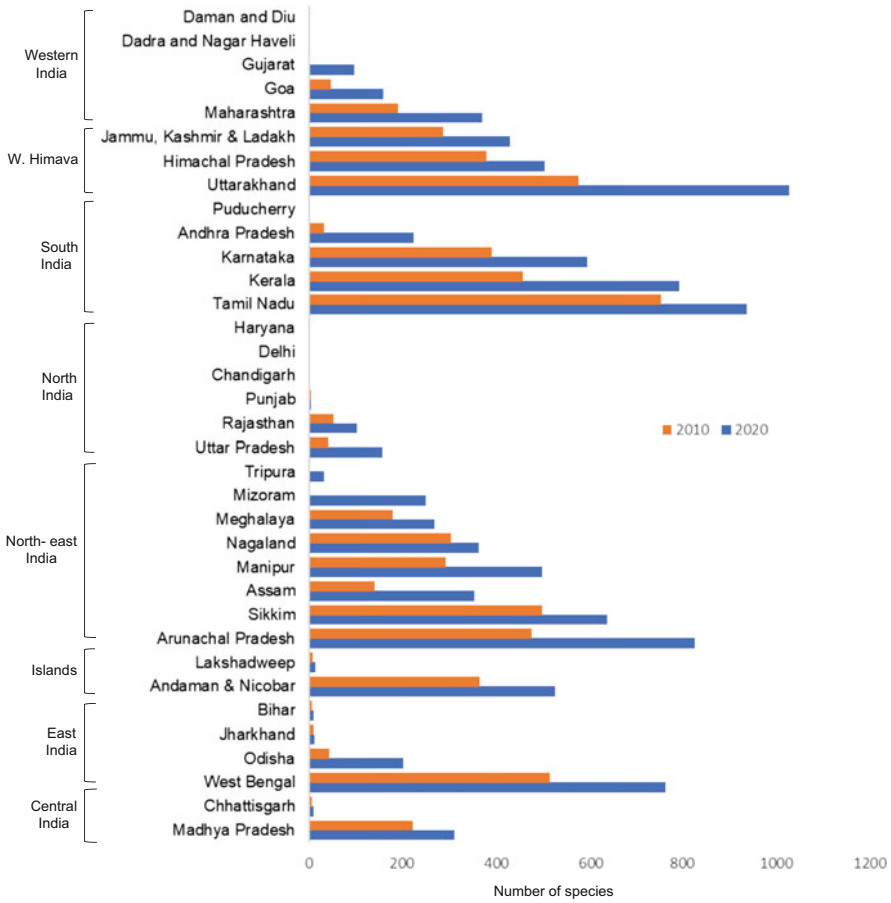

Fig. 6.3 Current status of lichen diversity in different states in comparison with Singh and Sinha (2010)

monitored in alpine and montane ecosystems (Grabherr et al. 2010) as the mountain species are unusually sensitive to the climate change. These species not only lose parts of their range but also suffer available land surface area with increasing altitude. Therefore, CSIR-NBRI, in collaboration with ISRO's Space Application Centre, has initiated climate change studies using cryptogams in alpine Himalaya of Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Sikkim and Uttarakhand. Here several permanent monitoring plots were established on mountain peaks, climate sensors are placed and changes happening in the plots are directly monitored by the satellite. As it is a long-term project, the results are yet to be yielded.

6.9.4 Biodeterioration Studies

In India, lichens on monuments are being studied since 1980s. Gayathri (1980) was first to describe the effects of lichens on granite statues in India. Thereafter, very few prominent monuments of Assam, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Uttar Pradesh and Uttarakhand have been studied (Table 6.5). The collection of more than 1000 specimens and systematic account of 112 species growing on these monuments are available (Bajpai and Upreti 2014). The methodology in assessing the lichen diversity, damage caused, and conservation are discussed (Bajpai and Upreti 2014; Choudhary et al. 2016a, b; Uppadhyay et al. 2016; Deshmukh et al. 2017). Studies have shown that climate change and increasing air pollution favours the growth of toxitolerant lichen species (Seward and Edwards 1997). These lichens cause much damage as the monuments provide excellent niche for growth of such lichens. Therefore, it is advised to remove the lichens and conserve the monuments. The lichens can be carefully scrapped in regular intervals and agricultural fungicides and herbicides (e.g. Butachlor 5%, Calixin 2%, Propachlor, Simazine 1% and Zebtane 5%) can be used in generous concentration to void lichens for longer period (Garg et al. 1995; Singh et al. 1999). Stone paints can also be applied to create a protective covering over the monuments to prevent fungus and algal growth. Apart from these, bio-formulations made of plant extracts such as neem (*Azadirachta indica* A. Juss.) and chukri (*Rumex hastatus* D. Don.) may also be attempted (Chandra et al. 2019).

6.9.5 Ethnolichenology

The lichens are the part of Indian culture since the time immemorial. The lichens are referred to as 'Shipal' in Atharveda (1500 BC), as 'Shailaya, Shilapushp' in Sushruta Samhita (1000 BC), Charak Samhita (300–200 BC) and several Nighantu (AD 1100–1800), as 'Charilla' in Ayurveda and used for medicine. The lichens are also used in Unani and Homoeopathy medicine. The lichens are also an important ingredient in 'hawan or homasamagri' (sacrificial fire), 'garam masala, meat masala and sambar masala' (various ground spices). The lichens are being used in the preparation of traditional perfume 'Otto, Hina Attar' in Kannauj, a town in Uttar Pradesh (Upreti et al. 2005a). Garhwal herdsmen use lichen *Buellia subsororioides* S.R. Singh & D.D. Awasthi as 'henna, mehndi' (natural dye); Nepalese of Sikkim use thalli of *Heterodermia diademata* (Taylor) D.D. Awasthi for protecting wounds from infection; Gond and Oran of Madhya Pradesh use *Parmotrema sancti-angelii* (Lynge) Hale for treating ringworm-like skin disease; Lepchas Sakyong valley, North Sikkim use *Peltigera polydactyla* (Neck.) Hoffm. to stop bleeding from cuts, *Stereocaulon himalayense* D.D. Awasthi & I.M. Lamb as antidote for urinary trouble; Bhotias of Nanda Devi Biosphere Reserve use smoke of *Thamnolia vermicularis* (Sw.) Schaer. as vermicide; and Bhotia and Garhwalis of Uttarakhand use *Usnea longissima* Ach. as stuffing material and ingredient of a poultice for bone setting (Upreti et al. 2005a); and Adi tribes of Arunachal Pradesh use *Leptogium*

Table 6.5 List of states that are explored for lichens growing on monuments and historical buildings (updated after Bajpai and Upreti 2014 with Choudhary et al. 2016a, b; Joshi et al. 2015, Uppadhyay et al. 2016, 2018; Behera et al. 2020)

	States	Districts	Localities
1.	Assam	Sonitpur	Da-Parbatia temple ruins, Singri Hillruins, Bhomoraguri Hill rock inscription, Garh Doul, Sakraswari Umatumuni Island, Biswanath Siva Linga, Bamgaon ruin, Bordol temple, Dhandi ruins, Basudev Doul, Nandikeshwar Devalaya, Mahabhairab temple, Chummery Compound sculptures
		Tezpur	Temple ruins of Bamuni Hills
2.	Karnataka	Mysore	Chamundi Hills, Chamundi temple, Somanthapur Keshava temple, Panchlingeswar temple, Srirangapatna Tipu's dungeons
		Chikmangalur	Baggavalli, Yohnarsimhaswami temple
		Hassan	Belur, Chennakeshava temple, Halebidu Hoysaleshwar temple, Hullekere Channakeshava temple
		Shimoga	Kavaledurga, Saivappa's fort Complex
		Tumkur	Venkateshswar temple
3.	Madhya Pradesh	Anoopur	Amarkantak, Patleshwar temple, Kapildhara, Kabirchabutra, Jweleshwar temple
		Ashoknagar	Kila Kothi, Chanderi fort, Khuni Darwaja, Chanderi fort, Kati Ghati, Digambar Jain temple, Jageshwari Mata temple, Chanderi Graveyard
		Chattarpur	Khajuraho, Parwati temple
		Datia	Panchamkavikipahadi, Chiviyapurkimata, Veer Singh palace, Karam Sagar dam, Dong Karera village, Udnukipahadi
		Dhar	Dhar fort, Lat ki Masjid, Bhojshala; Mandav—Lal Mahal, Bhangi Gate, Jali Mahal, Alamgir Gate, Jami Masjid, Delhi Gate, Jahagia Mahal, Sunset Point, Lohani Cave, Rani Roopmati Mahal, Chistikhan Mahal
		Dindori	Khukhuridadar, Kabir
		Guna	Bajranggarhfort, GaderkiGufa, Beesbhuji temple
		Gwalior	Chaturbhuj temple, Gujri mahal, Gwalior fort,Tansenki dargah, Devkho, Gupteshwar temple
		Hosangabad	Pachmari, Jatashankartemple, Apsara Vihar, Chote Mahadev, Pandav Cave Down Fall
		Jabalpur	Madan Mahal, Karia Pathar
		Raisen	Bhimbekta Cave, Jamunjhiri, Rang Mahal, Lakhajwar
		Shivpuri	Narwar fort, Survaya fort, Atal Sagar dam, Bhadaiyakund
		Tikamgarh	Orcha, Sheesh Mahal, Jahingir Mahal
4.	Maharashtra	Satara	Mahabaleshwar, Graveyard, Koteshwar temple, Panchganga temple, Old Mahabaleshwar temple, Pratapgarh fort
5.	Odisha	Khordha	Bhubaneswar, Kotitirtha temple, Rameshwar temple, Shatrughneswar temple, Bhairingeswar temple, Kapileswar temple, Lingaraj temple, Mukteswar temple,

(continued)

Table 6.5 (continued)

	States	Districts	Localities
			Parshurameswar temple, Raja Rani temple, Sukhmeswar temple
		Jaipur	Ratnagiri and Udayagiri Buddhist excavation site
		Puri	Konark, Sun temple, Chaurasi Amreshwar temple, Amreshwar temple
6.	Uttar Pradesh	Mahoba	Bukhar Pahar
		Lucknow	NBRI Boundary Walls, Dilkusha fort, Mahmudabad fort, Carlton Hotel Wall, Imambara, Tikatgunj temple, Udaygunj ShutarKhana, Moosa Bagh fort
		Faizabad	Gulabari Dome, Maqbara
		Agra	Buland Darwaja, Kaach Mahal
		Allahabad	Khushro Bagh Fort, Akbar's fort
		Kanpur	Bithoor area Monuments, fort of Nana Sahib
		Varanasi	Ram Nagar fort, BHU Hostel building
7.	Uttarkhand	Almora	Jageshwar temple

denticulatum Tuck. as a vegetable (Rout et al. 2010). Upreti et al. (2015) listed the ethnolichenological uses of 125 lichens that occur in India. Some of these lichens are validated for their claimed usage, while most of them have to be evaluated. Also, these lichens should be popularized through herbal formulations and their metabolites be used for drug development.

6.9.6 Bioprospecting Studies

As mentioned earlier, lichens are used in Indian traditional medicine since long. Chandra and Singh (1971) provided a detailed description of crude drug 'Chharila' sold in Indian markets which comprises three species of Parmelioid lichens—*Parmotrema chinense* (Osbeck) Hale & Ahti, *P. perforatum* (Ach.) Mass. and *P. sancti-angelii*. The drug has astringent, resolvent, laxative, carminative properties and is also supposed to possess aphrodisiac property. The drug is considered to be useful in dyspepsia, spermatorrhoea, amenorrhoea, calculi, diseases of blood and heart, stomach disorders, enlarged spleen, bronchitis, bleeding piles, scabies, leprosy, excessive salivation, soreness of throat, tooth ache and general pain. The smoke of 'Chharila' is believed to relieve headache. Acharya Balkrishna, a well-known Ayurveda practitioner of Patanjali Yogpeeth, Uttarakhand, has well narrated the uses of lichens for various ailments (Balakrishna 2020). However, the scientific validation of these claims has never been carried out, although there is some indirect evidence available (Upreti et al. 2015). Nayaka et al. (2010) listed 137 medicinally important lichens occurring in India based on ethnolichenological uses as well as biological activities reported throughout the world. The biological activities exhibited by lichens include antimicrobial (antibacterial, antiviral, antifungal), antioxidant, anti-inflammatory, antipyretic, anti-tyrosinase, analgesic,

anti-ulcer, antiproliferative and cytotoxic. Among these, antimicrobial and antioxidant studies are most common. Indian researchers have tested lichen extracts against cardiovascular disease (Mahadik et al. 2011), as anti-obesity (Anil et al. 2011; Shivanna et al. 2017), hepatoprotective (Verma et al. 2008) and probiotic (Gaikwad et al. 2012) agents, which can be considered as modern lifestyle problems. The lichen chemicals are peculiar and have many functional sites, and hence easy to develop the green nanoparticles. The biological activities of gold and silver nanoparticles synthesized by using lichen extracts are well experimented. These nanoparticles are found to be potential antimicrobial (Singh et al. 2015), quorum sensors (Singh et al. 2017a), antibacterial (Kumar et al. 2010; Rai and Gupta 2019) and antioxidant (Debnath et al. 2016). CSIR-NBRI has already developed antimicrobial nanoparticles from *Usnea* spp. and further studies on other lichen taxa are in progress (Prateeksha et al. 2019a). In the recent days, the endolichenic fungi (ELF) have attracted increased attention of researchers. The ecologist, taxonomist, chemist and agronomist showed their interest to investigate these unique organisms for their correct identity, novel biomolecules, enzyme production, biocontrol, plant growth promoting, bioremediation and biotransformation potential. From 2008 to March 2019, a total of 172 metabolites were isolated from ELF of which 99 are novel. These molecules exhibit activities such as anticancer, antifungal, antibacterial, antioxidant, anti-inflammatory, UV protectant, quorum sensing and miscellaneous activities (Singh et al. 2017b; Agrawal et al. 2019; Prateeksha et al. 2019b).

The lichen and their extracts have proven their potential as pharmaceutically important sources, but they are not being utilized to the fullest. Lichens produce more than 1000 secondary metabolites (Elix 2014); among them except for 50–60, the remaining are unique to lichens. Most of these secondary metabolites are responsible for biological activities and at least 35 such metabolites are experimentally validated. From India, numerous publications are available for various biological activities of lichens; however, identification of responsible bioactive molecules is lacking. Any bioprospecting studies require huge biomass for crude extraction and compound isolation. The lichens are slow-growing organisms, sensitive to climatic conditions, and, therefore, they cannot be cultivated. Hence most of the bioprospecting studies are restricted to lichens available in large quantities. Although, the mycobiont culture and production of target molecules are possible, it is one of the tedious experiments and the success rate is low in most of the cases.

6.9.7 Miscellaneous Studies on Lichens

Lichenometry is a useful technique for reconstructing palaeoenvironment, dating moraine ridges in the alpine areas and estimating the rate of glacier retreat. In a study carried out in Pindari glacier, using lichen *Rhizocarpon geographicum* (L.) DC it is estimated that the glacier retreated 1 km in 600 years (Joshi and Upreti 2010). Similar studies were carried out in Kupup and Thangu area of eastern Himalaya in Sikkim and Thajiwas glacier in Ganderbal district of north-western Himalaya of Jammu and Kashmir (Bajpai et al. 2016). Using the same technique, Gupta (2005)

estimated the age of landslides in Pawari landslide zone in Kinnaur district of Himachal Pradesh. The lichens are also used in forestry, especially as indicator of forest health (Dudani et al. 2015; Sonam et al. 2017). The lichens are tested for their dye yielding properties (Rawat et al. 2018; Sharda and Rastogi 2012).

6.10 Conclusions

Among 200 lichen researchers in India, about 50 are actively involved studying various aspects of lichenology. From the year 2010 to 2019, a total of 772 research papers have been published at an average of 77 papers per year (Fig. 6.4). Although maximum publications are from CSIR-NBRI, BSI and ARI, the contributions from other organizations cannot be ignored. In the recent days lichen research in India is leaning towards bioprospecting for various biological activities. Although some researchers are still involved in inventorying lichens from under explored areas, pure taxonomic investigations are declining. The superannuation of expert lichenologists in different centres is one of the main reasons for this. Further, change in publication policy of reputed journal requires revision of taxa at a larger geographical level and application of modern tools such as molecular systematics and distribution modelling. Indian researchers need to update their techniques for taxonomic as well as other aspects. Nonetheless, lichen research in India is prospering and now it is no more a neglected branch of botany.

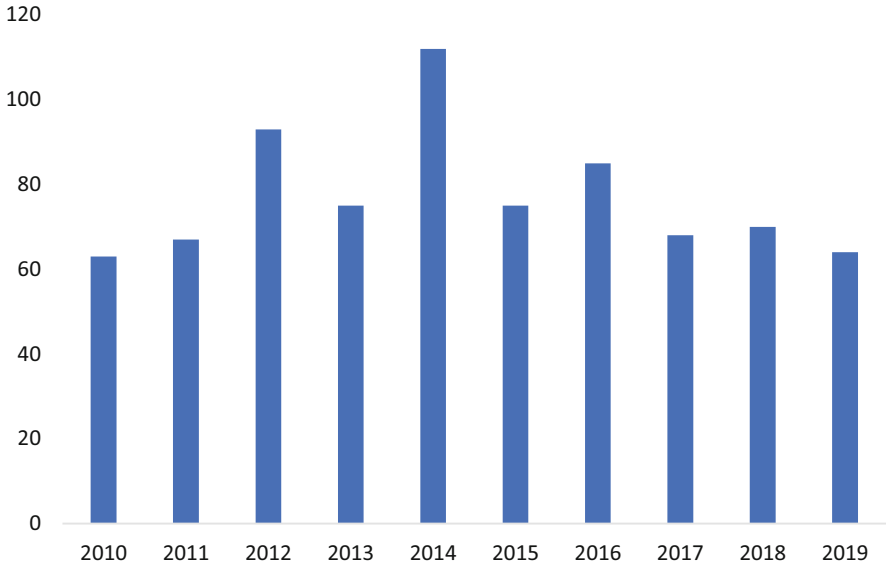

Fig. 6.4 Number of publications in lichenology from India since year 2010

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History and Development of Ectomycorrhizal Research in India

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T. N. Lakhanpal, Ashwani Tapwal, and Vaneet Jishtu

Abstract

This chapter attempts to trace the developments in ectomycorrhizal (ECM) research in India. The research on ECM in India was initiated at erstwhile FRI (now ICFRE) at Dehradun after independence in the late fifties by Dr. B.K. Bakshi and collaborators. They described the ECM in some important forest trees, viz., Pines, Deodar and high-altitude conifers. They isolated the mycorrhizal associates in pure culture and artificially inoculated the seedlings in a few cases. More impetus to ECM research was provided in the late seventies and early eighties from South, North and Eastern India. Natarajan, Raaman, Reddy and Mohan and their associates intensely investigated the ECM and its implications in seedling regeneration in *Pinus patula* and other pines. Sharma, Mishra and their students carried out similar studies on *P. kesiya* in the eastern Himalayas. Lakhanpal and collaborators studied the various aspects of ECM in Chir Pine, Blue Pine, Deodar, Fir, Spruce, Chilgoza Pine, Yew and apple plants in N.W. Himalaya. All these studies concerned characterization, identification, mycobiont association, physical and chemical status and artificial inoculation with selected mycobionts. Recently (2019, 2020) Atri and his students have carried out similar studies on ECM of sal trees in H.P. Tapwal and associates at HFRI, Shimla, are working on the ECM relationship of some important Himalayan forest tree species. The emphasis has gradually shifted now towards

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evaluation of field performance of the inoculated seedlings which is the primary aim of studies on this symbiotic association.

Keywords

Mycorrhiza · Ectomycorrhiza · Research · ICFRE · Pines · Deodar · Yew

7.1 Introduction

Forests have been the backbone of mankind throughout its long history of existence. Out of the 18 biodiversity hotspots in the world, two distinct regions are located in India: the Eastern Himalaya and the Western Ghats. The use of forests initially was need based but later turned commercial and the natural balance was upset due to over-exploitation. Many areas were threatened due to demographic processes, urbanization, agriculture and industrialization. Therefore after independence rehabilitation and revegetation of deforested and denuded areas became the first priority. This demanded introduction of new techniques to improve the planting stock, reduce the transplanting period and improve the survival and growth parameters of the planted stock. One such technology was the mycorrhizal biotechnology. Forest Research Institute (Now Indian Council of Forestry Research and Education) Dehradun was seen as a potential organization to develop technology for protection and rehabilitation of forests.

The term mycorrhiza literally means 'fungus-root' (Mykes- fungus or mushroom, Rhiza-root) in Greek and describes a mutually beneficial relationship between the feeder roots of plants and fungi (Frank 1885). It is perhaps an essential association for one or both partners, the fungus and the root, which is primarily responsible for nutrient transfer (Brundrett 2004). It is now regarded a universal association encompassing almost all groups of plants with only a few exceptions, and therefore it is often stated that the presence of mycorrhiza is a rule and absence an exception. The usefulness of this association is fully recognized now and it has become an acknowledged technology for sustainable development in forestry (Trappe 1962; Gerdemann 1968; Majestrik 1972).

Symbiosis is the most widespread mycorrhizal association involving root-inhabiting fungi and plant feeder roots. Mycorrhizae as presently recognized are categorized into: Ectomycorrhizae, Endomycorrhizae, Ectendomycorrhizae, Arbutoid, Monotropoid, Ericoid and Orchidaceous types (Harley and Smith 1983). Out of these, the ectomycorrhizae and endomycorrhizae (Arbuscular Mycorrhiza-AM) are reported to be the most widespread (Taylor and Alexander 2005; Smith and Read 2008). Ectomycorrhizae, as the name indicates, occupies the exterior of the root forming a loose or compact fungal sheath called mantle. The hyphae forming the mantle sheath penetrate through the epidermis and enter into the cortical region occupying the interstices in between the cortical cells perhaps replacing the middle lamella forming an interconnecting network called the Hartig net (named after the person who discovered it first). Mantle and Hartig net are the two characteristics of

ectomycorrhiza and serve to distinguish it from other types of mycorrhizae. Hartig net usually may extend up to endodermis or may be limited to one or a few layers of cortex. The ectomycorrhizal association is initiated by the spores or hyphae (propagules) of fungal symbionts which grow vegetatively over the feeder (short) root surface stimulated and attracted by root exudates. After colonization, there is change in colour and morphology of the feeder roots. The colour is usually determined by the colour of mycelium of the fungal symbiont and may be black, red, yellow, brown, white or blends of these. The root morphology varies from unforked, bifurcated, nodular, multiforked or coralloid to some other shapes. The colonization of the fungus does not spread beyond endodermis in a living root (Martin and Hilbert 1991).

Mycorrhizae are known to improve the growth parameters of the associated plants by manipulating the physiological processes such as increased absorption surface, selective ion absorption and accumulation (Jorgenson and Shoulders 1967; Shoulder and Jorgensen 1969; Harley 1959; Marks and Kozolowski 1973). Mycorrhizae also help the seedlings to resist infection by certain feeder root pathogens (Marx 1971). In addition, the mycorrhizae are known to improve feeder root health and increase disease resistance and tolerance to drought and to high soil temperature, soil toxins and extremes of soil pH (Marx 1980). The increase in surface area results from both the extensive vegetative growth of fungal hyphae and multi-branching habit of the ectomycorrhizae. The ectomycorrhizae are able to absorb and accumulate N, P, K and Ca in the fungal mantle more rapidly and for longer periods than non-mycorrhizal feeder roots. Ectomycorrhizae remain active for periods ranging from several months to 3 years (Marx and Shafer 1989; Manoharachary et al. 2009b). The characterization and identification of mycorrhiza has been standardized by prominent researchers like Melin (1927), Dominik (1956), Rambelli (1966), Ceruti and Bussett (1962), Trappe (1965), Zak (1969) and Agerer (1987–2012) in different plant species. Agerer (l.c.) has produced an excellent treatise in the form of a Colour Atlas of ectomycorrhiza which serves as an indispensable guide for identification and characterization of ectomycorrhizae in different species.

Mycobiont is the most integral component in the ectomycorrhizal association. Many species of fungi are usually involved in the ectomycorrhizal associations of a forest stand on a single tree species, on an individual tree or even a small segment of lateral root. A single fungal species can enter into mycorrhizal association with numerous tree species on the same site. Some fungi are apparently host specific while others have broad host range (Marx and Cordell 1988).

About 10% of the world flora enters into ectomycorrhizal associations. An estimated 20,000–25,000 species of fungi are reported to establish ectotrophic mycorrhizae with about 5000–6000 trees and woody plant species (Brundrett 2009; Rinaldi et al. 2008; Roy-Bolduc et al. 2016). These plant species belong mainly to families Pinaceae, Fagaceae, Gnetaceae, Betulaceae, Nothofagaceae, Fabaceae, Lepidospremoideae of Myrtaceae, Dipterocarpaceae, and Amhersteae of Caesalpiniaceae. Some tree genera such as *Alnus, Eucalyptus, Casuarina, Cupressus, Juniprus, Tilia, Ulmus* and *Arbutus* form both ectomycorrhizae and

endomycorrhizae depending on soil conditions and trees' age (Harley and Smith 1983). The ectomycorrhiza like associations have also been reported in some liverworts and Lycophytes but their implications are not yet fully understood (Bidartondo et al. 2003; Horn et al. 2013).

The genetic diversity of ectomycorrhizal basidiomycetes has been studied by Riviére et al. (2007) from African and Indian tropical rain forests. Among the basidiomycetous fungi, species of Hymenomycetes primarily belonging to genera *Boletus, Cortinarius, Suillus, Russula, Gomphidius, Hebeloma, Tricholoma, Laccaria* and *Lactarius*, and in Gastromycetes, to the genera *Rhizopogon, Scleroderma,* and *Pisolithus* form ectomycorrhizae. Certain Ascomyceteous members like *Cenococcum geophilum* (Eurotiales), truffles (Tuberales) and some members in Pezizales also form ectomycorrhiza (Trappe 1962). Many of the ectomycorrhizal fungi can be grown routinely in pure culture but they cannot exist saprophytically in nature without a plant host associate (Kendrick and Berch 1985; Manoharachary et al. 2009a, b).

General technical works for the identification of ectomycorrhizal fungi are those of Singer (1986), Arora 1986, Moser (1978), Ainsworth et al. (1973a, b), Watling and Gregory (1980), Kumar et al. (1990), Lakhanpal (1996), Natarajan and Raaman (1983), Lakhanpal et al. (2010) and Das and Sharma 2005. Over the past few years there has been a dramatic increase in the number of studies on ectomycorrhizal (ECM) fungi involving molecular identification of species and individuals (Horton and Bruns 2001; Horton 2002; Iotti et al. 2005; Toftegaard et al. 2010). Janowski et al. (2019) pointed out that morphological identification of the ectomycorrhizae often proves to be misleading and now a number of molecular methods that require isolation of nucleic acids are being used. They devised an effective molecular identification protocol for ectomycorrhizal root tips. They pointed out that Ascomycota were generally more difficult for DNA isolation than Basidiomycota.

In vitro synthesis of ectomycorrhiza has been successfully carried out in many countries by different workers. Melin (1921, 1922, 1923) successfully demonstrated for the first time that ectotrophic mycorrhizae could be produced in synthetic cultures by inoculating seedlings of *Picea abies*, *Pinus sylvestris* and *Larix europaea* with appropriate fungi. Hacskaylo (1953) substituted sand with vermiculite in vitro synthesis experiments. Marx and Zak (1965) further improved the substrate by stabilizing the acidity with an addition of finely ground sphagnum peat moss. Molina (1979) tested pure cultures of ectomycorrhizal fungi for mycorrhiza formation with red alder. Fortin et al. (1983) reviewed and evaluated the different methods used for synthesizing ectomycorrhizae.

Different types of inocula have been tried and tested for inoculation of the seedlings in the nurseries, e.g. soil inoculum, nurse seedlings, crushed fruit bodies and spores, and vegetative mycelium (Mikola 1970). All these inocula have some advantages and disadvantages. The most important initial step in any nursery inoculation programme is the selection of the mycobiont (Trappe 1977; Ruehle 1980). One criterion is host specificity. Another criterion is the ability of the selected fungi to grow in pure culture. Still another criterion is the ecological adaptation of the selected fungus to major types of sites on which the seedlings are to be planted.

The concept of improving field performance of tree seedlings by forming ectomycorrhizae on them in nurseries with specific fungi ecologically adapted to the planting site was originally developed by Moser (1958) in Austria. Using various modifications of Moser's technique and philosophy, Takacs (1967) in Argentina, Theodorou and Bowen (1970) in Australia, and Vazzo and Hacskaylo (1971) in the United States showed experimentally that field survival and growth of tree seedlings with specific ectomycorrhizae exceeded the performance of seedlings that lacked or had few natural ectomycorrhizae at planting. Marx and Schenck (1983) reviewed the potential of mycorrhizal symbiosis in agricultural and forest productivity.

Castellano (1992) compiled a list of outplanting performance of seedlings inoculated with ectomycorrhizal fungi from the world literature. Sixty species of ectomycorrhizal fungi have been used experimentally to form ectomycorrhizae on 49 tree species. Over 40% of the publications dealt with *Pisolithus tinctorius* on 29 different tree species. *Cenococcum geophilum, Hebeloma crustuliniforme, Laccaria bicolor, L. laccata, Suillus granulates, S. luteus,* and, *Thelephora terrestris* have been evaluated to a lesser extent on six or more tree species. Marx et al. (1991) produced *Pinus pinaster* seedlings with *Suillus granulates* ectomycorrhizae and outplanted them to produce edible fruit bodies of the fungus.

7.2 History of Ectomycorrhizal Research in India

The Forest Research Institute (now ICFRE) Dehradun was the pioneering institute to initiate mycorrhizal research in India soon after independence. A team of devoted scientists led by Dr. B.K. Bakshi geared up to meet this challenge. They started the investigations with some common and vulnerable forest trees species. In the beginning the studies on ectomycorrhiza were primarily for gaining understanding the basis of ectomycorrhizal association, tree species and the associated mycobionts.

The various tree species that have been worked over the years to ascertain the mycorrhizal relationship in India are *Pinus roxburghii* Sarg., *P. wallichiana* A.B. Jacks., *P. gerardiana* Wall. ex D. Don, *Cedrus deodara* (Roxb. ex D. Don) G. Don, *Shorea robusta* Gaertn., *Picea smithiana* (Wall.) Boiss., *Abies pindrow* (Royle ex D. Don) Royle, *A. spectabilis* (D. Don) Mirb., *Taxus wallichiana* Zucc. among the conifers, *Rhododendron arboreum* Sm., *Leucaena leucocephala* (Lam.) de Wit, *Casuarina, Alnus* sp., *Quercus incana* Bartram and *Malus domestica* Borkh. The developments in ectomycorrhizal research in India have been earlier reviewed by Lakhanpal (1989a, b, 1991), Raaman and Mahadevan (1988), Manoharachary et al. (2009a) and more recently by Tapwal and Lakhanpal (2015) and Kumar and Atri (2017).

The initial inputs by the group at FRI, Dehradun, laid a firm foundation for subsequent investigations on ECM. In fact it was Chaudhari (1945) who should be credited with the first ever study in the pre-independent India, on the mycorrhizal association in *Abies spectabilis* (D. Don) Spach., *Cedrus deodara* (Roxb. ex D. Don) G. Don, *Picea morinda* Link, *Pinus roxburghii* Sarg. and *Taxus wallichiana* Zucc. Dr. Bakshi later on consolidated and continued the studies more systematically on

different tree species with the aid of USDA, PL-480 project. With the incessant efforts of IDRC, Canada, and TERI, New Delhi, a joint programme for mycorrhizal research was formulated in 1985–1986 and for ectomycorrhizal research. TERI (Alok Adoleya), CAS in Botany, Madras University (Natrajan, Raaman and Mohan), Department of Biosciences, H.P. Univ. Shimla (T.N. Lakhanpal) and Department of Life sciences, NEHU (Mishra, G.D. Sharma) were identified as Nodal Centres (Jalali 2001). Under the aegis of this set-up, assessment of the research work carried out in India was made by organizing symposia from 1987 onwards at different places and universities, JNU, University of Madras, UAS Bangalore, Bangkok, Changmai (Thailand) and then TERI New Delhi. During the past few years Botany Dept. of Punjabi University, Patiala, has also become an active centre of mycorrhizal research (N.S. Atri). Similar mycorrhiza-related work is also being carried at HNB, Garhwal University, Srinagar, Uttarakhand (R.P. Bhatt), BSI (K. Das), HFRI, Shimla (A. Tapwal) and IFGTB, Coimbatore (V. Mohan).

M.S. Reddy and his team at Thapar Institute of Engineering & Technology are focusing on bioremediation of heavy metal toxicity from soils at molecular level. Cu and Cd stress-tolerant metallothionein genes, *LbMT1* and *LbMT2* from *Laccaria bicolor* (Reddy et al. 2014); *ShMT1* and *ShMT2* from *Suillus himalayensis*; *PaMT1* from *Pisolithus albus* (Reddy et al. 2016); and *HcMT1*and *HcMT2* from *Hebeloma cylindrosporum* (Ramesh et al. 2009), have been characterized as important ECM fungal determinants of metal detoxification. The metal tolerance associated novel genes and their possible mechanisms are being studied through metatranscriptomic approach that leads to the identification of novel metal tolerant genes (Leszczyszyn et al. 2013; Thakur et al. 2019).

7.3 Characterization and Identification

Morphological studies, characterization and identification of mycorrhizae have been accomplished in a number of tree species at the erstwhile FRI, Dehradun. Bakshi (1957) described the morphology of ectomycorrhizae in spruce, silver fir, sal and deodar. Subsequently Bakshi and Thaper (1960, 1966); Bakshi et al. (1968) and Mukerjee and Rehill (1962) described morpho-anatomical features of ectomycorrhizae of pines. In *Picea smithiana,* the roots are reported to be monopodial with typical ectomycorrhizal features and with two distinct types, the brown and yellowish white. In *P. gerardiana* the mycorrhizal roots are bifurcate, dichotomous or coralloid with three mycorrhizal types: white creamish, yellowish brown and black types (Kumar and Lakhanpal 1983; Lakhanpal and Kumar 1984; Lakhanpal and Chaudhary 1988; Kumar 1989). Sehgal and Sagar (2017) reported four types of mycorrhizae in the roots of *P. gerardiana.* But dark brown type and dark reddish types may represent two stages of development of the same. Similarly in *Abies pindrow* also two types of mycorrhizae have been observed (Sharma and Lakhanpal 1988).

Thakur (1990) further elaborated the morphology of mycorrhizae in *Abies pindrow* Royle, and *A. spectabilis* (D. Don) Spach., *Pinus roxburghii* Sarg. and

Taxus wallichiana Zucc. He observed the mycorrhizae to be brown type and pinnately or racemosely branched and yellowish type, either unbranched or pinnately branched in *A. pindrow*, monopodial type in *A. spectabilis,* forked, with repeated dichotomy and brown in colour in *T. wallichiana.* Mehrotra and Thapar (1990) described the morphological and anatomical details of mycorrhizae in *P. kesiya* and observed it to be typical ectomycorrhizal type. Sharma (2003) observed mycorrhizal roots of *T. wallichiana* to be light brown in colour bearing root hairs. Thick and smooth mantle was observed in the transverse section of young roots which perishes in mature roots. Both intercellular and intercellular penetration into cortical cells takes place.

Bakshi and Thaper (1960) reported in *T. wallichiana* that the short roots resemble the ectotrophic mycorrhizae in morphology; however the infection is typically endotrophic and appears only in the ultimate rootlets. Thakur (1990) also reported endotrophic type of root anatomy in *T. wallichiana.* Sharma (2003) later observed the development of mantle and Hartig net in the roots of *T. wallichiana*. She also observed hyphal coils in the cortical cells.

There are some overlaps in the observations of different researchers on the branching pattern of roots in different countries. The ectomycorrhizae have been reported to be simple unforked or monopodial type in spruce (Kumar and Lakhanpal 1983), fir and sal (Bakshi 1957; Sharma and Lakhanpal 1988) or pinnately and racemosely branched in silver fir (Harley 1969; Trappe 1967; Chivlers and Pryor 1965). They are reported to be bifurcate type in pines (Bakshi and Thaper 1966; Bakshi et al. 1968) and multiforked (coralloid) in deodar (Bakshi 1957).

The mycorrhizal roots of *Pinus wallichiana* have swollen tips and rounded apices (Sagar 1993), while conical apex has been reported in *T. wallichiana* (Thakur 1990). The mycorrhizal roots in *P. wallichiana* are initially creamish white, turning yellowish brown at maturity, and of coralloid type with no specific odour and taste and surrounding mycelium. The mycorrhizal roots possess a characteristic fungal mantle and Hartig net typical of ectomycorrhizal anatomy. In *C. deodara,* the mycorrhizae are racemosely branched initially and become coralloid later on. Anatomically the mycorrhizal roots do not possess a characteristic Hartig net, but an ephemeral mantle sheath. Kumar and Atri (2016) characterized for the first time the ectomycorrhizae of *Russula* and *Lactifluus* associated with *Shorea robusta* in the Shivalik ranges of outer Himalayas and provided the morpho-anatomical details of the mycorrhizal roots of sal trees. The ectomycorrhizal roots of sal associated with *Russula* spp. were light brown to greyish brown, with plectenchymatous gelatinized outer mantle layers having abundant cystidial elements. Ectomycorrhizae of *Lactifluus* species were reddish brown to light brown, lack cystidial elements and have thin-walled emanating hyphae.

Kumar and Atri (2019) worked out the morpho-anatomical details of mycorrhizal roots of *Shorea robusta* associated with three species of *Russula*. The ECM roots of *R. cremeoavallanae* were mostly greyish brown with almost plectenchymatous outer mantle layers having subcylindrical to oval-shaped cystidia, those of *R. romagnesiana* were reddish brown with silvery patches with purely plectenchymatous outer mantle layers beset with obpyriform to obclavate cystidia.

R. nigricans associated ectomycorrhizal roots have greyish brown to black mycorrhizal system and have almost pseudoparenchymatous dark brown mantle with capitate cystidial elements.

In still another study, Kumar and Atri (2020) investigated the ectomycorrhizal association of some other species of *Russula* with *Shorea robusta* and observed that the mycorrhizal roots of *R. chlorinosma* are irregularly pinnate to simple stringy to cottony with white to greyish brown surface, while those of *R. azurea* are irregularly pinnate to coralloid, smooth purple to greyish purple, whereas those of *R. cyanoxantha* are mostly simple to monodial pinnate with loosely short spiny and light brown surface.

Anatomically, a typical fungal mantle consists of a weft of interwoven hyphae enveloping variously coloured and well-developed 'Hartig Net'. *Cenococcum graniforme* forms a very characteristic mantle which consists of horizontally arranged palisade cells alternating with groups of small pseudo parenchymatous cells (Trappe 1964; Bakshi et al. 1966).

The 'Hartig Net' has been observed to be formed around the cortical cells in intercellular spaces by invasion of fungal hyphae; the hyphae may replace the middle lamellae completely. The Hartig Net formed by different fungi appeared similar but degree of development differed considerably (Bakshi 1974). Similar work on exotic pines and some other forest trees has been carried out by Thapar and co-workers at FRI, Dehradun, and on *Pinus patula* by Mahadevan, Natarajan, Raaman, and Mohan at Kodaikanal, Tamil Nadu. Similar ectomycorrhizal anatomy has been observed in *A. pindrow, A. spectabilis* and *P. roxburghii.*

A special mention may be made by the mycorrhizal association of apple plants, in H.P. orchards. Thankur (1998) carried out for the first time investigations on the mycorrhiza of apple plants. He observed that the roots in apple plants exhibit typical ectomycorrhizal associations in the initial stages of seedling growth, i.e. from third month to almost 12 month of age. Then the fungal hyphae enter into the cortical cells and gradually occupy them profusely, establishing endomycorrhizal association, which persists in the mature roots. The mycorrhizal roots possess swollen apices, are branched monopodially, then dichotomously and ultimately may become coralloid in the mature roots. The mycorrhizal roots are light orange, creamish white, pastel yellow and dull yellow (Kornerup and Wanscherr 1978), tasteless, odourless and with smooth fungal mantle, without surrounding mycelium and attached rhizomorphs. Anatomically the roots possess a conspicuous fungal mantle sheath from the age of 3 months to almost 12 months. Afterwards the mantle becomes greatly reduced or almost inconspicuous. The fully formed mantle consists of 3–4 layers. The Hartig net is not well developed, however.

7.4 Mycorrhizosphere Associations

The rhizosphere of mycorrhizal roots was named as the mycorrhizosphere by Marks and Foster (1976). Rambelli (1973) applied the name 'mycorrhizosphere' to describe the microbial ambience around the mycorrhizae. He distinguished rhizosphere and

mycorrhizosphere by the fact that the latter includes all those microorganisms located farther from the surface of the fungus cover and are influenced either very slightly or not at all by the action of the substances produced by the symbionts (Davey 1969). The rhizosphere community influences plant growth by drawing energy and releasing a complex of hormones, allo-chemicals and chelators that probably affect the plant both positively and negatively.

Eleven fungi and mycelium of some unidentified Basidiomycetes were isolated from mycorrhizosphere of *Picea smithiana* (Lakhanpal and Kumar 1984). In a similar study with *Abies pindrow,* Sharma and Lakhanpal (1988) isolated 21 species of fungi from its mycorrhizosphere. The fungi belonged to the traditional classes of fungi Zygomycetes, Ascomycetes and Deuteromycetes and two were sterile mycelial forms. Chaudhary and Lakhanpal (1988) isolated 13 fungal species and one unidentified basidiomycetous mycelium from the mycorrhizosphere of *Pinus gerardiana*. This sterile mycelium was later on used for inoculating the seedlings and it was found to be the mycelium of some mycorrhizal associate of this plant. Sehgal and Sagar (2017) recorded seasonal fungal diversity in the mycorrhizosphere of *P. gerardiana* and reported the presence of 12, 24, 32 and 17 species of fungi during winter, spring, rainy and autumn seasons, respectively. From the mycorrhizosphere of *T. wallichiana* 46 fungal species were isolated by Sharma (2003) and Gulati (2004) and from the mycorrhizosphere of *P. wallichiana* 28 fungi were isolated by Sagar (1993). Thakur (1990) isolated 21, 22, 14 and 27 species from the mycorrhizosphere of *A. pindrow, A. spectabilis, P. roxburghii* and *T. wallichiana*, respectively. The studies on mycorrhizosphere are limited only to the enumeration of rhizosphere microbes.

7.5 Physical and Chemical Status of Mycorrhizal Plants

It is a common observation that ectomycorrhizal association increases the absorptive area of plants greatly enhancing the uptake of nutrients, especially the uptake of phosphate. Melin et al. (1958) long back demonstrated with the use of radioisotopes that fungal symbionts are able to transfer carbon, nitrogen, phosphorus and calcium from nutrient solution into plant by considerably large quantities as compared to non-mycorrhizal plants. Analysis of physical and chemical status of mycorrhizal and non-mycorrhizal plants of *Picea smithiana* was carried out by Kumar and Lakhanpal (1983). The mycorrhizal plants were reported to attain better shoot/root ratio (Fresh weight and dry weight) and to exhibit higher shoot/root ratio compared to non-mycorrhizal plants. Marked differences in needle nutrient content have been observed with higher concentration of P, Ca and Mg. Similarly percentage of P, Ca and Mg was also considerably higher in both root and shoot of mycorrhizal plants but there was non-significant difference in nitrogen and magnesium. Almost similar results were obtained by Sharma and Lakhanpal (1988) with *Abies pindrow*. They observed no obvious differences in organic carbon, nitrogen, phosphorus and pH of the sterilized and unsterilized soil but there was marked differences in shoot height and shoot/root ratio of the mycorrhizal plants, however. Tapwal and Kapoor (2019)

carried out a detailed estimation of chemical status of inoculated and uninoculated seedlings of *P. gerardiana* and observed invariably higher concentration of each tested element (C, N, P, Ca, Mg, Mn, Cu, Zn, Fe) in the inoculated seedlings than the uninoculated ones. Further, the total C, N and P content was higher in the roots while the Ca, Mg, Cu and Fe content was higher in shoots of 21-month-old seedlings. Therefore it can be surmised that mycorrhizal roots have better and improved elemental composition.

7.6 Drought Resistance

Availability of water is one of the limiting factors in growth and survival of plants. The studies on the effect of water stress on mycorrhizal and non-mycorrhizal seedlings of *Abies pindrow, Picea smithiana* and *Pinus gerardiana* revealed that mycorrhizal seedlings tolerated higher levels of water stress than non-mycorrhizal seedlings. With slight variation, the mycorrhizal seedlings in these plants tolerated water stress of –6 atms against –4 atms by non-mycorrhizal seedlings for a maximum of 8 days (Lakhanpal and Kumar 1986; Lakhanpal and Sharma 1988). For exposing seedlings to stress artificially PEG 6000 was used. Increase in Water Saturation Deficit (WSD) has been found less significant in mycorrhizal seedlings than non-mycorrhizal seedlings. In seedlings subjected to water stress a colour change from lighter to darker in the fungal mantle has been observed, thereby supporting the assumption that mycorrhizal seedlings are more drought resistant. Nagrajan and Natarajan (1998) reported that salt stress influences the growth and mycological symbiosis in *Casuarina equisetifolia.*

7.7 Effect of Soil Fumigants/Biocides

Usually the nursery soils harbour soil pathogens, which cause mortality of seedlings. To counter this aspect, the soil needs to be treated beforehand to eliminate the soil pathogens. Therefore, soil fumigants are used to rid the soil of the root pathogens. An appropriate dose of fumigants/biocides needs to be calculated so that the pathogens are killed but the mycorrhizal development is not hindered or retarded. Commonly recommended soil fumigants are methyl bromide and formaldehyde.

Appropriate doses of fumigants, methyl bromide and formaline were used for *Abies pindrow* and *Picea smithiana* (Kumar and Lakhanpal 1986); formaline (38% formaldehyde) and methyl bromide, each in six different dosages, were applied 15 days before sowing the experimental plots. Seedlings with different treatments were lifted every month for studies. Percentage of ectomycorrhizal development was estimated visually (Wilcox 1968), whereas mycorrhizosphere analysis was done by dilution plate method. In the case of *Picea smithiana* higher germination percentage, increased seedling survival rate, higher root-shoot ratio and maximum development of mycorrhiza was observed in treatments with 210 mL per 4 lit of formaline and 130 mL per 4 lit of methyl bromide. In *Abies pindrow*, 210 mL formaline and

140 mL of methyl bromide per 4 lit of water were found to control the damping off of seedlings and at the same time did not suppress the mycorrhizal development. Dosage lower than these increases the disease severity and development of mycorrhiza-forming fungi and other beneficial microorganisms in rhizosphere (Lakhanpal et al. 1988). Damping off of seedlings is one of the major problems in the nurseries. But if biocides are used to control diseases or pests, there is retarding effect on the development of mycorrhiza (Hacskaylo and Palmer 1957). Reddy and Mishra (1970) screened nine chemicals and could control damping off disease effectively. However, all these chemicals are costly, whereas methyl bromide and formaline are cheaper.

7.8 Ectomycorrhizal Associates

It has been demonstrated by many workers that most fungi that form ectomycorrhiza with forest trees are members of Basidiomycetes, which produce mushrooms or puffballs as reproductive structures. However, certain Ascomycetes, such as truffles, have also been reported as mycorrhiza formers (Kormanik et al. 1977). Sharma and Lakhanpal (1981); Lakhanpal et al. (1987), Kumar et al. (1990) and Lakhanpal (1996) have reported about 72 species belonging to families Amanitaceae, Agariceae, Hygrophoraceae, Tricholomataceae, Russulaceae, Boletaceae, Strophariaceae, and Paxillaceae to be mycorrhizal with different trees in North-Western Himalayas. Singh and Thapar (1988) identified 29 fungal symbionts which were in intimate association with different plant species and presented a diagnostic key for their identification on the basis of mycorrhizal roots and mantle characters. Raaman and Mahadevan (1988) reported results on the selection of fungi for ectomycorrhizal inoculation in *Pinus patula* nursery at Kodaikanal. The trial seedlings were grown in three types of soil and with *Amanita muscaria, Laccaria laccata, Lycoperdon* and *Scleroderma citrinum.* Out of three soils tried, shale soil was reported to enhance mycorrhizal formation. From among the fungal species listed, *Laccaria laccata* was observed to form abundant mycorrhizae.

Raaman (1988) described succession of ectomycorrhizal fungi in the colonization of *Pinus patula* plantation and reported that *Scleroderma citrinum, Suillus brevipes, S. pallidiceps, S. punctatipes* and *S. subluteus* were dominant up to the age of 3; in the fourth year *Thelephora terrestris* was dominant; from seventh to tenth year *Tricholoma sejunctum* and *Russula parazurea* were abundant and beyond tenth year only *Lycoperdon perlatum* and *Amanita muscaria* were dominant. Sharma (2008) reported 62 ECM-forming mushroom species with forest trees. These belonged to genera *Russula* (2 spp.), *Lactarius* (9 spp.), *Cantharellus* (1 sp.), *Scleroderma* (6 spp.), *Pisolithus* (2 spp.), *Geaster* (5 spp.), *Leccinum* (2 spp.), *Amanita* (6 spp.) and *Boletus* (6 spp.) from Madhya Pradesh and Chhattisgarh. Mohan (2003) investigated the ECM-forming fungi in three species of *Acacia,* two species of *Casurina* and three species of *Eucalyptus* in Southern India and reported the association of *Lycoperdon perlatum, Pisolithus tinctorius, Scleroderma* sp. and *Thelephora ramaridides* with three tree species and observed their frequency

distribution at different plantations sites. Tapwal et al. (2021a) in a recent study could identify 22 species of fungi, the dominant among them being *Geastrum* (4 spp.), *Russula* and *Suillus* (3 spp. each), *Boletus, Ramaria* and *Scleroderma* (2 spp. each) associated with *Pinus gerardiana.* Natarajan et al. (2005) studied the diversity in ectomycorrhizal fungi of a dipterocarp forest in Western Ghats. Species associated with *Dipterocarpus indicus* were in the families Amanitaceae (3), Cortinariaceae (3), Boletaceae (1), Russulaceae (3), with *Hopea parviflora* associated species belonged to only Cortinariaceae (3) and with *Vateria indica*, the associated species belonged to Trichlolomataceae (2), Amanitaceae (2), Boletaceae (2), Russulaceae (9), Sclerodermataceae (1). They commented that the biodiversity of putative ectomycorrhizal fungi associated with dipterocarps occurring in the Uppangala forest in W. Ghats, Karnataka, differs considerably from that of fungi associated with *Pinus patula* plantations in the Nilgiri Hills of the Western Ghats.

Singh (2018) with R.P Bhatt at H.N.B. Gharwal University recorded 39 species to be ectomycorrhizal with different angiospermic and gymnospermic tree species in the Uttarakhand Himalaya. Most of them belonged to Basidiomycota and one to Ascomycota. In a similar study on genus *Russula* in Uttarakhand Himalaya, Ghosh (2019) with R.P. Bhatt and Kanad Das reported 56 species of *Russula* associated with different forest types and considered them to be probable mycorrhiza formers with different forest tree species. Kumar and Atri (2020) presented a detailed discussion on diversity of ectomycorrhizal fungi associated with *Shorea robusta* for the first time from the sal forest in the Shiwalik ranges in N.W. Himalaya. They described 50 mushroom species, out of which 22 ECM types were found directly associated with roots of *Shorea robusta.* Among these, the species of *Russula* were the most predominant ones. They utilized some of these species for artificial mycorrhizal synthesis and evaluation of growth parameters. Kumar (2010) reported four fungi associated with *P. gerardiana.*

7.9 Ecological Aspects of Ectomycorrhiza

Thakur (1990) attempted to correlate moisture percentage, temperature and pH variation with the extent of mycorrhizal development in some important forest tree species. It was observed that in *Abies pindrow* maximum root activity was during the month of July and August when the moisture percentage was 42.9% and 48.4% and the soil temperature ranged from 12.4–14 °C and 12.2–14.1 °C. In *A. spectabilis* maximum root activity was also in July and August like *A. pindrow* whereas in *P. roxburghii* it was in the month of August when the moisture percentage ranged from 32.1% to 37.5% and the temperature ranged from 16.0 °C to 21.4 °C. In *Taxus wallichiana* it was maximum in July and August when the moisture percentage ranged from 44.3% to 55.2% and the temperature ranged from 12.4 °C to 14.5 °C. The pH range was acidic in the first three plants whereas it ranged from acidic to slightly alkaline in *T. wallichiana*. With regard to the soil fractions A_2 (Humus) and A_3 (Decayed wood and charcoal) provided the most frequent substrate for the formation and activity of ectomycorrhizae over a wide range of site conditions.

These fractions of soil had high moisture levels in the organic materials (A_2 and A_3 fractions). The work of Harvey et al. (1976) and Worley and Hacskaylo (1959) support these observations.

Singh (1998) for the first time in India made a detailed investigation on the ecological aspects of mycorrhizal association among different tree species. The investigations were carried out in a pure forest of *P. roxburghii* and a mixed forest comprising different tree species: *C. deodara*, *Pinus wallichiana*, *Rhododendron arboreum*, *Aesculus indica*, and *Quercus leucotrichophora*. Singh and Lakhanpal (2005) observed that environmental factors and soil conditions influence the mycorrhizal association in different ecosystems. These associations are regulated by the features of the host plants and mycorrhizal fungus as well as soil conditions and environmental factors. There is a positive relationship between soil organic matter and ectomycorrhizal activity and the latter is closely related to soil moisture. pH influences the mycorrhizal development and higher mycorrhizal counts have been reported in seedlings at lower pH value. It appears that mycorrhizae form when there is a deficiency of N, P, K or Ca in the soil. The mycorrhizal roots generally lack or have less root hair development and depend upon mycotrophy for nutrient uptake.

7.10 In Vitro Mycorrhizal Synthesis

The studies on mycorrhiza ultimately aim at mass production of inoculum and artificially inoculate the seedlings so that they establish and perform better at disturbed planting sites. Such studies are of limited extent in India but whatever has been done indicates good prospects for future. For isolation of ectomycorrhizal fungi in pure culture sporophores tissue or spores and mycorrhiza have been used and different media tried the world over. The media tried are Mikola (1948), Norkran (1950), Melin and Das (1954), Moser (1958), Rawlings (1933), Fries modified (Pringle and Brawn 1957), and White's modified (Vasil 1959). In India isolation of mycorrhizal fungi was seen best in White's modified nutrient medium. Raaman (1988) reported mass production of ectomycorrhizal spawn of *Laccaria laccata* and *Amanita muscaria* on sorghum grains. Raaman and Thiagarajan (1988) reported 24 °C and 28 °C, respectively, as good spawn growth and 12 h light moderately good for their growth. Reddy and Natarajan (1996) achieved in vitro mycorrhizal synthesis between four pines, *P. patula*, *P. pseudostrobus*, *P. oocarpa* and *P. elliotii* and *Amanita muscaria*, *Laccaria fraterna, L. laccata*, *Pisolithus tinctorius*, *Rhizopogon luteolus*, *Scleroderma citrinum* and *Thelephora terrestris*. All except *A. muscaria* established mycorrhizal association within 2–6 weeks and the degree of colonization varied with different species. Verma et al. (2014) tested eight strains of *Suillus* species for in vitro mycorrhizal synthesis with *P. wallichiana* in N.W. Himalaya. All the strains showed improvement in different growth parameters. They also described a new species of *Suillus*, *S. indicus*, on molecular basis. On the basis of molecular characters Singla et al. (2004) established that the *Pisolithus* species in India is actually *P. albus*. Khosla et al. (2009) also observed that ectomycorrhizal plants were more resistant to aluminium toxicity in their study on *Populus deltoides*.

Sharma and Misra (1988) tested various ectomycorrhizal associates of *Pinus kesiya* for their efficiency in colonization, production and improvement in growth. They reported to have identified certain efficient isolates which could be raised as potential inocula for *P. kesiya* afforestation programmes. Chaudhary and Lakhanpal (1988) also reported results of artificial inoculation of *Pinus gerardiana* seedlings with mycorrhizal associates isolated from the mycorrhizosphere of the natural plants. The inoculated seedlings were reported to develop mycorrhiza after 6 weeks of inoculation and they attained better height and shoot/root ratio (Fresh and dry weight) and exhibited higher survival percentage (Kumar and Lakhanpal 1991). Similar were the results obtained with spruce. In vitro synthesis of ectomycorrhiza between *Pinus wallichiana* and its mycorrhizal associate *S. sibiricus* revealed improvement in different growth parameters, e.g. shoot height, root length, number of short roots, number and percentage of mycorrhizal roots, fresh and dry weight of shoot, root and seedlings, root/shoot ratio (on fresh and dry weight basis) and seedlings volume were recorded at 3-month, 6-month and 1-year sampling stages (Sagar 1993).

In vitro mycorrhizal synthesis between *C. deodara* and *Rhizopogon himalayensi*s revealed that inoculated seedlings/soils exhibited better growth and development of *C. deodara* seedlings. The number of short roots in all inoculated soils was significantly higher than respective uninoculated soils in almost all the sampling stages. Inoculated seedlings in different soils were also observed to attain greater shoot, root and seedling dry weight than uninoculated seedlings. Inoculated seedlings exhibited lower root/shoot ratio (on both fresh dry weight bases) than uninoculated seedlings in some of the sampling. Root collar diameter and seedling volume of inoculated seedlings were also observed to be more than uninoculated seedlings (Singh et al. 2020).

Bakshi (1974) studied the in vitro synthesis of mycorrhiza between *P. patula* and *Scleroderma geaster*. Kannan and Natarajan (1987, 1988) carried mycorrhizal synthesis between *P. patula* and *S. citrinum* and *A. muscaria*. Ray and Adholeya (2008) developed molecular markers for four heavy metal tolerant isolate of *Laccaria fraterna* and *P. tinctorius*.

Similar synthesis has been achieved by Tapwal et al. (2015, 2016) in *Shorea robusta* and *Dipterocarpus retusus* with *Russula amoena* alone and in combination with *Glomus* sp. and a significant increase in growth of colonized seedlings has been observed which is more in the case of *R. amoena*. Similar studies have been carried on the performance of *P. gerardiana* seedlings aseptically inoculated with *Scleroderma polyrhizum* and there has been recorded considerable improvement in all the growth parameters compared to control seedlings (Tapwal et al. 2021b).

Satyanarayanana et al. (1998) discussed the details of the problems and also prospects in the inoculum production of ectomycorrhizal fungi and after in-depth studies recognized *Laccaria laccata*, *Pisolithus tinctorius, Paxillus involutus, Suillus luteus, Hebeloma crustuliniforme* and *Coenococcum geophilum* as the suitable inoculants. Gupta (1998) tested 65 isolates of EM fungi belonging to 13 genera for mass inoculum production and physiological characterization (production of enzymes, siderophores and solubilization of inorganic phosphates and organic

matter). After a thorough screening she observed seven of the strains (2 in *Laccaria*, 3 in *Pisolithus* and 2 in *Rhizopogon*) to be more efficient and better performers based on temperature range, pH and salinity tolerance and recommended them for use in mass production of inoculum by submerged culture.

Jha et al. (2008) evaluated the effect of temperature, light and relative humidity on the mycorrhizal development of pine seedlings. It was observed that pine seedlings inoculated with *Pisolithus tinctorius* attained maximum growth compared to other inoculants under moderate and high light intensity at 25 °C. Relative humidity had little effect on mycorrhizal colonization but affected the seedling survival.

Rajak et al. (2003) reported *Cantharellus minor* forming ECM with *Dendrocalamus strictus*. This species seems to have been regarded as a new species later on, as *C. tropicales*, and Sharma et al. (2011) and Sharma (2008) synthesized in vitro mycorrhiza between these two and reported improvement in all growth parameters.

Pyasi et al. (2013) tested the effect of *Lycoperdon compactum* and *Russula michiganensis* on the seedlings of *Shorea robusta* and observed that only *L. compactum* forms mycorrhiza with the plant roots and also produced fruit bodies near the seedlings. Maximum growth indices were observed in ECM inoculations.

Rao et al. (1996) estimated the efficacy of different mycobionts by inoculating them into *P. kesiya* seedlings grown in forests and degraded soil. All the inoculants exhibited a promotory effect on the growth of pine seedlings in forest and degraded soils. A definite enhancement trend was observed in the seedling growth. In forest soil it was maximum in *Scleroderma aurantium*, and in degraded soil, it was maximum in *S. luteus* and minimum with *C. graniforme*.

7.11 Introduction of Exotic Conifers

Though the introduction of exotics is not encouraged for obvious environmental reasons, at times it may appear to be necessary, especially when fast-growing species are required. Most of our conifer species, especially Pines, are not tropical, and hence introduction of tropical pines has been tried in some places. These exotic species of conifers have been introduced in India to meet the increasing demand of industrial wood. Seth (1972) summarized the results of trials of tropical and subtropical pines in India and concluded that it is mainly the subtropical pines, namely, *Pinus patula, P. kesiya, P. pseudostrobus* and *P. caribeae*, which offer scope for introduction in India. Looking at the necessity of mycorrhizal requirements in afforestation/reforestation programmes, it is now recommended strongly that mycorrhizal inoculum be introduced in the nurseries for establishment and better growth of seedlings. Many workers have attributed the low survival of out plantings to inadequate mycorrhiza. Bakshi et al. (1972) observed that poor regeneration of *Abies pindrow* is due to poor development of mycorrhiza besides many other factors. For regeneration of conifers, nurseries have been established in natural forest zones. Workers have tried to introduce mycorrhiza by soil inoculum, mycorrhizal seedlings

and pure culture techniques. But their methods need standardization in order to inoculate nursery seedlings successfully.

7.12 Nursery Management Practices

It has been observed that nursery management practices carried out in the nursery influence the development of mycorrhiza in seedlings (Bakshi 1971). Many of such studies have been carried out by different workers. Thaper and Singh (1988) highlighted the effects of cultural practices in mycorrhizal development in pine seedlings. They reported that biocides added to the soil, e.g. blitox, zineb, cumin, captan, thride, brassicol, zinc oxide, feroxone and gammexena, had a retarding effect in the development of mycorrhiza which was offset with age. The shading of nursery beds was also reported to have adverse effect in mycorrhizal development. There was noticed a change in the fungal symbionts or different levels of soil moisture. Both mycorrhizal development and increased growth were obtained at 55% soil moisture.

It has been observed that there is a direct correlation between light intensity and mycorrhizal infection (Harley and Waid 1955; Hacskaylo and Snow 1959). Bakshi (1974) studied the effect of shading of nursery beds on the development of mycorrhiza in seedlings. They have shown that soluble carbohydrates in entire root system are directly correlated with light which again is correlated with the development of mycorrhiza.

Kumar et al. (1968) studied the effect of fertilizers on the development of mycorrhiza and concluded that addition of compost/fertilizers individually or in combination produced a depressing effect on the production of short roots and also their infection by mycorrhiza-forming fungi.

Reddy and Khan (1972) carried out experiments to find out whether soil inoculums from hardwoods could be used, for introducing mycorrhiza in conifers and vice versa. They concluded that even though mycorrhiza developed in all treatments, plants receiving the lower symbionts from the same showed better growth and this indicated that differences existed among lower symbionts as regards uptake of nutrients.

That many mycorrhizal fungi are host specific has been recognized (Trappe 1977). Some fungal species such as *Amanita muscaria*, *Pisolithus tinctorius* and *Laccaria laccata* are having broad host range, whereas certain *Suillus* and *Rhizopogon* species fruit only in association with particular host species.

7.13 Field Performance of Seedlings

Tapwal et al. (2021b) conducted field trials of *P. gerardiana* seedlings at two sites, one in Kinnaur district and the other in Lahaul Spiti. The former is a natural zone of the Chilgoza pine whereas the other is not. At both sites most of the seedlings survived winter season and the percent survival was higher in the inoculated

seedlings. The survival percentage was more in the natural zone than the other zone, but in both the places the growth parameters were greatly improved than the control. It is worth mentioning that till date most of the efforts on regeneration of *P. gerardiana* have not been successful. This speaks of the importance of mycorrhizal tailoring of the seedlings which have been tried for the first time.

Similar trials have been conducted on *Cedrus deodara*, *Pinus gerardiana* and *Taxus wallichiana* (Kumar, Singh and Lakhanpal, unpublished) and they all have performed very well at respective sites in district Mandi, Kinnaur and Badrinath, respectively. At Badrinath in Badrivan, under a project from G. B. Pant Institute of Environment and Development, more than 20,000 mycorrhizically tailored saplings of *Taxus wallichiana* were planted by the G.B. Pant Institute which have established very well and are performing quite well under field conditions and have grown more than 20–30 ft. during the last almost 20 years. They all were mycorrhizically tailored species (Singh and Lakhanpal, unpublished).

7.14 Future Strategies

The review of status on the mycorrhizal research in India indicates that we still know little about the mycorrhizal association/requirements of many of our important trees on country basis and there is a need for concerted and coordinated efforts at national level. The future studies should lay emphasis on both basic and applied aspects. There are limited studies on field performance. Techniques have been devised and standardized for production of mass inoculum of mycorrhizal fungi but have been little used at field level. Survey of fungi which are associated with different tree species is equally essential. It would then be required to select more efficient strains of the mycorrhiza-forming fungi and to evaluate them for physiological parameters, especially rate of growth under local conditions. This should be followed by defining methods and conditions to successfully inoculate nursery seedlings and to replace their wild inoculation (duff) presently being followed, by pure culture inoculations with ectomycorrhizal fungi. Further if by artificial inoculation the transplanting period of seedlings of different plants is reduced even by 1 year, there would be lot of saving in terms of time, money and energy. Molecular techniques for identification of mycorrhizal fungi in the absence of fruiting bodies and cultures need to be applied and standardized.

Though mycorrhizal synthesis and glasshouse trials have been successful, there is need to extend these to field trials and assess the performance of seedlings in the field. The basic studies are essential but implementing the lab trials on land is truly indicative of the success of nursery trials. There has been and still is less effort in this direction.

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History and Development of Arbuscular Mycorrhizal Research in India

8

D. J. Bagyaraj, T. Muthukumar, and R. Ashwin

Abstract

Frank coined the term "mycorrhiza" in 1885 and showed that it is a symbiotic association between plants and certain fungi. Prehistory is the report of this association earlier to coining of the term mycorrhiza. The prehistory and history of arbuscular mycorrhizal fungi (AMF) globally is covered briefly. Tracing the history of AMF in India, the occurrence of AMF was first reported by Bakshi from Forest Research Institute, Dehradun. Systematic studies on AMF were initiated in the 1970s by a few institutions in India. There was an upsurge of interest in 1980s and many laboratories started working on AMF. In the 1990s lesser number of institutions initiated work on AMF compared to the 1980s. From 2000 and later the number of laboratories initiating work on AMF further came down, though some of the institutions which initiated the work in the 1970s, 1980s, and 1990s still continue to work on AMF. The work of Indian scientists on AMF covered the areas like taxonomy, diversity, plant growth promotion, ecology, biological control of plant pathogens, alleviation of abiotic stresses, in vivo and in vitro mass production and development of microbial consortia with PGPR, thereby reducing the NPK fertilizer application to crops in the field. We hope this will be of interest to scientists working on AMF.

Keywords

AMF · Biocontrol · Crop productivity · Diversity · Prehistory · History

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8.1 Introduction

The term mycorrhiza, coined by a German botanist AB Frank in 1885, is derived from the combination of two Greek words which literally means fungus root (Frank 1885). There are mainly four different types of mycorrhiza. (1) ectomycorrhiza, (2) arbuscular mycorrhiza, (3) ericoid mycorrhiza, and (4) orchid mycorrhiza. The latter three types of mycorrhiza are jointly referred to endomycorrhiza by some scientists. In ectomycorrhiza the fungus grows intercellularly in the cortex of the plant root (called Hartig net), but never intracellularly. In the endomycorrhiza, mainly the arbuscular mycorrhiza, the fungus grows inter- and intracellularly and forms within the cortical cells specific fungal structures. In ectomycorrhiza often a thick hyphal mantle is formed around feeder roots and these roots are morphologically altered; this is a characteristic often used for identifying the type of mycorrhiza. Ectomycorrhiza are commonly associated with temperate forest tree species and nutrient cycling processes in forest ecosystems. In contrast to ectomycorrhiza, arbuscular mycorrhiza (AM) colonization does not change the morphology of the root. To determine AM colonization, microscopic observation of the roots after staining is necessary (Bagyaraj 2014).

The common endomycorrhizal association in most of the plants is the arbuscular type occurring in the majority of the agricultural crops and tropical tree species. Hence, this is dealt in detail. It is easier to list plant families that do not form AM than to list those that do. Families which do not form AM include Pinaceae, Betulaceae, Orchidaceae, Fumariaceae, Commelinaceae, Urticaceae, and Ericaceae. Some plant families rarely forming AM include the Brassicaceae, Chenopodiaceae, Polygonaceae, and Cyperaceae. Families forming both ectomycorrhizae and AM include Juglandaceae, Tiliaceae, Myrtaceae, Salicaceae, Fagaceae, and Caesalpiniaceae. Further the AM association is geographically ubiquitous and occurs in plants growing in arctic, temperate and tropical regions. It occurs over a broad ecological range from aquatic to desert environments. Arbuscular mycorrhizal fungi (AMF) belong to the phylum Glomeromycota with one class Glomeromycetes with four orders (Glomerales, Diversisporales, Paraglomerales, and Archaeosporales), 11 families and 25 genera (Redecker et al. 2013). The commonly occurring genera of AMF are *Glomus, Gigaspora, Scutellospora, Acaulospora,* and *Entrophospora.* These fungi are obligate symbionts and have not been cultured on nutrient media. AMF are not host-specific although evidence is growing that some of them exhibit preferential association with certain host plants. AMF produce large thick-walled resting spores called extramatricular chlamydospores in soil which can survive adverse conditions and germinate when conditions are favourable. The germ tubes die unless they encounter and successfully come in contact with the host root. After forming an appressorium on the root surface, the hypha penetrates the root and ramifies in the root cortex. Branches from the longitudinally running intercellular hyphae enter cortical cells and develop short haustoria-like branched hyphal structures called arbuscules. Vesicles are formed in the cortical cells, which are thin-walled structures of various sizes and shapes containing oil droplets and

function as storage organs. The presence of arbuscules and vesicles is the criteria for identifying AMF in the roots (Bagyaraj 2014).

The experiments conducted earlier in sterilized soil and under controlled conditions showed that AMF inoculation could improve plant growth. Since most of the natural soils usually harbour AMF, it was felt that plants may not respond to inoculation with selected AMF, in unsterile soils. Later investigations indicated that even in unsterile soils, plants do respond to inoculation with efficient strains of AMF. Now it is proved beyond doubt that AMF inoculation improves plant growth. The growth increase is favoured in soils with low to moderate fertility, especially phosphorus in limiting concentrations. It is well known that AMF are not host-specific. Though a particular AMF can infect and colonize many host plants, it has a preferred host, which exhibits maximum symbiotic response when colonized by that particular AMF. This led to the concept of "host preference" in AMF, and in turn the procedure for screening several AMF and selecting an efficient fungus for a particular host. This in turn led to the selection of inoculant AMF for many crops important in agriculture, horticulture, and forestry. AMF are obligate symbionts. Attempts to culture AMF on artificial media have met with little or no success. At the present time, the only method to produce these fungi is in association with the host plant root. Other techniques to produce AMF inoculum in an almost sterile environment through nutrient film technique, circulatory hydroponic culture system, root organ culture, and tissue culture are also available. However, for mass production, the most convenient method is by the traditional pot culture technique (Bagyaraj 2014; Chen et al. 2018).

AMF inoculum of suitably selected strains can be used for inoculation in the nursery bed. Growers only need to incorporate inoculum in the nursery beds or seedling trays at the appropriate rate manually. Seedlings thus raised will be colonized by the introduced fungus and then can be planted out in the field. There are several reports of increased growth and yield of food, fodder, and fuel crops because of inoculation with efficient AMF. These studies also brought out that because of inoculation, nearly 50% of phosphate fertilizer application could also be reduced. Some horticultural plants are propagated through cuttings. In such cases, the rooting of cuttings is important. Enhanced rooting of cuttings through inoculation with AMF has been reported. AMF-inoculated plants withstanding transplant shock has also been reported in cashew. Studies also showed a high percentage of grafting success in cashew. Soilless media like vermiculite, perlite, potting mixes, etc. are used for raising several horticultural crop plants. Such soilless media are usually fumigated or heat sterilized. Inoculation of such soilless media enhancing seedling growth and finally yield has been reported in woody ornamentals and asparagus. Inoculation of micropropagated plantlets with AMF after hardening also improved plantlet vigour and growth in several cultivated plants (Bagyaraj 2014; Chen et al. 2018).

8.2 Prehistory and History of Arbuscular Mycorrhiza

History is the recorded events of the past. Frank coined the term "mycorrhiza" in 1885 to denote the association between plant roots and fungi. Prehistory, then, means those events that occurred before the term "mycorrhiza" was coined.

8.2.1 Prehistory

Nägeli C in 1842 described fungal hyphae in the roots of *Iris* sp. Holle JG in 1875 reported the presence of certain endophytic fungi within fern roots (Holle 1875). Mollberg in 1884 recorded certain fungi in the roots of plants belonging to the family Liliaceae (Mollberg 1884). Treub in 1884 and Bruchmann in 1885 observed fungi in the roots of ferns and pointed out that they do not cause any harm to the host (Trappe and Berch 1985).

8.2.2 History: Global

Janse (1897) carried out an extensive survey of AMF association in Africa and Java. He was the first person to coin the term "vesicles" to the globular structures produced by the fungus in the root cortex. Gallaud (1905) named highly branched hyphal structures in the cortical cells as "arbuscules". Peyronel (1924) reported that the hyphae of AMF in soil are much thicker than the hyphae seen within the root. Barbara Mosse in the 1950s working at Rothamsted Experimental Station, England, contributed a great deal to AMF research in understanding morphology, physiology, host response to inoculation, inoculum production, etc., and hence referred to as "*Mother of mycorrhiza studies*" (Mosse 1953). Gerdemann JW in the 1960s from the USA contributed to different aspects of AMF symbiosis (Gerdemann 1961). He developed the technique for isolating AMF spores from the soil. Harley (1959) from the UK published the first book on "The Biology of Mycorrhiza". The second edition of the book was published in 1969. Philips and Hayman (1970) from the UK developed staining technique for observing mycorrhizal root colonization. Porter (1979) from Australia developed a method for determining the infective propagule numbers in the sample by MPN technique. Tinker PB in 1970s from the UK using ^{32}P labelled P showed that AMF hyphae can travel up to 8 cm away from the root system (Sanders and Tinker 1971). Bowen in the 1970s from Australia contributed a great deal on the ecology of AMF. Work of Schenck NC and Trappe JM; Walker C, Morton JB, and Benny GL; and Thaxter RA and Hall I in the 1980s and 1990s on taxonomy of AMF are commendable. Schüβler A (2001) used molecular data for studying taxonomy of AMF. The international culture collection of AMF (INVAM) in the USA and the International Bank for Glomeromycota (BEG) in Europe were established in 1985 (Koide and Mosse 2004). The classical work of Barbara Mosse from England; Baylis GTS and Powell CL from New Zealand; Gerdemann JW and Habte M from the USA; Abbott LK and Robson AD from Australia; Barea JM and

Azcón-Aguilar C from Spain; and Gianinazzi Pearson V from France in the 1970s clearly brought out that AMF help plant growth, nutrition and productivity through uptake of diffusion-limited nutrients from the soil. Similarly in the 1980s classical work on ecology of AMF by Barbara Hetrick, Allen M, Bethlenfalvay GJ, and Wright SF from the USA and van der Heijden MGA from the Netherlands brought out its role in ecosystem functioning. Harley JL and Smith SE brought out the book entitled "Mycorrhizal Symbiosis" in 1983. The first book devoted solely on AMF entitled "VA Mycorrhiza" was edited by Powell CL and Bagyaraj DJ in 1984 (Koide and Mosse 2004). Studies on AMF inoculation improving plant growth was initiated and studied extensively by several scientists. The names which deserve mention are Barbara Mosse and Christine Hepper from the UK; Trappe J, Menge J, Molina R, Gerdemann JM, Schenck NC, and Nemec S from the USA; Fortin A, Furlan V, and Shannon Berch from Canada; Gianinazzi Pearson and Garbaye J from France; Bowen G, Lynette Abbott K, and Robson AD from Australia; Siqueira JS from Brazil; Ogawa M from Japan; Paola Bonfonte-Fasolo from Italy; Wiemken A, Boller T and Mader P from Switzerland; Yenchai Vasuvat and Hyde KD from Thailand; Khan AG from Pakistan who moved to Australia later and Ikram A from Malaysia. The work of Karen Cooper from New Zealand and Tinker FE from the UK and Smith SE from Australia on physiology of AMF gave an impetus to scientists to pursue research in this area. Linderman RG, Hayman DS, Daft MJ and Barea JM studied the interaction of AMF with other soil organisms. Biological control of plant pathogens by AMF was investigated extensively by Schenck NC, Sikora RA, Menge J and Davis RM. Some of the scientists intensively working on different aspects of AMF currently include Joanna Dames from South Africa, Joyce Jefwa M from Kenya; Khasa D, Chantel Hamel, and Quoreshi A from Canada; Junling Zhang, Liang-Dong Guo and Cheng Qin from China; Nancy Johnson from the USA; Maarja Opik from Estonia; Rillig M from Germany; Sturmer S from Brazil; Ashraf M and Ahmed N from Pakistan and many others as it is difficult to mention all the names.

8.2.3 History: India

The work initiated in the 1970s and continued onwards: Mycorrhiza research in India was initiated in the 1950s under the leadership of Bakshi K at Forest Research Institute, Dehra Dun. Surveys to find out the occurrence of AMF were carried out. AMF association was observed in 8 conifers and 28 broad-leaved forest species. Their spore population was more in summer (Bakshi 1957). Thapar HS from the same institute found that exotic conifers and hardwoods established AMF with native endophytes (Thapar and Uniyal 1990). He reported that *Glomus macrocarpum* to be the most common AMF in forest soils. The work was further continued later by Kamala Uniyal, Harsh NSK, and Singh HP. They studied the role of AMF in establishing forest tree species in saline/alkaline soils and also the interaction between AMF and rhizobia in producing healthy vigorously growing forest tree seedlings (Harsh and Kumar 2015). In the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, systematic studies on practical aspects of AMF in the country was initiated in the year 1975 by Bagyaraj

DJ. AMF in several plants, including aquatic plants, for the first time in the world, was reported. AMF could improve plant growth through increased uptake of diffusion-limited nutrients like phosphorus (P) and water was described. The "host preference" in AMF was demonstrated which lead to screening and selecting efficient AMF for inoculating crop plants leading to saving of P fertilizer up to 50%. Considerable contribution was made on the ecology of AMF in tropics. The synergistic interaction between AMF and plant growth-promoting rhizobacteria (PGPR) improving crop productivity was shown under field conditions. A mycorrhiza helper bacterium (MHB) was isolated which enhanced the activity of AMF. Manjunath A, Mallesha BC, Harinikumar KM, Padmavathi T, and many others were associated with this work. The group developed microbial consortia (AMF+PGPR) for combating abiotic and biotic stress in plants. A protocol for mass production of AMF was also developed. A method for farmers to raise their own AMF inoculum in the field was also demonstrated (Godse et al. 1976; Bagyaraj 1984, 2013; Raghu et al. 2020).

Mishra RR in 1979 from North-Eastern Hill University, Shillong initiated work on AMF which was continued later by Sharma GD, Kayang H, and Jha DK (Jha et al. 1993; Panna and Kayang 2009). Extensive investigation on the ecology of mycorrhizal association in timber trees, weeds, and medicinal plants and their role in plant nutrient uptake was carried out by them. Further, they studied the effect of heavy metals on AMF association and the role of AMF in overcoming heavy metal toxicity. Tripartite interaction between AMF, *Frankia,* and *Alnus* was studied. Their work included the diversity of AMF associated with crop plants and coal mining areas of Meghalaya. *Pacispora* and *Ambispora* were recorded from this region. Mukerji KG in the 1970s from Delhi University established an excellent school on fine structure and taxonomy of AMF. He has extensively worked on various aspects of AMF symbiosis such as role of AMF in sustainable cultivation of many agricultural and horticultural crops, and their role in abiotic and biotic stress alleviation. He also worked on afforestation of arid and semi-arid regions of India using AMF primed multipurpose leguminous tree seedlings (Bhattacharjee et al. 1970, 1980; Mukerji and Kapoor 1986). Manju Gupta and Rupam Kapoor continued the work. Rupam Kapoor has shown increased secondary metabolite production in aromatic and medicinal plants because of AMF inoculation. It was observed that AMF enhanced the transcript levels of genes encoding upstream rate-limiting enzymes that facilitate the utilization of universal precursors. Her group has also worked on biochemical and physiological mechanisms involved in the amelioration of salt stress in mycorrhizal plants (Kapoor et al. 2017). Manju Gupta M has been studying intensively different aspects of AMF such as distribution, phylogeny, taxonomy, and its interaction between rhizosphere microorganisms (Gupta et al. 2017).

Jalali BL in the 1970s from Haryana Agricultural University surveying soils for AMF observed more root colonization and spores in nutrient-deficient soils. Inoculation of plants with efficient strains of AMF improved growth and P uptake. Fungicides like Brassicol, Emisan, and Bavistin depressed AMF. AMF reducing the severity of the disease caused by many root pathogens was also reported by him (Jalali and Domsch 1975; Jalali and Jalali 1991). Swaminathan K and Verma BC at

ICAR-Central Potato Research Institute, Shimla initiated the work in the late 1970s which was continued later by Rai RP. They surveyed the AMF associated with potato crop grown in hills of North India and also Indo-Gangetic plains. The results confirmed the association with three AMF genera (*Acaulospora*, *Glomus,* and *Gigaspora*), with *Glomus* being most dominant. Inoculation of potato with AMF brought out its role in improving plant growth through P nutrition (Swaminathan and Verma 1977; Rai 1989). Singh VK and Pandey S, Rajendra Agricultural University, Samasthipur, studied mycorrhizal symbiosis in litchi. They brought out that the evolution of CO_2 from the root is positively correlated with the intensity of mycorrhizal colonization (Pandey and Singh 1980).

The work initiated in the 1980s and continued onwards: Ajit K. Varma in the 1980s from Jawaharlal Nehru University, New Delhi, investigating the AMF of Aravalli Hills and xerophytes reported *Melia azadirachta* harboured *Gigaspora* sp. in the root system, while *Ficus bengalensis* and *Murraya paniculata* harboured *Glomus* sp., thus showing host preference by these fungi. A new species *Gigaspora coralloidea* occurring in xerophytes was reported. Later he moved to Amity University, Noida, and continued the work on AMF. He wrote a report on "History on mycorrhiza research in India—Current Status" in 1991 and also edited few books on AMF (Varma 1991, 1998). Sitaramaiah K from GB Pant University of Agriculture and Technology, Pantnagar, carried out extensive studies on the interaction between AMF and root-knot nematodes and the influence of AMF affecting the microorganisms in the rhizosphere. The work on different aspects of AMF was later continued by Johri BN, Anil K Sharma, and Singh HP. Their studies showed that AMF help in P and Zn nutrition, and also help to alleviate stress effects in water-stressed plants through stomatal regulation. Studies on the interaction between AMF and PGPR were also undertaken (Sharma et al. 1992; Singh et al. 1990a, b, c). Subba Rao NS and his associates Tilak KVBR and Singh CS from ICAR-Indian Agricultural Research Institute, New Delhi, studied the interaction between AMF and N-fixing bacteria, especially *Azospirillum.* Synergistic interaction between the two organisms was observed. They also studied the photoassimilate partitioning and translocation in sorghum colonized by AMF. The nitrate reductase activity of AMF suggested that it may play a role in nitrogen assimilation and translocation to the host plant (Rao et al. 1985, 1986).

Peter Dart and Krishna KR initiated the work on AMF at International Crops Research Institute for Semi Arid Tropics, Hyderabad. They found that different plant genotypes within a single species varied in their ability to harbour AMF. This suggested that during breeding programmes inheritance patterns of AMF colonization may be worth looking at (Krishna and Dart 1984). Kandasamy D and Santhanakrishnan P followed by Kumutha K and Balachander D from Tamil Nadu Agricultural University, Coimbatore, carried out Intensive studies on plant growth response to AMF inoculation. Other areas of study included the interaction of AMF with other beneficial soil microbes, biological control of soil-borne plant diseases using AMF, alleviation of salt and heavy metal stresses, and possibilities of reducing P fertilizer application through AMF inoculation. The molecular diversity of AMF in subtropical soils of Coimbatore was also carried out. Subramanian KS later studied

biofortification of Fe and Zn in cereals using AMF symbiosis (Kandasamy et al. 1986; Suchitra et al. 2012). Rohini Iyer and George V Thomas from Central Plantation Crops Research Institute, Kasargod, investigating the AMF association in plantation crops like coconut, cacao, cinnamon, and black pepper found *Gigaspora gilmori* to be the dominant fungal associate. Spores were abundant in poor soils and younger roots showed a higher percentage of root colonization. The occurrence of mycorrhiza in coconut was reported for the first time from Kayangulam Centre of this institute (Thomas 1988; Iyer and Moosa 1993).

Lakshminarasimhan C, Kannan K, and Selvaraj T from AVVM Sri Pushpam College, Poondi, studied the role of AMF in sugarcane and medicinal plants (Kannan and Lakshminarasimhan 1990). Ali SS and Nibha Gupta, Ravishankar University, Raipur, initiated studies on diversity and application of AMF in agriculturally important crops and forest tree species growing in different agroclimatic zones of Chhattisgarh. Incidence of AMF in mangrove and saline environment, tea gardens, and their application as plant growth promoters under different soil types and stress conditions have been investigated (Gupta and Ali 1996; Gupta et al. 2016). Sarma YR and Anandaraj M from ICAR—Indian Institute of Spices Research, Kozhikode, brought out a suppressive effect of AMF against root-knot nematodes in black pepper which was comparable to phorate treatment. They screened and identified the most effective AMF for inoculating black pepper and further developed a nursery technology with AMF to take care of foot rot disease caused by *Phytophthora capsici* which is a threat to black pepper cultivation. They also used AMF for inoculating other spice crops like ginger, clove, turmeric, etc. (Anandaraj and Sarma 1995).

Manoharachary C and Rama Rao P started the work on AMF at Osmania University, Hyderabad, and contributed to AMF diversity, occurrence, ecology, and taxonomy. Three new AMF species, viz., *Glomus hyderabadensis*, *G. indica,* and *Acaulospora terricola*, were reported. Extensive studies on AMF associated with oilseed crops especially in nutrient-deficient soils were carried out. The study conclusively proved that AMF inoculation improved not only crop growth and sustenance but also oil yield. The distribution of AMF in soils polluted with sewage and industrial effluent was investigated by them. They also studied the effect of AMF and rock phosphate on phosphatase activity in a few forest tree species. Bhadraiah B and Kunwar IK also contributed to these findings (Kanakadurga et al. 1990; Rani et al. 2004; Manoharachary 2004). Sudhir Chandra and Harbans Kehri K, University of Allahabad, Prayagraj, did extensive work on AMF inoculation in flowering, vegetable, and medicinal plants raised on metal-polluted and salt-affected soils. Improved performance of the plants because of AMF inoculation was observed. Dual inoculation of AMF with *Rhizobium* and *Azotobacter* improved the performance of legumes, oilseed crops, and medicinal plants. Work on AMF in curtailing the root pathogens in crop plants was also undertaken (Akhtar et al. 2019; Chandra et al. 2009). D Maiti, ICAR-Central Rainfed Upland Rice Research Station, Hazaribagh, studied the AMF responsiveness of upland rice varieties in nutrient-deficient soil. He also worked on the possibilities of enhancing the native AMF

association for the improvement of upland rice under rainfed agroecosystem (Maiti and Barnwal 2012).

Potty VP, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, investigated the interaction between AMF and phosphate-solubilizing bacteria in tuber crops. He developed AMF technology for enhancing the production of tropical tuber crops. Cassava was suggested as an alternate host for mass multiplication of AMF. Lignite slurry with AMF spores was suggested as a method for inoculating tuber crops (Potty 1985). Katiyar RS and Das PK, Central Sericulture Research and Training Institute, Mysore, clearly brought out that inoculation with efficient AMF can become a great boon to mulberry cultivation through curtailing the application of P fertilizer (Katiyar et al. 1990). Mehrotra VS, University of Allahabad, Prayagraj, did extensive studies on the taxonomy of AMF. In a newly described species *Glomus sterilum,* he showed the presence of sterile sporocarps, with well-developed pseudoparenchymatous peridium. He also described a new species *Glomus bagyarajii,* which was later found to be the best AMF symbiont for inoculating the medicinal plant *Coleus forskohlii.* The effect of single and mixed inoculation AMF on growth and yield of sunflower was also studied (Mehrotra 1997; Mehrotra and Baijal 1992). Sharma MP, ICAR-Indian Institute of Soybean Research, Indore, initiated his research on AMF at Maharana Pratap University of Agriculture and Technology, Udaipur. His main contributions are interaction between AMF and root-knot nematodes and role of AMF in the establishment of forest plants in arid lands and heavy metal-polluted soils. Currently, he is working on role of AMF and rhizobia in soybean productivity and crop and soil management practices with AMF to study soil C sequestration. High-throughput method for assessing fatty acid signature biomarkers for studying AMF live biomass and PLFA microbial community was standardized by him (Sharma et al. 1996, 2012).

Sujan Singh, Sunil Khanna, and Alok Adholeya at The Energy Research Institute, New Delhi, started studying the role of AMF in improving plant growth in many crop plants and forest tree species. They studied the possibility of cultivating *Jatropha curcas* in fly ash overburdens using AMF. Later production of AMF through root organ culture was developed at this institute. They also brought out that AMF is a potential tool for inoculating plants for the successful restoration of degraded ecosystems. Global assessment of AMF diversity revealed a very low endemism. Reena Singh and Sankar TP also joined in this contribution. Molecular characterization of a few AMF has been carried out. Industrial overburden reclamation technology using AMF developed by them is an important achievement. The institute also brings out the periodical newsletter "Mycorrhiza News". This institute has a mycorrhiza culture collection (Lal et al. 1990; Davison et al. 2015). At Punjab Agricultural University, Ludhiana, work on AMF was carried out by Singh RS, Kang MS, Singh V, Chahal PPK, and Chahal VPS. They studied the response of different crop plants important in agriculture and horticulture to inoculation with AMF. They also studied in detail the effect of fungicides on AMF and the role of AMF in the biological control of soil-borne pathogens. Later Gosal SK, Sharma S, and Kaur R started working on the role of AMF in crop productivity (Singh et al. 1990a, b). Laxmanan M and Gunasekaran P, Madurai Kamaraj University, Madurai,

studied the effect of pH, temperature, and nutrients on the germination of the AMF *Glomus fasciculatum* in vitro. The diversity of AMF associated with cereals was also studied. Extensive studies on the activation of phenol metabolism during AMF symbiosis was carried out by them (Gunasekaran et al. 1987).

Chakraborty BN and Chakraborty U, University of North Bengal, Siliguri, developed polyclonal antibody-based immunological diagnostic kits using indirect immunofluorescence for two dominant AMF associated with tea and mandarin. Based on 16S rDNA sequence analysis, *Bacillus flexus* and *Bacillus mycoides* have been identified as MHB associated with AMF. Induced immunity in tea and mandarine plants following successful AMF root colonization against root pathogens has been demonstrated. Immunogold localization of defence enzyme (chitinase) both in tea and mandarin roots following colonization with AMF and challenge inoculation with the fungal pathogen(s) was confirmed through transmission electron microscopy (Bhutia et al. 2012; Chakraborty and Chakraborty 2012). Venkateshwarlu B, ICAR—Central Research Institute for Dryland Agriculture, Hyderabad, studied the role of AMF in promoting the growth of different millet crops and also the effect of pesticides on AMF symbiosis (Manga et al. 1985). Sullia SR and Anusuya D, Bangalore University, Bengaluru, screened and selected the best AMF for inoculating many medicinal plants. Detailed investigations on the effect of different fungicides, insecticides, and herbicides on AMF association were carried out. The mutualistic symbiosis of AMF and *Trichoderma* on micropropagated flowering plants was also investigated (Sullia and Anusuya 1992). Kothandaraman R and Kochutheresiamma J from Rubber Research Institute of India, Kottayam, did extensive studies on screening and selecting the best AMF for inoculating rubber seedlings in the nursery. They also studied AMF association in cover crops in rubber plantations (Kochutheresiamma et al. 1988).

Chaudhuri S from Bidhan Chandra Krishi Viswa Vidyalaya, Kalyani, did extensive investigations on AMF associated with mangrove plants. He brought out that AMF is sensitive to salinity and inundation stress explaining the edaphic and physiographic differences between estuarine and maritime mangrove habitats. The importance of AMF in cultivating bamboo in laterite wasteland was brought out. His studies proved that mandarine orange is an AMF-dependent crop and the extent to which it is colonized is related to available P in soil (Panja et al. 1990; Sengupta and Chaudhuri 2002).

The work initiated in the 1990s and continued onwards: Sharma JK from Kerala Forest Research Institute, Peechi, initiated the work on AMF in forest tree species which was continued later sincerely by Sankaran K, Mohanan V, and Maria Florence. They studied the mycorrhizal status, diversity, and dependency of 23 plantation tree species grown in Kerala. A remarkable diversity of AMF and population in each host species was encountered. Soil pH resulted in around 35% of the variability in AMF root colonization in teak. Nursery trials brought out significant improvement in the planting stock of tree species through AMF inoculation. Mycorrhizal status of different species of bamboo cultivated in Kerala was also investigated (Sharma et al. 1995; Mohanan and Sebastian 1999). Reddy SM, Kakatiya University, Warangal, investigated the occurrence of AMF in soils polluted with tannery

and paper mill effluents. He also studied the mycorrhizal dependency of multipurpose tree species in coal mine spoils (Srinivas et al. 1998).

At Tropical Forest Research Institute, Jabalpur, Jamaluddin systematically started studying AMF in tropical forest trees. The role of AMF in tissue culture raised teak and bamboo, mass production of AMF, and its application in forest nurseries was highlighted. The work brought out the role of AMF in the development of plants important in forestry in disturbed low fertile soil (Chandra and Jamaluddin 1999). Subhashini DV, ICAR—Central Tobacco Research Institute, Rajahmundry, carried out extensive studies on the role of AMF in tobacco cultivation. The main contributions are (a) methods of inoculating tobacco with AMF and plant growth response, (b) phosphorus economy, yield, and quality of flue-cured tobacco, (c) genotype-dependent variation in AMF colonization, (d) interaction between AMF and PGPR in promoting tobacco growth and nutrition, and (e) biological control of damping-off disease caused by *Pythium aphanidermatum* and root-knot nematodes (Subhashini and Padmaja 2012). At Mahatma Phule Agricultural University, Rahuri, BK Konde did extensive studies on AMF in crop plants. Carrying out interaction studies between AMF and N-fixing bacteria it was concluded that dual inoculation is better in improving plant growth. The economic analysis suggested that dual inoculation resulted in a higher cost-benefit ratio over control and single inoculation (Konde et al. 1998).

Janardhanan KK, Central Institute of Medicinal and Aromatic Plants, Lucknow, studied the role of AMF in several medicinal and aromatic plants particularly Palmarosa and mint. AMF association significantly enhanced growth, biomass, and secondary metabolites in these plants. The diversity of AMF associated with these two plants was also investigated (Gupta and Janardhanan 1991). Currently, Kalra A, Khaliq A, Pandey R, and Singh R are investigating the role of AMF in improving the growth and productivity of many medicinal and aromatic plants. Further, the role of AMF in the biocontrol of soil-borne plant pathogens has given promising results (Gupta et al. 2000). Nair SK and Sivaprasad P, Kerala Agricultural University, Thiruvananthapuram, studied the role of AMF in plantation crops and forest tree species grown in Kerala. Extensive studies on the role of AMF in the cultivation of plantation crops with special reference to its role in the biocontrol of soil-borne plant pathogens were carried out (Sivaprasad et al. 1990). Lakshman HC, Karnatak University, Dharwad, studied AMF associated with medicinal plants and crops commonly grown in black cotton soils and the effect of season on their diversity. He also brought out the role of insects and domestic fowls in spore dispersal of AMF (Lakshman and Raghavendra 1990). Lizzie Nair N and Shinde BP of Poona University, Pune, studied the diversity of AMF and their role in improving crop productivity in Maharashtra. Physiological changes in the host because of AMF colonization were also studied which led to the role of AMF in protecting plants against water stress. They also contributed to the role of animals in the dissemination of AMF (Shinde and Nair 1996). Later Sujata Bhargava and Mahesh Borde continued the work and studied the influence of AMF on growth and biochemical changes in crop plants under salinity stress.

Rao AV and Tarafdar JC, ICAR-Central Arid Zone Research Institute, Jodhpur, found that *Glomus mosseae* is the best AMF for arid crops and *Glomus fasciculatum* to be the best for arid trees. For seedling propagation, *Glomus fasciculatum* was found to be the best with a spore load of 400 per polybag holding 1 kg soil. AMF can be very effective in transporting relatively immobile nutrients such as P, Zn, and Cu. Besides transporting of available inorganic P, AMF can release both phosphatases and phytase to mobilize unavailable organic phosphorus for plant nutrition. The status of AMF population under desert depends on soil depth, soil organic carbon, moisture content, and site of plantation. *Glomus* was the predominant AMF under Thar Desert environment. AMF improved the therapeutic value of safed musli tuber by stimulating the saponin content. It can also enhance drought tolerance of the plants (Tarafdar and Kumar 1996; Tarafdar and Rao 1997). Udaiyan K and Muthukumar T, Bharathiar University, Coimbatore, examined a large number of pteridophytes, gymnosperms, and angiosperms occurring in the Western Ghats region of Tamil Nadu for their AMF association. These investigations highlighted the rich diversity of AMF in the Western Ghats region of peninsular India. They have also reported the occurrence of the spore-in-spore syndrome and their seasonality in India and the ability of spore-forming sporiferous saccule of *Acaulospora* to germinate and act as propagule. Plant growth-promoting ability of AMF and the effect of manures and biocides on AMF association was also studied (Muthukumar and Udaiyan 1999, 2000).

Mahadevan A and Raaman N, University of Madras, Chennai, isolated several AMF species from rhizosphere soils, magnesite mine spoils, and polluted soils. These AMF are highly useful in phytoremediation and revegetation of wastelands with *Prosopis juliflora* and *Casuarina equisetifolia*. They also worked on axenic germination of AMF spores, mechanisms of induced systemic resistance brought out by AMF, and inoculation of micropropagated plantlets with AMF (Raaman and Mahadevan 1996; Raaman et al. 1993; Raman et al. 1994). At the University of Agricultural Sciences, Dharwad, Sreenivasa MN initiated work on AMF. He screened and selected the best AMF for inoculating chilli and tomato, the two major crops grown in the region. These fungi are mass-produced and made available to farmers. Further, these fungi also suppressed soil-borne pathogens especially *Sclerotium*. Jones Nirmalnath from the same university brought out the role of AMF in the control of plant-parasitic weeds, *Striga* in sugarcane, and Orabanche in tobacco. AMF diversity under elevated CO_2 and temperature and also its role in alleviating water stress were studied by him (Sreenivasa et al. 1992; Asha et al. 2016; Chimmalagi et al. 2018). Vyas SC of JN Agricultural University, Indore, worked on the effect of fungicides on AMF colonization and plant growth. Interaction between AMF and *Rhizobium* on soybean was also studied. The status of mycorrhizal association in the Malwa region of MP was also investigated (Vyas and Vyas 2000).

Sridhar KR, Mangalore University, Mangalore conducted many surveys in coastal sand dunes (CSD) of the Indian coast to record AMF occurrence, intensity, and diversity. AMF were found in the sands of vegetated and non-vegetated CSD. The AMF colonization of dune plant roots attained a peak during the post-monsoon

season. The AMF diversity in moderately disturbed dunes (MDD) and severely disturbed dunes (SDD) revealed the diversity to be lower in SDD than MDD. These studies brought out that AMF diversity, as well as species richness, are higher in CSD of tropics compared to temperate regions (Beena et al. 1997, 2000). Sukhada Mohan Das, ICAR-Indian Institute of Horticultural Research, Bangalore, proposed that finger millet (ragi) is an excellent host for mass multiplying AMF. The best AMF for colonizing root-stocks of mango was developed. Further studies revealed that AMF colonization helped in the efficient utilization of phosphate by enhancing acid and alkaline phosphatase activity in the roots which break down complex phosphates and increase the availability of phosphate to the plant (Mohandas 2012). Rodrigues B, University of Goa, Goa, carried out AMF diversity studies from various habitats, viz., mine wastelands, coastal dunes, mangroves, Khazan lands, plantation, and field crops, and in medicinal plants. Besides, he has worked on both in vitro and in vivo methods of inoculum production. More than 6 AMF species have been successfully cultured in vitro. Detailed standardization protocols for viable and efficient inoculum production have been undertaken. Besides a novel AMF species *Acaulospora soloidea* has been reported from Goa (Souza and Rodrigues 2013; Rodrigues and Rodrigues 2015). Mehtab Bukhari, Government College of Arts, Science & Commerce, Quepem, Goa, also contributed to AMF diversity in plants growing on iron ore mine wastelands, medicinal and mangrove plants, Pteridophytes, and ornamental plants. She also studied the growth response of vegetable and fruit crops to AMF raised in iron ore mine rejects (Bukhari and Rodrigues 2008).

V Mohan, Institute of Forest Genetics and Tree Breeding, Coimbatore, carried out extensive studies on the ecology and diversity of AMF in different forest tree species, medicinal plants, and agriculture crops in Western and Eastern Ghats of South India as well as in western India. Efficacy of AMF and PGPR individually and in combination on seed germination, growth, and disease control of commercially important fast-growing native tree species were evaluated under nursery as well as field conditions. AMF biofertilizer product named "IFGTB Tree Growth Booster" has been developed and is available to various stakeholders (Sundar et al. 2011; Mohan et al. 2011). Surendra Gopal, Kerala Agricultural University, Thrissur, screened and selected *Claroideoglomus etunicatum* as the best AMF for inoculating mahogany seedlings. Inoculation showed a positive influence on the rate of photosynthesis, stomatal conductance, transpiration rate, chlorophyll content, relative growth rate, and water potential of seedlings. *Glomus proliferum* at the time of transplanting showed maximum growth and seedling quality benefits in the nursery as compared to all other fungi used for *Tectona grandis*. The best treatment for activating mangosteen seedling growth was the combination of *Glomus fasciculatum* + *Azospirillum* + single super phosphate. Among the solanaceous crops, AMF population was minimum in tomato and maximum in brinjal. *Glomus* spp. was the most predominant in Thrissur and Palakkad districts (Ajeesh et al. 2017).

Deiveekasundaram M, Tholkappian P, and Stella D from Annamalai University, Chidambaram, studied extensively AMF in the mangrove ecosystem of Pichavaram.

They also studied the role of AMF in improving crop productivity through P nutrition. Interaction between AMF and N-fixing and P-solubilizing organisms was also investigated in crops like cassava, soybean, groundnut, and medicinal plant *Datura*. The results showed that 25% of N and P can be saved through such inoculations (Stella 2002; Tholkappian and Deiveekasundaram 2006). AMF occurrence among the *Avicennia* sp., in the mangrove ecosystem of the Pichavaram mangrove forest of Cuddalore district in Tamil Nadu, India, was recorded by Lingan et al. (1999). Tholkappian et al. (2000) reported that increased root colonization by AMF might augment the uptake of nitrogen, potassium, phosphorus, and other minor nutrients resulting in an increase in the yield of cassava (*Manihot esculenta* Crantz) in alluvial soils of coastal Tamil Nadu. Stella (2002) experimented the co-inoculation effect of *Bradyrhizobium japonicum* and *G. fasciculatum* and documented that this combination significantly increased yield components in soybean, thereby saving the minimum of 25% N and P fertilizer. Improved P utilization efficiency of mycorrhizal (*G. fasciculatum*) peanut in the coastal soils of South India was observed by Tholkappian and Deiveekasundaram (2006). Application of AMF and phosphobacteria to the medicinal plant *Datura metal* (L.) significantly increased root colonization of 58.9% over control with AMF spore number of 130.44/100 g of rhizosphere soil and further observed an increase in alkaloids content (Tholkappian et al. 2016).

The work initiated in 2000 onwards: Many scientists have joined the groups mentioned above and few of them started their research on AMF later. It can be seen that the majority of the scientists started their research on AMF in 1980s and later the number of scientists initiating work on AMF decreased. Some scientists initiated work on AMF around 2000 and later, and are actively working on AMF at present. They are Guatam HR and Savitha Verma of YS Parmar University of Horticulture and Forestry, Solan; Jha DK and Khan MH, University of Gauhati, Guwahati; Lakshmipathy R, Acharya NG Ranga Agricultural University, Bapatla; Dr. Bhowmik SN, ICAR-Indian Institute of Agricultural Research Institute, New Delhi; Singh VK from the University of Delhi; Shukla A from Dr. Harisingh Gour Central University, Sagar; Banerjee K from Gujarat Forest Research Institute, Gandhinagar; Chaurasia B from Guru Ghasidas University, Bilaspur; Sunita Chahar from NES Ratnam College of Arts, Science and Commerce, Mumbai; Saikia M and Shrivastava K of North-Eastern Regional Institute of Science and Technology, Nirjuli, and many others. It is difficult to mention all the names.

8.3 Diversity and Distribution of Arbuscular Mycorrhiza in India

After the first publication of 11 AMF spore morphotypes associated with around 25 Gymnosperm and Angiosperm tree species by Thapar and Khan (1973), Bakshi (1974) reported 14 spore morphotypes belonging to *Acaulospora*, *Gigaspora*, *Sclerocystis*, and *Endogone* (later removed from Glomeromycota) associated with forestry species in India. Later there was a surge in the reports on the diversity of AMF in India between 1980 and 2000. By 2005, around 105 AMF species were

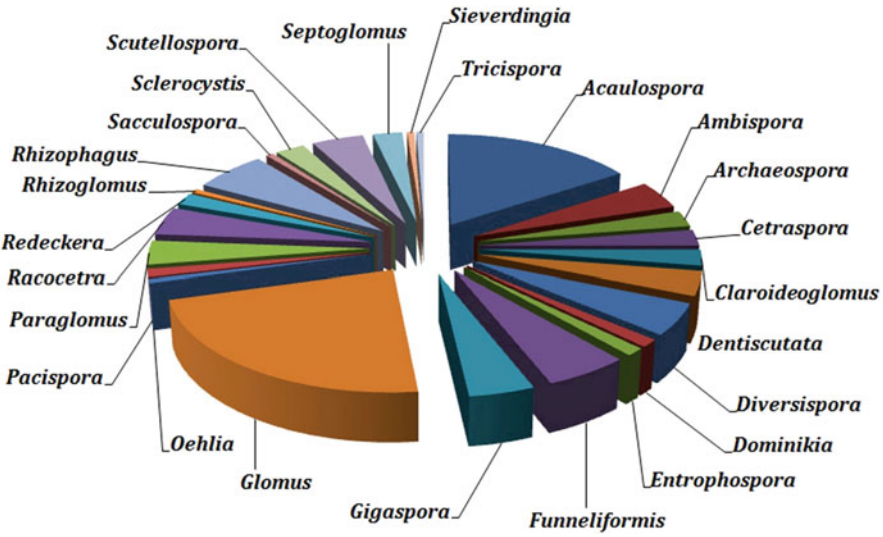

Fig. 8.1 Proportion of species in different genera of Glomeromycota reported from India

reported from different vegetation and soil types in India (Manoharachary et al. 2005). These studies indicated the widespread occurrence of species belonging to *Glomus* and limited distribution of species belonging to other taxa like *Acaulospora*, *Entrophospora*, *Gigaspora*, *Sclerocystis*, and *Scutellospora*. Gupta et al. (2014) consolidated a region-wise checklist of the distribution of AMF in different states of India after 2005. This study revealed the occurrence of 148 species of AMF from 21 genera and also created a searchable AMF diversity database (http://amfungi.aurobindo.du.ac.in now shifted to www.amfungi.in). Moreover, the study also indicated that *Funneliformis mosseae* (=*Glomus mosseae*) is more common in Indian soils compared to the previous assumption of *Glomus macrocarpum* and *Rhizophagus fasciculatus* (=*G. fasciculatum*) as the most widely distributed AMF species (Gupta et al. 2014). Among the different AMF genera reported from Indian soils, *Glomus* is represented by the maximum number of species followed by *Acaulospora* and *Scutellospora*. Yadav and Pandey (2016) reviewed the biodiversity of AMF in India and reported the occurrence of 120 species of AMF belonging to *Acaulospora*, *Dentiscutata*, *Entrophospora*, *Gigaspora*, *Sclerocystis*, and *Scutellospora*. The distribution and species diversity in this review resemble those of Gupta et al. (2014).

One of the authors (Muthukumar T) collected information on the diversity of AMF after 2014 and included the species listed by Gupta et al. (2014) and studies since 2010 that were not covered by Gupta et al. (2014). This analysis revealed the presence of 165 species of AMF belonging to 25 genera (Fig. 8.1) which is ~50% of the 334 validly described AMF species as in February 2020 (http://www.amf-phylogeny.com/amphylo_species.html). *Glomus* is the dominant genus represented by 37 species, followed by *Acaulospora* with 26 species, *Rhizophagus* with

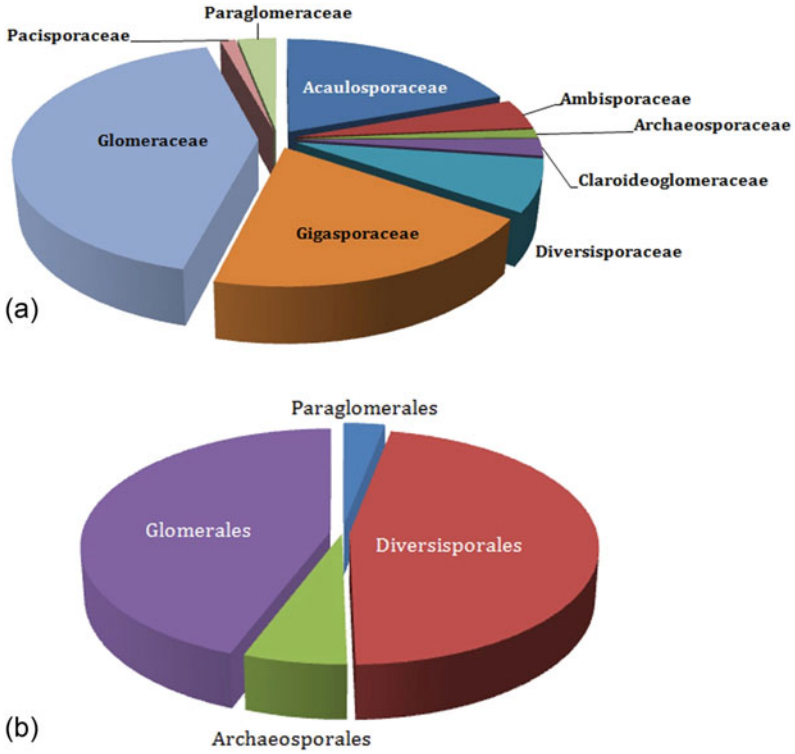

Fig. 8.2 Proportion of taxa in families (**a**) and orders (**b**) of Glomeromycota reported from India

10 species, *Funneliformis* with 9 species, and *Diversispora* and *Scutellospora* with 8 species each. The 3 genera *Ambispora*, *Gigaspora*, and *Racocetra* are represented by 7 species each. *Dentiscutata* and *Paraglomus* are represented by 6 species each and 5 genera (*Archaeospora*, *Cetraspora*, *Claroideoglomus*, *Sclerocystis*, *Septoglomus*) are represented by 4 species each. *Redeckera* is represented by 3 species, and *Dominikia*, *Entrophospora*, and *Pacispora* are represented by 2 species each. *Oehlia*, *Rhizoglomus*, *Sacculospora*, *Sieverdingia*, and *Tricispora* are represented by one species each. In India, only 9 of the 15 AMF families are represented, of which Glomeraceae is represented by 70 species followed by Acaulosporaceae and Gigasporaceae with 32 species each (Fig. 8.2a). Archaeosporaceae and Pacisporaceae are the least represented AMF in Indian habitats with 2 species each. Diversisporales are the most diverse order, followed by Glomerales (Fig. 8.2b). Eighteen AMF taxa new to science are reported from Indian soils (Table 8.1). Of these, *Gigaspora tuberculata* is synonymized with *Racocetra persica* (=*Scutellospora persica*) (Bentivenga and Morton 1995) and *Sclerocystis indica* is synonymized with *Sclerocystis rubiformis* (Almeida and Schenck 1990). However, the diversity of AMF in several habitats is small despite India being one of the mega diversity rich countries in the world.

Table 8.1 Arbuscular mycorrhizal (AM) fungal species reported new to science from India, their place of isolation and the vegetation/host from which they were reported

AM fungal species	Place of isolation	Vegetation/host	References
Acaulospora soloidea	Goa	*Murraya paniculata* (Rutaceae)	Vaingankar and Rodrigues (2011)
Acaulospora terricola	Andhra Pradesh	*Vallaris solanacea* (Apocynaceae)	Rani et al. (2003)
Dentiscutata nigerita	Kodar, Goa	*Carica papaya* (Caricaceae)	Khade (2010)
Entrophospora hexagoni	Osmanabad, Maharashtra	Medicinal plants	Rhatwal and Gandhe (2009)
Gigaspora candida	Wazirabad, Delhi	Wheat fields	Bhattacharjee et al. (1982)
Gigaspora tuberculata	Madhogarh Hills on the Alway-Jaipur road, Haryana	Desert grass *Cenchrus ciliaris* (Poaceae)	Neeraj et al. (1993)
Glomus bagyarajii	Allahabad	Contaminated pot culture	Mehrotra (1997)
Glomus delhiense	Old Delhi Ridge	Grassy area	Mukerji et al. (1983)
Glomus goaensis	Chorlem Ghat region of Goa	Wild banana	Khade (2009)
Glomus hyderabadensis	Hyderabad	*Allamanda cathartica* (Apocynaceae)	Rani et al. (2004)
Glomus indicum	Alappuzha, Kerala	*Euphorbia heterophylla* (Euphorbiaceae)	Błaszkowski et al. (2010)
Glomus multicaule	Dehradun, Uttar Pradesh	Hard wood stands	Gerdemann and Bakshi (1976)
Glomus multisubstensum	Old Delhi Ridge	*Maerua arenaria* (Capparaceae)	Mukerji et al. (1983)
Glomus reticulatum	Bangalore	—	Bhattacharjee et al. (1980)
Glomus sterilum	Allahabad	*Solanum tuberosum* (Solanaceae)	Mehrotra and Baijal (1992)
Sacculospora felinovii	Goa	Coastal sand dunes	Willis et al. (2016)
Sclerocystis indica	Uttar Pradesh	—	Bhattacharjee et al. (1980)
Sclerocystis sinuosa	Dehradun, Uttar Pradesh	Conifer and hard wood stands	Gerdemann and Bakshi (1976)

8.4 Role of Arbuscular Mycorrhiza in Crop Productivity in India

The benefits of AMF in agricultural ecosystems are now widely known. An increase in P uptake and biomass growth response because of AMF is well documented (Bagyaraj et al. 2015). AMF which supply plant nutrients, produce plant growth hormones, and control root pathogens can be better exploited in sustainable agriculture.

Role of AMF in plant growth: Improved plant growth due to inoculation of soil with AMF has been demonstrated especially under P deficient conditions (Bagyaraj et al. 2015). The growth improvement is mainly because of enhanced diffusion-limited nutrients like P, Zn, Cu, etc. AMF can also enhance tolerance to root pathogens and abiotic stresses such as drought, salt, and metal toxicity. Greater soil exploration by mycorrhizal roots beyond the P depletion zone as a means of increasing phosphate uptake is well established. The hyphae beyond this zone directly translocate nutrients from the soil to the root cortex. Experiments with ^{32}P-labelled phosphate indicate that AMF hyphae obtain their extra phosphate from the labile pool rather than dissolving soluble phosphate (Raj et al. 1981). Sparingly soluble rock phosphate is better utilized by the hyphae by closer physical contact with the ions dissociating at the particle surface. AMF also play a role in the formation of stable soil aggregates, building up of the macroporous structure of soil that allows penetration of water and air and prevents erosion (Chen et al. 2018). Glomalin, a soil protein produced by AMF, plays an important role in soil aggregation. Glomalin in soil is quantified operationally as glomalin-related soil protein (GRSP), which ranges as high as several mg/g soil, and GRSP is highly correlated with aggregate water stability. Plant hormones like cytokinins and gibberellin-like substances are also produced by AMF which enhances plant growth. AMF can tolerate a wide range of soil water regimes and also improve the water relations of many plants. Changes in the root exudations and altered rhizosphere microorganisms (which also affect plant growth) may result because of colonization of roots by AMF (Chen et al. 2018). Inoculation with AMF improving growth, nutrition, and yield of crop plants is well documented (Bagyaraj et al. 2015). These studies also brought out that application of P fertilizer can be reduced by 50% through inoculation with selected AMF. In medicinal and aromatic plants AMF inoculation not only increased crop yield but also that of the active ingredients (Lakshmipathy et al. 2019).

Role of AMF in Biocontrol: Many authors have suggested the ability of AMF colonized plants to withstand the attack from root pathogens better which can be ascribed to an increased nutritional status in the host plant due to the presence of the AMF. Consistent reduction of disease symptoms has been described for fungal pathogens such as *Phytophthora parasitica*, *P. vignae, Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *Chalara* (*Thielaviopsis*) *basicola*, *Rhizoctonia solani*, *R. bataticola*, *Sclerotium rolfsii, Pythium ultimum, P. splendens, Dothiorella gregania, Botrytis fabae, Ganoderma psuedoferreum,* and *Aphanomyces* spp., bacteria such as *Pseudomonas syringae* and *P. solanacearum,* and nematodes such as *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Tylenchulus semipenetrans*, *T. vulgaris, Pratylenchus dihystera* and *Radopholus similis* (Bagyaraj 2018). Most

of the studies on AMF-root pathogens suggest that AMF decreased or mitigated the disease severity. Consistent reduction of disease symptoms has been described for fungal, bacterial, and nematode pathogens. Studies conducted so far suggest that the mechanisms of suppression may be due to morphological, physiological, and biological alterations in the host. Thickening of the cell walls through lignification and production of other polysaccharides in mycorrhizal plants preventing penetration and growth of pathogens like *Fusarium oxysporum* and *Phoma terrestris* have been demonstrated. A higher concentration of orthodihydroxy phenols present in mycorrhizal plants compared to non-mycorrhizal plants was found to be inhibitory to the root rot pathogen *Sclerotium rolfsii* (Bagyaraj 2018). The activation of specific plant defence mechanisms as a response to AMF colonization is an obvious basis for the protective capacity of AMF. Among the compounds involved in plant defence studied in relationship to AMF colonization are phytoalexins, enzymes of the phenylpropanoid pathway, chitinases, peroxidases, pathogenesis related (PR) proteins, etc. Mycorrhizal plants harbour higher population of microorganisms in the rhizosphere, thus making it difficult for the pathogen to compete and gain access to the root. Further mycorrhizosphere supports a higher population of antagonists and siderophore producers. Thus, the possibility of biologically controlling the root pathogens with AMF looks promising (Bagyaraj 2018).

Interaction of AMF with other soil microorganisms: The presence of AMF is known to enhance nodulation and N fixation by legumes. Mycorrhizal and nodule symbioses often act synergistically on infection rate, mineral nutrition, and plant growth (Bagyaraj 1984). The increased P uptake conferred by the AMF symbiosis is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbiont, leading to increased N fixation and consequently promotion of root and mycorrhizal development. Several workers have reported that co-inoculation of AMF with mycorrhiza helper organisms (MHO) enhanced mycorrhizal colonization and in turn plant growth and yield. In high-tech agriculture, some crops are raised in disinfected soil/nursery. Disinfection kills several groups of organisms. In such soil/substrate mycorrhizal inoculation is a must. If MHO are also killed, then colonization by AMF will not be proper. It is suggested that milder disinfection treatments which selectively kill indigenous AMF and root pathogens but conserving MHO should be developed. It is probable that, in the near future, commercial mycorrhizal inoculum will contain MHO, improving the efficiency of inoculation in a wider range of conditions and reducing the quantity of inoculum needed. The information available on MHO is scanty. Extensive studies are needed in this area in order to find out MHO out of microorganisms occurring not only in soil but also in other environments. Recent studies have shown that inoculation with microbial consortia consisting of an efficient AMF together with a nitrogen fixer, P solubilizer, and PGPR carefully screened and selected for a particular plant is more beneficial than AMF alone in improving the growth, biomass, and yield of plants. These studies also brought out that 50% of NPK fertilizers can be saved through inoculation with selected microbial consortia with no adverse effect on crop yield (Thilagar et al. 2016; Desai et al. 2020). Thus, AMF together with PGPR play an important role in sustainable agriculture.

We have attempted to cover the historical development of research on AMF in India to the maximum extent possible. We might not have covered some institute or scientist, which is unintentional, and we apologize for the same. We hope this chapter will be of some interest to those who study AMF and it will serve the purpose of providing important historical context for the current researchers.

8.5 Conclusion and Future Perspectives

Research on AMF started 135 years ago globally, but in India, it was initiated only around 50 years back. In 50 years considerable work has been done on different aspects including diversity, plant growth promotion, ecology, alleviation of biotic and abiotic stresses, interaction with other soil microorganisms, and mainly its role in enhancing the growth of plants important in agriculture and forestry. Many habitats in India like the Eastern Ghats and the North-Eastern region are yet to be systematically assessed for their AMF diversity. As natural habitats are disappearing at an alarming rate in different parts of the country, it is necessary to conserve these AMF for their sustainable use in future. More information is required on the ecology of these fungi and mycorrhiza helper organisms which can promote the functional activity of AMF. Many AMF workers are happy with pot culture trials; it is important to extend these studies under field conditions so that the technology developed can be extended to the end-user.

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Developments in Endophytic Fungal Research in India

9

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Abstract

Endophytic fungi not only help in the development of the plants but also produce bioactive compounds that have various therapeutic applications. They also improve the health of crops, protect from various diseases, and enable them to tolerate the abiotic as well as biotic stresses. Earlier, the focus of researchers regarding endophytic fungi was mainly confined to study their biodiversity but at present, the center of attention also includes exploration of their various traits or to exploit various activities and also to produce the similar bioactive compounds for which host plants are known. Endophytic fungi are also considered as one of the best sources to produce bioactive compounds on a large scale and also use to enhance the production of bioactive compounds employing epigenetic modification as well as coculture methods. In the future, the endophytic fungi can be exploited for bioremediation and bioleaching purposes. Endophytic fungi could be used in producing the pigments as well as in the field of nanobiotechnology. Modern genomic approaches like genome editing tools may help in exploring the novel aspects related to endophytic fungi. It is suggested that endophytic fungi should be considered as the reservoir of various biotechnological applications which may help in enhancing the bio-economy of our country.

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Endophytic fungi · Bioactive molecules · Bioactivities · Epigenetics · Co-culture · Biodiversity · Bioremediation

9.1 Introduction

The term "endophyte" was first introduced in 1866 by de Barry in Germany, which means within the plants. Among all the microbes that reside in plant tissues, fungi are the most encountered species and do not cause any harm to the plants. They help the plants to tolerate environmental stress, prevent them from diseases caused by harmful insects and pathogens, and enhance the health and development of the host plants. In nature, most of the plants serve as hosts for the endophytes, but few endophytic fungi may either be host specific or specific to certain plant parts (Singh et al. 2017). They have a huge range of geographical and ecological distribution. Endophytic fungi are also responsible for the therapeutic values of its host plants as they produce a large number of therapeutically important biomolecules *in situ* on its own or help/induce the host plants to produce such compounds by activating the responsible silent gene(s). Although fungal endophytes are diverse, the clavicipitaceous (some grasses are infected by this group) and non-clavicipitaceous (can be isolated from angiosperms, conifers, allies and ferns) are the two major groups of the endophytic fungi. Endophytic fungi may be transferred to their concerned host plants horizontally (through natural openings or injuries during vegetative or clonal propagation and resides permanently in plants) as well as vertically (via seeds of the host plants) (Prasad and Harsh 2015).

Fungal endophytes are good candidates for exploiting biotechnological approaches as they enhance the ecological fitness of their host plant and also synthesize novel bioactive compounds as well as industrially important enzymes. A better understanding of the interaction of endophytic fungi with their host, other microbes and environment would help in exploiting its economic potential and strengthening the bio-economy of the country (Suryanarayanan 2019). The ability to produce bioactive compounds by endophytic fungi is well known, but unfortunately its exploitation is very less because of the earlier focus mainly confined to their distribution, diversity, ecology and taxonomical characterization related studies. To screen the targeted bioactive compounds, the information regarding the production of bioactive compounds from other fungal sources should be considered (Suryanarayanan et al. 2009). In addition, there is a need of exploring fungal endophytes in aspects of crop improvements by understanding its interaction with biotic and abiotic factors (Nataraja et al. 2019). It is still unclear how an endophytic fungus interacts with the plant without any harm to the host (Verma et al. 2009a). Also, an endophytic complex of a host changes due to age, environment, location and season (Priti et al. 2009). The development of molecular biology and genomics plays a vital role to unravel the various aspects of ecology and evolutionary relationships of fungal endophytes with other fungi. For better utilization of the

endophytic fungi for technological applications, it is important to consider endophytic fungi as an integral part of plants not just merely a fungus (Suryanarayanan 2013). To isolate various bioactive compounds, plants are being exploited ruthlessly due to which there is a danger of extinction of several plant species. In comparison to plants, the endophytic fungi may cater to and sustain the global demand of bioactive compounds. That's why endophytic fungi are considered a promising source for drug discovery and it may reduce the dependency and burden to plants. *Pestalotiopsis* sp. identified as a potential source of various metabolites and further genetic manipulation may lead to improve strain for better utilization (Deshmukh et al. 2017).

9.2 History and Developments

9.2.1 Biodiversity

Endophytic fungi are diverse, most are not host specific and also the physiochemical alterations in host plants lead to diversification of endophytic fungi as mentioned in Table 9.1. Plants of mangrove forests of Tamil Nadu, Andaman Island, Kerala, Arunachal Pradesh, Western Ghats and Nilgiri biosphere reserve are diverse in foliar endophytic fungi. Among all the isolates, *Colletotrichum* sp., *Glomerella* sp., *Paecilomyces* sp., *Phoma* sp., *Phomopsis* sp., *Phyllosticta* sp., *Sporomiella* sp., Sterile mycelium and *Xylaria* sp. are the dominant and not host- specific (Kumaresan and Suryanarayanan 2001; Pandey et al. 2003; Rajamani et al. 2018; Suryanarayanan and Kumaresan 2000; Suryanarayanan et al. 2018). Temperate and tropical rain forest in the Western Ghats also has the greatest diversity of endophytic fungi (Rajulu et al. 2013, 2016; Pandey et al. 2003; Suryanarayanan et al. 2002, 2011).

Endophytic fungi isolated from the *Adenocalymma alliaceum, Aegle marmelos, Azadirachta indica, Catharanthus roseus, Cinnamomum camphora, Eucalaptus citriodora*, *Mimusops elengi*, Nyctanthes arbor-tristis and *Terminalia cordifolia,* have the dominance of hyphomycetes, coelomycetes, ascomycetes, and mycelia sterilia (Gond et al. 2007, 2012; Kharwar et al. 2014; Verekar et al. 2017; Verma et al. 2007, 2011a). But some endophytic fungi isolated from medicinal plants of the Uttar Pradesh region also belong to zygomycetes (Kharwar et al. 2014). Interestingly, the endophytic fungal morphotypes isolated from the stem, leaf and bark of the *Tectona grandis* were mainly confined to ascomycetes (Murali et al. 2006) and in some cases confined to both ascomycetes as well as basidiomycetes (Singh et al. 2017). In addition, endophytic fungi isolated from the stem, leaf and bark of the *Rhododendron arboretum* Sm., *Taxodium distichum* Rich., *T. mucronatum* Ten. collected from Tamil Nadu were mainly belongs to coelomycetes (Kamalraj and Muthumary, 2013). Diverse endophytic fungi have also been isolated from members of Aizoaceae, Acanthaceae, and Chenopodiaceae dicotyledonous families (Suryanarayanan and Kumaresan 2000).

Table 9.1 Biodiversity of endophytic fungi reported from different sources

Source	Location	Endophytes\class	References
Cymbopogon citratus	Maharashtra	*Cladosporium cladosporioides* *Drechslera* sp. *Colletotrichum gloeosporioides* *Phyllosticta* sp.	Deshmukh et al. (2010)
Aegle marmelos *Adenocalymma alliaceum* *Azadirachta indica* *Catharanthus roseus* *Cinnamomum camphora* *Eucalyptus citriodora* *Nyctanthes arbor-tristis* *Terminalia cordifolia*	Uttar Pradesh	Hyphomycetes Ascomycetes Coelomycetes Mycelia sterilia	Gond et al. (2007, 2012); Kharwar et al. (2014); Verma et al. (2007, 2011a)
Mimusops elengi (Bakul)	Madhya Pradesh		Verekar et al. (2017)
Rhododendron arboretum Sm. *Taxodium distichum* Rich. *T. mucronatum* Ten.	Tamil Nadu	Coelomycetes	Kamalraj and Muthumary (2013)
Aegiceras corniculatum *Avicennia marina* *Althaea officinalis* *Bruguiera cylindrical* *Ceriops decandra, Excoecaria agallocha, Lumnitzera racemosa*	Tamil Nadu	Sterile mycelium *Phoma* sp. *Paecilomyces* sp. *C. gloeosporioides* *Glomerella* sp. *Phyllosticta* sp.	Kumaresan and Suryanarayanan (2001)
Tectona grandis	Tamil Nadu Karnataka Kerala, Arunachal Pradesh, Uttarakhand, Uttar Pradesh	Ascomycetes, Basidiomycetes	Murali et al. (2006); Singh et al. (2017)
Costus spicatus *Beloperone plumbaginifolia* *Lepisanthes tetraphylla* *Pleurostylia opposita* *Justicia gendarussa* *Sauropus androgynus* *Madhuca longifolia* *Vitex negundo*	Tamil Nadu	*Fusarium* sp. *Alternaria* sp. Mycelia sterilia	Palanichamy et al. (2018)

(continued)

Table 9.1 (continued)

Source	Location	Endophytes\class	References
Ocimum basalicum *Rauvolfia tetraphylla* *Glycosmis pentaphylla*			
Cordia wallichii *Dalbergia latifolia* *Anogeissus latifolia* *Cocos nucifera* *Erythroxylon monogynum* *Cinnamomum malabatrum* *Daphniphyllum neilgherrense* *Ixora nigricans* *Syzygium cumini* *Lumnitzera racemosa* *Careya arborea* *Elaeocarpus tuberculatus* *Rhodomyrtus tomentosa* *Stereospermum personatum* *Plumeria rubra* *Michelia nilagirica* *Lasianthus venulosus*	Western Ghats	*Phyllosticta* sp.	Pandey et al. (2003)
Acanthus sp. *Aegiceras* sp. *Avicennia* sp. *Bruguiera* sp. *Ceriops tagal* *Excoecaria agallocha* *Lumnitzera* sp. *Nypa fruticans* *Phoenix paludosa* *Rhizophora sp, Scyphiphora hydrophyllacea, Sonneratia alba, Xylocarpus granatum.*	Andaman Islands	*Phomopsis* sp. *Xylaria* sp. *Phyllosticta* sp. *Colletotrichum* sp.	Rajamani et al. (2018)
Aerides odorata *Arundina graminifolia* *Cymbidium aloifolium* *Cymbidium munronianum* *Dendrobium fimbriatum* *Dendrobium moschatum* *Eria flava*	Arunachal Pradesh	*Xylaria* sp.	Rajulu et al. (2016)

(continued)

Table 9.1 (continued)

Source	Location	Endophytes\class	References
Paphiopedilum fairrieanum *Pholidota imbricata* *Rhynchostylis retusa* *Vanilla planifolia*			
Anacardiaceae Boraginaceae Celastraceae Combretaceae Euphorbiaceae Fabaceae Mimosaceae Rhamnaceae Rubiaceae Rutaceae Sapindaceae Ulmaceae Verbenaceae	Western Ghats	*Xylaria* sp.	Rajulu et al. (2013)
Ficus benghalensis	Chennai	*Sporomiella minima* *Lasiodiplodia theobromae* *C. gloeosporioides* *Fusicoccum* sp. *Phoma* sp. *Phomopsis* sp. *Phyllosticta* sp. *Aspergillus* sp. *Aureobasidium pullulans* *Curvularia lunata* *Fusarium* sp. *Gliocladium* sp. *Paecilomyces* sp. *Penicillium* sp. *Phialophora* sp. *Trichoderma* sp.	Suryanarayanan and Vijaykrishna (2001)
Acanthaceae, Aizoaceae, Apocynaceae Aquifoliaceae Barringtoniaceae Bignoniaceae Boraginaceae Combretaceae Celastraceae Dipterocarpaceae Ebenaceae Elaeodendraceae Erythroxylaceae Euphorbiaceae Fabaceae	Western Ghats Southern India Arunachal Pradesh Andaman Island	*Colletotrichum* sp. *Phoma* sp. *Pestalotiopsis* sp. *Phomopsis* sp. *Phyllosticta* sp. *Xylaria* sp. *Sporomiella* sp.	Suryanarayanan and Kumaresan (2000); Suryanarayanan et al. (2002, 2011, 2018)

(continued)

Table 9.1 (continued)

Source	Location	Endophytes\class	References
Flacourtiaceae Lauraceae Loganiaceae Lythraceae Magnoliaceae Malvaceae Melastomaceae Mimosoideae Moraceae Myrtaceae Oleaceae Pedaliaceae Rhamnaceae Rubiaceae Rutaceae Sabiaceae Samydaceae Sapotaceae Staphyleaceae Sterculiaceae Symplocaceae Ternstromiaceae Tiliaceae Verbenaceae			
Cymodoceaceae Hydrocharitaceae	Tamil Nadu	*Aspergillus* sp. *Paecilomyces* sp. *Penicillium* sp.	Venkatachalam et al. (2015)

In many studies, among various parts of the plants like stem, leaf, bark and root; leaf harbored higher diversity of endophytic fungi across the different locations as well as seasons which indicates that host parts play important role in determining the composition of the endophytic fungi. On the other hand in some cases, colonization frequency of endophytic fungi was higher in rhizome or bark as compared to leaf (Venkatachalam et al. 2015; Gond et al. 2007). In some other cases, environmental factors played the important role in the distribution of endophytic fungi along with part of the host plant. Also, there is overlap in the distribution of the endophytic fungi among plant organs as many are non-tissue specific (Deshmukh et al. 2010; Kharwar et al. 2011a; Rajulu et al. 2016; Singh et al. 2017; Suryanarayanan and Vijaykrishna 2001; Suryanarayanan et al. 2011). It has also been established that mature tissues represent more diversity as compared to young ones (Kharwar et al. 2012).

Several studies reported different endophytes as dominant isolates like *Xylaria* species to orchids of Western Ghats (Rajulu et al. 2016), *Aspergillus* sp. to *A. indica* and seagrass species collected from Varanasi and Tamil Nadu, respectively (Venkatachalam et al. 2015; Verma et al. 2007). *Cladosporium cladosporioides*

and *Drechslera* sp. were dominant in *A. indica, Cymbopogon citratus* and *M. elengi* (Bakul) collected from Varanasi, Mumbai, and Madhya Pradesh, respectively (Deshmukh et al. 2010; Verekar et al. 2017; Verma et al. 2007). The species of *Trichoderma*, *Periconia,* and *Stenella* were also reported as dominant isolates in *A. indica* collected from Varanasi (Verma et al. 2007).

Phomopsis sp. and *Pestalotiopsis* sp. were dominant in *A. indica, M. elengi* (Bakul), and *Cinnamomum camphora* L. collected from Varanasi and Madhya Pradesh (Kharwar et al. 2014; Verekar et al. 2017; Verma et al. 2007). *Colletotrichum glocosporioides* and *Phyllosticta* sp. were dominant in *C. citratus* collected from Mumbai (Deshmukh et al. 2010). *Penicillium* sp., *Aspergillus* sp., and *Paecilomyces* sp. isolated from seagrass of Tamil Nadu have higher colonization frequency (Venkatachalam et al. 2015). *Chaetomium globosum* was dominant in *M. elengi* (Bakul) collected from Madhya Pradesh (Verekar et al. 2017). *Sporormiella minima, Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Fusicoccum* sp., *Phoma* sp., *Phomopsis* sp., *Phyllosticta* sp., *Aspergillus* sp., *Aureobasidium pullulans, Curvularia lunata, Fusarium* sp., *Gliocladium* sp., *Paecilomyces* sp., *Penicillium* sp., *Phialophora* sp., and *Trichoderma* sp. were reported from *Ficus benghalensis* especially residing in aerial root of the host collected from tropical forests of Western Ghats (Suryanarayanan and Vijaykrishna 2001). *Fusarium* sp., *Alternaria* sp., and mycelia sterilia were the most frequent fungi present in leaf of *Beloperone plumbaginifolia*, *Costus spicatus*, *Glycosmis pentaphylla*, *Justicia gendarussa*, *Lepisanthes tetraphylla*, *Madhuea longifolia*, *Ocimum basalicum*, *Pleurostylia opposite*, *Reuvolfia tetraphyll*a, *Sauropus androgynous* and *Vitex negundo* collected from Tamil Nadu (Palanichamy et al. 2018). Diversity of endophytic fungi studied to date showed the diverse distribution of endophytes that depends on plant parts/tissues and their physiochemical conditions, geography, and environmental factors. Much work has to be done to understand the diversity and distribution of endophytic fungi and their interaction with the host plant.

9.3 Potential Applications

Endophytic fungi produce various metabolites that are potent against a variety of diseases. Nowadays, these fungal endophytes are considered as a good source than their host plants because the strain can be manipulated easily to enhance the yield and production of some cryptic compounds which is more beneficial for the industrial applications. It also produces various enzymes, pigments and volatile compounds that can be used in the field of bio-remediation, bioleaching and biodiesel etc. It is the most promising approach in the agriculture field to improve the yield and quality of the crops.

Endophytic fungi are the most promising organisms as they produce secondary metabolites that show diverse bioactivities. Also, they are considered as a good candidate to produce the specific metabolites at a large scale with high yield by improving the strains with the help of various bio-techniques. In addition, there is a

requirement of novel antimicrobial agents that can be obtained from endophytic fungi as many chemical antimicrobial compounds induce resistance in pathogens and harm the host. Due to the long-term treatment of patients having a fungal infection, the anti-fungal compounds present in the market become less-effective. Anti-fungal compounds isolated from endophytic fungi can be used to treat life-threatening fungal infections, and also there is a need to bioprospecting the endophytic fungi for antifungal compounds that do not cause resistance with time (Deshmukh and Verekar 2012).

9.3.1 Applications of Endophytic Fungi

The endophytic fungi possess various biological properties e.g., antibacterial, antifungal, antiplasmodial, anticancer, and antioxidant; the details of these activities are depicted in Table 9.2.

Endophytic *Fusarium* sp. produces antibacterial compounds active against *Escherichia coli.* Another endophytic fungus *Alternaria* sp. *Colletotrichum* sp. and *Cladosporium* sp. registered the activity against *E. coli* and *Staphylococcus aureus* (Atri et al. 2020). Similarly, *Colletotrichum dematium* and *C. globosum* were active against a large number of bacteria including, *Shigella boydii, Salmonella paratyphi, Salmonella enteritidis,* and *Shigella flexneri*. *Colletotrichum dematium* was also

Table 9.2 Potential applications of endophytic fungi, isolated from different hosts

Endophytic fungi	Source	Bioactivities	References
Colletotrichum sp. *Cladosporium* sp. *Fusarium* sp. *Alternaria* sp.	*Moringa oleifera* *Withania somnifera*	Antibacterial	Atri et al. (2020)
Nigrospora oryzae *Chaetomium globosum* *C. dematium*	*Nyctanthes arbor-tristis*	Antibacterial Antifungal	Gond et al. (2012)
Aspergillus terreus	*Achyranthes aspera*	Antibacterial Antifungal	Goutam et al. (2016)
Fusarium sp.	*Citrus limon*	Antibacterial	Nivetha and Kharwar (2019)
Fusarium sp. *Nigrospora* sp.	Marine alga Western Ghats	Antiplasmodial	Kaushik et al. (2014)
Alternaria alternata *Penicillium* sp. *C. globosum*	*Adenocalymma alliaceum*	Antimicrobial	Kharwar et al. (2011a)
Pestalotiopsis sp. *Phomopsis* sp.	*Cinnamomum camphora* L.	Antimicrobial	Kharwar et al. (2012)
F. tricinctum	*Taxus baccata*	Anticancer Antioxidant	Vasundhara et al. (2016)
Nigrospora sp.	*Ginkgo biloba*	Antimicrobial Antioxidants	Pawle and Singh (2014)

active against a pathogenic fungus *C. lunata*. In addition, *Nigrospora oryzae* isolated from stem and leaf of *Nyctanthes arbor-tristis* was found active against *P. aeruginosa* and *Shigella* sp. (Gond et al. 2012). *Aspergillus terreus* also displayed the antibacterial and antifungal activities (Goutam et al. 2016). Endophytic *Fusarium* sp. isolated from *Citrus limon* also produces antibacterial compounds active against *S. aureus* (Nivetha and Kharwar 2019). *Fusarium* sp. isolated from various plants like a marine alga and *Nigrospora* sp. isolated from trees of Tamil Nadu produces antiplasmodial compounds active against *Plasmodium falciparum* (Kaushik et al. 2014). Out of 12 fungal endophytes recovered from leaves and petioles of *Adenocalymma alliaceum*, nine endophytic fungi (*Alternaria alternata*, *Penicillium* sp., *C. globosum,* and *C. lunata*) were found active with broad spectrum activity against several bacteria (*S. flexneri*, *S. enteritidis*, *S. paratyphi*, *P. aeruginosa,* and *Morganella morganii*). Thus, this plant represents a good source for endophytic fungi having antimicrobial potential (Kharwar et al. 2011a). *Pestalotiopsis* sp. showed inhibitory activity against *Pythium aphanidermatum* (54.5%), *Phytophthora cryptogea* (57.7%), and *Microsporum nanum* (51.4%) significantly, while *Phomopsis* sp. inhibited *P. aphanidermatum* moderately (Kharwar et al. 2012). Another endophytic fungus *Fusarium tricinctum*, isolated from *Taxus baccata*, was active against MCF-7, Hela, and PBMC with IC_{50} 225, 220, and 110μg/mL, respectively, and also showed free radical scavenging activity with IC_{50} 482 ± 9μg/mL (Vasundhara et al. 2016). *Nigrospora* sp. associated with *Ginkgo biloba* displayed antimicrobial activity against *E. coli* (R-2046), *S. aureus* (ATCC 6538), *Geotrichum* sp. (NFCCI-2521), *Klebsiella* sp. (R2434), and *Candida albicans* (NCIM 3471). The ethyl acetate extract of the fungus also showed antioxidant activity with IC_{50} value of 9.28μg/mL compared to the IC_{50} (1.74μg/mL) value of the standard ascorbic acid (Pawle and Singh 2014).

9.3.2 Bio-actives from Endophytic Fungi

Endophytic fungi are known to produce bioactive compounds with various biological activities; the endophytic fungi, their host plant isolated compounds, and their biological activities are presented in Table 9.3.

9.3.2.1 Anticancer Activity

The discovery of pharmaceutically important compounds from endophytic fungi generated the interest among mycologists that endophytic fungi may be a prospective source for anticancer compounds (Bedi et al. 2018; Kharwar et al. 2011b). Stierle and her co-workers discovered taxol-producing endophytic fungus *Taxomyces andreanae* from the *Taxus brevifolia* for the first time (Priti et al. 2009). Taxol is the most promising anticancer compound used worldwide. As cancer statistics revealed that cancer cases are increasing day by day, so the market demand for taxol is relatively high. This is a huge concern for the pharmaceutical industry and health departments not only because of its economics but also because of the risk involved in excessive exploitation of its scarcely distributed source tree *T. brevifolia*.

Table 9.3 Production of bioactive compounds from fungal endophytes

Compound Name	Endophytic fungus	Source	Bioactive Potential	References
Taxol	*Pestalotiopsis pauciseta*	*Cardiospermum helicacabum*	Anticancer	Gangadevi et al. (2008)
	P. terminaliae	*Terminalia arjuna*		Gangadevi and Muthumary (2009)
	Fusarium redolens	*Taxus baccata*		Garyali et al. (2014a, b)
	Alternaria brassicicola	*T. arjuna*		Gill and Vasundhara (2019)
	Phomopsis longicolla	*Mesua firrea*		Jayanthi et al. (2015)
	Phyllosticta tabernaemontanae	*Wrightia tinctoria*		Kumaran et al. (2009a)
	P. dioscoreae	*Hibiscus rosa-sinensis*		Kumaran et al. (2009b)
	Botryodiplodia theobromae, Lasiodiplodia theobromae	*Morinda citrifolia*		Pandi et al. (2010, 2011)
	Taxomyces andreanae	*T. brevifolia*		Priti et al. (2009)
	P. pauciseta	*Tabebuia pentaphylla*		Vennila and Muthumary (2011)
7-epi-10-deacetyltaxol	*P. microspora*	*Taxodium mucronatum*	Anticancer	Subban et al. (2017)
Vincristine and vinblastine	*F. oxysporum*	*Catharanthus roseus*	Anticancer	Kumar et al. (2013)
Depsipeptide	*Phomopsis glabrae*	*Pongamia pinnata*	Anticancer	Verekar et al. (2014)
Altersolanol	*Phomopsis* sp.	*Nyctanthes arbor-tristis*	Anticancer	Mishra et al. (2015)
Ophiobolin A	*Bipolaris setariae*	*Parthenium hysterophorus*	Anticancer	Bhatia et al. (2016)
Macrophin Rosellisin Methoxyphenoxyacrylic acid	*Phoma macrostoma*	*Glycyrrhiza glabra*	Anticancer	Nalli et al. (2019)
3-beta-hydroxy urs-12-en-28-oic acid	*Trichoderma viride*	*Ziziphus mauritiana*	Anticancer	Sheeba et al. (2020)

(continued)

Table 9.3 (continued)

Compound Name	Endophytic fungus	Source	Bioactive Potential	References
Secalonic acid derivative, F-7	*Aspergillus aculeatus*	*Rosa damascene*	Anticancer	Farooq et al. (2020)
Javanicin	*Chloridium* sp.	*Azadirachtaindica*	Antimicrobial	Kharwar et al. (2009)
4-(2,4,7-trioxa-bicyclo[4.1.0] heptan-3-yl) phenol	*P. mangiferae*	*Mangifera indica*	Antimicrobial	Subban et al. (2013)
Alternariol methyl ether	*Alternaria* sp.	*Vitex negundo*	Antimicrobial	Palanichamy et al. (2018)
Terrein, 4,5-Dihydroxy-3-(1-propenyl)-2-cyclopenten-1-one	*Aspergillus terreus*	*Achyranthes aspera*	Anticancer Antimicrobial	Goutam et al. (2017, 2020)
6-(heptacosa 18′Z enyl)-2-(18″hydroxy l-1″enyl-19″oxy)-3hydroxybenzoquinone 3β-5α-dihydroxy-6β-phenyl acetyloxy-ergosta-7,22-diene	*Chaetomium cupreum*	*M. luteola*	Anticancer Antimicrobial Antioxidant	Shylaja and Sathiavelu (2019)
Ergoflavin	*Ascomycetes* sp.	*M. elengi* (Bakul)	Anticancer Anti-inflammatory	Deshmukh et al. (2009)
Trichalasins (E, F and H)	*Diaporthe* sp.	*T. baccata*	Anticancer Antioxidant	Vasundhara et al. (2017)
Piperine	*C. gloeosporioides*	*P. nigrum*	Anticancer Antimicrobial Antidepressant Anti-inflammatory Antioxidant	Chithra et al. (2014)
Resveratrol	*F. equiseti*	*Vitis* sp.	Antioxidant	Dwibedi and Saxena (2019)

Endophytic fungi act as a dependable source of the taxol with a high yield that can further be enhanced by the strain improvement with the help of genetic modifications and optimization of physicochemical parameters of culture conditions (Kharwar et al. 2011b). Taxol is a billion-dollar drug to treat the various types of cancer. Fungal endophytes serve as potential microbes for the production of taxol to meet the demand for clinical and scientific research. A large number of endophytic fungi having the ability to produce taxol were reviewed and identified with morpho-molecular techniques (Sonaimuthu and Johnpaul 2010). Therefore, taxol-producing endophytic fungi are considered as the best alternative candidates to meet the expectations of market demand and cost-effectiveness. Nowadays, taxol is obtained from the endophytic fungi associated with *Taxus* as well as non-*Taxus* plants.

Pestalotiopsis sp., an endophytic fungus with taxol-producing ability was isolated from leaves of *Cardiospermum helicacabum* and *Terminalia arjuna* (Gangadevi et al. 2008; Gangadevi and Muthumary 2009). *Phyllosticta* sp., an endophytic fungus, was isolated from the leaves of *Wrightia tinctoria* and *Hibiscus rosa-sinensis* possesses the taxol-producing ability (Kumaran et al. 2009a, b). These results indicate that taxol-producing endophytic fungi are not only confined to taxol-producing plants but also associated with non-taxol-producing plants. Garyali et al. (2014a, b) screened 60 fungal endophytes from the bark of *T. baccata* collected from Jammu and Kashmir, Doda, Shimla, and Almora for their taxol-producing potential. Based on molecular markers such as 10-deacetylbaccatin III-10-*O*-acetyl transferase (DBAT) and Baccatin III-3-amino, 3-phenylpropanoyltransferase (BAPT), *Fusarium redolens* was identified as taxol-producing endophytic fungus with the highest yield.

Taxol also detoxifies the aflatoxin secreted by *Aspergillus flavus* in sunflower seeds (Banu and Muthumary 2010). Taxol obtained from fungal endophytes *Lasiodiplodia theobromae* and *Botryodiplodia theobromae* isolated from leaves of *Morinda citrifolia* showed a cytotoxic effect against MCF-7 breast cancer cell line with an IC_{50} value of 300μg in Sprague Dawley rats induced by 7,12-dimethyl benz (*a*) anthracene (Pandi et al. 2010, 2011). In recent years, a large number of endophytic fungi associated with medicinal plants were screened for the taxol-producing ability.

In a study seventy-seven coelomycete endophytic fungi from five different medicinal plants (*Mesua ferrea* L., *Rauvolfia tetraphylla* L., *Vitex negundo* L., *Anisomeles malabarica* L., and *Piper nigrum* L.) were screened for the taxol-producing ability. Of these, only *Phomopsis longicolla* was the promising endophytic fungus having taxol-producing ability isolated from *Mesua ferrea* (Jayanthi et al. 2015). *Alternaria brassicicola,* an endophytic fungus isolated from *T. arjuna*, could also produce the taxol (Gill and Vasundhara 2019). Vennila and Muthumary (2011) and Vennila et al. (2012) reported the taxol-producing endophytic fungus *Pestalotiopsis pauciseta* VM1 isolated from *Tabebuia pentaphylla* Hemsl. This taxol showed a high degree of anticancer activity against MCF-7, a human breast cancer cell line. Taxol derivatives also exhibited anticancer effects, for example; a taxol derivative 7-epi-10-deacetyltaxol was produced from fungal endophyte *Pestalotiopsis microspora* isolated from the bark of *Taxodium mucronatum*. It

shows a cytotoxic effect against HepG2 cells by activating the MAPK pathway (Subban et al. 2017).

During 2009–2018, a large number of endophytic fungi having the ability to produce anticancer compounds were discovered from terrestrial and mangrove plants (Bedi et al. 2018; Deshmukh and Verekar 2014; Deshmukh et al. 2018). Vinblastine and vincristine are anticancer compounds obtained from an endophytic fungus *Fusarium oxysporum* isolated from Indian *Catharanthus roseus* plant (Kumar et al. 2013). A novel depsipeptide showed anticancer activity with mean IC_{50} of 0.089μM against 40 human cancer cell lines. This compound was produced by *Phomopsis glabrae*, an endophytic fungus associated with the leaves of *Pongamia pinnata* (Verekar et al. 2014). Altersolanol, an anthraquinone obtained from the endophytic fungus *Phomopsis* sp. isolated from plant *Nyctanthes arbor-tristis* was studied for anticancer activity against 34 human cancer cell lines and showed mean IC_{50} and IC_{70} value of 0.005μg/mL and 0.024μg/mL, respectively. This compound is a kinase inhibitor that cleaves caspase 3 and 9 and decreases the antiapoptotic protein expression and hence induced apoptosis (Mishra et al. 2015). Ophiobolin A, obtained from an endophytic fungus *Bipolaris setariae*, showed an inhibitory effect against phosphorylation of S6, ERK, and RB (effector proteins of PI3K/mTOR, Ras/Raf/ERK, and CDK/RB pathways) with IC_{50} 1.9 ±μM, 0.28 ± 0.02μM, and 1.42 ± 0.1μM, respectively, and hence induced apoptosis in MDA-MB-231 cancer cells (Bhatia et al. 2016). At IIIM (Jammu), a group of workers under the leadership of the Director, was involved in dealing with different aspects of fungal endophytes from structural to functional diversity (Arora et al. 2019). Four metabolites, macrophin, rosellisin, 2-(2-hydroxy-5-6-methoxy-3-methylene-1,4-benzodioxin-2(3*H*)-one, and methoxyphenoxyacr-ylic acid were isolated, for the first time, from an endophytic fungus, *Phoma macrostoma* inhabiting the inner tissue of medicinal plant *Glycyrrhiza glabra* L., with an impressive cytotoxic activity (Nalli et al. 2019).

A compound identified as 3-beta-hydroxy urs-12-en-28-oic acid obtained from the crude extract of *Trichoderma viride*, an endophytic fungus of *Ziziphus mauritiana*, was active against the HeLa cell line with an IC_{50} value of 23.57μg/mL (Sheeba et al. 2020). A new secalonic acid derivative F-7, isolated from the endophytic *Aspergillus aculeatus* MBT 102, is associated with the plant *Rosa damascena*. The compound showed strong cytotoxic activity against triple-negative breast cancer (TNBC) cells. It was observed to induce apoptosis (induced mitochondrial damage and the reactive oxygen species-mediated apoptosis) arresting the G1 phase of the cells in a dose-dependent manner. Also, the compound causes a significant microtubule disruption in TNBC cells. Subsequently, it restricted the cell migration leading to the concomitant increase in the expression of cleaved caspase and PARP (Farooq et al. 2020).

Terrein, 4,5-dihydroxy-3-(1-propenyl)-2-cyclopenten-1-one obtained from *A. terreus*, a fungal endophyte associated with *Achyranthes aspera,* showed cytotoxic effect against A-549 cancer cell line with an IC_{50} value of 121.9 ± 4.821μg/mL (Goutam et al. 2017, 2020). *Chaetomium cupreum*, an endophytic fungus isolated from *Mussaenda luteola* plant was found to produce two anticancer compounds

which showed 52% and 49% cytotoxic effect against MCF-7 at a concentration of 100μg/mL. These compounds were identified as 6-(heptacosa-18′ Z enyl)-2-(-18 ″hydroxyl-1″ enyl-19″ oxy)-3 hydroxy benzoquinone and 6-(3β-5α-dihydroxy 6-β-phenyl acetyloxy ergosta-7,22-diene) (Shylaja and Sathiavelu 2019). Ergoflavin, a dimeric xanthene isolated from Ascomycetes isolated from *M. elengi* (bakul), exhibited cytotoxic activity against ACHN, H460, Pancl, HCT16, and Calu1 cancer cell lines with the IC_{50} value of 1.2, 4.0, 2.4, 8.0, and 1.5μM, respectively, and also displayed anti-inflammatory activity against TNF-α and IL-6, with IC_{50} value of 1.9 and 1.2μM, respectively (Deshmukh et al. 2009). Trichalasins (E, F and H) present in the crude extract of *Diaporthe* sp. associated with *T. baccata* was characterized by an ultra-high-performance liquid chromatography-quadrupole time-of-flight analysis. It displays anticancer activity against HeLa and MCF-7 cancer line with IC_{50} value of 1257 ± 80μg and 1058 ± 44μg, respectively (Vasundhara et al. 2017).

Some other compounds are obtained from endophytic fungi which show anticancer activity, but after their mechanisms of action studied, they were proved to be unsuitable anticancer compounds for human applications. For instance, koningic acid, a glycolysis inhibitor produced by *Trichoderma virens,* an endophytic fungus of *A. indica* (collected from Mumbai). It was observed that koningic acid is not a suitable option for human cancer therapy (Rahier et al. 2015).

9.3.2.2 Antimicrobial Activity

After the discovery of penicillin in 1929, scientists all over the world continued investigation on the natural antimicrobial compounds. As the impact of endophytes in our lives has been realized, endophytic fungi have attracted the attention of scientists for the discovery of new antimicrobial agents which are less prone to resistance and cost-effective (Deshmukh et al. 2015). Javanicin, a naphthaquinone isolated from *Chloridium* sp. associated with neem root was effective against *Pseudomonas* sp. (Kharwar et al. 2009). A novel compound 4-(2,4,7-trioxa-bicyclo[4.1.0]heptan-3-yl) phenol had been isolated from *Mangifera indica* L., associated endophytic fungus *Pestalotiopsis mangiferae*. This compound has antimicrobial activity against *Klebsiella pneumoniae, Micrococcus luteus, C. albicans, Bacillus subtilis, E. coli*, and *P. aeruginosa* (Subban et al. 2013). Antimicrobial alternariol methylether is a compound obtained from an endophytic fungus *Alternaria* sp. (Palanichamy et al. 2018).

Terrein 4,5-dihydroxy-3-(1-propenyl)-2-cyclopenten-1-one obtained from *A. terreus*, a fungal endophyte associated with *A. aspera,* also reported to have antimicrobial activity against *Bipolaris sorokiniana* (a causative organism for many plant diseases) (Goutam et al. 2017, 2020). Compounds 6-(3β-5α-dihydroxy-6-β-phenyl acetyloxy-ergosta-7,22-diene) and 6-(heptacosa-18′ Zenyl)-2-(-18″ hydroxyl-1″ enyl-19″ oxy)-3 hydroxy benzoquinone are isolated from endophyte *C. cupreum* and *Myrmica luteola* and are active against *Mycobacterium* sp. at 25μg/mL and 6.25μg/mL, respectively (Shylaja and Sathiavelu 2019). Thus, endophytic fungi are considered as good sources of antimicrobial compounds without developing resistance with time.

9.3.2.3 Other Activities

Compounds 6-(3β-5α-dihydroxy-6β-phenylacetyloxy-ergosta-7,22-diene) and 6-(heptaco-sa-18′ Zenyl)-2-(-18″ hydroxyl-1″ enyl-19″ oxy)-3 hydroxy benzoquinone isolated from *C. cupreum* and *M. luteola* showed free radical scavenging activity of 71.63 ± 1.40% and 72.07 ± 1.95%, respectively (Shylaja and Sathiavelu 2019). Trichalasins (E, F, and H) are present in a crude extract of *Diaporthe* sp. and associated with *T. baccata* also exhibited free radical scavenging activity of IC_{50} 482 ± 9μg/mL (Vasundhara et al. 2017). Another compound resveratrol is isolated from *Fusarium equiseti*. This strain could be a good candidate for the commercial production of resveratrol (Dwibedi and Saxena 2019). Resveratrol was reported to have antioxidant property. An alkaloid compound piperine was obtained from fungal endophyte *C. gloeosporioides* isolated from the *P. nigrum* from local farms of Kerala (Chithra et al. 2014).

9.3.2.4 Fungal Extracellular Enzymes

Since fungi are also known to produce various enzymes that are necessary for the colonization of plant tissues, therefore endophytic fungi can also produce extracellular commercially important enzymes. Thus, a large number of endophytic fungi were screened for the extracellular enzyme-producing ability as presented in Table 9.4. Among various endophytic fungi, *Pseudofusicoccum adansoniae* isolated from *Tinospora cordifolia* showed amylase, protease, and lipase activities (Mishra et al. 2019). *Alternaria* sp. from *W. somnifera* exhibited glutaminase free L-asparaginase activity (Nagarajan et al. 2014). Also, various endophytic fungi associated with trees of forests of Western Ghats exhibits chitinolytic enzyme-producing ability (Rajulu et al. 2011). *Talaromyces stipitatus*, an endophytic fungus obtained from root of *Avicennia marina*, can produce chitosanases and chitinases enzymes (Paranetharan et al. 2018). *Xylaria* species associated with the evergreen and dry forests of Western Ghats have the potential to produce cellulases, laccases, and lipases (Rajulu et al. 2013).

9.3.2.5 Fungal Pigments

As synthetic colors have carcinogenic and immunosuppressive side effects, there is a need for alternative biological sources to produce various pigments. There are many pigments that are isolated from fungi and were evaluated safe. Endophytic *Fusarium*, *Monascus*, *Laetiporus, Aspergillus*, *Penicillium,* and *Trichoderma* species were able to produce anthraquinones, rubropunctatin, monascin, quinines, rubropuntamine, ankaflavin, and β-carotene. These pigments possess many bioactivities. The production of pigments is affected by pH, aeration, carbon source, and type of fermentation (Mukherjee et al. 2017). Biopigments are also produced by the addition of some precursor chemicals to growth media of endophytic fungi associated with *Clerodendrum viscosum* (Mugesh et al. 2014). The pigment melanin was produced by the fungal endophyte *Phyllosticta capitalensis* (Suryanarayanan et al. 2004).

Table 9.4 Production of enzymes from fungal endophytes

Enzymes	Endophytic fungus	Source	References
Cellulases, laccases and lipase	*Xylaria* or *Nemania*	Anacardiaceae Boraginaceae Celastraceae Combretaceae Euphorbiaceae Fabaceae Mimosaceae Rhamnaceae Rubiaceae Rutaceae Sapindaceae Ulmaceae Verbenaceae	Rajulu et al. (2013)
Amylase, protease, lipase	*Pseudofusicoccum adansoniae*	*T. cordifolia*	Mishra et al. (2019)
L-asparaginase	*Alternaria* sp.	*W. somnifera*	Nagarajan et al. (2014)
Chitosanases Chitinase	*Talaromyces stipitatus*	*Acaryochloris marina*	Paranetharan et al. (2018)
Chitinolytic enzymes	*Alternaria* sp. *Colletotrichum* sp. *Fusarium* sp. *Nigrospora* sp. *Pestalotiopsis* sp. *Phomopsis* sp. *Phyllosticta* sp. *Xylaria* sp.	Western Ghats	Rajulu et al. (2011)

9.3.2.6 Green Synthesis of Nanoparticles

Endophytic fungi have the potential to synthesize metal nanoparticles (NPs) and this is an emerging approach in the field of nanobiotechnology (Verma et al. 2009b). Although much work needs to be done on these aspects of endophytic fungi, some of them have been used to synthesize silver and gold nanoparticles very efficiently. In India, for the first time, mycosynthesis of metal nanoparticles has been attempted using fungal endophytes by Ahmad et al. (2003) and has synthesized different metal nanoparticles for various application (Shankar et al. 2004). Silver nanoparticles were synthesized by treating cell filtrate of *Phomopsis helianthi*, an endophytic fungus isolated from *N. arbor-tristis*, with 1 mM solution of silver nitrate. The average size of the nanoparticles was 35.05 nm, which displayed the antimicrobial activity against each *E. coli* and *P. aeruginosa* with a 14 mm zone of inhibition (Gond et al. 2019). Silver nanoparticles are also synthesized by treating cell filtrate of *A. clavatus*, an endophytic fungus isolated from *A. indica*, with 1 mM solution of silver nitrate. The average size of nanoparticles observed was 10–25 nm that also showed an impressive antimicrobial activity against *E. coli*, *Pseudomonas fluorescens,* and *C. albicans* (Verma et al. 2010). By treating cell filtrate of *A. clavatus*, an endophytic fungus isolated from *A. indica,* with chloroaurate ions,

gold nanoparticles was also synthesized with the average size of 20–35 nm (Verma et al. 2011b). Similarly, a foliar endophyte *C. globosum* isolated from *T. grandis* carried out controlled synthesis of nanoparticles of gold and silver with a wide range of temperature and pH. The biologically synthesized silver nanoparticles showed better antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, and *Proteus mirabilis* as compared to ionic silver (Singh et al. 2018).

9.3.3 Genomic Studies and Plant Interactions

An endophytic fungus having potent enzymatic activities has been isolated from the roots of the medicinal plant *T. cordifolia*. When partial gene sequence similarity was performed, then this fungus was identified as *P. adansoniae* with 100% 18S RNA and ITS sequences (Mishra et al. 2019). A total number of 48 fungal endophytes were isolated from the leaves of *C. indica*, *A. marmelos,* and *M. oleifera*. These endophytes were further characterized by the analysis of ITS2 sequence–secondary structure. These structures are elucidated with minimum free energy method (MFOLD version 3.1), and for each genus, consensus structure has been generated with the help of 4SALE. The generation of the phylogenetic trees based on ITS2 was done by ProfDistS (Gokulraj et al. 2014). By using an ion 530chip ExT kit and IonTorrent platform for sequencing, the genome sequence assembly of *F. tricinctum* was reported which has 42,732,204 bp, with a median read length of 386 bp, from a total 6.62 Gb (Meena et al. 2018). Latha et al. (2004) revealed a wide range of diversity in genetic makeup among *Acremonium* endophytes of warm-season grasses on the basis of RAPD markers. Therefore, genomic studies play an important role to identify the endophytic fungi; these studies also help in altering the gene expressions to improve the strain for enhanced production of commercially important compounds on large scale.

Muscodor species are proficient producers of bioactive volatile organic compounds (VOCs) with many potential applications. The members of this genus are poorly explored for the production of soluble compounds (extrolites). Epigenetic modifiers (suberoylanilidehydroxamic acid (SAHA) and 5-azacytidine) have induced the over-expressing PKS genes. Each variant produced a different set of VOCs distinct from the wild type, and several VOCs including methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)hexane-2,4-diol and 2-carboxymethyl-3-*n*-hexylmaleic, the production of which could be attributed to the activity of otherwise silent PKS genes. The bioactive extrolite brefeldin A was isolated and characterized from the wild type. Two other products were isolated and characterized as ergosterol and xylaguaianol C (Qadri et al. 2017). Hence, *Muscodor yucatanensis* has the genetic potential to produce several previously undetectable VOCs and organic solvent-soluble products.

Endophytic fungi reside in plants and interact with it, and thus it is hypothesized that it could communicate with the plant to prevent it from harmful microorganisms, and further help in triggering some gene activation to tolerate biotic or abiotic stress. The endophytic fungi residing in seeds of maize crop are responsible for the

improvement of maize as it interacts with the maize at a genetic level and positively affects its metabolism (Chowdhury et al. 2019). Endophytic fungi play an important role in enhancing the growth and development of plants. An endophyte triggers Indian popcorn plant to synthesize defense molecules against *Fusarium moniliforme*. This defense is dependent on jasmonic acid (Gond et al. 2015). Interaction of endophytic fungi with host as well as non-host plants plays an important role in the agriculture to improve the crop quality, to enhance the yield, and also to prevent the crops from various diseases.

9.3.4 Enhanced Production of Bioactive Compounds

Endophytic fungi can be manipulated by various methods to enhance the production of bioactive compounds. The most commonly used method is the optimization of culture conditions. Terrein, an antimicrobial compound, is a fine example isolated with high yield after optimization process from a fungal endophyte *A. terreus* isolated from *Achyranthes aspera*. By fixing the carbon source (4% dextrose) and modifying the nitrogen source (1% casein) in the culture media, the yield of terrein was higher than that in the control. This compound also has better activity against *Aeromonas hydrophila*, *S. aureus,* and *Enterococcus faecalis* (Goutam et al. 2020). Optimization of culture growth conditions are not always used to enhance the production of bioactive compounds; sometimes it can also be used to inhibit the production of undesirable compounds. The fermentation period of 96 h yield the maximum production of L-asparaginase in *Alternaria* sp., while a high concentration of glucose in the medium inhibited the production of L-asparaginase (Nagarajan et al. 2014). The presence of salicylic acid enhanced the lipid peroxidation of unsaturated fatty acids and reactive oxygen species in *P. microspora* which resulted in a high yield of taxol. The oxidation reaction triggers the geranylgeranyl pyrophosphate synthase gene expression and stimulates the isoprene biosynthetic pathway resulting in higher taxol production (Subban et al. 2019). Endophytic fungi especially *Fusarium* sp. associated with *Aloe vera, Ocimum sanctum, Cassia alata, Semecarpus anacardium*, *P. pinnata, Desmodium pulchellum, Zingiber roseum, R. tetraphylla*, *M. citrifolia,* and *Mentha arvensis* are capable of producing plant growth-promoting Indole Acetic Acid (IAA). Yeast extract, broth medium with neutral pH, incubation period of 7 days, temperature at 35 °C and 0.3% L-tryptophan supplementation are some optimized parameters for the enhanced production of IAA (Gangwar et al. 2014; Pradhan and Tayung 2019).

Various approaches like epigenetic modifications (by the use of DNA methyltransferase (DNMT) and procainamide and/or histone deacetylase (HDAC) inhibitors), one-strain-many-compounds (OSMAC), coculture technique (based on intercommunication of two organisms and competition for the nutrients), gene-editing, and biotransformation are being used to explore various applications of endophytes in the production of therapeutic agents. A large number of metabolites are also reported to be produced from endophytic fungi. Some culture optimization strategies are needed for the cultivation of fungus involved in the One-Factor-at-a-

Time (OFAT) approach, Response Surface Methodology (RSM), and Plankett-Burman design. These approaches help in reducing the number of trials and also in predicting the most promising factors which support in enhancing the productivity (Deshmukh 2018).

Response Surface Methodology is a technology which limits the trial steps by using the One-Factor-at-a-Time (OFAT) approach. This approach is used to enhance the production of taxol from *F. redolens* (Garyali et al. 2014b). Change in some growth conditions (PDB with -0.1% starch) of *Emericella quadrilineata,* a fungal endophyte associated with leaf of *Pteris pellucida*, induces the production of many bioactive compounds, some of which are active against *A. hydrophila* and *S. aureus*. In addition, 25 days incubation period leads to the production of maximum bioactive compounds. On incubation for 21 days at 26 °C, the fungus becomes salt-tolerant (Goutam et al. 2014). *C. gloeosporioides*, fungal endophyte associated with the root of *A. indica* and leaves of *Syzygium cumini*, respectively, were treated with 5-azacytidine at different concentrations for epigenetic modifications. After epigenetic modification, it develops activity against *E. faecalis, S. flexneri, S. aureus, S. typhi,* and *A. hydrophila* (Kumar et al. 2016; Sharma et al. 2017a). Also *C. gloeosporioides,* an endophytic fungus associated with *S. cumini*, treated with extracts of turmeric and grape skin showed the increase in the production of bioactive compounds by 174.32% and 272.48%, respectively (Sharma et al. 2017b). Fungal endophyte may also play an important role in biotransformation as vinblastine transformed into vincristine by *F. oxysporum* isolated from the *C. roseus*. Vinblastine dissolved in sterile water with mycelia mat of *F. oxysporum* was successfully biotransformed into more useful vincristine at room temperature (Kumar and Ahmad 2013).

9.4 Conclusions

Endophytic fungi reside inside healthy plant tissues and do not cause any harm to them. Various biodiversity studies showed that endophytic fungi distributed in host plants irrespective of their nature, plant part, location, and environmental conditions. Although some endophytic fungi overlap among plant parts, host plants, and locations, they are very less in number. The chemical therapeutic compounds available in the market are unable to meet the demands because they face a resistance surge in pathogens. Again, plants are not considered as an evergreen source to extract the bioactive compounds because they are prone to various microbial infections, necessary maturation period, age, environmental conditions, soil type, and also they may be extinct due to overexploitation. Thus, endophytic fungi become the emerging candidates to produce various bioactive compounds having different activities as they can be exploited at a large scale to meet the current demands. Being the producers of many host mimetic compounds, they could be utilized as an alternative of their hosts. Studies of the endophytic fungi at the genomic level may help to edit their genome, which may assist in manipulating the strain for better applications. As endophytic fungi reside in plants as an integral

part, it can be assumed that they have a better understanding of the metabolism of their host plant, and thus endophytes can be used in agricultural fields in crop improvements.

9.5 Future Perspectives

Despite impressive progress in the area of fungal endophyte research in India, there is still a need of understanding the mechanism by which endophytic fungi produce secondary metabolites of host origin and non-host phytochemicals. There are some studies, however inconclusive, regarding the mutual horizontal gene transfer, which encodes dual origin bioactive compounds to explain the dual synthesis of compounds. There is also scope for the study of physiology and biochemistry of endophytic fungus and plant interaction. Some endophytic fungi do not produce secondary metabolites in culture medium even after employing advanced fermentation techniques; this attenuation may be due to the lack of stimulus or signaling provided by their respective hosts (Priti et al. 2009). There is a need to focus on whole-genome sequencing and bioinformatics data which may help in predicting the gene(s) responsible for secondary metabolites production.

Genome editing tool CRISPR/Cas9 is a promising tool that can be used to manipulate the genome of the endophytic fungi to explore its further potential. Although a very few studies exist related to genome editing of filamentous fungi (Deshmukh 2018). More work should be done in co-culture techniques and epigenetic modifications to enhance the yield of bioactive compounds and also to produce the cryptic compounds. Although work has been reported in the literature on strain improvement by altering growth conditions of fungal endophytes, there should be approaches to understand the mechanism behind the changes occurring in the fungal strain. Fungal endophytes should be explored for the production of pigments as biopigments are safe in use and can be a better candidate for industrial applications compared to the pigments produced chemically. Fungal endophytes can also be exploited in bioremediation, bioleaching, and biodiesel processes. Thus, fungal endophytes can be better explored and exploited in the areas of the pharmaceutical industry, textile industry, pollution control, biofuel, agriculture, and nanobiotechnology with the understanding of their complete genetic makeup.

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Fungal Endophytes of Mangroves: Diversity, Secondary Metabolites and Enzymes

10

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Abstract

The culturable fungal endophytes of mangrove plants so far investigated appear to be not distinct from those associated with the terrestrial plants. The pattern of distribution of endophytes in a leaf and its species composition is similar in plants of mangrove and other ecosystems. This reflects the ecological success of a few fungal species to lead an endophytic life in plants of different environment and taxonomic affiliation. Despite this commonality, endophytes of mangroves are distinct in possessing certain traits which enable them to survive in the harsh mangrove environment. Their ability to produce novel bioactive compounds and enzymes make them attractive candidates for bioprospecting. Considering the endophyte-mediated improvement of performance of plants of other ecosystems, more studies are needed on mangrove endophytes addressing their role in abiotic and biotic stress tolerance of mangroves. Their functions in mangrove ecosystem including litter degradation and nutrient recycling, as well as their enzyme arsenal and secondary metabolite spectrum, need to be studied in detail in order to improve our understanding of these unique plant endosymbionts. Information gleaned on these aspects may aid in the protection and restoration of deteriorating mangrove vegetation.

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Endobiome · Fungal diversity · Foliar endophytes · Forest restoration

10.1 Introduction

Mangrove forests or tidal forests are found along tropical and subtropical ocean coastlines. Mangrove ecosystem is unique since the life here is adapted to high salinity, hypoxia, tidal fluctuations, strong ultraviolet light, anaerobic soils and high tidal interference (Rothschild and Mancinelli 2001; Sandilyan and Kathiresan 2012) (Plate 10.1). According to Global Mangrove Watch (GMW), the global cover of mangroves for 2010 was 137,600 km^2 (Bunting et al. 2018) with around 75% of mangroves being located in merely 15 countries (Giri et al. 2011). Mangrove ecosystems are biodiversity rich and highly productive; they provide many valuable ecosystem services including nutrient cycling, carbon sequestration, bioremediation of waste and contribution to food security (Lee et al. 2014; Malik et al. 2015; Richards and Friess 2016). They afford protection against shoreline erosion (Alongi 2014) and natural calamities like floods and tsunamis (Menéndez et al. 2020). As a blue carbon reservoir, the mangroves account for 10–15% of global carbon storage (Alongi 2014).

Plate 10.1 Pichavaram Mangrove Forest, Tamil Nadu

10.2 Mangrove Habitats in India

The coastline of India, which is about 7516.6 km^2 including the Island territories (Anonymous 1984), has a mangrove cover of about 4975 km^2 (FSI 2019) (Plate 10.1). The state of West Bengal has the largest mangrove cover of 2112 km^2. The East Coast or the Deltaic Mangrove habitat has larger and more widespread cover when compared to the West Coast Mangroves (Estuarine and Back Water Mangrove Habitat) and Andaman and Nicobar Islands (Insular Mangroves) because of its distinctive geo-morphological setting (Ragavan et al. 2016). Indian mangrove forests harbour a large number of floral and faunal wealth with over 1600 plant and 3700 animal species (Ghosh 2011). As far as the number of obligate or true mangrove plant species are concerned, the estimate varies from thirty (Mandal and Naskar 2008) to thirty-nine (Kathiresan 2008).

10.3 Mangrove Fungi

A mangrove ecosystem supports a variety of microbes including bacteria, protists, microalgae and fungi. Fungi occur on the prop roots, pneumatophores, decaying leaves, roots, and wood of mangrove plants, as well as on drift wood, intertidal grasses, algae, sediments, soil, crustaceans, corals, and calcareous tubes of mollusc shells of the mangrove forests (Kohlmeyer and Kohlmeyer 1979; Hyde et al. 1998; Jones and Mitchell 1996; Sarma and Hyde 2001). The fungal communities associated with a mangrove species of distantly located individuals are more dissimilar than those of closely occurring individuals; furthermore, the fungal associates of the aerial parts of mangroves, which are never submerged, are less diverse than those occurring in the submerged parts of the plants (Lee et al. 2019). Generally terrestrial fungi are associated with the aerial parts of mangroves, while marine fungi (obligate and facultative) appear to dominate the flooded parts (Lee et al. 2019). Such a preferential distribution of these ecological groups of fungi is a reflection of their adaptations to different environmental niches offered by mangrove ecosystem.

10.4 Mangrove Endophytes

Apart from the fungi mentioned above which are associated with various tissues of mangrove plants, some fungi occur as endophytes in their leaves and roots. Endophytes (Class 3 type—Rodriguez et al. 2009) live inside the living tissues of all plants as mutualists or commensals for short or long periods. Latent pathogens also occur as endophytes until environmental signals induce them to switch over to a pathogenic phase and cause diseases (Wheeler et al. 2019). One of the earliest studies on the fungal endophytes (FE) of mangroves is that of Suryanarayanan et al. (1998) which addresses the endophyte assemblages in the leaves of *Rhizophora apiculata* and *R. mucronata*. This study showed that, like in terrestrial plants, the leaves are densely colonised by many FE species, only one or two of them are

dominant (Suryanarayanan et al. 2018). Rajamani et al. (2018), in one of the largest surveys of mangroves for their foliar endophytes, studied twenty mangrove species of the Andaman Islands and found that *Phomopsis/Diaporthe* occurs as endophyte in all the plants and *Xylaria*, *Colletotrichum* and *Phyllosticta* are endophytic in most of the plants. Here we collate the available data for the last 22 years (from the year 1998 to 2020) to identify the dominant endophytes (non-sterile and culturable) of mangrove leaf endophyte communities. A total of thirty-eight mangrove plant species from different parts of the world are included in this analysis (Table 10.1). *Guignardia* spp. were present in 38% of the plant hosts, while species of *Glomerella* (*Colletotrichum*) (Plate 10.2a), *Xylaria* (Plate 10.2b) and *Diaporthe* (*Phomopsis*) infected 21%, 18% and 16% of the mangrove species, respectively (Fig. 10.1).

Since specific adaptations are required to survive, only some plant species grow in a mangrove environment; thus, the species diversity of plants in mangrove forests is low, and consequently the density of individual species is high (Gilbert et al. 2002). Considering this low host plant diversity and the fact that the environment would act as a filter in selecting fungi tolerant to the harsh conditions prevailing there, it is conceivable that the diversity of foliar endophytes of mangroves would not be high. Rajamani et al. (2018) concluded that endophytes of 'mangroves are not unique with reference to their species diversity and frequency of occurrence when compared to those of terrestrial plants'. This conclusion is confirmed by the work of Suryanarayanan et al. (2018) in which 224 angiosperm plants of 60 families (including mangroves) were screened for their foliar endophyte assemblages. They showed that species of *Colletotrichum*, *Phyllosticta*, *Phomopsis* and *Xylaria* occurred as endophytes in the leaves of many plant hosts including those that were taxonomically not closely related. A few other studies also endorse the wide host range of these fungi as foliar endophytes (Pandey et al. 2003; Jeewon et al. 2004; Murali et al. 2006; Wei et al. 2007; Tejesvi et al. 2009; Govindarajulu et al. 2013). Such a host generalism is also observed among other guilds of tropical fungi such as wood rotting fungi (Parfitt et al. 2010), mycorrhizal fungi (Zhao et al. 2003; Tedersoo et al. 2010) and epifoliar fungi (Gilbert and Webb 2007). One explanation for such a lack of host specificity among tropical plant-associated organisms is the existence of high plant species diversity in the tropics that results in a non-continuous distribution of hosts (May 1991; Novotny et al. 2002). However, it is not known how such broad host range endophytes are adapted to encounter the different secondary metabolites and co-occurring microbes of different plant species (Suryanarayanan 2013, 2020; Schulz et al. 2015).

Generally, FE of plants of terrestrial habitats exhibit some degree of tissue specificity (Su et al. 2010; Wearn et al. 2012). A similar tissue specificity is also present among mangrove FE. The endophyte assemblages of the bark, petiole and the propagule in *Rhizophora apiculata* differ significantly indicating tissue preference among mangrove FE (Kumaresan et al. 2002).

Table 10.1 Dominant foliar fungal endophytes of mangrove plants

Host	Location	Dominant foliar endophyte(s)	References
Acanthus ebracteatus	Andaman Island, India Luzon Island, Philippines	*Colletotrichum gloeosporioides* *Aspergillus niger*	Rajamani et al. (2018) Ramirez et al. (2020)
Acanthus ilicifolius	Nethravathi Mangrove, Karnataka, India	*Cladosporium* sp.	Maria and Sridhar (2003)
	Ranong Province, Thailand	*Phyllosticta* sp. 1	Chaeprasert et al. (2010)
	Andaman Island, India	*Colletotrichum* sp.	Rajamani et al. (2018)
A. ilicifolius var. *xiamenensis*	Lieyu Township, Kinmen County, Taiwan	*Drechslera dematioidea* and *Fusarium oxysporum*	Chi et al. (2019)
Acrostichum aureum	Nethravathi Mangrove, Karnataka, India	*Acremonium* sp. and *Paecilomyces* sp.	Maria and Sridhar (2003)
Aegiceras corniculatum	Beilun Estuary National Reserve, South China	*Leptosphaerulina chartarum*	Li et al. (2016)
	Andaman Island, India	*Diaporthe pseudomangiferae*	Rajamani et al. (2018)
	Zhanjiang Mangrove National Nature Reserve, South China	Dothideomycetes and Tremellomycetes	Yao et al. (2019)
	Luzon Island, Philippines	*Nigrospora* sp. 2	Ramirez et al. (2020)
Aegiceras floridum	Luzon Island, Philippines	*Cladosporium* sp.	Ramirez et al. (2020)
Avicennia alba	Chanthaburi Province, Thailand	*Phyllosticta* sp.1	Chaeprasert et al. (2010)
Avicennia marina	Pichavaram Mangrove, Tamil Nadu, India	*Phoma* sp. 2	Kumaresan and Suryanarayanan (2001)
	Beilun Estuary National Reserve, South China	*Phyllosticta capitalensis*	Li et al. (2016)
	Andaman Island, India	*Diaporthe pseudomangiferae*	Rajamani et al. (2018)
	Zhanjiang Mangrove National Nature Reserve, South China	Tremellomycetes	Yao et al. (2019)
	Luzon Island, Philippines	*Phialophora* sp.	Ramirez et al. (2020)

(continued)

Table 10.1 (continued)

Host	Location	Dominant foliar endophyte(s)	References
Avicennia officinalis	Pichavaram mangrove, Tamil Nadu, India	*Paecilomyces* sp.	Kumaresan and Suryanarayanan (2001)
	Andaman Island, India;	*Diaporthe pseudomangiferae*	Rajamani et al. (2018)
Avicennia schaueriana	Itamaracá Island, Brazil	*Colletotrichum gloeosporioides*	Costa et al. (2012)
Bruguiera cylindrica	Pichavaram Mangrove, Tamil Nadu, India	*Colletotrichum gloeosporioides*	Kumaresan and Suryanarayanan (2001)
	Andaman Island, India	*Xylaria* sp. 1	Rajamani et al. (2018)
Bruguiera gymnorrhiza	Beilun Estuary National Reserve, South China	*Neofusicoccum australe*	Li et al. (2016)
	Andaman Island, India	*Phyllosticta capitalensis*	Rajamani et al. (2018)
	Zhanjiang Mangrove National Nature Reserve, South China	Dothideomycetes and Tremellomycetes	Yao et al. (2019)
Bruguiera parviflora	Andaman Island, India	*Xylaria* sp. 1	Rajamani et al. (2018)
Ceriops decandra	Prachuap Khiri Khan Province, Thailand	*Phyllosticta* sp. 1	Chaeprasert et al. (2010)
	Luzon Island, Philippines	*Penicillium* sp. 3	Ramirez et al. (2020)
Ceriops tagal	Andaman Island, India	*Xylaria* sp. 1	Rajamani et al. (2018)
	Luzon Island, Philippines	*Penicillium* sp. 3	Ramirez et al. (2020)
Excoecaria agallocha	Pichavaram Mangrove, Tamil Nadu, India	*Glomerella* sp.	Kumaresan and Suryanarayanan (2001)
	Andaman Island, India	*Phyllosticta capitalensis*	Rajamani et al. (2018)
	Zhanjiang Mangrove National Nature Reserve, South China	Dothideomycetes	Yao et al. (2019)
	Luzon Island, Philippines	*Phialophora* sp.	Ramirez et al. (2020)
Kandelia candel	Mai Po Nature Reserve, Hong Kong	*Phomopsis* sp., *Pestalotiopsis* sp., *Guignardia* sp. and *Xylaria* sp.	Pang et al. (2008)
	Beilun Estuary National Reserve, South China	*Phyllosticta capitalensis*	Li et al. (2016)

(continued)

Table 10.1 (continued)

Host	Location	Dominant foliar endophyte(s)	References
	Zhanjiang Mangrove National Nature Reserve, South China	Dothideomycetes and Tremellomycetes	Yao et al. (2019)
Laguncularia racemosa	Itamaracá Island, Brazil	*Guignardia* sp.	Costa et al. (2012)
Lumnitzera littorea	Chanthaburi Province, Thailand	*Phyllosticta* sp. 1	Chaeprasert et al. (2010)
	Andaman Island, India	*Phyllosticta capitalensis*	Rajamani et al. (2018)
Lumnitzera racemosa	Pichavaram mangrove, Tamil Nadu, India	*Phyllosticta* sp. 4	Kumaresan and Suryanarayanan (2001)
	Andaman Island, India	*Phyllosticta capitalensis*	Rajamani et al. (2018)
Nypa fruticans	Andaman Island, India	*Xylaria* sp. 1	Rajamani et al. (2018)
	Luzon Island, Philippines	*Phialophora* sp.	Ramirez et al. (2020)
Osbornia octodonta	Luzon Island, Philippines	*Phialophora* sp.	Ramirez et al. (2020)
Phoenix paludosa	Andaman Island, India	*Nodulisporium* sp. 1	Rajamani et al. (2018)
Rhizophora apiculata	Pichavaram Mangrove, Tamil Nadu, India	*Phyllosticta* sp. MG 90 and *Sporormiella minima*	Suryanarayanan et al. (1998); Kumaresan and Suryanarayanan (2002)
	Chanthaburi, Prachuap Khiri and Ranong Province, Thailand	*Phyllosticta* sp. 2 and *Cladosporium* sp. 1	Chaeprasert et al. (2010)
	Andaman Island, India	*Aspergillus fumigatus*	Rajamani et al. (2018)
Rhizophora mangle	Itamaracá Island, Brazil	*Phyllosticta* sp.	Costa et al. (2012)
Rhizophora mucronata	Pichavaram Mangrove, Tamil Nadu, India	*Sporormiella minima*	Suryanarayanan et al. (1998)
	Chanthaburi and Ranong Province, Thailand	*Phyllosticta* sp.2 and *Pestalotiopsis* sp.1	Chaeprasert et al. (2010)
	Matang Mangrove Forest Reserve, Malaysia	*Pestalotiopsis* sp.	Hamzah et al. (2018)
	Andaman Island, India	*Diaporthe discoidispora*	Rajamani et al. (2018)
	Hainan Island, China	*Neofusicoccum*	Zhou et al. (2018)
	Luzon Island, Philippines	*Penicillium* sp. 4	Ramirez et al. (2020)

(continued)

Table 10.1 (continued)

Host	Location	Dominant foliar endophyte(s)	References
Rhizophora stylosa	Andaman Island, India Hainan Island, China Zhanjiang Mangrove National Nature Reserve, South China	*Xylaria* sp. 1 *Pestalotiopsis* sp. and *Seiridium* sp. Dothideomycetes	Rajamani et al. (2018) Zhou et al. (2018) Yao et al. (2019)
Scyphiphora hydrophyllacea	Andaman Island, India	*Phyllosticta capitalensis*	Rajamani et al. (2018)
Sesbania bispinosa	Nethravathi Mangrove, Karnataka	*Aspergillus niger*	Anita et al. (2009)
Sonneratia alba	Chanthaburi Province, Thailand Andaman Island, India Luzon Island, Philippines	*Phyllosticta* sp. 1 *Pestalotiopsis* sp. *Phialophora* sp.	Chaeprasert et al. (2010) Rajamani et al. (2018) Ramirez et al. (2020)
Sonneratia apetala	Hainan Province, China	*Phomopsis* sp. 3	Xing et al. (2011)
Sonneratia caseolaris	Hainan Province, China	*Stemphylium solani*	Xing et al. (2011)
Sonneratia ovata	Hainan Province, China	*Glomerella* sp.	Xing et al. (2011)
Sonneratia paracaseolaris	Hainan Province, China	*Phoma* sp.	Xing et al. (2011)
Suaeda microphylla	Songyuan Guaibodian, Jilin, China	*Alternaria alternata*	Sun et al. (2011)
Suaeda corniculata	Songyuan Guaibodian, Jilin, China	*Alternaria alternata*	Sun et al. (2011)
Xylocarpus granatum	Ranong Province, Thailand Andaman Island, India	*Colletotrichum* sp. 3 *Colletotrichum gloeosporioides*	Chaeprasert et al. (2010) Rajamani et al. (2018)
Xylocarpus moluccensis	Ranong Province, Thailand	*Phyllosticta* sp.2	Chaeprasert et al. (2010)

10.5 Adaptations of Mangrove Foliar Endophytes

The core endophyte species of the leaves of mangrove plants are not unique as those of terrestrial plants. Considering the distinctiveness of mangrove environments, they should exhibit at least some trait difference to survive in this ecosystem. Mangroves have evolved mechanisms to tolerate a wide range soil salinities (Reef and Lovelock 2015). The leaves of *Aegiceras* and *Avicennia* have salt glands on their epidermis

Plate 10.2a *Glomerella* sp. (*Colletotrichum*) endophytic in leaves of many mangrove species

Plate 10.2b *Xylaria* sp.

through which the excess salt absorbed by the plant is secreted. Leaves of *Bruguiera* and *Kandelia* do not possess salt glands on their leaves and sequester the salt in their vacuoles. Endophytes thus need to be salt tolerant to survive in mangrove leaves. Kumaresan et al. (2002) showed that several mangrove endophytes tolerate 7% NaCl in the growth medium which is equal to twice the concentration of salt present in seawater. It is known that in *Cirrenalia pygmea*, a fungus associated with mangrove roots, polyols regulate turgor and the activities of its polyol metabolism enzymes increase with salinity (Ravishankar and Suryanarayanan 1998). Additionally, this fungus also uses amino acids as compatible solutes for turgor regulation (Ravishankar et al. 1996). Exposure to salinity decreases the unsaturation index of

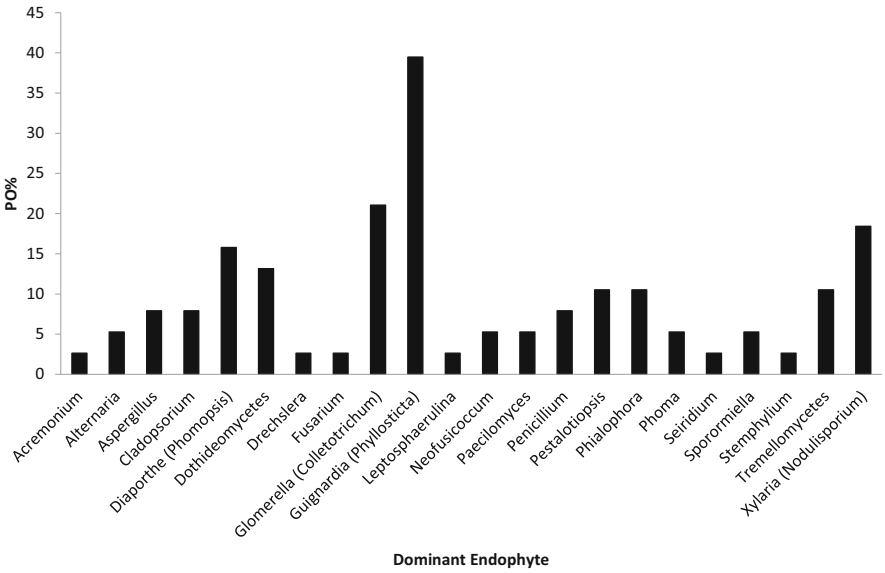

Fig. 10.1 Percentage Occurrence (PO%) of dominant endophytic genera in 38 mangrove hosts

fatty acids in this fungus suggesting a reduction of membrane fluidity for turgor regulation (Ravishankar et al. 1994). It is likely that such alterations at the metabolic level work in the mangrove endophytes also for survival in a saline milieu. Leaves of many mangrove plants contain tannins (Lin et al. 2007) which are antifungal in nature (Anttila et al. 2013). Mangrove leaf endophytes tolerate and even degrade tannins indicating that they are adapted to survive in mangrove leaves (Kumaresan et al. 2002). Dominant mangrove endophytes such as *Phyllosticta* have melanised hyphal walls and melanin protects fungi from sudden osmotic shocks (Ravishankar et al. 1995).

10.6 Bioactive Compounds of Mangrove Endophytes

FE produce an array of secondary metabolites with several exploitable bioactivities (Suryanarayanan et al. 2009; Prado et al. 2013). In a few cases, the endophytes produce their host plant's secondary metabolites in culture (Mohana Kumara et al. 2012; Gandhi et al. 2015). Reciprocal influence of the plant host and its endophyte could result in the production of novel metabolites (Ludwig-Müller 2015). Mangrove fungal endophytes too are a promising source of novel chemical scaffolds which could lead to the synthesis of many useful drugs. The environmental conditions of the mangrove ecosystem determine the composition of its microbes; thus, some of these microbes, even though are not taxonomically different from other ecosystems, exhibit trait difference. This raises the hope of identifying novel bioactive compounds from mangrove endophyte assemblages. A majority of the

850 new bioactive compounds characterised from mangrove-associated fungi in the last decade are produced by FE (Ancheeva et al. 2018). Many mangrove endophytes produce novel metabolites exhibiting a variety of activities including antibacterial, anti-inflammatory, antiviral, α-glucosidase inhibitory, acetylcholinesterase inhibitory, anticancer, antiproliferative, cytotoxic, COX-2 and Protein kinase G (PknG) inhibitory activities (Table 10.2).

10.7 Enzymes of Mangrove Endophytes

It is well known that fungal enzymes find use in various industrial processes. As the demand for such enzymes increases, enzymes vested with higher activity and stability are being sought. Although directed evolution along with site-directed mutagenesis (Piscitelli et al. 2011) and protein engineering are being used for obtaining better enzymes (Böttcher and Bornscheuer 2010); the search for novel biocatalysts from hitherto unexplored microbes would be profitable. FE qualify for being such a novel source, since they have hardly been explored for enzymes (Suryanarayanan et al. 2012). FE of plants of extreme environments such as the mangroves may produce enzymes adapted to such harsh conditions which may find use in industrial applications. A few studies endorse this because endophytes produce novel pharmaceutically important enzymes (Govinda Rajulu et al. 2011; Thirunavukkarasu et al. 2011; Nagarajan et al. 2014) and enzymes for food and biofuel production (Suryanarayanan et al. 2012). Marine-derived endophytes produce ionic liquid-tolerant xylosidases which could find use in conversion of lignocellulosic biomass to biofuel (Sengupta et al. 2017). Some FE utilise toxic furaldehydes, the most abundant volatiles produced during biomass conversion to biofuel (Govinda Rajulu et al. 2014). Since furaldehydes inhibit the downstream process in biomass to biofuel conversion, these FE could find use in improving the efficiency of the process. Mangrove endophytes produce amylase, cellulase, laccase, lipase, pectate transeliminase, protease and tyrosinase (Kumaresan et al. 2002; Kumaresan and Suryanarayanan 2002; Maria et al. 2005) (Table 10.3). Paranetharan et al. (2018) reported that *Talaromyces stipitatus,* an endophyte of the root of the mangrove tree *Avicennia marina*, elaborates salt-tolerant chitinase and chitosanases. This endophyte is halotolerant and produces such chitin-modifying enzymes even in the presence of a high concentration of NaCl in the growth medium, and NaCl induced the production of isoforms of chitinase and chitosanase by this fungus. Mangrove fungal endophtes have hardly been explored for their enzyme arsenal to be used in various industries. Modern methodologies such as genome mining and metagenomics should be employed for identifying enzymes of non-culturable endophytes as has been done for bacteria (Kennedy et al. 2008).

Table 10.2 Various secondary metabolites of mangrove endophytes and their bioactivities

Endophytes	Host and Location	Compounds	Activity tested	References
Alternaria longipes	*Avicennia officinalis*, India	2,4,6–triphenylaniline	Antidiabetic activity	Ranganathan and Mahalingam (2019)
Annulohypoxylon sp.	*Rhizophora racemosa*, Cameroon	Daldinone H, Daldinone I, Daldinone J, Daldinone C, Hypoxylonol C, Daldinone B, 3,4-dihydro-3,4,6,8-trihydroxy-l(2*H*)-naphthalenone, (*R*)-scytalone, 1-hydroxy-8-methoxynaphthalene	Cytotoxic activity	Liu et al. (2017)
Aspergillus flavus	*Kandelia obovata,* Guangdong Province, China	Diphenyl ethers and Phenolic bisabolane sesquiterpenoids	α- glucosidase inhibitory activity	Wu et al. (2018a)
Aspergillus flavus	*Sonneratia alba*, Kupang, East Nusa Tenggara Province, Indonesia	Kojic acid	Antibacterial activity	Ola et al. (2020)
Aspergillus sp.	*Acanthus ilicifolius,* Hainan Island, China	Aspergisocoumrin A–B, 8-dihydroxyisocoumarin-3-carboxylic acid and Dichlorodiaportin	Anticancer activity	Wu et al. (2018b)
Aspergillus sp.	*Avicennia marina,* Red Sea coast close to Hurgada, Egypt	1-(2′,6′-dimethylphenyl)-2-*n*-propyl-1,2-dihydropyridazine-3,6-dione and Dioxoauroglaucin	Antiploriferative activity	Elissawy et al. (2019)
Basidiomycetous fungus XG8D	*Xylocarpus granatum,* Samutsakorn province, Thailand	Chamigrane sesquiterpenes: Merulinol A–F	Cytotoxic activity	Choodej et al. (2016)

Botryosphaeria sp.	*Kandelia candel,* Guangdong Province, China	Botryosphaerin A, Orthosporin, 1*RS* 2*SR*,4*SR*-1,2,3,4-tetrahydronaphthalene-1,2,4,5-tetrol, 1*RS*,2*RS*,4*RS*1,2,3,4-tetrahydronaphthalene-1,2,4,5-tetrol, 11-epiterpestacin and Fusaproliferin	Antimicrobial activity, COX-2 inhibitory and Cytotoxic activity	Ju et al. (2016)
Campylocarpon sp.	*Sonneratia caseolaris*, China	Campyridone A–D and Ilicicolin H	Anticancer activity	Zhu et al. (2016)
Cladosporium sp.	*Ceriops tagal*, South China Sea	4-*O*-α-D-ribofuranose-3-hydroxymethyl-2-pentyl- phenol, (-)-trans-(3*R*,4*R*)-3,4,8-trihydroxy-6,7-dimethyl-3,4-dihydronaphthalen-1(2*H*)-one, (3S)-3,8-dihydroxy-6,7-dimethyl-α-tetralone, (-)-trans-(3*R*,4*R*)-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-napthalenone, (-)-(4*R*)-regiolone, 1,8-dimethoxy naphthalene, (2*S*)-5-hydroxy-2-methyl-chroman-4- one and (2*R**,4*R**)3,4-dihydro-5- methoxy-2-methyl-1(2*H*)-benzo pyran-4-ol	Cytotoxic activity and Antibacterial activity	Wu et al. (2019)
Cladosporium sp.	*Ceriops tagal,* South China	1,1′-dioxine-2,2′-dipropionic acid and 2-methylacetate-3,5,6-trimethylpyrazine	Antibacterial activity	Bai et al. (2019)

(continued)

Table 10.2 (continued)

Endophytes	Host and Location	Compounds	Activity tested	References
Clonostachys rosea	*Bruguiera gymnorrhiza, Santolo Garut Beach, West-Java, Indonesia*	(-)-dihydrovertinolide, Clonostach acids A–C And (-)-Vertinolide	Antimicrobial activity	Supratman et al. (2019)
Cytospora sp.	*Ceriops tagal*, China	Seiricardine D, Xylariterpenoid A, Xylariterpenoid B, Regiolone, 4-hydroxyphenethyl alcohol, (22*E*, 24*R*)5, 8-epidioxy-5a, 8a-ergosta-6,22*E*-dien-3ß-ol, (22*E*, 24*R*)5, 8-epidioxy-5a, 8a-ergosta-6,9(11), 22-trien-3 ß-ol, ß-sitosterol and Stigmast-4-en-3-one	Antimicrobial activity	Deng et al. (2020)
Daldinia eschscholtzii	*Bruguiera sexangula* var. *rhynchopetala*, South China Sea	Cytochalasin: [11]-cytochalasa-5(6),13-diene-1,21-dione-7,18-dihydroxy-16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*R**), [11]-cytochalasa-*n*(12),13-diene-1,21-dione-7,18-dihydroxy16,18-dimethyl-10-phenyl-(7*S**,13*E*,16*S**,18*R**), 1-(2,6-dihydroxyphenyl)butan-1-one and 1,8-dimethoxynaphthalene	Antibacterial activity	Yang et al. (2018)
Diaporthe phaseolorum	*Acanthus ilicifolius*, China	Alkaloids: Diaporphasine A–D Meyeroguilline A, Meyeroguilline C, Meyeroguilline D, 5-deoxybostrycoidin and Fusaristatin A	Cytotoxic and Growth inhibitory activity	Cui et al. (2017)

Diaporthe sp.	*Rhizophora stylosa*, Sanya City, Hainan Province, China	Octaketides (Dothiorelone O, (15*R*)-acetoxydothiorelone A) Chromone (Pestalotiopsone H), Phthalides ((±)-microsphaerophthalide H, microsphaerophthalide I) and α-pyrone (methyl convolvulopyrone)	Anti-influenza A virus (H1N1)	Luo et al. (2018a)
Diaporthe sp.	*Rhizophora stylosa*, Sanya city, Hainan Province, China	Isochromophilones A−F, Azaphilone derivatives	Cytotoxic activity	Luo et al. (2018b)
Diaporthe sp.	*Bruguiera sexangula,* South China	Sesquiterpenoids, 1-methoxypestabacillin B, 11-nor-8,9*R*-drimanediol, chrodrimanin type meroterpenoids	Antiviral activity	Luo et al. (2019)
Eupenicillium sp.	*Xylocarpus granatum,* South China Sea	Penicilindole A–C	Cytotoxic activity and Antibacterial activity	Zheng et al. (2018)
Eupenicillium sp.	*Xylocarpus granatum*, South China Sea	Phenol derivative, 3-chloro-5-hydroxy-4-methoxyphenylacetic acid methyl ester, Methyl 4-hydroxyphenylacetate, Cytosporone B, (*R*)-striatisporolide A, (*R*)-butanedioic acid and Ergosterol	Insecticidal activity	Mei et al. (2020)
Eurotium rubrum	*Suaeda salsa,* BoHai, China	Rubrumol	Anticancer activity	Zhang et al. (2017a)
Fusarium solani	*Avicennia officinalis*, Dive agar and Shrivardhan, Maharashtra, India	3-Pyridylacetic acid, Aloe-emodin, Antipyrine, Mitoxantrone and Sulfabenzamide. 2, 4, 6-Trimethylacetophenone	Anticancerous compounds, Antioxidant activity, Anti-inflammatory activity and Antimicrobial activity	Sonawane et al. (2020)

(continued)

Table 10.2 (continued)

Endophytes	Host and Location	Compounds	Activity tested	References
		Imine and Daidzein. Anabasamine, Desethylhydroxychloroquine and Mometasone Furoate. Antipyrine, Dihydrodeoxystreptomycin, Phenylacetic acid and Phenylpyruvic acid		
Fusarium sp.	*Kandelia candel*, Dongzhai mangrove, Hainan, China	Cyclic peptide: Beauvericin	Anticancer activity	Tao et al. (2015)
Lasiodiplodia sp.	*Excoecaria agallocha*, Gaoqiao, Zhanjiang city, Guangdong Province, China	Lasidiplodins: 12*E*,15*R*-5-hydroxy-3-methoxy-16-methyl-8,9,10,11,14,15-hexahydro-1*H*-benzo[c][1] oxacyclodocecin-1-one, Ethyl 2,4-dihydroxy-6-(8-oxononyl)benzoate, (*R*)-Zearalane, 2,4-dihydroxy-6-nonylbenzoateand (*R*)-de-*O*-methyllasiodiplodin	Cytotoxic activity	Huang et al. (2017)
Lasiodiplodia theobromae	*Acanthus ilicifolius,* Zhanjiang Mangrove Nature Reserve, Guangdong Province, China	Chloropreussomerin A-B Preussomerin M, Preussomerin K, Preussomerin H, Preussomerin G, Preussomerin F, Preussomerin D, Preussomerin C, Preussomerin A	Cytotoxic and Antibacterial activity	Chen et al. (2016a)

Lasiodiplodia theobromae	*Acanthus ilicifolius*, South China Sea	Lasiodiplactone A	Anti-inflammatory activity and α-glucosidase inhibitory activity	Chen et al. (2017a)
Mucor irregularis	*Rhizophora stylosa,* Hainan Island, China	Rhizovarin A–F Penitrem A, Penitrem C, Penitrem F	Cytotoxic activity	Gao et al. (2016)
Neosartorya udagawae	*Avicennia marina,* Hainan Province, China	Neosartoryadin A–B and Fumiquinazoline	Anti-influenza A virus (H1N1)	Yu et al. (2016)
Penicillium brocae	*Avicennia marina*, China	Penicibrocazine A–F	Antimicrobial activity	Meng et al. (2015)
Penicillium brocae	*Avicennia marina,* Hainan Island, China	Spirobrocazine A–C and Brocazine G	Anticancer activity	Meng et al. (2016)
Penicillium chermesinum	*Heritiera littoralis,* Samut Sakhon province, Thailand	2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6*H*-benzo[c] chromen-6-one	Anticancer activity	Darsih et al. (2017)
Penicillium chrysogenum	*Myoporum bontioides* (Semi-mangrove) Leizhou Peninsula, China	Penochalasin I, Penochalasin J, Chaetoglobosin G, Chaetoglobosin F, Chaetoglobosin C, Chaetoglobosin A, Chaetoglobosin E, Armochaetoglobosin I and Cytoglobosin C	Cytotoxic activity and Antifungal activity	Huang et al. (2016)
Penicillium citrinum	*Bruguiera sexangula* var. *rhynchopetala*, South China Sea	4-chloro-1-hydroxy-3-methoxy-6-methyl-8-methoxycarbonyl-xanthen-9-one and 2'-acetoxy-7-chlorocitreorosein	Antibacterial activity	He et al. (2017)

(continued)

Table 10.2 (continued)

Endophytes	Host and Location	Compounds	Activity tested	References
Penicillium citrinum	*Bruguiera sexangula* var. *rhynchopetala*, South China Sea	Penibenzophenone A–B, (*E*)-*tert*-butyl (3-cinnamamidopropyl)carbamate, Culochrin, Asterric acid and *n*-butyl asterrate	Antibacterial activity and Cytotoxic activity	Zheng et al. (2019)
Penicillium janthinellum	*Sonneratia caseolaris,* Province, China	Penicisulfuranol A–F	Cytotoxic activity	Zhu et al. (2017)
Penicillium sp.	*Ceriops tagal,* Hainan Province, China	Penicieudesmol B	Anticancer activity	Qiu et al. (2018)
Penicillium sp.	*Bruguiera gymnorrhiza*, China	2-deoxy-sohirnone C, 5*S*-hydroxynorvaline-*S*-Ile, 3*S*-hydroxylcyclo(*S*-Pro-*S*-Phe) and Cyclo(*S*-Phe-*S*-Gln)	Antibacterial activity	Jiang et al. (2018)
Penicillium sp.	*Kandelia candel,* Guangxi province, China	3-epiarigsugacin E, Arisugacin D, Arisugacin B, Territrem C, Terreulactone C	Inhibitory activities against acetylcholinesterase	Ding et al. (2016)
Pestalotiopsis clavispora	*Rhizophora harrisonii,* Port Harcourt, Nigeria	Pestalpolyol I, Pestapyrones A, Pestapyrones B, (*R*)-(-)-periplanetin D, Pestaxanthone, Norpestaphthalide A and Pestapyrone C	Anticancer activity	Hemphill et al. (2016)

Pestalotiopsis coffeae	Fishtail palm, Xinglong Hainan Province, China	Isocoumarin derivatives: 6,8-dihydroxy-7-methyl-1-oxo-1*H*-isochromene-3-carboxylic acid, 6,8-dihydroxy-3-methoxy-3,7-dimethylisochroman-1-one (*R*)-periplanetin D, (*R*)-5,7-dihydroxy3-((*S*)-1-hydroxyethyl)- isobenzofuran-1(3*H*)-one, (*S*)-5,7dihydroxy-3-((*S*)-1-hydroxyethyl)-isobenzofuran-1(3*H*)-one, (*R*)-5,7-dihydroxy-3-((*S*)-1-hydroxyethyl)-6-methylisobenzofuran1(3*H*)-one and (*S*)-5,7-dihydroxy-3-((*S*)-1-hydroxyethyl)-6 methylisobenzofuran-1(3*H*)-one	Not mentioned	Wang et al. (2018)
Pestalotiopsis sp.	*Rhizophora mucronata*	Dimethylincisterol, Flufuran, Ergosta-5,7,22-trien-3-ol, Stigmast-4-en-3-one, Demethylincisterol A3, Ergosta-5,7,22-trien-3-ol, Stigmastan-3-one, Stigmast-4-en-3-one, Stigmast4-en-6-ol-3-one, Similanpyrone B, (2-cis, 4-trans)-abscisic acid and 5, 8-epidioxy-5, 8-ergosta-6, 22*E*-dien-3-ol	Anticancer activity	Zhou et al. (2017)

(continued)

Table 10.2 (continued)

Endophytes	Host and Location	Compounds	Activity tested	References
Pestalotiopsis sp.	*Rhizophora stylosa,* Dong Zhai Gang-Mangrove, China	Pestalotiopisorin B, (*R*)-(-)- mellein methyl ether, Pestalotiopyrone G, (*R*)-mevalonolactone, Pestalotiollides A, Pestalotiollides B	Antibacterial activity	Xu et al. (2020)
Phomopsis longicolla	*Bruguiera sexangula var. rhynchopetala,* South China Sea	Biphenyl derivative 5,5′-dimethoxybiphenyl-2,2′-diol	Antibacterial activity	Li et al. (2017)
Phomopsis sp.	*Acanthus ilicifolius*, South China Sea, Hainan Province, China	Phomopyrone A, Acropyrone and Ampelanol	Antibacterial activity	Cai et al. (2017a)
Phomopsis sp.	*Kandelia candel*, Mangrove Nature Conservation Area, Fugong, Fujian Province, China	Polyketides: *Mycoepoxydiene* Deacetylmycoepoxydiene, Phomoxydiene A, 2,3-dihydromycoepoxydiene, Phomoxydiene B and Phomoxydiene C	Cytotoxic activity and Activity against of AMPK	Zhang et al. (2017b)
Phomopsis spp.	*Xylocarpus granatum,* Trang Province, Thailand	Phomopsichalasin D–G	Anticancer activity	Luo et al. (2016)
Phyllosticta capitalensis	*Bruguiera sexangula,* China	Meroterpenes guignardone A, 12-hydroxylated guignardone A, Guignardone J, Guignardone M, and four Polyketides: Xenofuranone B, 6,8-dihydroxy-5-methoxy-3-methyl-1*H*-isochromen-1-one, Regiolone and 3,4-dihydroxybenzoic acid	Antimicrobial activity	Xu et al. (2019)

Pleosporales sp. SK7	*Kandelia candel*, Shankou Mangrove Nature Reserve, Guangxi Province, China	Sesquiterpene: (10*S*,2*Z*)-3-methyl-5-(2,6,6-trimethyl-4-oxocyclohex-2-enyl) pent-2-enoic acid, Methyl 2-(2-carboxy-4hydroxy-6-methoxylphenoxy)-6-hydroxy-4-methylbenzoate, Asterric acid, Methyl asterrate and Methyl 3-chloroasterric acid	Cytotoxic activity	Wen et al. (2019)
Pseudopestalotiopsis theae	*Rhizophora racemosa,* Lagos	Polyketide derivatives: Pestalotheols I–Q and Cytosporins O–W	Cytotoxic activity and Antibacterial activity	Yu et al. (2020)
Rhytidhysteron rufulum	*Bruguiera gymnorrhiza,* Prachuab Kiri Khan Province, Thailand	Rhytidchromones A–E	Anticancer activity	Chokpaiboon et al. (2016)
Talaromyces amestolkiae	*Kandelia obovata*, Zhanjiang Mangrove Nature Reserve, Guangdong Province, China	Isocoumarins and Benzofurans: {6-hydroxy-8-methoxy-3,4-Dimethylisocoumarin, *S*-(_)-5-hydroxy-8-methoxy-4-(10-hydroxyethyl)-isocoumarin, 5,6-dihydroxy-3-(4-hydroxypentyl)-isochroman-1-one. 5-hydroxy-7-methoxy-2-methylbenzofuran-3-carboxylic acid and 1-(5-hydroxy-7-methoxybenzofuran-3-yl) ethan-1-one	α-glucosidase inhibitory and antibacterial activities	Chen et al. (2016b)
Talaromyces sp.	*Kandelia obovata,* Guangdong Province, China	Talaramide A	Protein kinase G (PknG) inhibitor activity	Chen et al. (2017b)

(continued)

Table 10.2 (continued)

Endophytes	Host and Location	Compounds	Activity tested	References
Talaromyces stipitatus	*Acanthus ilicifolius,* Shankou Mangrove, China	Talaromyone A–B	Antibacterial activity and α-glucosidase inhibitory	Cai et al. (2017b)
Trichoderma sp.	*Xylocarpus granatum,* Hainan Island, China	(9*R*,10*R*)-dihydro-harzianone	Anticancer activity	Zhang et al. (2016)
Zasmidium sp.	*Laguncularia racemosa,* Juan Diaz, Panama	Triglyceride and Dehydrocurvularin	α- glucosidase inhibitory activity	Lopéz et al. (2019)

Table 10.3 Extracellular enzymes from fungal endophytes of mangrove plants

Host	Endophytes	Extracellular enzymes	References
Rhizophora apiculata	*Chaetomium globosum, Glomerella* sp. MG 108 *Pestalotiopsis* sp. MG 98, *Sporormiella minima* and Sterile form MG 168	Amylase, Cellulase, Laccase, Lipase, Pectate transeliminase, Pectinase, Protease, Tyrosinase	Kumaresan et al. (2002); Kumaresan and Suryanarayanan (2002)
Avicennia marina, A. officinalis, Bruguiera cylindrica, Ceriops decandra, Lumnitzera racemosa	*Colletotrichum* sp. MG 295, *Paecilomyces* sp. MG 208, *Phoma* sp. MG 190, *Phomopsis* sp. MG 186, *Phyllosticta* sp. MG 123 and Sterile form MG 302.	Amylase, Cellulase, Laccase, Lipase, Pectate transeliminase, Pectinase, Protease, Tyrosinase	Kumaresan et al. (2002)
Acanthus ilicifolius and *Acrostichum aureum*	*Acremonium* sp. *Alternaria chlamydospora, Alternaria* sp., *Aspergillus* sp. 2, *Aspergillus* sp. 3, *Fusarium* sp. and *Pestalotiopsis* sp.	Amylase, Cellulase, Lipase, Protease	Maria et al. (2005)
Avicennia marina	*Talaromyces stipitatus*	Chitinase/ chitosanase	Paranetharan et al. (2018)
Mangroves form Cananeia mangrove forest, Brazil	*Diaporthe* sp., *Fusarium sambucinum Fusarium* sp., *Hypocrea lixii* and *Trichoderma camerunense*	Endo-cellulase, Endo-xylanase, Lignin peroxidase, Manganese peroxidase and Laccase	Martinho et al. (2019)

10.8 Mangrove Endophytes: Not an Insignificant Biotic Component

FE have evolved with the plants and are a constant entity of a plant microbiome. They are an inevitable constituent of a plant and their role in the growth, performance and reproduction of their host plants are being unravelled. Endophytes increase the abiotic tolerance of the plants they colonise. The abiotic stresses include salinity, nitrogen limitation and drought (Rho et al. 2018; Sampangi-Ramaiah et al.

2020). These abilities of endophytes are being viewed as a new avenue for improving crop performance especially under the predicted climate change scenario (Suryanarayananan and Uma Shaanker 2020). Endophytes of plants from extreme habitats which are adapted to the harsh conditions could be inoculated into crops to enhance their stress tolerance. For example, FE of plants of the Antarctic increased salt stress tolerance of lettuce and tomato plants (Molina-Montenegro et al. 2020). Similarly, an endophyte from a salt-tolerant plant confers salt tolerance to salt-sensitive rice (Sampangi-Ramaiah et al. 2020). Mangrove roots have salt-tolerant genes (Basyuni et al. 2011; Krishnamurthy et al. 2017), and leaves have salt glands with such genes to exude salt (Jyothi-Prakash et al. 2014). Endophytes associated with these tissues could be screened for their salt tolerance and selected for their performance in crop plants.

Litter degradation is critical to the nutrient budget of any forest ecosystem. Fungi among the litter degrading organisms play a fundamental role here as deconstructors of recalcitrant biopolymers such as cellulose and lignin in the biomass. It is now established that some FE continue to survive in fallen leaves and contribute to litter degradation by swapping to saprotrophic mode of existence (Unterseher et al. 2013; Yuan and Chen 2014; Prakash et al. 2015). There is a major lacuna with reference to the contribution of FE of mangroves in litter degradation. In mangroves, leaf endophytes of *Rhizophora apiculata* including species of *Glomerella*, *Pestalotiopsis* and *Phialophora* continue to grow as saprotrophs after the leaf fall (Kumaresan and Suryanarayanan 2002). They also elaborate biomass degrading enzymes such as cellulases, xylanases, laccases and pectinases suggesting that they could contribute to mangrove litter degradation (Kumaresan and Suryanarayanan 2002). The persistence of FE in fallen leaves and their ability to elaborate biopolymer degrading enzymes would render the biomass fit for the subsequent saprotrophic players to complete the process of degradation (Voříšková and Baldrian 2013; Prakash et al. 2015) (Fig. 10.2). However, their performance under salinity, a major stress in mangrove ecosystem (Virgulino-Júnior et al. 2020), is not known. Information on the role of FE in litter decomposition may be useful for estimating nutrient recycling and predicting carbon sequestration in mangrove ecosystems.

10.9 Conclusions

The culturable foliar FE of mangrove so far studied are not taxonomically unique when compared to those of plants of other ecosystems. However, they possess unique traits which aid in their survival in the extreme environment of the mangrove ecosystem. It can be expected that the sustained interactions of endophytes with mangrove plants, co-occurring microbes supported by these plants and the unique environment would set these fungi apart from their conspecific endophytes of other ecosystems. Mangrove cover is being lost or fragmented to a great extent mainly due to aquaculture and rice cultivation (Bryan-Brown et al. 2020). Additionally, climate change is predicted to have negative influence on mangrove vegetation due to increased temperature, storminess and salinity (Ward et al. 2016). Restoration of

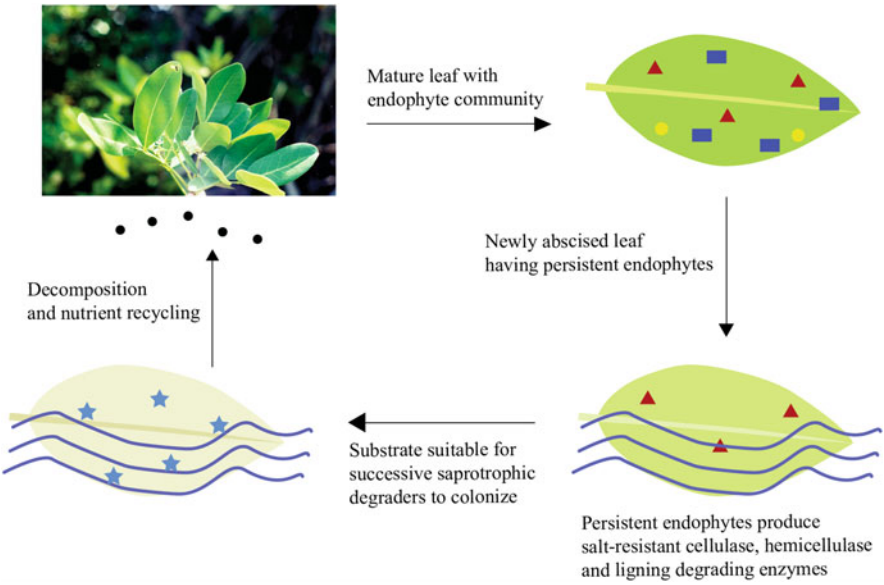

Fig. 10.2 Possible role of leaf endophytes in litter degradation in a mangrove ecosystem

mangrove cover is usually done by propagules planting and/or transplantation of nursery-raised plants (Thivakaran 2017). Although the functional roles played by fungal associates in the maintenance of mangrove ecosystem are hardly understood, the general appreciation that the microbiome (including the endophytes) associated with a plant contribute to its survival and performance (Suryanarayanan 2020) that should motivate studies on endophytes in maintaining mangrove health.

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Insect Pathogenic Fungi and Their Applications: An Indian Perspective

11

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Abstract

The use of insect pathogenic fungi is an attractive eco-friendly option for pest control in agriculture. In India, incorporating entomopathogens as a part of integrated pest management (IPM) programs has led to a notable increase in their use. As of now, more than 250 products based on 15 different species of entomopathogenic fungi, *viz., Beauveria bassiana* (~90 formulations), *Metarhizium anisopliae* (~40 formulations), *Lecanicillium lecanii* (~70 formulations), *Hirsutella thompsonii* (3 formulations), and others, are registered for their use against various pests and pathogens in India. On the contrary, despite having the potential, many entomopathogenic fungi could not reach the commercial level because of a lack of strategies for identifying the best sources to isolate and select the most virulent strains, resulting in the high cost and poor performances in the field. Other concerns are, of course, the shelf life and field stability of the formulations. In this scenario, defining a strategy for the knowledge-based development of mycoinsecticides is crucial to gain more acceptance of entomopathogens in Indian agriculture. This chapter summarizes the various steps to obtain more virulent strains of entomopathogens, either from the natural environment or by strain improvement. The journey of mycoinsecticides from laboratory to commercialization, globally and with particular reference to the Indian scenario, is also documented. The next step in making the fungal

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entomopathogens technologically and commercially successful is to establish such virulent strains in crops susceptible to insect pests as bodyguards, which is also discussed.

Keywords

Beauveria bassiana · Endophytic entomopathogens · Horizontal gene transfer · *Metarhizium anisopliae* · Mycopesticide · Strain improvement

If one way be better than another that you may be sure is Nature's way.
—Aristotle (384–322 BC)

11.1 Introduction

Insect pathogenic fungi are one of the key regulators of insect populations in nature. Some genera such as *Beauveria* and *Metarhizium*, with a broad host range, are being used as mycoinsecticides for the control of forest and agricultural insect pests. However, the long-term persistence of the released strains and the effect on non-target hosts and native fungal populations need careful consideration. The coevolution of pathogen and host, either balancing selection or the effect of selected species, has been suggested by many researchers. According to Mei et al. (2020), in the case of plant-pathogen interactions, the effect of selective sweeps is considered to be prominent than the evolution of balancing selection. For instance, the introduction of exotic strains was reported to drive the population shift of the wheat yellow stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici.* It was reported that though insect pathogens, *Beauveria* and *Metarhizium* species, were closely related to plant pathogens and endophytes, the evolutionary pattern of fungus-insect interactions is unclear (Shang et al. 2016; Wang and Wang 2017). Moreover, the effect of biocontrol organisms introduced in the field on the evolution of the local population structure is still unknown. According to Hywel-Jones (2002), insect pathogenic fungi range across highly coevolved *Cordyceps*, a broad-range opportunistic pathogen *Metarhizium*, and opportunistic necrotrophic *Conidiobolus coronatus*.

The hyphomycetous fungi (reproduce asexually by conidia or by hyphal aggregations) are the main players associated with insects. Bassi and Metchnikoff's work triggered interest in these fungi as potential pest controllers (Subramanian 1983). The most commonly occurring entomopathogenic genera are *Acremonium, Aspergillus, Beauveria, Fusarium, Hirsutella, Metarhizium, Nomuraea, Paecilomyces,* and *Verticillium* (Vidhate et al. 2013). One of the reasons could be that conidia of these fungi are produced in large numbers that act as infective propagules. Though a number of species are saprophytic, there are a few ecto- or endoparasites on flies, beetles, termites, and even ants. For instance, *Antennopsis*

gallica is an ectoparasite on termites. However, they are not being used as biocontrol organisms, which could be due to their restricted association with insects.

11.2 Research on Entomopathogenic Fungi in India

It has been seen that earlier work on insect pathogenic fungi was more focused on short communications on their occurrence and identification. However, some attempts were also made to understand fungus-insect interactions (Phadke 1983). Though the fungi like *Beauveria, Hirsutella, Metarhizium, Nomuraea,* and *Verticillium* were recorded in India, they were not being used to control the insect pests in the fields. Instead there were restrictions to use them in the field for unknown fear of possible harmful effects on useful insects. Phadke (1983) extensively surveyed the literature for the reports on entomopathogenic fungi. In 1886, Berkeley recorded two species of *Cordyceps*, *C. falcata* and *C. racemosa*, on a dead caterpillar from Assam. However in 1931, Butler and Bisby reported *Entomophthora muscae* from the housefly from Meerut, UP. Eventually, *Cephalosporium lecanii* (*Verticillium lecanii*) on coffee scale pest (*Coccus viridis*) was also reported from Karnataka state. In 1952, Kamat and Dhande reported *Metarhizium anisopliae* infecting sugarcane hoppers (*Pyrilla perpusilla*). Other fungi reported during that period were *Hirsutella abietina* on *Pyrilla pusana, Aschersonia coffeae* on the unidentified larva, and *Entomophthora brahminae* on chestnut beetles, to name a few. In the 1970s, *Beauveria bassiana*, one of the important insect pathogens, was reported, which was further studied along with *Metarhizium* for practical utility in a "Biological Control Programme" (Phadke 1983). A full account of various insect pathogenic fungi, primary and secondary colonizers, on insect pests, and their respective host plants reported in India was given by Phadke (1983). The notable species recorded were *Aspergillus (A. flavus, A. fumigatus,* and *A. parasiticus*), *Beauveria* (*B. bassiana* and *B. brongniartii*), *Cephalosporium* (*C. indicum* and *C. lecanii*), *Cordyceps* (*C. falcata* and *C. racemosa*), *Entomophthora* (*E. grylii, E. lecanii, E. muscae, E. aphidis,* and *E. brahminae*), *Hirsutella* (*H. abietina, H. nodulosa,* and *H. versicolor*), *Isaria* sp., *M. anisopliae, Mucor hiemalis, Nomuraea rileyi,* and *Paecilomyces farinosus*, to name a few.

Eventually, researchers started working on the field conditions, which could be conducive to pest and pathogens too. Khan and Rajak (1987) reported the role of humidity on the infectivity of *B. bassiana* to the I to VI instar larvae of a gram pod borer, *Helicoverpa armigera*. Earlier Nayak et al. (1978) did an interesting experiment to mimic field conditions to study the pathogenicity of *B. brongniartii* to the rice skipper, *Parnara mathias*. They allowed healthy larvae to crawl on a sporulating culture of *B. brongniartii* and then confirmed pathogenicity by isolating the same fungus from the diseased larvae. Vimala Devi et al. (2003) observed that *N. rileyi* isolates from different geographical locations showed different virulence levels against *H. armigera* and *S. litura*.

Natural fungal epizootics were reported on *H. armigera* and *Spodoptera litura* in cotton, pulse, and peanut fields, especially during winter in South India.

Abbaiah et al. (1988) observed epizootics of *B. bassiana* on *H. armigera* during 1984–1987, while epizootics of *Nomuraea rileyi* on lepidopteran pests have been observed since 1995 by Uma Devi et al. (2003). It is indeed worth pursuing whether such shift was due to the development of resistance in insect community to a specific fungus, which might be unlikely because multiple factors are involved in fungus-insect interaction. The other likely possibilities include the host density-dependent resistance, variation in the virulence of a fungus towards insect or inoculum densities of different fungi, and climatic variations (Thorvilson 1984). Extensive work on the insect pathogenic fungi for technology development was carried out by Deshpande and co-workers (2004). Using soil dilution and Galleria bait methods, a number of *Metarhizium* and *Beauveria* strains were isolated and tested against *H. armigera* in bioassays. The isolation of *N. rileyi* from naturally infected larva of *S. litura* in a sugar beet field was also reported by Deshpande et al. (2004). Further, Yadav and Deshpande (2012) reported effective use of the same isolate for controlling *S. litura* in sugar beet field. Dutta et al. (2013) reviewed the reports of entomopathogenic fungi from North East India. It was seen that *Arthrinium, Aschersonia, Aspergillus, Beauveria, Conidiobolus, Fusarium, Geotrichum, Metarhizium, Mucor, Nomuraea,* and *Verticillium* are common in agriculture fields. Notable observations were *B. bassiana* on banana leaf beetle (*Nodostoma subcostatum*) reported in 1979 from Jorhat, Assam; major coleopteran pest rice hispa (*Dicladispa armigera*) on rice, sugarcane, wheat, and maize infected with *Aspergillus, Beauveria, Fusarium, Mucor,* and *Penicillium* species; and potato pest cutworm or greasy surface caterpillar *Agrotis ipsilon* infected with *Metarhizium* to name a few (Dutta et al. 2013). Thakur and Sandhu (2010) reported periodic surveys of insect pathogenic fungi from Madhya Pradesh and Chhattisgarh forest areas. During 1999–2005, 21 surveys were conducted and 500 plus cadavers were collected. The fungal genera mainly were *Beauveria, Nomuraea,* and *Paecilomyces*, which showed a broad host range, while *Metarhizium, Lecanicillium,* and *Isaria* had been reported with lower distribution frequencies.

The following sections will discuss about the journey of mycoinsecticides: Concept to commercialization globally and with particular reference to Indian scenario too.

11.3 Fungus-Insect and Fungus-Fungus Interactions

As compared to bacteria and viruses, fungi infect a broader range of insects, such as lepidopterans (moths and butterflies), homopterans (aphids and scale insects), hymenopterans (bees and wasps), coleopterans (beetles), and dipterans (flies and mosquitoes). One of the reasons could be that the viral and the bacterial control agents infect insects *via* their digestive tract while fungi act by contact. The other important feature of fungus-insect interaction is not known to show resistance in the insect community as multiple virulence factors contribute to the host killing process. More than 350 fungi from over 40 species and 27 genera are reported to be insect pathogens. The commonly encountered fungal genera are *Beauveria, Lecanicillium,*

Metarhizium, Paecilomyces, and *Tolypocladium*, while opportunistic insect pathogens are from a variety of genera like *Aspergillus, Cladosporium, Fusarium, Geomyces, Mortierella, Mucor, Penicillium,* and *Pestalotiopsis,* to name a few. The genera *Absidia, Chaetomium, Penicillium, Rhizopus, Talaromyces,* and yeast like *Williopsis* species are reported to be secondary colonizers (Chavan et al. 2006, 2008).

The fungi exhibit various morphological forms that work as infective propagules in fungus-insect interactions (Deshpande 1999). The hypha and unicellular yeast (blastospores) and chlamydospores (thick-walled hyphal cells) are vegetative growth forms, while conidia of deuteromycetes and sporangiospores of zygomycetes are produced asexually. The sexually produced zygospores (zygomycetes), oospores (oomycetes), or even ascospores (ascomycetes) were also reported to be useful as infective propagules.

As fungi are effective by contact, considerations such as adhesion of an infective propagule to the insect body, physical entry, and growth of fungus into host, and finally the killing using enzymatic and non-enzymatic components, are important factors to know. For instance, in *Beauveria*, *Hirsutella*, *Metarhizium*, *Nomuraea*, and *Verticillium*, conidia are infective propagules. Therefore, preinfection stages are adhesion and germination of conidia and differentiation into appressoria. The conidia are either dry hydrophobic, as in the case of *Beauveria, Metarhizium,* and *Nomuraea,* or sticky hydrophilic, as observed in *Hirsutella* and *Verticillium* species. The adhesion is either passive (adsorption) or active host-specific (germination and penetration) phenomenon. The toxic lipids, and cuticle phenolic compounds and chitinases produced during molting, affect the effectiveness of adhesion.

Once the fungus breaks through the cuticle with the help of appressorium and cuticle-degrading enzymes such as chitinases, proteases, and lipases, it may grow profusely in the hemolymph by forming the hyphal bodies/yeast-like blastospores, resulting in insect death as a result of starvation or physiological/biochemical disruption brought about by the fungus. The secondary metabolites of the attacking fungus may contribute to the demise of the insect. As a result, the sporulated mycelial growth of the fungus on the cadaver of the host is seen.

Several insect pathogens show either the mycoparasitism towards fungi or anti-fungal activity due to fungal cell wall-degrading enzymes, and the latter is commonly observed. One of the important reasons could be the common structural features of the protective covers, *viz.,* insect cuticle and fungal cell wall. Vidhate et al. (2015) reported the use of conidia of *M. anisopliae*, singly and sequentially with hydrolytic enzymes of *Myrothecium verrucaria* to control both pest and pathogens in the grape field. Yun et al. (2017) observed that *M. anisopliae* and *B. bassiana* were effective against green peach aphid (*Myzus persicae*) and showed that the control of *Botrytis cinerea*, a fungal pathogen which affects vegetables and fruit crops, could be due to the hydrolytic enzymes. On the other hand, *Verticillium (Lecanicillium) lecanii*, a pathogen of aphids, mealybugs, and mites, can parasitize rust fungi (*Uromyces appendiculatus* bean rust, *Uromyces dianthi* carnation rust, *Puccinia recondita f. sp. tritici* wheat leaf rust, *Puccinia striiformis* stripe rust of wheat, and *Phakopsora pachyrhizi* soybean rust) (Spencer and Atkey 1981).

Benhamou (2004) reported mycoparasitism of *V. lecanii* on *Penicillium digitatum*, a causative agent of citrus fruit green mold. The ultrastructural observations suggested the changes and damage in *P. digitatum* hyphae.

Some fungi show antagonism among themselves. A parasitic fungus recognizes the chemical signals originating from the host fungus. *Trichoderma* exhibits necrotrophic parasitism, *i.e.,* attachment by coiling, penetration, and lysis by cell wall hydrolytic enzymes. del Rio et al. (2002) reported biotrophic mycoparasitism of *Sporidesmium sclerotivorum* in response to the contact with the host, *Sclerotinia sclerotivorum*. The haustoria produced in the hyphae of a host, *S. sclerotivorum*, trigger the production of cell wall lytic activities by the host itself (del Rio et al. 2002). Similarly, the biotrophic specificity of *S. mycoparasitica* among the tested strains of *F. avenaceum*, *F. oxysporum*, *F. proliferatum*, and *F. sporotrichioides* was also reported towards *F. avenaceum* and *F. oxysporum.*

Similarly, the primarily mycoparasitic *Trichoderma harzianum* can parasitize the elm bark beetle, *Scolytus* p. (Jassim et al. 1990). Coppola et al. (2019) reported the indirect way of insect pest control using *Trichoderma atroviride* tomato seed treatment. It negatively affected the development of *Spodoptera littoralis* (Boisduval) larvae and the aphid *Macrosiphum euphorbiae* (Thomas) longevity. According to the researchers, *Trichoderma* induced transcriptional changes of a wide array of defense-related genes in the tomato plant. The impact on aphids was correlated with the upregulation of genes involved in the oxidative burst reaction. Such observations in the laboratory might open a new area of biocontrol strategy.

The dual activity of some fungi towards insects and fungi has been attributed to the cuticle/cell wall-degrading enzymes, which facilitate the pathogen's entry in the respective hosts. A common structural component of protective covers of fungi and insects is chitin, a β-1,4-linked *N*-acetylglucosamine polymer, which is almost 25–50%. Other components are wax, lipids, protein in insect cuticle, and fungal cell walls that have glucans in addition to chitin and mannoproteins. Thus, the cuticle-degrading enzymes (CDEs) mainly include proteases, lipases and chitinases, and enzymes such as chitinases, proteases, and glucanases that are the main components of the mycolytic enzyme (ME) complex. As chitin is the main structural component, chitinases contribute significantly to the degradation of protective covers. Therefore, most of the studies for the production of CDEs and MEs and their use in the field are centered around chitinases (Chavan and Deshpande 2013).

Several studies on the appearance sequence of extracellular enzymes in *Metarhizium* and *Beauveria* cultures were reported. The cuticular structure, *per se*, influences the extracellular appearance of CDEs. In general, *in vitro*, proteolytic enzymes such as esterase, endopeptidase, aminopeptidase, and carboxypeptidase are produced within the first 24 h of growth. *N*-acetylglucosaminidase appears next, and endo-chitinase that randomly attacks chitin polymer is produced in significant quantities after 4 days. Lipases are detectable after 5 days. Based on this appearance sequence of extracellular CDEs, St. Leger et al. (1986) assigned a major role to protease in cuticle degradation by *M. anisopliae*.

Furthermore, cuticle melanization makes it more resistant to chitinolytic enzymes (Nahar et al. 2004). In view of this, Nahar et al. (2004) suggested an alternate

mechanism for the hydrolysis of chitin, a main structural component of the cuticle. *M. anisopliae* produced constitutively two enzymes, chitin deacetylase and chitosanase. The chitin deacetylase converts chitin to chitosan, a glucosamine polymer, which is further hydrolyzed by chitosanase. Thus, these two enzymes initiate the process in the entomopathogens, which show low or delayed chitinase production.

11.4 Production of Infective Propagules of Entomopathogens

11.4.1 Isolation of Entomopathogens

The rhizospheric soils are mostly used for the isolation of entomopathogens. If the chemical insecticides are not routinely sprayed, one can expect several potent isolates. Nahar et al. (2003) observed that *Metarhizium* isolates from fields of *H. armigera* host plants such as tomato, okra, and other vegetables heavily sprayed with chemicals were less virulent to *H. armigera* than the isolates from the custard apple field rarely sprayed with chemicals. Usually, soil dilution method is used to isolate entomopathogenic fungi. The addition of antibacterials such as streptomycin, tetracycline, cycloheximide, and dodine increases the possibility of appearance of relatively slow-growing entomopathogens (Kulkarni et al. 2008). For the isolation of specific insect pathogens, *Galleria* bait method is used. The method is more effective for the isolation of *Beauveria* strains than other entomopathogens such as *Metarhizium* and *Nomuraea* (Nahar et al. 2003). In the fields, usually natural fungal epizootics on *H. armigera* and *S. litura* were reported. These entomopathogens can be isolated as potential biocontrol organisms (Uma Devi et al. 2003). Alternately, the dead insects in the field with hard bodies can be a potential source to isolate entomopathogenic fungi (Nahar et al. 2003). Interestingly, if infected with an entomopathogen, the live insect larvae show jerky movement and lost orientation can also be a potential source of entomopathogen (Kulkarni et al. 2008). Sahayaraj and Borgio (2009) reported the isolation of 21 *M. anisopliae* strains from the different agricultural field of Tamil Nadu, India, and tested their potential against the red cotton bug *Dysdercus cingulatus*.

11.5 Strain Improvement of Entomopathogenic Fungi

The selection of parent strain is crucial for strain improvement. The ability to produce different levels of various cuticle-degrading enzymes plays an important role in strain selection (Chavan et al. 2008). Interestingly, the spore-related characteristics such as the size, viability, production, speed of germination, relative hyphal growth, and their response to the environmental perturbations are important for strain selection. It was reported that in the case of *P. fumosoroseus* the spore size and virulence towards diamondback moth, *Plutella xylostella* were correlated with each other (Altre et al. 1999). On the other hand, Sugimoto et al. (2003) reported

relatedness among genetic properties, conidial morphology, and virulence of *V. lecanii* towards different insect hosts. Thus, based upon the insect bioassay and level of cuticle-degrading enzyme activities and other characteristics, selection of the fungal strain for strain improvement is possible.

Temperature, humidity, and solar radiations affect the efficiency of entomopathogens in the field (Zimmermann 2007). Attempts have been made to improve the strains for better performance in the field. Isolation of variants of local strains is a popular and acceptable method. Chandra Teja and Rahman (2016) reported the importance of the ability of entomopathogens to grow and produce conidia under wide temperature ranges and further used them in semiarid climates of Telangana and Andhra Pradesh. They have isolated two variants of *Metarhizium* that can grow at 35 °C. Further, Kulkarni et al. (2008) evaluated 63 *Metarhizium* isolates, from soil (53) and insect hosts (10), for their ability to produce extracellularly cuticle-degrading enzymes (CDE) and demonstrated correlation of CDE activities with *H. armigera* mortality in the laboratory bioassay. Based on higher conidia production, faster sedimentation time (indicates hydrophobicity), lower median lethal conidial concentration, and lethal time (LC_{50} and LT_{50}) against *H. armigera*, three strains were reported to have commercial potential.

Wongwanich et al. (2017) reported that three isolates of *B. bassiana* were mutagenized with ethyl methanesulfonate for obtaining a strain that can survive and grow at higher temperatures (33–35 °C). The pathogenicity of one of the mutant strains against the brown plant hopper, *Nilaparvata lugens* (Stal), was prominent.

The number of candidate genes coding for toxins, enzymes, physiological regulators, and hormones was evaluated to increase virulence of entomopathogens and improve their tolerance to environmental stress (Karabörklü et al. 2017; Zhao et al. 2016). The overexpression of genes coding for cuticle-degrading enzymes, *viz.,* chitinases and proteases, is a strategy employed to improve the efficacy of entomopathogenic fungi. For instance, *M. anisopliae* expressing the additional copy of *Pr1* showed improved insecticidal activity against *M. sexta*. The overexpression of an additional copy of chitinase (*CHIT1*) in *B. bassiana* improved its virulence by 23% (Fang et al. 2005). Similarly, *M. anisopliae* strain overexpressing chitinase (*CHI2*) was more effective against the cotton stainer bug, *Dysdercus perivianus* (Merzendorfer (2013). Further, the *Trichoderma koningii* strain was engineered with chitinase from *M. anisopliae* that showed the improved insecticidal activity against the silkworm (*Bombyx mori*) and Asian corn borer (*Ostrinia furnacalis*). Pinnamaneni et al. (2010) engineered *B. bassiana* with exochitinase, which expressed higher pathogenicity towards lepidopteran pests. Peng et al. (2015) reported that overexpression of *ATM1* (coding for acid trehalase) produced eight-fold more virulent *M. acridum* strain. In addition to these, genes coding for esterases, protein kinase A, osmosensor MOS1, a perilipin MPL1, benzoquinone oxidoreductase, CDEP1: Bbchit1 (protease: chitinase hybrid-protein), etc. were also demonstrated to improve the efficacy of fungal entomopathogens (Fang et al. 2009; Zhao et al. 2016).

Overexpressing the regulators of insect hormones or key physiological processes is another strategy explored by the researchers to improve the efficacy of

entomopathogenic fungi. These regulators disrupt insects' normal physiological functions and make them more susceptible to entomopathogens (Ortiz-Urquiza et al. 2015). The insecticidal potency of *B. bassiana* was improved by engineering such regulators. For instance, *B. bassiana* strain overexpressing the diuretic hormone (MSDH) that disrupts the water-salt balance in *M. sexta* showed improved efficacy against *M. sexta*, *Galleria mellonella*, and *Anopheles aegypti* (Fan et al. 2012). The genes coded by insect protease inhibitors and neuropeptides were also evaluated to improve the virulence of entomopathogenic fungi (Zhao et al. 2016). Recently, RNA interference (RNAi) technique has been used for genetic modification of entomopathogen to enhance their efficacy against insect pests. Chen et al. (2015) constructed the *Isaria fumosorosea* strain expressing the dsRNA against *TLR7* (coding for the immune-related gene) in whitefly *Bemisia tabaci*. When infected with this strain, *TLR7* expression was knocked down in whitefly nymphs. The attempts were also made to genetically engineer the entomopathogenic fungi to improve their tolerance towards abiotic stresses such as ultraviolet (UV) radiation, high temperature, and low water activity. Despite the number of advantages provided by recombinant entomopathogens over non-recombinants, socioeconomic concerns associated with their use have brought several controversies including but not limited to its potential adverse effects on the environment, gene flow to wild strains, evolution of resistance in target insect pests, etc.

Interestingly, the insect toxin genes from different orthopods (scorpion, spider, etc.) have been engineered to produce the recombinant entomopathogens. Wang and St. Leger (2007) engineered the *M. anisopliae* strain with *AaIT1* (coding for the sodium channel blocker), obtained from *Androctonus australis*. This recombinant strain was 22-fold more potent at 40% reduced LT_{50} than the parent strain against *M. sexta*. It was more effective against mosquitoes (at 9-fold lower LC_{50}) and coffee berry borer beetle (at 16-fold lower LC_{50}) than the parent strain. The scorpion toxin gene *AaIT1* was also engineered in *B. bassiana* to improve its virulence against insect pests. This recombinant strain was highly virulent against *Aedes albopictus* (Deng et al. 2017, 2019), *Dendrolimus punctatus* (Masson pine caterpillar), and *G. mellonella* (the wax moth) than the parent strain (Lu et al. 2008). However, co-expression of *Pr1* ((subtilisin-like protease) and *AaIT* in *B. bassiana* to further improve its virulence was not successful as *Pr1* digested the *AaIT* in hemolymph (Lu et al. 2008). *L. lecanii* expressing insect toxin gene *BmKit* from *Berthella martensi* showed approximately seven-fold lower LC_{50} with 25% reduced LT_{50} than the parent strain against the cotton aphid, *Aphis gossypii* (Pava-Ripoll et al. 2008; Xie et al. 2015). In addition to *AaIT*, genes coding for various spider toxins, i.e., *u-HXTX-Hv1a* (coding for insect voltage-gated calcium channel blocker) from *Atrax robustus*, *k-HXTX-Hv1c* (coding for $Ca2^{+}$- activated K^{+} channel inhibitor) and hybrid toxin gene (coding for CaV and KCa channel blocker) from *Hadronyche versuta*, *LqhIT2* (coding for insect-specific neurotoxin) from the Israeli yellow scorpion, and *BjaIT* from *Buthotus judaicus* were also evaluated to enhance the effectiveness of mycoinsecticides (Fang et al. 2014).

However, the use of genetically engineered organisms in the fields has raised a number of concerns. It has been recently demonstrated that naturally transformed

fungal entomopathogens with insecticidal traits from insecticidal plants improved virulence against insect pests (Pathan and Deshpande 2019). In 2003, Nahar et al. (2008) observed that *M. anisopliae* strains isolated from soils associated with *Annona squamosa* (custard apple) had higher virulence than strains isolated from other plants' rhizosphere and the insect cadavers. Pathan and Deshpande (2019) attributed this improved virulence to the horizontal transfer of insecticidal traits from *A. squamosa* to *M. anisopliae* strains. The two insecticidal cyclopeptides of *A. squamosa* origin were reported from *M. anisopliae* strains that led to higher virulence. Further, *M. anisopliae* strains showed 20 genes of *A. squamosa* origin, some of which are involved in the biosynthesis of insecticidal peptides. These genes were explicitly present in *M. anisopliae* strains isolated from the custard apple field and were absent in the strains isolated from other crop plants' rhizosphere. Phylogenetic analysis further confirmed that these genes were closer to those from *A. squamosa* than to those from fungi (Fig. 11.1). It was suggested that these strains could have established an endophytic relationship with *A. squamosa* and have come into the soil from the plant debris. In other words, the higher virulence could be attributed to the genes horizontally transferred to *M. anisopliae* during its endophytic existence in *A. squamosa.* Similarly, *Metarhizium* strains associated with *Capsicum annuum* (chili), *Azadirachta indica* (neem), and *Carica papaya* (papaya)—plants with insecticidal properties—were also reported to have higher virulence (Pathan and Deshpande 2019). A genome-wide analysis of different *Metarhizium* strains suggested that such type of horizontal gene transfer from bacteria, archaea, arthropods, plants, and even vertebrates might be increasing the host range of *Metarhizium* strains (Hu et al. 2014; Zhang et al. 2019).

11.6 Entomopathogenic Fungi as Endophytes

The fungi and bacteria that can grow within plant tissues without causing any noticeable disease symptoms to the plant are known as endophytes. Interestingly, the endophytic fungi, in general, can be entomopathogenic, and, in particular, can retard the developmental rate of the pest, inhibit insect food consumption rate, reduce larval survival, and decrease reproduction rate. Indeed, many unknown mechanisms may exist in fungus-plant-insect interactions (Mantzoukas and Eliopoulos 2020).

Fungal entomopathogens can establish themselves as endophytes, both naturally and in response to various inoculation methods. Different inoculation methods such as soil drenches, seed coatings and immersions, radical dressings, root and rhizome immersions, stem injection, foliar sprays, and flower sprays were reported (Parsa et al. 2013). Biswas and co-workers (2012) introduced *B. bassiana* as an endophyte in jute (*Corchorus olitorius*) through seed treatment. Endophytic colonization was detected with sequence-characterized amplified region (SCAR) markers in all the plants grown from treated seeds. It was expected to control infestation by jute semilooper (*A. sabulifera*) and Bihar hairy caterpillar (*S. obliqua*). Russo et al. (2015) evaluated the effectiveness of three inoculation methods (foliar spray, seed

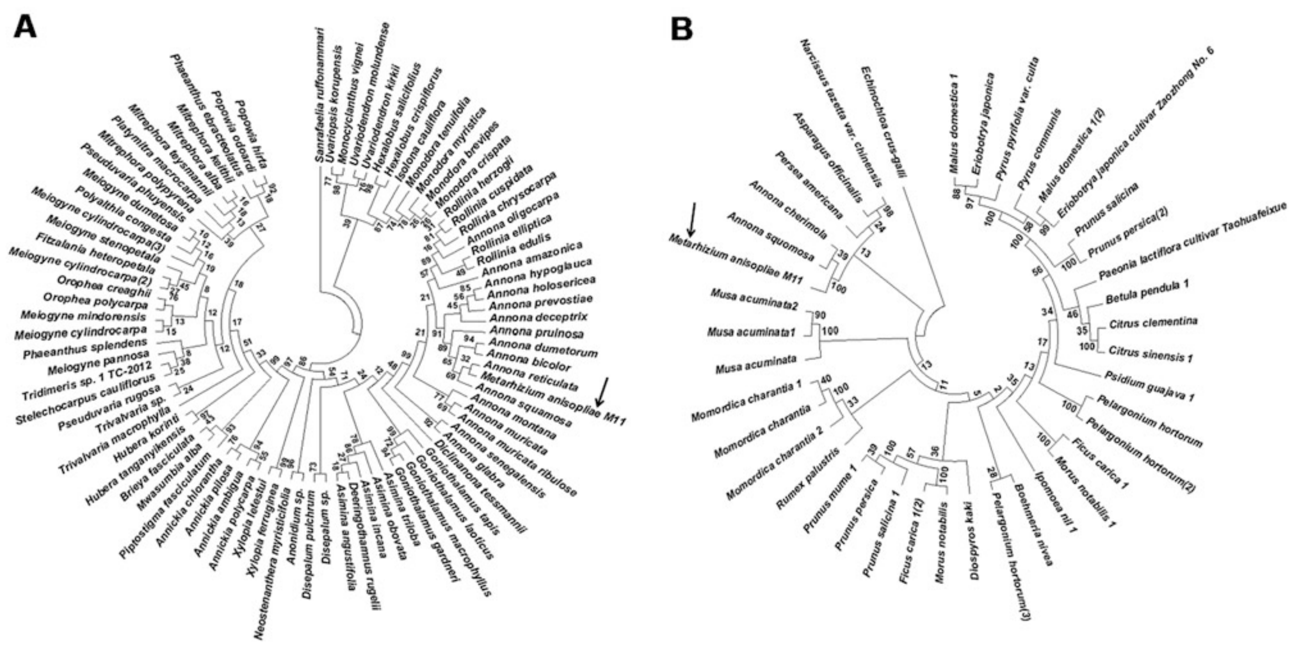

Fig. 11.1 Phylogenetic analysis based on the genes coding for (**a**) eliciting plant response like protein and (**b**) protein phosphatase 2C isolated from the *M. anisopliae* M34311 (M11) strain. The evolutionary history was inferred using the maximum parsimony method. The trees were obtained using the subtree-pruning-regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The coding data was translated assuming a genetic code table. Evolutionary analyses were conducted in MEGA6. In both the trees, M11 was grouped with the *Annona* and other insect-resistant plant species, based on these gene sequences. The black bold arrow indicates the position of the M11 strain in phylogeny. The analysis suggested the horizontal transfer of these genes from *Annona squamosa* to *M. anisopliae* strain M11, isolated from *A. squamosa* field

immersion, and root immersion) in establishing *B. bassiana* as an endophyte in tobacco, corn, wheat, and soybean. Though all the methods were useful for colonization in all four plant species, decrease in the efficacy with time (after 7, 14, 21, and 28 days) was different for different methods. Lopez and Sword (2015) reported a negative impact on the survival of *Helicoverpa zea* larvae and enhancement in cotton yield due to endophytic *B. bassiana* and *Purpureocillium lilacinum.* Pathan and Deshpande (2019) established *M. anisopliae* as an endophyte in custard apple by foliar spray and covering roots with spore suspension and *in planta* presence was checked with fluorescent markers specific for fungal organisms. Earlier, Nahar et al. (2008) reported that repeated subculturing on the artificial medium affects the virulence of *M. anisopliae* towards *H. armigera*. For instance, the 40th subculture had increased (1.6 times more) LT_{50} and 15 times more LC_{50} than the first subculture of *M. anisopliae*. With five passages in insect host, 40th subculture regained the virulence similar to 1st subculture. Interestingly, Pathan and Deshpande (2018) reported that if 40th subculture conidia were established in custard apple as an endophyte, it regained its virulence.

11.6.1 Mission Mode Collection of Entomopathogenic Fungi

One of the mission mode collections of entomopathogenic fungi is developed by the US Department of Agriculture-Agriculture Research Service (USDA-ARS). One of the important features for such collections is the maintenance of the virulence for effective field performance. This collection contains the most diverse cultures of fungal pathogens of insects, mites, spiders, nematodes, and other invertebrates comprising 6000 plus isolates.

The environmental parameters such as temperature, relative humidity, or nutrient availability control the biology as well as physiology of entomopathogenic fungi. Furthermore, in fields, per se, the understanding of interactive behavior of entomopathogens with host with respect to environmental factors is of great significance. In view of this, homogeneity of infective propagules and their virulence are essential characteristics to develop any mycoinsecticide for the field performance. The maintenance and repeated subculturing decrease the virulence and also develop variation in entomopathogenic fungi (Nahar et al. 2008). In most of the cases, the virulence can be regained by host passages for few times. Alternately, as mentioned earlier, development of entomopathogenic fungi as endophytes in plants having insecticidal traits can also be useful to increase their virulence. The pests having some part of their life cycle in soil can be maintained regularly with the respective hosts along with entomopathogens. Thus, in situ preservation can be one of the methods to supply active strains.

11.7 Entomopathogenic Fungi from Laboratory to Field: Practical Considerations

The cost of production of infective propagules, in most cases conidia, is solely dependent on the yield of viable conidia per kg of solid substrate, their rapid germination on the insect host, and virulence as evidenced by LC_{50} and LT_{50}, which have greater potential as effective biocontrol agents. Furthermore, apart from the killing potential, the stability of the strain under natural environmental perturbations of temperature and relative humidity and broad host range (including fungal hosts) are also important characteristics (Tupe et al. 2017). The steps involved in the knowledge-based development of entomopathogens as mycoinsecticides (from laboratory to field) were compiled and presented in the form of video by Tupe et al. (2017).

To investigate interactions with other soil inhabitants, the molecular tools that efficiently monitor mycoinsecticide in the field are necessary. Various researchers have reported the microsatellite markers for strain-specific identification of entomopathogens. These markers allow monitoring the persistence of applied mycoinsecticide and genetic diversity of indigenous species-related flora with the culture-based method. It is also possible to use the microsatellite markers to identify applied strains in bulk soil DNA extracts. Indeed, it is possible to use these markers to protect the strains and intellectual property. However, the microsatellite markers are from non-coding regions; thus these markers cannot differentiate between saprophytically growing strain and virulent strain. In this regard, markers that can correlate virulence are more important.

11.8 Future Perspectives

The use of entomopathogenic fungi as mycoinsecticides, especially in developing countries, will be economically sustainable if agriculture universities have tie-ups with the small-scale industries. The research inputs are necessary mainly with respect to the increasing shelf life of the existing formulations, identification of the fast pest-killing strains, use of consortium (one or two strains in combination), and combination with cuticle-degrading enzyme formulation prepared using novel nanotechnology (Deshpande 2019).

The regulation of target-specific genes is one of the important areas in developing effective mycopesticides. Wang and St. Leger (2007) identified a tissue-specific promoter in *M. anisopliae*, which is expressed specifically in insect hemocytes and provided a suitable way to regulate the expression of target gene in entomopathogenic fungi. Similarly, Liao et al. (2009) reported the use of ethanol-inducible *alc* system for regulating gene expression in *B. bassiana*.

Isolating the naturally transformed entomopathogens can be an alternative to genetically modifying the organisms to increase their potency. Given the social and cultural resistance against the acceptance of transgenic crops and genetically modified insect pathogens universally, the use of indigenous entomopathogens

naturally transformed into more virulent strains is a viable and cost-effective strategy to save crops from insect pests.

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Historical Perspectives of Rusts in India

12

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Abstract

Rusts, being an important group of pathogenic fungi, are responsible for substantial economic losses and they are known to pass through five different spore stages in their life cycle. Diversity and documentation of rusts in India have been attempted in the pre-Butler's, Butler's, and post-Butler's era. However, there has been a drastic decline in rust research in India in the recent past owing to possibly a lack of interest of few research groups. Historically, Butler laid the foundation for the study of rusts in India. The post-Butler's era was ruled by several Indian scientists (Mehta, Prasada, Thirumalachar, Mundkur, Ramakrishna, Pavgi, Ramachar, Bagyanarayana, and others) through significant contribution towards the rust biodiversity. Several new rust genera and species new to science were discovered in this phase. However, with the advent of recent molecular tools and techniques, many countries have involved meticulously to document rich diversity of rusts and maintained their own catalogues for future pathological studies. From the Indian perspective, there is enormous scope for enhancing the knowledge on biodiversity of rusts on plant systems based on recent molecular tools and sequencing platforms. This review attempts to provide an overall picture of past and present scenario on rust research in India with future economic concern of these pathogens.

Keywords

Biodiversity · Crop rusts · Forest tree rusts · Butler · Systematics · Taxonomy

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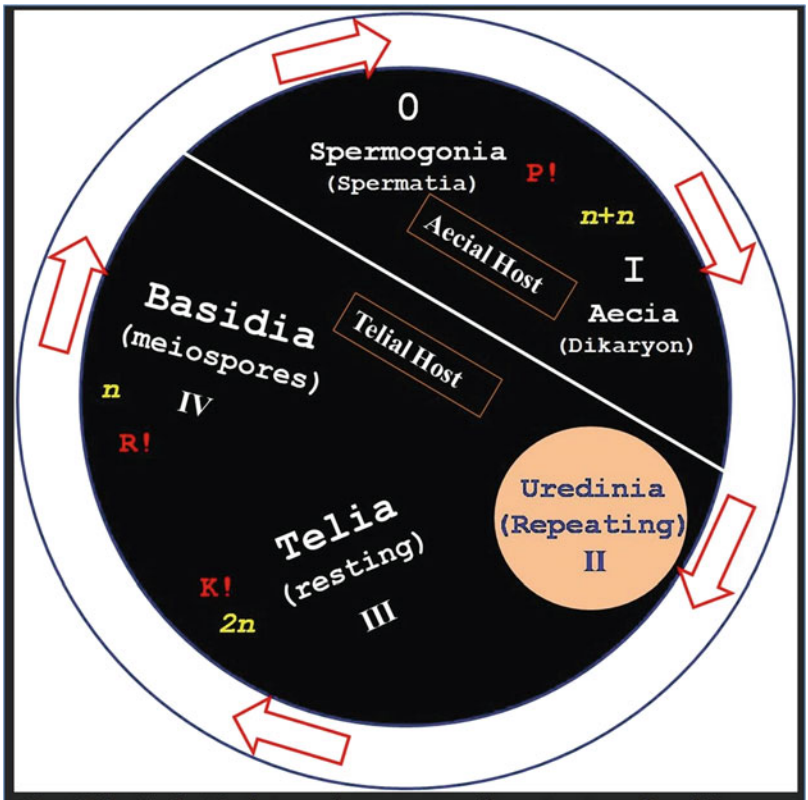
12.1 Introduction

Rust (phylum, Basidiomycota; subphylum, Pucciniomycotina; order, Pucciniales) is one of the largest groups of Basidiomycota. They show variable and complicated life cycles. Sato and Kakishima (1982) and Ono (2008) discussed the importance of assessment of life cycles of rusts at length. More than 7800 species (166 genera in 14 families) of rusts have been reported worldwide (Hooker 1967; Cummins and Hiratsuka 2003; Aime 2006; Aime et al. 2006; Kirk et al. 2008; Toome and Aime 2012). These are considered as the most species-rich category of obligate pathogens of plants that possess four unique characteristics. Firstly, single species usually possess five spore stages (spermogonium, aecium, uredinium, telium, and basidium) which differ functionally as well as morphologically; the spores of each state are designated as spermatia, aeciospores, urediniospores, teliospores, and basidiospores, respectively. Urediniospores are capable of producing repeatedly the same spore state on infection of the same host plants. Dual terminology and morphological and ontogenic schemes have been given to these spore states. The morphological system highlights the morphological characteristics of spores to designate the states (e.g., Laundon 1967; Holm 1973), whereas the ontogenic characteristics deal with spore position in different stages of life cycle (Arthur 1905; Hiratsuka 1973, 1975; Cummins and Hiratsuka 2003). Both systems have advantages as well as disadvantages that lead to difficulty in selecting any one of them. Owing to their morphological variability, the morphological approach is difficult to define the spore state. Positions of the spores in the life cycle represent their biological features. But some of the characteristic features (e.g., basidium development and repeated yield) need to determine states of spore ontogeny from live source owing to lack of evidence by the dead herbarium specimens. Precise identification of biological characteristics with clarity is an important skill necessary for a plant pathologist. Hence, we adapted the ontogenic system in our review. Additional information is available in the literature (Hiratsuka 1975; Hiratsuka and Sato 1982; Cummins and Hiratsuka 2003).

The second feature is that a rust species may need two host plant species which are not phylogenetically identical. The spermogonial as well as aecial states are borne on one of the hosts, while the remaining spore stages are showed on another host. Such a pattern of life cycle is designated as heteroecious (Fig. 12.1). Rest of the rust species produce all states of spores on one of the hosts known as autoecious. Many species in their life cycle exhibit all five spore states designated as macrocyclic. Some others are devoid of uredinial stage or both the aecial and uredinial stages designated as demicyclic and microcyclic, respectively. Some of the microcyclic rusts have morphologically similar teliospores (similar to aeciospores), either macrocyclic or demicyclic species; such a kind of life cycle is known as endocyclic. Identification of spore states as well as life cycle of a specific rust is important to apply ecological methods of control. Disparity in life cycles influences the nature as well as locations of inoculum source. For instance, the destruction of alternate hosts near the fields is one of the efficient methods to control the states of spermogonial and aecial hetero-macrocyclic and hetero-demicyclic rusts like *Gymnosporangium*



Rust Life Cycle: For heteroecious macrocyclic rusts, stages 0 and I are on the aecial host, and stages II and III on the telial host; stage IV develops on germination of teliospores. Meiosis (reduction division, R!), producing haploid (n) spores follows immediately after the fusion of nuclei (karyogamy, K!; 2n) in germinating teliospores; plasmogamy (P!), leading to dikaryotic (n + n) cells, follows after the transfer of spermatia to compatible receptive hyphae in the spermogonia; For Autoecious rusts remain on only one host throughout; hemicyclic rusts omit stages 0 and I; demicyclic rusts omit stage II; microcyclic rusts omit stages I and II; endocyclic rusts produce stage III (telia) morphologically resembling stage I and omit stage II. (modified from Helfer, 2014)

Fig. 12.1 Typical life cycle of a rust

asiaticum (Japanese pear rust) (Umemoto et al. 1989) and *Coleosporium eupatorii* (pine needle rust). Infections of host plants (spermogonial and aecial) are due to only basidiospores produced from the teliospore germination. Eradication of the telial hosts (*Juniperus* spp. for *G. asiaticum*; *Eupatorium* spp. for *C. eupatorii*) will be very effective. Such measure for common barberry (*Berberis vulgaris*), an alternate host of *Puccinia graminis* (wheat stem rust), was studied extensively in the USA

subsequent to an epidemic during 1916. Urediniospores were transmitted from the southern states to spring grain areas; however eradication of barberry influences the frequency as well as severity of wheat stem rust outbreak (Roelfs 1985). In addition, some rusts could persist a long duration as a perennial mycelium after successful infection of host, irrespective of their monokaryotic or dikaryotic mycelia.

The third unique characteristic is that rusts usually have a precise host range. In contrast, some species of rusts possess several hosts. Those may be composed of special significance as subspecies are host specific or comprise several species (designated as cryptic species) which are morphologically identical. There are some exceptions, especially *Phakopsora pachyrhizi* (Asian soybean rust) and *Puccinia psidii* (myrtle rust).

The fourth feature is that rusts are obligate on host plants. They continue in nature on live plant species and live on dead host as a resting state. Williams et al. (1966) were successful in establishing the axenic cultures (*P. graminis* f. sp. *tritici*) that attracted global attention. In Japan, some of the rusts were cultured successfully on semi-defined media following the methods by Katsuya et al. (1978). Because of these efforts, about 30 spp. have been grown axenically on semi-defined media (Maclean 1982; Williams 1984; Yamaoka and Katsuya 1984; Yamaoka 2002). Thus, rusts may not live like saprophytes as they are obligate parasites, and do not have a tendency to kill their host plants immediately after infection which is responsible for severe economic losses.

In spite of many studies on rusts in India, their diseases have not drawn much attention of plant pathologists. Research on the rusts in India was established by Butler when he was the Imperial Mycologist. Agricultural board was established to chalk out the plan to control the wheat rust disease in major wheat-growing regions of India. Since then, many mycologists developed interest and documented the diverse rusts from India. Rusts cause serious damage to many commercial crops, vegetables, fruits, and forest trees. The purpose of this review is to consolidate studies carried out on rusts in India since the era of Butler and to draw the attention of future researchers towards the fascinating field of rusts.

12.2 The Background

Rusts constitute important ecological communities in the ecosystem. Their unique lifestyle as biotrophic with complicated life cycles made them susceptible to global changes in the environment (Helfer 2014). As biotrophs, they are crucial as drivers for community dynamics as well as diversification by coevolution with their hosts (Gilbert 2002). They are capable of influencing the composition of vegetation and in turn plant community structure (Dobson and Crawley 1994) by affecting the photosynthetic ability of their hosts and by rerouting the photosynthate in their biomass leading to reduction in the carbon sequestration of host plants. In energy plantations for example, yield of dry matter loss has been estimated to be more than 40% by rust infection (Dawson et al. 2005). Based on Cummins and Hiratsuka (2003), among the 14 families of rusts, 13 are accepted. Recent molecular phylogeny of rusts (18S and

28S rDNA) revealed 8 out of 13 families and 3 suborders (Aime 2006). Being obligate parasites of vascular plant species (ferns, gymnosperms, and angiosperms), rusts also cause serious damage to the crops (vegetables, fruits, and trees, such as wheat, corn, soybean, pear, and pines). Biology as well as phytopathology of rusts have been studied extensively and catalogued from different geographic locations as early as the last century (Japan: Berkeley and Curtis 1859; Ito 1938, 1950; Australia: McAlpine 1906; North America: Arthur 1934; former USSR: Kuprevich and Tranzschel 1957; Europe: Gäumann 1959; Hiratsuka 1960; Wilson and Henderson 1966). There is, however, a clear knowledge gap in the rich biodiversity of rusts recorded or any reports so far from earlier mycologists are very limited. Extensive studies have to be carried out to document already reported rust taxa as well as new rusts using molecular methods to develop a national catalogue of rusts and studies on disease control and prevention in future.

12.3 Milestones of Rust Research in India

Research and development in the field of rust mycology was initiated from E.J. Butler in India. Before Butler's era, the research activities of rust mycology and identification and assessment of diseases were very meagre. However, Butler's contribution to Indian rust mycology provided a solid platform. After Butler's departure from India, several mycologists showed interest in studying the rusts and documented several new genera and species. Thus, we can envision three distinct phases of rust research in India: (1) pre-Butler's era (prior to 1900); (2) Butler's era (1902–1928); and (3) post-Butler's era (1930 onwards).

12.3.1 Before Butler's Era (Prior to 1900)

During the pre-Butler's era, contributions came chiefly from the overseas mycologists (e.g., Berkeley and Currey in England; Leveille and Montagne in France), who identified or named specimens from India, which were sent to them by the missionaries (Koenig and Jacquemont) or others like SulpisKurz (then Curator of the Royal Botanic Garden, Calcutta) and Hooker and Wight. The early studies in India were the pioneering studies of two British Medical Officers DD Cunningham and A Barclay. Cunningham pioneered the research on aerobiology and aeromycology with contributions to our knowledge on the Uredinales, Mucorales, and Ustilaginales. Barclay (1885 onwards) made significant contributions on the Uredinales. Cunningham published his research between 1871 and 1897. He carried out his studies in this tenure especially on Mucorales and Uredinales from Calcutta, Eastern India. In 1885, Barclay started his research on rusts near Shimla. Barclay's main contributions include life histories of Himalayan Uredinales. Dietel (1890) contributed to the acquaintance of rusts of Himalaya. *Gymnosporangium cunninghamianum* was named after him by Major A. Barclay in 1890, who found it in Shimla, but received illustrations matching them made by

Cunningham from Almora during 1874 (Berkeley and Broome 1874; Barclay 1889, 1890, 1891).

12.3.2 Butler's Era (1902 to 1928)

The development of mycology in India owes a great deal to Butler, who came to India in 1901 and tried to identify the fungi collected by the early missionaries and others. Butler's contributions to mycology and applied mycology were not only basic and fundamental, but also relevant to the needs of the country, where a balanced development of the twin disciplines of mycology and plant pathology took place. Butler's monograph (1907) of the genus *Pythium* and his book *Fungi and Disease in Plants* (Butler 1918) are still classics to set the quality for future studies (Subramanian 1986). Most of the research pertaining to rusts is available in the publication authored jointly with Sydow and Sydow, where most of the new species to India are published and well documented in his book "The Fungi of India" (Sydow et al. 1906, 1907, 1912). Apart from initiating research in pathology, forest pathology and fungal diseases of the Indian crop plants were also covered. Thus, several new species of fungi were new to science from India. He published the new species of Indian fungi (including rusts) jointly with H. Sydow and P. Sydow with the title "The Fungi Indiae Orientalis" (Sydow et al. 1906, 1907, 1912). Butler also wrote "The Fungi of India" jointly with G.R. Bisby. It is a carefully compiled enumeration of fungal species known from the Indian subcontinent till that time (Butler 1914; Butler and Bisby 1931). His visions for the field of mycology led to the establishment of culture collection center. Butler set up a herbarium of fungi at the Imperial Agricultural Research Institute (then in Pusa, Bihar), currently known as the Herb. crypt. Indiae orientalis and located at the Indian Agricultural Research Institute, New Delhi. He started preserving the specimens collected across the country and now Herbarium Cryptogamae Indiae Orientalis (HCIO) hosts the largest collection in the country contributed by several mycologists from India and other countries.

Butler travelled across the country and collected fungal specimens (diseased specimens) from his visits. Careful and scientific study led to the establishment of a string of backbone mycological investigations in India. To have a clear understanding of his quantum of information and contribution made by Butler, let us take the preserved specimen in HCIO collected by Butler. The HCIO, a national herbarium established in 1905, not only serves as an educational resource for the National Agricultural Research System (NARS), but also conserves fungal biodiversity. Most of the Indian precious collections of the rusts are housed in Indian Agricultural Research Institute (IARI) and HCIO herbaria. In order to serve the needy and projects, the available herbarium collections and catalogues were brought out by HCIO authorities. The important one that deals with the rusts is the checklist of *Puccinia* species by Deeba-Kamil et al. (2013). Although the general catalogue is having entry of other rust members including *Puccinia* spp., many were holotypes and serving as different types of materials to the needy taxonomists in the country

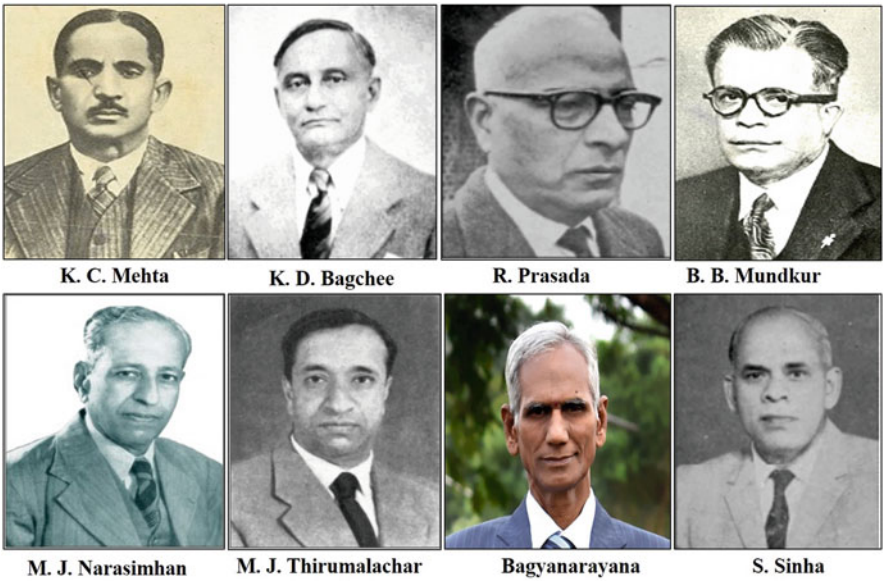


Fig. 12.2 Stalwarts of Indian mycologists who contributed to the knowledge of rusts of India

and it hosts a large number of specimens of Indian rusts collected by various stalwarts of Indian mycology (Uma-Maheswari et al. 2012) (Fig. 12.2). The check-list of *Puccinia* spp. available from HCIO (deposited) showed that among 2700 specimens available, over 700 specimens were deposited by Butler (it is a collection of *Puccinia* genus; there are several other rusts collected and deposited at HCIO) and the details are presented in Table 12.1. The list contains several new species which were published (Sydow et al. 1906, 1907, 1912) and several new collections from the country which were identified and named previously.

12.3.3 Post-Butler's Era (1930 Onwards)

Mycologists associated with Butler followed the legacy left behind by Butler after 1928. The major contributions to the study of rusts of India are collected and presented. There are several stalwarts who contributed to the study of rusts in India and their major achievements are presented below:

Bagchee KD. Bagchee initiated his career in 1927 (Forest Research Institute, Dehradun) and carried out pioneering research on forest pathology with special emphasis on the rust biology and pathology of conifers, soilborne diseases, and their control especially decay and control of timbers (Misra et al. 2016).

Mehta KC. After the strong foundation laid by Butler for the study of mycology in India and wheat rust research in particular, he had created a pool of young scientists and ignited the spark to explore the unexplored area of mycology including

Table 12.1 List of *Puccinia* species collected by E.J. Butler and their respective specimens available at HCIO, New Delhi

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia absinthii	Artemisia sp. (HCIO 1529), *A. absinthium* (HCIO 1530),	Kashmir	1908	
Puccinia anthistiriae	*Anthistiria anathera* (HCIO 5940)	Kashmir	1908	
Puccinia apludae	*Apludae aristata* (HCIO 5883)	Dehradun	1903	New species[a]
Puccinia artemisiella	*Artemisia vulgaris* (HCIO 1534), HCIO 1573, HCIO 5897	Kashmir	1908, 1909	
Puccinia arthraxonis	*Arthraxon lanceolatus* (HCIO 764, HCIO 765, HCIO 1535)	Dehradun, Himalaya,	1902, 1907, 1908	New species[b]
Puccinia arundinellae	*Arundinella braziliensis* (HCIO 758), *A. wallichii* (HCIO 759, HCIO 1536, HCIO 5901)	Dehradun, Assam, Burma	1905,1908	
Puccinia barbeyi	*Asphodelus fistulosus* (HCIO 531)	Punjab	1906	
Puccinia burmanica	*Themeda triandra* (HCIO 1532, HCIO 5953)	Burma	1908	New species[c]
Puccinia butleri	*Launaea asplenifolia* (HCIO 5954, HCIO 5955, HCIO 5957)	Pusa, Kanpur	1903,1905, 1906, 1908	New species[a]
Puccinia cacao	*Rottboellia compressa* (HCIO 1537, HCIO 5962)	Pusa	1905	
Puccinia calosperma	*Darlingia celosioides* (HCIO 544), *Caulibus floribusque* (HCIO 5969)	Dehradun	1903, 1902	
Puccinia carthami	*Carthamus oxyacanthae* (HCIO 535), *C. tinctorius* (HCIO 1538)	Punjab, Pusa	1905, 1911	
Puccinia centaureae	*Centaurea calcitrapa* (HCIO 1539)	Kashmir	1908	
Puccinia cephalandrae-indicae	*Cephalandra indica* (HCIO 548, HCIO 6002, HCIO 6004)	Bombay, Pusa	1905, 1906, 1911	New species[a]
Puccinia chrysanthemi	*Chrysanthemum indicum* (HCIO 518)	Nilgiris	1904	
Puccinia chrysopogi	*Jasminum* sp. (HCIO 1473), *Andropogon gryllus* (HCIO 1540, HCIO 6015)	Kashmir	1908	
Puccinia cichorii	*Cichorium intybus* (HCIO 1549, HCIO 6017)	Kashmir	1908	
Puccinia cipurae	*Cipura paludosa* (HCIO 824)	Calcutta	1903	New species[b]
Puccinia citrulli	*Citrullus colocynthis* (HCIO 1580)	Madras	1910	New species[c]

(continued)

Table 12.1 (continued)

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia congesta	*Polygonum* sp. (HCIO 6064)	Burma	1908	
Puccinia coronata	*Poa flexuosa* (HCIO 1541), *Stipa* sp. (HCIO 1542), *Agropyron* sp. (HCIO 6095), *Rhamnus procumbens* (HCIO 6103)	Kashmir, Mussoorie	1908, 1911, 1912	
Puccinia curculiginis	*Curculigo orchioides* (HCIO 517, HCIO 6115)	Malabar, Dehradun	1904, 1914	
Puccinia cynodontis	*Cynodon dactylon* (HCIO 505, HCIO 6118, HCIO 6132), *Cynodontis dactyli* (HCIO 6122)	Saharanpur, Bombay, Pusa, Kashmir	1904, 1907, 1908, 1912	
Puccinia cypericola	*Cyperus rotundus* (HCIO 562), *Cyperus capitatus* (HCIO 564), *Cyperus tuberosus* (HCIO 570), *Cyperus subcapitatus* (HCIO 7021)	Dehradun, Surat, Madras, Pune	1903, 1904, 1923	
Puccinia dactylidina	*Dactylis glomerate* (HCIO 1582, HCIO 10281)	Kashmir, Simla	1908, 1943	
Puccinia dovrensis	*Erigeron alpine* (HCIO 848)	Himalaya	1907	
Puccinia droogensis	*Berberis aristata* (HCIO 519)	Nilgiris	1905	New species[c]
Puccinia duthiae	*Andropogon pertusa* (HCIO 6165, HCIO 6168)	Karachi Dehradun	1902, 1903, 1908	
Puccinia epilobii	*Epilobium* sp. (HCIO 6193)	Kashmir	1908	
Puccinia expallens	*Hypoxis aurea* (HCIO 825)	Dehradun	1905	New species[b]
Puccinia ferruginosa	*Artemisia* sp. (HCIO 6224)	Assam	1905	
Puccinia flavipes	*Fimbristylis miliacea* (HCIO 778)	Mysore	1905	New species[b]
Puccinia geranii-silvatici	*Geranium nepalense* (HCIO 6246, HCIO 6248)	Kashmir	1908	
Puccinia glumarum	*Hordeum vulgare* (HCIO 497, HCIO 6255), *Brachypodium sylvaticum* (HCIO 6252), *Phalaris minor* (HCIO 6265), *Triticum vulgare* (HCIO 6274, HCIO 6282)	Bihar, Simla, Kanpur, Hisar, Dehradun, UP, Ajmer	1904, 1903, 1905, 1907	
Puccinia gracilenta	*Bambusa* sp. (HCIO 6312)	Darjeeling	1909	New species[c]

(continued)

Table 12.1 (continued)

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia graminis	*Festuca gigantea* (HCIO 1546), *F. kashmiriana* (HCIO 1547), *Brachypodium sylvaticum* (HCIO 1548), *Berberis lyceum* (HCIO 6327)	Kashmir, Kasauli Pusa UP, Pusa, Jaipur	1905, 1908	
Puccinia heterospora	*Sida mysorensis* (HCIO 510, HCIO 6470, HCIO 6471), *Sida* sp. (HCIO 6455), *S. humilis* (HCIO 6464), *S. spinose* (HCIO 6472)	Pune, Coorg, Dehradun, Mysore, Dharwad	1902, 1903	
Puccinia hieracii	*Hieracium crocatum* (HCIO 1550), *Cichorium intybus* (HCIO 6480)	Kashmir	1908	
Puccinia himalensis	*Rhamnus davurica* (HCIO 843, HCIO 6482, HCIO 6484)	Kumaon, Kashmir	1907, 1908	
Puccinia hydrocotyles	*Hydrocotyle polycephala* (HCIO 566)	Assam	1905	
Puccinia inayati	*Launaea nudicaulis* (HCIO 6509)	Burma	1908	New species[b]
Puccinia incompleta	*Ischaemum ciliare* (HCIO 1600, HCIO 6515, HCIO 6513)	Wayanad, Chittagong	1907, 1909	New species[c]
Puccinia invenusta	*Phragmites karka* (HCIO 888, HCIO 6523, HCIO 6525)	Bihar, Pusa	1907	New species[b]
Puccinia iridis	*Iris* sp. (HCIO 6530), *Iris kashmiriana* (HCIO 6535)	Kashmir	1908	
Puccinia komarovii	*Impatiens* sp. (HCIO 6549)	Mussoorie	1912	
Puccinia kozukensis	*Andropogon micranthus* (HCIO 1553)	Burma	1908	
Puccinia kuehnii	*Saccharum spontanei* (HCIO 6557), *S. spontaneum* (HCIO 6577)	Pusa, Kanpur, Burma	1906	New species
Puccinia lateripes	*Ruellia* sp. (HCIO 1554)	Bihar, Pusa	1911	
Puccinia lateritia	*Hedyotis vestita* (HCIO 801), *H. Auricularia* (HCIO 802)	Assam	1905	
Puccinia leptodermidis	*Leptodermis lanceolata* (HCIO 1555, HCIO 6594)	Punjab, Kasauli	1908, 1914	
Puccinia leucophaea	*Colquhounia coccinea* (HCIO 1581)	Mussoorie	1911	New species[c]
Puccinia lolii	*Avena sativa* (HCIO 6606)	Pusa	1907	
Puccinia maydis	*Zea mays* (HCIO 6644)	Mangri	1905	
Puccinia melanocephala	*Arundinaria* sp. (HCIO 512)	Assam		New species[b]

(continued)

Table 12.1 (continued)

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia menthae	*Calamintha umbrosa* (HCIO 153, HCIO 509), *C. clinopodium* (HCIO 1556), *C. umbrosa* (HCIO 6659)	Ranikhet, Mussoorie Kashmir	1905, 1907, 1908	
Puccinia mysorensis	*Kyllinga triceps* (HCIO 513), *K. tricipitis* (HCIO 6720, HCIO 6721)	Mysore, Pune	1902, 1903	New species[a]
Puccinia nakanishikii	*Andropogon nardus* (HCIO 534)	Mysore	1903	
Puccinia nepalensis	*Rumex* sp. (HCIO 1557, HCIO 6726), *R. orientalis* (HCIO 1559)	Kasauli, Kashmir, Mussoorie	1908, 1912	
Puccinia neyraudiae	*Neyraudia madagascariensis* (HCIO 1610)	Darjeeling	1912	New species[c]
Puccinia nitida	*Polygonum* sp. (HCIO 1560, HCIO 6731)	Kashmir, Mussoorie	1908, 1912	
Puccinia obscura	*Luzula campestris* (HCIO 782)	Cherrapunji	1905	
Puccinia oligocarpa	*Stipa* sp. (HCIO 1571)	Kashmir	1908	New species[c]
Puccinia oplismeni	*Oplismenus compositus* (HCIO 526, HCIO 6756)	Mussoorie, Kumaon	1903, 1907	New species[a]
Puccinia oryzopsidis	*Oryzopsis molinoides* (HCIO 760)	Kumaon	1907	New species[b]
Puccinia pachypes	*Spodiopogon albidus* (HCIO 1609)	Wayanad	1912	New species[c]
Puccinia paspali	*Panicum sanguinale* (HCIO 529, HCIO 1514)	Dehradun, Bihar	1904, 1907	
Puccinia penniseti	*Pennisetum typhoideum* (HCIO 525)	Pune, Nagpur	1905	
Puccinia pimpinellae	*Pimpinella diversifolia* (HCIO 6830)	Mussoorie, Kashmir	1912	
Puccinia polliniae	*Strobilanthes* sp. (HCIO 618, HCIO 6861)	Dehradun, Mussoorie	1903, 1905	
Puccinia polygoni-amphibii	*Polygonum persicaria* (HCIO 1562)	Kashmir	1908	
Puccinia prainiana	*Smilax aspera* (HCIO 511, HCIO 1564), *S. elegans* (HCIO 1563)	Mussoorie, Himalaya	1903, 1907	
Puccinia princeps	*Pogostemon* sp. (HCIO 532)	Dehradun	1904	
Puccinia propinqua	*Andropogon* sp. (HCIO 745)	Kumaon	1907	New species[b]
Puccinia prunicolor	*Andropogon serratus* (HCIO 506)	Dehradun	1902	New species[a]

(continued)

Table 12.1 (continued)

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia pruni-spinosae	*Persica vulgaris* (HCIO 514), *Prunus communis* (HCIO 1566)	Pusa, Kashmir	1906, 1908	
Puccinia pulvinata	*Echinops cornigerus* (HCIO 1568)	Kashmir	1908	
Puccinia purpurea	*Sorghum vulgare* (HCIO 520)	Coimbatore	1904	
Puccinia pusilla	*Andropogon assimilis* (HCIO 6983)	Karnataka	1905	New species[a]
Puccinia romagnoliana	*Cyperus rotundus* (HCIO 768, HCIO 7020)	Pusa, Assam Mysore	1903, 1906	
Puccinia roscoeae	*Roscoea alpine* (HCIO 1569)	Mussoorie	1911	
Puccinia ruelliae	*Ruellia prostrate* (HCIO 538)	Mysore	1903	
Puccinia rufipes	*Imperata arundinacea* (HCIO 528)	Dehradun	1902	
Puccinia schirajewskii	*Serratula pallida* (HCIO 1575)	Kashmir	1908	
Puccinia scirpi	*Scirpus barbara* (HCIO 14)	Madras	1904	
Puccinia simplex	*Hordeum vulgare* (HCIO 5874)	Pusa	1916	
Puccinia solmsii	*Polygonum chinense* (HCIO 806)	Mysore	1903	
Puccinia sonchi	*Launaea nudicaulis* (HCIO 49, HCIO 797)	Srinagar, Mysore	1903, 1908	
Puccinia sorghi	*Zea mays* (HCIO 6645)	Kashmir	1908	
Puccinia spongiosa	*Webera corymbosa* (HCIO 516)	Mysore	1903	
Puccinia tanaceti	*Artemisia vulgaris* (HCIO 102), *Taraxacum officinale* (HCIO 114)	Kashmir	1908, 1909	
Puccinia thwaitesii	*Justicia gendarussa* (HCIO 134, HCIO 508)	Comilla, Mysore	1903, 1913	
Puccinia tinctoriae	*Serratula pallida* (HCIO 148)	Kashmir	1908	
Puccinia triticina	*Triticum vulgare* (HCIO 167)	Kanpur	1903	
Puccinia urticae	*Urtica* sp. (HCIO 1576)	Kasauli	1908	
Puccinia versicolor	*Andropogon* sp. (HCIO 227), *A. contortus* (HCIO 515)	Burma, Dharwad	1903, 1908	
Puccinia wattiana	*Clematis* sp. (HCIO 263), *C. gouriana* (HCIO 265)	Dehradun Mussoorie	1903, 1910	

(continued)

Table 12.1 (continued)

Rust	Host and HCIO Accession	Locality	Year	Remarks
Puccinia xanthopoda	*Scleria* sp. (HCIO 774)	Ranikhet	1907	New species[b]
Puccinia xanthosperma	*Bambusa* sp. (HCIO 539)	Mussoorie	1903	New species[a]

New species by E.J. Butler published in the following references are in association with Sydow and Sydow:
[a]Sydow, H.; Sydow, P.; Butler, E.J. 1906. Fungi Indiae orientalis Pars I. 4:424–445
[b]Sydow, H.; Sydow, P.; Butler, E.J. 1907. Fungi Indiae orientalis Pars II. 5:485–515
[c]Sydow, H.; Sydow, P.; Butler, E.J. 1912. Fungi Indiae orientalis Pars IV. 10(3):243–280

rusts. Impact of temperature on the viability of uredospores of wheat (black, brown, and yellow rusts) had been studied by Mehta. These rusts were killed owing to the high temperature in the plains. For investigation of their sources, Mehta designed laboratories at Agra and Shimla to study in warm as well as cooler climates. He established that the wind from hills to the plains blows spores of rusts, and they could be managed through eradication of season wheat as well as barley in the hilly regions by replacement of oats (Misra et al. 2016). As early as 1907, rusts associated with cereals (e.g., wheat) received prime attention due to a severe outbreak of rust in Punjab (Raychaudhuri et al. 1972). Butler collected and investigated the rusts of wheat during his stay as Imperial Mycologist in India. Knowledge on understanding the epidemiology and spread of wheat rust was contributed by Mehta (1929, 1931a, b, 1937, 1941). Investigations on the rust epidemiology and methods were started by Mehta (1929) by a series of experiments on the recurrence of wheat black stem rust in India. Most of his experimental results on the wheat black stem rusts were published in the form of a monograph.

Prasada R. Prasada is another important personality, who contributed to the knowledge of Indian rusts significantly. He established the Cereal Rust Research Laboratory in Shimla and carried out research with meticulous planning and forethought. Until 1946, he was in charge of the laboratory prior to shifting IARI to New Delhi. Prasada's laboratory became an important station to monitor virulence of rusts beyond national boundaries. He contributed a lot to the epidemiology of rusts of cereals in collaboration with Mehta that resulted in the publication of monographs by ICAR during 1940–1952 (Prasada 1946, 1947, 1960). Prasada contributed significantly to cereal rusts and other rusts (grasses, maize, millets, legumes, oilseeds, and fiber crops) (Prasada 1951). Other examples of his contributions include blight of linseed and cucurbits (*Alternaria*), wheat and pea (powdery mildews), and pearl millet (blight). Prasada was in touch with breeding rust-resistant varieties of wheat since 1935. He also contributed to the improvement of high-yielding and rust-resistant varieties of linseed and its wide cultivation. During his Australian visit in 1956, for the first time, he found natural occurrence of barberry-infected wheat stem rust in Tasmania. This observation was responsible for insight into the origin of new races of rusts in Australia and their epidemiology (Misra et al. 2016).

Thirumalachar MJ. Description of new genera of rusts like *Acervulospora, Kernella,* and *Hiratsukamyces*; new genera of smuts *Mundkurella, Franzpetrakia, Zundelula,* and *Georgefischeria*; establishing the genus *Sclerophthora* for the downy mildew disease causing "crazy top" on corn and other graminaceous hosts based on its *Phytophthora*-like asexual stage; and establishing the morphological basis for differentiating *Entomophthora* from *Conidiobolus* on the basis of cultural studies and studies on the life cycle of an edible rust-causing malformation of floral parts on *Acacia eburnea* described by Barclay as *Aecidium esculentum* to establish its autoecious nature and identify it as *Ravenelia esculenta* are some of the important examples of Thirumalachar's contribution. Many of the contributions from Thirumalachar have been published with header noteworthy rusts (Box 12.1). The major geographical areas covered by Thirumalachar and coworkers in Karnataka state include Bangalore, Mysore, and Coorg. Their contributions include the addition of new species along with elucidating different life cycles or rusts.

Thirumalachar critically examined the genus *Phakopsora*, added several new species from a wide range of hosts (Thirumalachar and Kern 1949), and detailed morphological examinations of parasitism exhibited by *Hamelia* species (Thirumalachar and Narasimhan 1947). The culture of sporogenous tissues on basal medium and developing a staining technique for observation of teliospores are still valid for the study of rusts (Thirumalachar 1940; Thirumalachar and Cummins 1949). He also authored the genera of rusts along with Mundkur (Thirumalachar and Mundkur 1950). His contributions towards new species and genus include *Puccinia leocarpum* (Thirumalachar 1941a); *Hedyotis stylosa* (new comb.) (Thirumalachar 1942); *Masseeella breyniae* (Thirumalachar 1943); *Acervulospora* (new genus), *Acervulospora ichnocarpi*, *Puccinia volutarellae, Puccinia bellurensis*, and *Puccinia boerhaviaefoliae* (Thirumalachar 1945); *Kernia* (new genus) (Thirumalachar 1946); *Hemileia mysorensis*, *Mainsia pterocarpi*, *Corbulopsora cumminsii*, *Scopella fici*, *Cerotelium wagateae*, *Puccinia bulbostylidicola*, and *Aecidium memecyli* (Thirumalachar 1947a); *Hapalophragmium mysorense* and *Hapalophragmiopsis* (new genus), *Hapalophragmium ponderosum* (new combination), *Uredo carissae* (Thirumalachar 1950). Critical analysis and reassessment of species of rusts reported by various workers through detailed investigations were published in a series of papers titled "Critical Notes on Some Plant Rusts" (Thirumalachar 1949). *Didymosporella* (new genus), *Didymosporella toddaliae* revisit to the structure of *Nothoravenelia* (Thirumalachar 1951), *Chrysomyxa simplex* (new combination), *Cerotelium terminaliae-paniculatae*, *Stereostratum lagerhamianus* (new combination) (Thirumalachar 1960), and appended many rust species are his critical observations (Thirumalachar and Whitehead 1954).

Box 12.1: Studies Carried Out on Rusts by M.J. Thirumalachar

- A method for germinating and staining Teleutospores (Thirumalachar 1940)
- A new species of Puccinia on *Ocimum adscendens* (Thirumalachar 1941a, b)
- *Hapalophragmium ponderosas* Syd. On *Acacia leucophlaea* Wild (Thirumalachar 1941)
- Morphological and cytological study of the rust on *Hedyotis stylosa* [*Chrysocelis ascotela* (Syd.) comb. nov.] (Thirumalachar 1942)
- Contributions to the flora of Nandi Hills (Thirumalachar et al. 1942)
- *Masseeella breyniae*—A new species of rust (Thirumalachar 1943)
- Some noteworthy rusts—I (Thirumalachar 1945)
- Two new genera of rusts on Bignoniaceae (Mundkur and Thirumalachar 1945)
- *Kernia*, a new genus of the Uredinales (Thirumalachar 1946)
- Revisions of and additions to Indian Fungi (Mundkur and Thirumalachar 1946a)
- Revisions of and additions to Indian fungi. I. (Mundkur and Thirumalachar 1946b)
- Some noteworthy rusts—II (Thirumalachar 1947a)
- Brief notes on genera Stereostratum Magn. and Anthomycetella Syd. (Thirumalachar 1947b)
- Studies on the morphology and parasitism of *Hemelia* species on Rubiaceae in Mysore (Thirumalachar and Narasimhan 1947)
- Critical note on some plant rusts (Thirumalachar 1949)
- Notes on some species of *Phakopsora* and *Angiopsora* (Thirumalachar and Kern 1949)
- Genera of rusts (Thirumalachar and Mundkur 1949a)
- Genera of rusts 11 (Thirumalachar and Mundkur 1949b)
- The taxonomic significance of sporogenous basal cells in the Uredinales (Thirumalachar and Cummins 1949)
- Some noteworthy rusts—III (Thirumalachar 1950)
- Genera of rusts III (Thirumalachar and Mundkur 1950)
- Critical notes on same plant rusts—II (Thirumalachar 1951)
- Critical notes on some plant rusts—III (Thirumalachar and Narasimhan 1951)
- On the validity of the genera Coleopuccinia and Coleopucciniella (Uredinales) (Thirumalachar and Whitehead 1954)
- Critical notes on some plant rusts—III (Thirumalachar 1961)
- Some new or interesting rusts from Maharashtra, India (Patil and Thirumalachar 1971)

Mundkur BB. Mundkur is one of the prominent mycologists who significantly contributed to the development of mycology in India and reported several new taxa of rusts in association with Thirumalachar. He was a mycologist in several institutes (Indian Agricultural Research Institute, New Delhi; Deputy Director of Plant Diseases, Directorate of Plant Protection, Ministry of Food and Agriculture; Professor of Botany, University of Poona, Pune). Mundkur carried out extensive studies on plant-associated fungi. He studied the smuts on wheat, barley, and oats and found several smut-resistant varieties. The outstanding contribution of Mundkur is the publication of the two classic books "The Ustilaginales of India" and "Fungi and Plant Disease." Although much of his research was addressed on smuts, Mundkur also contributed to the knowledge of rusts. Through the research papers published with Thirumalachar (1947a, b, 1950), Mundkur brought out all the information available on almost all rusts prevalent in India. They recorded new occurrences; proposed new species, combinations, and varieties; and corrected the wrong identifications made earlier by several workers (Mundkur 1936, 1938, 1943; Mundkur and Prasad 1938; Mundkur and Kheswalla 1943a, b; Mundkur and Thirumalachar 1945, 1946a, b; Thirumalachar and Mundkur 1949a, b).

Ramakrishnan TS. Ramakrishnan is one of the major contributors to the Indian mycology and rusts in particular from the regions of Madras (earlier Madras Presidency included: Karnataka, Kerala, and Tamil Nadu) (Box 12.2). Although we are not aware of region-specific data, Ramakrishnan and coworkers collected rusts from Karnataka as well as Tamil Nadu. Ramakrishnan was in the mycology section of Agricultural Research Institute, Coimbatore (Tamil Nadu), as professor and government-assigned mycologist. Series on rusts of Madras Presidency by Ramakrishnan and coworkers have been published from the Indian Academy of Sciences with highlights of new fungi. Additions to the Fungi of Madras—I to XVII series included several rusts new to India as well as science. Since these papers dealt with other fungi, careful examinations revealed that there were several new species of rusts in their contributions: *Puccinia linkii* and *Xenostele neolitsea* (Ramakrishnan and Ramakrishnan 1947a); *P. solani-gigantea* (Ramakrishnan and Ramakrishnan 1947b); *Aecidium terminaliae, Hapalophragmium anamalaiensis*, *Puccinia luculenta*, *P. vernoniae-monosis*, and *Uredo amomi* (Ramakrishnan and Ramakrishnan 1948a); *Dasturella divina, Puccinia thomasiana*, *P. tweediana*, *P. tricholana*, *Uraecium nothopegia*, *Uromyces loculiformis*, and *U. wellingtonica* (Ramakrishnan and Ramakrishnan 1948b); *Aecidium marsdenia, Cronartium fici*, *Chrysocelis indica*, *Goplana indica*, *Melampsora stereospermi*, *Puccinia jasminicola*, *Uredo malabarica*, and *U. terminalia-paniculata* (Ramakrishnan and Ramakrishnan 1949); *Bubakia indica* and *Phakopsora kirganeliae* (Ramakrishnan and Ramakrishnan 1950a); *Uromyces nilagiricus* (Ramakrishnan and Ramakrishnan 1950b); *Xenostele indica* (Ramakrishnan 1951); *Kuehneola trichosanthes*, *Phakopsora chorisandrae*, *P. mangalorica*, and *Ravenelia coimbatorica* (Ramakrishnan 1952); *Aecidium meliosma-wightii*, *A. anaphalis-leptophylla*, *A. pavonia-odorata*, *Puccinia chloris-incompleta*, and *P. gymnopetalum-wightii* (Ramakrishnan et al. 1952); *Aecidium cuspidatum*, *Puccinia kunthiana*, and *P. pectiniformis* (Ramakrishnan et al. 1953); *Aecidium gymnematis*,

A. walayarense, and *Puccinia curcumae* (Ramakrishnan and Sundaram 1953); *Phakopsora vitis*, *Puccinia tiliaefolia*, and *Uromyces anotis-monospermae* (Ramakrishnan and Sundaram 1955a); and *Phakopsora grewiae* and *P. zizyphi-vulgaris* (Ramakrishnan and Sundaram 1955b).

Box 12.2: Studies Carried Out on Rusts by T.S. Ramakrishnan

- *Puccinia linkii*, *Xenostele neolitsea* in additions to fungi of Madras—I (Ramakrishnan and Ramakrishnan 1947a)
- *Puccinia solani-gigantea* in additions to fungi of madras—II (Ramakrishnan and Ramakrishnan 1947b)
- A new rust on *Dalbergia paniculata* (Ramakrishnan and Ramakrishnan 1947c)
- *Aecidiun terminaliae, Hapalophragmium anamalaiensis, Puccinia luculenta, P. vernoniae-monosis,* and *Uredo amomi* in additions to fungi of Madras—IV (Ramakrishnan and Ramakrishnan 1948a)
- *Dasturella divina*, *Puccinia thomasiana*, *P. tweediana*, *P. tricholana*, *Uracium nothopegia, Uromyces loculiformis* and *U. weillingtonica* in additions to fungi of Madras—V (Ramakrishnan and Ramakrishnan 1948b)
- *Aecidium marsdenia*, *Cronartium fici*, *Chrysocelis indica*, *Goplana indica, Melampsora stereospermi, Puccinia jasminicola, Uredo malabarica* and *U. terminalia-paniculatae* in additions to fungi of Madras—VI (Ramakrishnan and Ramakrishnan 1949)
- *Bubakia indica* and *Phakopsora kirganeliae* in additions to fungi of Madras—VII (Ramakrishnan and Ramakrishnan 1950a)
- *Uromyces nilagiricus* in additions to fungi of Madras—VIII (Ramakrishnan and Ramakrishnan 1950b)
- *Xenostele indica* in additions to fungi of Madras—X (Ramakrishnan 1951)
- *Kuehneola trichosanthes*, *Phakopsora chorisandrae*, *P. mangalorica* and *Ravenelia coimbatorica* in additions to fungi of Madras—XII (Ramakrishnan 1952)
- *Aecidium meliosma-wightiae, A. anaphalis-leptophylla, A. pavonia-odoratae*, *Puccinia chloridis-incompleta* and *P. gymnopetali-wightiae* in additions to the fungi of Madras—XIII (Ramakrishnan et al. 1952)
- *Aecidium gymnematis*, *A. walayarense* and *Puccinia curcumae* in additions to fungi of Madras—XV (Ramakrishnan and Sundaram 1953)
- *Aecidium cuspidatum, Puccinia kunthiana* and *P. pectiniformis* in additions to fungi of Madras—XIV (Ramakrishnan et al. 1953)
- *Phakopsora vitis*, *Puccinia tiliafolio* and *Uromyces anotis-rnonospermae* in additions to the fungi of Madras—XVII (Ramakrishnan and Sundaram 1955a)
- *Phakopsora grewiae* and *P. zizyphi-vulgaris* (Ramakrishnan and Sundaram 1955b)

Pavgi MS. Pavgi and his associates (Hiremath RV and Singh UP) in 1980s contributed largely to the smut fungi of India (Box 12.3). However, Pavgi and Hiremath studied rusts and different life stages of various *Ravenelia* species and significantly contributed to the knowledge of rusts in northern India. Most important studies included studies on Indian *Ravenelia* species associated with various hosts and their developmental stages were recorded along with his associates. A wide range of rust research papers published by Pavgi and his team are (1) Growth of telial head in *Ravenelia* (Hiremath and Pavgi 1976a); (2) Dikaryotization in some of the *Ravenelia* species (Hiremath and Pavgi 1976b); (3) Growth of pycnium in the *Ravenelia* genus (Hiremath and Pavgi 1975a); (4) Germination and cytological aberrations of teliospores in *Puccinia sorghi* (Hiremath and Pavgi 1975b); (5) Respiration in *Ravenelia breyniae* infected by *Melanthesa rhamnoides* (Hiremath et al. 1974a); (6) Metabolism of minerals in *M. rhamnoides* infected by *Ravenelia breyniae* (Hiremath et al. 1974b); (7) Morphology and taxonomic studies of *Puccinia* on corn and *Sorghum* (Pavgi 1972); (8) Assay of aureofungin against rusts in vitro (Hiremath and Pavgi 1971); (9) Metabolism of carbohydrate and nitrogen in *M. rhamnoides* infected by *R. breyniae* (Singh et al. 1970); (10) Morphological studies of *Pycnia* of some species of *Ravenelia* (Pavgi and Hiremath 1969, 1970); (11) Morphology as well as genetics of pathogenicity of *Puccinia sorghi* (Pavgi 1969); (12) *Ravenelia taslimii pycnia* (Hiremath and Pavgi 1969); (13) *Pycnia* morphology of *Ravenelia* species (Pavgi and Flangas 1965); (14) Cytology of Puccinia Sorghi (Pavgi et al. 1960); and (15) Validity of the rust genus *Kernkampella* (Hiremath and Pavgi 1978).

Box 12.3: Studies Carried Out on Rusts by M.S. Pavgi

- Cytology of *Puccinia Sorghi* (Pavgi et al. 1960)
- Electron microscopic observations on the spore forms of three Puccinia species (Pavgi and Flangas 1965)
- Morphology and genetics of pathogenicity of *Puccinia sorghi* (Pavgi 1969)
- *Pycnia* of *Ravenelia taslimii* (Hiremath and Pavgi 1969)
- Morphology of *Pycnia* of some *Ravenelia* species (Pavgi and Singh 1969)
- Morphology of *Pycnia* of some *Ravenelia* species—II (Pavgi and Hiremath 1970)
- In vitro assay of aureofungin against some rusts (Hiremath and Pavgi 1971)
- Morphology and taxonomy of the *Puccinia* species on corn and sorghum (Pavgi 1972)
- Respiration of *Melanthesa rhamnoides* infected by Ravenelia *breyniae* (Hiremath et al. 1974a)
- Mineral metabolism of *Melanthesa rhamnoides* infected by *Ravenelia breyniae* (Hiremath et al. 1974b)
- Development of pycnium in the genus *Ravenelia* (Hiremath and Pavgi 1975a)

(continued)

Box 12.3 (continued)

- Teliospore germination and cytological aberrations in *Puccinia sorghi* (Hiremath and Pavgi 1975b)
- Development of telial head in *Ravenelia* species (Hiremath and Pavgi 1976a)
- Dikaryotization in some species of *Ravenelia* (Hiremath and Pavgi 1976b)
- Validity of the rust genus *Kernkampella* (Hiremath and Pavgi 1978)
- Carbohydrate and nitrogen metabolism of *Melanthesa rhamnoides* infected by *Ravenelia breyniae* (Singh et al. 1970)

A large number of *Ravenelia* species had been documented from India. Even before Butler, many scientists have collected and cited the origin of samples from India (Cooke 1880; Cunningham 1889; Barclay 1890; Parker 1886; Sanwal 1951; Rajendren 1967). They were characterized by having compound telial heads (Fig. 12.3). A special attention is needed towards the new genus *Kernkampella* erected by the Indian mycologist Rajendran due to differences in the spore morphology and splitting behavior of the teliospores (Rajendran 1970a). Rajendran erected a new species from *Ravenelia* to *Kernkampella* (Rajendran 1970a). Though Pavgi and Hiremath argued that they cannot be separated because those characters considered by Rajendran are not of significance to erect as a new species (Hiremath and Pavgi 1978), presently, Laundon's (1975) critical evaluation of the spore morphology was accepted and agreed with the facts of Rajendran, and *Kernkampella* status had been regained. Other notable contributions include understanding the biology and developmental stages of *Ravenelia* and *Kernkampella* (Rajendran 1970a, b).

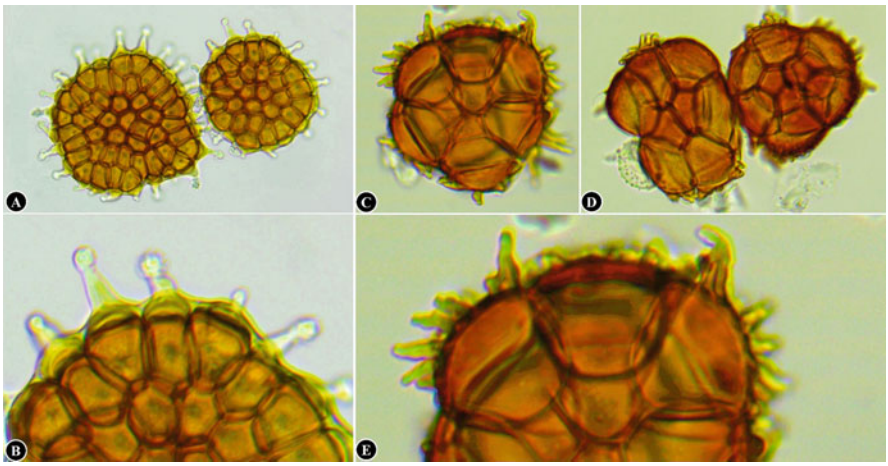


Fig. 12.3 Spore morphology of *Kernkampella* sp. (**a**, **b**) and *Ravenelia* sp. (**c**–**e**) showing the morphological differences between two spores

Ramachar P. In recent years, Ramachar along with Bagyanarayana contributed to the knowledge of rusts in India (Ramachar and Salam 1954; Salam and Ramachar 1955, 1956; Cummins and Ramachar 1958; Ramachar and Bagyanarayana 1977). They erected several new species and critically examined different rusts occurring in India including different stages of rusts. Their contributions to the knowledge of rusts mainly come from the state of Andhra Pradesh (Ramachar and Bagyanarayana 1976, 1982; Ramachar et al. 1978). They revised and provided several nomenclatural changes to the Uredinales (Ramachar and Bagyanarayana 1976, 1978). Ramachar and coworkers discovered spermogonial and aecial stage of *Kernkampella kirganeliae* (Bagyanarayana and Ramachar 1985). Some of the new species of rusts reported include the following: to reinstate the genus status for *Mehtamyces* (Uredinales) was a detailed morphological examination (earlier it was rejected to provide the genus status) (Ramachar and Sudhakararao 1981); *Physopella hiratsukae* (Ramachar and Bagyanarayana 1976); and a new species *Puccinia ctenolepidis* on *Ctenolepis* from India (Ramachar et al. 1985). Recently, Bagyanarayana continued to investigate the rusts of Andhra Pradesh and added several new species like *Puccinia cannacearum* on *Canna indica* (Bagyanarayana and Ramesh 1999). Further the legacy was continued by Bagyanarayana and his colleagues in his active service at Osmania University, Hyderabad (Bagyanarayana et al. 1998; Bagyanarayana and Ramesh 1999).

12.4 Important Rusts in India

In India, several agriculturally important crops are associated with rusts that cause severe economic losses. Most important crop plants affected by rusts are wheat (*Puccinia triticina*, leaf rust/brown rust; *Puccinia graminis* f. sp. *tritici*, stem rust/black rust; *Puccinia striiformis* f. sp. *tritici*, stripe rust/yellow rust), groundnut (*Puccinia arachidis*), coffee (*Hemileia vastatrix*), soybean (*Phakopsora pachyrhizi*), common bean (*Uromyces appendiculatus*), maize (*Puccinia sorghi*, common rust; *Puccinia polysora*, southern corn rust), sorghum (*Puccinia sorghi*), cowpea (*Uromyces vignae*; *U. appendiculatus*), pearl millet (*Puccinia substriata*), and other weed crops. Research on rusts of these crops by various researchers from conventional universities and agricultural universities has contributed to knowledge and advanced our research strategies for the crop improvement through breeding strategies. In the subsequent section, important crop rusts have been described in a nutshell. Further, rusts associated with forest trees also gained attention from several rust specialists who added several new species and genera. Generalists' view of rusts of Karnataka, Kerala, and other states had been detailed. The documentation of the rusts from several other states is, however, missing (though studies have been conducted) and many are yet to be explored.

12.4.1 Wheat Rusts

Wheat is one of the prime staple food crops, which feeds over 50% of global population, and it serves as a vital food crop of India. This crop is known to be affected by three different rust diseases (leaf rust, stem rust, and stripe rust) (Fig. 12.4). Among these, leaf rust is a serious threat to the wheat production at global level (Bhardwaj et al. 2019). Serious problems were faced in wheat production in India during 1970–1980 owing to the rust epidemics; it was managed later by adapting resistant varieties. The resistant varieties released in India consisted of race-specific resistance. Indian wheat production during 1970–1980 suffered the loss due to rust disease in spite of advances in science and technology. The rust problem for wheat production persisted. The damage of wheat by rusts was extensive and incomparable with other cereal rusts. In 1905, the Board of Agriculture directed the mycologists of the country and addressed that "The mycologists will study plant life in the soil, and all fungal diseases of plants among which may be mentioned wheat rust, linseed rust, potato blight, the pepper vine disease, red rot in sugar cane, the wilt disease of pigeon pea, rusts of millet, smuts of cereals, paddy diseases, the opium poppy blight, diseases of ginger, turmeric, and eggplant; all these cause a great loss to the cultivator."

The occurrence of wheat rust was seen in the wheat-growing regions of India from Punjab to Bengal and Madras. This triangle was formed by joining Bombay, Shimla, and Calcutta and it was endemic and highly destructive. Field after field

Fig. 12.4 Yellow rust of wheat collected from Haryana showing the severity of leaf infection and the extent of damage it causes to wheat (Courtesy: Dr. Ravindra Kumar, Scientist, ICAR, Karnal, Haryana)

became red owing to release of uredospores under the stalks of wheat. Subsequently, every year the rust spores were killed by the extreme summer heat in the plains. Later, for about 6 months in the year, no live wheat plant could be found on the ground. The question that remained was about the origin of such a sudden surge of the rust and it was a challenge to the breeders to bring out resistant varieties of wheat. All these efforts laid the way forward for other economic botanists at Pusa (Bihar) to improve the Indian wheat (Mason 1943).

12.4.2 Soybean Rusts

Soybean is used as one of the prime sources of fats and proteins of plant origin and has a major influence on Indian cuisine. However, its adaptation in the diet has made a tremendous impact on its cultivation across India. Even though its nutritional values are not limited to mankind, it attracts a wide range of pathogens that can easily establish their life cycle. One such devastating disease is soybean rust, which is caused by a biotrophic fungus *Phakopsora pachyrhizi* and made its first impact at the global level in Japan with the yield loss of up to 90% in 1903 (Anonymous 1991). In India, the disease was first recorded in the Uttaranchal state followed by West Bengal and Uttar Pradesh during the 1970s (Singh and Thapliyal 1977). There were no reports available on the incidence of the disease between 1974 and 1993, but in 1994 all of a sudden it reappeared with its sporadic form causing loss of up to 80% of the yield in the northern Karnataka regions, Maharashtra, and Madhya Pradesh (Anahosur et al. 1995; Patil and Basavaraja 1997). The symptoms on the abaxial surface of the leaf start with the development of yellow lesions with a brown speck at the center which later develop into brown to dark pustules (Singh and Thapliyal 1977).

Managing the threats posed by the rusts of soybean is a major challenge and in the pursuit of different management strategies, the development of resistant verities paved the way to successful management (Bhor et al. 2014). The discovery of resistant genes in the soybean referred to as R genes with the loci containing *Rpp* 1-6 genes made a breakthrough in developing the resistant cultivars (Bromfield and Hartwig 1980; McLean and Byth 1980; Bromfield and Melching 1982; Hartwig and Bromfield 1983; Hartwig 1986; Garcia et al. 2008; Li et al. 2012). These cultivars have the drawback of resistance to a particular rust isolate and to overcome this problem, gene pyramiding process was employed, which was more effective in suppressing the rusts than the cultivars of single *Rpp* genes (Hartman et al. 2005; Garcia et al. 2008; Lemos et al. 2011; Francis et al. 2012; Maphosa et al. 2012; Yamanaka et al. 2013, 2015; Bhor et al. 2014). The molecular markers were used as an effective means to identify the resistance genes and transfer them to develop new resistance soybean cultivars (Song et al. 2004). With the advantage of molecular marker linkage map, soybean rust-resistant genes were easily mapped and identified (Garcia et al. 2008). Some of the notable cultivars highly resistant to soybean rusts are SJ-1, JS-19, PK-838, EC 241778, EC 241780, Ankur, PI-200492, and EC-389149 (Ramteke et al. 2004; Verma et al. 2004; Patil et al. 2004).

12.4.3 Coffee Rusts

Historically, important coffee leaf rust research gained momentum in Butler's era. In the Kent's estate at Doddengooda, the first *Coffea arabica* showing resistance to *Hemileia vastatrix* was recorded during 1911 in Coorg (Mysore, India) (Fig. 12.5). The selfed seeds of this resistant plant resulted in a well-known Kent cultivar, which was used in large-scale planting (1918–1920) in order to replace the Coorg cultivar which was heavily attacked by the rust (Bhat et al. 2013). The Kent's coffee was imported by many countries in spite of later susceptibility to the rust; its role was vital in the improvement of *C. arabica*. Evidence on the selection pressure by coffee, resistance genes on the origin, and distribution of pathotypes was evaluated by Várzea and Marques (2005). The highest number of rust races was registered in India. This resulted in identifying resistant genotype CLR in India (Prakash et al. 2015). Coffee research station established at Balehonnur, Karnataka, conducted several experiments to document the rust resistance genotypes and screening. Now this station is under the control of the Central Coffee Board.

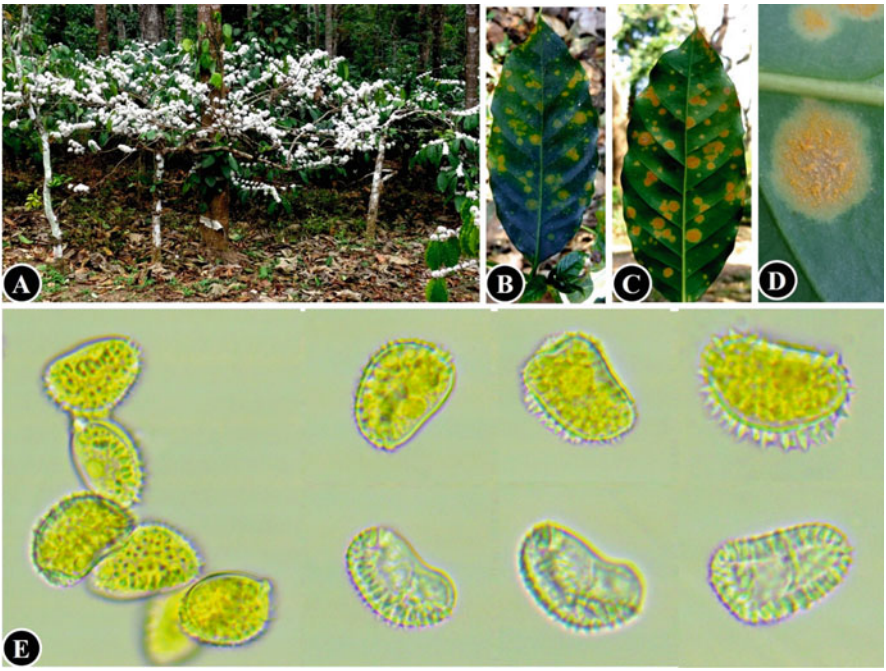


Fig. 12.5 Coffee rust caused by *Hemileia vastatrix*—a serious problem for coffee growers in Coorg and south Karnataka

12.4.4 Groundnut Rusts

Rust associated with groundnut (*Arachis hypogaea*) is a major severe menace to the farmers in all major peanut-growing regions. The groundnut rust disease is caused by *Puccinia arachidis* (Fig. 12.6). The disease was first recorded in India by Chahal and Chohan (1971) from Punjab during 1969 and now it is in all major groundnut-growing regions of the country (Subrahmanyam et al. 1979). During 1970s and 1980s, several researchers assessed and reported its wide occurrence and considered it as a major pathogen of groundnut crops in India (Goswami 1974; Mallaiah 1976; Mayee et al. 1977; Subrahmanyam et al. 1979). Many agricultural universities in association with research institutes (ICRISAT, Patancheru, Hyderabad; NIPGR and NBPGR, New Delhi; NABI, Mohali) are working to explore the possibility of obtaining better performing varieties. Agricultural universities in India carried out research to supply disease-free seeds to the farmers. However, the UAS, Dharwad, in association with ICRISAT developed several improved varieties of peanut and they were subjected to large-scale field trials. The newly developed varieties, hybrids, may perform better, but their performance is always under check as there is a fair chance of exit for host and pathogen and they may break the resistance and become susceptible. Hence, the breeding program should continue to produce new varieties or hybrids as per the demand of the farmers' community. Genetic resources are important for traits of breeding varieties and serve as reservoirs of many useful genes for future groundnut germplasm improvement programs. Researchers are exploiting the host genome and utilizing the various advanced molecular platforms to find a

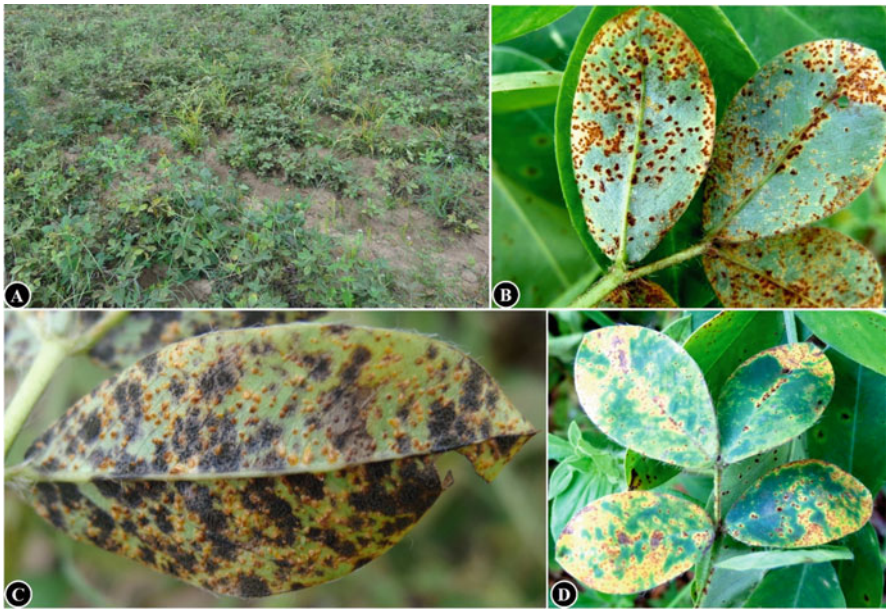


Fig. 12.6 General characteristics of *Puccinia arachidis* rust associated with *Arachis hypogaea*

solution for rust pathogens. Indian researchers are looking for resistant genes from wild sources. Chaudhari et al. (2019) evaluated 340 diverse genotypes of peanut from the gene bank of ICRISAT. Suitable breeding lines and popular cultivars were evaluated for leaf spot/rust resistance as well as yield across three locations in India. Under natural as well as artificial epiphytotic conditions of disease, investigations revealed a significant variation in the genotypes of leaf spot/rust resistance in different environments.

12.4.5 Forest Tree Rusts

In India, studies on rust disease affecting forest tree species were initiated towards the end of the nineteenth century. Since then, many workers have contributed to the knowledge on this subject (Thirumalachar and Mundkur 1949a, b, 1950; Ramakrishnan 1950). Bakshi and Singh (1967) collated available information on rusts affecting the forest tree species and mostly covered the forests in northern India. From the southern and central part of India, during 1950s and 1960s many researchers have contributed towards the knowledge on rusts (Mundkur 1943; Mundkur and Thirumalachar 1945; Ramakrishnan 1950). Studies on rusts of the Western Ghats are rather meagre. Sharma et al. (I985) initiated the work on disease survey in forest plantations in Kerala and brought out information on rusts affecting teak (*Olivea tectonae*), *Bombax ceiba* (*Uredo bombacis*), and *Dalbergia latifolia* (*Uredo sissoo*). Mohanan et al. (1997) generated information on rusts affecting different bamboo species in the Western Ghats as well as in bamboo-growing countries in Asia. In a recent study carried out in Kerala State of the Western Ghats, Mohanan (2004) recorded different rust genera, *Aecidium, Crossopsora, Olivia, Puccinia, Ravenelia, Uredo, Uromyces zaghouani,* and so on, affecting a large number of forest tree species in different forest ecosystems.

Information on rusts affecting the forest tree species since years in Kerala state is very meagre. The available information was made during 1940s and 1950s by Ramakrishnan, which became a knowledge base of this group of obligate pathogenic fungi. Little effort has been made subsequently to contribute to the knowledge on rusts, especially those occurring in forest ecosystems (Nair 1971; Sharma et al. 1985; Harsh et al. 2006). Since most rusts exhibit host specificity and seasonality in occurrence, long-term systematic study is required to understand their life cycle, ecology, and host-pathogen relationship. Mohanan (2010) made a systematic disease survey of the rusts causing diseases in teak, rosewood, and cotton tree in the forest nurseries as well as plantations of Kerala state. A few more rusts were gathered from the forest ecosystems during the biodiversity survey on plant pathogenic fungi in the Western Ghats of Kerala. Mohanan (2010) from Kerala Forest Research Institute (Peechi) has published a list of rusts recorded in the form of book series "*Rust Fungi of Kerala*," which consists of more than 95 rusts associated with various tree species in different forest ecosystems. A total of 95 rust species (25 genera) associated with 117 host species (80 genera in 43 families) were collected and studied, which resulted in 15 hitherto undescribed rust species.

Karnataka state is a part of biodiversity hot spot in Western Ghats and harbors a great diversity of mycobiota. However, occurrence of rusts from Karnataka was least documented. However, Karnataka Biodiversity Board in association with Botanical Survey of India has enlisted the reported fungi from Karnataka region. Although Thirumalachar and Ramakrishnan documented several taxa from Karnataka state, there are many yet to be discovered and documented from the forest and agroecosystems. Karnataka represents ten different agroecological regions. Common bean rust, maize rust, groundnut rust, sorghum rust, pearl millet rust, and onion rust are the most common rusts in different agroecological regions. However, there are a large number of wild plants affected and associated with rusts including weeds, forest trees, and other plants. Mahadevakumar and Janardhana (2014) reported the diversity of rusts occurring in Mysore region of Karnataka state associated with wild plant species and food crops. A total of 19 species of rusts associated with 21 host species belonging to 10 genera were recorded. Important genera include *Puccinia* (8 spp.), *Uromyces* (3 spp.), *Hemileia* (1 sp.), *Ravenelia* (1 sp.), *Phakopsora* (1 sp.), *Sphaerophragmium* (1 sp.), *Melampsora* (1 sp.), *Aecidium* (1 sp.), *Olivea* (1 sp.), and *Coleosporium* (1 sp.).

Rusts of Maharashtra had also been studied and documented by Thirumalachar and his associates (Patil and Thirumalachar 1971). Although Maharashtra is in the belt of Western Ghats and many mycologists have tried to uncover the hidden diversity of rusts (Sathe 1965, 1968, 1969; Yadav 1968; Chavan 1969; Chavan and Patil 1974). much is yet to be known about the rusts. Similarly, there are only two reports on the records of rusts of Rajasthan (Jaipur) (Tyagi 1967, 1973; Tyagi and Prasad 1972). This is a big challenge for the current and subsequent generations to take up and document the rusts that are unexplored.

12.4.6 Reviews and Articles on Rusts

Rusts associated with *Dalbergia latifolia* and other *Dalbergia* species had received little taxonomic conflict. Earlier Ramakrishna and Ramakrishna identified a new species of rust called *Scopellopsis*. But Thirumalachar placed this genus under *Maravalia* (transferred to *Maravalia*). But morphological examination presented by Rajendran clearly stated that the genus status of *Scopellopsis* was reinstated based on the type specimen examination and a comparative account of both genera. Recently, Gowtham has presented a review (checklist) of rusts from Himachal Pradesh. They provided an updated aspect on the rust diversity through field surveys and mycological data analysis in Himachal Pradesh (Gautam and Avasthi 2019). Further, checklist of *Puccinia* species from Himachal Pradesh was reported by Gautam and Avasthi (2016a, b). Many conventional Indian universities have conducted research and contributed to the knowledge of rusts. Thesis searches through Shodhganga showed nine entries in the online database. However, there may be some omission, but many of the universities have theses dealing with different aspects of rusts. Similarly, online repository of M.Sc. and Ph.D. theses of agricultural universities revealed the availability of theses and most of the theses

were from the University of Agricultural Sciences, Bengaluru, and UAS, Dharwad, dealing with maize, peanut, and soybean rusts. The details of entries collected from Krishikosh are presented in Table 12.2 (the data presented in this table are representative and do not include all the rust-related theses).

12.5 Recent Rust Research in India

Recently, several new rusts have been reported from India. Some of the new rusts are *Puccinia mysuruensis* on *Psychotria nervosa* wild coffee from Karnataka (Mahadevakumar et al. 2016), *Stereostratum corticioides* on *Phyllostachys bambusoides* (Tangjang et al. 2018), *Puccinia duthiae* on *Dichanthium foveolatum* from Maharashtra (Pawar et al. 2018), *Puccinia jabalpurensis* on *Lagascea mollis* from Jabalpur (Bhanu 2009), *Puccinia himachalensis* on *Clematis grata* from Himachal Pradesh (Gautam and Avasthi 2016b), *Uromyces umiamensis* on *Momordica cochinchinensis* from Meghalaya (Berndt and Baiswar 2009), and *Puccinia bagyanarayanii* on *Justicia betonica* from Madhya Pradesh (Kumar et al. 2017). These are very scanty compared to the rust research and discovery of new and known species from other countries like the USA, Japan, and China. It may be due to the fact that the facilities and research personnel working on rusts in India are very few and need to create workforce who can reliably contribute to the knowledge of rusts of India.

12.5.1 Rust Resistance Through Breeding

Recent research on rusts in India (mainly in agricultural universities, ICAR institutes, and breeding institutes) is actively engaged to find the solution for rust pathogens. Since the pathogen is obligate parasite, it has evolutionary significance. Henceforth, it is quite a difficult task to find the solution that we usually find for other fungal diseases. Though there are wide opportunities to look into the aspects, breeding for resistance is the only method available for the scientists since 1990s. Scientists are constantly thriving to find better germplasm which exhibits resistance to rust pathogens. In the event of climate change, several pathogenic fungi are changing and there are several new pathotypes of rusts expected to evolve. As a result, pathologists, breeders, and policy makers are trying their best to keep the crop plants healthy through breeding for resistance. However, due to constant fight between the host and pathogen, at any point of time, a resistant breed may turn into a susceptible one.

The ICAR institute (Indian Institute of Wheat and Barley Research) has supported various breeding programs through institutional funding and All India Coordinated Research Projects connecting two or more institutions and agricultural universities. University of Agricultural Sciences (UAS), Bangalore, and UAS, Dharwad, have contributed towards understanding various rusts and initiated breeding strategies for development of rust resistance in maize, soybean, groundnut

Table 12.2 List of Ph.D. and M.Sc. theses/dissertations on rusts of various crop plants from the Indian agricultural universities and institutes

Year	Title	Host	Thesis	University/institute
1985	Studies on Leaf Rust Resistance in Wheat	Wheat	Ph.D.	College of Agriculture, Solan
1991	Genetics of Slow Leaf Rusting in Wheat	Wheat	Ph.D.	Chaudhary Charan Singh, Haryana Agricultural University, Hisar
2004	Epidemiology, Crop Loss Assessment and Management of Soybean Rust in Karnataka	Soybean	Ph.D.	University of Agricultural Sciences GKVK, Bangalore
2006	Genetics for Slow Leaf-Rusting and Yield Related Traits in Bread Wheat (*Triticum aestivum* L.)	Wheat	Ph.D.	Junagadh Agricultural University, Junagadh
2006	Studies on Leaf Rust of Wheat Caused by *Puccinia recondita* Rob. Ex. Desm. f. sp. *tritici*	Wheat	Ph.D.	Sardarkrushinagar Dantiwada Agricultural University, Dantiwada
2008	Genetic and Molecular Analysis of Stem Rust (*Puccinia graminis* f. sp. *tritici*) Resistance Genes in Synthetic Hexaploid Lines of Wheat (*Triticum aestivum* L.)	Wheat	Ph.D.	Indian Agricultural Research Institute, New Delhi
2012	Identification and Validation of Rust Resistance Genes in Wheat Through Molecular Markers	Wheat	Ph.D.	Indian Agricultural Research Institute, New Delhi
2013	Mapping and Characterization of Leaf Rust Resistance Gene Transferred from *Triticum monococcum* L. to Wheat (*Triticum aestivum* L.)	Wheat	Ph.D.	Punjab Agricultural University, Ludhiana
2013	Genetic Mapping of Resistance to Southern Corn Rust (*Puccinia polysora* Underw.) of Maize	Maize	Ph.D.	University of Agricultural Sciences, Bangalore
2014	Mapping QTLS and Determining Relationships Among Resistances to Multiple Foliar Pathogens of Maize (*Zea mays* L.)	Maize	Ph.D.	University of Agricultural Sciences, Bangalore
2015	Studies on Groundnut Rust Caused by *Puccinia arachidis* Speg.	Groundnut	Ph.D.	University of Agricultural Sciences, Dharwad
2015	Marker Assisted Selection for Rust Resistance in Soybean	Soybean	Ph.D.	Mahatma Phule Krishi Vidyapeeth, Rahuri
2017	Fine Mapping and Identification of Candidate Genes for Stripe and Leaf Rust Resistance Transferred from *Aegilops umbellulata* to Bread Wheat (*Triticum aestivum*)	Wheat	Ph.D.	Punjab Agricultural University, Ludhiana
2019		Wheat	Ph.D.	

(continued)

Table 12.2 (continued)

Year	Title	Host	Thesis	University/institute
	Genetic Analysis of Leaf Rust Resistance in NP Series Dicoccum Varieties of Wheat			Indian Agricultural Research Institute, New Delhi
1969	Studies on Maize rust *Puccinia sorghi* Schweinitz in Mysore State	Maize	M.Sc.	University of Agricultural Sciences, Bangalore
1976	Studies on Groundnut Leaf Rust Caused by *Puccinia arachidis* Speg.	Groundnut	M.Sc.	Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur
1987	Studies on Rust (*Puccinia penniseti* Zimm) of Pearl Millet [*Pennisetum americanum* (L) Leeke]	Pearl millet	M.Sc.	Acharya NG Ranga Agricultural University, Hyderabad
1996	Studies on Rust of Soybean *Glycine max* (L.) Caused by *Phakopsora pachyrhizi* Syd.	Soybean	M.Sc.	University of Agricultural Sciences, Bangalore
2004	Perpetuation, Physiologic Specialization and Management of Black Stem and Leaf Rusts of Wheat	Wheat	M.Sc.	University of Agricultural Sciences, Dharwad
2005	Epidemiology and Management of Soybean Rust (*Phakopsora pachyrhizi* Syd.)	Soybean	M.Sc.	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
2006	Crop-Weather-Pest Relationship for Yellow Rust Occurrence and Aphid Incidence in Wheat	Wheat	M.Sc.	Punjab Agricultural University, Ludhiana
2006	Studies on Rust (*Puccinia arachidis* Speg.) of Groundnut (*Arachis hypogaea* L.)	Groundnut	M.Sc.	Junagadh Agricultural University, Junagadh
2008	Host Resistance and Yield Loss Assessment Due to *Puccinia polysora* Underw. Rust of Maize (*Zea mays* L.)	Maize	M.Sc.	University of Agricultural Sciences, Bangalore
2008	Genetics of Leaf and Yellow Rust Resistance in Interspecific Derivatives of Wheat (*Triticum aestivum* L.)	Wheat	M.Sc.	Indian Agricultural Research Institute, New Delhi
2009	Epidemiology of Maize Rust (*Puccinia polysora* Underw.) and Inheritance of Resistance	Maize	M.Sc.	University of Agricultural Sciences, Bangalore
2009	Genetics of Rust Resistance in SEL.T3336 and WR95 in Wheat (*Triticum aestivum* L.)	Wheat	M.Sc.	Indian Agricultural Research Institute, New Delhi
2009	Studies on Groundnut Rust Incited by *Puccinia arachidis* (Speg.)	Groundnut	M.Sc.	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
2011	Screening for Resistant Sources and Assessment of Yield Loss in	Maize	M.Sc.	University of Agricultural Sciences, Bangalore

(continued)

Table 12.2 (continued)

Year	Title	Host	Thesis	University/institute
	Rust Disease of Maize (*Zea mays* L.) Incited by *Puccinia polysora* Underw.			
2011	Molecular Cytogenetic Characterization of Wheat—*Aegilops umbellulata* Leaf Rust and Stripe Rust Resistant Introgression Lines	Wheat	M.Sc.	Punjab Agricultural University, Ludhiana
2011	Studies on Loss Assessment and Management of Common Rust of Maize Caused by *Puccinia sorghi* Schw.	Maize	M.Sc.	University of Agricultural Sciences, Dharwad
2011	Studies on Molecular Variations in *Puccinia arachidis* Speg. Causing Rust of Groundnut	Groundnut	M.Sc.	University of Agricultural Sciences, Dharwad
2013	Studies on Epidemiology and Integrated Management of Soybean Rust Caused by *Phakopsora pachyrhizi* (Syd.).	Soybean	M.Sc.	Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
2014	Molecular Characterization of Bread Wheat (*Triticum aestivum* L.) Genotypes Conferring Stripe Rust Resistance	Wheat	M.Sc.	Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir
2014	Genetics and Molecular Mapping of Rust Resistance in Wheat	Wheat	M.Sc.	Indian Agricultural Research Institute, New Delhi
2015	Epidemiological Study of Rust (*Puccinia purpurea*) of Sorghum	Sorghum	M.Sc.	Mahatma Phule Krishi Vidyapeeth, University Library, Rahuri
2016	Studies on Epidemiology and Management of Pearl Millet Rust Incited by *Puccinia substriata* var. *penicillariae*	Pearl Millet	M.Sc.	Chaudhary Charan Singh Haryana Agricultural University, Hisar
2017	Evaluation of Wheat (*Triticum aestivum* L.) Germplasm Using Most Prevalent Races for Brown Rust (*Puccinia triticina*) Resistance	Wheat	M.Sc.	Banaras Hindu University, Varanasi
2018	Molecular Mapping of Leaf and Stem Rust Resistance Genes in a Wheat-Rye Recombinant "SELECTION 212"	Wheat	M.Sc.	Indian Agricultural Research Institute, New Delhi
2019	Over-Summering Behaviour of *Puccinia striiformis* f. sp. *tritici* Erikss., the Incitant of Yellow Rust of Wheat and Its Management	Wheat	M.Sc.	Punjab Agricultural University, Ludhiana

(continued)

Table 12.2 (continued)

Year	Title	Host	Thesis	University/institute
2019	Management of Stripe Rust of Wheat Incited by *Puccinia striiformis* f. sp. *tritici* Westend	Wheat	M.Sc.	Chaudhary Charan Singh Haryana Agricultural University, Hisar

(peanut), and many other important crops. The UAS, Dharwad, has released several new varieties of crops, which were resistant to rust. For example, during 2012, a new hybrid maize (GH-0727) survived up to 120 days with the ability to yield 74 quintals per hectare. This high-yielding as well as high-starch-containing variety is rust resistant. During 2016, additional three popular varieties of groundnut (ICGV 91114, TAG 24, and JL 24) were improved for rust resistance using molecular breeding methods in association with ICRISAT, Hyderabad. Aggarwal et al. (2018a, b) reported stripe rust pathogens based on molecular methods. These stripe rust isolates were characterized by their phenotypic response on hosts by different Yr genes. Ten different pathotypes were characterized by virulence/avirulence status (70S0-2, 67S64, 70S4, 66S0, 70S64, 66S64-1, 38S102, 47S102, 46S119, and 78S84). Aggarwal et al. (2018a, b) also used these pathotypes of *P. striiformis* f. sp. *tritici* along with additional 38 pathotypes of rust species (*P. graminis tritici* and *P. triticina*) to assess their phylogenetic relationship. The sequence data of Asian isolates are clustered in a distinct lineage compared to those obtained from the USA. Manjunatha et al. (2018) developed a PCR and loop-mediated isothermal amplification (LAMP) methods for detection of *Puccinia triticina* in wheat. Recently, Kumar et al. (2019) published based on mining of the Indian wheat leaf rust-resistant germplasm. They performed field trials across ten locations, followed by molecular screening to elucidate the presence of APR genes (Lr34+, Lr46+, Lr67+, and Lr68) in wheat germplasms. For field trials, 190 wheat accessions were chosen from 6319 accessions based on leaf tip necrosis (LTN), severity of disease, and coefficient of infection. Molecular trials revealed 73% of the accessions consisting of APR genes. The resistance by APR genes in the view of high-yielding cultivars is expected to reveal a high-level nonspecific resistant durable race (Kumar et al. 2019).

Several new varieties were released by the wheat research institute from India. Recently, in IARI Regional station (Wellington, Tamil Nadu), well-designed wheat improvement strategies were adapted. This attempt resulted in developing a variety of high-yielding semidwarf disease-resistant dicoccum wheat (HW 1098) designated as "Nilgiri Khapli" (Sivasamy et al. 2014). Recently, Indian scientists decoded the draft genome of *Puccinia triticina* known to cause wheat rust in India. A team of scientists from ICAR decoded the genomes of 15 different strains of *P. triticina*. Recent molecular data based on OMICS approach will help the breeders and scientists of different domains to understand the dynamic behavior of the rusts which is regarded as the severe constraint to wheat production in major wheat-growing regions in India and the world.

Sharma from ICAR-NRCPB, New Delhi, coordinated a DBT project on *Puccinia triticina* (leaf rust pathogen) de novo genome sequencing in three ICAR institutes

(National Research Centre on Plant Biotechnology, New Delhi; Indian Institute of Wheat and Barley Research, Shimla; Indian Agricultural Research Institute, Wellington) with two of the state agricultural universities (Punjab Agricultural University, Ludhiana; Tamil Nadu Agricultural University, Coimbatore). In this study, the main focus was laid on understanding the molecular mechanism of variability in rusts through decoding the genomes of one of the highly variable Race77 and its 13 biotypes with a stable Race106. The Race106 was identified in 1930 and deposited in the national collection at Shimla, which did not mutate during the last 85 years, while the Race77 was first found in 1954 in Pusa (Bihar) and evolved into 13 biotypes in wheat breeding programs. Hence, understanding the molecular mechanism underlying virulence and adaptability within Race77 is important in unraveling the molecular basis of fast evolution as well as the stability of Race106 genome. Next-generation sequencing (NGS) technology was adapted in the ICAR-NRCPB to decode the genomes of 15 wheat strains (~1500 MB data) susceptible to leaf rusts. The draft genome (~100 Mb) sequence of Race77 with 33X genome predicted that 27,678 protein-coding genes are involved in different functions. The detailed studies provided more information about *P. triticina* on the genome structure, organization, molecular mechanism of variation, and pathogenicity. Such genomic information will be of immense help and seems to be the landmark in India for the wheat improvement programs.

12.5.2 Knowledge Gap in Rust Research

Rust research in India has been highly remarkable during 1940–1960; thereafter, there was a waning in the number of research personnel as well as research activities towards training to document the diversity of rusts from different regions. There are many universities that conduct research on rusts, but the actual data is not available on the database. Lack of information of scholars who worked under renown mycologists (like MJ Thirumalachar, TS Ramakrishnan, and MS Pavgi) and their further contribution to science (to rusts of India) is creating a large vacuum and efforts are needed to fill this gap by promoting and encouraging scientific investigations across the country. Collaboration and exchange of information are very much essential for the documentation of biodiversity of rusts. The availability of biodiversity data on rusts from India so far is not complete and not even attempted to explore and analyze as per recent trends of knowledge and methods. Although there were some exceptional studies conducted by certain institutions, we are still far behind in estimating and exploiting the biological diversity or rusts. From the last three decades, we have not seen any major progress in the field of rust research. The dearth of information on regional rust data leads to ineffective attempts to tackle the diseases. Although rusts of Maharashtra were well studied by various mycologists, we could not trace them owing to non-digitalization. In order to fill such vacuum, serious attempts are called for.

Creating a national rust catalogue is still a dream for many rust mycologists. Compilation of rusts reported from India as a new species, genus, or a new record

needs to be well documented, which may serve as a reference source for upcoming rust investigators. Countries like Australia, the USA, Japan, and Russia have their own well-documented rust data and the quality of rust research carried out in these countries is exemplary. There is an urgent need to create a platform to teach and train the young minds to pursue their careers by enriching their knowledge on rust diversity through inventories and molecular analysis. The fundamental research on these aspects will throw light on several basic questions like evolution, divergence, and genetic mechanism underlying the development of resistance to a particular gene, which is very important to the scientists who intend to develop new disease-resistant genetic variants.

12.6 Future Perspectives

Considering rusts as an important group of fungi with their different spore stages and host preference, several challenges could be tackled to face the threat posed by them. Since they are obligatory biotrophs, they are in need of living host for survival and perpetuation. However, association of rusts with agriculturally important crop plants may cause severe loss of production leading to havoc in food security. Best example is the wheat rust (which caused loss of up to 70% in wheat-growing countries like the USA, Israel, and others). Scientists have a huge task to develop resistant varieties of crop plants to rust diseases to safeguard the quality food production. Recently, we have collected more than 100 rusts from crop plants, tree species, and wild plants in Karnataka (S. Mahadevakumar, unpub. data). Among them, there are many species that need to be renamed and reassessed for their validity and their occurrence in specific geographic setup. In Karnataka state alone, without getting into the forest regions, we could collect a huge number of rusts. Rust research in India is solely based on morphology and host preference. In the recent taxonomic developments, by applying molecular tools and techniques, a wide range of rusts could be named or evaluated more precisely, which has bearing on developing the control measures. The application of morphological studies complemented with molecular techniques should be encouraged for basic and applied aspects of rusts in India.

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Biocontrol Research in India

13

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Abstract

Management of agricultural production systems on a sustainable basis is one of the basic challenges confronting the eventual fate of mankind as the total population is rising extraordinarily. Protection against pests and diseases in crops has an apparent role to play to meet the rising demand for food quality and quantity. The yield losses incurred due to various pests, pathogens, and weeds altogether are estimated to be ranging between 20% and 40% of global agricultural production. To cope up with the growing food demand, synthetic pesticides were applied indiscriminately; but extreme use of synthetic pesticides has led to degradation of land, air, and water contamination along with resistance development in pest and pathogens as well as adverse impacts on natural enemies and humans. The growing demands to address food security with the maintenance of environmental safety have resulted in searching for alternative options such as biological control. From the mid-1970s, considerable research has been carried out in the field of biocontrol. Biocontrol agents such as *Trichoderma*, *Beauveria*, *Verticillium*, *Pseudomonas,* and *Bacillus* have brought a new change in the field of management of phytopathogens. A large number of biopesticides available in the market proves the tale of their immense popularity. Moreover, there are many success reports of pest and weed control by natural enemies. So overall

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it can be stated that biocontrol research in India has been carried out on a wide avenue and will reach extreme heights in future.

Keywords

Biological control · *Trichoderma* · *Pseudomonas* · *Bacillus* · Biopesticide

13.1 Introduction

The last few decades have witnessed the monopoly of chemical pesticides as a plant protection agent. Agriculture has observed a major revolution in the behavior of crop protection chemicals that commenced in the late 1800s with the introduction of chemicals such as DDT and Bordeaux mixture. The effect of chemical pesticides on agriculture has been dramatic as chemical pesticides have become a synonym for agriculture. However, apart from their beneficial effects, there are issues of residual effect, adverse effect to non-targeted organisms, etc. that have raised serious environmental complications. Moreover, the emergence of resistance in many vector species against a particular chemical leads to the concern of pest survival and the need for new chemical insecticides. It was comprehended later that as long as it is dependent on the sole use of chemicals, pest control is not safe anymore. Thus, it paved the path for the search for some better alternatives in crop protection. The introduction of natural enemies such as pathogens, predators, and parasites has attracted the attention of researchers for exploring the management of pests through them.

Biological control/biocontrol is considered as an environmentally friendly and holistic tactic for plant disease management along with quality crop production endeavor (Mukhopadhyaya 1994; Singh et al. 2012, 2016a, b; Mishra and Arora 2016). According to Garret (1965) "Biological control may be defined as a practice whereby survival or activity of a pest or pathogen is reduced through the activity of any living organism." However, Cook (1988) defined biological control as the "use of natural or modified organisms, genes, or gene products to reduce the effects of pests and diseases." In another attempt, Wilson (1997) in his own words redefined biological control as "the control of plant pests and disease with a natural biological process or the product of the same process." In biological control, a wide range of microbes have been commercially released as biocontrol agents and this provides an alternate strategy to chemical disease management. Biocontrol strategy mainly depends on the synergistic relationship among the different genera of the microbial community acting together to confront the abiotic and biotic stresses to which plants are subjected (Saxena et al. 2016; Ram et al. 2018; Ram and Singh 2017).

Biocontrol usually involves the management of one organism by another (Singh et al. 2004). This control might be communicated as either a more extended populace of the pest and pathogen (DeBach and Hagen 1964) or a limitation or counteraction of the seriousness or frequency of irritation harm regardless of the nuisance populace (Cook and Baker 1983). Biocontrol relies upon the information of natural

cooperation in the environment and living organisms at cellular levels and frequently is more scrambled to manage in contrast to physical and chemical control methods.

Biocontrol is now being considered for increasing crop production by minimizing crop losses and managed ecosystems. The introduction of new tools like recombinant DNA technology and crop simulation modeling has facilitated the pest control methods.

The monetary losses incurred as a result of pests and diseases in India have been accounted to be around 42.66 million dollars (Subash et al. 2017). Not many synthetic pesticides are accessible in the Indian pesticide market which could provide good results along with safeguarding of the environment. So, there is an urgent need to search for some better alternatives and technologies dependent on natural procedures for pest management (Arora and Mishra 2016). Biopesticides seem to be an appropriate contender in this regard; however, developing countries like India and others face various obstacles in their commercialization (Singh et al. 2004; Dutta 2015). Despite biological controls having been used in agriculture for several decades, still, it is in its infancy as an industry. The major constraint is the moderate activity and lower shelf life, higher production input, and lack of awareness among the growers. Besides this, the inadequate knowledge regarding benefits offered by biopesticides, absence of technical skill in the later phases of improvement, trouble in quality control testing time of these bioactive products before enlistment, and commercialization are few hindrances in their successful registration and commercialization (Keswani et al. 2014; Singh et al. 2016a, b). Due to undiscriminating use of chemical pesticides, the rich biodiversity of various biodiversity hot spots in the Indian subcontinent has deteriorated to a great extent and we are at an alarming level where many of the biocontrol agents may extinct. So, the existing situation demands the exploration of more alternative methods of pest control which do not pose any threat to the environment along with flora and fauna.

This chapter comprehensively discusses biological control with its history and the need for sustainable agriculture. We have tried to highlight the present status of research on biological control and few stories of successful biocontrol in India. The possible prospects and general recommendations that could enhance the promotion and acceptance of biopesticides in boosting agriculture in India have also been included in the chapter.

13.2 Necessity for Biological Control in India

India is mainly agriculture based and a majority of the population is dependent on it for their livelihood. According to a survey, the production of food grain should increment to 250 million tons continuously in 2020 to address the issues of the growing populace of our country. To feed such a huge population a large thrust has been laid to enhanced production under limited sources. Pests and diseases contribute about 20% of overall crop losses. So as a plant protection strategy, farmers and growers frequently depend intensely on synthetic chemicals and pesticides to minimize yield losses. In any case, the environmental pollution brought about by

indiscriminate use and overuse of agrochemicals, just as dread mongering by certain adversaries of pesticides, has prompted impressive changes in individuals' perspectives towards the utilization of pesticides in agriculture. A rise in the population of pests and diseases has brought about the expanded utilization of toxic components for their administration. The population of species resistant to pesticides and fungicides has expanded. As of late after signing a petition with trade and tariffs more accentuation is given to the utilization of biopesticides for crop protection in the light of their least toxic nature, low degrees of disease resistance, and low residual issues. In any case, biological controls ought to be integrated with other control measures because various strategies are viable in various situations and areas under changing stress conditions.

13.3 History and Development of Biological Control: An Indian Perspective

Biological control of plant diseases and use of bioagents for control or regulation of pests have been reported to be in practice since the thirteenth century. The introduction of vedalia lady beetle from Australian subcontinent to the Americas and to other parts of the world was the first documented example of widespread use of a particular biocontrol agent. Such pest control practices gave impetus to the use of pest control agents in agricultural practices across the globe. After the Second World War, chlorinated hydrocarbons and other chemicals such as cyclodienes, carbamates, organophosphates, and synthetic pyrethrins, which had potent pest control efficiency, came subsequently in regular use as pesticides leading to decrease in biological control programs throughout the world. The famous Green Revolution of India in the 1960s and 1970s promoted large-scale utilization of chemical fertilizers along with varied chemical pesticides for pest control activities which resulted in diseases and deaths of farm animals and farmers. The publication of "Silent Spring" by Rachel Carson in the early 1960s created public awareness of the environmental damage that synthetic pesticides can cause. Search for alternative methods to synthetic chemicals led to a reemergence of biological control. Neem tree (*Azadirachta indica*) and its derivatives, i.e., extract from neem leaves, neem oil, and neem seed cake, are generally used for controlling pathogen infestation in crops and also for reducing risks of postharvest losses in stored cereals (Brahmachari 2004).

In India, the inception of a systematic research program in the field of biological control of crop pests dates back to late 1950s when the "CAB International Institute of Biological Control," formerly known as Commonwealth Institute of Biological Control, set its office in India in 1957. After the realization of the negative impact of chemical agents for pests and pest disease control, the importance of biological agents for pest control and use of eco-friendly methods for control of insects and plant pathogens gained momentum. In this context, the Indian Council of Agricultural Research (ICAR) started All India Coordinated Research Project (AICRP) on Biological Control of Crop Pests and Weeds (AICRP-BC&W) in the year 1977. In the year 1989, the Department of Biotechnology (DBT), Govt. of India, launched a

National Bio-control Network Programme for 5 years starting initially with 10 R&D projects, which was later extended with the inclusion of 200 projects implemented at various national institutes and state agricultural universities (SAUs) (Wahab 2004). Because of long-term agricultural sustainability and profitability to the farming community, the Govt. of India included an integrated pest management program in the National Policy Statement in the year 1985. Thereafter, intending to strengthen and widespread establishment of biocontrol research at regional levels, the Ministry of Agriculture launched a scheme on "Strengthening and Modernization of Pest Management Approach in India" in 1991–1992. Further, in the eighth 5-year plan of India, an independent Project Directorate of Biological Control (PDBC) was established in the year 1993 as an up-gradation of AICRP on Biological Control and Weed Control.

Varied forms of biological control methods have been developed in the twentieth century. Several biocontrol formulations for managing pests have been developed using natural plant products, nematodes, and effective strains of bacteria, fungi, and viruses. In the Indian context, research work on bioagents for pest control started with the identification and use of entomopathogenic nematodes (EPNs) in the late 1960s. The first attempt for use of EPN was made in 1966 for biocontrol of insect pests of rice, sugarcane, and apple in field conditions by Dr. T.M. Manjunath, the former director of Monsanto's R&D Center in India. Research efforts to identify other potent bioagents in the form of entomopathogenic bacteria and fungi were started simultaneously thereafter by different research groups in India at various national research institutes, namely Indian Agricultural Research Institute (IARI), New Delhi; Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala; Sugar Breeding Institute (SBI), Coimbatore, Tamil Nadu; Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu; Rajasthan Agricultural University (RAU), Udaipur, Rajasthan; Anand Agricultural University (GAU), Gujarat; National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, Karnataka; Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh; Directorate of Rice Research (DRR), Hyderabad, Andhra Pradesh; Central Institute for Cotton Research (CICR), Nagpur, Maharashtra; and several others.

In India, research work on biopesticides was largely restricted to government research laboratories till 1970s and 1980s before the first initiative taken by Dr. T.-M. Manjunath to introduce public-private partnerships in biocontrol research in India with emphasis on integrated plant protection practices using bioagents (Manjunath 1992). In the 1990s, some private companies with established pesticide market in India like Monsanto seeds and Bayer Industries started working on the development of biopesticides such as *Bacillus thuringiensis* and *Trichoderma harzianum* as an alternative to the chemical pesticides for commercial scale production under public-private partnership with govt. research labs. Thereafter many small private companies also started on the same lines. Due to initial competition in the market without any defined legislation for biopesticide production and quality control, the market used to get flooded with low-quality ineffective biopesticide formulations also.

Table 13.1 List of fungi included in the schedule of the Insecticides Act 1968 for the production of biopesticides in India

1.	*Gliocladium* spp.
2.	*Trichoderma* spp.
3.	*Beauveria bassiana*
4.	*Metarhizium anisopliae*
5.	*Verticillium lecanii*
6.	*Nomuraea rileyi*
7.	*Hirsutella* species
8.	*Verticillium chlamydosporium*
9.	*Ampelomyces quisqualis*
10.	*Candida oleophila*
11.	*Fusarium oxysporum* (nonpathogenic)
12.	*Coniothyrium minitans*
13.	*Pythium oligandrum*
14.	*Phlebia gigantea*
15.	*Paecilomyces lilacinus*
16.	*Penicillium islanidicum*, *Alcaligenes* spp.
17.	*Chaetomium globosum*
18.	*Aspergillus niger*—strain AN27
19.	Vesicular arbuscular mycorrhizae (VAM)
20.	*Photorhabdus luminescens akhurstii* strain K-1
21.	*Piriformospora indica*

To address the above concerns and to help farmers to choose the right product, the Central Insecticides Board and Registration Committee (CIBRC), Govt. of India, included some legislation for production and distribution of biopesticides under the Insecticides Act of 1968 and Insecticides Rules of 1971. Currently 21 fungi are included in the Gazette of India for commercial production of biopesticides (Table 13.1).

13.4 Present Status

Biological control agents used in India are mainly categorized under four categories, namely microbial biopesticides, plant protectants (also called botanical pesticides), pheromones, and other natural insect growth regulators. Microbial biopesticides include formulations made by using strains of bacteria, fungi, viruses, or entomopathogenic nematodes. Fungal biopesticides have the maximum percentage share among the biopesticides with most of such formulations involving the use of *Trichoderma* strains as an active ingredient. Currently, about 350 biopesticide products are available in the Indian market for field applications. Among bacterial biopesticides, strains of *Pseudomonas fluorescence* are more popular in India as

compared to other licensed products based on *Bacillus* strains such as strains of *Bacillus thuringiensis, Bacillus sphaericus,* and *Bacillus subtilis* which are more popular in overseas markets. Certain other non-spore-forming bacteria such as *Serratia entomophila* and *Chromobacterium subtsugae* with potent biocontrol potential against a wide range of insect pests have not been much tested for formulation development (Jackson et al. 1992; Martin et al. 2007). Similarly, some other effective strains like *Yersinia entomophaga* and *Pseudomonas entomophaga* with proven entomopathogenic role have also not been given much emphasis as biopesticides (Vodovar et al. 2006; Hurst et al. 2016). Among virus-based biopesticides, only nucleopolyhedrosis viruses (NPVs) are being used for biocontrol of *Helicoverpa armigera* in India and their market share is very low. Another entomopathogenic virus, granulovirus (GV) that infects larvae of sugarcane pests, has abundant natural occurrence in the Indian subcontinent but its mass multiplication and production at commercial scale have not been given priority (Easwaramoorthy and Jayaraj 1987). Among commonly used entomopathogenic nematodes (EPNs), *Heterorhabditis* and *Steinernema* are the two most effective EPNs that are being used under field conditions against different soilborne pests but registered products based on EPNs are not yet available in the market.

Among plant-based biopesticides, use of neem-based products is most common although other plant products with biocontrol potential such as eucalyptus leaf extract, *Pyrethrum*, and *Cymbopogon* are also available for field-level application (Isman 1997; Walia et al. 2017; Dougoud et al. 2019).

Among 85 genera of entomopathogenic fungi, only three species are included in the Gazette of India (March 26, 1999) for commercial production of biopesticides in India. They are *B. bassiana*, *V. lecanii*, and *M. anisopliae.* These entomopathogens are being assessed against several agricultural and urban insect pests. Several insect species belonging to the order Lepidoptera (Hussain et al. 2009), Coleoptera (Ansari et al. 2006), Isoptera (Hussain et al. 2010, 2011), Diptera (St. Leger et al. 1987), and Hemiptera (Leite et al. 2005) are susceptible to various fungal infections. This has led to several attempts for application of entomopathogenic fungi for pest control with varying degrees of success.

Application of pheromones or other natural insect growth regulators is also a very important component considering the adoption of an integrated pest biocontrol approach and pheromone-based products are already available in the market as traditional biopesticide products.

Cotton, sugarcane, and rice are the three major crops where pheromone technology is being utilized to control the pest population.

Some pilot projects have also started in other parts of India for promoting the use of pheromone application technology (PAT) in the management of crop pests.

13.4.1 Success Stories of Biological Control in India

- Prickly pear, *Opuntia elatior* Miller, *O. stricta* (Haworth), and *O. vulgaris* Miller (Cactaceae), has emerged as a serious pest in South India. The first successful classical biological control of this insect was done in India with the insect *Dactylopius ceylonicus* which was introduced from Brazil in 1795. Currently, *D. ceylonicus* has emerged as a successful biocontrol agent in the management of *O. vulgaris*, reducing it from a state of widespread abundance to that of virtual extinction in parts of southern India and northern Sri Lanka.
- Water fern, *Salvinia molesta*, has been a nuisance for water bodies of Asia, Africa, and Australia. Salvinia was first observed in 1955 in Vole Lake (Kerala) and has assumed pest status since 1964. In Kuttanad area of Kerala, the weed has brought havoc by occupying some 75,000 acres of canals and another 75,000 acres of paddy fields. In extreme cases, it has choked rivers, canals, and lagoons and caused hindrance in operations such as navigation, irrigation, fishing, and shell collection. The exotic weevil *C. salviniae* was found to be an effective biocontrol agent against *Salvinia* plant. About 2000 sq km area of the weed has been cleared by *C. salviniae* in Bengaluru region. In Kerala, the control of *Salvinia* has brought the aquatic flora back to its natural days when weed infestation has not occurred.
- Water hyacinth (*Eichhornia crassipes*) was first brought into Bengal as a decorative plant around 1896; later on the weed has spread all over India and occupied more than 200,000 ha of water surface. Water hyacinth is viewed as the most detrimental aquatic weed in India. It grows wherever it starts moving from freshwater lakes, tanks, lakes, stores, streams, and waterways to water system channels. Likewise, it has become a serious problem in overflowed rice fields. For the administration of weed, natural enemies were introduced in India, viz., hydrophilic weevils *Neochetina bruchi* (Ex. Argentina) and *N. eichhorniae* (Ex. Argentina) and Galumnid bug *Orthogalumna terebrantis* (Ex. South America), from their natural geographic area. Till date, more than 450,000 weevils have been delivered at various areas in 15 states which brought about significant control of water hyacinth over a large area.
- *Icerya purchasi* (origin: Australia) was presumably presented as imported orchard stock or blooming plants from Sri Lanka and it spread to developed wattles, flower brambles, and citrus. It was first revealed from Nilgiris in 1928 as a nuisance of developed wattle, *Acacia decurrens*, and another *Acacia* spp. With the spread of the bug in Karnataka, Kerala, and Maharashtra, the citrus harvest of these states was genuinely undermined. It has been recorded from 117 host plants. The coccinellid beetle, *Rodolia cardinalis* (origin: Australia), has a magnificent history for the suppression of cottony cushion scale. *I. purchasi* was acquainted with India in 1926 through the USA. The beetle was delivered in the Nilgiris in 1930 and it successfully controlled the pest. In 1941, the bug reached

considerable extent and spread to upper Palani slopes (Tamil Nadu); however, the attack was lowered by releasing *R. cardinalis*.

- Mealybug (*Planococcus citri*), pineapple mealybug (*Dysmicoccus brevipes*), grape mealybug (*Maconellicoccus hirsutus*), oriental mealybug (*Planococcus lilacinus*, *P. pacificus, P. robustus*), and striped mealybug (*Ferrisia virgata*) are delicate-bodied sucking insects which cause serious damage and reduce the yield and quality of the produce. To the extent to which biological control of bugs in India is concerned, maybe the first natural enemy was the coccinellid hunter *Cryptolaemus montrouzieri* which was introduced in Tamil Nadu. The predator is discovered to be successful in controlling mealybugs on mango, pomegranate, citrus, guava, grapes, mulberry, espresso, custard apple, ber and green shield scale, sapota, etc.
- San Jose scale is a serious pest of apple in northwestern India. It likewise attacks different deciduous trees, poplars, and willows. Around 50 hosts have been recorded in India. The scale bug colonizes all parts of the plant. Aphelinid parasitoid, i.e., *Encarsia perniciosi* strain from California, was introduced in 1958 and Illinois, Chinese, and Russian strains were presented in 1960 for the biological management of San Jose scale in India. Russian strain gave 89% parasitism in Himachal Pradesh and showed excellent results. The parasitoid was found very effective in the management of San Jose scale and restricted the threat of the pest to a considerable extent.
- Woolly aphid (*Eriosoma lanigerum*) is a local pest of Eastern USA. It was presumably coincidentally acquainted with India from England as demonstrated from its record in Shimla region of Himachal Pradesh where nursery stocks were imported. Presently it has spread to all the apple-growing regions of the country. For the control of woolly aphid, aphelinid parasitoid, *Aphelinus mali*, a local of North America, was introduced from England to Saharanpur (Uttar Pradesh). *C. septempunctata* demonstrated good results in controlling the bug. Moreover, management of woolly aphid of sugarcane was successfully done by application of bioagents, viz. *Dipha aphidivora*, *Chrysoperla* spp., coccinellid bugs, syrphid flies, and a few spiders in the regions of Maharashtra and Karnataka, where its occurrence during 2003–2004 had made significant monetary losses to the growers. In a nutshell, *A. mali* has emerged as a prime biocontrol agent for the management of *E. lanigerum*.
- *Parthenium hysterophorus* is one of the most serious weeds prevalent in India. It can be commonly seen growing all over ranging across the vast majority of the empty and marginal grounds. In India, *P. hysterophorus* was first recorded from Pune in 1955. The weed is known to suppress local vegetation by the release of growth-inhibiting chemicals through leaching, root exudates, decaying of residues, etc. It covers no-man's-land as well as attacks developed fields and represents a threat to yields, for example, cereals, vegetables, fruits, and oil seeds. In 1983, a chrysomelid beetle *Zygogramma bicolorata* was imported from

Mexico for parthenium control. Host specificity tests were conducted with 40 plant species comprising 25 families and the beetle was regarded to be harmless to economic plants. The beetle has demonstrated excellent results in the management of parthenium all over the Indian subcontinent.

- *Lantana camara*, a weed from Central and South America, was introduced into India in 1809 as an ornamental plant. It spread soon into open regions in forestland, and pastured framing thick shrubberies. It is an enduring, straying bush with thorny stems, spreading by seed, however regrowing vivaciously after cutting. For the management of the pest, tingid ribbon bug, *Teleonemia scrupulosa*, was introduced from Australia in 1941. *T. scrupulosa* does not attack teak or some other economic plants in India under field conditions. The adequate populace of *T. scrupulosa* does not permit lantana to overgrow and assumes appropriate control over the weed. The bug acted as a relief to the growers who were irritated by the menace of *L. camara.*
- A serious epidemic of sugarcane Pyrilla occurred during 1972–1973 in the region of UP, Bihar, Punjab, and Haryana, which was effectively constrained by the use of potential biocontrol agents like egg parasitoid *Tetrastichus pyrillae* and nymphal predator *Epipyrops melanoleuca*. Losses around a sum of 11.00 crores was saved. Correspondingly, again epidemic of this pest occurred in 1987 in some sugarcane-developing regions, when again use of its potential bioagents spared a total of Rs. 16.00 crores. Besides in 1994, epidemics in Karnataka were likewise effectively constrained by the potential biocontrol agents.
- In India, polyphagous insect pests were successfully managed with the use of nuclear polyhedrosis infection (NPV) on cotton, pulses, vegetables, oilseeds, etc.
- Research on the management of phytopathogens has been carried out extensively in different parts of the country. G.B. Pant University of Agriculture and Technology, Pantnagar; ICAR-IARI, New Delhi; Tamil Nadu Agricultural University, Coimbatore; Banaras Hindu University, Varanasi; UAS, Bengaluru; AAU, Anand, Gujarat, etc. are the leading institutes where work on biocontrol of phytopathogens has been conducted and success is achieved (Singh et al. 2009) (Table 13.2). BHU developed a very economical technology for mass production of *Trichoderma* on cow dung for use by the resource-poor farmers which has been popularized in eastern districts of Uttar Pradesh. Similarly, in ICAR-NBAIM, Mau research is being carried out in the exploration of agriculturally important microorganisms (AIMs) for promoting plant growth and managing important diseases of field crops.
- A representative list of leading research groups working on the biocontrol of phytopathogens is given in Table 13.3 and marked in Fig. 13.1.

Table 13.2 Biocontrol agents (BCAs) used in India on different crops for the management of diseases of a variety of crops

Crop	Disease	Pathogen	Biological control agents (BCAs)
Cereals			
Barley	Foot and root rot	*Sclerotium rolfsii*, *Fusarium, Curvularia, Pythium, Aspergillus, Penicillium*	*Trichoderma viride*
Rice	Blast	*Pyricularia oryzae*	*T. pseudokoningii*
	Bunt	*Neovossia indica*	*Trichoderma* spp.
	Karnal smut	*Tilletia barclayana*	*T. harzianum, T. viride, T. virens T. deliquescens*
	Sheath blight	*Rhizoctonia solani*	*T. harzianum, T. viride, T. virens, P. fluorescens, P. putida T. harzianum, T. viride, T. virens*
	Brown spot	*Drechslera oryzae*	*Aspergillus niger* AN 27, *T. viride*
	Bacterial leaf blight	*Xanthomonas oryzae*	*Bacillus* spp.
	Sheath rot	*Sarocladium oryzae*	*P. fluorescens*
Wheat	Karnal bunt	*Neovossia indica*	*T. viride, T. harzianum, T. pseudokoningii, T. koningii*
	Loose smut	*Ustilago segetum T. koningii*	*T. viride, T. harzianum*
	Root rot	*S. rolfsii, F. oxysporum*	*T. harzianum*
	Spot blotch	*Drechslera sorokiniana*	*T. reesei, T. pseudokoningii T. viride*
	Take-all	*Gaeumannomyces graminis* var. *tritici*	*T. harzianum*
	Spot blotch	*Drechslera sorokiniana*	*Chaetomium globosum*
Maize	Charcoal rot, blight	*Macrophomina phaseolina, R. solani*	*Trichoderma* spp.
Sorghum	Charcoal rot	*M. phaseolina*	*A. niger* AN27
Pulses			
Pigeon pea	Wilt	*Fusarium udum*	*T. viride, T. harzianum, T. koningii, B. subtilis*
Chickpea	Seedborne disease Wilt	*F. oxysporum* f. sp. *ciceri*	*Xanthomonas campestris* pv. *vinae, T. viride, T. harzianum*

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
	Root rot	*Rhizoctonia/M. phaseolina*	*T. viride, T. harzianum*
	Collar rot	*Sclerotium rolfsii*	*T. viride, T. harzianum, P. fluorescens*
	Gray mold	*B. cinerea*	*Trichoderma* sp.
	Stem rot	*S. sclerotiorum*	*T. harzianum*
Black gram	Dry rot	*M. phaseolina*	*T. harzianum*
	Damping-off	*S. rolfsii*	*T. viride*
	Wilt	*F. udum*	*A. niger* AN27
Cowpea	Wilt	*F. oxysporum*	*T. viride*
	Charcoal rot and wilt	*M. phaseolina, F. oxysporum* f. sp. *tracheiphilium*	*T. harzianum, T. koningii, T. pseudokoningii*
Soybean	Dry root rot	*M. phaseolina*	*T. viride, T. harzianum*
Horse gram	Damping-off	*M. phaseolina*	*T. viride*
Lentil	Wilt complex	*R. solani, F. oxysporum*	*T. viride*
	Collar rot	*S. rolfsii*	
Month bean	Blight	*M. phaseolina*	*T. harzianum, T. viride*
Mung bean	Root rot	*M. phaseolina*	*T. harzianum, T. viride*
Ornamental crops			
Gladiolus	Yellow and corm rot	*F. oxysporum gladioli*	*T. viride, T. harzianum*
Jasmine	Root rot	*M. phaseolina*	*T. harzianum, T. viride*
Rose	Gray mold	*Botrytis cinerea*	*T. harzianum, T. viride*
China rose	Wilt	*F. oxysporum*	*A. niger* AN27
Carnation	Wilt	*F. oxysporum* f. sp. *dianthi*	*Trichoderma* sp., *A niger*
Oilseed crops			
Groundnut	Crown rot	*Aspergillus niger*	*T. T. harzianum, B. subtilis*
	Stem and pod rot	*Sclerotium rolfsii*	*T. harzianum, Rhizobium* sp.
	Late leaf spot	*Cercospora personata*	*Penicillium islandicum P. fluorescens T. harzianum, B. subtilis*
	Root and stem rot	*R. solani*	*T. virens, T. longibrachiatum*
	Rust	*Puccinia arachidis*	*Verticillium lecanii, T. harzianum*
Castor	Wilt complex seed rot	*S. rolfsii, F. solani, F. oxysporum*	*T. harzianum, T. virens*
	Wilt		*T. virens*

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
		Fusarium oxysporum f. sp. *ricini*	
	Gray mold	*Botrytis cinerea*	*T. T. viride, P. fluorescens*
Mustard	Damping-off	*Pythium aphanidermatum*	*T. harzianum*
Sunflower	Root rot	*M. phaseolina*	*T. viride* AN27
	Wilt	*F. oxysporum* f. sp. *carthami*	*A. niger* AN27
Sesamum	Blight	*Phytophthora* sp.	*T. harzianum, T. viride*
	Wilt	*F. oxysporum* f. sp. *sesami*	*A. niger 27*
	Root rot	*M. phaseolina*	*Trichoderma* sp., *Gliocladium* sp., *B. subtilis*
Sunflower	Blight	*Alternaria helianthi*	*T. virens*
	Root/collar rot	*S. rolfsii, R. solani, S. sclerotiorum*	*T. harzianum, T. hamatum*
Sunhemp	Wilt	*Fusarium oxysporum*	*A niger* AN27
Vegetable crops			
Bean	Seeding rot	*Pythium* sp., *S. sclerotiorum, B. cinerea, R. solani*	*T. koningii*
Bottle gourd	Wilt	*F. oxysporum*	*A. niger* AN27
	Root	*R. solani*	*A. niger* AN27
	Collar rot	*S. sclerotiorum*	*A. niger* AN27, *T. viride, T. harzianum, T. koningii*
Cabbage	Damping-off		*T. koningii, A niger*
	Alternaria blight	*Alternaria brassicicola*	*T. virens, T. longibrachiatum*
Cauliflower	Damping-off	*Rhizoctonia solani P. aphanidermatum*	*T. harzianum A. niger* AN27
	Stalk rot	*S. sclerotiorum*	*A. niger* AN27
Chili	Root rot	*S. rolfsii*	*T. harzianum*
	Fruit root and dieback	*Colletotrichum capsici*	*T. viride, T. harzianum, T. koningii, T. pseudokoningii T. pileatus*
Cucumber	Seedling disease	*Phytophthora sp. Pythium* sp., *Fusarium oxysporum* f. sp. *cucumerinum*	*A. niger* AN27
Eggplant	Wilt. Damping-off	*F. solani P. aphanidermatum*	*T. viride, T. harzianum T. koningii*
	Collar rot	*S. sclerotiorum*	*T, viride*
Fenugreek	Root rot	*R. solani*	*T. viride P. fluorescens*
French bean	Root rot	*R. solani*	

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
			T. viride, T. harzianum T. hamatum, T. viride
Okra	Wilt	*Pythium* spp.	*A niger* AN27
Pea	Seed and collar rot	*Pythium* sp. *R. solani*	*T. harzianum T. hamatum*
	White rot	*S. sclerotiorum*	*T. viride, T. harzianum, T. hamatum, T. viride*
Potato	Black scurf	*R. solani*	*T. viride, T. harzianum, T. koningii, Bacillus cereus*
	Bacterial wilt	*Ralstonia solanacearum*	*Bacillus cereus, B. subtilis*
	Charcoal rot	*M. phaseolina*	*A niger* AN27
	Late blight	*P. infestans*	*Trichoderma* spp.
Radish	Seedling rot, Damping-off	*Pythium* sp., *R. solani*	*T. harzianum, T. hamatum*
Tomato	Damping-off and wilt	*P. indicum F. oxysporum* f. sp. *lycopersici*	*T. viride, T. harzianum, P. fluorescens*
	Gray mold	*B. cinerea*	*T. harzianum*
	Root rot	*Meloidogyne incognita, M. javanica*	*T. harzianum*
	Cucumber mosaic Virus (CMV)		*P. fluorescens*
Plantation crops			
Areca nut	Fruit rot	*Phytophthora* spp.	*Trichoderma, P. fluorescens*
	Foot rot and root rot	*Ganoderma lucidum*	*T. harzianum*
Black pepper	Foot rot and root rot	*Phytophthora capsici*	*T. harzianum T. virens/VAM*
Cardamom	Capsule rot	*Phytophthora meadii, P. nicotianae*	*T. virens*
Coconut	Stem bleeding	*Thielaviopsis paradox*	*T. virens*
	Basal stem rot Leaf rot	*Ganoderma/G. applanatum Phytophthora* sp.	*T. harzianum Bacillus* spp.
	Bud rot	*P. palmivora*	*Trichoderma* spp.
Coffee	Collar rot	*R. solani*	*T. harzianum*
Ginger	Rhizome	*Pythium*	*T. harzianum*
Mulberry	Leaf spot	*Cercospora moricola*	*T. harzianum, T. viride*
	Stem canker and dieback	*Botryodiplodia* spp.	*T. harzianum, T. virens, T. pseudokoningii*

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
	Cutting rot	*F. solani*	*T. harzianum, T. virens, T. pseudokoningii*
	Collar rot	*Phoma sorghina*	*T. harzianum, T. pseudokoningii*
Rubber	Brown rot	*Phellinus noxius*	*T. harzianum. T. virens*
Fruit crops			
Apple	Bark splitting	*R. solani*	*A. niger* AN27
Apple	White root rot	*Dematophora necatrix*	*T. viride, T. harzianum T. virens*
	Blue mold	*Penicillium expansum*	*Candida* spp.
Banana	Panama disease	*Fusarium oxysporum* f.sp. *cubens*	*P. fluorescens/T. viride/ A. niger* AN27
Citrus (mandarin)	Root rot *P. colocasiae*	*Phytophthora nicotianae* pv. *parasitica*	*T. harzianum/T. viride/T. virens*
Guava	Wilt	*Gliocladium roseum/ Fusarium solani*	*Penicillium citrinum/ Aspergillus niger/ Trichoderma* spp., *Penicillium citrinum*
	Anthracnose	*Colletotrichum gloeosporioides, Pestalotia psidii*	*T. harzianum*
	Fruit rot	*Lasiodiplodia theobromae, C. gloeosporioides, Pestalotiopsis versicolor, Phomopsis psidii, Rhizoctonia arrhizus*	*T. harzianum*
Mango	Fruit rot	*Lasiodiplodia theobromae, Rhizopus*	*Trichoderma* sp. *T. harzianum*
	Bacterial canker	*Xanthomas campestris*	*B. coagulans*
Melon	Wilt	*F. oxysporum*	*T. viride*
Mulberry	Stem cancer	*Botryodiplodia theobromae*	*Trichoderma* spp.
	Root rot	*F. solani, F. oxysporum*	*Trichoderma harzianum*
	Cutting rot	*F. solani*	*Trichoderma pseudokoningii*
	Collar rot	*Phoma mororum/P. sorghina*	*Trichoderma pseudokoningii*
	Root rot	*M. incognita*	*Verticillium chlamydosporium*
Muskmelon	Wilt	*F. oxysporum F. solani, R. solani*	*T. harzianum*
Orange blue	Blue mold	*Penicillium italicum*	*T. harzianum*

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
Strawberry	Gray mold	*B. cinerea*	*T. harzianum*
Watermelon	wilt	*F. oxysporum* f. sp. *solani*	*T. viride* *A. niger* AN27
Plantation crops			
Cotton	Bacterial blight	*Xanthomas campestris* pv. *malvacearum* *Verticillium dahliae*	*T. viride T. harzianum* *A niger* AN27
	Root rot	*Rhizoctonia* sp./*M. phaseolina*	*T. viride/T. harzianum/T. virens*
	Wilt	*F. oxysporum* f. sp. *vasinfectum, S. rolfsii, R. solani*	*T. harzianum*
Sugarcane	Red rot	*Colletotrichum falcatum*	*P. fluorescens, P. putida* *T. harzianum*
	Root rot	*Pythium graminicola*	*T. viride*
	Sett rot	*Ceratocystis parados*	*Trichoderma* sp.
	Seedling rot	*Pythium* sp.	*T. viride/T. harzianum*
	Wilt	*F. moniliforme*	*T. viride, T. harzianum, T. longibrachiatum*
	Damping-off	*P. aphanidermatum*	*T. harzianum*
	Root/rot	*S. rolfsii*	*T. harzianum*
Condiments, spices, narcotics, stimulant crops			
Black pepper, foot rot	Foot rot	*Phytophthora*	*T. harzianum, T. viride, P fluorescens, Bacillus*
	Slow decline	*Radopholus similis* *Meloidogyne incognita* *P. capsici*	*T. harzianum, T. virens, Pochonia chlamydosporia Lilacinus, Pasteuria penetrans*
	Collar rot	*Phytophthora capsici*	*Trichoderma* spp.
	Anthracnose	*Colletotrichum gloeosporioides*	*P. fluorescens*
	Root knot	*M. incognita*	*Trichoderma* spp.
Cardamom	Capsule rot	*Phytophthora*	*T. harzianum, T. viride, T. hamatum, P. fluorescens*
	Damping-off	*F. moniliforme, P. aphanidermatum*	*T. harzianum*
	Clump rot	*Pythium vexans* *R. solani* *M. incognita*	*T. harzianum*

(continued)

Table 13.2 (continued)

Crop	Disease	Pathogen	Biological control agents (BCAs)
	Rhizome rot	*Pythium vexans, R. solani, F. oxysporum*	*T. harzianum, T. viride, Laetesaria arvalis, B. subtilis*
Coriander	Wilt	*F. oxysporum* f. sp. *corianderii*	*T. viride, T. harzianum Streptomyces*
Cumin	Wilt	*F. oxysporum* f. sp. *cumin*	*Trichoderma/Gliocladium*
Ginger	Rhizome rot	*F. oxysporum* f. sp. *zingiberi Pythium pleroticum P. aphanidermatum P. myriotylum*	*T. harzianum, G. virens, T. viride, P. fluorescens*
	Yellowing	*F. oxysporum* f. sp. *zingiberi*	*T. harzianum, T. virens*
	Bacterial wilt	*Ralstonia solanacearum*	*P. fluorescens (endophytic)*
	Dry root rot	*Pratylenchus coffeae, Fusarium complex, M. phaseolina*	*T. harzianum*
Turmeric	Rhizome rot	*P. graminicola Fusarium* sp. *P. aphanidermatum M. incognita, R. similis*	*T. harzianum, T. viride*
Poppy	Downy mildew	*Peronospora arborescens*	*Trichoderma* spp.
	Sclerotinia rot	*S. sclerotiorum*	*T. harzianum, T. viride*
Tobacco	Damping-off	*Pythium aphanidermatum*	*P. fluorescens CHAO*
Vanilla	Damping-off	*P. meadii*	*T. harzianum, P. fluorescens*
Other crops			
Betelvine	Foot and root rot	*Phytophthora parasitica* var. *piperina*	*T. viride*
	Collar rot	S. *rolfsii*	*T. harzianum*
Casuarina root rot	Root rot	*R. solani*	*Trichoderma* sp.
Chirpine	Pre- and post-damping-off	*F. solani*	*T. viride*
Menthol mint	Stolon decay	S. *sclerotiorum*	*T. harzianum, T. viride*
Month bean	Blight	*M. phaseolina*	*T. harzianum, T. viride*
Passion fruit	Collar rot	*R. solani*	*T. harzianum, Trichoderma*
Pearl millet	Ergot	*Claviceps fusiform*	*T. viride, T. harzianum*

Table 13.3 Representative research groups on biological control of plant pathogens and pests working in India

S. No.	Name of research group leaders	Work address in India
1.	A. N. Mukhopadhyay, U. S. Singh, J. Kumar, H. S. Chaube	GB Pant Univ of Agric & Tech, Pantnagar, India
2.	R. S. Vasudeva, Bineeta Sen, K. Annapurna, Rashmi Agarwal, Biswajeet Paul	IARI, New Delhi
3.	Prabhu Patil	CSIR-IMTECH, Chandigarh
4.	T. Prameela Devi	ITCC, IARI, New Delhi
5.	Surendra Singh	Central University of Haryana, Mahendragarh
6.	Anil Kumar Saxena, Alok Srivastava, D. P. Singh, D. K. Arora	ICAR-NBAIM, Mau
7.	R. S. Upadhyay, Bharat Rai, R. S. Dwivedi, H. B. Singh, B. K. Sarma	B.H.U., Varanasi, India
8.	Arup Mukherjee, Sudhmoy Mondal, M. K. Bag	NRRI, Cuttack
9.	Prasun K. Mukherjee	BARC, Mumbai
10.	Kishore Packnikar	Agarkar Research Institute, Pune
11.	Yogesh Shouche	NCCS, Pune
12.	D. Ponnuswamy, B. Thangavel, Pramila Devi	Indian Institute of Oilseed Research, Hyderabad
13.	P. Sreerama Kumar, A. N. Shylesha, Mahesh Yandigeri	ICAR-NBAIR, Bengaluru
14.	S. Sriram, M. S. Rao	ICAR-IIHR, Bengaluru
15.	S. Gnanamanickam, D. Lalithkumari	University of Madras, Chennai
16.	Vinayak Hegde, Murali Gopal, Alka Gupta	CPCRI, Kasaragod, Kerala
17.	R. Jayarajan	TNAU, Coimbatore
18.	R. N. Pandey	AAU, Gujrat
19.	C. Manoharachary	Osmania University, Hyderabad
20.	Apparao Podile	University of Hyderabad
21.	C. S. Nautiyal, P. S. Chauhan, Poonam C. Singh	CSIR- NBRI, Lucknow
22.	Bhusan L. Jalali	HAU, Hisar
23.	K. G. Mukherjee	University of Delhi
24.	S. P. Singh	NBAII, Bengaluru
25.	S. R. Niranjan	University of Mysore
26.	L. Daiho, Tiameren A. O.	Nagaland University
27.	A. Haseeb, M. R. Khan	AMU, Aligarh
28.	D. K. Maheshwari	Gurkul Kangri University, Haridwar
29.	Alok Kalra, R. Pandey	CSIR-CIMAP, Lucknow
30.	N. K. Arora	BBAU, Lucknow
31.	K. K. Pandey	ICAR-IIVR, Varanasi
32.	C. Sen	BCKV. Kalyani

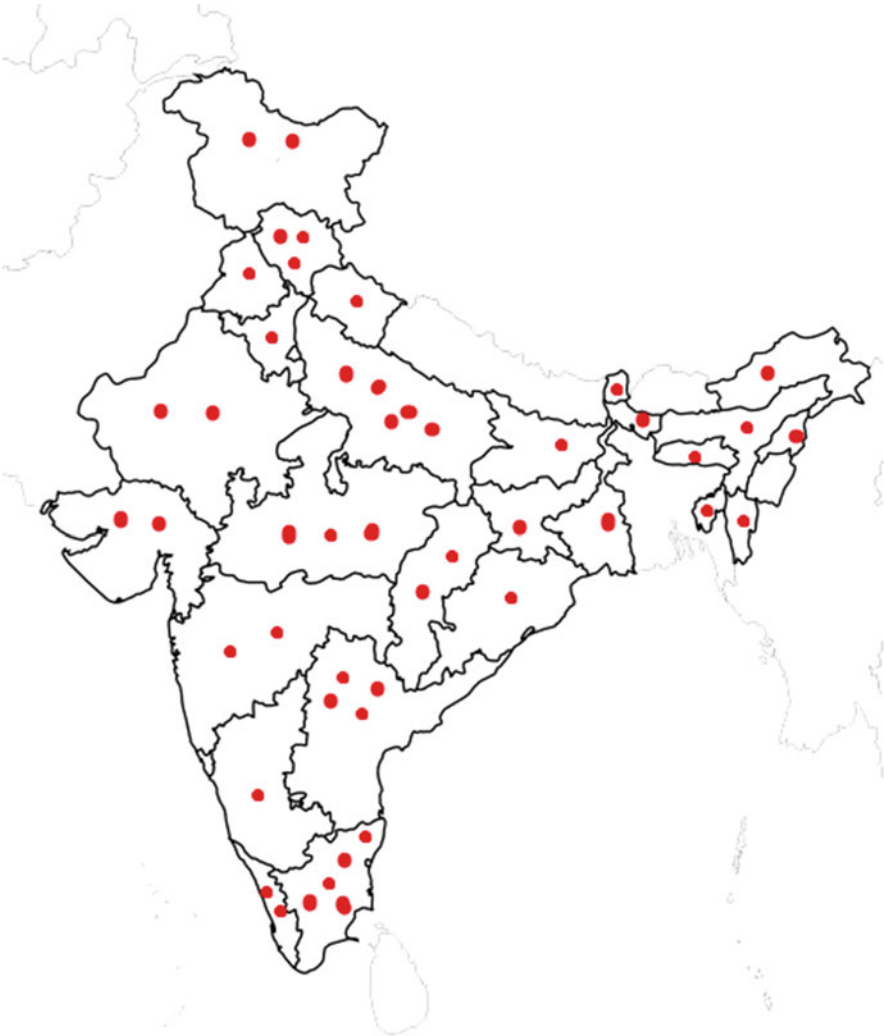

Fig. 13.1 The location of some of the institutes/universities in India, where research work on biological control of plant pathogens is going on. Red dots represent active research groups in different states of India

13.5 Current Status of Biocontrol Laboratories in India

In India, currently, 361 biocontrol laboratories are operating under different schemes. The aims and objectives of different functional biocontrol laboratories are as follows:

- Mass multiplication of bioagents in the laboratory and subsequent release in the farmer's fields against their target pests
- Conservation and augmentation of bioagents already existing at the farmer's field
- Proper monitoring and surveillance of insect pests, disease, weeds, and bioagents for various crops
- Training to the trainers and farmers in the identification, production, utilization, and evaluation of different biocontrol agents
- Standardization of protocols for mass production of pathogens, parasitoids, and predators

Thirty-five biocontrol laboratories are functional under Central Integrated Pest Management Centres (CIPMCs) while state biocontrol laboratories (SBCLs) count for 98. There are 38 state biocontrol laboratories (SBCLs) in states/UTs established under Grants-in-Aid by the Government of India during X^{th} and XI^{th} Plan. The number of laboratories under ICAR is 49, whereas 141 private laboratories are functional (www.ppqs.gov.in) (Table 13.4).

Table 13.4 Number of biocontrol laboratories in India

Sl. No.	Type of lab	Number of labs
1.	CIPMCs	35
2.	SBCL (grant-in-aid)	38
3.	ICAR	49
4.	SBCL	98
5.	Private	141
Total		**361**

13.6 Potential Applications and Future Prospects

The global food production must keep pace with the continuously growing population of the world, which envisages the increased use of chemical pesticides, consequently for quick and effective control of crop pests to cover large swaths of affected areas in a minimal time frame. However biological management of plant diseases has opened a new window of opportunity for microbial products to replace the chemical pesticide markets. However, the commercial application of the biological control agents is slow mainly due to the variable performance of the agent under the variable environmental condition in the field. To improve the commercialization of the biocontrol technology we must develop biopesticides with higher efficacy. In this direction, we have to accelerate more research on some underdeveloped aspects of biocontrol including the effect of environmental parameters on the activity of biocontrol agents, development of formulations in which bioagent can survive for a longer period, mass production of biocontrol agents, and use of the advanced technique for improvement of mechanisms and strategies of biocontrol.

Despite their vast potential, biopesticides are yet to be employed as major catalysts of organic pest management programs. Even the government-based schemes at national and state levels have not proven to be very effective for the promotion of biopesticides. Most of the research being carried out in biocontrol has provided effective results at the laboratory level. Development of biopesticides having broad-spectrum activity, long shelf life, and a high degree of tolerance towards environmental factors is a cumbersome task (de la Cruz et al. 2019). In India, research on biocontrol still contains a few technical issues, which results in the development of low-quality products. Field testing, registration, and licensing consume a lot of time before a new product is commercially available. Hence, the current registration system needs to be updated in such a way that it should favor the applicants. This will surely lead to sustainability and fulfillment of the targets of sustainable agricultural development (Arora et al. 2018).

In India, most of the firms involved in the production of biopesticides contain only single microbial strain which gives them limited applications and narrow spectrum. Biopesticide involving microbial consortium (MC) comprises the symbiotic association of two microbial groups and even more (Clark et al. 2009), for improved yield development. The prime objective of any consortia is fulfilling the gap created by one microorganism by the other and it is assumed that if one fails to bestow its features, then the next one will demonstrate its action to its full potential. The strategy is quite effective and such developed products are economical and have proven more trustworthy among the end users. Shorter shelf life of active ingredients in biopesticides has remained a major concern for years that affected their commercial production. So, the technological breakthroughs in this segment are most awaited which need to be solved by a microbial consortium. Few reports suggest that coating of the active ingredient with suitable biopolymer boosted the shelf life of formulation (Aziz Qureshi et al. 2015; Sharma et al. 2019).

Arora and Mishra (2016) suggested that adding some additives, viz. secondary metabolites and precursor molecules, along with suitable carriers resulted in the

extension of shelf life and activity of biocontrol products. However, unfortunately, the commercialization of these techniques has not been achieved so far (Mishra and Arora 2018). While talking in terms of patent landscaping, India is lagging behind other countries where biologicals are being applied on a large scale (Mittal and Singh 2006). Moreover, in the last 10 years, there have been a lesser number of Indian patents employing bioagents. However in contrast, countries such as China, the USA, and other European countries have made much progress in patenting biocontrol products (Saenz-de-Cabezon et al. 2010). These countries have also initiated producing nanotechnology-based biopesticide formulations, whereas India is far behind where research is only at its early stage and no biosafety guidelines are yet in force (Chhipa and Joshi 2016; Hashem et al. 2018).

India is trying to have a clear perspective for using genetically engineered microorganisms (GEMs) in the form of biocontrol products. The plant-incorporated protectants employ the use of GEMs. A series of guidelines related to the safety assessment of genetically modified organisms (GMOs), i.e., research, food safety, and environmental risk assessment, are now jointly included under Rules, 1989, notified under the Environment (Protection) Act, 1986 (Ahuja 2018). Limited efforts have been made on the application of GMO in the field of biocontrol, and only Bt cotton is a commercial success (Shukla et al. 2018). Hence, it can be stated that in the light of the above facts, the status of biopesticides in India still requires a lot of sustenance and improvement to completely eliminate out chemical pesticides.

13.7 Conclusion

India, being a mega diversity country, has played a pivotal role in popularizing classical biological control on a global scale. However, it can be ascertained that the classical biological control of weeds in the world had its beginning in India. Overall, the classical biocontrol measures offer high effectiveness and eco-friendly solutions to the threat of pests and invasive alien weeds. Currently, 361 biocontrol laboratories are operating in our country and their main objective is the mass production of bioagents for management of pests and diseases. These bioagents serve as an essential and vital part of integrated pest and disease management programs. A solid public and provincial approach is needed to expand the movement of compelling usage of biocontrol programs in our nation. It has usually been seen that the natural enemies of many major pests are present in one area/locality; hence suitable approaches should be made to transfer from one region to another. In addition to pests, biocontrol of phytopathogens is equally important. Biocontrol agents such as *Trichoderma, Pseudomonas, Bacillus,* and *Burkholderia* have emerged as effective natural enemies in the management of phytopathogens. In addition to disease management, these bioagents also play a crucial role in growth promotion attributes. Development of agriculture as a whole has faced a few new difficulties, making further development conceivable just if these difficulties are met suitably and opportune. Rise in crop production from the advanced accessible cultivating strategies for arriving at a level in creating nations including India is a cumbersome task. The issues related to the environment which arise due to disproportionate use of

chemical pesticides need to be resolved for creating a pollution-free healthy and safe ecosystem. So biological control, if exercised to its full potential, can play as an alternate system in achieving the goal of agriculture.

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Fungal Aerobiology and Allergies in India: An Overview

14

A. B. Singh and Chandni Mathur

Abstract

Today more than 30% of the world population is known to suffer from one or the other allergic ailments such as bronchial asthma, allergic rhinitis, and atopic dermatitis. Major causative agents are pollen grains, fungal spores, dust mites, insect debris, etc. Detailed information on the daily seasonal and annual variation of different causative agents in the atmosphere is a prerequisite for effective diagnosis and therapeutic management of allergic ailments. Aerobiological investigations have been carried out in different parts of the country to ascertain aerial concentration and seasonality of fungal allergens. An attempt has been done to review the important fungal allergens prevalent in different parts of the country during the last few decades. The studies carried out under the All India Coordinated Project on "Aeroallergens and Human Health" have revealed the quantitative and qualitative prevalence of aerosols in different parts of the country. The prevalent fungal spores in both outdoor and indoor air are Aspergilli, Penicilli, *Cladosporium,* ascospores*, Alternaria, Drechslera, Epicoccum, Nigrospora, Candida albicans,* and some others. Other clinically important fungal allergens are from different species of *Aspergillus, Ganoderma, Mucor mucedo, Fusarium solani, Curvularia, Nigrospora, Scopulariopsis brevicaulis, Alternaria alternata,* and others.

Keywords

Aerobiology · Airborne fungi · Indoor fungi · Occupational fungi · Allergy · Allergens

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14.1 Introduction

Rapid industrialization and urbanization though have resulted in booming of the economy of the country, it has also contributed significantly to enhancing the problems of patients suffering from respiratory disorders as the quality of air deteriorated due to addition of large number of pollutants. The incidence of respiratory allergy is increasing all over the world. This is evident by the epidemiological data available from different parts of the world. The prevalence of respiratory allergy has been reported to be 15–30% across the globe (O'Neil et al. 1987; Pekkanen et al. 1997; Chhabra et al. 1998; Anonymous 2000; Anthracopoulos et al. 2001; Woolcock et al. 2001). An in-depth analysis of aerobiological perspective of indoor and outdoor fungal allergens has been done by Singh and his students (Singh and Chandni 2012; Singh 2017).

Air carries a large number of bioparticles (biopollutants) and chemical pollutants and these pose a burden on the respiratory tract of humans. The bioparticles include pollen grains, fungal spores, insect debris, plant parts, animal dander, mites, etc. These materials of biological origin are known to be causative agents of respiratory disorders like asthma, allergic rhinitis, and atopic dermatitis.

Of the various agents, fungal biopollutants are the major source of morbidity among sensitive individuals. Detailed information on their daily, seasonal, and annual variation shows that the air carries a large number of bioparticles (biopollutants) and chemical pollutants, and these pose a burden on the respiratory tract of humans. The bioparticles include pollen grains, fungal spores, insect debris, plant parts, animal dander, mites, etc. These materials of biological origin are known to be causative agents of respiratory disorders like asthma, allergic rhinitis, and atopic dermatitis.

India is blessed with multilingual, multicultural, and multireligious populations of more than 1.35 billion (1/5th of the world population). It comprises one of the richest flora on the earth. India is one of the countries where aerobiological studies were initiated as early as the nineteenth century. Studies on various aspects of aerobiology have progressed rapidly especially during the last 30 years. An attempt has been made in this chapter to review important airborne fungal allergens prevalent in different parts of the country.

14.2 Earlier Aerobiological Studies

In India, Cunningham (1873) from Calcutta was the pioneer to establish relationship between the airborne organisms and the so-called zymotic diseases. There was a conspiracy of silence on aerobiological studies for about half a century when two important centers started aerobiological investigations at Jaipur and Delhi (Kasliwal and Solomon 1958; Kasliwal et al. 1959; Shivpuri et al. 1960; Dua and Shivpuri 1962; Shivpuri 1964). Aerobiological studies in Kolkata were initiated again at Bose Institute by Chanda and his students who also prepared fungal calendars for Calcutta, Falta, and Kalyani (Chanda 1973; Mandal and Chanda 1979; Chanda

and Mandal 1980; Mandal and Chanda 1980). From Assam monthly fungal concentration and their seasonality have been reported by Sharma et al. (2010, 2014). The aerial fungal diversity and its clinical significance in allergic diseases in India had been critically reviewed by Singh (2012).

Fungal spores were first reported to be an important cause of hypersensitivity in 1726 (Floyer 1726). Blackley (1873) suggested that *Chaetomium* and *Penicillium* spp. are associated with "bronchial catarrh." Nearly half a century later in 1924, van Leumen reported *Aspergillus, Mucor,* and *Penicillium* to be responsible for allergic reactions. Subsequently many scientists throughout the world carried out aerial surveys using different sampling devices to identify important fungal types, and studied fungal sensitivity in hypersensitive individuals (Aukrust 1980; Shivpuri 1980; Agarwal et al. 1982; O'Neil et al. 1987; Singh et al. 1998; Gupta et al. 1993; Anonymous 2000; Singh 2012, 2017).

In India, aeromycological studies were initiated in 1959 with the work of T. Sreeramulu at Vishakhapatnam. The studies spread further in other parts and many centers came into existence. The center at Aurangabad was initiated by S.T. Tilak in 1966 and the Mysore center was started by A. Ramalingam in 1965. Aerobiological studies at Madras (now Chennai) were initiated by B.P.R. Vittal in 1976. Shivpuri and his students initiated work on fungal allergy in Delhi and the work is being carried out extensively by Singh and his students (Shivpuri 1980; Singh et al. 1987a, b; Singh et al. 1998; Singh et al. 1990; Gupta et al. 2000; Sharma et al. 2011a, b).

An All India Coordinated Project on aerobiology sponsored by CSIR, New Delhi, was coordinated by Nair and Joshi (1980–1983) and many new centers spread over 20 states carried out qualitative analysis of airborne fungal spores. The work carried out in different centers was compiled in the form of a book entitled "Airborne pollen, spores and other plant material of India - A survey" (Nair et al. 1983). Two decades later, an All India Coordinated Project on "Aeroallergens and Human Health" was sponsored by the Ministry of Environment and Forests, Govt. of India, which was undertaken and successfully completed by AB Singh and his collaborators (Anonymous 2000). This provided up-to-date information on seasonal and annual concentration of airborne spores of indoor and outdoor air from different ecogeographical regions of India.

14.2.1 Aerial Fungal Diversity

Fungi are ubiquitous in nature and cosmopolitan in distribution. There are more than 80,000 species of fungi and these have evolved by elaborate mechanisms for their dispersal. The spores produced form a normal component of outdoor air and also of indoor environment such as storehouses, hospitals, libraries, and residential buildings. Due to their small size, spores remain suspended in the atmosphere for a long time. When inhaled by susceptible individuals, they cause respiratory disorders.

14.2.1.1 Monitoring Airborne Fungi

For effective and efficient diagnosis and treatment of respiratory allergies caused by aeroallergens, information on diurnal, seasonal, and annual variations, and the type and concentration of airborne fungal spores, is a prerequisite. Aerobiological sampling is, therefore, carried out to achieve this aim through various sampling devices currently used for airborne fungal allergens.

The recognition of aero-fungal allergens is divided into two phases: (1) collection of material and (2) sample analysis (Singh 2012).

Different methods employed to achieve the objectives generally exploit the following basic regimes of collection:

1. Fallout on a fixed surface through gravitational force
2. Impaction on rapidly moving surface
3. Impaction through suction of air
4. Filtration technique
5. Immunochemical assays

And for analysis of sampled data, three procedures are followed:

1. Microscopic enumeration of individual fungal spores
2. Tally of colonies produced in culture or semisolid media
3. Immunochemical assay for bulk reservoirs

14.3 Sampling Devices

The various sampling devices and their principle of working have been enumerated as follows:

14.3.1 Gravimetric Samplers

This is based on the principle that bioparticulates settle down on a surface due to gravitational force. The Durham gravity sampling device consists of two horizontal discs with a diameter of 22.1 cm and 8.1 cm. The upper disc protects the slide from rain and sun. Nevertheless this method presents certain disadvantages and thus has been discontinued in many parts of the world. It does not give diurnal variation, is dependent on wind velocity, and favors large pollen grains. In spite of these drawbacks, it is economic, simple, independent of power, and still used in the Indian subcontinent by several workers. The slides are exposed daily at a fixed hour, coated with adhesive glycerine jelly. After exposure, the slides are mounted in a drop of molten glycerine jelly, for various allergen types trapped. The results are expressed as number/unit area. However, this method being qualitative is no more popular but still in some developing countries is being used.

14.3.2 Impaction Samplers

In impaction sampler particles are sampled passively from air on adhesive-coated stationary or rotating rods.

14.3.2.1 Rotorod Sampler

Rotorod sampler developed by Perkins (1957) was the first such device to receive wide attention. Adhesive-coated clear Lucite rods of 1–3 mm width are used as a collection surface. Collection efficiencies vary with the speed of travel and inversely with the width of the sampling area. This is a lightweight portable and DC-operated sampler. It has an arm which holds two cubical rods coated with silicone grease. The exposure time can be increased or decreased as required. The rods are mounted on a grooved slide in cotton blue stain and scanned under microscope. Fungal spore particles recovered are counted microscopically to generic level based on the morphology of spores by transmitted light and results are easily expressed as "particles recovered/unit volume" of air sampled.

14.3.3 Suction Samplers

The method requires suction of certain volume of air according to a known velocity and for a chosen duration on a trapping. There are several instruments currently in use. The Hirst spore trap (Hirst 1952) is the most commonly used sampler. Trapping of fungal spore particles is done on slides coated with glycerine jelly and slides are replaced each day with fresh slides.

14.3.3.1 Burkard Seven-Day Sampler

The above device was modified to Burkard trap in which slides were replaced with a drum which can rotate and run continuously for 7 days with a definite speed of 10 L/mt (Fig. 14.1). Burkard continuous 7-day sampler uses a glycerine-coated tape mounted on a rotating drum. The drum is connected to a timer and rotated at constant speed. The tape is changed every 7 days. Exposed tape is cut in 7 strips corresponding to 7 days and mounted on a slide. This is a very good sampler to study diurnal, daily, or seasonal trends for airborne fungal allergens.

14.3.3.2 Burkard Personal Slide Sampler

Burkard personal slide sampler is compact, battery operated, 10 cm in height, and 8 cm in diameter (Fig. 14.2). It has a rectangular orifice at the top and a slit on the side to insert the microslide coated with glycerine jelly. The sampler takes in 10 Lit/min air particles that got impacted on the slide in the form of a streak. It is mounted in glycerine and scanned. The exposed slides/trap are scanned for different/spore types and counts are expressed as number/m^3.

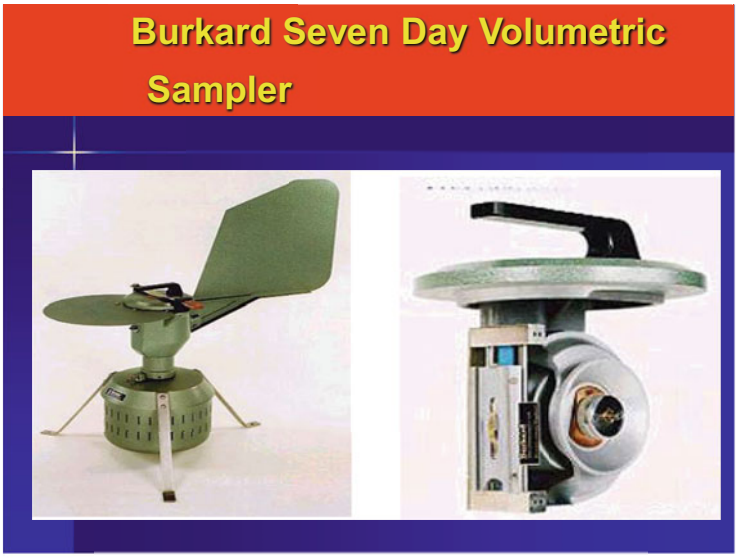

Fig. 14.1 Burkard 7-day volumetric sampler for spore counts with suction rate of 10 L/mint. Its disc moves with uniform speed for seven continuous days and each day's data mat represents 24-h counts

14.3.3.3 Burkard Petri Plate Sampler

Burkard petri plate sampler is similar to the above sampler except that it has a stage to hold a petri plate containing nutrient agar media instead of slides. These two samplers are appropriate for spot sampling in both indoor and outdoor air (Fig. 14.2).

14.3.3.4 Andersen Sampler

The best device for estimating culturable spores in indoor and outdoor environments is the Andersen six-stage/two-stage sampler (Fig. 14.3). It utilizes petri plates containing nutrient media kept under sieves of different pore sizes, each having 400 pores. Circular orifice takes up air at the rate of 28.3 L/min and passes it through the sieves kept in the order of decreasing pore size. Finally air passes out after impacting on the last petri plate. Exposed petri plates are incubated at 28–30 °C to allow the colonies to grow and later on number and types of colonies are counted.

14.3.4 Filtration Devices

Filters of definite pore size offer volumetric potential especially appropriate for smaller aerosol classes and where ambient velocities are low. Bioparticulates are sucked through filter and impinged on the media in a holder. Both media and holder are relatively inexpensive. But these are not widely used, as in morning air they may not faithfully align with the prevailing airstream.

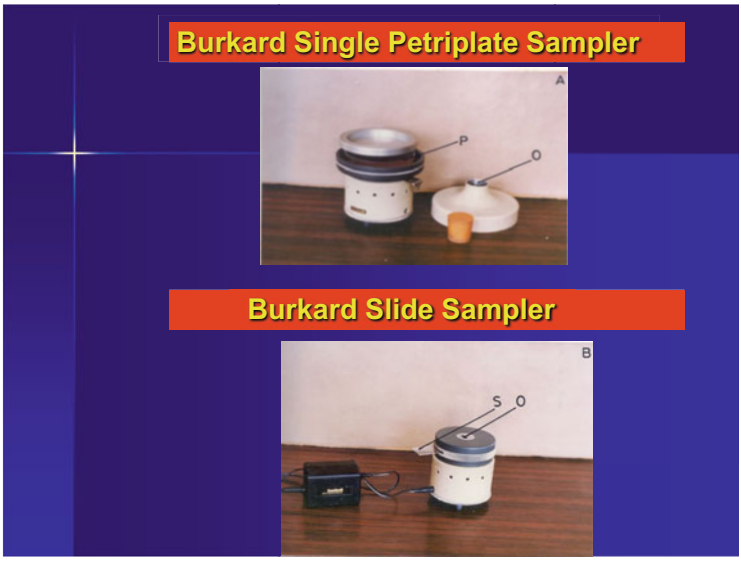

Fig. 14.2 Burkard portable spot sampler for culturable petri plate exposure containing suitable media for fungi and portable sampler for spore counts. Both suck air at 10 L/mt

Fig. 14.3 Andersen six-stage sampler with six petri plate containing sterile media. The larger to smaller fungal spores deposited on the first to last sixth stage representing human trachea as large particles are deposited on upper respiratory tract while smaller spores of less than 3μm are deposited on the last (sixth) stage

The cultured samples may be examined microscopically after clearing the filters with immersion oil and other compatible fluids. Specific stains may be applied first. Alternatively filters exposed with aseptic precautions may be collected on liquid culture media for colony growth and colony-forming units can be identified.

14.3.5 Immunochemical Assays

Recently described immunochemical assays for airborne allergens relied on large (e.g., 20 × 25 cm) fibre glass filters exposed for a 24-h period in high-volume (hi vol) sampler. They are rated for continuous operation and adapted to receive filter support. Hi vol devices are developed to study total suspended particles and devices are operated traditionally with filter surface directed upwards.

14.4 Analysis of Exposed Samples

Regardless of their method of collection, samples of mixed biologic aerosols are analyzed by one of the techniques.

14.4.1 Direct Microscopy

The microscopic identification of distinctive particles is an approach validated by years of practical applications of both gravimetric and volumetric samplers (Fig. 14.4). A variety of particles, including certain basidiospores, ascospores, and spores of rust, smuts, downy mildew, and primitive green plants, are recognizable, which fail to grow on most laboratory media. Therefore, it is the most dependable way to identify most of the pollen and fungal spores.

14.4.2 Culture Analysis

This includes the tally of colonies produced in culture or semisolid media. This technique of analysis is employed to identify the culturable fungal spores on the basis of their colony characteristics which otherwise cannot be identified under the microscope.

The culture plates, after exposure, are incubated at appropriate temperature and impacted spores are allowed to grow for a couple of days till colonies start forming. Each colony represents one spore and is considered a colony-forming unit.

Fig. 14.4 Microscopic slide exposed in portable slide sampler and examined under microscope for spore and other bioparticle identification

14.4.3 Immunochemical Assay

Immunochemical analysis following descending elution offers an analytic approach to dust without potential or defined form (e.g., dander, seed pomace, arthropod effluvia). If micronic aerosols carry pollen allergens, these fractions are also accessible to immunoassay in bulk samples obtained by filtration. This procedure is based on immunological inhibition assay such as ELISA and immunoblot inhibition assay.

14.5 Aerobiological Surveys in India

Aeromycological studies in India had progressed along three different lines. These include (1) study of airborne fungal spores in the atmosphere of different places, (2) study of fungal spores present in indoor environments, and (3) study of aeromycoflora over crop fields.

14.6 Outdoor Aerial Fungal Diversity

Studies on aerial fungal diversity have been carried out in different cities and towns of India. Various techniques like gravity settling method (settle plates, gravity slides), impaction techniques (Rotorod sampler and aeroscope), and volumetric devices (Burkard trap, Andersen samplers) have been commonly used.

Cladosporium spp. have been reported as the most predominant fungi by most of the investigators, and basidiospores and ascospores are the second dominant group.

From Northern India, Aspergilli-Penicilli, *Cladosporium, Helminthosporium, Epicoccum,* and *Drechslera* are reported to be important fungi in ambient air (Table 14.1). Singh et al. (1987a, b) carried out aerial surveys at Dehradun for 2 consecutive years. *Cladosporium, Alternaria,* smut spores, *Curvularia,* Ascospores, *Nigrospora,* Asp-Penicilli, and *Epicoccum* were the dominant forms reported. July to October was the period of high spore catch. Gupta et al. (1993) reported 98 fungal types from the atmosphere of Delhi as a result of 2 years of aerobiological survey at five different locations in Delhi metropolis. *Cladosporium* contributed 25–40% to the total airborne fungal load followed by *Ustilago* (24%), *A. flavus* (10–13%), *Alternaria* (11%), and *A. niger* (8%).

Aeromycological studies revealed important fungi prevalent in the atmosphere of Jabalpur (Verma and George 1997). From Himachal Pradesh (Solan) 17 fungal types and from Lucknow 40 fungal types were recorded. The dominant types were Aspergilli-Penicilli, *Cladosporium*, *Helminthosporium*, *Epicoccum*, and *Drechslera* (Anonymous 2000).

Aerobiological surveys carried out in Eastern India revealed Aspergilli/Penicilli and *Cladosporium.* Ascospores, rust and smut spores, *Nigrospora*, *Periconia*, *Ganoderma*, and *Rhizopus* are major fungal types (Anonymous 2000). Sinha et al. (1998) carried out aerial survey for aero-fungi at Jamshedpur and reported 23 fungal genera with 40 species. Members of Deuteromycetes (14 genera) were dominant followed by Phycomycetes (5) and Ascomycetes (4). The important fungal types present in the atmosphere of Imphal (Manipur) had been identified by Singh and Singh (1998). Aeromycoflora at the foothills of Eastern Himalayas has been studied by Majumdar and Battacharya (2000). A total of 18 fungal spore types were identified and the predominant types were *Alternaria,* Aspergilli, *Cercospora, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium,* etc. *A. flavus, A. fumigatus, A. niger, P. citrinum,* and *M. hiemalis* were the dominant fungi recorded from the atmosphere of greater Silchar area in Assam (Sharma et al. 2004).

From Western India, Tilak (1980) reported Deuteromycetes to contribute 70% to the total fungal aerospora in the atmosphere of Aurangabad. The other common types recorded are *Alternaria, Curvularia, Nigrospora,* Aspergilli-Penicilli, *Drechslera, Periconia, Pithomyces, Stachybotrys, Memnoniella, Torula,* etc. Tilak (1991) reported spore types belonging to 37 ascomycete genera to be common in air. He classified them into four types based on their diurnal periodicity. He also suggested that there exists a close relationship between rainfall and release of ascospores. Recent survey reported 18 fungal types and 22 fungal types from the atmosphere of Aurangabad and Pune, respectively. *Cladosporium,* Aspergilli-Penicilli, *Curvularia, Rhizopus,* and *Helminthosporium* were the common fungal types encountered (Anonymous 2000).

From Southern India, studies on seasonal periodicity of fungal spores in Bengaluru, the Garden City of India, were conducted. Maximum concentration of dry spores was reported in Northeast Monsoon whereas ascospores and basidiospores were dominant during Southwest Monsoon (Agashe and Sudha

Table 14.1 Important airborne fungi (% concentration) recorded from different places of India

Name of allergen	Calcutta	Chandigarh	Delhi	Trivandrum
Alternaria alternata	–	3.5	1.1	
A. candidus	4.9	–	5.4	
A. clavatus	–	–	4.48	
A. flavus	5.46	5.22	1.75	
A. fumigatus	4.9	2.61	–	
Aspergillus japonicus	3.12	4.76	3.51	1.6
A. nidulans	9.8	3.22	–	–
A. ochraceous	4.0	1.2	2.2	–
A. oryzae	4.9	–	5.47	–
A. sydowii	6.08	3.57	–	–
A. terreus	7.69	5.95	3.48	–
A. versicolor	10.99	–	2.2	–
Cladosporium cladosporioides	5.1	4.76	–	1.6
Cladosporium herbarum	3.65	–	4.48	–
Chaetomium	–		2.0	–
Curvularia pallescens	8.0	1.74	–	–
Epicoccum nigrum	10.0	3.57	–	–
Fomes pectinatis	7.14	4.76	–	–
Fusarium roseum	6.86	1.74	1.75	–
Ganoderma lucidum	6.86	2.6	6.2	1.6
Neurospora implicata	10.5	3.22	3.51	–
Neurospora sitophila	3.1	2.6	3.51	–
Paecilomyces variotii	9.1	3.22	4.17	–
Sphacelotheca cruenta	14.3	3.22	6.94	–
Sporotrichum pruinosum	6.67	6.45	5.55	–
Rhizopus nigricans	5.88	1.74	–	–
Uromyces aloe	1.23	6.45	5.56	–
Ustilago cynodontis	4.87	3.2	–	–
Ustilago scitaminea	2.7	9.68	2.8	–
Ustilago tritici	1.98	–	2.98	–

1990). *Nigrospora* has been reported to be the dominant fungus from Madras renamed as Chennai now (Vittal and Krishnamoorthi 1998). Of the 34 fungal types identified from Visakhapatnam, Aspergilli-Penicilli, *Cladosporium*, *Curvularia*, Basidiospores, and Uredospores were the dominant types from Chennai. Of the 50 fungal types identified, *Periconia, Curvularia, and Ganoderma* were the dominant types. *Tetraploa* was reported to be dominant along with other fungal types from Trivandrum (Anonymous 2000). Adhikari et al. (2004a, b, c) carried out the study of airborne fungal spores in rural agricultural areas of India for 2 consecutive years. The concentration of viable fungi varied from 72 to 1796 colony-forming units per cubic meter of air in the first year and 155–1256 (CFU/m^3) in the second sampling year.

14.7 Prevalence of Indoor Fungi

Human beings are exposed to both outdoor and indoor environment. Environment of the workplace plays a crucial role in hypersensitive individuals with the symptoms increasing during working hours and reducing afterwards. However, in some cases, symptoms prevail throughout the day. Children employed in various industries are also exposed to occupational allergens.

Studies on mycoflora of indoor environment are relatively few in India when compared with outdoors. The occupational areas surveyed under the All India Coordinated Project on Aeroallergens coordinated by the author (A.B. Singh) included hospitals, poultry farms, libraries, bakeries, farmhouses, domestic houses, grain stocks, leather storehouses, etc. *Aspergillus, Penicillium, Cladosporium,* and some moniliaceous fungi are predominant in the air of most of the indoor environment surveyed. In Assam, an attempt was made to evaluate the qualitative and quantitative fungal burden (load) in five different working environments of South Assam and the possible risks of indoor fungi to employees and stored products. Fungal concentrations in different working environments were studied using a Burkard personal petri plate sampler (Sharma et al. 2011a, b). The fungal floras observed in various working and occupational environments in India are briefly described here.

14.8 Occupational Indoor Fungi

14.8.1 Bakeries

Different sections of a bakery in Delhi were surveyed and 74 fungal types belonging to 33 genera were isolated (Singh et al. 1990). Aspergilli/Penicilli (69.2%) were the dominant spore type in bakery (Table 14.2) with a peak in October followed by smut spores (28.5%) with a peak in February–April. *Aspergillus flavus* was the dominant fungal type in both packaging and storage section (Singh et al. 1990, 1995). Allergenically significant fungal aerosols have been shown to be prevalent in a rural bakery of West Bengal (Adhikari et al. 2000).

14.8.2 Granaries

Aspergillus spp. are the predominant fungi followed by *Cladosporium*; *A. flavus* had two distinct seasons from September to November and May to June (Table 14.2). *Cladosporium* was prevalent during winter months. Other important contributors were *Rhizopus, Curvularia*, *A. versicolor, A. fumigatus, Epicoccum nigrum,* and *Alternaria* (Pandit et al. 1995). Fungal diversity present in the dust of grain storage godowns had been studied by Pugalmaran and Vittal (1999).

14.8.3 Poultries

Different sections of poultry were surveyed and 130 fungal types were identified (Singh and Singh 1996). In hatchery section, Aspergilli-Penicilli spores were dominant. In poultry shed area, *Cladosporium, Candida albicans, A. flavus, A. niger, Scopulariopsis brevicaulis, P. nigricans,* and *Alternaria* sp. had been reported to be predominant fungi in Delhi area (Table 14.2). An analysis of the aeromycospora of a poultry farm in Kerala has been done and important fungal types have been identified by Jothish and Nayar (2003).

14.8.4 Sugar Industries

The high concentration of *Cladosporium* coincided with the crushing season in the sugar industry. *Cladosporium* is reported to be present in high concentration (60%) from November to March in both bagasse storage and cane-cutting sections of the industry (Table 14.2). *A. fumigatus, Epicoccum, Saccharomyces,* and smut spores are other major contributors in bakeries (Pandit and Singh 1992).

Table 14.2 Airborne fungi in different occupational locations

DOMINANT FUNGAL FORMS IN DIFFERENT OCCUPATIONAL SITES		
SUGAR INDUSTRY	VEGETABLE MARKET	GRANARY
CLADOSPORIUM	CLADOSPORIUM	CLADOSPORIUM
A. FLAVUS	P. FREQUENTANS	A.NIGER
A.NIGER	A.FUMIGATUS	A.FLAVUS
PENICILLIUM	EPICOCCUM	PENICILLIUM
A.VERSICOLOR	A.NIGER	RHIZOPUS
RHIZOPUS	A.SYDOWI	A.JAPONICUS
ALTERNARIA	PENICILLIUM	A.SYDOWI
A.SYDOWI	A.FLAVUS	NEUROSPORA
A.OCHARACEOUS	FUSARIUM	A.OCHARACEOUS
A.JSPONICUS	PAECILOMYCES	FUSARIUM

14.8.5 Libraries

High concentration of *Cladosporium, Penicillium, Paecilomyces,* and *Aspergillus* sp. had been reported to be dominant in library environment. After agitation of books, concentration of *A. niger*, *Penicillium* sp., and *Cladosporium* was found to increase severalfold (Singh et al. 1990). Nadimuthu and Vittal (1995) reported airborne fungi to be present in low concentration in air-conditioned libraries when compared with conventionally ventilated libraries. Mycoflora of library dust in Jalgaon (Maharashtra) was studied with reference to deterioration of books. Deuteromycotina members were found to be very common and showed luxuriant growth in the dust from stored books (Rane and Gandhe 2005).

14.8.6 Cattle Sheds

A study conducted in two different sections of a large rural indoor cattle shed revealed 35 fungal types prevalent in air. *A. niger*, *A. flavus*, and *Cladosporium cladosporioides* were found to be the dominant fungal types (Adhikari et al. 2004a, b, c).

14.8.7 Residential Houses

Cladosporium and Aspergilli were found to be predominant in the residential houses of allergy patients in Bengaluru (Agashe 1994). A total of 17 fungal antigens were tested on patients with respiratory allergy in Agra (Shalini and Chauhan 1999). *Rhizopus nigricans* showed maximum (20–95%) sensitivity, followed by *Fusarium solani* (14.80%) (Chauhan et al. 2004). Due to the aerial fungal concentration in residential houses of Delhi, children were affected with asthma and allergy (Fig. 14.5), as recorded by Sharma et al. (2011a, b) in comparison with control air outside the house.

Important fungi isolated and quantified were the species of *Aspergillus*, *Cladosporium,* and *Penicillium*. Most of the fungi showed significantly (>0.05) higher concentration than immediate outdoor air. An association between indoor fungi in Delhi homes and sensitization in allergic children was established (Sharma et al. 2012).

14.8.8 Fungi of Allergenic Significance

Fungal allergy is a worldwide problem. Many fungal species are known to cause severe respiratory and cutaneous allergic diseases. Several investigators from different parts of India have identified potential fungal allergens of their area. Shivpuri and his colleagues initiated clinical studies in Delhi in 1970s and found 19 fungal

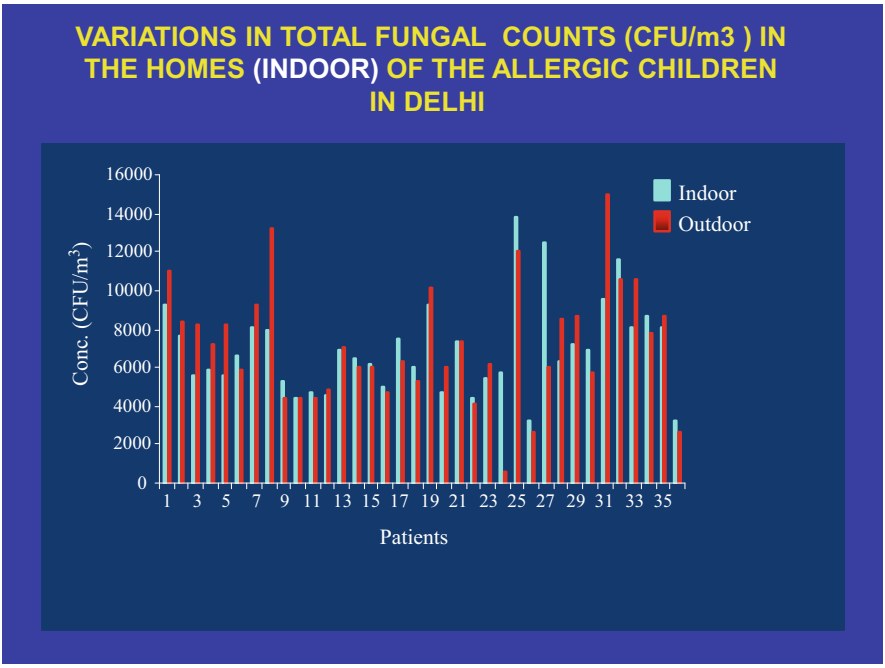

Fig. 14.5 Histogram showing fungal colony counts (CFU/CM) from houses of allergic and asthmatic patients in Delhi indoor and outdoor air, to act as control for clinical correlations of allergic symptoms

extracts to be of allergenic significance. He reported *C. herbarum, A. niger,* and *A. fumigatus* to be important fungal allergens (Shivpuri 1980).

Important fungal allergens causing sensitization in patients of naso-bronchial allergy of hilly regions have been identified (Singh et al. 1987a, b). *Ganoderma lucidium* has been reported to induce sensitization in hypersensitive patients. Skin test results with spore and whole-body extracts of *Ganoderma* showed 28.48% and 17.44% of patients to be positive to respective extracts (Singh et al. 1995). Gupta et al. (1993) observed through ELISA high level of specific IgE against *Fomes pectinatis* in the sera of exposed population. Common environmental allergens responsible for respiratory allergy had been reviewed by Singh and Kumar (2002). From Bengaluru, *Mucor mucedo, Fusarium solani, Curvularia,* and *Nigrospora* were found to be allergenically significant (Agashe and Anand 1982). *A. flavus, Helminthosporium, Neurospora, Candida, and Cladosporium* had been reported as the important allergenic fungi in AP (Acharya 1980).

14.8.9 Occupational Fungal Allergens

Epidemiological surveys for respiratory diseases were carried out among agricultural industrial workers such as bakeries, poultry farms, granaries, and sugar industry. About 40–59% of workers in different work environments suffered from one or more respiratory ailments; *Aspergillus* was found to be the major contributor. The sensitization pattern of workers in a bakery environment revealed high level of IgE and IgG antibodies to six species of *Aspergillus* (Singh et al. 1998). *Scopulariopsis brevicaulis* spores could sensitize poultry workers and this was evident by significantly high levels of IgE antibodies in the workers (Singh et al. 1998). The work done by various investigators with respect to fungal allergens in both outdoor and indoor environment had been reviewed by Singh and Deval (2005).

Clinical studies were carried out at different places under AICP on "Aeroallergens and Human Health." The study provided important information on potential fungal allergens of different places. The important fungal allergens identified including *A. ochraceous, A. japonicus, Cladosporium, Alternaria alternata, A. versicolor, A. ochraceous, A. Japonicus, Uromyces, Ustilago, Neurospora sitophila,* and *Sporotrichum* had been reported to be allergenic for the first time (Anonymous 2000).

14.8.10 Fungi of Crop Fields

Aerobiological studies with respect to plant diseases of different crops have been carried out by various investigators. Tilak and Babu (1984) investigated certain diseases of bajra crop. Aerial dissemination of urediniospores of groundnut rust was studied by Mallaiah and Rao (1982). Aerobiological and epidemiological studies with respect to groundnut rust have also been carried out by Murdhankar and Pandey (1991). Aeromycoflora of crop fields like cereals, pulses, vegetables, oilseeds, and cash crops has been conducted in Imphal. A close relationship between meteorological factors, growth stage of crop, and spore load in the air over the field was observed. In the maize crop, spores of pathogenic fungi were reported to be abundant in the air 4–5 weeks prior to the appearance of disease (Singh and Doryanthra 1991). *Alternaria alternata* was reported to be the causal organism of leaf and stem spot disease of sunflower. The conidia were trapped from the air when the crop was in flowering stage (Ramachander Rao 1993).

Day-to-day variations in the concentration of *Alternaria porri* conidia over onion field infected with purple blotch were studied by Chawla and Rajasab (1994). The conidia were present in high concentration in the air when the crop was at 7–8-leaf-stage state. Studies over a carrot field revealed that the concentration of conidia of *Alternaria davuhi*, the causative agent of leaf blight, increased with the progress of the disease (Channabasavari et al. 1994). The uredospores of *Periodispora mori* causing mulberry rust appeared in the air from August onwards and spore concentration gradually increased up to December corresponding with the disease severity (Prasad et al. 1994).

Aerophyllo-mycoflora of some solanaceous crop plants in Bhilai Nagar (MP) showed fungal population to exhibit wide variation at different stages of crop development. Maximum numbers of microorganisms were recorded during senescent stage and minimum number was observed during seedling stage (Sahu 1998) Aerial surveys over cotton field at Ahmedpur showed *Ramularia areola*, *Cercospora* sp., *Helminthosporium* sp., and *Alternaria* sp. to be pathogenic to the cotton crop (Jagannath and Gaikwad 1998). Hedge and Koycarnis (2002) carried out studies at Ugar Khurd and Dharwad (Karnataka), and reported a load of uredospores of *Phakopsora pachyrhizi* to be maximum in August–September which also coincided with the critical stage of infection at flowering and pollination stages. They also developed prediction model for the disease based on the severity of disease and environment factors. Ugar Khurd has been shown to be the source of infection and hot spot for soybean rust outbreak in Karnataka (Hedge and Kulkarni 2002).

Many more investigators have made significant contributions to the knowledge towards aerobiology of fungal plant pathogens which had been reviewed by Vittal (2005).

14.9 Conclusions

The progress made in the field of aerobiology has been very impressive in the last 25 years. It was not possible to cover each and every paper published in this chapter but an attempt has been made to review selected publications from different geographical locations of India.

14.10 Future Priorities

Allergy has been known for more than a century but the subject has suffered due to lack of knowledge on basic allergic mechanism and poor diagnostic procedures. In the last two decades, a fast growth has been observed in the field of aerobiology. A large number of fungal allergens have been identified from different geographical locations of India. However, due to urbanization and industrialization, the fungal flora of the region is continuously changing. Regular atmospheric surveys are, therefore, recommended in order to study the seasonal and annual variations of different fungal allergens in outdoor and indoor environments of the region.

Till date most of the surveys conducted are mainly in urban areas. Rural area should be included in the study as a major fraction of our population live in these areas. This is also important as maximum information on indigenous allergens is essential for the diagnosis and treatment of allergic disorders.

Aerobiological investigations should be correlated with clinical studies in order to establish a relationship between the concentration of allergens and the patients' syndrome. The increase in the prevalence of allergic diseases should be studied in detail.

Efforts should be made to standardize the antigenic extracts prepared from various allergens. Another important aspect that is to be studied in detail is the cross-reactivity among different allergens. This will be helpful in minimizing the suffering of patients to a great extent.

The rapid development in the field of aerobiology has resulted in the understanding of prevalence of different allergens. This information should be linked with immunological and clinical studies so that it is of direct significance to both the patients and allergy practitioners.

14.11 Prevention of Fungal Allergens

1. The source and environmental conditions that help the fungi in getting airborne in significant concentrations in air should be identified.
2. The substrate on which these microorganisms grow and flourish should be removed.
3. The kitchen should be kept clean and dry as it contains plenty of substrate for the growth of fungi.
4. Bathrooms should also be kept clean and dry. Seepage on the walls must be avoided.
5. As fungi are occupational hazards, the working environmental conditions should be improved.
6. Workplaces should be well ventilated and hygienic.
7. Reduction of moisture inside the workplaces is recommended.
8. The quality of air should also be improved by periodic maintenance of air treatment plant, fumigation, and application of antifungal agents.
9. Personal fitter masks of the pore size sufficient to stop the entry of respirable sized microbes should be used.
10. Regular health checkup of patients is recommended.

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Keratinophilic Fungi: Diversity, Environmental and Biotechnological Implications

15

Jitendra Kumar, Itisha Singh, and R. K. S. Kushwaha

Abstract

Keratinophilic fungi colonize keratinous substrates and convert them to the constituent components of low molecular weight. These fungi can be distinguished from others in their characteristic that they are adapted to consumption of keratinous proteins as sources of carbon and nitrogen. These tend to utilize proteins, peptides, and amino acids as carbon sources even in the presence of sugars. They also exhibit a great diversity in their mode of nutrition and physiology. This chapter critically reviews not only the distribution of keratinophilic fungi but also their social and environmental implications. Recycling of keratin contributes to nutrients in soils, pollution control, and solid waste management.

Keywords

Keratinophilic fungi · Keratin · Keratinase · Peptides · Amino acids · Feather composting

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15.1 Introduction

Keratin is a natural fibrous protein forming the outermost keratinized layer of humans and animals. Various keratinous substrates occur in nature in various forms such as hair, wool, feathers, nails, claws, quills, scales, horns, hooves, and tortoise shell and in the outer layer of skin. Keratinophilic fungi colonize different keratinous substrates and degrade them to components of low molecular weight. These fungi are reported from the sites inhabited and frequented by humans and animals, and are saprophytic in nature and occasionally cause infection in humans and animals. These fungi can be divided into (1) keratinophilic fungi (grow on keratinic materials), (2) keratinolytic fungi (capable of decomposing keratin completely), and (3) dermatophytes (belong to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*). Keratinophilic fungi include a variety of filamentous fungi mainly belonging to the order Onygenales and other saprophytic fungi of hyphomycetes, and several other taxonomic groups. All the dermatophytes are keratinolytic in nature. Based on infection, these can be anthropophilic (infecting humans), zoophilic (infecting animals), and geophilic (reported from soil).

15.2 Distribution of Keratinophilic Fungi

The first report of the occurrence of *Microsporum gypseum* (Bodin) was from soils of Dibrugarh district of Assam (Dey and Kakoti 1955). Later on *M. gypseum* was also reported from the soil in Delhi (Randhawa et al. 1959), Dehra Dun and Calcutta (Puri 1961), and Poona (Padhye 1961), and the perfect state of fructification of *Microsporum gypseum* (Sarkar 1962). Mohapatra and Gugnani (1964) reported on the morphology, pathogenicity, and perfect state of strains of *M. gypseum* isolated from soil. The isolation of *Trichophyton mentagrophytes* was reported from soil (Puri 1961), two new species of *Trichophyton* (Sarkar 1962), and *Keratinomyces ajelloi* (Padhye and Thirumalachar 1962). *Trichophyton indicum* and *T. evolceanui* were reported from soil (Randhawa and Sandhu 1963). *Keratinophyton terreum* is the perfect state of *Trichophyton indicum* (Randhawa and Sandhu 1964), *Chrysosporium lucknowense* (Garg 1966).

Randhawa and Sandhu (1965) comprehensively surveyed keratinophilic fungi from Indian soils, and reported *Keratinophyton terreum*, *Microsporum gypseum*, *Ctenomyces serratus*, *Trichophyton evolceanui*, *Keratinomyces ajelloi*, *Chrysosporium tropicum*, and *Microsporum cookei*. The soil samples were collected from Delhi, Uttar Pradesh, Punjab, Maharashtra, Bengal, Madhya Pradesh, Jammu and Kashmir, Kerala, Rajasthan, Andhra Pradesh, Mysore, Orissa, Bihar, and Himachal Pradesh. Garg (1966) reported *Arthroderma quadrifidum*, *Chrysosporium evolceanui*, *C. keratinophilum*, *C. lucknowense*, *C. tropicum*, *Ctenomyces serratus*, *Keratinomyces ajelloi*, *Microsporum canis*, *M. cookie*, *M. gypseum*, and *Trichophyton mentagrophytes* from the soils collected from Rajasthan, Jammu, Uttar Pradesh, Punjab, Andhra Pradesh, Delhi, Gujarat, Madhya Pradesh, Mysore, and West Bengal.

Padhye et al. (1966) recovered *Trichophyton mentagrophytes, T. rubrum, Microsporum gypseum*, *M. nanum*, *Chrysosporium indicum*, *C. tropicum*, *C. evolceanui*, *Arthroderma tuberculatum*, and *Ctenomyces serratus* from the soils of Poona, and later *Chrysosporium tropicum*, *C. indicum*, *Microsporum gypseum*, and *Ctenomyces serratus*, from marine habitats of Mumbai, Maharashtra (Padhye et al. 1967). *Arthroderma simii* is a teleomorph of *Trichophyton simii* and had been reported from India (Padhye and Thirumulachar 1967).

Professor GJF Pugh visited Madras University and recorded *Chrysosporium* sp. and *Ctenomyces serratus* from birds' nests near Madras in India (Pugh 1966).

Roy et al. (1972) recorded these fungi from the soils of some parts of Orissa. Sur and Ghosh (1980a) also reported these fungi from soils. These fungi were recorded from house sparrows (*Passer domesticus*) (Sarangi and Ghosh 1991), *Lonchura striata* (spotted munia), *L. malacca* (black-headed munia), *Pycnonotus cafer* (Indian bulbul), *Copsychus saularis* (Oriental magpie-robin), *Acridotheres tristis* (common myna), *Sturnus contra* (pied myna), *Turdoides striatus* (jungle babbler), quail (*Coturnix* species), *Dinopium benghalensis* (black-rumped flameback), and *Gallus domesticus* (Sur and Ghosh 1980b).

During this period at the University of Saugar, the research was carried out under the guidance of Professor S.C. Agrawal: Dr. R.K.S. Kushwaha, Dr. P.C. Jain, K.V. Singh, G.B. Singh, S.K. Deshmukh, R. Rathore, and S.K. Agnihotri carried out the work extensively. Dr. Kushwaha moved to Christ Church College, Kanpur; Dr. Deshmukh moved to Mumbai; and Dr. Jain continued the work at Saugar. The recoded fungi included *Chrysosporium crassitunicatum*, *C. evolceanui*, *C. indicum*, *C. lucknowense*, *C. merdarium*, *C. tropicum*, *Keratinomyces ajelloi*, *Malbranchea pulchella*, *M. aurantiaca*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, and *Trichophyton terrestre* (Kushwaha and Agrawal 1976; Kushwaha and Agrawal 1977a; Jain and Agrawal 1977; Deshmukh and Agrawal 1983; Singh and Agrawal 1983).

Kushwaha and his associates have extensively worked on keratinophilic fungi from Kanpur and its vicinity. A large number of fungi were recorded from soil: *Diamargaris* sp., *Mucor pusillus*, *Aspergillus* spp. (Nigam and Kushwaha 1989), *Arthroderma flavescens*, *A. gertleri*, *Aphanoascus terreus*, *C. tuberculatum*, *C. indicum*, *C. tropicum*, *Trichophyton flavescens*, *T. vanbreuseghemii* (Nigam and Kushwaha 1990). Many reports had been recorded belonging to all habitats of soil (Kushwaha et al. 1985), indoor environment (Nigam and Kushwaha 1984, 1985), forest soil and grassland (Nigam and Kushwaha 1990), Andaman (Dixit and Kushwaha 1990), house dust (Nigam and Kushwaha 1986, 1990), leather (Nigam et al. 1994), Mediterranean sea beach (Katiyar and Kushwaha 1997), water sediments (Katiyar and Kushwaha 2000), soil and birds (Kushwaha and Gupta 2004, Tripathi and Kushwaha 2005a), potted plants (Singh et al. 2009a, b), public parks (Singh and Kushwaha 2010), and sewage slug (Kushwaha 2014). Kushwaha and co-workers reported keratin-degrading capacity of these fungi on various substrates such as peacock feather (Kushwaha 1983), keratin degradation (Kushwaha 1998), feather (Parihar and Kushwaha 1999), hair (Parihar and Kushwaha 2000), and human hair (Katiyar and Kushwaha 2012). Kushwaha

(2000) reported physiology of *Chrysosporium* and biotechnological applications. Other group working at CDRI, Lucknow, recorded a large number of fungi from Lucknow and its vicinity (Jain et al. 1985). Another group working in Agra reported these fungi from Ghana Bird Sanctuary/Keoladeo National Park, Bharatpur (Singh et al. 1994, 1996), and from the soils of Agra (Saxena et al. 2004). They have also recorded keratinophilic fungi from domestic chicken (*Gallus domesticus*), domestic pigeon (*Columba livia*), house sparrow (*Passer domesticus*), house crow (*Corvus splendens*), duck (Arias sp.), and rose-ringed parakeet (*Psittacula krameri*) (Dixit and Kushwaha 1991). Kushwaha and his associates have reported several new species found in Indian soil, e.g., *Acrodontium album* (Kushwaha and Agrawal 1976), *Botryotrichum keratinophilum* (Kushwaha and Agrawal 1976), *Chrysosporium crassitunicatum* (Kushwaha and Agrawal 1977b), *Chrysosporium geophilum* (Kushwaha and Shrivastava 1989), *Chrysosporium christchurchicum* (Tripathi and Kushwaha 2005b), and *Chrysosporium kanpuranse* (Tripathi and Kushwaha 2005c).

Deshmukh and associates recorded keratinophilic fungi from beaches of Goa (Deshmukh and Agrawal 1983), soils from Mumbai (Deshmukh 1999), salt pans (Deshmukh 2004), meteoritic crater (Deshmukh and Verekar 2006), Usar soil (Deshmukh and Verekar 2011), bird sanctuaries (Deshmukh and Verekar 2011), public parks (Deshmukh and Vereker 2012), Kaziranga National Park (Deshmukh et al. 2017), and Sambhar Lake (Deshmukh et al. 2018). These fungi were also recorded from the states of Kerala (Deshmukh 2002), Himachal Pradesh (Deshmukh and Verekar 2006), Jammu and Kashmir (Deshmukh 2002), Jammu (Deshmukh and Agrawal 2003), Ladakh (Deshmukh et al. 2010), Karnataka (Deshmukh et al. 2000), Uttarakhand (Deshmukh et al. 1985), (Deshmukh 1985), and Chhattisgarh (Deshmukh and Shukla 2000–2001). They have also recorded these fungi from pigeon (Deshmukh 2004) and emu (Deshmukh et al. 2021). More recently Sharma and Souche (2020) recorded keratinophilic fungi from various parts of Maharashtra and reported two new genera *Currahmyces indicus* gen. et sp. nov. and *Canomyces reticulatus* gen. et sp. nov., and a new species *Ctenomyces indicus* sp. nov.

While working with keratinophiles, a large number of fungi had been isolated from salt pans in Mumbai. It was observed that a large number of keratinic materials (hair, pieces of blankets, feather, etc.) get collected and decomposed during the formation of salt (Deshmukh 2004).

Prof. Geeta Sumbali and her students reported keratinophilic fungi from poultry farm soils in Jammu and recoded *Chrysosporium keratinophilum*, *C. queenslandicum*, *C. tropicum*, *C. pannorum*, *Malbranchea flava*, *Scopulariopsis brevicaulis*, *Microascus manganii*, and *Gliocladium virens* from poultry farm soils (Kaul and Sumbali 1997, Kaul and Sumbali 2000). Later *Chrysosporium inops*, *C. merdarium*, *C. queenslandicum*, and *Chrysosporium* anamorph of *Gymnoascus demonbreunii*, along with other saprophytic fungi, were isolated from the soils collected from Khardung La (Kotwal and Sumbali, 2016). *Chrysosporium tropicum*, *C. indicum*, *Ctenomyces serratus*, *Gymnoascus* sp., *Microsporum gypseum*,

M. audouinii, *Trichophyton simii*, *T. terrestre*, *T. mentagrophytes*, *T. verrucosum*, and *Trichophyton* sp. were recorded from the soils of *Rajasthan* (Jain and Sharma 2012). Previously Jain and Sharma (2011) *reported keratinophilic fungi* with particular reference to soil pH. These fungi were also recorded from schools and college playground soils of Jaipur (Sharma and Sharma 2010).

More recently Kumawat et al. (2020) reported *Aphanoascus arxii, Arthroderma multifidum, Chrysosporium indicum, Ch. queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Ctenomyces serratus, Malbranchea saccardo, Microsporum audouinii, Microsporum canis, Trichophyton equinum, Trichophyton erinacei, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton terrestre, Trichophyton verrucosum, and Uncinocarpus queenslandicus* from the semiarid region of Rajasthan.

Keratinophilic fungi were also reported from the soils of Bihar and Jharkhand (Verma et al. 1982) and piggeries of Ranchi (Kumar et al. 2012), garbege waste soil (Kumar et al. 2013). *Microsporum gypseum, M. nanum, Trichophyton mentagrophytes, T. terrestre, Chrysosporium keratinophilum, C. pannorum, C. anam. A. cuniculi,* and *C. tropicum* were isolated from primary schools and public parks in the city of Madras (Ramesh and Hilda 1999). Anbu et al. (2004) reported these fungi from poultry farm in Tamil Nadu, and recorded *Chrysosporium keratinophilum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Myceliophthora vellerea*, teleomorph of *Chrysosporium* (*Arthroderma tuberculatum*), and *Geomyces pannorum* along with non-dermatophytic fungi. Ghosh and Bhatt (2000) recorded *Chrysosporium zonatum*, *Malbranchea aurantiaca*, *Aphanoascus fulvescens* from Chilka lake soil Orissa.

Chrysosporium evolceanui, Chrysosporium indicum, Microsporum fulvum, Microsporum gypseum, and *Trichophyton rubrum* along with other saprophytic fungi were isolated from the soils of Jabalpur (Pandey et al. 1990). Later *Chrysosporium indicum*, *Geotrichum candidum*, *Gymnoascoideus petalosporus*, *Scopulariopsis brevicaulis,* and *Talaromyces trachyspermus* were recovered from gelatin factory in Jabalpur (Rajak et al. 1991). Sharma et al. (2006) reported *Amauroascus queenslandicus, Aphanoascus durus, A. reticulosporus, A. terreus, Arthroderma ciferri, Arthoderma gypsea, A. incurvata, Auxarthron conjugatum, A. umbrinum, Ctenomyces serratus, Gymnoascus citrinus, G. petallosporus, and Nanniziopsis vreesii* from the soils collected from Madhya Pradesh and Chhattisgarh.

Researchers at VP Chest Institute, New Delhi, isolated *Chrysosporium tropicum, C. keratinophilum, Keratinophyton terreum, Anixiopsis stercoraria, Pseudoarachniotus hyalinosporus, Auxarthron zuffianum, Ctenomyces serratus, Trichophyton simii, T. mentagrophytes var. granulare,* and *Microsporum gypseum* from the small mammals, viz. *Tatera indica, Rattus rattus, Suncus murinus, Mus platythrix, Funambulus palmarum, Bandicota bengalensis, Nesokia indica, Millardia meltada, Meriones hurricane,* and *Mus musculus*, in India (Gugnani et al. 1975).

Table 15.1 New keratinophilic fungi reported from India

Sr. No.	Genus and species	Reference
1.	*Chrysosporium indicum*	Randhawa and Sandhu (1963); Garg (1966)
2.	*Chrysosporium evolceanui*	Randhawa and Sandhu (1963); Garg (1966)
3.	*Chrysosporium lucknowense*	Garg (1966)
4.	*Gymnoascus petalosporus*	Orr et al. (1977a, b)
5.	*Gymnascella aurantiaca, G. citrina, and G. kamyschkoi*	Orr et al. (1977a, b)
6.	*Arachniotus candidus*	Orr et al. (1977a, b)
7.	*Pseudoarachniotus desertormn, P. flavoluteus, P. roseus, P. ruber,* and *P. trochleosporus*	Orr et al. (1977a, b)
8.	*Acrodontium album*	Kushwaha and Agrawal (1976)
9.	*Botryotrichum keratinophilum*	Kushwaha and Agrawal (1975)
10.	*Chrysosporium crassitunicatum*	Kushwaha and Agrawal (1977b)
11.	*Verticillium saksenii*	Kushwaha (1980)
12.	*Chrysosporium geophilum*	Kushwaha and Shrivastava (1989)
13.	*Chrysosporium christchurchicum*	Tripathi and Kushwaha (2005b)
14.	*Chrysosporium kanpuranse*	Tripathi and Kushwaha (2005c)
15.	*Chrysosporium aquaticum*	Gupta and Kushwaha (2012)
16.	*Auxarthronopsis bandhavgarhensis*	Sharma et al. (2013)
17.	*Gymnoascus verrucosus*	Sharma and Singh (2013)
18.	*Matsushimamyces bohaniensis*	Sharma et al. (2015a, b)
19.	*Nannizzia graeserae*	Sharma and Shouche (2018)
20.	*Currahmyces indicus, Canomyces reticulatus,* sp. nov., *Ctenomyces indicus*	Sharma and Souche (2020)

Chrysosporium indicum, C. evolceanui, Chrysosporium state of Arthroderma tuberculatum, Trichophyton mentagrophytes var. mentagrophytes, and *Microsporum gypseum* along with species of *Aspergillus, Penicillium, Paecilomyces, Fusarium, Chrysosporium, Acremonium, Rhizopus, Mucor, Geotrichum, Trichosporon,* and *Rhodotorula* were isolated from the soils from burrows of rats in different parts of India and Nepal (Gugnani et al. 2007). The new fungi recorded from Indian soils are shown in Table 15.1.

15.3 Keratin Degradation: Environmental Implications

Keratin is a natural fibrous protein that forms the outermost keratinized layer of humans and animals and its appendages. Keratin can be classified into α-keratin and β-keratin based on the secondary protein structure. Keratinolytic process may involve two steps: sulfitolysis (reaction that breaks disulfide bonds of the keratin fiber) and proteolysis. Fungal keratinases are mainly from *Trichophyton rubrum*, *T. simii, Microsporum canis*, *Chrysosporium indicum, C. keratinophilum, C. pannicola, C. pannorum, C. queenslandicum, C. tropicum, Malbranchea flava,* and *Malbranchea chrysosporoidea* (Singh 1997; Kaul and Sumbali 1997, 1999; Raju et al. 2007; Kumar and Kushwaha 2014). It is also produced by other fungi, viz. *Acrodontium album, Aspergillus ustus, A. quercinus, Botryotrichum keratinophilum, Chaetomium globosum, Curvularia indica, Gliocladium agrawalii, G. roseum* (Kushwaha 1983)*, Geotrichum candidum* (Rajak et al. 1991), *Absidia cylindrospora* and *Rhizomucor pusilus* (Rajak et al. 1992), *Scopulariopsis brevicaulis* (Anbu et al. 2005), *Microsporum gypseum* (Raju et al. 2007), *Acremonium strictum* (Kumar and Kushwaha 2012, 2014), *Cunninghamella echinulata* (More et al. 2013), *C. indicum, C. tropicum, C queenslandicum (*Kumar and Kushwaha 2014), *Aspergillus fumigates* (Paul et al. 2014), *Aspergillus flavus* (Mini et al. 2015), and *Scopulariopsis brevicaulis* (Satyalakshmi et al. 2015). Keratinase is immobilized on chitosan and chitosan-grafted-β-cyclodextrin matrix (for the improvement of enzyme properties) with immobilization final yields of 90% and 93%, respectively (Srivastava et al. 2020).

Keratinase production is induced by non-keratinous substrates, for example sugarcane bagasse, shrimp cell powder, soybean meal, skimmed milk, soy flour, wheat bran, gelatin, casein, and sodium caseinate. The addition of different carbon sources, such as glucose, fructose, sucrose, maltose, mannitol, lactose, and starch, and additional nitrogen sources like yeast extract, beef extract, polypeptone, soy peptone, malt extract, casein, tryptone, urea, ammonium sulfate, ammonium nitrate, ammonium chloride, and potassium nitrite into the keratin cultivation medium leads to a high level of keratinase production (Srivastava et al. 2019).

Keratinases can be used to decrease pollution load from the environment caused mainly due to the increased processing of the poultry, leather, textile, and detergent industries. Keratinolytic enzymes have a wide variety of applications in solid waste treatment, detergents, food industry, leather industry, silk fiber processing, drug delivery, and biopolymers.

The conversion of feathers into feather meals, by applying physical and chemical methods, results in the loss of nutritionally essential amino acids such as methionine, lysine, and tryptophan and also in the formation of nonnutritive amino acids like lysine, alanine, and methionine. Therefore, currently the poultry feathers are converted into feather meal, a digestible dietary protein, for animal feed using keratinases. The microbial production of L-lysine is an expanding branch of manufacturing biotechnology.

Biodegradation of feather waste is an alternative avenue for creating a viable end product with visible benefits to the primary producers in the environmental and

economic strategies. Kumar et al. (2015) supplemented filtrates obtained from feather degradation by keratinophilic fungi and found better growth of pea plant due to increase in nutritional value of soil. Kumar et al. (2017) developed low-cost manure from degraded feathers and observed positive effect on maize and pea plants. Kumari and Kumar (2020) used *A. tenuissima* for degradation of feather in soil and increased soil nutritional value. Compost was found to be a better root and shoot enhancer in chickpea plant. Kumar et al. (2020) used *C. indicum* for the development of feather compost and studied the effect on maize plant. The investigations on keratin decomposition proved that keratinophilic fungi are significant in the recycling of very hard keratin protein and conversion into constituent amino acids which are easily available for plants. This could be a pioneering technology that could be used to control the mechanism to a specific solid waste.

15.4 Distribution Patterns and Potential Biotechnologies

In this discourse, keratinophilic fungi have been shown to occur in many natural and man-made habitats, and reported about 28 new fungi from the Indian subcontinent (Table 15.1). These habitats are frequented by humans and animals and add the keratin materials into soil. This habitat includes cattle farms, poultry farms, caves, schools, gardens, water bodies, crop fields, and others. The frequency and density of these fungi in soil also depend upon the keratinic material added to the soil. *Chrysosporium indicum* and *M. gypseum* were the most distributed fungi in Indian soils that indicates their adaptation to warmer conditions of India (Deshmukh and Agrawal 1983). The less frequency of *C. indicum* was observed in hilly areas of Jammu and Kashmir in comparison with plains (Garg 1966). The low frequency of the other species of the genera *Chrysosporium*, *Malbranchea*, *Microsporum*, and *Trichophyton* with restricted growth on keratin baits indicates that these fungi are poor keratin colonizers.

The occurrence and distribution of keratinophiles are influenced by various factors such as pH, temperature, moisture content, deft of soil profile, and quantity and composition of organic matter. The effect of pH on the occurrence of keratinophilic fungi was emphasized for the first time by Marples (1964). Later on the distinct correlation between the pH of birds' nests and the presence of keratinophilic fungi was reported by Pugh (1966). It was observed that alkaline soil (pH 9.0) favors the growth of *T. terrestre*, while other keratinophilic fungi prefer to grow at a pH in the range of 6.5–8.0; certain keratinophilic fungi grow at particular pH (Jain and Sharma 2011). In the previous study it was reported that these fungi are most frequently reported from low acidic to low alkaline soil (Bohme and Ziegler 1969). Its range of pH, neutral to weak alkaline, favors the growth of these fungi, and the growth of these fungi is inhibited at pH below 4.0. The absence of these fungi in high-acidic soils is due to low enzyme activity (Srivastava et al. 1990). Various factors like atmosphere, humidity, temperature, and nature and water retention of soil affect fungal growth. It was also observed that increased number of *T. ajelloi* was reported at higher humidity, while *T. terrestre* was reported in soils with low

humidity (Chmel et al. 1972). *Aphanoascus fulvescens*, *Chrysosporium carmichaelii*, *Chrysosporium* sp., *Geomyces pannorum v pannorum*, *G. pannorum v. vinaceus*, *Malbranchea gypsea*, *Malbranchea* sp., *Microsporum gypseum*, *Trichophyton mentagrophytes,* and *Trichophyton terrestre* were isolated from the soil samples collected from Antarctica having extremely low temperature considering their adaptability and tolerance to low temperature (Mercantini et al. 1989, 1993). However the minimum temperature required for their growth has been reported to be 20 °C and 10 °C, respectively (Oorschot 1980).

Survival of keratinophilic fungi is also affected by the biotic factors including the presence of keratinous substrates (hair, nails, hooves, and skin of humans and animals) and some microorganisms, viz. bacteria, actinomycetes, and other fungal species which may exert antagonistic effects on the occurrence of fungi. Geophilic dermatophytes (*M. gypseum* complex, *T. ajelloi*, and *T. terrestre*) survive and proliferate in unsterilized soil, while zoophilic and anthropophilic fungi were lysed and destroyed by soil microorganisms (Grin and Ozegovic (1963).

The ability to decompose the keratin enables them as pathogens in humans and livestock. Most of these fungi are nonpathogenic in nature but there are some reports of mycoses in humans caused by this group of fungi, for example *Myriodontium keratinophilum* (Maran et al. 1985), *Gymnascella dankaliensis* (De Hoog and Guarro 1995), *C. zonatum* (Sigler et al. 1998; Roilides et al. 1999), *G. hyalinospora* (Iwen et al. 2000), *Geomyces pannorum* var. *pannorum* (Gianni et al. 2003), *C. pannorum* (Zelenková 2006), and *C. queenslandicum*, *C. sulfureum*, *C. tropicum*, *Malbranchea pulchella*, and *M. keratinophilum* (Lysková 2007). The species of *Chrysosporium* are also known to cause infection in the range of reptile species. The species of *Chrysosporium* like *C. keratinophilum*, *C. tropicum*, and *Chrysosporium* spp. are the causal agents of cutaneous and systemic mycoses in reptile species (Paré and Jacobson 2007). *Chrysosporium guarroi* was isolated from pet green iguanas (*Iguana iguana*) [Abarca et al. 2010; Kahraman et al. 2015], *Chrysosporium queenslandicum* from a garter snake (Vissiennon et al. 1999), and *Chrysosporium ophiodiicola* from black rat snake (Rajeev et al. 2009). *Chrysosporium* anamorph of *Nannizziopsis vriesii* is found in multiple reptile species such as chameleons (Paré et al. 1997), snakes (Bertelsen et al. 2005; Nichols et al. 1999), saltwater crocodiles (Thomas et al. 2002), bearded dragons (Bowman et al. 2007; Hedley et al. 2010), green iguanas (*Iguana iguana*) (Han et al. 2010), and girdled lizards (Hellebuyck et al. 2010). Saidi et al. (1994) reported *Chrysosporium tropicum* from the comb lesion in two breeds of chicken in India. These reports suggest that the species of *Chrysosporium* can be opportunistic pathogens.

Keratinophilic fungi can degrade keratinic materials into small peptides and amino acids. The enzyme is responsible for hydrolyzing insoluble keratin and belongs to the class of serine protease. Serine proteases are alkali stable. These enzymes are stable in highly alkaline environments, while being not very heat resistant. A number of dermatophytes/keratinophilic fungi and some saprophytic fungi are responsible for degradation of keratin (Qiu et al. 2020). Keratinolytic enzymes possess a wide variety of applications in solid waste treatment, detergents, food industry, leather industry, fiber modification, drug delivery, and biopolymers

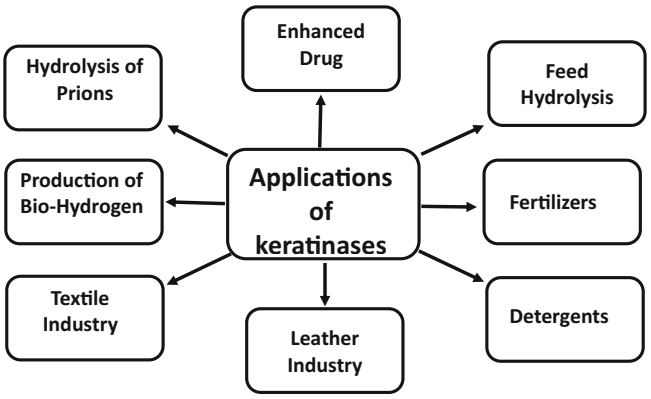

Fig. 15.1 Potential applications of keratinases

and need further investigations (Srivastava et al. 2020) (Fig. 15.1). Since most of the purified keratinases known to date cannot completely solubilize native keratin, their exact nature and uniqueness for keratinolysis are still an enigma in the world of proteases.

15.5 Conclusions and Future Perspectives

Keratinophilic fungi are reported in various part of India, but the numbers of samples collected were limited in comparison to the area of country; hence there is a need for systemic survey for prevalence of these fungi. From India only 28 new fungi were recorded so far but the systematic survey will definitely add significant numbers to the list of new fungi. The molecular approach for identification will also help to obtain novel fungi. The whole-genome sequencing and transcriptome analysis will aid in identifying genes that encode keratinases. Recombinant strains containing potent keratinase genes will decompose feathers or other keratinous waste in soil and increase nutritional profile. It will be beneficial to decompose feather waste in agricultural fields in a short duration. There are a few reports on bacterial strain development carrying multiple keratinase gene copies in the chromosome of *Bacillus licheniformis* for increasing the production of keratinase (Wang et al. 2004). *Bacillus licheniformis* keratinase gene was expressed under T7 promoter in *E. coli* and xylose-inducible expression system in *B. megaterium*. Optimization of the process parameters using RSM resulted in a threefold higher level of keratinase production by the recombinant *B. megaterium* (pWHK3) than the native strain *B. licheniformis* MKU3 (Radha and Gunasekran 2007). Such strains will help in converting the poultry/pig industry residues into higher quality amino acids that can be supplemented to poultry, pig, ruminant, and fish feeds.

Keratin degradation potential of these fungi opens a new feature in the recycling of keratin protein. Keratinophilic fungal provisions improve the degradation of

feather waste by keratinase activities and increase nutrient availability in soil. Crop-specific research is necessary on these aspects. Keratinases from these fungi could be used as additives in detergent application for cleaning of clogged drains. Fungal enzymes can be useful in the dehairing process of leather. These fungi could be major pollution controllers for the leather industry because all waste of leather treatment contains keratinous material. Partially hydrolyzed keratin can be used in making glue, biodegradable films, and coating from keratinous waste. Partially hydrolyzed feathers are good adsorbents of heavy metals. This could aid in pollution abatement.

Keratinophilic fungi survive in various extreme environmental conditions and are known to produce a number of bioactive metabolites including enzymes, indole compounds, and antimicrobial, antiviral, cytotoxic, cytoprotective, and other substances. Compounds produced by keratinophilic fungi and dermatophytes with the above properties include fusidic acid (Elander et al. 1969); floccosin and floccosic acid (Blank et al. 1969); cryscandin and pannorin (Yamashita et al. 1984; Ogawa et al. 1991); aranorosin, aranorosinol A, aranorosinol B, aranochlor A, and aranochlor B (Roy et al. 1988, 1992; Mukhopadhyay et al. 1998); zaragozic acids D and D2 (Dufresne et al. 1993); malbranchin (Chiung et al. 1993); rumbrin (Yamagishi et al. 1993); gypsetin (Shinohara et al. 1994); and 1-hydroxy-2-oxoeremophil-1(10), 7(11),8(9)-trien-12(8)-olide (Martinez-Luis et al. 2005). The overall metabolic potential of keratinophilic fungi is immense because of their specific nature. Keratinophilic fungi, if explored extensively for metabolite production, can be novel sources of many promising molecules of medical, biotechnological, and agrochemical utility.

There is a need for systematic sampling of large areas to recover forms that have very restricted distribution. Selection of keratin-rich habitats is also a key in obtaining rare onygenalean forms, many of which are still to be described.

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Part III

Different Groups of Fungi

Taxonomy and Ecology of Soil Fungi in India: Aspects and Prospects

16

Manoharachary Chakravarthula

Abstract

Soil is a complex and dynamic medium. Soils are diversified and support a variety of microbes, fungi and other biotic communities. Soils differ in their physico-chemical composition. In spite of the constellation of soil physico-chemical factors, the dynamic equilibrium of microbes and fungi is maintained. Forest soils support a variety of fungi including new fungi. Cultivated soils harbour some soil-borne and root-borne pathogens along with other fungi. Quantitatively and qualitatively forest soils are rich in fungi followed by rhizosphere soils, wild soils, cultivated soils, usar and desert soils. Further semi-aquatic habitat supports less number of fungi, but fungi possessing perennating structures and melanin pigment bearing fungi dominate. Further among various physico-chemical factors, organic matter content, total nitrogen, moisture, pH, and vegetation have been considered as the most influencing factors affecting the distribution, phenology, quantitative and qualitative composition of fungi. Semi-aquatic habitats suffer from less oxygen besides having more organic matter. The most commonly isolated fungi from different soils are represented by *Alternaria, Aspergillus, Chaetomium, Curvularia, Drechslera, Fusarium, Humicola, Memnoniella, Mucor, Nigrospora, Penicillium, Rhizopus, Sordaria Trichoderma* and others. Desert and usar belong to extremophilic habitat and have harboured few fungi including thermophilic and alkalitolerant fungi. It is important to mention that diversified soils of India were surveyed for fungal taxa from time to time. All such fungi were indexed in "Fungi of India" volumes. However, very few new fungal taxa have been reported from soils of India indicating that there is unexplored wealth of fungi in soil habitats. Since Waksman's (Sci N S 44:

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320–322, 1916) study "Do fungi live and produce mycelium in the soil," many research contributions have been made all over the world, but scanty information is available on the ecology of soil fungi from India. Soil fungi are important in agriculture, biotechnology, industry, pharmaceuticals, natural cycling of elements, transformation, bioremediation, waste management and other such activities concerning human welfare. Further, critical and exhaustive studies are essential on the taxonomy and ecology of soil fungi from India.

Keywords

Conservation · Diversity · Ecology · Fungi · Physico-chemical factors · Significance · Soil · Taxonomy

16.1 Introduction

It is a wonder to observe the presence of "unseen" living organisms in the environment. The late C. Mishra has suspected the presence of infectious organisms in soil. As early as 1577 A.D., the discovery of microscope in the seventeenth century has also unravelled the fungi present in soil along with other microbes. Soil is a dynamic medium which supports fungi and maintains their quantitative and qualitative composition in spite of the constellation of physico-chemical factors. Diversified soils all over the world harbour countless microbes and fungi. Saprophytic, pathogenic and symbiotic groups of fungi occur in varied types of soils. They compete with each other and also with other microbes for their nutrition, survival, growth and multiplication. However, mankind is keen to know both harmful and beneficial fungi. It is interesting to note that the international agreements on intellectual property rights and the provision for patenting have influenced researchers, scientists and R&D organizations to take up studies on the biodiversity of microbes including fungi, plants and other living biotic communities. Mother earth supports a number of plants including agricultural crops and forest plants. All the necessary nutrients are supplied to the plants by soil. Besides plants other biotic communities also live in the soil. Agriculture plays an important role in the country's economy besides offering food security and nutritional security through increased crop productivity. In the last few years, agriculture has shown phenomenal growth curve the world over due to the application of biotechnological approaches and innovations in the control of plant diseases and pests. Crop yields have increased in cereals, millets, vegetables, fruit crops and other crops which offer food and nutritional security. However, the arrival of invasive pathogens, weeds, insects and others has changed the climate and agriculture scenario. Non-judicious use of chemical fertilizers, pesticides, fungicides and other mechanisms increased losses in crops due to loss of soil fertility, development of resistance in plant pathogens, shift in monsoon and other reasons. The crop yields got minimized leading to hunger which immediately caused the downfall of economy and increase in poverty. Therefore, there is a need to reduce the crop yield losses using biofertilizers, biopesticides, growth promoters, disease resistant

varieties and plant breeding programmes along with biotechnological methods and also gene transfer. Therefore, in recent decades it has become increasingly important to conduct biodiversity studies on soil microbes and fungi all over the world to protect the interest of every country so as to have the prosperity and progress of every nation through advanced agriculture achieving record crop productivity. Soil is an important and frequently exploited habitat for fungi. The fungi bring about transformation of the dead and decayed plant and animal materials into organic matter which enriches soil fertility. The soil is a complex medium and its profile has several strata beginning from non-decayed litter on the top to un-weathered parent rock at the bottom; mineral layer is derived from the latter. Soils of tropics and temperate climates not only differ in their physico-chemical setup, vegetation and temperature but also differ in the quantitative and qualitative composition of microbes and fungi.

Fungi being highly specialized in their organization, colonization, nutrition and role vary from soil to soil and also in the same soil with an increase in depth. Another important feature being the distribution and composition of mycoflora, which depends mostly upon the extent, availability and utilization of nutrients like organic matter in the soil and influence of other factors. Soils the world over have been analysed for the quantitative and qualitative composition of fungi, distribution of fungi, activity of fungi, seasonal variation and other related aspects besides being the study on soil physico-chemical factors since the discovery of soil fungi by Waksman in 1905. It is in this context the soil fungi received considerable attention in view of their role of human welfare. It is the mother earth which supports diversified flora and fauna that helps the sustenance of humanity.

16.2 Taxonomy of Soil Fungi

Nature is the resource of diversified living organisms including fungi. A total of 2.0–3.1 million fungi have been considered as an approximate estimate (Hawksworth and Lucking 2017). Soil fungi have become a central point for a long time as they can be estimated, cultured and studied under laboratory conditions which can be employed in industry, agriculture, pharmaceuticals, biotechnology, fermentation, transformation and several other processes for the benefit of mankind. Earlier, efforts were made to identify and classify the fungi based on morphological, anatomical, ultrastructure, cell wall composition, physiological, biochemical and reproductive features. The peculiar features like presence of chitin cell wall, absorptive nutrition, varied reproductive structures and biochemical properties paved the way to treat fungi as a separate kingdom designated as Mycota or Mycetae. It needs a mention that microbiologists, botanists and animal scientists failed to embrace and include fungi in their kingdoms and classifications, although old literature shows the inclusion of fungi in the plant kingdom. In recent times, it has been evidenced that fungi are important for sustenance of life as they are essential in the creation of oxygen-rich atmosphere which supports life, besides being present in different strata

of fossil formations. Naturalists of fifteenth and sixteenth centuries have studied fungi in relation to their habitats and classified them as below.

1. Terrestrial fungi—growing in soil.
2. Hypogean fungi—growing under the ground.
3. Epiphytic fungi—growing on plants.

Later a number of mycologists have proposed different classifications (Manoharachary et al. 2014). Schüβler et al. (2001) proposed five phyla, namely Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota and Basidiomycota. Blackwell et al (2007) recognized Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota under fungi and Oomycota in Chromista.

Of late techniques in molecular biology have been making major contributions in the understanding of fungal biology and their relationship. Several molecular tools such as DNA-DNA hybridization, DNA fingerprinting, DNA probes, percentages of G+C contents, PCR reaction, RFLP, RAPD, DNA barcoding, DNA editing and others are employed to characterize, identify and classify the fungi. Kirk et al. (2008) have proposed a detailed and complicated classification of fungi. Therefore, it is advisable to follow a classification which is not vague and scientifically accurate that helps in the separation of fungi without any confusion. Mycologists have agreed since times immemorial that species is the unit of classification. Therefore, species identification and its relationships are important. Taxonomy is the mother of all sciences and has been dealing with classifying, assigning and identifying the fungal species and others. In recent times, taxonomists have become endangered species and only a few fungal taxonomists are now available to help in taxonomic research of fungi. Therefore, teachers, researchers, students and others who are interested in the taxonomy of fungi may choose a classification that is comfortable, helpful and easy to identify and segregate fungi so that the science of fungi and their taxonomy can grow and flourish. It is important to mention that identification and assigning the fungus to a taxonomic group and species epithet are essential. From India, Manoharachary (1972, 1976a, b, 1979, 1980, 1983, 1986), Manoharachary and Rama Rao (1973a, b, 1974, 1975, 1977), Manoharachary et al. (1975), Manoharachary et al. (2014), Nagamani et al. (2006), and Manoharachary et al. (1990) have attempted to provide research contributions, monographs, and manuals on soil fungi to help in the identification and also to study the ecology of soil fungi from India. The soil fungi which are described from time to time have been indexed in "Fungi of India" volumes published by the earlier researchers (Bilgrami et al. 1981, 1991a, b; Butler and Bisby 1961; Gouri Rane 2017; Jamaluddiin and Ojha 2004; Kamat et al. 1971; Mukerji and Juneja 1976; Rangaswami et al. 1970; Rao 1965; Sarbhoy et al. 1975, 1980, 1986a, 1986b; Subramanian 1971, Tandon and Sudhir 1973; Tilak and Rao 1968).

16.3 Some Noteworthy Fungi from Soils of India

Soil is bountiful of fungi and one's lifetime is not enough to study the millions of fungi that are present in soils. The noteworthy fungi include new taxa, new additions to the fungi of India, and fungi new to Telangana and Andhra Pradesh and new to India. There are not many new and noteworthy soil fungi reported from India. All such soil fungi have been listed in the indices of fungi (Bilgrami et al. 1979, 1981, 1991; Butler and Bisby 1961; Jamaluddiin and Ojha 2004; Kamat et al. 1971; Madhusudhan Rao and Manoharachary 1981; Manoharachary 1974a, b, 1976a, b; Manoharachary and Bilolikar 1979; Manoharachary and Rama Rao 1974, 1975, 1977; Manoharachary and Reddy 1975; Manoharachary et al. 1975; Manoharachary et al. 1990; Mukerji and Juneja 1976; Nagamani et al. 2006; Rama Rao 1962; Rama Rao and Manoharachary 1990; Rangaswami et al. 1970; Rao 1962, 1965; Reddy and Manoharachary 1977; Sarbhoy et al. 1975, 1980, 1986a, 1986b; Subramanian 1971; Tandon and Sudhir 1973; Tilak and Rao 1968; Vaidehi 1973).

Some photographs of soil fungi are provided as Figs. 16.1, 16.2, 16.3, 16.4, 16.5, 16.6, 16.7, 16.8, 16.9, 16.10, 16.11, and 16.12).

In my 45 years of investigations on soil fungi of Telangana and Andhra Pradesh (India), I have isolated, identified and provided taxonomic account of 332 fungal species (Nagamani et al. 2006). A total of 480 soil fungi are reported from India. Table 16.1 shows new fungal species and new additions to the fungi of India which are isolated and identified by me. The source is from 30 types of soils collected from Telangana and Andhra Pradesh during 1970–2020. A total of 332 fungi which are encountered during my research studies along with my students are published as *Handbook of Soil Fungi* (2006). Since Waksman's (1916) discovery about the

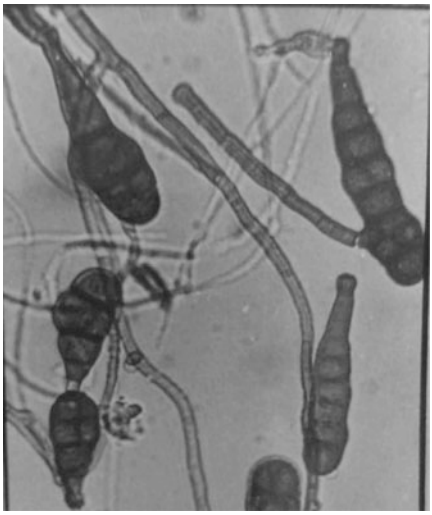

Fig. 16.1 *Alternaria infectoria* (× 180)

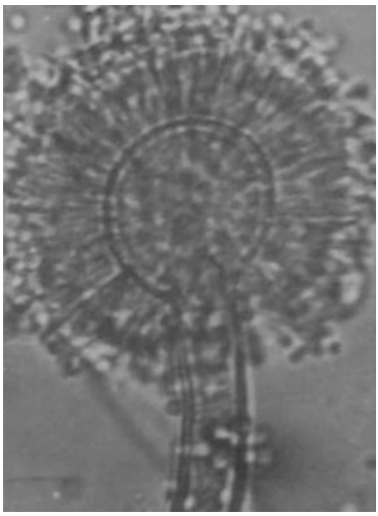

Fig. 16.2 *Aspergillus Candidus* (× 180)

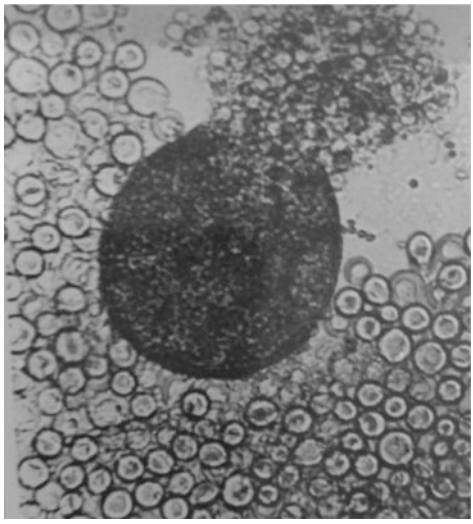

Fig. 16.3 *Aspergillus nidulans* (× 80)

presence of soil fungi, a lot of research has been done and voluminous literature has accumulated since then.

Forest soils, cultivated soils and desert soils were surveyed by many, but mud soils of ponds, rivers, lakes, paddy fields, mangroves and oceans have not yet been studied extensively. Hence there is very little information available about fungi occurring in semi-aquatic habitats, as these fungi are helpful to mankind in different ways.

Fig. 16.4 *Curvularia maculans* (× 180)

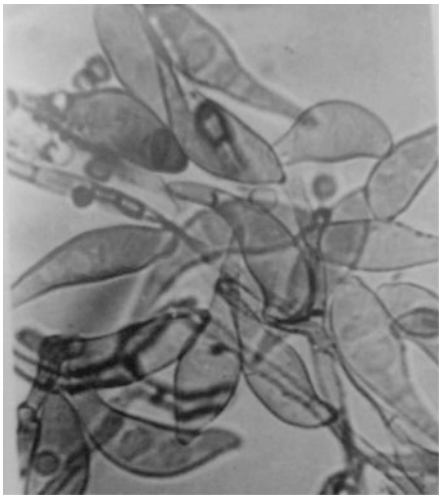

Fig. 16.5 *Drechslera sivanesanii* (× 180)

16.4 Fungi in Diversified Soils and Semi-aquatic Habitats

16.4.1 Soil Profile

The soil is a dynamic medium. The top most layer of the Earth's crust is made up of organic minerals and rock particles. The rock-soil particles do support life. The vertical cross section of the soil which is of some layers running parallel to the surface is known as soil profile. The layers are also called as horizons of the soil. The following are the horizons:

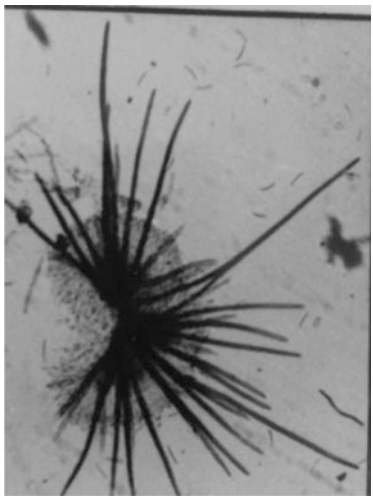

Fig. 16.6 *Colletotrichum capsici* (× 100)

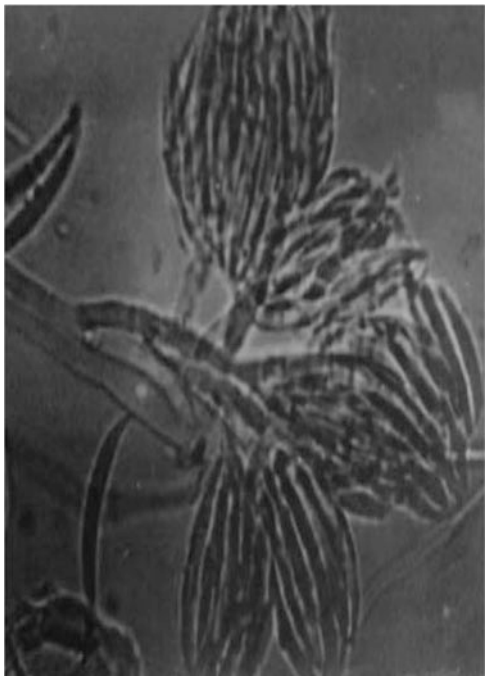

Fig. 16.7 *Fusarium semitectum* (× 180)

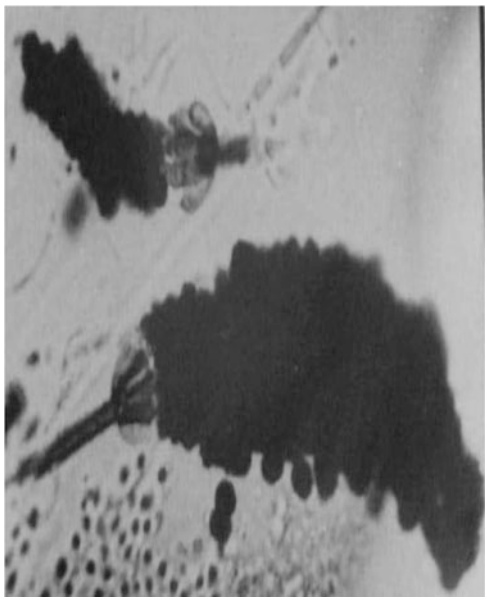

Fig. 16.8 *Memnoniella echinata* (× 180)

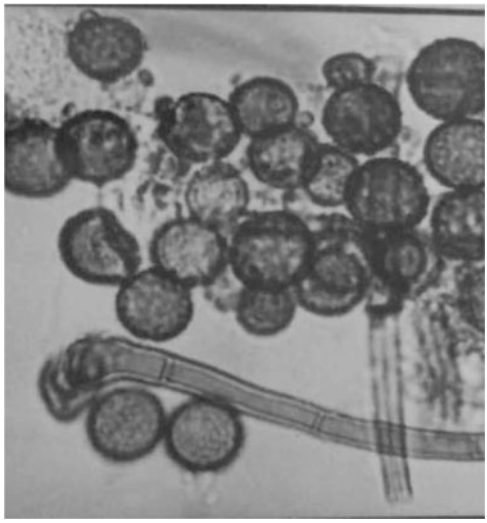

Fig. 16.9 *Periconia byssoides* (× 180)

1. 0-Horizon—consists of organic materials
2. A-Horizon—denotes top soil.
3. B-Horizon—present below "A" is called subsoil.
4. C-Horizon—bedrock or rocky layer.

All the horizons are identified based on soil colour, texture, thickness and other factors (Fig. 16.13).

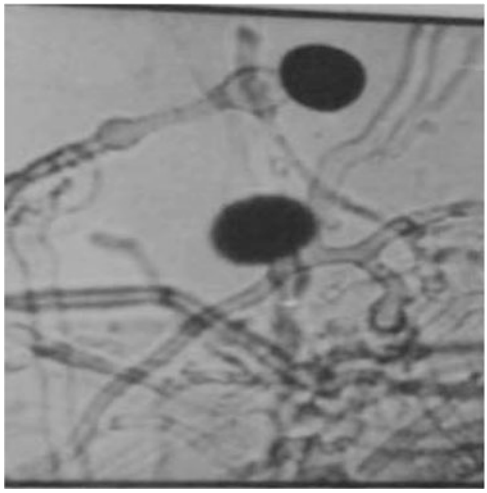

Fig. 16.10 *Nigrospora Oryzae* (× 180)

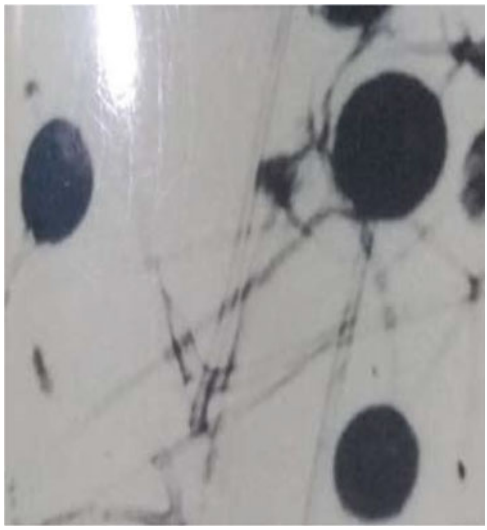

Fig. 16.11 *Rhizopus nigricans* (× 25.2)

Three categories of soil particles, namely sand, silt and clay, were established based on their particle size. An ideal soil contains 25% air, water 25%, minerals 45% and organic matter 5%. The soil types present in India include Entisols, Vertisols, Alfisols, Oxisols, Forest-Mountain soils, Arid-Desert soils, Saline-Alkaline soils and Peaty and Marshy soils.

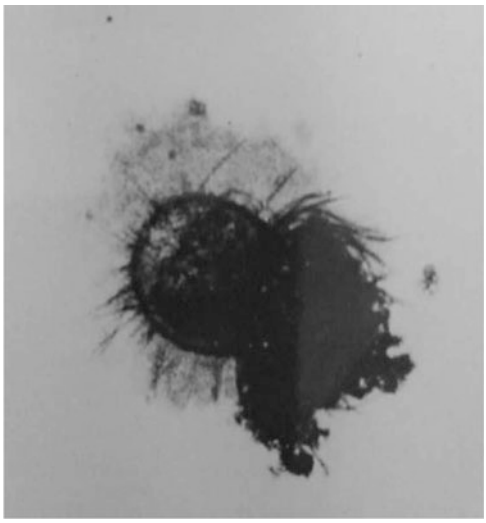

Fig. 16.12 *Chaetomium fusisporale* (× 180)

Table 16.1 List of noteworthy fungi from Indian soils (author's data)

1.	*Achaetomium salami* Rama Rao
2.	*Acremonium strictum* Gams
3.	*Aspergillus Deflectus* Fennel & Raper
4.	*Aspergillus restrictus* Smith
5.	*Beltraniella humicola* Rama Rao
6.	*Byssochlamys nivea* Westling
7.	*Botryotrichum state of Chaetomium* Kunje
8.	*Chondroplea saksenii* Manohar & V.T. Reddy
9.	*Coniochaetidium boothii* (Manohar & Rama Rao) VonArx
10.	*Chaetomium nigricolor* Ames
11.	*Drechslera rostrata (Drechsler)* Richardson & Fraser
12.	*Doratomyces purpureafuscus (Fr)* Morton & Smith
13.	*Eleutherascus lectardii* (Nicot)VonArx
14.	*Hendersonia punithalingamii* V.R.T Reddy and Manohar
15.	*Nectria humicola* Rama Rao
16.	*Periconia saraswatipurensis* Bilgrami
17.	*Periconia atropurpurea* (Berk & Curt) Lit
18.	*Perisporium funiculatum* Preus
19.	*Perisporiopsis melioloides* (Berk & Curt) VonArx
20.	*Phoma fimeti* Brun
21.	*Pithomyces maydis (Sacc.)* Ellis
22.	*Robillarda sessillis* Sacc.
23.	*Robillarda suxenii* Manohar
24.	*Sphaeronema allahabadensis* Chandra & Tandon
25.	*Stemphyliomma terricola* Manohar & Rama Rao
26.	*Trimmatostroma terricola* Manohar et al.
27.	*Tritirachium roseum* VonBeyan

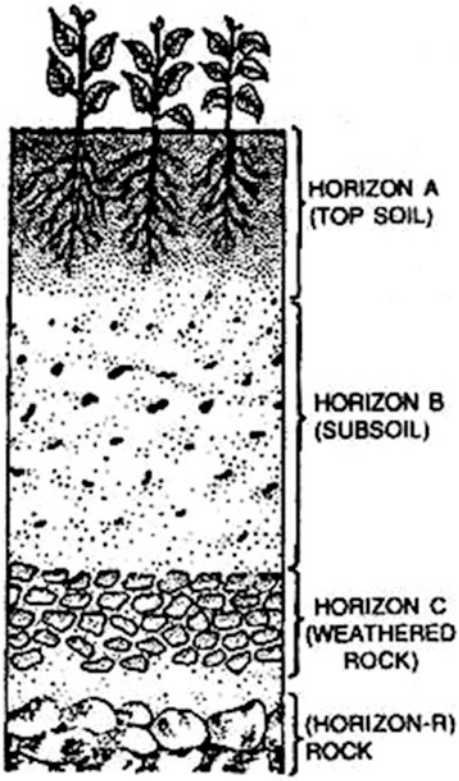

Fig. 16.13 Soil profile (author's diagram)

16.5 Rock Soils and Rock Crevices: Fungi

Copious literature is available on fungi associated with diversified habitats. Little or no information is available on microbes and fungi associated with rock surfaces and soil of rock crevices (Manoharachary 1986).

Rocks form one of the source materials for soil formation. Fungi and other microbes attack rock surfaces and live on the debris deposited on rock surfaces and rock crevices. These fungi and microbes play a major role in the breakdown, mineralization and weathering of rocks which leads to soil particle formation on rock surfaces and soil accumulation in rock crevices. Manoharachary (1986) has surveyed soil samples of rock crevices and rock surfaces from ten regions and isolated the fungi following Wakman's dilution plate and Warcup's soil plate techniques. The fungi that are isolated, cultured and identified from soils of rock surface and rock crevices are as follows:

Alternaria alternata, Aspergillus niger, Aspergillus terreus, Mucor varians, Rhizopus nigricans, Aspergillus terreus, Chaetomium aureum, Cladosporium cladosporioides, Drechslera hawaiiense, Humicola grisea, Nigrospora oryzae, Penicillium turbatum, Trichoderma viride, Fursarium solani and *Memnoniella indica.*

Possibly these fungi might have been involved in the mineralization of the rock materials and soil formation.

16.6 Soil Fungal Ecology

Soil is an important and dynamic medium supporting several living biota including fungi. It is important to mention that soil is composed of minerals like C, N, P, K, Calcium, Sodium, Chlorides, Silica, Fe (iron), Mn, Zn, Cu, Mg and others besides the organic matter. Soil is formed after weathering and breakdown of rocks which is followed by mineralization, humification and other processes. Microbes and fungi play an important role in the decomposition of litter; the decomposed litter and other resultant products enter into the soil. Among layers of the soil profile, A1 Horizon which is approximately of 10 cm harbours a wealth of fungi. The deeper horizons of the soil will not harbour many fungi, and their number along with species composition gets dwindled. Earlier Manoharachary (1977), Manoharachary et al. (1990), Mukerji (1966), Nilina et al. (2007), Ramdayal and Gupta (1968), Ramakrishna et al. (2017), Rao (1965), Saksena (1955, 1967), Subramanian (1973), Subramanya et al. (2016), Uma Maheshwari and Komalavalli (2013), and Vaidehi (1973) have contributed to the ecology of soil fungi along with listing of fungal taxa that they have isolated and identified. Manoharachary et al. (2014) have reported 348 fungal species from diversified soils and soil related habitats from Telangana and Andhra Pradesh (India) (Table 16.2). The above researchers have enlisted the fungi after careful observation and identification.

There are no monographs available from India but for the above handbook and manuals of soil fungi. In view of the above, the author has attempted to collect various soil samples from all over the country and processed them for isolation and identification. Most of such fungi have been enlisted in Table 16.2.

16.7 Fungi in Semi-aquatic Habitats (Muds)

Semi-aquatic habitats include freshwater pond muds, river muds, lake muds, paddy field soils and others. Though they are terrestrial for some time, they get submerged under water for longer periods. Mud stands as a transient stage with aquatic and terrestrial habitats experiencing both anaerobic and aerobic environments. Fungi occurring in these habitats vary depending upon the availability of oxygen/carbon dioxide along with nutrients, rainfall, velocity of water flow, depth of water body, aquatic vegetation and other related factors. Fungi vary both quantitatively and qualitatively in marginal muds and deep mud samples. Very little information is available on such fungi and their ecology from semi-aquatic habitats (Alivelumangamma et al. 1996; Dutta and Ghosh 1965; Madhusudhan Rao and Manoharachary 1981; Rangaswami and Venkatesan 1966). Estuary is the region where the freshwater of the river and the sea meets with each other.

Table 16.2 List of fungi from diversified soils and soil-related habitats from Telangana and Andhra Pradesh

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
1.	*Absidia cylindrospora* Hagem	Soil	2011, Hyderabad	Kunwar	OUFH 830
2.	*Absidia fusca* Linnemann	Pond mud	1983, Hyderabad	Manohar	OUFHS 1
3.	*Absidia glauca* Hagem	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 2
4.	*Absidia spinosa* Lenden.	Forest soil	1973, Vikarabad	Manohar	OUFHS 4
5.	*Achlya debaryana* Humphrey	Pond mud	1979, Hyderabad	Manohar	OUFHS 5
6.	*Achyla recurva* Cornu	Pond mud, soil	1981, Mahaboobnagar	Madhusudan Rao	OUFHS 6
7.	*Acremonium chrysogenum* (Thirum. & Sukap.) Gams	Soil	2005, Mulugu	Kunwar	OUFH 574
8.	*Acremonium implicatum* (J.C. Gilman & E.V. Abbott) W. Gams	Soil	2004, Narsapur	Manohar	OUFHS 7
9.	*Acremonium strictum* W. Gams	Cultivated soil	1984, Ananthagiri hills	Manohar	OUFHS 8
10.	*Acrophialophora fusispora* (S.B. Saksena) Samson	Soil (eggplant)	1977, Hyderabad	Manohar	OUFHS 9
11.	*Actinocladium rhodosporum* Ehrenberg	Soil	2006, Mulugu	Kunwar	OUFH 575
12.	*Allomyces anomalus* R. Emers	Pond mud	1983, Karimnagar	Manohar	OUFHS 10
13.	*Allomyces arbuscula* Butler, E.J. Butler	Forest soil	1981, Maha-boobnagar	Madhusudan Rao	OUFHS 11
14.	*Alternaria alternata* (Fr.) Keissl	Soil (*Sesamum*)	1977, Hyderabad	Manohar	OUFHS 12
15.	*Alternaria brassicicola* (Schwein.) Wiltshire	Rhizosphere soil (Cluster bean)	1978, Hyderabad	Manohar	OUFHS 13
16.	*Alternaria humicola* Oudem	Pond mud	1976, Vikarabad	Manohar	OUFHS 14
17.	*Alternaria radicina* Meier	Soil	2002, Vikarabad	Kunwar	OUFH 004
18.	*Alternaria tenuissima* (Kunze) Wiltshire	Forest soil	1981, Mahaboobnagar	Manohar	OUFHS 15
19.	*Amorphotheca resinae* Pabery	Forest soil	1989, Mannanur	Manohar	OUFHS 16

20.	*Arachniotus* sp.	Soil	2006, Papikonda	Nagaraju	OUFH 429
21.	*Ardhchandra selenoides* (de Hoog) Subram. & Sudha	Rhizosphere soil	2008, Vikarabad	Kunwar	OUFH 573
22.	*Arthrinium euphorbiae* Ellis	Soil	2006, Mothugudem	Kunwar	OUFH460
23.	*Arthrinium phaeospermum* (Corda) M.B.Ellis	Field soil (cabbage)	1970, Hyderabad	Padma	OUFHS 18
24.	*Arthrobotrys foliicola* Matsuchima	Soil	2008, Narsapur	Kunwar	OUFH 572
25.	*Ascochyta graminicola* Saccardo	Forest soil	1973, Vikarabad	Manohar	OUFHS 19
26.	*Aspergillus aculeatus* Lizuka	Rhizosphere soil (*Sorghum*, cotton)	2001, Narsapur	Kunwar	OUFH 025
27.	*Aspergillus amstelodami* (Mangin) Thom & Church	Forest Soil	2004, Vikarabad	Manohar	OUFHS 21
28.	*Aspergillus awamori* Nakaz	Soil (Tamarind)	2005, Hyderabad	Kunwar	OUFH 340
29.	*Aspergillus brunneouniseriatus* Singh & Bakshi	Forest soil	1989, Amrabad, Mannanur	Manohar	OUFHS23
30.	*Aspergillus caespitotus* Raper & Thom	Mud soil	2000, Karimnagar	Shantha Devi	OUFHS 24
31.	*Aspergillus candidus* Link	Pond mud	1979, Nizamabad	Manohar	OUFHS 25
32.	*Aspergillus clavatus* Desm.	Polluted pond mud	1993, Hyderabad	Narendra	OUFHS 27
33.	*Aspergillus deflectus* Fennell & Raper	Forest, wild, cultivated soils	1974, Anantagiri hills	Manohar	OUFHS 28
34.	*Aspergillus fischeri* var. *spinosus* Raper & Fennell	Pond mud	1983, Kakinada	Manohar	OUFHS 30
35.	*Aspergillus flavipes* (Bainier & Sartory) Thom & Church	Forest, wild, cultivated soils	1974, Vikarabad	Manohar	OUFHS 31
36.	*Aspergillus flavus* Link	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 32
37.	*Aspergillus flavus* var. *columnaris* Raper & Fennell	Soil	2001, Hyderabad	Manohar	OUFHS 33
38.	*Aspergillus flavus* var. *oryzae* (Ahlb.) Kurtzman, Smiley, Robnett & Wicklow	Cultivated soil (castor)	1981, Hyderabad	Ramarao	OUFHS 34
39.	*Aspergillus fumigatus* Fresen.	Forest, wild, cultivated soils	1974, Anantagiri hills	Manohar	OUFHS 35
40.	*Aspergillus funiculosus* Sm.	Soil (spinach)	1995, Medak	Padma	OUFHS 36

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
41.	*Aspergillus humicola* Chaudhuri & Sachar	Soil (spinach)	1995, Medak	Padma	OUFHS 37
42.	*Aspergillus japonicus* Saito	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 38
43.	*Aspergillus kanagawaensis* Nehia	Scrub jungle forest soil	1977, Vikarabad	Manohar	OUFHS 39
44.	*Aspergillus nidulans* (Eidam) G. Wint.	Mud Soil	1996, Khammam	Manohar	OUFHS 40
45.	*Aspergillus nidulans* var. *echinulatus* Fennell & Raper	Forest soil	2004, Narsapur	Manohar	OUFHS 41
46.	*Aspergillus niger* Tiegh	Forest soil	1974, Anantagiri hills	Manohar	OUFHS 42
47.	*Aspergillus nidulans* var. *latus* Thom & Raper	Soil	2001, Hyderabad	Kunwar	OUFH 009
48.	*Aspergillus ochraceus* Wilh.	Soil (spinach)	1995, Rangareddy distt.	Manohar	OUFHS 44
49.	*Aspergillus niveus* Blochwitz	Soil	2002, Hyderabad	Kunwar	OUFH 049
50.	*Aspergillus parasiticus* Speare	Soil	2004, Hyderabad	Manohar	OUFHS 45
51.	*Aspergillus repens* (Corda) Sacc.	Pond mud	1983, Hyderabad	Manohar	OUFHS 46
52.	*Aspergillus restrictus* Sm.	Forest, wild, cultivated soils	1989, Amrabad	Manohar	OUFHS 47
53.	*Aspergillus ruber* Thom & Church	Forest soil	2004, Vikarabad	Manohar	OUFHS 48
54.	*Aspergillus rugulosus* Thom & Raper	Soil	2009, Bhradachalam	Nagaraju	OUFH 646
55.	*Aspergillus stellatus* Curzi	Forest soil	1981, Mahaboobnagar	Manohar	OUFHS 49
56.	*Aspergillus stellatus* Curzi var. *stellatus*	Soil	2001, Hyderabad	Kunwar	OUFH 024
57.	*Aspergillus sulphureus* Desm.	Mud soil	2003, Karimnagar	Shantha Devi	OUFHS 50
58.	*Aspergillus sydowi* (Bainier & Sartory) Thom & Church	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 51
59.	*Aspergillus tamarii* Kita	Forest soil	1989, Mannanur	Manohar	OUFHS 52

60.	*Aspergillus terreus* Thom	Pond mud	1979, Vikarabad	Manohar	OUFHS 53
61.	*Aspergillus unguis* (Weil & L. Gaudin)	Forest, wild, cultivated soils	1974, Anantagiri hills, Vikarabad	Manohar	OUFHS 54
62.	*Aspergillus ustus* (Bain.) Thom & Church	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 55
63.	*Aspergillus versicolor* (Vuill.) Tiraboschi	Forest, wild, cultivated soils	1984, Anantagiri hills, Vikarabad	Manohar	OUFHS 56
64.	*Athelia rolfsii* (Curzi) Tu & Kimbr. (= *Sclerotium rolfsii*)	Field soil	2004, Hyderabad	Manohar	OUFHS 57
65.	*Aureobasidium pullulans* (de Bary) Arnaud	Forest soil	1989, Amrabad	Manohar	OUFHS 58
66.	*Bartalinia robillardoides* Tassi	Forest soils	1984, Vikarabad	Manohar	OUFHS 59
67.	*Beltrania rhombica* Penz.	Wild soil	2002, Narsapur	Narsimha-charyulu	OUFHS 60
68.	*Beltrania santapaui* Piroz. & S.D. Patil	Soil	2006, Narsapur	Kunwar	OUFH 535
69.	*Betraniella humicola* Ramarao	Soil	2008, Bayyaram	Kunwar	OUFH 569
70.	*Betraniella odinae* Subram.	Rhizosphere soil (*Vitis vinifera*)	1965, Hyderabad	Rafia Mehdi	OUFHS 62
71.	*Bipolaris papendorfii* (Aa) Alcorn	Rhizosphere soil (peanut)	1978, Hyderabad	Ravindra-nath	OUFHS 63
72.	*Blakeslea trispora* Thaxt.	Forest soil	1981, Amrabad	Reddy	OUFHS 64
73.	*Bloxamia nilagirica* (Subam.) Nag Raj & W. B. Kendr.	Forest soil	1981, Mahaboobnagar	Manohar	OUFHS 65
74.	*Botryotrichum piluliferum* Sacc. & March.	Forest soil	1989, Mannanur	Manohar	OUFHS 66
75.	*Byssochlamys nivea* Westling	Forest soil	1984, Vikarabad	Manohar	OUFHS 67
76.	*Candida albicans* (C.P. Robin) Berkhout	Polluted pond mud	1993, Hyderabad	Narendra babu	OUFHS 68
77.	*Cephaliophora irregularis* Thaxter	Forest soil	1981, Mahaboobnagar	Manohar	OUFHS 69
78.	*Cephalotrichum microsporum* (Sacc.) P.M. Kirk (=*Doratomyces microsporus*)	Soil (Spinach)	1995, Hyderabad	Padma	OUFHS70

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
79.	*Ceratocystis paradoxa* (Dade) C. Moreau (=*Thielaviopsis paradoxa*)	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 71
80.	*Chaetomella raphigera* Swift	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 72
81.	*Chaetomium abuense* Lodha	Soil	2004, Hyderabad	Kunwar	OUFH 237
82.	*Chaetomium amberpetense* Ramarao & Ram Reddy	Cultivated soil (castor)	1994, Hyderabad	Manohar	OUFHS73
83.	*Chaetomium atrobrunneum* Udagawa & Takada	Forest soil	1994, Mahaboobnagar	Manohar	OUFHS 74
84.	*Chaetomium arcuatum* Rai & Tewari	Soil	2009, Vikarabad	Kunwar	OUFH 562
85.	*Chaetomium aureum* Chivers	Pond mud	1983, Hyderabad	Manohar	OUFHS 75
86.	*Chaetomium bostrychoides* Zopf.	Soil	2001, Hyderabad	Kunwar	OUFH 017
87.	*Chaetomium ciliatum* Bonord	Soil	2001, Hyderabad	Kunwar	OUFH 018
88.	*Chaetomium convolutum* Chivers	Soil (coriander)	1994, Hyderabad	Manohar	OUFHS 77
89.	*Chaetomium erraticum* Ames	Soil	2008, Mulugu	Kunwar	OUFH 565
90.	*Chaetomium funicola* Cooke	Polluted pond mud	1993, Hyderabad	Narendra Babu	OUFHS 78
91.	*Chaetomium fusisporale* Rai & Mukerji	Soil	2008, Mulugu	Kunwar	OUFH 568
92.	*Chaetomium globosum* Kunze	Paddy field soil	1979, Nanded, Nizamabad	Manohar	OUFHS 79
93.	*Chaetomium gracile* Udagawa	Soil (*Sesamum*)	1977, Hyderabad	Manohar	OUFHS 80
94.	*Chaetomium homopilatum* Omvik	Forest soil	1989, Mannanur	Manohar	OUFHS 81
95.	*Chaetomium indicum* Corda	Polluted pond mud	1993, Hyderabad	Narendra Babu	OUFHS 82
96.	*Chaetomium mollicellum* L.M. Ames	Soil	1994, Guntur	Manohar	OUFHS 83
97.	*Chaetomium nigricolor* L.M. Ames	Soil	1994, Amrabd	Manohar	OUFHS 84

98.	*Chaetomium osmaniae* Rama Rao & Ram Reddy	Soil	1967, Hyderabad	Ramarao	OUFHS 85
99.	*Chaetomium pachypodioides* L.M. Ames	Forest soil	1994, Bhadrachalam	Manohar	OUFHS 86
100.	*Chaetomium reflexum* Skolko & J. W. Groves	Forest soil	1994, Paloncha	Manohar	OUFHS 87
101.	*Chaetomium salami* Rama Rao	Dune Soil	1985, Chirala Coast	Ramarao	OUFHS 88
102.	*Chaetomium spirale* Zopf.	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 89
103.	*Chaetomium subterraneum* Swift & Povah	Rhizosphere soil (grape)	1965, Hyderabad	Rafai Mehdi	OUFHS 90
104.	*Chaetomium trilaterale* Chivers	Cultivated soil	1994, Warangal	Manohar	OUFHS 91
105.	*Choanephora cucurbitarum* (Berk. & Ravenel) Thaxt.	Rhizosphere, soil (spinach)	1995, Hyderabad	Padma	OUFHS 92
106.	*Circinella muscae* (Sorokin) Berl. De Toni	Cultivated soil (castor)	1981, Hyderabad	Ramarao	OUFHS 93
107.	*Circinella simplex* Tiegh.	Silty, clay, loamy, cultivated soil	1979, Nanded, Nizamabad	Manohar	OUFHS 94
108.	*Circinotrichum maculiforme* C.G. Nees ex Persoon	Soil	2008, Warangal	Kunwar	OUFH 584
109.	*Cladosporium cladosporioides* (Fresen.) de Vries	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 95
110.	*Cladosporium herbarum* (Pers.) Link	Rhizosphere soil (*Ocimum abscendens*)	1975, Hyderabad	Manohar	OUFHS 96
111.	*Cladosporium macrocarpum* Preuss	Polluted pond mud	1993, Hyderabad	Narendra Babu	OUFHS 97
112.	*Cladosporium oxysporum* Berk. M.A. Curtis	Pond mud	1983, Hyderabad	Manohar	OUFHS 98
113.	*Cladosporium sphaerospermum* Penz.	Rhizosphere soil (spinach)	1995, Hyderabad	Padma	OUFHS 99
114.	*Cladosporium spongiosum* Berk. & M.A. Curtis	Polluted pond mud	1993, Hyderabad	Narendra Babu	OUFHS 100
115.	*Cladosporium variabile* (Cooke) G.A. de vries	Polluted pond mud	1993, Hyderabad	Narendra Babu	OUFHS 101
116.	*Cochliobolus australiensis* (Tsuda & Ueyama) Alcorn (=*Drechslera australiensis*)	Forest, wild, cultivated soils	1984, Ananthagiri hills	Manohar	OUFHS 102

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
117.	*Cochliobolus geniculatus* R. Nelson (=*Curvularia geniculata*)	Soil (cashew nuts)	1980, Hyderabad	Ramarao	OUFHS 103
118.	*Cochliobolus hawaiiensis* Alcorn (=*Drechslera hawaiiensis*)	Coastal soil	1969, Chirala	Lakshmi-narsim-ham	OUFHS 104
119.	*Cochliobolus lunatus* R.R. Nelson & Haasis (=*Curvularia lunata*)	Pond mud	1974, Hyderabad	Manohar	OUFHS 105
120.	*Cochilobolus nodulosus* Luttr. (=*Helminthosporium nodulosum*)	Forest soil	1989, Amrabad, Mannanur	Manohar	OUFHS 106
121.	*Cochliobolus spicifer* R.R. Nelson	Riverbed soil	1979, Gadwal	Manohar	OUFHS 107
122.	*Cochliobolus tuberculatus* Sivan (=*Curvularia tuberculata*)	Rhizosphere soil (castor)	1981, Gadwal	Ramarao	OUFHS 108
123.	*Colletotrichum capsici* (Syd.) E. J. Butler & Bisby	Rhizosphere, nonrhizo. soil (castor)	1981, Hyderabad	Ramarao	OUFHS 109
124.	*Colletotrichum dematium* (Pers.) Goove	Soil (*Brassica*)	1981, Hyderabad	Ramarao	OUFHS 110
125.	*Coniochaetidium boothii* (Manohar & P.Rama Rao) Dania García, Stchigel & Guarro	Pond mud soil	1973, Vikarabad	Manohar	OUFHS 111
126.	*Corynascus sepedonium* (C.W. Emmons) Arx	Soil	1964, Hyderabad	Ramarao	OUFHS 112
127.	*Coryneum terrophilum* (Goos & Morris) Sutton (=*Murogenella terrophila*)	Rhizosphere soil (paddy)	1967, Visakapatnam	Ramarao	OUFHS 113
128.	*Cunninghamella blakesleeana* Lendner	Pond mud	1976, Hyderabad	Manohar	OUFHS 114
129.	*Cunninghamella echinulata* (Thaxt.) Thaxt. ex Blakeslee	Cultivated soil	1976, Nanded	Manohar	OUFHS 115

130.	*Curvularia brachyspora* Boedijin	Forest soil	1989, Amrabad	Manohar	OUFHS 116
131.	*Curvularia clavata* B.L. Jain	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 117
132.	*Curvularia lunata* var. *aeria* (Bat. J.A. Lima & C.T. Vasconc.) M. B. Ellis	Forest, wild, cultivated soils	1984, Ananthagiri hills	Manohar	OUFHS 118
133.	*Curvularia prasadii* Mathur & Mathur	Soil (spinach)	1989, Hyderabad	Manohar	OUFHS 119
134.	*Curvularia trifolii* (Kauffman) Boedijin	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 120
135.	*Cylindrocladium parvum* P.J. Anderson	Forest soil	1990, Kurnool	Kunwar	OUFH 126
136.	*Dendryphiella vinosa* (Berk. & M. A. Curtis) Reisinger	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 122
137.	*Dictyoarthrinium sacchari* (J.A. Stev.) Damon	Pond mud soil	1974, Vikarabad	Manohar	OUFHS 123
138.	*Didymosphaeria igniaria* Booth	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 124
139.	*Didymosphaeria sadasivanii* (T.K.R. Reddy) Kowalski	Dryland soil	1979, Medak	Ramarao	OUFHS 125
140.	*Eleutherascus lectardii* (Nicot) Arx	Pond mud	1972, Vikarabad	Manohar	OUFHS 126
141.	*Emericellopsis minima* Stolk	Forest, garden soil	1977, Khammam	Manohar	OUFHS 128
142.	*Epicoccum nigrum* Link	Forest soil	1989, Amrabad	Manohar	OUFHS 129
143.	*Eupenicillium brefeldianum* (B.O. Dodge) Stolk & D. B. Scott	Soil	1974, Mahaboobnagar	Manohar	OUFHS 130
144.	*Eurotium chevalieri* Mangin	Soil (cashew nut)	1980, Hyderabad	Ramarao	OUFHS 132

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
145.	*Fennellia nivea* (B.J. Wiley & E.G. Simmons) Samson	Forest, wild, cultivated soils	1984, Anantagiri hills	Manohar	OUFHS 133
146.	*Fusarium chlamydosporum* Willenw. & Reinking	Riverbank soil	1984, Gadwal	Manohar	OUFHS 134
147.	*Fusarium incarnatum* (Desm.) Sacc.	Forest soil	1981, Mahaboobnagar	Madhusu-dan Rao	OUFHS 135
148.	*Fusarium javanicum* Koord.	Rhizosphere soil, soils (castor)	1981, Hyderabad	Ramarao	OUFHS 136
149.	*Fusarium lateritum* Nees	Soil	2004, Anantagiri	Kunwar	OUFH 243
150.	*Fusarium oxysporum* Schlecht	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 137
151.	*Fusarium poae* (Peck) Wollenw.	Rhizosphere, soil (castor)	1981, Hyderabad	Ramarao	OUFHS 138
152.	*Geosmithia lavendula* (Raper & Fennell) Pitt	Soil	2005, Hyderabad	Manohar	OUFHS 140
153.	*Geosmithia putterillii* (Thom) Pitt	Soil	2008, Vikarabad	Kunwar	OUFH 582
154.	*Geotrichum candidum* Link	Polluted mud	1993, Karimnagar	Narendra Babu	OUFHS 141
155.	*Gibberella acuminata* Booth	Forest Soil	1983, Guntur	Manohar	OUFHS 142
156.	*Gibberella avenacea* Cook	Polluted mud	1993, Karimnagar	Narendra Babu	OUFHS 143
157.	*Gibberella fujikuroi* (Sawada) Wollenw.	Rhizosphere soil, soils (cluster bean)	1978, Hyderabad	Manohar	OUFHS 144
158.	*Gibberella indica* B.Rai & R.S. Upadhyay (= *Fusarium udum*)	Soil	2002, Warangal	Kunwar	OUFH 074

159.	*Gibberella intricans* Wollenw.	Pond mud	1979, Vikarabad	Manohar	OUFHS 146
160.	*Gibberella pulicaris* (Fr.) Sacc.	Forest soil	1989, Mannanur	Manohar	OUFHS 147
161.	*Gibberella zeae* (Schwein.) Petch	Herbicide treated soil	2003, Hyderabad	Sailaja	OUFHS 148
162.	*Gilmaniella humicola* G. L. Barron	Scrub jungle forest soil	1989, Mannanur	Manohar	OUFHS 149
163.	*Gliocladium deliquescens* Sopp	Mud	2000, Karimnagar	Shantha Devi	OUFHS 150
164.	*Graphium penicillioides* Corda	Forest, wild, cultivated soils	1984, Ananthagiri hills	Manohar	OUFHS 152
165.	*Graphium putredinis* (Corda) S. Hughes	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 153
166.	*Graphium terricola* Manohar Rag. Rao, Rehana & Rama Rao	Sea shore soil	1975, Bheemilipatnam	Manohar	OUFHS 154
167.	*Gymnascella dankaliensis* (Castell) Currah	Cultivated soil (pearl millet), forest soil	1977, Gadwal, Mahaboobnagar	Manohar	OUFHS 155
168.	*Haematonectria haematococca* (Berk. & Broome) Samuels & Rossman (= *Fusarium solani*)	Soil	2002, Khammam	Kunwar	OUFH 073
169.	*Hendersonia punithalingamii* Reddy & Manohar	Forest soil	1989, Mannanur	Manohar	OUFHS 156
170.	*Heterocephalum aurantiacum* Thaxt.	Soil	1984, East Godavari	Manohar	OUFHS 157
171.	*Humicola fuscoatra* Traaen	Soil (cashew nut, castor)	1980, Hyderabad	Ramarao	OUFHS 158
172.	*Humicola grisea* Traaen	Riverbank, cultivated soils	1977, Gadwal	Reddy	OUFHS 159

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
173.	*Humicola nigriscens* Omvik	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 160
174.	*Hyphodiscosia jaipurensis* Lodha & K.R.C. Reddy	Soil	2010, Paloncha	Nagaraju	OUFH 690
175.	*Khuskia oryzae* H.J. Huds.	Deciduous forest soil	1981, Mannanur	Reddy	OUFHS 161
176.	*Lasiodiplodia theobromae* (Pat.) Griffiths & Maubl.	Forest soil	1977, Hyderabad	Manohar	OUFHS 162
177.	*Lewia infectoria* (Fuckel) M. E. Barr & E.G. Simmons	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 163
178.	*Lichtheimi corymbifera* (Cohn) Vuill. *(Absidia lichtheimi)*	Soil	2008, Hyderabad	Kunwar	OUFH 527
179.	*Macrophomina phaseolina* (Tassi) Goid.	Rhizosphere, soils (cluster bean)	1978, Hyderabad	Manohar	OUFHS 164
180.	*Magnaporthe grisea* (T.T. Hebert) M.E. Barr	Soil	1968, Nellore	Tilak	OUFHS 165
181.	*Magnaporthe salvinii* (Catt.) R.A. Krause	Pond mud, sea shore soil	1983, Hyderabad Anakapalli	Manohar	OUFHS 166
182.	*Mariannaea elegans* var. *elegans* (Corda) Samson	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 167
183.	*Memnoniella echinata* (Rivolta) Galloway	Pond mud	1983, Hyderabad	Manohar	OUFHS 168
184.	*Microascus brevicaulis* S.P. Abbott	Forest soil	1981, Mahaboobnagar	Madhusudan Rao	OUFHS 169
185.	*Microdochium dimerum* (Penz.) Arx	Forest, wild, cultivated soil	1984, Vikarabad	Manohar	OUFHS 170
186.	*Monatosporella rhizoidea* Vasant Rao & de Hoog	Soil	2008, Vikarabad	Kunwar	OUFH 576
187.	*Monodictys fluctuata* (Tandon & Bilgrami) M. B.Ellis	Pond mud	1979, Vikarabad	Manohar	OUFHS 172

188.	*Monodictys putredinis* (Wall.) S. Hughes	Soil	2003, Hyderabad	Kunwar	OUFH 100
189.	*Mucor bacilliformis* Hesselt.	Soil (peanut)	1965, Tirupati	Manohar	OUFHS 173
190.	*Mucor circinelloides* f. sp. *circinelloides* Tiegh.	Forest soil	2004, Narsapur	Manohar	OUFHS 174
191.	*Mucor fragilis* Bainier	Forest soil	2004, Vikarabad	Manohar	OUFHS 175
192.	*Mucor hiemalis* Wehmer	Soil (cluster bean)	1978, Hyderabad	Manohar	OUFHS 176
193.	*Mucor hiemalis* f. sp. *silvaticus* (Hagem) Schipper	Soil (peanut)	1965, Tirupati	Rao	OUFHS 177
194.	*Mucor lausanensis* Lendn.	Forest soil	2004, Vikarabad	Manohar	OUFHS 178
195.	*Mucor racemosus* Fresen.	Pond mud	1976, Hyderabad	Manohar	OUFHS 180
196.	*Mucor varians* Povah	Riverbank soil	1977, Gadwal	Reddy	OUFHS 181
197.	*Myrothecium cinctum* (Corda) Sacc.	Cultivated soil	1980, Hyderabad	Manohar	OUFHS 182
198.	*Myrothecium gramineum* Lib.	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 183
199.	*Myrothecium leucotrichum* (Peck) M.C. Tulloch	Forest soil	1984, Vikarabad	Manohar	OUFHS 184
200.	*Myrothecium roridum* Tode	Forest, cultivated soils	1984, Ananthagiri	Manohar	OUFHS 185
201.	*Myrothecium verrucaria* (Alb. & Schwein.)	Rhizosphere soil (*Datura fastuosa*)	1975, Hyderabad	Manohar	OUFHS 186
202.	*Nannizzia gypsea* (Nann.) Stockdale	Soil (Poultry farm)	2001, Hyderabad	Sowjanya	OUFHS 187

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
203.	*Nectria hematococca* Berk. & Broome	Forest, wild, cultivated soils	1984, Hyderabad	Manohar	OUFHS 188
204.	*Nectria humicola* Rama Rao	Mazie field soil	1969, Narsapur	Ramarao	OUFHS 189
205.	*Nemania serpens* var. *serpens* (Pers.) Gay	Riverbank soil	1983, Gadwal	Manohar	OUFHS 190
206.	*Neocosmospora vasinfecta* E. F. Sm.	Paddy field soil	1977, Karimnagar	Manohar	OUFHS 191
207.	*Neocosmospora vasinfecta* var. *africana* (Arx) P.F. Cannon & D. Hawksw.	Soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 192
208.	*Neosartorya glabra* (Fennell & Raper) Kozak	Forest soil	2004, Narsapur	Manohar	OUFHS 193
209.	*Paecilomyces lilacinus* (Thom.) Samson	Pond mud	1983, Hyderabad	Manohar	OUFHS 194
210.	*Paecilomyces variotii* Bainier	Soil (cashew nut)	1980, Hyderabad	Ramarao	OUFHS 195
211.	*Paraphoma fimeti* (Brunnaud) Gruyter, Aveskamp & Verkley (= *Phoma fimeti*)	Soil	2006, Hyderabad	Kunwar	OUFH 381
212.	*Penicillium adametzi* Zaleski	Soil	2004, Hyderabad	Manohar	OUFHS 196
213.	*Penicillium aurantiogriseum* Dierckx	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 197
214.	*Penicillium chrysogenum* Thom	Forest soil	1981, Mahaboobnagar	Madhusu-dan Rao	OUFHS 198
215.	*Penicillium citreonigrum* Dierckx	Forest soil	2002, Vikarabad	Narsimha-charyulu	OUFHS 199

216.	*Penicillium citrinum* Thom	Cultivated soil	1979, Nanded	Manohar	OUFHS 200
217.	*Penicillium coeruleum* Sopp	Soil	2011, Vikarabad	Kunwar	OUFH 800
218.	*Penicillium commune* Thom	Forest, wild, cultivated soils	1984, Ananthagiri	Manohar	OUFHS 201
219.	*Penicillium corylophyllum* Dierckx	Soil (cashew nut)	1980, Hyderabad	Ramarao	OUFHS 202
220.	*Penicillium decumbens* Thom	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 203
221.	*Penicillium digitatum* (Pers. & Fr.) Sacc.	Mud soil	1996, Karimnagar	Alivelu-manga-mma	OUFHS 204
222.	*Penicillium duclauxii* Delacr.	Forest soil	2004, Narsapur	Manohar	OUFHS 206
223.	*Penicillium funiculosum* Thom	Forest, wild, cultivated soils	1984, Ananthagiri	Manohar	OUFHS 207
224.	*Penicillium glabrum* (Wehmer) Westling	Forest soil	2004, Narsapur	Manohar	OUFHS 208
225.	*Penicillium herquei* Bainier & Satory	Rhizosphere, soil (spinach)	1995, Hyderabad	Padma	OUFHS 209
226.	*Penicillium humuli* J.F.H. Beyma	Rhizosphere soil	2010, Karimnagar	Kunwar	OUFH 696
227.	*Penicillium implicatum* Biourge	Mud	2000, Karimnagar	Shantha Devi	OUFHS 210
228.	*Penicillium islandicum* Sopp	Forest, wild, cultivated soils	1984, Vikarabad	Manohar	OUFHS 211
229.	*Penicillium italicum* Stoll	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 212
230.	*Penicillium lividum* Westling	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 214

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
231.	*Penicillium miczynskii* K.M. Zaleski	Maize field soil	2002, Vikarabad	Narsimha-charyulu	OUFHS 216
232.	*Penicillium novae-zeelandiae* Beyma	Forest soil	2002, Vikarabad	Narsimha-charyulu	OUFHS 217
233.	*Penicillium olivicolor* Pitt	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 218
234.	*Penicillium oxalicum* Currie & Thom	Rhizosphere & nonrhizo. soils (spinach)	1995, Hyderabad	Padma	OUFHS 219
235.	*Penicillium purpurogenum* Stoll	Seashore soil (*Casuarina*)	1996, Anakapalli	Chandra Mohan	OUFHS 220
236.	*Penicillium restrictum* Gilman & Abbott	Forest soil	1981, Mahaboobnagar	Madhus-udan Rao	OUFHS 221
237.	*Penicillium roseopurpureum* Dierckx	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 222
238.	*Penicillium rubrum* Stoll	Forest, wild, cultivated soils	1984, Ananthagiri	Manohar	OUFHS 223
239.	*Penicillium rugulosum* Thom	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 224
240.	*Penicillium simplicissimum* (Oudem.) Thom	Soil	1965, Tirupati	Rao	OUFHS 225
241.	*Penicillium spinulosum* Thom	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 226
242.	*Penicillium thomii* Maire	Mud soil	2000, Karimnagar	Shantha Devi	OUFHS 227
243.	*Penicillium turbatum* Westling	Pond mud	1979, Vikarabad	Manohar	OUFHS 228

244.	*Penicillium variabile* Wehmer	Rhizosphere & nonrhizo. soils (castor)	1981, Hyderabad	Ramarao	OUFHS 229
245.	*Penicillium vinaceum* Gilman & Abbott	Pond mud soil	1974, Hyderabad	Manohar	OUFHS 230
246.	*Penicillium viridicatum* Westling	Forest soil	2004, Narsapur	Manohar	OUFHS 231
247.	*Periconia atropurpurea* (Berk. M.A. Curtis) M.A Litv.	Pond mud	1977, Vikarabad	Manohar	OUFHS 232
248.	*Periconia hispidula* (Pers.) E.W. Mason & M.B. Ellis	Forest soil	2004, Bhadrachalam	Manohar	OUFHS 233
249.	*Periconia saraswatipurensis* Bilgrami	Pond mud	1974, Vikarabad	Manohar	OUFHS 234
250.	*Pestalotiopsis glandicola* (Castagne) Steyaert	Rhizosphere soil (grape)	1964, Hyderabad	Ramarao	OUFHS 235
251.	*Pestalotiopsis mangiferae* (Henn.) Steyaert	Soil	1964, Hyderabad	Ramarao	OUFHS 236
252.	*Phaeoisariopsis pubescens* (Cooke & Ellis) M.B. Ellis	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 237
253.	*Phoma eupyrena* Sacc.	Pond mud	1977, Gadwal	Reddy	OUFHS 238
254.	*Phoma fimeti* Brunaud	Pond mud soil	1975, Vikarabad	Manohar	OUFHS 239
255.	*Phoma glomerata* (Corda) Wollenw. & Hochapfel	Soils (*Datura fastuosa*)	1979, Nanded	Manohar	OUFHS 240
256.	*Phoma herbarum* Cooke	Freshwater tank mud	1996, Karimnagar	Alivelu-manga-mma	OUFHS 241
257.	*Phoma hibernica* Grimes, M. O'Connor & Cummins	Freshwater tank mud	1996, Karimnagar	Alivelu-manga-mma	OUFHS 242

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
258.	*Phoma humicola* J.C. Gilman & F.V. Abbott	Deciduous forest soil, garden soil	1975, Nanded	Manohar	OUFHS 243
259.	*Phoma nebulosa* (Pers.) Berk.	Seashore soil (*Casuarina*)	1996, Anakapalli	Chandra Mohan	OUFHS 244
260.	*Phoma terricola* (=*Pyrenochaeta decipens*) Boerema	Pond mud soil	1974, Hyderabad	Manohar	OUFHS 245
261.	*Phytopthora palmivora* (E.J. Butler) E.J. Butler	Field soil (*Colocasia*)	1980, Hyderabad	Satya Prasad	OUFHS 246
262.	*Pithomyces maydicus* (Sacc.) M.B. Ellis	Pond mud soil	1974, Hyderabad	Manohar	OUFHS 247
263.	*Pithomyces sacchari* (Speg.) M.B. Ellis	Pond mud	2000, Karimnagar	Shantha Devi	OUFHS 248
264.	*Pithomyces terricola* (Manohar & Ramarao) P.M. Kirk	Pond mud	1979, Vikarabad	Manohar	OUFHS 249
265.	*Preussia funiculata* Fuckel (=*Perisporium funiculatum*)	Forest soil	1981, Mahaboobnagar	Madhusu-dan Rao	OUFHS 250
266.	*Pseudeurotium ovale* Stolk	Rhizosphere soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 251
267.	*Pseudocochliobolus eragrostidis* Tsuda & Ueyama (Anamorph *Curvularia eragrostidis*)	Forest soil	1989, Amrabad	Manohar	OUFHS 252
268.	*Pseudocohliobolus pallescens* Tsuda & Ueyama (Anamorph *Curvularia pallescens*)	Forest, wild, cultivated soils	1984, Ananthagiri	Manohar	OUFHS 253
269.	*Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (=*Paecilomyces lilacinus*)	Soil	2005, Hyderabad	Kunwar	OUFH 280
270.	*Pythium acanthicum* Dechsler, J. Wash.	Scrub jungle soil	1975, Vikarabad	Manohar	OUFHS 255

271.	*Pythium aphanidermatum* (Edson) Fitzp.	Pond mud	1976, Hyderabad	Manohar	OUFHS 256
272.	*Pythium butleri* Subram.	Scrub jungle soil	1975, Vikarabad	Manohar	OUFHS 257
273.	*Pythium carolinianum* Matthews	Pond mud	1983, Hyderabad	Manohar	OUFHS 258
274.	*Pythium debaryanum* R. Hesse	Forest soils	1975, Vikarabad	Manohar	OUFHS 259
275.	*Pythium echinulatum* Matthews	Forest soil, pond mud	1975, Vikarabad	Manohar	OUFHS 260
276.	*Pythium elongatum* Mathews	Pond mud	1978, Hyderabad	Manohar	OUFHS 261
277.	*Pythium mamillatum* Meurs	Deciduous forest soil	1975, Pakhal	Manohar	OUFHS 262
278.	*Pythium middletoni* Sparrow	Pond mud soil	1975, Vikarabad	Manohar	OUFHS 263
279.	*Pythium myriotylum* Drechsler	Scrub jungle soil	1975, Vikarabad	Manohar	OUFHS 264
280.	*Pythium spinosum* Sawada	Riverbank soil	1981, Gadwal	Reddy	OUFHS 265
281.	*Rhizomucor miehei* (Cooney & R. Emers.) Schipper (=*Mucor miehei*)	Soil	1984, Warangal	Veugopal Rao	OUFHS 266
282.	*Rhizomucor pusillus* (Lindt) Schipper (=*Mucor pusillus*)	Soils	1984, Warangal	Venugo-pal Rao	OUFHS 267
283.	*Rhizopus arrhizus* A. Fish. var. *arrhizus*	Rhizosphere soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 268
284.	*Rhizopus microsporus* var. *chinensis* (Saito) Schipper & Stalpers	Forest soil	2004, Vikarabad	Manohar	OUFHS 269
285.	*Rhizopus stolonifer* var. *stolonifer* (Ehrenb.) Vuill.	Pond mud	1976, Vikarabad	Manohar	OUFHS 270

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
286.	*Robillarda sessilis* (Sacc.) Sacc.	Pond mud soil	1974, Vikarabad	Manohar	OUFHS 271
287.	*Robillarda suxenae* Manohar & Rama Rao	Forest soil	1973, Vikarabad	Manohar	OUFHS 272
288.	*Sagenomella diversispora* (van Beyma) W. Gams	Soil	2004, Hyderabad	Manohar	OUFHS 273
289.	*Saprolegnia monoica* Pringsheim	Maize field soil	1964, Hyderabad	Ramarao	OUFHS 274
290.	*Scolecobasidium constrictum* E.V. Abbott	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 275
291.	*Scolecobasidium humicola* G.L. Barron & L. V. Busch	Soil (spinach)	1995, Hyderabad	Padma	OUFHS276
292.	*Scolecobasidium tshawytschae* (Doty & D.W. Slater) Mcginnis & Ajello	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 277
293.	*Scopulariopsis brumptii* Salv.-Duval	Rhizosphere soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 278
294.	*Setosphaeria rostrata* K.J. Leonard (=*Drechslera halodes*)	Forest, wild, cultivated soil	1984, Anantagiri hills	Manohar	OUFHS 279
295.	*Sordaria fimicola* (Oberge ex. Desm.) Ces. & De Not.	Pond mud	1977, Vikarabad	Manohar	OUFHS 280
296.	*Spegazzinia lobulata* Thrower	Pond mud	1974, Vikarabad	Manohar	OUFHS 281
297.	*Sphaeronema allahabadensis* Chandra and Tandon	Uncultivated soil	1975, Hyderabad	Manohar	OUFHS 282
298.	*Sphaeronema spinella* Kalchbrenner	Rhizosphere soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 283
299.	*Sporotrichum pruinosum* J.C. Gilman & E.V. Abbott (=*Chrysosporium pruinosum*)	Field soil (maize)	2004, Hyderabad	Manohar	OUFHS 287

300.	*Stachybotrys atra* Corda	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 288
301.	*Stachybotrys bisbyi* (Sriniv.) G.L. Barron	Forest soil	1981, Mahaboobnagar	Madhusudan Rao	OUFHS 289
302.	*Stachybotrys chartarum* (Ehrenb.) S. Hughes	Cultivated soil (castor)	1981, Hyderabad	Ramarao	OUFHS 290
303.	*Stachybotrys cylindrospora* C.N. Jensen	Soil	1974, Hyderabad	Manohar	OUFHS 291
304.	*Stachybotrys parvispora* S. Hughes	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 292
305.	*Stachybotrys pulchra* Speg.	Polluted soil	1993, Hyderabad	Narendra Babu	OUFHS 293
306.	*Stachylidium bicolor* Link	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 294
307.	*Stachylidium extorrea* var. *majus* Berl.	Rhizosphere soil	1988, Hyderabad	Mahmood	OUFHS 295
308.	*Staphylotrichum coccosporum* Meyer & Nicot	Soil	1965, Tirupati	Rao	OUFHS 296
309.	*Synephalastrum racemosum* Cohn ex. J. Schrot.	Cultivated soil	1979, Nanded	Manohar	OUFHS 297
310.	*Talaromyces funiculosus* (Thom) Samson, Yilmaz, Frisvad & Seifert (= *Penicillium funiculosum*)	Soil	2010, Hyderabad	Kunwar	OUFH 688
311.	*Talaromyces luteus* (Zukal) C.R. Benjamin (Anamorph *Penicillium leuteum*)	Soil (castor)	1981, Hyderabad	Ramarao	OUFHS 298
312.	*Talaromyces trachyspermus* (Samson & Abdel-Fattah) Yaguchi	Forest soil	2004, Narsapur	Manohar	OUFHS 299
313.	*Talaromyces varians* (G. Sm.) Samson, Yilmaz & Frisvad (= *Penicillium varians*)	Lemon rhizosphere soil	2006, Hyderabad	Kunwar	OUFH 398
314.	*Thanatephorus cucumeris* (A.B. Frank) Donk	Maize field soil	1969, Hyderabad	Laxminarsimhan	OUFHS 300

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
315.	*Thermoascus aurantiacus* Miehe	Soil	1984, Rampachoda-varam	Venugo-pal Rao	OUFHS 301
316.	*Thermomyces lanunginosus* Tsikl.	Soil	1984, Vijayanagaram	Venugo-pal Rao	OUFHS 302
317.	*Thielavia terricola* (Gilman & Abbott) Emmons	Forest soil	1981, Amrabad	Madhusu-dan Rao	OUFHS 303
318.	*Torula caligans* (Batista & H.P. Upadhyay) M.B. Ellis	Soil	1974, Adilabad	Singh	OUFHS 304
319.	*Torula herbarum* (Pers.) Link	Soil (cluster bean)	1978, Hyderabad	Manohar	OUFHS 305
320.	*Trichoderma asperellum* Samuels, Lieckfeldt, & Nirenberg	Herbicide treated soil	2003, Hyderabad	Sailaja	OUFH 071
321.	*Trichoderma atroviride* karst.	Polluted soils	2003, Hyderabad	Satyavani	OUFHS 307
322.	*Trichoderma aureoviride* Rifai	Forest soil	2002, Vikarabad	Narasimh-acharyulu	OUFHS 308
323.	*Trichoderma citrinoviride* Bisset	Forest soil	2002, Vikarabad	Narasimh-acharyulu	OUFHS 309
324.	*Trichoderma fasciculatum* Bisset	Soils (*Arachis hypogeae*)	2004, Chittor	Srilakshmi	OUFHS 310
325.	*Trichoderma fertile* Bisset	Polluted soils, soils (*Arachis hypogeae*)	2004, Chittor	Srilakshmi	OUFHS 311
326.	*Trichoderma hamatum* (Bonord.) Bainier	Soil	2002, Vikarabad	Kunwar	OUFH 059
327.	*Trichoderma harzianum* Rifai	Forest soil	2001, Chittor	Srilakshmi	OUFHS 312
328.	*Trichoderma konilangbra* Samuels, Petrini & Kubicek	Polluted soil	2003, Hyderabad	Satyavani	OUFH 187
329.	*Trichoderma koningii* Oudem	Forest soil	2001, Chittor	Srilakshmi	OUFHS 314

330.	*Trichoderma longibrachiatum* Rifai	Soil	2004, Perantalapalli, Papikondalu	Nagamani	OUFH 070
331.	*Trichoderma piluliferum* Webster & Rifai	Cultivated soil (pigeon pea)	2001, Hyderabad	Satyavani	OUFHS 316
332.	*Trichoderma polysporum* (Link & Pers.) Rifai	Soil	2004, West Godavari	Nagamani	OUFHS 317
333.	*Trichoderma pseudokoningii* Rifai	Soil	2004, Perantalapalli, Papikondalu	Nagamani	OUFH 069
334.	*Trichoderma reesei* E.G. Simmons	Polluted soil	2003, Hyderabad	Satyavani	OUFHS 319
335.	*Trichoderma strictipilis* Bissett	Polluted soil	2003, Hyderabad	Satyavani	OUFHS 320
336.	*Trichoderma virens* (Miller, Giddens & Foster) von Arx	Forest soil	2004, Vikarabad	Nagamani	OUFHS 321
337.	*Trichoderma viride* Pers. (=*Trichoderma lignorum*)	Soil (*Datura fastuosa*)	1975, Hyderabad	Manohar	OUFHS 322
338.	*Trichothecium roseum* (Pers.) Link	Pond mud	1984, Hyderabad	Manohar	OUFHS 323
339.	*Trichurus spiralis* Hasselbr.	Forest soil	2004, Narsapur	Manohar	OUFHS 324
340.	*Trimmatostroma indicum* Manohar Rag. Rao & Rama Rao	Soil (coffee)	1977, Ananthagiri	Manohar	OUFHS 325
341.	*Tritirachium dependens* Limber	Soil	2004, Hyderabad	Manohar	OUFHS 326
342.	*Tritirachium roseum* J.F.H. Beyma	Soil	1974, Hyderabad	Manohar	OUFHS 327
343.	*Verticillium puniceum* Cooke & Ellis	Soil (spinach)	1995, Hyderabad	Padma	OUFHS 328
344.	*Verticillium terrestre* (Pers.) Sacc.	Rhizosphere soil (grape)	1965, Hyderabad	Rafia Mehdi	OUFHS 329

(continued)

Table 16.2 (continued)

Sl. No.	Fungal species	Substance	Year and place of collection	Collected by	Accession no.
345.	*Westerdykella multispora* (Saito & Minoura ex. Cain) Cejp & Milko	Pond mud	1983, Hyderabad	Manohar	OUFHS 330
346.	*Wojnowicia hirta* Sacc.	Forest soil	2004, Vikarabad	Manohar	OUFHS 331
347.	*Zygorhynchus moelleri* Vuill.	Cultivated soil	1979, Nanded	Manohar	OUFHS 332
348.	*Zygosporium masonii* Hughes	Forest soil	2005, Vikarabad	Kunwar	OUFH 282

Source: Manoharachary et al. 2014. J Indian Bot.Soc. 93 (1–2): 16–34
OUFHS Osmania University Fungal Herbarium–Soil, *Manohar.* Manoharachary

Table 16.3 Fungi from semi-aquatic habitats (author's data)

1.	*Allomyces Arbuscular* Butler
2.	*Achlya debaryana* Humphrey
3.	*Achlya recurva Cornu*
4.	*Alternaria alternata* (FR.) Keissl
5.	*Amorphotheca resinae* Pabery
6.	*Asochyta graminicola* Sacc.
7.	*Aspergillus awamori* Nakaz
8.	*Aspergillus ruber* Thom & Church
9.	*Aspergillus stellatus* Curz
10.	*Aspergillus terreus* Thom
11.	*Chaetomium globosum* Kunze
12.	*Chaetomium indicum Corda*
13.	*Cladosporium macrocarpum* Preuss
14.	*Cladosporum spongiosum* Berk & Curtis
15.	*Coniocheata boothii* (Manohar & Rama Rao) Garcia et al.
16.	*Cunninghamella blakesleeana* Lendener
17.	*Dictyoarthrinium sacchari* (Stev.) Damon
18.	*Geotrichum candidum* Link
19.	*Gibberella avenacea* Cook
20.	*Gliocladium deliquesens* Sopp
21.	*Humicola grisea* Traaen
22.	*Magnaporthe salvinii* (Catt.) Krause
23.	*Mucor racemosus* Fresen
24.	*Nemania serpens (Pers)* Gay
25.	*Neocosmospora vasinfecta* F.F. Sm
26.	*Penicillium restrictum* Gilman & Abbott
27.	*Penicillium turbatum Westling*
28.	*Periconia Sp.*
29.	*Phoma eupyrena* sacc.
30.	*Pithomyces sacchari (Speg)* M.B. Ellis
31.	*Pithomyces terricola* (Manohar & Rama Rao) P.M. Krik
32.	*Pythium carolinianum* Matthews
33.	*Pythium middletoni* Sparrow
34.	*Rhizopus arrhizus* Fish
35.	*Robillarda sessilis* (Sacc.) Sacc
36.	*Sordaria fimicola (Oberge ex Desm) Ces & Denot*
37.	*Spegazzinia lobulata* Thrower
38.	*Trichothecium sp.*
39.	*Zygorhynchus moelleri* Vuill

It is a peculiar habitat where fungi which got adapted to freshwater and seawater are present, thus form amphibic fungi. Fungi listed in Table 16.3 play an important role in the decomposition of litter, cycling of elements, water body productivity,

disease production and food chain cycle. Semi-aquatic habitats being transient get disturbed very frequently because of monsoon rains and water currents; hence the same mycoflora may not exist. Further estuary mud also gets disturbed due to forcible water currents from river and seawater currents of high speed. Therefore, estuary habitat is highly dynamic in its nature. The fungi associated with semi-aquatic habitats particularly from riverbank soil, estuary soil, river mud and estuary mud are listed in Table 16.3.

16.8 Marine and Mangrove Mud Fungi

Marine habitats are known to possess deep mud and marginal mud and also high saline water. A variety of fungi representing hyphomycetes, coelomycetes, ascomycetes, chydrits and a few basidiomycetes are known to occur in saline waters. Marine fungi are pantropical or pan-temperate in distribution. Marine mud fungi are the major decomposers of woody, herbaceous materials followed by degradation of cellulose, lignin and pectin by fungi. The marine and mangrove mud ecosystem possess halophilic condition and also adapted to anaerobic situation to some extent. Mangroves are tropical and subtropical and also are characterized by plant species that are adapted to high temperatures and organic matter content. Mangrove muds possess fluctuations in salinities and oxygen levels. Mangroves and oceans provide ecosystem services of great social, economical and environmental importance. Sulphur and iron are abundant and characterized by reducing conditions and highly variable salinities. Marine and mangrove mud fungi remain as one of the few under-explored resources of natural products, and marine fungi remain as a primary source for unexplored chemicals. Marine and mangrove mud fungi can be isolated through deep-sea sediment collection using core sampler, besides soil plate and dilution plate methods. Fungi have been obtained from sediments which are estimated to be of a million years. Fungi have been recovered mostly in nonsporulating conditions. Sarma and Vittal (2001) have obtained mostly species of *Aspergillus* and *Penicillium,* which are of frequent occurrence. Borse et al. (2016), Raghu Kumar (2017) and Sarma and Vittal (2001) have recorded *Verrucaria enalia, Cirrenlia pygmea, Cryptosphaeria mangrovei, Lophiostoma mangrovei, Lulworthia sp., Phomopsis mangrovei,* and *Hypoxylon* sp., and others have been isolated from marine and mangrove mud samples. Some Indian mycologists have reported fungi from marine and mangrove mud (Borse et al. 2012, 2016; Chinnaraj and Untawale 1992; Patil and Borse 1983; Raghu Kumar and Chandra Latha 2012; Sarma and Vittal 2001, 2004).

However, it has been observed by the author that there are a lot of missing links in understanding marine and mangrove mud fungi, and biodiversity cum taxonomy studies seems to be incomplete.

16.9 Usar and Desert Soil Fungi

These soils are apparently lifeless, stress tolerant and mostly sandy with little or no moisture. These soils are unproductive, impermeable and hard due to the presence of undesirable elements on the surface. These soils are grouped as unproductive, alkaline and with little or no life soils. These soils enjoy a pH range of 7.4–11.0. Fungi are mostly confined to the upper surface only. Fungi showed progressive decrease as the soil depth increased. Fungi mostly appear during monsoon. Subramanya et al. (2016) have evaluated microbial diversity in soil, sand dune and rock substrates of Thar monsoon desert. The above workers have concluded that more fungi were present in soil than in sand dunes and rocky substrates. Desert soils that are mostly sandy (90–95%) receive low rainfall, possess low nitrogen and organic matter and have high calcium carbonates and phosphates besides being non-fertile. It is interesting to note that these soils possess high temperature and pH-tolerant fungi, which include some fungi belonging to *Aspergillus, Acremonium* spp., *Gliocladium cibotic, Phialophora geniculata, Stilbella annulata* and others. Mukerji (1966) reported the fungi and also ecological data of usar soils. In conclusion, both usar and desert soils are known to possess a few fungi and these habitats are non-fertile with unfavourable conditions for the growth of fungi. Some fungi do appear on the upper surface during monsoon and these fungi might have arrived due to surface run-off and also might have been the droppings of birds in which the fungi were present. It is also possible that the debris of xerophytic vegetation might have been deposited along with the fungi growing on them. It is also felt necessary that in-depth studies are essential both in usar and desert soils so as to find out typical fungi as they experience adverse climatic conditions. Such fungi will be of great potential in pharmaceuticals and other industries.

Thermophilic fungi which tolerate temperature above 60 °C are also known to colonize desert and usar soils (Johri et al. 2013; Satyanarayana and Johri 1984). The most common thermophilic fungi that occur in tropical desert and usar soils include *Chaetomium thermophile*, *Humicola insolens, Talaromyces thermophilus, Thermoascus aurantiacus* and *Thermomyces lanuginosus.* The thermotolerant white-rot fungus *Phanerochaete chrysosporium* is also common that degrades lignin.

16.10 Rhizosphere Soil Fungi

The rhizosphere is a specialized ecological niche present around plant roots as influenced by root exudates. The microbes and fungi are present in the rhizosphere complex environment. Further the microbes and fungi not only affect the plant but also interact with each other and also are interdependent.

Root exudates of diversified plants and breakdown products attract microbes, feed them and in turn the plant often benefits from the microbes and fungi. Rhizosphere microorganisms and fungi affect plant growth, development and productivity in varied soils under different plant covers. Most of the rhizosphere fungi serve as

growth promoters and enhance plant productivity. Several microbial interactions such as antagonism, competition and synergism are the important processes that occur simultaneously in soil and rhizosphere. Generally, the rhizosphere possesses greater microbial activity than normal soil. Though some fungi are common to rhizosphere soils of cereals, millets, fruit crops, vegetables, oil seed crops, fibre crops, forest plants and others, some specific fungi/groups of fungi are known to occur in such rhizosphere soils. Generally rhizosphere soils not only harbour more fungi but also specific fungal associations are noticed under plant cover of each crop and forest plants. For example Penicillia are abundantly present in the rhizosphere of *Eucalyptus* plantations. Similarly most of the crop plants support Aspergilli, mucorales and fungi imperfecti. The rhizosphere soils of some wild and forest vegetation support interesting and new fungi. Several aspects of rhizosphere have been discussed and elaborated by Mukerji et al. (2006). The most common fungi that occur in rhizosphere are species of *Alternaria*, *Aspergillus, Chaetomium, Curvularia, Drechslera, Emericella, Fusarium, Penicillium, Rhizoctonia, Rhizopus, Sordaria, Stachybotrys, Trichoderma,* and others. The overall activity of the rhizosphere is higher than the soil microorganisms, which is further away from the root. Further only some selected groups of fungi which compete and establish a conducive relationship with root system can only multiply and become predominant in rhizosphere. These rhizosphere fungi can be isolated by means of soil dilution plate method and soil plate methods. Further, it is known that the nature of the plant greatly affects the fungal population. It has been observed that fungi present in the rhizosphere of leguminous plants were less than crucifers, which can be attributed to different rooting habits, nodule formation and different root metabolites. It is also observed that Arbuscular mycorrhizal fungi form a dominant group in the rhizosphere soils of various plants. Indeed the rhizosphere fungi play an important role in helping mankind as documented earlier by Mukerji et al. (2006). Though rhizosphere and its microbial ecology has completed 100 years of research activity, still it is a specialized and significant microbial habitat which is of utmost importance in agriculture, industry, pharmaceuticals, disease management and biotechnology. Therefore, the studies on rhizosphere soil fungi must be continued in the coming years.

16.11 Soil Fungi in Amended and Unamended Soils

Organic and inorganic amendments have profound effect on soil fungi. Earlier Bagyaraj and Rangaswami (1967) and Khalish and Manoharachary (1985) have employed various amendments in different forms in order to know changes in microbes and fungi besides evolving suitable control measures for soil-borne diseases.

The amended soils are known to possess many fungi which will possess antagonism and biocontrol mechanisms. Some of the fungi which appear newly have potential for biotechnological purposes. Some of the perennating fungi germinate, proliferate and multiply in amended soils, which are also important from an

economic point of view. However, unamended soils will have normal fungal flora, which occur naturally and multiply in the soil. Amendments play an important role in plant health; hence this kind of methodology has to be employed for sustenance of agriculture. Nishat Khalish and Manoharachary (1985) have studied quantitative and qualitative changes in mycoflora of oil cake amended soils supporting coriander crop in relation to carbon dioxide evolution, organic matter content and other factors. Fungal number showed an increase by 25th day of crop amended with oil cake than non-amended soils. Species of *Aspergillus*, *Curvularia*, *Drechslera*, *Mucor*, *Penicillium* and *Rhizopus* were isolated. However, *Fusarium solani,* wilt and root rot causing fungus disappeared from amended soils. Interestingly, *Trichoderma viride* which was not present in normal soil has been found in amended soil along with *Penicillium stecki* which have proved as biological control agents. In view of the above, there is a need to conduct research on soil fungi in amended and unamended soils using various organic and inorganic substances, plant extracts, minerals, etc., so as to find out the possibilities of controlling the plant diseases and pests for sustenance of agriculture.

16.12 Mycorrhizal Fungi in Soil

The soil supports the growth and multiplication of Ectomycorrhizal and Arbuscular mycorrhizal fungi besides other groups of mycorrhizae which are also root and plant symbionts. The mycorrhiza, composite organs of roots and fungi, is formed in most species of angiosperms and gymnosperms and other lower plants. Initially, Frank (1885) who coined the term mycorrhiza was of the opinion that mycorrhizae represent the beneficial association between the roots of trees and fungus. Mycorrhizae are responsible for plant growth and nutrient uptake in the plants. A lot of information has accumulated on the taxonomy, ecology, physiology and beneficial activities of mycorrhiza in the last many years. Scientists have started exploiting them for human welfare. Many ways were devised to use them as tools for improving the productivity in plants and/or making the plants resistant to metal ions, diseases and pests. Ecomycorrhizal species belonging to genera *Amanita, Boletus, Lepiota, Russula, Rhizopogon* and *Scleroderma* grow on plant root cum soil and litter mixture in various forests zones. Mycorrhizas in abundance have been reported with *Pinus patula*. Arbuscular mycorrhizal fungi are associated with rhizosphere soils and non-rhizosphere soils of all types. AM fungi are characterized by the presence of structures called arbuscules and vesicles. Arbuscules are produced by the internal mycelium intracellularly in the form of highly ramified minute arborescence. Hyphae penetrate mechanically and enzymatically into cortical cells.

As the arbuscule hyphae bifurcate repeatedly they get enveloped by a host-derived encasement layer and continuously invaginating host plasmalemma and ensure an extremely wide contact between the two symbionts, but are short lived and digested by host, few days after their formation. Vesicles are also produced by their internal mycelium but mostly intercellularly. They are regarded as storage structures and are absent in some cases. Arbuscular mycorrhizal association is

generally ubiquitous and occurs in soils along with plants growing in arctic, temperate and tropical regions. There are six AM fungal genera, namely *Glomus, Gigaspora, Acaulospora, Scutellospora, Entrophospora* and *Sclerocystis.* AM fungi are known to help in conserving and using phosphorus efficiently, besides reducing the use of phosphatic fertilizers by the farmers. Mycorrhizal benefits are immense and most obvious under low fertilizer input conditions that exists in developing countries. EM fungi help in the establishment of forest seedlings. AM fungi help in developing disease resistance in plants, productivity of plants, abiotic stress tolerance and other related useful activities. Further, in recent times, AM fungal biofertilizers are developed by different industries all over the world. Ectomycorrhizae also play an important role in afforestation and also help in heavy metal tolerance. AM fungi play an important role in the establishment of plants, enhances the uptake of nutrients by plants, improves soil fertility, soil aggregation and management of disturbed soils. All the mycorrhizal fungi are not only associated with plants but also present in the soils in the form of mycelium and also survive as perennating structures which germinate and come out during the rainy season (Manoharachary 2004).

16.13 Soil and Mushrooms

Mushrooms belong to a higher group of fungi called Basidiomycotina, and most of the mushrooms also grow on humid soils. Several species of mushrooms are known. Fungi like *Amanita* which also grow on soil are known to be poisonous but are also ectomycorrhizal. Similarly edible mushrooms like *Agaricus, Morchella*, *Tuber* and other such edible fungi do occur on humid soils. Mushrooms are a rich source of protein and fibre besides having all essential amino acids and metabolites useful for increasing immunity. Mushrooms like *Morchella* which grow under apple orchards on humid soils are highly prized for their taste, flavour and nutritional qualities. Some fungi are also known to be of medicinal importance and they grow on humid soils. There is a lot of literature accumulated on mushrooms growing on soil in India and is difficult to enlist all such data here. However, Manoharachary and Nagaraju (2017) have enlisted higher fungi from Telangana state.

16.14 Techniques of Isolation

Several isolating techniques have been proposed to isolate soil and related habitats by a number of researchers and these include Dilution Plate Technique (Waksman 1916), Soil Plate Method and modified Soil Plate Method (Warcup 1950), Agar Film Method (Jones 2011), Soil Immersion Tube Method (Chester 1945), Immersion Plate Technique (Thornton 1952), Baiting Technique (Butler 1907) Dilution Frequency Method, Direct Microscopic Observation (Conn 1918), Uncoated Glass Slide Technique (Chlodony 1936) and Root Maceration and Burial Technique (Nagamani et al. 2006). Soil core sampling techniques and various other techniques

as mentioned above have been employed to isolate soil, rhizosphere soil fungi and also from related ecological habitats. It is essential to employ both conventional and nonconventional techniques so as to project a comprehensive and complete picture of fungi present in soil and also in related habitats. In recent times, molecular techniques such as metagenomics, transcriptomics and others have been employed and these have paved the way for an in-depth analysis of fungi from soil and related habitats.

The media that are employed are of synthetic, semi-synthetic and natural media types. For purification of fungal cultures, single spore isolation has to be employed. Mites are attracted by the smell of fungi, hence damage of the cultures by feeding on them. Therefore, mites can be avoided by applying few drops of 2% $HgCl_2$ mixed in absolute Ethanol–Glycerine. Several mounting media and stains are commonly used for staining fungi (Nagamani et al. 2006). The maintenance of cultures and their storage are very important so as to observe them from time to time. Periodic transfers, long-term storage of fungal cultures at 4 °C, cryopreservation, preservation in sterile mineral oil, storage in water, storage in silica gel, storage in sterile soil, lyophilization and vacuum drying and also storage in liquid nitrogen are some of the preservation methods. Therefore, both ex situ and in situ techniques are to be employed. There are more than 46 media available to isolate fungi from soils and semi-aquatic habitats, and all these media will give you an improved picture of the total fungal species present in the soil sample (Nagamani et al. 2006).

16.15 Significance of Soil Fungi

Vast mycoflora of diversified groups exists in the soil and also in the soil type of habitats. Probably mouldy earth is the appropriate epithet for our mother Earth. Fungi being ubiquitous and cosmopolitan in their distribution do occur in virgin soils, cultivated soils, desert soils, usar soils, thermal soils, muds, ecotonic soils and soils of semi-aquatic habitats including the soils of rock crevices and sphagnum bogs. The majority of soil fungi are microscopic but mushrooms and ectomycorrhizal fungi which grow on humid soils are macroscopic. Fungi that live in soil are saprophytes, parasites, biotrophs and symbionts.

Fungal species representing *Fusarium, Ophiobolus, Pythium, Phytopthora, Verticillium, Rhizoctonia, Sclerotium and Macrophomina* live as pathogens and at times these fungi grow as biotrophs. Fungi representing *Aspergillus, Penicillium, Rhizopus, Mucor, Drechslera, Curvularia, Chaetomium, Emericella* and several other such fungi live as saprophytes in different soils. Some species of *Penicillium* and *Trichoderma* are considered as antagonistic fungi as they inhibit the growth of some pathogenic, non-pathogenic fungi and bacteria. It is also known that the soil fungi are of paramount importance in biotechnology, agriculture, pharmaceuticals, food industry waste recycling, bioremediation and other such activities. High temperature tolerating fungi which are known as thermophiles and extremophilic fungi elaborate a number of enzymes and possess potential for elaboration of several secondary metabolites which are of biotechnological importance. Soil yeasts are of

utmost importance in biotechnology and not much is known from India about this group. Soil fungi such as *Chaetomium spp.* are cellulolytic. *Microsporum* spp. are considered as keratinophilic and mycotic. Chytrids are known to be chitiophilic. Species of *Pythium* are known to produce peptolytic enzymes. Mushrooms are known to serve as food besides elaborating several metabolites of medicinal importance. *Aspergillus* spp. are well known phosphate solubilizers besides elaborating several enzymes. *Emericella nidulans* is known for its ubiquitous occurrence and has been known for discovering parasexuality in that fungus. In a broader sense several soil fungi have great potential in performing various activities for human welfare, hence became important since times immemorial. Ecto- and endo- (AM) mycorrhizal fungi are the symbionts which mobilize nutrients such as phosphorus, Zn, iron and others from soil through mycorrhizal roots besides helping plant growth. Garrett (1953) gave an exhaustive account of root-infecting fungi and soil-inhabiting fungi, besides emphasizing the role of such fungi in disease production and their ecological behaviour. Saprophytic fungi of the soil are well known to play a significant role in the natural cycling of elements and are also known to produce substances of industrial and agricultural importance, besides being important in medicine, pharmaceuticals, bioremediation, waste recycling and transformation of elements and in other useful activities of human welfare.

16.16 Ecology of Soil Fungi

Soil fungi, in relation to gross habitat factors as reflected by their geographical range, have been made by a few researchers. Some aspects of fungi occurring in Indian soils in relation to soil factors have been worked out by some researchers (Alivelumangamma et al. 1996; Chaudhari and Sachar 1934; Dwivedi 1965; Gouri Rane 2017; Manoharachary 1977; Manoharachary et al. 1990; Mukerji 1966; Ramdayal and Gupta 1968; Ramakrishna et al. 2017; Rama Rao 1970; Saksena 1967; Saksena S.B. 1955; Shety 1954; Subramanian 1973; Uma Maheshwari and Komalavalli 2013).

Ecology of soil fungi deals not only with the occurrence of fungi but also with their distribution, phenology, qualitative composition, impact of factors such as pH, soil moisture, organic matter, vegetation, N, P, K, soil type, etc. Soil ecological approach to the study of soil fungi has taken root in the systematic studies made by the pioneer mycologists. Earlier researchers were mostly concerned with the distribution, estimation and qualitative composition of fungi in different soils so as to bring out the differences between soil types and soil fungi. I along with a batch of 50 Ph.D. students (1970–2020) worked on soil fungi in relation to gross habitat factors on more than 50 types of soils as reflected by their geographical range. Fungi occurring in Indian soils in relation to soil factors have also been worked out by some other researchers besides us. The data of soil physico-chemical factors and the statistical correlation data of factors with soil fungal numbers as studied by the authors have been presented in Tables 16.4 and 16.5.

Table 16.4 Physico-chemical factors of different soils and mud (author's data)

Average values of factor	Soil-1	Soil-2	Soil-3	Mud soil
Fungal number 10^3	112	114	90	60
Percentage moisture	10.3	14.0	9.0	32.0
Soil/mud temperature (in degree cent.) °C	38	28	26	8.0
pH	6.8	7.0	7.6	8.0
% Organic matter	0.6	3.1	0.3	6.0
Available "P" in PPM	0.3	3.2	1.8	5.0
Available "K" in mg per 100 g soil	12.4	20.4	18.0	22.0
Available "Ca" in mg per 100 g soil	50.0	62.0	102.0	180.0
Total "N" in mg per 100 g soil	13.0	20.0	6.0	14.0
Total soluble salts in mg per 100 g soil	8.0	18.0	14.0	22.0

[a]Note: Chlorides are in traces
Soil 1 = Scrub jungle soil; S2 = Dry deciduous soil; Soil 3 = Cultivated soil; Mud soil = Pond mud

Table 16.5 Correlation co-efficient (*r* and their calculated *t*) values obtained between soil factor and fungal number (author's data)

	Soil-1		Soil-2		Soil-3		Mud	
Factor	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Moisture	*0.706	3.1	0.335	1.10	*0.900	5.2	0.425	1.2
temperature	–0.404	1.4	0.012	0.039	–0.014	0.045	0.088	0.27
pH	–0.628*	2.4	–0.56	2.1	0.235	0.76	0.332	1.07
Organic matter	0.034	0.10	0.57	2.2	0.405	1.403	0.718*	2.45
Phosphorus	–0.187	0.601	-0.320	1.02	0.514	1.89	0.591*	2.357
Potassium	0.029	0.092	0.485	1.75	0.214	0.69	0.481	1.520
Calcium	0.109	0.347	0.530	1.97	0.45	1.5	0.436	1.53
Total Nitrogen	0.716*	3.241	0.732*	3.24	0.060	0.191	0.540	2.55
Total TSS	–0.229	0.760	0.335	1.12	0.249	0.817	0.418	1.4

*Significant at 5% level

16.17 Soil Fungi and pH

pH represents hydrogen ion concentration of a particular habitat. Soil is the dynamic and stable medium for fungi and other microbes and also covers a large portion of earth's crust. Fungi being represented by different Phylogenetic groups show different responses to soil pH. pH may affect fungi by physiological constant on survival and growth of soil fungi. Some fungi may fail to grow in a soil medium where the pH is very high or very low. Soil fungi normally prefer an acidic medium (4.0 to 6.5 pH). However, some other fungi like *Mortierella* and *Peziza may* prefer neutral to moderately alkaline pH. Soils from high altitude of cold climate or glaciers generally prefer pH 8.0-9.0. Members of verrucariaceae (*Myrothecium* sp., *Volutella* sp.*)* are known to prefer high pH and calcareous sites. Many saprobic fungi may prefer pH 7.0–8.5, while ectomycorrhizal fungi were found to grow in humid soils

associated with plant roots having a pH 5.0–6.0. In our studies, pH range 6.0–8.0 supported many fungi of different Phylogenetic groups. Therefore, pH forms a key factor in determining fungal community composition, their distribution and phenology. In recent times, many molecular tools have been employed by researchers to determine the soil fungi and ecological functions in different soils (transcriptome analysis). However, different researchers of different climates from India working on soil fungal ecology were of the opinion that soil pH is a decisive factor affecting soil fungi.

16.18 Soil Fungi and Soil Moisture

Soil moisture along with pH and organic matter serves the purpose of determining changes in soil fungal community. Soil moisture, as an ecological factor, plays an important role in the survival, distribution, phenology and growth of soil fungi. The variations in soil moisture have major influence on the respiration during growth.

In our experiment with different soils having different water holding capacities of 20, 40 and 60 percent respectively showed that the fungal counts varied along with the water holding capacity of the soil. Soil moisture at optimum level (25%) has allowed abundance of soil fungi and their growth. As expected, fungi developed at a moderate moisture level. However, 60% of maximum water holding capacity existing in semi-aquatic habitats (muds, etc.) did not favour the growth of the fungi abundantly. The marginal muds and ecotonic soils showed abundance of soil fungi as they were getting eroded by water currents repeatedly and the soils were getting replaced by the surrounding new organic matter which has become a new source for the appearance of new fungi along with some old fungi. The desert soils which were mostly sandy have shown few fungi at less moisture levels. Moisture in such kind of habitats has become an important factor. After rains, the desert and usar soils showed minimum levels of moisture, thus encouraging the growth of few soil fungi. Therefore, it is concluded that soil moisture determines the fungal structure, its distribution, phenology along with their biochemical activity in soil to some extent. Water holding capacity has also influenced the soil fungi.

16.19 Soil Fungi and Organic Matter

Soil type, soil properties and activity of soil fungi determine the health of soils. Such soils support numerous fungi differing qualitatively. Fungi are efficient and help in the decomposition and recycling of plant wastes that lead to the contribution of soil organic matter formation and soil fertility. Fungi are the efficient organisms which breakdown cellulose and lignin efficiently. Soil organic matter exists as fulvic acid and humic acid soluble at moderate alkaline pH, and also water soluble humin. Both fulvic acid and humic acid are utilized by fungi, but humin seems to be resistant for utilization by fungi. Organic matter is important in binding soil particles together to form a soil structure besides refracting the soil type formation and texture. The

decomposition of organic matter is dependent on C:N ratio and its decomposition by fungi is great. If the C:N is about 13:1, the nitrogen gets released due to the fact that the organic carbon is converted to carbon dioxide. Studies from India have indicated that organic matter is statistically positively correlated with fungi. Organic matter varies from soil to soil and the forest soil is rich in organic matter due to the presence of thick vegetation of diversified plants. However, cultivated soils harbour specific groups of fungi as it supports single or double crop and contains organic manure added into the soil. Therefore, members of Mucorales, Aspergilli, some soil-borne and other groups of fungi will be present. Diversified fungal species will be present in forest soils. Uncultivated and wild soils support different groups of fungi but the number and composition of fungi will be less than forest soils. Usar and desert soils having less moisture, high pH andless organic matter support not only less vegetation, but also very few fungi.

Further these soils receive scanty rainfall and soil type being sandy with little no carbon and nitrogen. The above data indicates that soils are known to support diversified groups of soil fungi but variations occur in the quantitative and qualitative composition of soil fungi in the soils mentioned above. It is important to mention that the authors have isolated *Curvularia tuberculata* from river sandy soils and this indicates that the soils mentioned above may contain new and interesting fungi if studied in depth (Nagamani et al. 2006).

16.20 Soil Fungi and Vegetation

Soil fertility is an important factor that affects not only growth and survival of soil fungi but also encourages plant growth as the soil supplies nutrients to vegetation. Soil also serves that purpose of nutrients and water holding capacity, and thus, allows anchoring plant roots. Thus, soils and vegetation possess a reciprocal relationship. Various forests and diversified crop plants along with variance in meteorological conditions, soil types and altitudes determine the quantitative and qualitative association of soil fungi, their phenology, distribution and activity. The plant diversity of tropical and temperate regions has greater influence on survival, growth and differentiation of soil fungal communities. Our studies with soil fungi have been greatly influenced by scrub jungle forest, dry deciduous forests, desert vegetation, temperate vegetation and some crop plants. Soil fungi play a crucial role in volatilization, nutritional cycling and other process. Soil fungal communities differ and show variations depending upon the vegetation type. In India, four major types of forests are identified namely tropical evergreen forests, tropical deciduous forests, tropical thorn forests, montane and swamp forests. Tropical deciduous forests of South India showed the presence of fungi mostly belonging to fungi imperfecti followed by few Ascomycetes and Mucorales. Similar biosphere reserves of Odisha having a pH of 5.0–6.0, high organic matter and moisture showed good fungal population. *Aspergillus niger* was the dominant fungal species present in Simplipal forests. Aspergilli followed by Penicillia, Mucorales, Ascomycetes, Fungi Imperfecti and few Basidiomycetes have occurred in the sequence as mentioned

above in almost all forest types. However, there are some indicator soil fungal species or a group of indicator fungal species for each forest type. However, from India such studies are very few or negligible. Therefore, in-depth studies are essential to be taken up by the researchers of this country which has different soil types and varied forest types. Forests are the hotspots for biodiversity and the prominent hosts for abiotic and biotic components wherein complex relationships exist between flora/fauna and soil fungi that maintains the structural richness of the habitat.

16.20.1 N, P, K and Soil Fungi

Nitrogen is an important element that affects the soil fungi. In our studies, we have found total nitrogen has been positively correlated with soil fungi occurring in scrub jungle forests, Telangana, India.

In Araku Valley soils, the nitrites were negatively correlated. In general, many soils collected from different areas of India showed that total nitrogen had a positive effect in the quantitative and qualitative composition of fungi. Maybe nitrates also is important in this regard. Available phosphorus has got great influence on the distribution and phenology of soil fungi. In our studies, available phosphorus though present in the soils has been significantly correlated with fungi occurring in cultivated soils and wild soils than in forest soils. Similarly, available potassium did not exhibit significant correlation with soil fungi. It is important to mention that insignificant does not mean that there is no influence of the factors such as potassium and others. Statistically, to derive a correlation we need a large number of samples and if the sample size is small then there is a possibility that the result as denoted significant or insignificant differs. Therefore, in our studies this kind of situation might have been the major factor to arrive that it is statistically significant or insignificant. In India, scanty data is available on soil fungal ecological aspects; hence concerted efforts are to be made in future.

16.21 Calcium, Total Soluble Salts and Chlorides in Relation to Soil Fungi

Calcium, total soluble salts and chlorides have been worked out by our group, and the results indicate that they are not positive factors in influencing the soil fungi. However, the calcium seems to be correlated significantly with the soil fungi associated with calcareous sites or calcium-rich sites. This clearly indicates that in India we have diversified soil types and some rare soils distributed in deeper territorial zones of the country. This includes caves, soils around sulphur springs, soils around specific ecological habitat and related sites. Therefore, the researchers have to concentrate on such soils where extremophilic fungi will be present and such fungi are of great importance in biotechnology and industry.

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Five Decades of Research on the Freshwater Hyphomycetes in India

17

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Abstract

Aquatic hyphomycetes, an important mitosporic fungal community, are involved in organic matter processing and energy flow in the lotic ecosystems worldwide. They have been studied widely in the past century and present on their morphology, taxonomy, phylogeny, anamorph-teleomorph connections, distribution, ecology, physiology, metabolites, and role in the food web. Owing to the community structure, the fascinating field of aquatic hyphomycetology attracted the attention of scientists throughout the world. The Indian subcontinent is of special significance owing to its unique geographical setup (tropical, subtropical, and temperate climatic conditions) with innumerable number of water bodies. The first report on aquatic hyphomycetes in India was in the year 1953 without the acquaintance that they are natives of lotic habitats. The real study in India was initiated five decades ago in a stream located in the northern region of Tamil Nadu followed by various studies on morphology, taxonomy, diversity, distribution, ecology, and detritus breakdown. Major studies have been carried out from the lotic habitats of Western Ghats and Himalayas. The current scenario in the Indian subcontinent reveals the growth of literature on aquatic hyphomycetes pertaining to basic aspects followed by their ecology, while applications are being initiated. This review encompasses historical perspectives, literature resource, habitats, methods of evaluation, diversity, distribution, ecology, and human interference with future perspectives.

Keywords

Ingoldian fungi · Diversity · Distribution · Ecology · Evaluation · Human interference

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17.1 Introduction

Mycological investigations in aquatic habitats received less attention compared to the terrestrial habitats (Bärlocher and Boddy 2016). Major fungal phyla represented in the freshwaters include Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and stramenopiles or heterokonts. They are capable of perpetuating in different aquatic niches such as detritus, sediments, biofilms, and live plant tissues (Shearer et al. 2007). They have adapted many trophic strategies mainly saprophytism, parasitism, and mutualism for their survival and dissemination (Jobard et al. 2010). Aquatic hyphomycetes are polyphyletic fungi known for the production of two major conidial shapes with interesting conidial ontogeny (staurosporus, multiradiate; scolecosporus, sigmoid); they are also designated as "freshwater hyphomycetes" or "Ingoldian fungi." Initial studies on aquatic hyphomycetes came from the aquatic habitats of temperate regions mainly North America and Europe (Bärlocher 1992a). Later, it was realized that they have wide geographic distribution (Ingold 1975). Despite their occurrence in different climatic and geographical conditions, the evolutionary forces faced by them in lotic habitats seem to be consistent (e.g., flow of water, availability of substrate, and physico-chemical factors).

Goh and Hyde (1996) proposed four subgroups among freshwater hyphomycetes: (a) Ingoldian fungi—grow on plant detritus in tree-lined lotic bodies and well-aerated lakes; (b) aeroaquatic fungi—normally grow in stagnant waters (ponds and puddles) or in slow-flowing streams that provide semiaquatic conditions; (c) terrestrial aquatic fungi—conidial fungi that live in rain drops on plant parts (leaf surface and trunk surface); and d) submerged aquatic fungi—heterogeneous fungi occurring on submerged decaying plant debris. A recent global assessment reveals occurrence of 335 morphospecies of aquatic hyphomycetes on a wide range of substrates (Duarte et al. 2016). Among geographic regions, Europe is the most studied continent followed by North America, South America, and Asia. However, less attention has been received by the African continent as well as polar regions. Among the 15 geographic regions examined, up to 50% goal of survey has been fulfilled by North America, temperate Europe, and tropics (western and eastern), and this shows the lacuna in our knowledge. Surprisingly, wide geographic regions share similar climatic conditions that possess high similarity in aquatic hyphomycete populations (Wood-Eggenschwiler and Bärlocher 1985; Duarte et al. 2016). Geographically, aquatic hyphomycete community has been influenced by narrow spatial scale (3–100 km) (Pascoal et al. 2005; Duarte et al. 2016). Thus, there is a wide scope for extensive inventory of aquatic hyphomycetes from various geographic locations.

The Indian subcontinent owing to its strategic geographic location offers a variety of climatic conditions (tropical, subtropical, and temperate) and ecosystems (plains, mountains, valleys, forests, coasts, semiarid regions, desert, and marine zones) with numerous water bodies (Sridhar 2020). The main geographic regions that drew attention of researchers to study aquatic hyphomycetes were the Western Ghats and Western Himalayas (the major hot spots of biodiversity). Although the earliest

report on aquatic hyphomycetes was available in India during the mid-twentieth century (Bhattacharya and Baruah 1953), the study was initiated by Ingold and Webster (1973). Various facets of studies in the Indian subcontinent include morphology, description, diversity, distribution, colonization, periodicity, ecology, mutualism, decomposition, physiology, and impact of pollution. The purpose of this review is to consolidate investigations carried out on aquatic hyphomycetes in the Indian subcontinent for the last five decades and to draw the attention of future researchers towards the fascinating field aquatic hyphomycetology with environmental, agricultural, and industrial relevance.

17.2 Historical Perspectives

17.2.1 Background

Studies on aquatic hyphomycetes were initiated more than a century ago (Bärlocher 1992a). They had drawn the attention of mycologists during the late eighteenth century (Saccardo 1880; Hartig 1880; de Wildeman 1893, 1894, 1895). *Heliscus lugdunensis* was the first fungus with clove-shaped conidia described by Saccardo (1880) on the pine bark found in Lyon (France) as well as in Northern Italy. It was followed by description of parasitic fungus *Cercospora acerina* on maple seedlings by Hartig (1880). Further advances were made by de Wildeman (1893, 1894, 1895) by the addition of three tetraradiate (*Tetracladium* spp.) and one sigmoid conidia of aquatic hyphomycetes associated with algae, leaves of willow, and a macrophyte (*Hippuris vulgaris*) in aquatic and semiaquatic habitats. Rostrop (1894) found *Tetracladium maxilliforme* (as *Titaea maxilliformis*) from the stems of red clover (*Trifolium pratense*). There was some confusion in the early twentieth century on the identification of *Tetracladium marchalianum* as an algal member (of the genera *Asterothrix* and *Cerasteria*) based on the drawings by de Wildeman (1893) (Huber-Pestalozzi 1925). Subsequently, it has been resolved that the spores of *T. marchalianum* belonged to a fungus (Lowe 1927; Karling 1935). Investigations on aquatic hyphomycetes were continued by Kegel (1906) by the discovery of *Varicosporium elodeae* on dead shoots of a perennial macrophyte (*Elodea canadensis*). This study was followed by the description of *Tetracladium setigerum* (as *Tridentaria setigera*) that appeared on the leaves of a flowering plant (*Angelica sylvestris*) and *Casaresia sphagnorum* on peat moss (*Sphagnum*) (Fragoso 1920).

After about two decades, Ingold (1942) found out the exact habitat of aquatic hyphomycetes based on his elegant observations on scum samples collected from an alder-lined stream in England. He further confirmed the growth of mycelia, conidiophores, and conidia of aquatic hyphomycetes by submerging decaying leaves of alder and willow in shallow water for 2 days. Further, he also established the pure cultures, and the culture strips on incubation in distilled water that produced huge crop of conidia. Ingold (1975) in his monograph very well illustrated the conidial ontogeny of multiradiate and sigmoid conidia of several aquatic hyphomycetes. Ingold has interpreted three important functions of unusual configuration of conidia

of aquatic hyphomycetes: (a) reduces sedimentation rate of conidia leading to successful dispersal, (b) tips of conidia adhere to the substrate firmly, and (c) complicated spore shape prevents conidial ingestion by aquatic fauna. Further studies took a different shape owing to the fundamental discovery by Ingold (1942); it was led by a zoologist H.B.N. Hynes (1963) to discover starlike structures of conidia of aquatic hyphomycetes in the gut content of stonefly larvae (Plecoptera). That was the beginning to understand the importance of aquatic hyphomycetes in the aquatic food web owing to their intermediary role between leaf detritus and leaf-shredding macroinvertebrates (Hynes 1963; Kaushik and Hynes 1971). Subsequently, Bärlocher (1981) demonstrated survival of conidia of aquatic hyphomycetes through gut passage of an amphipod crustacean (*Gammarus pulex*).

Webster (1959) proved experimentally the concepts of Ingold (1942) about the significance of conidial shape. Further, Webster and his associates studied the ecology of aquatic hyphomycete in greater detail (see Bärlocher 1992b). They established the planktonic role of conidia of aquatic hyphomycetes in dispersal, anchorage, and resistance against downward stream flow. Many scientists studied the dynamics and seasonal occurrence of aquatic hyphomycetes on autumn-shed deciduous leaves, twigs, and conifer needles (Arnold 1970; Triska 1970; Newton 1971; Bärlocher and Kendrick 1974; Bärlocher et al. 1978; Bärlocher 1982). Earlier studies were on the degradation of plant polymers (Tubaki 1957; Thornton 1963; Nilsson 1964) which were thoroughly investigated by Suberkropp and Klug (1980). Ever since the discovery of aquatic hyphomycetes by Ingold (1942), many investigators studied the biodiversity and geographical distribution throughout the world (Wood-Eggenschwiler and Bärlocher 1981; Webster 1987; Sridhar et al. 1992; Shearer et al. 2007; Bärlocher and Marvanová 2010; Duarte et al. 2012, 2015; Fiuza et al. 2017; Moro et al. 2018; Seena et al. 2019). Further advances took place on ecological services (Shearer 1992; Bärlocher 2005; Pascoal and Cássio 2008; Bärlocher and Sridhar 2014) and human interference (Sridhar and Raviraja 2001; Krauss et al. 2008; Schlosser et al. 2008; Canhoto et al. 2015) on aquatic hyphomycetes. Similar to taxonomy, diversity, and ecological studies, several traditional, biochemical, and molecular methods have been developed to study the aquatic hyphomycetes and their role more precisely (Gessner et al. 2003; Graça et al. 2005; Descals 2008; Suberkropp 2008; Bärlocher 2010; Ghate and Sridhar 2015b). Thus this group of fungi, being an important community in the lotic aquatic ecosystem, drew the attention of mycologists, ecologists, limnologists, biochemists, botanists, and zoologists.

17.2.2 Indian Scenario

Attention of the Indian mycologists was drawn towards the study of aquatic hyphomycetes after a decade of Ingold (1942) breakthrough. The first aquatic hyphomycete recorded was *Varicosporium elodeae* on submerged leaves by Bhattacharya and Baruah (1953) in Assam (northeast India). Later, Subramanian and Lodha (1964) described *Speiropsis hyalospora* occurring on horse dung in Uttar

Pradesh. *Articulospora tetracladia* as dark powdery colonies were found on decaying bamboo culms (*Bambusa* sp.) in Pune, Maharashtra (Patil and Rao 1972). Two years later, from a freshwater stream passing through scrubs at Kambakkam hills (80 km north of Chennai, Tamil Nadu), Ingold and Webster (1973) found additional conidia of four aquatic hyphomycetes (*Condylospora spumigena, Ingoldiella hamata, Lunulospora curvula,* and *Triscelophorus monosporus*) based on the assessment of submerged leaf litter, scum, and foam samples. This study was followed by reports of 5 and 12 species of aquatic hyphomycetes from Maharashtra (Thakur 1977; Patil and Kapadnis 1979). Further, Saikia and Sarbhoy (1980) described *Flabellospora octacladia* occurring on the stem of *Citrus aurantifolia* from Assam. Similar to Tamil Nadu, Ingold (1973) reported *Ingoldiella hamata* in foam samples in Andhra Pradesh followed by some more aquatic hyphomycetes that were reported from Andhra Pradesh (Rao and Manoharachary 1980; Manoharachary and Murthy 1981). The major massive report up to 30 species of aquatic hyphomycetes (in 23 genera) comes from Subramanian and Bhat (1981) based on the assessment of foam samples collected from 12 altitudinal lotic locations of the Western Ghats. This study was supported by another report of about 23 species of aquatic hyphomycetes from the River Payaswini in the Western Ghats based on leaf litter, foam, and water analysis (Sridhar and Kaveriappa 1982). A review by Sridhar et al. (1992) consolidated the studies on aquatic hyphomycetes focused on distribution (78 species in 45 genera) in 32 aquatic/semiaquatic habitats with regional differences, substrates, survival outside the streams, and additional studies carried out from different ecological regions of the Indian subcontinent.

Besides the diversity and distribution (in water, leaf litter, and foam), further studies on aquatic hyphomycetes have been continued on different ecological aspects including the occurrence on woody litter (Sridhar et al. 2010; Sudheep and Sridhar 2011), association with sediments (Sudheep and Sridhar 2012; Ghate and Sridhar 2015a; Karun et al. 2016), occurrence in estuarine habitats (Sridhar and Kaveriappa 1988), seasonal fluctuations (Sridhar and Kaveriappa 1984; Mer and Sati 1989; Chandrashekar et al. 1990), diurnal periodicity (Sridhar and Sudheep 2010; Ghate and Sridhar 2016), occurrence outside the streams (Sridhar 2009c; Chauvet et al. 2016), leaf litter decomposition (Raviraja et al. 1996b; Sudheep and Sridhar 2013a; Sridhar et al. 2013), palatability of leaf litter (Chandrashekar et al. 1989; Sridhar and Sudheep 2011a), mutualistic association (Raviraja et al. 1996a; Pathak and Sati 2017; Ghate and Sridhar 2017), production of lignocellulosic enzymes (Chandrashekar and Kaveriappa 1988), antimicrobial activity (Sridhar 2012; Singh and Sati 2019), nutritional requirements (Sati and Belwal 2005; Sati and Bisht 2005), phosphate solubilization (Sati and Pant 2018), plant growth enhancement (Sati and Arya 2010a, b), impact of human interference (Raviraja et al. 1998a; Chandrashekar and Kaveriappa 1989), and trapping conidia on latex-smeared slides (Ghate and Sridhar 2015b).

17.2.3 First Attempts

Several studies have been initiated in India from the mid-twentieth century. Box 17.1 highlights different facets on aquatic hyphomycetes initiated for the first time from 1953 onwards. Interestingly, similar to the global scenario, in the Indian subcontinent two reports on aquatic hyphomycetes initially came mainly from the terrestrial habitats (e.g., Subramanian and Lodha 1964; Patil and Rao 1972). The first authentic study was carried out in a stream in Tamil Nadu by the two British scientists Cecil T. Ingold and John Webster (1973) during their visit to C.V. Subramanian's laboratory in Madras University. Again the first taxonomic description of *Flabellospora octacladia* comes from the terrestrial habitat of Assam (Saikia and Sarbhoy 1980). With excellent illustrations, Subramanian and Bhat (1981) published a voluminous report from the Western Ghats, which stimulated several workers in the field. It is almost equivalent to the monograph published by Ingold (1975) based on his expeditions in different parts of the world. Aquatic hyphomycete-colonized leaf litter becomes a nutrient source. Rao and Manoharachary (1982) demonstrated the preference of conditioned leaf discs to juvenile fish and prawns against unconditioned leaf discs. The first seasonal dynamics comes from a coastal stream of southwest of Karnataka (Sridhar and Kaveriappa 1984). Laboratory in vitro studies by Sridhar and Kaveriappa (1986) demonstrated the impact of pesticides on aquatic hyphomycetes. Production of extracellular enzymes by aquatic hyphomycetes has been studied by Chandrashekar and Kaveriappa (1988). Chandrashekar et al. (1991) studied the occurrence of aquatic hyphomycetes in a thermal sulfur spring for the first time. Besides occurrence of aquatic hyphomycetes in water, scum, foam, and plant detritus in streams, their unnoticed ecological niche as endophytes in riparian roots extended into streams was demonstrated by Raviraja et al. (1996a). The important function of aquatic hyphomycetes is the breakdown of dead leaf litter; it was studied in the Western Ghats and southwest Karnataka by Raviraja et al. (1996b). Although aquatic hyphomycetes prefer unpolluted streams, they also continue leaf litter decomposition in polluted habitats too; it has been demonstrated by Raviraja et al. (1998a). The first genus of aquatic hyphomycete *Synnematophora* sp. growing on the mango leaf litter was reported from the Sampaje stream in the Western Ghats by Sridhar and Kaveriappa (2002). The growth response of aquatic hyphomycetes on different nutrients in vitro was studied by Sati and Bisht (2005). The first study of occurrence in tree canopy comes from the Southwest Karnataka (Sridhar et al. 2006). The first diurnal fluctuation of aquatic hyphomycete conidia in streams of the Western Ghats and west coast was studied by Sridhar and Sudheep (2010). Besides the basic function of aquatic hyphomycetes in energy flow, they also promote plant growth; this was demonstrated using endophytic hyphomycetes by Sati and Arya (2010a). Aquatic hyphomycetes also possess antimicrobial activity against bacteria and fungi (Sati and Arya 2010b; Arya and Sati 2011). Occurrence of aquatic hyphomycetes in sediments had been assessed by Sudheep and Sridhar (2012). Based on the growth of aquatic hyphomycetes on latex (Sridhar and Kaveriappa 1987a), Ghate and Sridhar (2015b) designed a conidial trap technique in streams on latex-smeared slides. To support the plant growth promotion, Singh

and Sati (2017) demonstrated the ability of phosphate solubilization by endophytic aquatic hyphomycetes.

Box 17.1: Studies Carried Out on Aquatic Hyphomycetes for the First Time in India

- Report of *Varicosporium elodeae* in Assam (Bhattacharya and Baruh 1953)
- Investigation in freshwater stream of Tamil Nadu (Ingold and Webster 1973)
- Description of *Flabellospora octacladia* in Assam (Saikia and Sarbhoy 1980)
- Major report in foam samples of Western Ghats (Subramanian and Bhat 1981)
- Palatability to fish and prawn (Rao and Manoharachary 1982)
- Seasonal study in southwest coast (Sridhar and Kaveriappa 1984)
- Impact of agrochemicals (Sridhar and Kaveriappa 1986)
- Enzymes (Chandrashekar and Kaveriappa 1988)
- Thermal sulfur spring in Western Ghats (Chandrashekar et al. 1991)
- Endophytes in riparian roots in Western Ghats (Raviraja et al. 1996a)
- Leaf litter breakdown in Western Ghats and south west coast (Raviraja et al. 1996b)
- Leaf litter breakdown in polluted river stretch in Western Ghats (Raviraja et al. 1998a)
- Report of genus *Synnematophora* in Western Ghats (Sridhar and Kaveriappa 2002)
- Growth response to nutrients (Sati and Bisht 2005)
- Report in tree canopy of south west coast (Sridhar et al. 2006)
- Colonization on woody litter in Western Ghats (Sridhar et al. 2010)
- Diurnal periodicity in Western Ghats and south west coast (Sridhar and Sudheep 2010)
- Report on plant growth promotion (Sati and Arya 2010a)
- Antifungal activity (Sati and Arya 2010b)
- Antibacterial activity (Arya and Sati 2011)
- Occurrence in hyporheic zones of Western Ghats (Sudheep and Sridhar 2012)
- Conidial trap technique on latex smear in southwest coast (Ghate and Sridhar 2015b)
- Phosphate solubilization (Singh and Sati 2017)

17.3 Literature Source

17.3.1 Reviews and Articles

Indian mycologists have invested their efforts on various aspects of aquatic hyphomycetes from different ecoregions of the Indian subcontinent. Table 17.1 provides a broad outline of selected topics studied. Overviews on aquatic hyphomycetes consolidated the studies carried out mainly from the Western Ghats and Himalayas. Major emphasis was to understand the diversity and distribution based on the assessment of water filtration, incubation of submerged leaf litter, and foam scanning. Besides screening submerged leaf litter, some studies evaluated submerged wood samples in streams and rivers (e.g., Sridhar et al. 2010, 2011a; Sridhar and Sudheep 2011b; Sudheep and Sridhar 2011, 2013b). Recently a new aquatic hyphomycete *Bactrodesmium aquaticum* has been described from the submerged woody debris (Borse et al. 2019a). Some studies have tried to understand the pattern of colonization of aquatic hyphomycetes through baiting specific leaf litter in streams of the west coast and Western Ghats (Sridhar and Kaveriappa 1989b; Sudheep and Sridhar 2013a). Sati and Tiwari (1992a) studied colonization pattern on the chir pine (*Pinus roxburghii*) needle litter in freshwater streams of Kumaun, Himalayas. In addition, two other studies evaluated the association of aquatic hyphomycetes in stream sediments (Sudheep and Sridhar 2012; Ghate and Sridhar 2015a).

Ecological studies on aquatic hyphomycetes in India include seasonal and diurnal periodicity, thermal sulfur springs in the Western Ghats, and stream sediments in relation to water qualities. Seasonal periodicity of aquatic hyphomycetes has been studied mainly from the Western Ghats and Himalayas (Sati and Pant 2006; Sati and Belwal 2009; Sridhar and Kaveriappa 1984, 1989a). Diurnal periodicity of conidia in streams has been studied in the Western Ghats as well as the southwest coast (Sridhar and Sudheep 2010; Ghate and Sridhar 2016). Occurrence and functions of aquatic hyphomycetes depend on the organic matter as well as the water quality (Rajashekhar and Kaveriappa 2003; Sati and Arya 2009). Besides organic debris (leaf and woody litter), several aquatic hyphomycetes have a mutualistic association as endophytes mainly in the riparian roots extended into the streams (Ghate and Sridhar 2017; Sati et al. 2009b). Besides stream environment, aquatic hyphomycetes occur outside the stream habitats (terrestrial litter, tree holes, stemflow, and throughfall) (Sridhar 2009c).

Organic matter decomposition is one of the major functions of aquatic hyphomycetes in the lotic ecosystem. Some investigations have been carried out on the leaf and woody litter in the streams of the Western Ghats and west coast (Raviraja et al. 1998a; Sridhar et al. 2011a; Sudheep and Sridhar 2013a, b). Decomposition of leaf litter in streams by aquatic hyphomycetes improves the nutritional quality (e.g., proteins and lipids), which attracts the stream fauna, and they prefer the colonized than fresh litter (Chandrashekar et al. 1989; Sridhar and Sudheep 2011a). However, the growth of aquatic hyphomycetes in the streams as well as on the medium depends on the nature of carbon and nitrogen sources (Chandrashekar and

Table 17.1 Selected literature dealing with aquatic hyphomycetes in India (see Table 17.3 for new species reported from India)

	Feature	References
Overviews	Western Ghats and Himalayas	Sridhar et al. (1992), Sati et al. (2002), Belwal and Sati (2006), Ramesh and Vijaykumar (2006), Sridhar (2009a, 2010, 2017, 2019), Arya and Sati (2012)
Diversity and distribution	Water, leaf litter, woody litter, and foam	Subramanian and Bhat (1981), Sridhar and Kaveriappa (1982, 1989a, 1992), Rajashekhar and Kaveriappa (2003), Belwal et al. (2006), Sati and Pant (2006), Maddodi et al. (2009), Sridhar et al. (2010, 2011a), Sudheep and Sridhar (2011), Sridhar and Sudheep (2011b), Sudheep and Sridhar (2013b), Sati et al. (2014), Chaudhari et al. (2016), Nemede et al. (2016), Pant and Sati (2018), Pant et al. (2019), Prashar et al. (2019)
Colonization	Leaf litter colonization	Sridhar and Kaveriappa (1989b), Sati and Tiwari (1992a, 2006), Sati and Pant (2006), Sudheep and Sridhar (2013a)
	Wood litter colonization	Sridhar et al. (2010), Sudheep and Sridhar (2011, 2013b)
	Sediments	Sudheep and Sridhar (2012), Ghate and Sridhar (2015a)
Ecology	Seasonal periodicity	Sridhar and Kaveriappa (1984, 1989a, 1989b), Chandrashekar et al. (1990), Sati and Pant (2006), Sati and Tiwari (2006), Sati and Belwal (2009), Sudheep and Sridhar (2013a, 2013b), Sreekala and Bhat (2016)
	Diurnal periodicity	Sridhar and Sudheep (2010), Ghate and Sridhar (2016)
	Thermal sulfur spring	Chandrashekar et al. (1991), Rajashekhar and Kaveriappa (1996)
	Sediments	Sudheep and Sridhar (2012), Karun et al. (2016)
	Water quality	Rajashekhar and Kaveriappa (2003), Sati and Arya (2009)
Mutualistic association	Endophytes (live roots of trees, grass, and ferns)	Raviraja et al. (1996a), Sati and Belwal (2005), Sati et al. (2009b), Ghate and Sridhar (2017), Sati and Pathak (2017), Pant and Sati (2018), Pant et al. (2019)
Occurrence outside the streams	Terrestrial leaf litter	Sridhar and Kaveriappa (1987b)
	Stemflow and throughfall	Sridhar and Karamchand (2009), Ghate and Sridhar (2015c)
	Tree holes	Karamchand and Sridhar (2008), Sridhar et al. (2013)
Decomposition	Leaf and woody litter	Raviraja et al. (1996b, 1998a), Sridhar et al. (2011a, 2011b, 2013), Sudheep and Sridhar (2013a, 2013b)

(continued)

Table 17.1 (continued)

	Feature	References
Animal preference	Prawns and fish	Rao and Manoharachary 1982; Chandrashekar et al. (1989), Sridhar and Sudheep (2011a)
Nutrition and physiology	Carbon and nitrogen sources	Chandrashekar and Kaveriappa (1988, 1991), Sati and Bisht (2005, 2006)
	Temperature, light, and pH	Rajashekhar and Kaveriappa (1996, 2000), Sati et al. (2012)
Human interference	Organic pollution	Raviraja et al. (1998a)
	Heavy metals	Raghu et al. (2001)
	Pesticides	Sridhar and Kaveriappa (1986), Chandrashekar and Kaveriappa (1989)
Techniques	Conidial count and conidial trap	Sati and Belwal (2009); Ghate and Sridhar (2015b)
Applications	Enzymes	Chandrashekar and Kaveriappa (1988, 1991)
	Antimicrobial activity	Arya and Sati (2011, 2012), Sati and Singh (2014), Singh and Sati (2019)
	Phosphate solubilization and plant growth promotion	Sati and Arya (2010a)Singh and Sati (2017), Sati and Pant (2018)
	Biomonitors/bioindicators	Raviraja et al. (1998b), Dubey (2016)

Kaveriappa 1988, 1991; Sati and Bisht 2005, 2006). Besides nutrient sources, aquatic hyphomycetes are also influenced by the impact of temperature, light, and pH (Rajashekhar and Kaveriappa 2000; Sati et al. 2012).

Human interference is one of the major impacts on the aquatic ecosystems. Studies have been carried out on the impact of organic pollution, heavy metals, and pesticides in Indian lotic habitats (Raviraja et al. 1998a; Raghu et al. 2001; Chandrashekar and Kaveriappa 1989). Some of the organic matter leads to decline in aquatic hyphomycetes in Indian lotic bodies between 50% and 83% (Sridhar and Raviraja 2001). For assessment of diversity, colonization, and functions of aquatic hyphomycetes, various methods are necessary. Those methods could be adapted from other microbial studies or should be designed specifically depending on the needs (spore production, spore count, spore impaction, and extent of colonization) (Descals 2005; Sati and Belwal 2009; Ghate and Sridhar 2015b). The knowledge gained by any basic research needs to be carried forward towards applications. Likewise, some studies have been performed in India on aquatic hyphomycetes (cellulolytic enzymes, antimicrobial activity, and biomonitoring) (Chandrashekar and Kaveriappa 1991; Raviraja et al. 1998b; Sati and Singh 2014; Sati and Arya 2010b). Interestingly, some aquatic hyphomycetes showed phosphate solubilization ability, which is important in plant nutrition and helpful in growth promotion (Sati and Pant 2018; Singh and Sati 2019). The most fascinating application comes from a study of plant growth promotion of root endophytic aquatic hyphomycetes studied by Sati and Arya (2010a). Two test host plants on inoculation of three endophytic fungi did not show any disease symptoms. Among the endophytes, two species

showed significant impact on growth and biomass of test plants studied. Nevertheless, aquatic hyphomycetes are important biomonitors of water quality of streams and rivers; similarly some of them are good candidates of bioindicators (Raviraja et al. 1998b; Dubey 2016).

17.3.2 Books and Theses

Substantial literature availability leads to publication of books and promotes further studies. Table 17.2 provides titles of books and theses published from India. All the authors of books belong to the academic institutions rather than private organizations. However, the content of six books partially deals with aquatic hyphomycetes. Studies dealt with aquatic hyphomycetes in the books include description, diversity, distribution, physiology, ecology, and checklists.

Nearly 35 theses have dealt with aquatic hyphomycetes either partially (20) or completely (15). The highest number of theses came from the North Maharashtra University, Jalgaon, Maharashtra (15), followed by the Mangalore University, Karnataka (9), and Kumaun University, Nainital, Uttarakhand (7). Major studies have been undertaken in the Western Ghats (23) compared to Himalayan region (7). Different aspects dealt in the theses included diversity, distribution, ecology, decomposition, endophytes, physiology, techniques, and enzymes (Fig. 17.1). Diversity and distribution of aquatic hyphomycetes have attracted more attention (47%) followed by ecology (25%) and rest of the aspects fall below 10%. Some of the theses have interesting topics like enzymes (Chandrashekar 1988), biochemical aspects (Chandrashekar 1988; Raviraja 1996; Maddodi 2002; Sudheep 2011), occurrence outside the streams (Sridhar 1984; Karamchand 2008), antimicrobial activities (Arya 2009; Singh 2014), endophytes (Singh 2014; Ghate 2016; Pant 2020), and plant growth promotion (Singh 2014).

17.4 Methods of Examination

Inventory of aquatic hyphomycetes needs specific methods of evaluation. A list of classical, biochemical, and molecular techniques has been given in the review by Sridhar et al. (2020). Some of the techniques have been followed by the researchers in India. Routine inventory needs assessment of stream water by filtration through Millipore filters, collection of submerged organic matter (leaf and woody litter) and incubation of sections (in shallow damp chamber or distilled water) in the laboratory (for a few weeks), or bubble chamber incubation with forced aeration using aquarium aerators (up to 48 h) followed by filtration through Millipore filters (porosity, 5 or 8μm). To get an overall idea of aquatic hyphomycete community, randomly collected leaf litter on incubation in distilled water in the laboratory for 4–5 days and collect the incubated water in beakers to induce foam on addition of a small quantity of detergent (e.g., sodium lauryl sulfate) followed by aeration to assess the accumulated conidia (Chandrashekar et al. 1986). Several aquatic hyphomycetes

Table 17.2 Titles of books and theses on aquatic hyphomycetes from India (arranged year-wise)

Book	Content	References
Recent Mycological Researches	Partial	Sati (2006)
Frontiers in Fungal Ecology, Diversity and Metabolites	Partial	Sridhar (2009b)
Microbes - Diversity and Biotechnology	Partial	Sati and Belwal (2012)
Freshwater Higher Fungi of India	Partial	Borse et al. (2016)
Freshwater and Marine Fungi of India	Partial	Borse et al. (2017)
Common Zoosporic and Water-Borne Conidial Fungi	Partial	Manoharachary and Kunwar (2018)
Thesis		
Aquatic Fungi of Maharashtra	Partial	Kapadnis (1980)
Studies on Water-Borne Fungi of Dakshina Kannada and Kodagu Regions	Complete	Sridhar (1984)
Some Aspects of Water-Borne Fungi and Their Enzymes	Complete	Chandrashekar (1988)
Taxonomy and Species Composition of Hyphomycetes in Forested Streams and Their Colonization Pattern on Tree Leaves in Nainital, Central Himalaya	Complete	Tiwari (1992)
Studies on Water-Borne Hyphomycetes of Some of the Rivers in Karnataka with Special Reference to Cauvery and Kali Rivers	Complete	Rajashekhar (1994)
Studies on Water Borne Fungi of Uttara Kannada Region	Partial	Vijayakumar (1995)
Ecological and Biochemical Studies on Aquatic Hyphomycetes of Western Ghats and West Coast of India	Complete	Raviraja (1996)
Studies on Water-Borne Conidial Fungi in the Running Fresh Water Bodies of Kumaun Himalaya	Complete	Belwal (2002)
Studies on Diversity, Ecology and Biology of Aquatic Fungi of Some Freshwater Streams of Western Ghats Forests in Goa state, India	Complete	Sreekala (2002)
Role of Aquatic Hyphomycetes in the Streams and Rivers of the Western Ghats, Karnataka - Ecological and Biochemical Studies	Complete	Maddodi (2002)
Studies on Root Endophytic Fungi Including VAM on Riparian Forest Plants	Partial	Pargain (2005)
Response Pattern of Aquatic Hyphomycetes Along Physicochemical Gradients	Complete	Bisht (2006)
Studies on Freshwater Filamentous Fungi of Western Ghats and West Coast of India	Partial	Karamchand (2008)
Studies on Aquatic Fungi from Dhule District	Partial	Patil (2008a)
Studies on Non-Zoosporic Fungi from Jalgaon District	Partial	Patil (2008b)
Studies on Aquatic Fungi from Satpura Range in Dhule District	Partial	Pawara (2008)
Diversity of Water-Borne Conidial Fungi and their Antimicrobial Activities in Kumaun Himalaya	Complete	Arya (2009)
Biodiversity of Salt Water Fungi from Lonar Lake and Freshwater Fungi from Buldhana District	Partial	Patil (2010)

(continued)

Table 17.2 (continued)

Book	Content	References
Diversity, Ecology and Bioprospecting of Fungi of Kaiga Environs of the Western Ghats	Complete	Sudheep (2011)
Studies on Aquatic Fungi from Melghat Wildlife Sanctuary, Amravati District	Partial	Nemede (2012)
Macrofungi and Aquatic Hyphomycetes of the Western Ghats and West Coast of India	Partial	Karun (2014)
Diversity of Aquatic Fungi in Shimoga District of Karnataka	Complete	Raju (2014)
Bio-Prospecting of Root Endophytic Aquatic Fungi as Plant Growth Promoters and Antimicrobial Potential	Complete	Singh (2014)
Effect of Environmental Factors on Diversity of Aquatic Fungi in Chikmagalur District, Karnataka	Complete	Suresha (2014)
Studies on Aquatic Fungi from Dang District, Gujarat	Partial	Ahire (2016)
Biodiversity of Aquatic Fungi from Ahmednagar District, Maharashtra	Partial	Borade (2016)
Studies on Macrofungi and Aquatic Fungi of Selected Wetlands of the Southwest India	Partial	Ghate (2016)
Studies on Aquatic Fungi from Nandurbar District	Partial	Wagh (2016)
Biodiversity of Aquatic Fungi from Thane District, Maharashtra	Partial	Gosavi (2017)
Biodiversity of Aquatic Fungi from Pune District, Maharashtra	Partial	Jagdale (2018)
Biodiversity of Aquatic Fungi from Khandwa District, Madhya Pradesh	Partial	Patil (2018a)
Biodiversity of Aquatic Fungi from Tapi District, Gujarat	Partial	Patil (2018b)
Studies in Aquatic Fungal Biodiversity from Maharashtra	partial	Sindhe (2018)
Biodiversity of Aquatic Fungi from Pachmarhi Biosphere Reserve, Madhya Pradesh	Partial	Chaudhari (2020)
Evaluation of Himalayan Root Endophytic Aquatic Hyphomycetes for Bioactivity and Phosphate Solubilization	Complete	Pant (2020)

also sporulate on damp incubation in the laboratory (Sridhar et al. 2020). Accumulated foam could be collected from the streams fixed in formalin-acetic-alcohol and examination in the laboratory will also facilitate to follow the aquatic hyphomycete community. Quantitative estimation of conidia has been attempted by Sati and Belwal (2009).

Similar to the study of leaf litter, woody litter was also studied by damp incubation and bubble chamber incubation (Sridhar et al. 2010; Sridhar and Sudheep 2011b). Using damp chamber incubation of woody litter (bark and cambium) collected from 12 high-altitude streams of the Western Ghats, 30 species of aquatic hyphomycetes were recorded (bark 20 spp.; cambium 18 spp.). Naturally submerged soft and hard woody litter (diam 1.5 cm; length 3 cm) were divided into nine vertical sections by Sridhar and Sudheep (2011b). Similar sections were simultaneously

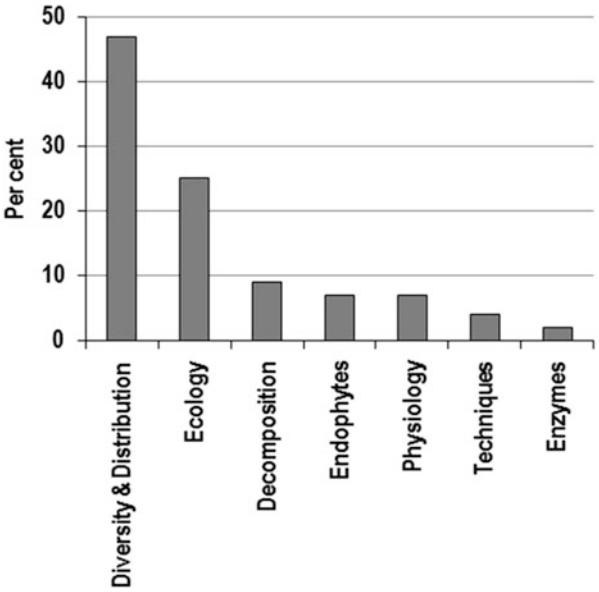

Fig. 17.1 Different aspects studied on aquatic hyphomycetes in India

assessed by damp chamber incubation (4 months) as well as bubble chamber incubation (72 h). Ten and 26 species of aquatic hyphomycetes were found in hardwood and softwood, respectively. In addition, Sridhar and Sudheep (2011b) mapped the occurrence of aquatic hyphomycetes in different sections of soft- and hardwoods. A single submerged leaf (e.g., banyan) or a woody litter may support up to 10–25 species of aquatic hyphomycetes, which could be detected conventionally. However, no attempts have been made so far in India to assess the phylogeny of aquatic hyphomycetes in water, foam, and detritus using molecular techniques although such attempts have been made for the last two decades elsewhere (Box 17.2).

Box 17.2: Studies Carried Out on the Molecular Phylogeny of Aquatic Hyphomycetes (Source: 2002–2012 – Duarte et al. 2013)

- 2002 – Sequencing 18s rDNA of 5 *Tetracladium* spp.
- 2003 – Anamorph-teleomorph connection by sequencing 18s rDNA
- 2005 – Sequencing 18S rDNA partial sequences of 22 new species
- 2006 – Partial sequencing of 28s rDNA of 8 species
- 2006 – Sequencing of l8S and 28S rDNA partial sequencing ITS of 11 species
- 2006 – ITS sequencing to establish endophytic and aquatic phases of *Dwayaangam*
- 2009 – Sequencing 28S rDNA partial sequences of 22 species

(continued)

Box 17.2 (continued)
- 2010 – Sequencing partial ITS for single conidia
- 2010 – Selection of best barcode COX1, ITS and DI/D2 of the genus *Tetracladium*
- 2012 – ITS barcodes for intraspecies diversity of *Articulospora tetracladia*
- 2014 – New DNA barcodes (Duarte et al. 2014)
- 2014 – ITS phylogenetic analysis of 18 species of *Campylospora* (Marvanovà and Laichmanovà 2014)
- 2015 – 454 Pyrosequencing (Duarte et al. 2015)
- 2016 – Metabarcoding to assess diversity in coarse and fine particulate organic matter (Wurzbacher et al. 2016)
- 2018 – LSU and ITS sequence data of *Nawawia* and *Neonawawia* (Yang et al. 2018)
- 2020 – Multigene (15 genes) analysis of ITS phylogeny of *Tricladiaceae* (Johnston and Baschien 2020)

A new conidial trapping technique has been designed by Ghate and Sridhar (2015b). On baiting six latex-smeared slides with plain slides as control in a stream of southwest coast up to 18 h, slides smeared with banyan (*Ficus benghalensis*) ranked first in trapping conidia of different species of aquatic hyphomycetes. Banyan latex-smeared slides showed the highest diversity of aquatic hyphomycetes followed by the latex of *Plumeria rubra*. The simplicity and novelty of this technique are that the slides with latex smears could be easily transported to different locations in slide boxes, and could be exposed to flowing water for various periods (e.g., 1–4 h). In the intervals, one could collect leaf litter, woody litter, and foam samples for comparison. After different periods of exposure of slides, water on latex smear is drained, stained with aniline blue in lactophenol, fixed with the cover glass, and easily transferred into slide boxes for conidial assessment in the laboratory.

Sudheep and Sridhar (2012) emphasized the assessment of aquatic hyphomycete population in sediments using baiting technique. The defined amount of sediment (fine particulate matter) could be inoculated to pre-weighed sterile leaf discs to allow aquatic hyphomycetes to colonize on shaking (150 rpm) for 2 weeks. After retrieving the incubated leaf discs, they should be incubated again for 48 h in sterile distilled water in bubble chambers to generate conidia. This indirect method provides information on those aquatic hyphomycete conidia or mycelia in the sediment samples. Assessment of dry mass of sediments and leaf discs in parallel samples facilitates to roughly guess the fungal biomass in sediments. This technique was also followed by Ghate and Sridhar (2015a) to assess aquatic hyphomycetes in an intermittent stream in southwest India.

Morphological observations are the fundamental aspect for identification of fungi. Aquatic hyphomycetes produce two major types of conidia: staurosporus (branched) and scolecosporus (sigmoid). However, some conidia have conventional cylindrical or oval shapes, while aeroaquatic fungi produce helicosporous spores

(coiled in different dimensions). Such shapes of the conidia facilitate dispersal in water, attachment to the organic substrate, and prevention of consumption by aquatic fauna. In addition to conidial morphology, conidial developmental stages (ontogeny) are also helpful in authentic identification. Although conidial ontogeny is simple in some scolecosporus species, it is a bit complex in staurosporus species (Sridhar and Kaveriappa 1987c, 1989d). A sample of sigmoid, helicosporous, multiradiate, and oval shaped conidia reported from the Western Ghats is shown in Fig. 17.2.

17.5 New Species from India

Up to 17 new species and 1 new genus have been described from different parts of India (Table 17.3). Description of ten new species and one genus came from the Western Ghats, while five new species were found in Himalayas. The rest two species were one each from the Cauvery river in Karnataka and Purna River in Gujarat. The first two species (*Flabellospora octacladia* and *Chaetospermum indicum*) were described from Assam and Maharashtra, respectively (Saikia and Sarbhoy 1980; Talde 1981). After a gap of 5 years, *Triscelophorus konajensis* was reported from the southwest coast of Karnataka (Sridhar and Kaveriappa 1987d). After 4 years, three more species were described from the Western Ghats and Himalayas (1991–1993). Eight years later, additional five species were described from the school of D.J. Bhat, Goa University (2001–2002). It was followed by description of a new synnematous genus *Synnematophora* on the mango leaves (*Mangifera indica*) in Payaswini river of the Western Ghats (Sridhar and Kaveriappa 2002). Seven years later, a root endophytic fungus *Tetracladium nainitalense* was described by Sati et al. (2009a) from Nainital in Himalayas. After a decade, from Nainital Sati and Pant et al. (2019) described a new *Catenomycopsis vinayaka*. From Maharashtra and Gujarat two and one species were described by B.D. Borse and his school, respectively (Borse et al. 2019a, b). Three species were described from the terrestrial habitats (*Flabellospora octacladia, Phalangispora bharathensis,* and *Speiropsis rogergoosensis*). However, *F. octacladia* and *P. bharathensis* were later found in the foam samples of Amalibari stream and Panzara river (Maharashtra), respectively (Patil et al. 2014). The sporodochial fungus *P. bharathensis* was recorded again on the fallen leaves of mango (*Mangifera indica*) in Tamhini Ghats of Maharashtra and included additional characteristics (Rajeshkumar 2014). Two new species *Tetracladium nainitalense* and *Kumbhamaya jalapriya* were described as aquatic root endophytes; the former was found with roots of *Eupatorium odenophorum* and *Colocasia* sp. in Nainital, while the latter with roots of *Hopea ponga* in Goa.

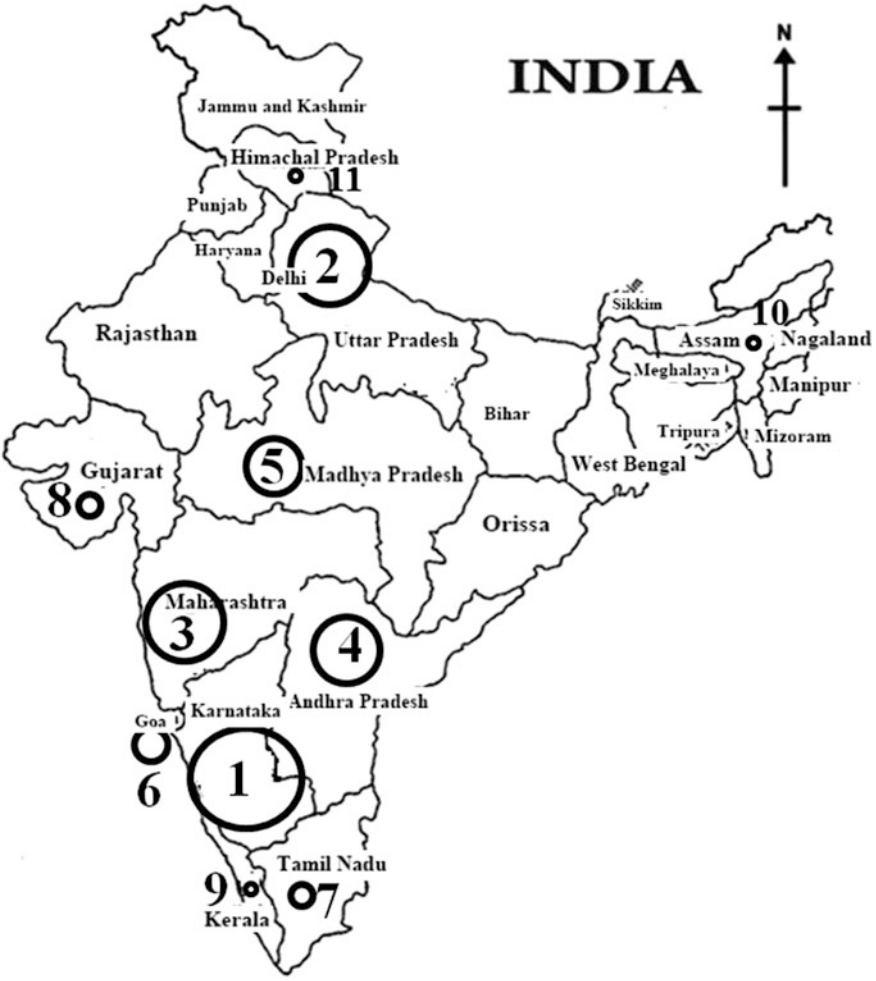

Fig. 17.2 Extent of literature resource on aquatic hyphomycetes in different parts of India

17.6 Habitats, Diversity, and Distribution

17.6.1 Habitats

The Western Ghats and Himalayas are the two ecoregions that attracted the attention of researchers to carry out studies on aquatic hyphomycetes. These two mega diverse habitats being major hot spots of biodiversity provide innumerable aquatic ecosystems and niches for diverse species of aquatic hyphomycetes. Most of the

Table 17.3 Freshwater hyphomycetes and allied species described from India (arranged year-wise) [*, new genus]

	Substrate	Location	References
Flabellospora octacladia Saikia & A.K. Sarbhoy	On stems of *Citrus aurantifolia*	Tinsukia, Assam	Saikia and Sarbhoy (1980)
Chaetospermum indicum Talde	Submerged stem of *Fimbristylis quinquangularis*	Purna and Dudhana rivers, Maharashtra	Talde (1981)
Triscelophorus konajensis K.R. Sridhar & Kaver.	Submerged leaves of *Ficus benghalensis*	Mangalore, Karnataka	Sridhar and Kaveriappa (1987d)
Vermispora cauveriana Rajash., Bhat & Kaver.	Submerged leaves of *Ficus religiosa*	Srirangapatna, Karnataka	Rajashekhar et al. (1991)
Tricladium indicum Sati & N. Tiwari	Submerged conifer needles of *Pinus roxburghii*	Niglat, Uttarakhand	Sati and Tiwari (1992b)
Pestalotia submersa Sati & N. Tiwari	Submerged leaves and needles of *Pinus roxburghii*	Niglat, Uttarakhand	Sati and Tiwari (1993)
Trinacrium indica Lekha, S.K. Nair & Bhat	Submerged leaves of *Coffea arabica*	Somwarpet, Karnataka	Soosamma et al. (2001)
Dendrospora yessemreddea S.K. Nair & D.J. Bhat	Freshwater foam	Bondla Wildlife Sanctuary, Goa	Sreekala and Bhat (2002)
Kumbhamaya jalapriya S.K. Nair & Bhat	Submerged live roots of *Hopea ponga*	Mollem Wildlife Sanctuary, Goa	Sreekala Nair and Bhat (2002)
Phalangispora bharathensis T.S.K. Prasad & Bhat	Fallen decaying *Holigarna arnottiana* leaves	Cotigao Wildlife Sanctuary, Goa	Prasad and Bhat (2002a)
Speiropsis rogergoosensis T.S.K. Prasad & Bhat	Dead leaves of *Artocarpus hirsutus* on soil	Kumara Parvatha, Karnataka	Prasad and Bhat (2002b)
**Synnematophora constricta* K.R. Sridhar & Kaver.	Submerged leaves of *Mangifera indica*	Payaswini river, Karnataka	Sridhar and Kaveriappa (2002)
Tetracladium nainitalense Sati & P. Arya	Root endophyte in *Eupatorium odenophorum* and *Colocasia* sp.	Nainital, Uttarakhand	Sati et al. (2009a)
Bactrodesmium aquaticum B.D. Borse, N.S. Pawar & S.Y. Patil	Submerged herbaceous and woody debris	Nakana Dam, Maharashtra	Borse et al. (2019a)
Setosynnema limnetica B.D. Borse & N.S. Pawar	Submerged leaves	Tapti river, Maharashtra	Borse et al. (2019b)
Tripospermum limneticum S.Y. Patil, N.S. Pawar & B.D. Borse	Submerged leaves of *Eucalyptus* sp. and *Polygonum glabrum*	Purna River, Gujarat	Patil et al. (2019)
Catenomycopsis vinayaka Sati & Pant	Root endophyte in *Eupatorium adenophyllum*	Nainital, Uttarakhand	Sati and Pant (2019)

studies come from the streamlets, streams, waterfalls, rivers, and rarely dams at different altitudinal ranges of the Western Ghats and Himalayas (see Tables 17.1 and 17.3). Interestingly, two thermal sulfur springs harbored aquatic hyphomycetes in the Western Ghats (Chandrashekar et al. 1991; Rajashekhar and Kaveriappa 1996). Recently, Chaudhari et al. (2016) listed occurrence of up to 45 species (in 29 genera) of aquatic hyphomycetes from Madhya Pradesh based on water, foam, plant detritus, stem flow, and live roots. Six species appeared to be endophytic in roots, while ten species were found in stem flow of tree species.

Including water samples, various substrates screened were leaf litter, woody litter, conifer needles, foam, scum, sediments, and damp terrestrial litter. In addition, the live roots of riparian trees or vegetation also supported aquatic hyphomycetes as endophytes in the Western Ghats and Himalayas (Raviraja et al. 1996a; Sati and Belwal 2005). Recently, studies have been carried out on the occurrence of aquatic hyphomycetes outside the streams: terrestrial leaf litter, stemflow, throughfall, decaying epiphytes (ferns), tree holes (dendrotelmata), and crown humus in the Western Ghats and southwest coast (Sridhar and Kaveriappa 1987b; Sridhar 2009c) (see more details in Table 17.1). These observations justify early reports on the occurrence of typical aquatic hyphomycetes in different substrates in terrestrial habitats in India and elsewhere. A recent study also revealed occurrence of 31 species of aquatic and aeroaquatic hyphomycetes on damp leaf litter of ten tree species during southwest monsoon in the scrubland of southwest Karnataka (Sridhar et al. 2020). However, the extent of occurrence is low in terrestrial habitats as compared to aquatic habitats, and their functions in terrestrial habitats appear to be different than aquatic habitats.

17.6.2 Richness and Diversity

Majority of studies in the Indian habitats concentrated on the species richness. In addition, it is necessary to provide the frequency of occurrence, conidial output, and substrate preference if any. Such approaches improve our knowledge to provide diversity, evenness, rarefaction indices (expected number of species), core-group fungi, keystone species, cryptic species, and so on. Minimum water quality parameters provide additional value to the study undertaken on aquatic hyphomycetes. Molecular approaches will direct us on colonized fungi in the substrates; these are usually missed by conventional methods of study. Such approaches emphasize seasonal fluctuations, succession, decomposition, mass loss of organic matter, food web, and pattern of energy flow.

Diversity of aquatic hyphomycetes is higher in the streams of the Western Ghats compared to the foothill, coastal region, and plains (Sridhar and Kaveriappa 1984, 1989a; Chandrashekar et al. 1990; Rajashekhar and Kaveriappa 2003; Sati and Tiwari 2006; Sati and Pant 2006; Maddodi et al. 2009; Sudheep and Sridhar 2013a). The diversity in the Western Ghat streams could be comparable to the streams of Himalayas based on the studies by S.C. Sati and coworkers (Sati and Tiwari 2006; Sati and Pant 2006). They concluded that the dense riparian vegetation

and abundance of substrate in Himalayas are responsible for high diversity of aquatic hyphomycetes. Mountain streams in the Western Ghats showed lower species richness (10 spp.) compared to the mid-altitude streams (20 spp.); gradual decrease was seen towards foothill streams (14 spp.) and coastal streams (12.5 spp.) (Sridhar and Kaveriappa 1989c; Raviraja et al. 1998b). Decrease in the species richness towards foothill and coastal region has been predicted due to the impact of reduced forest area as well as changes in water quality by human interference (e.g., agricultural chemicals and sewage input). This view has been further supported by Rajashekhar and Kaveriappa (2003) with strong correlation between the vegetation and aquatic hyphomycetes. In addition, besides temperature, other water parameters due to human interference have negative impact on the richness and diversity of aquatic hyphomycetes. Sreekala and Bhat (2016) have also evaluated the seasonal variation in species richness and diversity in three streams of the wildlife sanctuaries of Goa (123–280 m asl), and concluded that species density is dependent on rainfall, riparian vegetation, and substrate availability. Such precise comparisons could not be given for other ecoregions in India owing to lack of such studies.

In addition to assessing water, foam, and leaf litter, some studies have examined submerged woody litter in streams or rivers of the Western Ghats (Sridhar et al. 2010; Sudheep and Sridhar 2011, 2013b). Woody litter from 12 high-altitude streams of the Western Ghats yielded 30 aquatic hyphomycetes with higher species in the bark (20 spp.) than cambium (18 spp.) (Sridhar et al. 2010). Assessment of 350 woody litter naturally deposited in Kali river and Kadra Dam in the Western Ghats on bubble chamber incubation yielded 15 and 11 spp., respectively (Sudheep and Sridhar 2011). On immersion of two woody litter (*Anacardium occidentale* and *Terminalia paniculata*) in Kaiga stream and Kadra Dam in the Western Ghats, *T. paniculata* yielded 17 and 16 spp. of aquatic hyphomycetes in the stream and dam, respectively, whereas *A. occidentale* yielded 17 and 14 spp., respectively (Sudheep and Sridhar 2013b).

Similar to leaf litter and woody litter, live roots of riparian trees were also assessed for the occurrence of aquatic hyphomycetes (Raviraja et al. 1996a; Sati et al. 2009b; Sati and Arya 2010a; Ghate and Sridhar 2017). In the Western Ghats, about 22 species of aquatic hyphomycetes were endophytic in riparian roots (Raviraja et al. 1996a; Ghate and Sridhar 2017), while 29 species were endophytic in the Himalayan region (Sati and Pathak 2017). The diversity of aquatic hyphomycetes in the mid-altitude stream was higher than high-altitude stream, which is similar to the diversity found in water, foam, and detritus (Raviraja et al. 1998b; Ghate and Sridhar 2017). Chaudhari et al. (2016) claimed that there are six species of root endophytic aquatic hyphomycetes in Madhya Pradesh. This indicates that the riparian roots constitute an excellent ecological niche of aquatic hyphomycetes for their survival as well as perpetuation.

Patil and Borse (2015) in their checklist of aquatic mitosporic fungi in India conducted investigations on different substrates. Considering some additional reports on the occurrence of aquatic hyphomycetes on woody litter (Sridhar et al. 2010, 2011b; Sridhar and Sudheep 2011b; Sudheep and Sridhar 2011, 2013b),

submerged leaf litter stands first in the number of species colonized followed by woody litter, foam samples, water samples, and roots.

17.6.3 Distribution

Based on the extent of literature in the Indian subcontinent, Karnataka state stands first (37%) in the Western Ghats followed by Uttarakhand (21%) in Himalayas, Maharashtra (20%) in the Western Ghats, Andhra Pradesh (9%), Madhya Pradesh (5%), Goa (3%) in the Western Ghats, Gujarat (2%), Tamil Nadu (2%), Kerala (1%) in the Western Ghats, and Assam and Himachal Pradesh (<1%) in Himalayas (Fig. 17.3). Patil and Borse (2015) in their checklist classified distribution of freshwater mitosporic fungi in different states of India. The pattern of distribution is almost similar to the extent of reports presented above. The recent checklist by Borse et al. (2017) in the Indian subcontinent represents about 191 species of aquatic hyphomycetes (in 66 genera). Considering a recent report by Duarte et al. (2016), global occurrence is 335 morphospecies; thus India represents up to 57% of aquatic hyphomycetes. This is not surprising because the Indian subcontinent represents tropical, subtropical, and temperate climatic conditions. Further, it provides immense scope for studies in water bodies owing to ten biogeographic zones (trans-Himalayas, Himalayas, desert, semiarid zone, Western Ghats, Deccan Peninsula, Gangetic Plain, coasts, north-east zone, and islands) (Singh and Chaturvedi 2017). However, the vast ecoregions of the Indian subcontinent have not been inventoried for aquatic hyphomycetes (see Fig. 17.3). Although Goh and Hyde (1996) classified aquatic mitosporic fungi into four different groups, the actual aquatic hyphomycetes consisting of staurosporus (multiradiate), scolecosporus

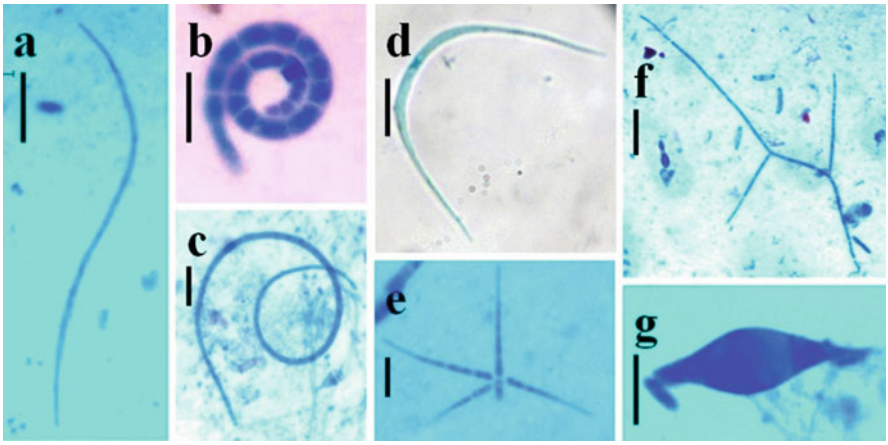

Fig. 17.3 Different conidia of aquatic hyphomycetes in the streams of Western Ghats: (**a**) *Anguillospora longissima*, (**b**) *Helicoma* sp., (**c**) *Helicosporium* sp., (**d**) *Lunulospora curvula*, (**e**) *Triscelophorus acuminatus*, (**f**) *Tricladium* sp., and (**g**) *Tumularia aquatica* (scale bar, 20μm)

(sigmoid), helicosporous (coiled), and conventional cylindrical and oval/spherical conidial shapes depict their adaptation to flowing waters. It will be convenient to consider typical aquatic hyphomycetes with the above spore features rather than including all those found in aquatic habitats. Other fungi found in aquatic bodies will be transients/immigrants or transferred through colonized substrate or accumulated in foam by terrestrial runoff leading to overlap with true aquatic hyphomycetes. Another advantage of such restrictions leads to focused attention and progress in future on aquatic hyphomycetes more precisely. It is not surprising that several aquatic hyphomycetes are found in tree canopies and terrestrial damp litter due to continuous wet conditions for a long period (4–6 months) especially in the Western Ghats. Shearer et al. (2007) rightly pointed out that "it is highly likely that species from aquatic habitats in the tropics also occur in the tropical rainforest habitat due to the very moist conditions."

17.7 Ecological Perspectives

Aquatic hyphomycetes are dependent mainly on the detritus material available from the riparian vegetation and transfer of coarse particulate organic matter from the terrestrial runoff. Their phenology coincides with the phenology of surrounding vegetation, rainfall, and physicochemical features of aquatic habitats. Further, their activity depends on the chemistry of the detritus material as well as the presence of consumer population. Availability of stagnant refuge and heterogeneous nature of stream bottom are the most important requirements. Depending on these factors, aquatic hyphomycetes show seasonality, succession, and decomposition of organic matter. Keystone, core-group, and cryptic nature of aquatic hyphomycetes in a given lotic body also depend on various factors. Under unfavorable situations, aquatic hyphomycetes need to face stressful conditions for survival and perpetuation.

17.7.1 Seasonal Studies

Two-year seasonal study of aquatic hyphomycetes in streams (coastal, foothill of the Western Ghats and mid-altitude of the Western Ghats) revealed increase in species richness and conidia during the post-monsoon season (September–December) followed by monsoon and summer seasons (Sridhar and Kaveriappa 1989a). Baiting five leaf litters in a southwest coastal stream also revealed similar seasonal fluctuation in a number of species (Sridhar and Kaveriappa 1989b). Such fluctuations are coinciding with increased rainfall and decreased water temperature. Further studies in the Western Ghats also showed similar results (Chandrashekar et al. 1990; Sudheep and Sridhar 2013a). On the contrary, in the two Himalayan streams near Nainital, species richness was higher during rainy and autumn seasons (July–September/October) than summer season (Sati and Tiwari 2006). Owing to decline in water temperature during winter in Himalayan streams, the species richness reduced substantially. In the Western Ghats, *Campylospora chaetocladia,*

C. filiformis, Flagellospora curvula, F. penicillioides, Lunulospora curvula, Phalangispora constricta, Synnematophora constricta, Triscelophorus acuminatus, T. monosporus, and *T. konajensis* were the most frequent core-group species (Sridhar and Kaveriappa 1989a; Chandrashekar et al. 1990). In Himalayan streams, *Alatospora acumulata, Anguillospora longissima, Clavariopsis aquatica, Lunulospora curvula, L. cymbiformis, Tetrachaetum elegans, Triscelophorus acuminatus,* and *T. monosporus* were the core-group fungi (Sati and Tiwari 2006; Sati and Pant 2006). Among the core-group fungi, *L. curvula, T. acuminatus,* and *T. monosporus* were common to the Western Ghats and Himalayas. Based on the assessment of aquatic hyphomycetes in three streams in Goa, Sreekala and Bhat (2016) concluded that the best season to recover aquatic hyphomycetes is the monsoon season.

17.7.2 Diurnal Studies

As the post-monsoon season in the Western Ghats and southwest coast of India is the more productive season for aquatic hyphomycetes, Sridhar and Sudheep (2010) studied the diurnal periodicity of drift conidia at 3-h intervals. Two peaks of drift conidia were observed during 9 am and 9 pm in both streams. Higher species richness and diversity were seen during the day in Western Ghats stream, while it was during the night in the coastal stream. The top five species in this study simulate similar to the seasonal studies. Another diurnal study on banyan latex-smeared slides (*Ficus benghalensis*) in the Western Ghats and coastal streams was done at 3-h intervals during the post-monsoon season (Ghate and Sridhar 2016). Among the drift conidia (water samples), control slides (without latex smear), and experimental slides (with latex smear), the latter method showed higher conidial trapping efficiency with higher conidial richness, species richness, and diversity. The peak richness and diversity were seen during 12 am to 3 am in the Western Ghats stream, while 3 am to 6 am in the coastal stream. As seen in the first diurnal study (Sridhar and Sudheep 2010), the top five species coincide with annual studies. There was no bias in the entrapment of staurosporus and scolecosporus conidia on latex smears. Latex entrapment method serves as a method of choice in assessing aquatic hyphomycetes in streams (Ghate and Sridhar 2016).

17.7.3 Decomposition

Litter decomposition is one of the basic ecosystem services in the aquatic ecosystems and many life activities are connected directly or indirectly with this function (Bärlocher et al. 2020). Decomposition of four-leaf litters *Acacia auriculiformis*, banyan (*Ficus benghalensis*), cashew (*Anacardium occidentale*), and *Eucalyptus globulus* up to 16 weeks in two streams of the Western Ghats in relation to leaf and water chemistry was studied by Raviraja et al. 1996b). The highest conidial production was seen in banyan leaf litter. Raviraja et al. (1998a) further studied the

decomposition of banyan and eucalypt leaf litter in an organically polluted stretch of Netravati river in the Western Ghats in relation to leaf and water chemistry. Relatively lower species richness and diversity of aquatic hyphomycetes were seen compared to other pristine streams in the Western Ghats. Decomposition of immersed leaf litters of teak (*Tectona grandis*) and Marwa (*Terminalia paniculata*) in two sites of the Kali river (reference site and impoverished site) up to 20 weeks yielded 24 species of aquatic hyphomycetes (Sridhar et al. 2011b).

A study on the decomposition of woody litters of teak and cashew wood (*Anacardium occidentale*) in Kaiga stream and Kadra Dam in the Western Ghats for 12 months showed higher diversity of aquatic hyphomycetes in stream than dam site (Sudheep and Sridhar 2013b). Aquatic hyphomycetes overcome the loss of their mycelial biomass or conidia by unidirectional flow of water in streams and rivers by colonizing the long-lasting substrates like woody debris and live roots of riparian vegetation. In addition, conidial survival in the intestine of fishes facilitates replenishment to the upper reaches. Moreover, many aquatic hyphomycetes survive in semiaquatic habitats of forest floor litter and in canopy (leaves, twigs, humus, stemflow, and throughfall) that facilitates their input into the nearby lotic bodies.

17.7.4 Physiology

A few studies have concentrated on the nutrition and physiology of aquatic hyphomycetes. Rajashekhar and Kaveriappa (2000) studied the impact of temperature and light on aquatic hyphomycetes. On exposure of nine species to different temperature ranges (5–35 °C), maximum growth was attained between 20 and 30 °C. *Vermispora cauveriana* showed the highest growth, while it was lowest in *Tetracladium setigerum*. On exposure to continuous dark, normal light-dark conditions and continuous light, sporulation of 6, 16, and 17 species, respectively, indicted the promotion of sporulation by light. Sati et al. (2012) studied the effect of temperature, pH, and light on the growth of aquatic hyphomycetes. For five aquatic hyphomycetes, optimum temperature is in the range of 20–25 °C for growth; optimum pH was in the range of 6.5–8.5. White as well as red light sources were favorable for growth, while blue light and darkness were inhibitory.

Chandrashekar et al. (1991) worked on the physiology of aquatic hyphomycetes using the water samples from a sulfur thermal spring in the Western Ghats (Bendre Thirtha). On incubation of coffee leaves naturally colonized in a Western Ghats stream in spring water at different temperatures (16 °C, 22 °C, 28 °C, 34 °C, and 40 °C), a gradual decrease in sporulating species was seen, i.e., 10, 8, 9, 1, and 0, respectively. The sulfide content (0.1–3.1 mg/L) did not affect the sporulation of aquatic hyphomycetes. In another study in a different sulfur thermal spring (Panekal) in the Western Ghats, Rajashekhar and Kaveriappa (1996) showed that sulfide concentration at 4 mg/L inhibited the colony growth of five aquatic hyphomycetes. Incubation of aquatic hyphomycetes colonized leaves in spring water at different temperatures (15, 20, 25, and 30 °C), and sporulating species were 0, 7, 7, and 2 spp., respectively, and in control, 9, 21, 19, and 7 spp., respectively, while only one

species sporulated at 35 °C and no sporulation at 40 °C. In the spring, synergistic effect of temperature and sulfide was seen.

Glucose and sucrose were the suitable carbon sources among eight carbon sources tested on four aquatic hyphomycetes, while fructose served as a good carbon source for two species and starch supported the growth of three species (Sati and Bisht 2006). The ammonium ions were the most preferred nitrogen source for four aquatic hyphomycetes followed by nitrates (Sati and Bisht 2005). Cystine served as a good source of nitrogen to all species studied, while asparagine was preferred by two species and proline was preferred by two species.

17.8 Ecosystem Services and Applications

Although some key information is available on aquatic hyphomycetes in India, it is necessary to link them towards ecosystem functions. One of the major ecosystem services of aquatic hyphomycetes is the breakdown of coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) and dissolved organic matter (DOM) (Suberkropp and Klug 1980). Being highly adapted to the lotic ecosystems, they serve as intermediaries between detritus and aquatic fauna (e.g., macroinvertebrates and vertebrates). To augment such transformation, they must have evolved to produce strong extracellular enzymes for degradation of lignocellulosic biomass (lignocelluloses, cellulose, hemicellulose, and pectin) and other plant polymers.

17.8.1 Enzymes

A few studies have been carried out to follow the capability of aquatic hyphomycetes to produce extracellular enzymes in India (e.g., Chandrashekar and Kaveriappa 1988, 1991). *Ingoldiella hamata* and *Phalangispora constricta* showed pyrocatechol oxidase activity, while amylase activity by *Triscelophorus acuminatus* (Chandrashekar and Kaveriappa 1988). *Lunulospora curvula* possesses high cellulase and amylase activities, while *P. constricta* had triacyl glycerol-hydrolyzing lipase activity. Among the carbon sources, carboxymethyl cellulose and ammonium sulfate are the excellent carbon and nitrogen sources to stimulate cellulase production in 12-day-old cultures of *L. curvula* as well as *Flagellospora penicillioides* under the optimal pH (5.2) with temperature (28 °C) (Chandrashekar and Kaveriappa 1991). The highest amylase production was achieved in *L. curvula* and *P. constricta* in a medium containing starch and ammonium sulfate with optimum pH (5.3) and temperature (28 °C) (Chandrashekar and Kaveriappa 1992). Starch and ammonium sulfate were the best sources of carbon and nitrogen for the production of amylase by *L. curvula* and *P. constricta* at pH (5.2) and temperature optima (28 °C), while amylase production in both species was reduced in the presence of glucose and sucrose.

17.8.2 Food Web

On the onset of enzymes, aquatic hyphomycetes enrich the detritus which is palatable to aquatic fauna (as shredders). Enzymes of aquatic hyphomycetes also serve their functions in the intestine of aquatic fauna. In addition to enzymes and proteins, ergosterol, the filamentous fungal sterol, has been identified as an important signature compound to assess the fungal activity. Besides, ergosterol is essential in the metamorphosis of arboreal insects (those that lay eggs in aquatic habitats as part of life cycle) and palatability to crustaceans and fish (Rao and Manoharachary 1982; Chandrashekar et al. 1989; Sridhar and Sudheep 2011a). Two approaches pertain to the interactions of aquatic hyphomycetes with detritus shredders that have been proposed by Bäerlocher (1992): (a) enrichment of quality litter by fungi into easily digestible state and (b) fungal enzymes continue to function in the gut of detritus shredders. To support these hypotheses, the juvenile fish and prawns preferred leaf discs colonized by aquatic hyphomycetes against control leaf discs (Rao and Manoharachary 1982). In another study, rubber leaf litter (*Hevea brasiliensis*) enriched by aquatic hyphomycetes was preferred against the control leaf litter by the fish (*Oreochromis mossambicus*) in a laboratory mesocosm study owing to enhancement of nutritional quality (Chandrashekar et al. 1989). The sequence of preference of rubber leaves colonized was by *Lunulospora curvula, Flagellospora penicillioides, Wiesneriomyces laurinus, Helicosporium* sp., *Triscelophorus acuminatus, Ingoldiella hamata,* and *Phalangispora constricta*. Sridhar and Sudheep (2011a) have demonstrated the occurrence of conidia of aquatic hyphomycetes in the feces of three fishes occurring in the Western Ghats (*Aplocheilus lineatus, Puntius filamentosus,* and *Rasbora daniconius*). They also showed the viability of aquatic hyphomycete spores using baiting technique as described for sediments (see Sect. 17.4).

17.8.3 Endophytes

Generally endophytic fungi are resourceful owing to their versatile properties (e.g., antimicrobial activity and metabolites). Nearly 40 species of aquatic hyphomycetes are known as endophytes in Indian waters (e.g., Ghate and Sridhar 2017; Sati and Pathak 2017). Endophytic *Anguillospora longissima* showed antibacterial activity against Gram-positive and Gram-negative bacteria (e.g., *Agrobacterium tumefaciens, Bacillus subtilis, Erwinia chrysanthemum, Escherichia coli,* and *Xanthomonas pseudomonas*) (Sati and Singh 2014). Same endophytes were antagonistic against plant pathogens like *Colletotrichum falcatum*, *Fusarium oxysporum*, *Pyricularia oryzae*, and *Tilletia indica* (Singh and Sati 2019).

Endophytic aquatic hyphomycetes are of agricultural significance owing to their excellent phosphate solubilization ability and plant growth enhancement. Endophytic fungi, *Anguillospora longissima*, and *Cylindrocarpon aquaticum* were potent phosphate solubilizers (Singh and Sati 2017). Among them, *A. longissima* was more potent than *C. aquaticum* with phosphate solubilization index 1.23 and 1.19,

respectively. Another endophytic fungus *Tetracladium setigerum* served as a potent phosphate solubilizer with phosphate solubilization index 1.3–1.5 on 7-day incubation in Pikovskaya's (PVK) agar medium with the highest phosphate solubilization (3.5 mg/L) in PVK broth after 21 days of incubation (Sati and Pant 2018). Among the endophytic *Heliscus lugdunensis*, *Tetrachaetum elegans*, and *Tetracladium nainitalense*, the first two showed growth promotion of two test plants (*Hibiscus esculentus* and *Solanum melongena*) through increased fresh weight, dry weight, length of shoots, and length of roots, while the latter had no such impacts (Sati and Arya 2010a). Further, inoculation of former two species did not show any disease symptoms in the host plants indicating their growth promotion traits under natural conditions.

17.8.4 Environmental Monitoring

Another important environmental application of aquatic hyphomycetes is the use of individual species, community, and their functions (e.g., conidial output, leaf mass loss, and ergosterol content) as indication of pristine or perturbation of lotic habitats. On an average, 20–25 species could be recorded in a moderate stream in India in one sampling. If the number of species falls below 10, there seems to be some factor responsible for decreased species richness (e.g., Raviraja et al. 1998a; Raghu et al. 2001). Water chemistry will depict the perturbations like pH, salinity, oxygen concentration, and biochemical oxygen demand. Organic pollution severely hampers the diversity as well as their conidial output (Raviraja et al. 1998a). Identification of pollution-tolerant and -sensitive indicator species will also help assessing the impact of pollution. For example, *Lunulospora curvula* and *Triscelophorus monosporus* were tolerant to heavy metal pollution in a stream near iron-ore mine (Raghu et al. 2001). Precise monitoring of risks of pollution in aquatic habitats could also be monitored by switching immersed leaf litter between unpolluted and polluted streams. Similarly, the lotic habitats could be classified as pristine, moderately polluted, and severely polluted based on species richness, diversity, conidial output, and mass loss of plant litter.

17.9 Human Interference

17.9.1 Deforestation and Urbanization

Freshwater biodiversity is hit by five major human interferences such as flow modification, habitat degradation, overexploitation, pollution, and invasion of exotic species (Dudgeon et al. 2006). The most important threat is channeling by removal of obstructions for agricultural or recreational purpose. Such interference reduces retention of detritus, which in turn destroys the potential ecological niches of aquatic hyphomycetes. Urbanization, deforestation, and removal of riparian vegetation lead to change in the course of the streams, which hampers the critical ecological

functions of aquatic hyphomycetes. The richness and diversity of riparian vegetation have been correlated to the increased species richness and diversity of aquatic hyphomycetes (Raviraja et al. 1998b; Rajashekhar and Kaveriappa 2003). Other human interference includes input of agricultural chemicals (e.g., pesticides and heavy metals), sewage, industrial wastes, and so on. In some regions, natural or artificial forest fires have additional threats to functions of aquatic hyphomycetes. Interestingly, urban runoff of southwest coastal city (Mangalore) possesses 35 species of aquatic and aeroaquatic hyphomycetes indicating their survival and role in urban habitats (Ghate and Sridhar 2018).

17.9.2 Pollution

Among ten major threats on earth, as many as seven hit the freshwaters (Rockström et al. 2009). Organic pollution, heavy metals, and pesticides are known to impoverish the stream fungi (Raviraja et al. 1998a; Sridhar and Raviraja 2001; Raghu et al. 2001). Organically polluted stretch of Netravati river in the Western Ghats resulted in 83% decline of aquatic hyphomycetes (Raviraja et al. 1998a). In Sitabhumi river, adjacent to Kudremukh iron-ore mine in the Western Ghats, heavy metal pollution reduced the diversity (6 spp.) as well as spore output (<1/mg leaf mass) of aquatic hyphomycetes (Raghu et al. 2001). However, a few species tolerated the extent of heavy metal pollution (e.g., *Lunulospora curvula* and *Triscelophorus monosporus*).

Growth of *Flagellospora penicillioides, Lunulospora curvula,* and *Phalangispora constricta* was not influenced by herbicides and fungicides up to 5 mg/L (Chandrashekar and Kaveriappa 1989). Organochlorine insecticides did not influence the growth at <10 mg/L, while inhibition of sporulation was seen between 5 and 10 mg/L (Sridhar and Kaveriappa 1986). In laboratory experiments incubation of aquatic hyphomycetes colonized leaf litter with Bordeaux mixture and benzene hexachloride inhibited sporulation of many species at 5–10 mg/L (*Campylospora chaetocladia, C. filicladia, Flabellospora verticillata, Flagellospora curvula, F. penicillioides, Lunulospora curvula, L. cymbiformis, Triscelophorus acuminatus, T. konajensis,* and *Wiesneriomyces laurinus*) (Sridhar and Kaveriappa 1986). Similarly, herbicides (Paraquat and 2,4-dichlorophenoxy butyric acid) and fungicides (Dihane M-45 and Captafol) have no growth inhibition up to 5 mg/L (*F. penicillioides* and *L. curvula*) (Chandrashekar and Kaveriappa 1989). Some of the pesticides (Bavistin, Captafol, 2,4-DB Fernoxone, Malathion, Mancozeb, Paraquat, Thodan, and Tridemorph) did not inhibit sporulation ability of aquatic hyphomycetes up to 5 mg/L, while conidial germination was inhibited at the same concentration (Chandrashekar and Kaveriappa 1994). Thus, the reproductive phase and germination of conidia seem to be more sensitive to herbicides/pesticides compared to vegetative phase and help in the recovery of activities of aquatic hyphomycetes on dilution of pollutants. Function of some valuable species of aquatic hyphomycetes (as keystone and core-group species) in the lotic ecosystem may be severely affected by human interference and elimination of such species will

lead to severe impoverishment (retarded decomposition and energy flow) of lotic habitats.

17.10 Future Perspectives and Conclusions

Considering aquatic hyphomycetes as an important mycota in lotic ecosystems, several challenges could be projected in view of environmental protection and safety. Beyond doubt the aquatic hyphomycetes serve as a model community to assess ecological services (food web and energy flow), perturbations or risk assessment (reduction in diversity and detritus breakdown), and restoration (rehabilitation and reinstatement) of lotic habitats. This could be achieved by monitoring structural (richness and diversity) and functional (palatability, enzyme/metabolites/growth factors) attributes of aquatic hyphomycetes. Assessment of leaf litter breakdown as a simple and cost-effective approach helps to evaluate structural (pattern) as well as functional (processes) phases of aquatic hyphomycetes in lotic ecosystem. Besides species richness and diversity approaches, systematics, anamorph-teleomorph connections, interaction with detritus shredders, energy flow, and stream productivity are other potential aspects of interest.

Based on the investigations carried out in the Western Ghats, mid-altitude streams possess the highest diversity of aquatic hyphomycetes. For example, Sampaje stream (~500 m asl) consists of about 90% of aquatic hyphomycetes (up to 80 spp.) reported from the whole Western Ghats region. As compared to the global richness (335 spp.), nearly 25% of species occur in a single location of the Western Ghats. Designation of such important lotic habitats in the rest of the Western Ghats, Himalayas, and other important ecoregions in India helps to impose conservation measures.

It is possible to classify the research carried out on aquatic hyphomycetes in India into three phases: phase 1—morphology, diversity, and distribution; phase 2—ecological studies; and phase 3—applications. Studies on phases 1 and 2 have been fulfilled to some extent, while phase 3 has been initiated. In application front, production of enzymes, secondary metabolites, growth factors, antimicrobial potential, medicinal potential, importance in agriculture, and inland fisheries are some of the aspects worth exploring to attract the funding agencies. Gaps in our knowledge in different aspects like richness, diversity, ecology, phylogeny, culture collections, new methods, molecular approaches, and applied aspects could be accomplished by well-designed national networks.

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Progress of Mushroom Research in India

18

Narender Singh Atri and Mridu

Abstract

The present communication deals with the details of progress of research work done on fleshy mushrooms in India over a period of time with special emphasis on systematics, biochemical, cultivation, and sociobiological aspects. The beginning of mushroom research in India dates back to nineteenth century. Since then lot of advancement has been made on various aspects. Earlier exploratory work on fleshy mushrooms was done following traditional techniques; however at present taxonomical work is being done on modern lines following both classical and latest molecular techniques. Besides this, mushrooms are also being evaluated for bioactive constituents which make them an excellent culinary option as functional food. The cultivation of mushroom is another aspect which has also started flourishing in the country. Over a period of time it has become a flourishing side venture for the entrepreneurs mainly in the states of Himachal Pradesh, Haryana, Punjab, Chhattisgarh, North Eastern States, etc. which offer high profit with relatively low investment. Mushrooms are also being evaluated for their therapeutic relevance in the treatment of some of the common ailments including diabetes, cancer, AIDS, hypertension, hypercholesterolemia, etc. In India, mushrooms remain an open field with innumerable opportunities for the researchers to explore and undertake researches in the areas of inventorization, evaluation, and domestication. Simultaneously, entrepreneurs need to take up the challenge of domestication, commercialization, and popularization of newer strains of fleshy mushrooms from the wild with application in human food and medicine.

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Mushrooms · Mycorrhiza · Systematics · Sociobiology · Cultivation · Biochemical studies

18.1 Introduction

India is one of the unique landmasses on this earth witnessing assorted elements of nature. From cold mountains to arid deserts, vast green plains to the wide seashores, there is a lot to explore in between. The diverse topography, physiognomy, and seasonal variations make beautiful landscapes and provide suitable home to a great range of life forms. The mushrooms are one of those precious creations of God offering multifarious benefits. Due to this, mushrooms have been cherished by Indians since old times for various purposes including food and medicine. Not only this, there is also a mention about the mushrooms in the ancient literatures such as Rig Veda, where the decoction of mushrooms has been called as "somrus" (Wasson 1968). People of India have been collecting wild edible mushrooms on the basis of their practical knowledge gained from the elders. This practice is followed even today and is being passed on from one generation to the next. But in the nineteenth century, Indian botanists started working on these wild tiny fascinating organisms scientifically. Since then, most of the states are being explored and time to time additions are being made to the mushroom biota of India and the world. In early period, the mushroom taxonomy was primarily based on the classical or traditional concepts, classifications, and techniques. But with the passage of time and advancement of technology, the methodology of investigating mushrooms and the concepts also changed which impacted the classification and the generic and species concepts. Nowadays, along with the classical method, molecular techniques are also being used for taxonomic investigations and for working out phylogeny and evolutionary relationships various digital tools and software are being used. The generated molecular sequences of the investigated taxa are submitted to online databases which become universally available and can be accessed from anywhere. According to a recent report, there is an estimate of 2.2–3.8 million species of fungi occurring on this earth (Hawksworth and Lücking 2017), out of which about 7000 identified species belong to mushrooms (Cannon and Kirk 2007). As compared from India, about 1920 taxa belonging to wild *Agaricales* Underw., *Russulales* Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David, and *Boletales* E.-J. Gilbert have been reported so far (Upadhyay et al. 2017). The work involving investigations on the systematics of mushrooms started slowly and gradually in various parts of the country. Not only this, simultaneously scientists also started showing their keen interest in studying the biochemical aspects of mushrooms as well as their medicinal properties. Besides this, cultivation of mushrooms also started in the beginning of the twentieth century on firm footing adopting scientific approach. As a consequence of the progress made in the cultivation of mushrooms, it has become one of the important horticulture crops among the Indian farmers. In this chapter attempt has been made to throw light

on the history as well as present-day status of researches on fleshy mushrooms in India with special reference to systematics, sociobiology, biochemical aspects, and cultivation.

18.2 Systematics and Mycorrhizal Studies

The earliest records of fleshy mushrooms in India are reported to have been documented in "Vedas" as early as 1200 B.C. (c.f. Ainsworth 1976). Wasson (1968) claimed that the "Soma" mentioned in the Hindu Rig Veda is the extract of *Amanita muscaria* (L.) Lam. However, the real beginning of the taxonomy and nomenclature of fungi dates back to Linnaeus in the eighteenth century, who appears to have been the first person to name few mushrooms from India (c.f. Purkayastha and Chandra 1976). A Danish Missionary Koenig, a pupil of Linnaeus, is reported to have collected a fungus sample from Tamil Nadu and sent it to Linnaeus (1753), and he named it *Lycoperdon pistillaris* {current name *Podaxis pistillaris* (L.) Fr.}. *Lentinus alopecinus* Fr. is reported to be another available record of the earliest validly identified mushroom from India (Fries 1838). Besides this, many other persons like Jacquemont (missionary), Sulpiz Kurz (Curator of the Royal Botanic Garden, Calcutta, at that time), Thompson, Hooker, and Wight are reported to have collected some samples and sent those to Berkeley, Currey, Leveille, and Montagne for identification (c.f. Subramanian 1986). Afterwards, Hooker and Thompson are reported to have collected a large number of samples from Northeast India, mainly Sikkim and Khasi Hills, and some from Kashmir and Plains of India, the records of which were published by Berkeley (1844–1856, 1867, 1876, 1882). These investigators laid the foundation stone for taxonomic studies in the field of mycology in general and mushroom in particular in India in the real sense. The actual work on collection and systematic studies on mushrooms began in India in the nineteenth century. Whatever contributions were made in this regard have been compiled in Fungi of India by Butler and Bisby (1931), which was first in the series and included the first authentic list of *Agaricales*. It was later revised by Vasudeva (1960). In due course of time additional compilations under the title "Fungi of India" were also brought out by Bilgrami et al. (1979, 1991). Sarbhoy et al (1986) released its sixth supplement enlisting 1805 species of fungi belonging to 650 genera. Up to this period, most of the work was based on the classification given by Saccardo (1882–1931). Later Jamaluddin et al. (2004) brought out another compilation of "Fungi of India" in which Indian fungi including mushrooms published between 1989 and 2001 were enlisted. In the later descriptions, due emphasis was laid on detailed macroscopic and microscopic characters along with the camera lucida drawings while describing the specimens taxonomically. In the meantime, many investigators including Sathe and Rahalkar (1975) published the updated list of agarics from India. Manjula (1983) revised and updated the list of agaricoid and boletoid fungi from India and Nepal along with the key to 538 species belonging to 115 genera and 20 families. Natarajan et al. (2005) updated the list which included 617 species of 105 genera of agarics and boletes. Amandeep et al. (2015a) enlisted

coprophilous agarics of India including 135 species belonging to 27 genera. Sharma and Atri (2015) gave an annotated checklist of genus *Lentinus* Fr. from India including 20 valid species. Recently the data on Agaricomycetous fungi of India has been compiled by Upadhyay et al. (2017) documenting 1920 taxa belonging to 35 families published from India from time to time. This checklist has recorded 168 genera representing 20 families of *Agaricales*, 32 genera representing 12 families of *Boletales*, and 7 genera representing 3 families of *Russulales*. Saini et al. (2018) documented 126 taxa of genus *Agaricus* L. from India. Verma and Pandro (2018) enlisted 80 species of amanitaceous mushrooms from India. The pioneer research on mycorrhizal fungi was undertaken in India by a few workers who published some reports on associations of fungi with *Abies spectabilis* (D. Don) Spach, *Cedrus deodara* (Roxb.) G. Don, *Picea morinda* (Wall.) Boiss., *Pinus roxburghii* Sarg., and *Taxus baccata* L. (Chaudhuri 1945; Bakshi 1957) The taxonomists in various regions of the country are working on different groups of agarics at a good pace which is discussed in the ongoing account.

North Indian Region: From Jammu and Kashmir, first survey of macrofungal diversity was most likely undertaken by Berkeley (1876). There are scattered reports about documentation of mushrooms from North India. As many as 63 mushroom species belonging to 23 genera were recorded by Hennings (1900, 1901) from Saharanpur in UP, 6 species were recorded from plains of India by Graham (1915), 11 agarics were documented from Punjab Plain by Rea (1922), 4 species of agarics from Kashmir by Murrill (1924), 6 species of agarics by Ginai (1936) from Punjab, and 25 species belonging to 13 genera by Mehrotra and Singh (1974) from Allahabad in Uttar Pradesh. Few more contributors from North India include Ahmad (1945) from Punjab, Pracer and Chahal (1962) from Punjab, Munjal et al. (1974) from Solan in Himachal Pradesh, Rath (1962) from Lucknow in Uttar Pradesh, Sohi et al. (1964) from Himachal Pradesh, etc. Watling and Gregory (1980) recorded 119 species of higher fungi from Jammu and Kashmir. Abraham, Kaul, and Kachroo were the other great contributors who along with their co-investigators studied the mushroom diversity of Kashmir, which was published in a series of documents (Abraham 1991, 1993; Abraham and Kaul 1985, 1988, 1990; Abraham and Kachroo 1989; Abraham et al. 1981, 1984). Concurrently, a series of North Indian *Agaricales* was published by Saini, Atri, and their co-investigators (Saini and Atri, 1982a, b, 1984, 1985, 1989a, b; 1995; Saini et al. 1982, 1988, 1989). Watling and Abraham (1992) documented 77 taxa of mycorrhizal macrofungi from Kashmir. Dar et al. (2002) reported 175 species of basidiomycetous fungi belonging to 61 genera from Kashmir Himalaya. Kumar and Sharma (2011a, b) identified 66 taxa belonging to 33 genera of wild edible lamellate and non-lamellate mushrooms from this region. Pala et al. (2012) described 14 species of macrofungi belonging to genus *Russula* Pers. and *Amanita* Pers. from Hirpora Wildlife Sanctuary of Southern Kashmir. Itoo and Reshi (2014) documented putatively ectomycorrhizal species from Kashmir Himalayas and recorded 19 of them associated with *Cedrus deodara* and 32 associated with *Pinus wallichiana* A. B. Jacks., while 25 taxa were documented associated with both of them. Many ectomycorrhizal species of Kashmir Himalayas were characterized by barcoding ITS region (Itoo and Reshi 2014; Itoo et al. 2015).

Dorjey et al. (2017) described 8 coprinoid species from Ladakh. Sharma et al. (2017) published new records of three species of genus *Gymnopilus* P. Karst. from India collected from Jammu and Kashmir. Farooq et al. (2017) gave an account of 25 mushroom species including ascomycetous and basidiomycetous members of Kashmir Himalayan forests which have been identified by studying morphological characters and using molecular technique as well. According to a recent data, 548 species of fungi including 182 species of basidiomycetous fungi have been documented from Jammu and Kashmir (Wani et al. 2020).

Contributions of Professor TN Lakhanpal and his associates from Department of Biosciences, H.P. University, Shimla, on the exploration of mushrooms of Northwest Himalayas are noteworthy. In his Presidential address in the Plant Sciences section (XIV) of 98th Indian Science Congress, held at SRM University, Chennai, Tamil Nadu, from January 3 to 7, 2011, Professor Lakhanpal presented an overview of the work done on various aspects of mushrooms done by him and his associates from 1976 onwards (Lakhanpal 2011). More than 150 publications and monographs from his lab dealing with mushroom systematics, mycorrhiza, ethnomycology, and ecology from this region speak volumes about the contributions made (Lakhanpal et al. 1988, 2010; Kumar et al. 1990; Bhatt and Lakhanpal 1990). Lakhanpal and Kumar (1984) published a study on mycorrhiza and mycorrhizosphere of *Picea smithiana*. Lakhanpal and his co-investigators have reported 72 species of fungi belonging to 15 genera of mushrooms and toadstools from Northwest Himalayas as an outcome of a vast study of 12 years (Kumar and Atri 2016). Saini and Atri (1984) gave an account of 31 species belonging to family *Russulaceae* Lotsy from Northwest Himalayas. Lakhanpal (1995) published a list of 190 species of *Agaricales* documented from this region. The ectomycorrhizal fungal species associated with Western Himalayas were reported by Pande et al. (2004). Studies on mycorrhiza and mycobionts of *Picea smithiana* were undertaken by Sagar et al. (2009). Semwal et al. (2014) described 23 edible wild mushroom species from Uttarakhand and Himachal Pradesh; 2 among these were ascomycetous fungi and others were basidiomycetous. Kaur and Singh (2014) documented 30 species belonging to family *Pluteaceae* Kotl. & Pouzar from North West India. Singh and Kaur (2015) recorded two new species of genus *Amanita* section *Caesareae* from Himachal Pradesh and Uttarakhand. Kumar and Atri (2016, 2019, 2020) reported the ectomycorrhizal association of russulaceous mushroom species with *Shorea robusta* from Indian Shiwaliks. With the advancement in techniques, Sharma et al. (2018a) studied the genetic diversity of 21 *Pleurotus* spp. from Himachal Pradesh using RAPD fingerprints.

An exhaustive account of russulaceous mushrooms of North India was published by Atri and Saini (1986, 1990a, b) and Atri et al. (1991, 1993, 1997) in a series of publications. Mushroom flora of Punjab was published by Saini and Atri (1995) enlisting 94 taxa spread over 24 genera. Besides this, an illustrated account of 6 species of *Bolbitius* Fr. (Amandeep et al. 2013a), 12 species of genus *Coprinopsis* P. Karst., 16 species of genus *Panaeolus* (Fr.) Quél., 12 taxa of coprophilous mushrooms (Amandeep et al. 2013b, 2014, 2015a, b), and 16 species of *Conocybe* (Amandeep et al. 2015c) was published from Punjab. Kaur et al. (2014) documented three species of genus *Panaeolus* from Punjab for the first time from India. Four new

species of the genus *Psilocybe* (Fr.) P. Kumm. were reported by Kaur and Kaur (2015) from Punjab. Besides this, Kaur et al. (2016) described six new taxa of genus *Agaricus* from Punjab. A detailed account of 6 taxa of family *Bolbitiaceae* Singer and 6 taxa of genus *Lepiota* (Pers.) Gray from Punjab was published by Atri et al. (1996, 2000). Atri and Kaur (2004) gave an illustrated account of genus *Coprinus* Pers. from Punjab. Das and Sharma (2005) documented family *Russulaceae* from Kumaon Himalaya. Vishwakarma et al. (2012) reported 40 taxa of macrofungi from Garhwal Himalayas. Joshi et al. (2012) published an exhaustive checklist of russulaceous mushrooms of Uttarakhand including 105 taxa, 55 of genus *Lactarius*, and 50 of *Russula*. Joshi et al. (2013) gave an account of six edible and medicinally important species of *Lactarius* from Garhwal Himalayas. Singh et al. (2017a) identified 21 wild edible mushroom species spread over 15 genera and 13 families from high elevations in Garhwal Himalayas. Bhatt et al. (2019) reported 8 new species of macrofungi from Uttarakhand. Sharma et al. (2018) while publishing a catalogue of russulaceous mushrooms of India documented 158 validly documented species of *Russula*, 83 species of *Lactarius*, 29 species of *Lactifluus* (Pers.) Roussel, 2 species of *Boidinia* Stalpers & Hjortstam, and 1 species each of *Multifurca* Buyck & V. Hofstetter and *Gloeopeniophorella* Rick.

East Indian Region: North East India is also not behind in the study of mushroom diversity. It was Berkeley to begin with (1844–1856), who initiated the taxonomic investigations on the materials collected from Sikkim and Khasi Hills of Assam. Currey (1874), Bose (1918, 1920, 1921, 1923, 1949), Bose and Bose (1940), Bose and Chatterjee (1950), Banerjee (1947), and Chakraborty and Purkayastha (1976) are some of the prominent earlier contributors from Bengal whose contributions are documented in the "Fungi of India" published from time to time (Vasudeva 1960; Bilgrami et al. 1979, 1991). Purkayastha and Chandra (1976, 1985) published a compilation of Indian edible mushrooms reported from time to time along with their own contribution. Sharma et al. (1988) documented mycorrhizal fungi from subtropical forest ecosystem of Meghalaya. Verma et al. (1995) compiled the list of 95 species of higher fungi published till then from the region. From North East, the contributions from the Lab of Dr. Kanad Das of Botanical Survey of India and Professor Krishnendu Acharya from the Department of Botany, University of Calcutta, Kolkata, are noteworthy. Besides using traditional tools, they are among the pioneers in India to use molecular tools in mushroom systematics. In a study on wild mushrooms of Sikkim, 120 wild species including 101 belonging to *Basidiomycota* Whittaker ex R.T. Moore have been reported by Das (2009). This count was updated to 126 species by Das (2010). Dr. Das is one of the most prolific contributors, who including his work on russulaceous mushrooms from Kumaon Himalayas has added more than 100 new taxa to the list of Indian mushrooms using both traditional and molecular techniques. As many as 151 species of *Agaricales* belonging to 42 genera were documented by Acharya et al. (2010) from the Darjeeling and hilly areas of Sikkim Himalaya. Das et al. (2013) described three new species of *Russula* (*R. sharmae* K. Das, Atri & Buyck; *R. dubdiana* K. Das, Atri & Buyck; and *R. sikkimensis* K. Das, Atri & Buyck) from Sikkim. Khaund and Joshi et al. (2013) reported 11 species of wild edible mushrooms

belonging to 9 genera and 8 families from Khasi Hills of Meghalaya. Dutta et al. (2013) enlisted 62 macrofungal species from Sundarbans. Kumar et al. (2013) described 15 wild edible mushroom species from Nagaland. Fourteen species belonging to 8 genera and 6 families along with their ethnobotanical information were documented by Sachan et al. (2013) from Similipal Biosphere Reserve, Odisha. Kalita et al. (2016) reported 22 species of wild edible mushrooms from Meghalaya. Pradhan et al. (2016) undertook an exhaustive survey of Eastern Himalayas and identified 98 species representing 72 genera spread over 47 families, out of which 58.16% were saprotrophs, 17.34% were ectomycorrhizal, and 10.2% were parasitic. A study on eco-diversity, productivity, and distribution frequency of mushrooms was undertaken in Gurguripal Eco-forest, West Bengal, from where 71 species of mushrooms belonging to 41 genera (Singha et al. 2017) were documented. Debnath et al. (2019b) documented 11 species of mushrooms from Tripura.

Central and West Indian Region: The diversity of agarics from Maharashtra has been studied by Uppal et al. (1935), Trivedi (1972), Sathe and Rahalkar (1975), Sathe and Sasangan (1978), Sathe and Deshpande (1982), and Patil and Thite (1978). Doshi and Sharma (1997) gave a list of wild mushrooms of Rajasthan comprising 173 species belonging to 95 genera. Patil (1978) enlisted 231 taxa of mushrooms including 63 genera of *Agaricales* from the state. The diversity of ectomycorrhizal mushrooms occurring in tropical forests of Madhya Pradesh and Chhattisgarh was studied by Sharma et al. (2009) and Pyasi et al. (2012). In all, 67 taxa representing 38 taxa of *Agaricales*, 17 taxa of *Boletales*, and 12 taxa of *Sclerodermatales* G. Cunn. were documented. Recently, Senthilarasu (2014) published a checklist of agarics of Maharashtra including 178 species belonging to 68 genera. Singh et al. (2006) undertook molecular characterization of 18 species of specialty mushrooms of Rajasthan. Borkar et al. (2015) documented 29 species of wild mushrooms occurring in Konkan region of Maharashtra. Three new records of genus *Russula* and *Amanita* were described from Sal Forest of Central India by Verma and Pandro (2018) and Verma et al. (2019, 2020).

South Indian Region: Noteworthy contributions have been made on mushroom systematics and exploration of other aspects of mushrooms including ecology, mycorrhiza, etc. from the laboratories of Late Professor K. Natarajan of CAS in Botany, University of Madras, Guindy Campus, Chennai; Professor P. Manimohan from Calicut University; and Dr. K.B. Vrinda and Dr. C.K. Pradeep from TBGRI Palode, Kerala. A series of descriptions of South Indian *Agaricales* were published by Natarajan (1975, 1977, 1978, 1995), Natarajan and Raman (1981, 1983), and Natarajan et al. (2005) along with the camera lucida drawings of microscopic structures. Natarajan (1995) gave an account of South Indian *Agaricales* (except Kerala). He recognized 230 species of agarics and boletes belonging to 67 genera. The state of Kerala was also explored exhaustively for the gilled fungi in the last two decades. Devi (1995) gave a list of 134 species belonging to 45 genera of mushrooms. ECM association of *Amanita muscaria*, *Laccaria laccata* (Scop.) Cooke, and *Suillus brevipes* (Peck) Kuntze with *Pinus patula* Schldl. was described by Mohan et al. (1995). A descriptive account of 9 species belonging to genus *Lentinus* (Manimohan et al. 2004) and 19 coprophilous species specifically

associated with elephant dung (Manimohan et al. 2007) were published from Kerala. Agaric flora of Western Ghats of Kerala has been studied identifying 409 species of agarics belonging to 100 genera under 19 families (Pradeep and Vrinda 2007). In a study on ectomycorrhizal fungal diversity of forests of district Thiruvananthapuram, it was reported that maximum number of ECM species belong to family *Russulaceae* followed by *Cortinariaceae* R. Heim. and *Amanitaceae*. From a total of 160 collections, 88% are reported to be associated with evergreen forests, 20% with both evergreen and deciduous forests, and 12% with exotic plantations (Pradeep and Vrinda 2010). Pushpa and Purushothama (2012) recorded 90 species of mushrooms including 80 species belonging to the order *Agaricales* from Karnataka. A checklist of 616 species of 112 genera from Kerala state was published by Farook et al. (2013). Sridhar (2018) documented 124 species of macrofungi from South West Coast of Karnataka, India. Few new species of crepidotoid agarics from Kerala were described along with their phylogenetic analysis by Kumar et al. (2018a, b).

18.3 Biochemical Studies

As discussed above, Indian scientists have been collecting and documenting the wild mushrooms for many decades. Not only this, there is a lot of ethnomycological data representing the diversity of mushrooms occurring in India which includes a good number of edible and medicinally useful mushrooms. Scientists have also been estimating the wild as well as cultivated mushrooms for their nutritionally and nutraceutically important components. Gopalakrishnan and Pruthi (1977) studied the nutritional quality of *Volvariella volvacea* (Bull.) Singer., while the proximate composition and nutritional value of *Volvariella esculenta* (Massee) Singer were estimated by Mohan and Jeyarajan (1978). Bano and Rajarathnam (1986) determined the vitamin content in four cultivated species of *Pleurotus*, namely *P. flabellatus* Sacc., *P. eous* (Berk.) Sacc., *P. sajor-caju* (Fr.) Singer, and *P. florida* Singer. Bisaria et al. (1987) studied amino acid composition of *P. sajor-caju* cultivated on different agro-residues. The nutritional composition of *P. citrinopileatus* Singer was documented by Ghosh et al. (1991). Longvah and Deosthale (1998) assessed *Schizophyllum commune* Fr. and *Lentinula edodes* (Berk.) Pegler from North East India for their nutritional composition. Till this period the biochemical studies on mushrooms in India almost revolved around the analysis of the components of nutritional value. But of late scientists also started showing their curiosity towards the medicinally important or nutraceutical components. Agrahar-Murugkar and Subbulakshmi (2005) studied the nutritional value of seven wild edible mushroom species from Khasi Hills of Meghalaya. Mallavadhani et al. (2006), while undertaking the chemical and analytical screening of three edible mushrooms *Volvariella volvacea*, *Agaricus bisporus* (J.E. Lange) Imbach, and *Calocybe indica* Purkayastha & A. Chandra, isolated various nutraceutically important compounds. Maiti et al. (2008) studied the antiproliferative and immunostimulatory protein fraction in some wild and cultivated mushroom

species. Kavishree et al. (2008) published the fat and fatty acid content of 23 wild edible species of mushrooms. Various species of culinary-medicinal termitophilous mushrooms were investigated for their chemical composition as well as vitamin content (Atri et al. 2012a, b). The amino acid composition of five species of genus *Lentinus* was evaluated by Sharma et al. (2012). Giri et al. (2012) studied the antimicrobial activity of various mushroom species belonging to genera *Amanita*, *Russula*, *Ramaria* Fr. ex Bonord., and *Pleurotus*. Atri et al. (2013) documented the nutritional and nutraceutical composition of five edible and medicinal species of genus *Pleurotus*. Sharma and Atri (2014) studied nutraceutical composition of wild species of *Lentinus*. Atri et al. (2014) documented the nutritional and nutraceutical potential of termitophilous and lepiotoid mushrooms of northwest India. Three species of macrolepiotoid mushrooms from North India were characterized for their nutritional and nutraceutical potential by Kumari and Atri (2014). Various wild edible species of mushrooms collected from east Khasi Hills, Meghalaya, were screened for the functional nutraceutical profiling including antioxidant, antimicrobial, and anti-inflammatory assay (Khaund and Joshi 2015). Sharma and Gautam (2016) studied nutritional, nutraceutical, and antioxidant potential of eight wild edible mushroom species from northwest Himalayas. Atri et al. (2016a) presented the nutritional profile of 20 North Indian mushrooms. In another investigation, antioxidant, antimicrobial, and antiproliferative activities of three wild mushrooms from Jammu and Kashmir were studied by Khan et al. (2016). While reviewing the work done on russulaceous mushrooms, Atri et al. (2016b) presented the nutritional profile and antioxidant properties of four species of *Russula*, two species of *Lactarius*, and one species of *Lactifluus* from North India. *In vivo* and *in vitro* antidiabetic activity of extracts of the three mushrooms was evaluated by Singh et al. (2017c). In another study, three wild edible mushroom species, viz. *Calocybe gambosa*, *Lentinus squarrosulus* Mont., and *Podaxis pistillaris* (L.) Fr., were estimated quantitatively for their nutritionally and nutraceutically important components (Mridu and Atri 2017). In a study, *Macrocybe lobayensis* was analyzed qualitatively and quantitatively for its antioxidant potential as well as metabolite profiling of its polyphenol-rich fraction (Khatua et al. 2019). Ao and Deb (2019) studied the nutritional and antioxidant potential of ten wild edible mushrooms from Nagaland. In a recent investigation, crude polysaccharides have been isolated from two wild edible mushrooms *Russula alatoreticula* K. Acharya, S. Khatua, A.K. Dutta & S. Paloi and *R. senecis* S. Imai and it is suggested that these mushrooms have excellent immunostimulating properties mediated by TLR/NF-κB pathway (Khatua and Acharya 2020). All these investigations show that presently the research on the biochemical parameters of mushrooms has reached up to a point that the bioactive components are studied along with their functions and physiological levels for which even *in vivo* studies are also being undertaken. Indian society is becoming more health conscious with the changing lifestyle and by adding mushrooms to their menu. Therefore, scientists are trying to get to the heart of the matter.

18.4 Studies on Mushroom Cultivation, Diseases, and Other Aspects

Mushroom cultivation is one of the fast-growing ventures nowadays in India. Being a crop with various benefits, low investment, and high profits, it attracts the farmers and scientists. The pioneers of mushroom cultivation in India were a few scientists of nineteenth century including N.W. Newton who attempted to grow a few mushrooms in 1886. Then in 1921, Lt. Col. Kirtikar initiated the cultivation of mushrooms in Kolkata (Suman and Sharma 2007). Bose (1921) published the possibilities of the mushroom industry in India based on the successful cultivation of two agarics on a medium composed of dung. Two reports on the cultivation of straw mushrooms *Volvariella volvacea* and *V. diplasia* (Berk. & Broome) Singer were published by Su and Seth (1940) and Thomas et al. (1943), respectively, in which they described the protocol of spawn preparation and cultivation. Further, following this as a basic protocol, various scientists kept on improvising the method for the enhanced growth of mushrooms by adding various supplements (Asthana 1947; Rath 1961; Ramakrishnan et al. 1968; Gupta et al. 1970; Purkayastha et al. 1980; Bisaria et al. 1987; Murugesan et al. 1995; Banik and Nandi 2004; Das and Mukherjee 2007; Panjikkaran and Mathew 2013; Naraian et al. 2016; Kataki et al. 2019). The cultivation of *Agaricus bisporus* (white button mushroom) started when a project was launched at Solan (Himachal Pradesh) with the collaboration of Indian Council of Agricultural Research and Government of Himachal Pradesh in 1961. Under this project, an initial effort of growing white button mushroom on cow dung was almost a failure but after that a successful cultivation was done on horse manure compost in 1964. Bano and Srivastava (1962) reported a higher yield in the cultivation of *Pleurotus flabellatus* on paddy straw. In the year 1971, Indian Council for Agricultural Research, New Delhi, started coordinated projects throughout India for mushroom cultivation. A successful commercial technology for the growth of *P. sajor-caju* was developed by Jandaik and Kapoor (1976) at the Indian Agriculture Research Institute, New Delhi. Madan et al. (1987) tried cultivation of *P. sajor-caju* on the leaves and stems of *Morus alba* L. and *Ricinus communis* L. from Himachal Pradesh and New Delhi; slowly the mushroom cultivation at commercial level extended to the other states such as Jammu and Kashmir, Punjab, Haryana, Chandigarh, Uttar Pradesh, Madhya Pradesh, Maharashtra, and Gujarat. With time, the cultivation of button mushroom also spread its feet to West Bengal and North East Hill (NEH) region (Chadha 1992). The production of button mushroom increased at a good rate mainly in Himachal Pradesh (8000 tons/year), Punjab, and Haryana (8000 tons/year) (Suman and Sharma 2007). Besides this, being a business with low investment and less space requirement, mushroom cultivation became a good source of income as well as self-reliance for rural and tribal women of India (Karwa and Rai 2005). There are many reports of involvement of women in this activity in Rajasthan (Paul and Panjabi 2003), Uttar Pradesh (Kunwar 2002), Orissa (Hossain and Mishra 2002), Haryana (Sharma et al. 2007a, b), Chhattisgarh (Khare et al. 2009), West Bengal (Biswas 2014), Bihar (Kushwah and Chaudhary 2016), and Punjab (Singla and Goel 2016). By the year 2007, the production of white button

mushroom (*Agaricus bisporus*), being the most accepted mushroom, reached about 60,000–70,000 tons/year while that of dhingri (*Pleurotus ostreatus*) reached 7200–8500 tons/year (Suman and Sharma 2007). Thakur (2014) reported the annual production of *P. ostreatus* at 15–20,000 metric tons and that of *Volvariella volvacea* and *Calocybe indica* at 10,000 tons. According to a report, from 2010 to 2017 the mushroom industry has grown with a pace of 4.3% per annum. In the year 2016, white button mushroom production was estimated at 94,676 metric tons (Sharma et al. 2017). Among all the Indian states, Punjab leads with a total production of 18,000 metric tons of mushrooms followed by Haryana (15,100 metric tons) and Maharashtra (12,050 metric tons) (Sharma et al. 2017).

India also started the export trade of mushrooms. In the year 1991, India exported only 790 kg of mushrooms (Singh et al. 2011). In 1994, India became the second largest exporter of canned mushrooms in the world, while by the year 2001–2002, total exports increased to 11.8 million kg (Singh et al. 2011; Sharma et al. 2017). As per the statistics published by Directorate General of Commercial Intelligence and Statistics, Ministry of Commerce and Industry, Government of India, in years 2008–2009, 15.1 million kg of processed mushrooms as well as 0.06 million kg of fresh mushrooms were exported. In 2016–2017, 1054 quintals of frozen and canned white button mushrooms were exported which generated a revenue of Rs. 7282.26 lakhs. As compared to 2018–2019 the quantity of mushrooms exported increased to 2913.19 quintals generating an estimated income of Rs. 83,98,274.

In view of the importance of mushrooms as a side avocation for strengthening the economic well-being of the common people, the National Centre for Mushroom Research and Training (NCMRT) was established at Chambaghat, Solan, Himachal Pradesh, in 1983 under the overall control of the Indian Council of Agricultural Research (ICAR), New Delhi. Under the auspices of NCMRT, All India Coordinated Research Project (AICRP) on Mushroom was sanctioned. In view of its contribution over a period of time in the establishment of the mushroom industry in India, this center was upgraded to ICAR-Directorate of Mushroom Research. Under All India Coordinated Research Project (AICRP) on Mushroom of this Directorate, at present 23 coordinating and 9 cooperating centers located in 27 states of the country are exclusively working on the mushroom research and development. Through the dedicated work being done under this project by various centers, the Directorate has developed an array of technologies for the cultivation of different nutritionally and medicinally important mushrooms in various agroclimatic regions of the country. Under the overall patronage of the Directorate all the coordinating centers work for a common mandate of germplasm collection of native edible mushrooms, multilocation evaluation of varieties and technologies, and training and supply of spawn to the growers. In view of the efforts made, India has registered a staggering 20-fold increase in the production of mushrooms in the last two decades. Presently it has reached up to 1.55 lakh tons (Kamal and Sharma 2018–2019). Out of the total production in India, button mushroom (*Agaricus bisporus*) accounts for 73% of production. The introduction of the other tropical mushrooms like oyster (*Pleurotus* spp.), paddy straw (*Volvariella volvacea*), milky mushrooms (*Calocybe indica*), and shiitake (*Lentinula edodes*) has brought the

much-needed diversification in the mushroom portfolio of the country leading to 12% share each of oyster mushroom and paddy straw mushroom, 2% share of milky mushroom, and 1% share of shiitake mushroom in the total mushroom production of the country (Srivastava et al. 2019).

Ever since the establishment of the Directorate of Mushroom Research lot of work is being done on all aspects of mushrooms including training, popularization, cultivation, recipes, postharvest technology, and mushroom products. As a part of popularization objective and extension activity, a series of handy cheap technical bulletins in English and Hindi language have been brought out by the Scientists of the Directorate. Some of these include Cultivation of Oyster Mushroom by Upadhyay (1990), Mushroom Recipes by Mehta Kiran (1990) and Verma and Rai (2005), Postharvest Technology of Mushrooms by Saxena and Rai (1990), Diseases of Mushrooms and their Management by Sharma (1994), Cultivation of Summer White Button Mushroom (*Agaricus bitorquis*) by Dhar and Verma (1996), Cultivation of White Button Mushroom (*Agaricus bisporus*) by Vijay and Gupta (1997), Cultivation Technology of Paddy Straw Mushroom (*Volvariella volvacea*) by Ahlawat and Tewari (2007), Cultivation Techniques of Shiitake by Annepu et al. (2019), etc. In the Status Report on Mushroom Products published by Srivastava et al. (2019) from the Directorate, complete details of the work being done on nutritional and nutraceutical evaluation of mushrooms; mushrooms as functional food; processing of mushrooms through freezing, canning, pickling, and drying; and mushrooms as a source of food and dietary supplements have been presented. Some significant contributions in this regard are by Harsh et al. (1993a), Harsh and Joshi (2008), Rai and Arumuganathan (2008), Mehta et al. (2011), Patel et al. (2012), Rahman and Choudhury (2012), and others.

Mushrooms like any other living organism are attacked by several pests and diseases. Since these are grown indoors on specific substrates, their quality and production are adversely affected by a large number of biotic and abiotic factors. The common biotic causes are parasitic and antagonistic fungi, bacteria, viruses, nematodes, mites, and insect pests. Very little work on pests and diseases affecting mushrooms has been carried out in India (Sharma 1994). Some such pioneering works on these aspects have been done by Sohi (1986), Garcha et al. (1987), Tewari and Singh (1984), Mallesha and Shetty (1988), Vijay and Sohi (1989), Kumar and Sharma (1998), Sharma (1991), etc. Work on white bubble disease of white button mushroom was undertaken by a number of investigators including Sharma and Kumar (2000). Sharma et al. (2007a, b) gave a detailed account of various fungal, viral, and bacterial diseases of button mushroom, paddy straw mushroom, and oyster mushrooms and their management in a technical bulletin published by ICAR-Directorate of Mushroom Research, Chambaghat, Solan. Keshari and Kranti (2020) gave an account of the management of phytopathogenic nematodes infesting mushroom. There is a long list of investigators who have contributed significantly to this area of research.

There are other major aspects also on which significant contributions are being made so as to increase the quality, productivity, and shelf life of mushrooms. Lot of work is being done on the evaluation of physical and biochemical parameters,

substrate evaluation, casing formulations, strain improvement, strain evaluation, crop protection, postharvest studies, etc. under the overall guidance of ICAR-Directorate of Mushroom Research, Chambaghat, Solan, by the scientists working at different coordinating centers (Annual Report 2018–2019) with the sole purpose of identifying the growing conditions so as to improve the qualitative and quantitative parameters of mushrooms for making this commodity remunerative for the stakeholders engaged in mushroom trade.

18.5 Studies on Sociobiology and Ethnomycology of Mushrooms

As discussed above, mushrooms are health-promoting fungal organisms which have been in use traditionally as a source of food and medicine. Most of the scientific investigations in this regard are provoked by the fact that common people have been collecting wild mushrooms since old times and are getting benefits by using them as a food and medicine as well as a source of livelihood. It is a routine activity for the indigenous people of India especially the rural and tribal populace of most of the states of the country in monsoon season. They have their own traditional ways of use, the knowledge of which has passed on from one generation to the next. However, the dependence on wild products has reduced nowadays because of easy availability in the markets as many of the commonly consumed mushrooms are being cultivated commercially. The local communities of different regions have many unheard stories with them. These experiences are the treasures for mankind which should be recorded and utilized. The idea of documenting this information came to the minds of a few scientists of India during 1970s. But this aspect of mycological research could not get much attention till the year 2000. There are a few reports which were published between 1970 and 2000 having such information regarding wild mushrooms. The main contributors of that period were Prof. T.N. Lakhanpal and his co-investigators from Himachal Pradesh (Lakhanpal 1986a, b, 1994; Lakhanpal and Shad 1986; Lakhanpal and Kaisth 1987; Sagar and Lakhanpal 1989; Shad and Lakhanpal 1991). Lakhanpal and Shad (1986) reported ethnomycology and trade of *Morchella* spp. in Himachal Pradesh including local names and recipes while ethnomycological information of two species of *Lactarius* was documented by Lakhanpal and Kaisth (1987). Sagar and Lakhanpal (1989) documented the edible species of Boletes with some ethnic information from North Western Himalayas, Dr. TN Kaul and Prof. JL Kachroo documented common wild edible mushrooms from Jammu and Kashmir (Kaul 1971; Kaul and Kachroo 1974), Dr. Aindrila Chandra and Prof. RP Purkayastha from Kolkata published "Manual of Indian edible mushrooms" (Purkayastha and Chandra 1985), and Dr. NSK Harsh and Prof. BK Rai from Madhya Pradesh recorded forest fungi and their ethnic uses among the local people and tribal communities of the state (Harsh et al. 1993b, 1999; Rai et al. 1993). Bahl (1986–1987) published the role of mushrooms in pharmacology. Later with the beginning of the twenty-first century, the researchers started giving more emphasis on the sociobiological and ethnomycological aspects of the

mushrooms of different regions. It includes the information regarding vernacular names in various languages and dialects, traditional ways of using them as food and medicines, and recipes, myths, and beliefs connected to the mushrooms. Till date, there are one or more records carrying ethnomycological and sociobiological information from almost every state of India from North to South and from West to the East except for a few (Atri and Mridu 2018).

From Jammu and Kashmir and Ladakh, Prof. Yash Pal Sharma and co-investigators have contributed a lot by surveying the local markets and recording recipes prepared by local people including Gaddi and Sippi tribes (Kumar and Sharma 2009, 2011a, b; Yangdol et al. 2014). In this region, *Cantharellus cibarius* Fr., *Geopora arenicola* (Lév.) Kers., *Ramaria formosa* (Pers.) Quél., *Sparassis crispa* (Wulfen) Fr., *Laetiporus sulphureus* (Bull.) Murill, etc. are some commonly used wild fungi. The ethnomycological information of *Morchella esculenta* from Kashmir Himalayas has been documented by Sayeed et al. (2018). The states of Punjab and Himachal Pradesh have been explored well by Prof. NS Atri and his associates from Patiala (Atri et al. 2005, 2010, 2012b, 2014; Kumari et al. 2012) and Prof. Anand Sagar from Shimla (Sagar et al. 2005, 2007, 2017). These publications include the local names of species of *Macrolepiota* Singer, *Termitomyces*, *Coprinus*, etc.; recipes; and market sale prices. Similar data specifically from the district Kinnaur was reported by Chauhan et al. (2014). The ethnomycology of northwestern Himalayas including Uttarakhand and Himachal Pradesh was documented by Semwal et al. (2014), Kumar et al. (2017), Singh et al. (2017c), Atri et al. (2019), Kaul et al. (2019), etc. In another northern state, Haryana, the collection of wild mushrooms is a common activity although the forested area of the state is very less surveyed for the documentation of the sociobiological data (Mridu and Atri 2015; Atri and Mridu 2018). Similarly, from Uttar Pradesh, there are just a few reports on ethnomycological uses of mushrooms (Vishwakarma et al. 2017; Vishwakarma and Tripathi 2019).

There are many tribal communities in the eastern states of the country which are somehow depending on the wild products for their food and livelihood. There are several reports of the traditional uses of mushrooms by the local people of Assam (Sarma et al. 2010; Gogoi and Sarma 2012; Basumatary and Gogoi 2016; Borah et al. 2018; Hansepi and Teron 2018; Nath and Sarma 2018; Swargiari and Buragohain 2018; Paul et al. 2019), Sikkim (Panda and Swain 2011; Pradhan 2016), Nagaland (Tanti et al. 2011; Kumar et al. 2014); Meghalaya (Khaund and Joshi 2013; Das et al. 2014; Kalita et al. 2016), Manipur (Apshahana and Sharma 2018), Tripura (Das et al. 2017), Arunachal Pradesh (Deb and Singh 2013; Singh et al. 2017b), West Bengal (Pradhan et al. 2010; Dutta and Acharya 2014; Singha et al. 2017, 2020), Odisha (Sachan et al. 2013; Panda and Tayung 2015), Jharkhand (Srivastava and Soreng 2014), Chhattisgarh (Rajak and Rai 2005; Tiwari et al. 2009; Kumar 2019), and Bihar (Manna and Roy 2014). Thus, the eastern region of India also bears a good potential to be explored for the documentation of sociobiological data as there are just a few reports from most of the eastern states.

There are only few documents available concerning the traditional practice of uses of wild mushrooms in the western states of India, i.e., Gujarat (Lahiri et al. 2010),

Rajasthan (Sharma and Doshi 1996; Doshi and Sharma 2007), and Maharashtra (Pusadkar et al. 2004; Tagade and Kawale 2014). The central Indian state Madhya Pradesh has also been explored up to some extent for the sociobiological aspects (Harsh et al. 1999; Rajak and Rai 2005; Thakur et al. 2015; Verma et al. 2019). Pandey and Veena (2012) documented the edible mushrooms along with some ethnomycological information from Western Ghats stretching from lower slopes of the Nilgiri Hills to the Goa and Maharashtra tri-border. The ethnic knowledge of wild mushrooms of Western Ghats was also recorded by Karun and Sridhar (2017).

The collection of wild mushrooms is a traditional activity in southern states also. There are reports of sociobiological uses of mushrooms from Karnataka (Karun and Sridhar 2013; Pavithra et al. 2015; Santhosh et al. 2016), Tamil Nadu (Johnsy et al. 2011; Davidson et al. 2012; Venkatachalapathi and Paulsamy 2016), and Kerala (Varghese et al. 2010; Shahina et al. 2019). However, there is hardly any ethnomycological information from Andhra Pradesh and Telangana. The available ethnomycological data from various regions of the country have been compiled by Atri and Mridu (2018) and Debnath et al. (2019a). Ethnomedicinal practices of wild mushrooms by the local tribes from various states of the country were documented by Debnath et al. (2019b). In view of the diverse landscape in terms of climate, tribal population, culture, and eating habits there is a lot of scope for ethnomycological investigations in India.

18.6 Conclusions and Future Prospects

In India serious efforts are being made for the documentation of mushroom wealth of the country, but still there is a long way to go as in many states including Haryana, Bihar, Chhattisgarh, Rajasthan, Gujrat, etc., not much has been done in this regard. These areas need more attention for inventorization of mushrooms since unexplored areas may be having many hidden species or varieties useful for the mankind. Exploration and evaluation of mushrooms from the wild and conservation of utility mushrooms are important areas of research which requires concerted effort on pan-India basis. Studies on mycorrhizal fungi also offer much scope for investigations.

The mushroom cultivation is also a good option with a great potential for sustainable agriculture. India has a major problem of agro-waste disposal for which mushroom cultivation is one of the good solutions. Being capable of digesting cellulosic and lignolytic substrates, mushrooms can be grown on agricultural residues. Farmers who are traditionally growing cash crops are slowly adopting mushroom cultivation; however still it is an underutilized activity in India. Some of the reasons behind this include lack of awareness of its benefits and some people have myths and false notions about mushrooms as a nonvegetarian food, etc. (Atri and Mridu 2018). There is also lack of knowledge about the edible species other than common white button mushroom due to which mushroom growers are less oriented towards the cultivation of other species of mushrooms. Government of India through

institutes like Directorate of Mushroom Research, Solan, and various agricultural universities is actively promoting mushroom cultivation by conducting training programs and also providing financial aids as well as insurance to the mushroom growers. In a nutshell, research on the various aspects of mushrooms has a bright future in India. Indian scientists, young scholars, and entrepreneurs should shoulder the responsibility to explore the mushroom biota of the country and domesticate the newer strains for utilization in the food and medicine industry in the welfare of the society.

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Developments in Thermophilic Fungal Research

19

T. Satyanarayana

Abstract

Thermophilic fungi have been isolated from a great variety of natural and man-made environments. These fungi grow in simple media containing carbon and nitrogen sources and mineral salts. Polyamines are essential for their growth and synthesized in these moulds. The composition of lipids varies considerably, predominantly containing palmitic, oleic and linoleic acids with low levels of lauric, palmitoleic and stearic acids. Thermophilic moulds are capable of efficiently degrading organic materials by secreting a battery of thermostable enzymes, which are useful in the bioremediation of industrial wastes and effluents that are rich in oil and heavy metals, and anti-nutritional factors such as phytic acid and polysaccharides. These fungi display synthesis of several antimicrobial substances and biotechnologically useful enzymes. The analysis of genomes of thermophilic fungi reveals high G:C contents, shorter introns and intergenic regions with lesser repetitive sequences, and further confirms their ability to degrade agro-residues efficiently. The genomes of thermophilic fungi are smaller than their mesophilic counterparts. Genetic engineering aids in ameliorating the characteristics of the enzymes. This chapter focuses on the biology and potential biotechnologies of thermophilic fungi, and developments in research in India.

Keywords

Thermophilic fungi/moulds · Lignocellulose · Composting · Bioremediation · Biotransformation · Bioethanol · Thermostable enzymes · Genomics

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19.1 Introduction

Fungi are members of the group of eukaryotic microbes that includes organisms like yeasts, moulds and mushrooms; these are classified as the kingdom fungi. Fungi are heterotrophs because they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Although all fungi are heterotrophs, the fungal kingdom comprises a wide range of life strategies ranging from saprotrophy through mutualism to parasitism. Among the estimated 3.8–5.1–12 million fungal species on Earth (Hawksworth and Lücking 2017; Blackwell 2011; Wu et al. 2019), close to 1,40,000 species have so far been characterized and named (Timthy et al. 2020).

Our fascination for this kingdom is natural because of their roles in organic matter cycling in ecosystems; the production of a variety of foods and beverages and even as a source of food themselves; their global ecological impact as the cause of devastating infections of humans, animals and plants including many crops grown around the world; and their roles as fundamental model systems in genetics and biological research.

Approximately 60 fungal species are capable of growth in the temperature range of 40–60 °C (Mouchacca 2000; Johri et al. 1999; Salar 2018). Probably because of their moderate degree of thermophily, habitats for isolating such fungi are not exotic as those of prokaryotes. Thermophilic fungal species display rather broad cardinal (minimum, maximum and optimum) temperatures that extend from 20 to 50 °C (Cooney and Emerson 1964). A thermophilic fungus is one that does not grow below 20 °C, but is capable of good growth at or above 50 °C. This definition has been used as a working model along with that of Crisan (1973) as those growing optimally at or above 40 °C. While thermotolerant fungi are those that can grow up to 10–12 °C (minima), but also occupy a niche with maxima around 50 °C (Cooney and Emerson 1964).

Thermophilic and thermotolerant fungi are ubiquitous in a great variety of substrates and environments (Cooney and Emerson 1964; Singh et al. 2016; Johri et al. 1999; Salar 2018). Their occurrence in natural (soils, composts and decomposing plant materials and others) and man-made (coal spoil tips, thermal effluent of nuclear reactors, washing machines, water heaters) environments had been well documented. Among approximately 1,40,000 species of fungi described and characterized till date, how are 60 species capable of growth at elevated temperatures, while others are not? The thermophily was extensively and intensively investigated (Sumner and Morgan 1969; Crisan 1973; Wright et al. 1983; Hammonds and Smith 1986). Thermophilic and mesophilic fungi are almost similar in many characteristics. When thermophilic fungi are cultivated at different temperatures, they display a greater saturation of lipids at high temperatures (Satyanarayana et al. 1992). Saturation of lipids in thermophilic fungal membranes appears to play an important role in thermophily, besides macromolecular thermostability (proteins and enzymes, ribosomes, etc.). The physical state of membrane lipids cannot, however, be viewed in isolation because their interaction with proteins

and sterols could also influence membrane fluidity and fungal growth (Hammonds and Smith 1986).

Thermophilic fungi play an important role in the ecology by degrading organic residues in soils, and in making compost for mushroom cultivation and promoting the growth of *Agaricus bisporus*. Other well-known applications of these fungi are in bioremediation; bioconversion of sterols and organic compounds; production of single-cell protein, industrially useful enzymes and bioactive compounds such as antibiotics and metal nanoparticles; and saccharification of lignocellulosics in bioethanol production. Several attempts have been made to sequence genomes of a few thermophilic fungi (*Myceliophthora thermophila*, *Thielavia terrestris*, *Rhizomucor miehei*, *Chaetomium thermophilum*, *Scytalidium thermophilum*, *Thermomyces lanuginosus*). This chapter focuses on the biology and potential biotechnological applications of thermophilic fungi with a thrust on the work done in India.

19.2 Thermophilic Fungal Research in India

Approximately 60 fungal species are capable of growth in the temperature range of 40–60 °C (Mouchacca 2000; Johri et al. 1999; Salar 2018). Probably because of their moderate degree of thermophily, habitats for isolating such fungi are not as exotic as those of prokaryotes. Thermophilic fungal species display rather broad cardinal (minimum, maximum and optimum) temperatures that extend from 20 to 50 °C (Cooney and Emerson 1964). A thermophilic fungus does not grow below 20 °C, whereas good growth occurs at or beyond 50 °C. This definition has been used as a working model along with that of Crisan (1973) as those capable of growth optimally at or beyond 40 °C. While thermotolerant fungi are capable of growth up to 10–12 °C (minima), it can also occupy a niche with maxima around 50 °C (Cooney and Emerson 1964).

Thermophilic and thermotolerant fungi are ubiquitous in a great variety of substrates and environments (Cooney and Emerson 1964; Singh et al. 2016; Johri et al. 1999; Salar 2018). Their occurrence in natural (soils, composts and decomposing plant materials and others) and man-made (coal spoil tips, thermal effluent of nuclear reactors, washing machines, water heaters) environments had been extensively documented. Among approximately 1,44,000 species of fungi characterized and described so far, 60 species are capable of growth at elevated temperatures, while others are not. The phenomenon of thermophily was extensively and intensively investigated by several workers (Sumner and Morgan 1969; Crisan 1973; Wright et al. 1983; Hammonds and Smith 1986). Thermophilic and mesophilic fungi are almost similar in many characteristics. When thermophilic fungi are cultivated at different temperatures, they display a greater saturation of lipids at high temperatures (Satyanarayana and Johri 1992). Saturation of lipids in thermophilic fungal membranes appears to play a key role in thermophily, besides macromolecular thermostability (proteins and enzymes, ribosomes, etc.). Hammonds and Smith (1986) opined that the physical state of membrane lipids

cannot, however, be viewed in isolation because their interaction with proteins and sterols can also influence membrane fluidity and fungal growth.

Thermophilic fungi play an important role in the ecology by degrading organic residues in soils, and in making compost for mushroom cultivation and promoting the growth of *Agaricus bisporus*. Other well-known applications of these fungi are in bioremediation; bioconversion of sterols and organic compounds; production of single-cell protein, industrially useful enzymes and bioactive compounds such as antibiotics and metal nanoparticles; and saccharification of lignocellulosics in bioethanol production. Several attempts have been made to sequence genomes of a few thermophilic fungi (*Myceliophthora thermophila*, *Thielavia terrestris*, *Rhizomucor miehei*, *Chaetomium thermophilum*, *Scytalidium thermophilum*, *Thermomyces lanuginosus*). This chapter focuses on the biology and potential biotechnological applications of thermophilic fungi with special emphasis on the work done in India.

Research on thermophilic fungi in India was initiated by B.N. Johri in 1970s at the Department of Botany, University of Sagar (now: Dr. H.S. Gour Central University), Sagar, and later continued at Bhopal and Pantnagar. Satyanarayana and Johri (1984) studied temperature and nutritional relationships of thermophilic fungi. Thermophilic fungal strains have been isolated from coal mine soils of Madhya Pradesh, and have been characterized and identified (Thakre and Johri 1976). Satyanarayana et al. (1977) reported seasonal variation in the occurrence of thermophilic fungi in nesting materials of birds. These fungal strains were isolated from composting paddy straw and their role in decomposing paddy straw is confirmed by producing extracellular enzymes (Johri and Satyanarayana 1986; Sharma and Johri 1992, Satyanarayana and Johri 1983b; Satyanarayana et al. 1985, 1988). Johri et al. (1999) published a book entitled 'Thermophilic Moulds in Biotechnology' in 1999 (Johri et al. 1999). Satyanarayana and Johri published several reviews in books as well as journals on thermophilic fungi (Johri and Satyanarayana 1984, 1986; Satyanarayana et al. 1987, 1988, 1992; Singh et al. 2016). Johri and his students carried out research on the production, characterization and applications of thermophilic fungal enzymes like amylase (Roy et al. 2000), lipase (Johri et al. 1990, 1991), protease (Satyanarayana and Johri 1983a; Prakash et al. 1982), xylanase (Satanarayana and Johri 1983b; Dubey and Johri 1987; Chaudari et al. 1988) and cellulase (Singh et al. 1990) and others (Satyanarayana et al. 1985).

Ramesh Maheshwari carried out investigations on different aspects of thermophilic fungi at the Department of Biochemistry, Indian Institute of Science, Bangalore. Maheshwari and Kamalam (1985) isolated *Melanocarpus albomyces* from soil and compost samples and studied factors that influenced the production of xylanase. Rajasekaran and Maheshwari (1993) assessed the potential of growth of thermophilic fungi in soils; the presence of thermophilic fungi in soils could be due to the result of aerial dissemination of propagules from composting plant materials. Maheshwari and his students carried out detailed investigations on the thermophilic fungal enzymes such as amylase, xylanase, cellulase, polygalacturonase, trehalase and others (Prasad and Maheshwari 1978; Maheshwari and Kamalam 1985; Mishra and Maheshwari 1996; Basha and Palanivelu 1998; Sathish Kumar and Palanivelu

1998; Prabhu and Maheshwari 1999). Physiological aspects and enzymes of thermophilic fungi have been reviewed elegantly by Maheshwari et al. (2000).

R.P. Thakre (Department of Botany, Nagpur University, Nagpur) and Borkar reported eco-physiology of thermophilic fungi isolated from diverse habitats in Vidarbha region of Maharashtra. The list included *Rhizomucor pusillus*, *Rhizopus microsporus*, *Rhizopus rhizopodiformis*, *Emericella nidulans*, *Chaetomium thermophile* var. *dissitum*, *Myriococcum albomyces*, *Thermoascus aurantiacus*, *Aspergillus fumigatus*, *Malbranchea pulchella var. sulfurea*, *Sporotrichum thermophile*, *Humicola insolens* and *Thermomyces lanuginosus* (Borkar and Thakre 2013; Borkar 2017).

At the Department of Microbiology (University of Delhi South Campus, New Delhi), T. Satyanarayana and his students have attempted to study polyamines (Singhania et al. 1991), and production and applications of cellulase, xylanase and pectinase (Banerjee et al. 1994, 1995; Kaur and Satyanarayana 2004a, b; Kaur et al. 2004; Kamra and Satyanarayana 2004, Phadtare et al. 2017), phytase (Singh and Satyanarayana 2006a,b; a, b, 2009, 2011; Altaff et al. 2008), and glucoamylase (Kaur and Satyanarayana 2004a; b; Kumar and Satyanarayana 2003, 2007, 2009) and others (Satyanarayana et al. 1985). Phytase-encoding gene of *S. thermophile* was cloned and expressed in *Escherichia coli* (Ranjan and Satyanarayana 2015) as well as *Pichia pastoris* (Ranjan and Satyanarayana 2016; Maurya et al. 2017). Bioprocesses for the production of enzymes in solid state and submerged fermentations have been developed.

R.C. Kuhad and his students (Department of Microbiology, University of Delhi South Campus, New Delhi) studied the production of cellulase by *Thermoascus aurantiacus* (Jain et al. 2016, 2018).

B.S. Chadha and his students at the Department of Microbiology (Guru Nanak Dev University, Amritsar) have been working on the isolation and molecular characterization of thermophilic fungi and their utility in the production of industrially important enzymes like amylase, phytase, xylanases, cellulases, pectinase and auxiliary enzymes (Chadha et al. 2019; Soni et al. 2008; Sharma et al. 2010; Kaur et al. 201; Basotra et al. 2019). The studies included purification, characterization and applications of enzymes. Potentially important mutant strains have been generated with deregulated hyper-enzyme-producing ability by employing a combination of classical (rational mutagenesis and screening, and protoplast fusion) and molecular approaches based on systems biology tools (proteome and genome). Agrawal et al. (2019) have recently cloned two lytic polysaccharide monooxygenase (LPMO)-encoding genes from *Scytalidium thermophilum* and *Malbranchea cinnamomea* and expressed in *Pichia pastoris*. An increase in saccharification of paddy straw was recorded when a commercial enzyme Cellic CTec2 was supplemented with the recombinant LPMOs.

K.R. Aneja and R.K. Salar at the Department of Microbiology (Kurukshetra University, Kurukshetra) isolated 19 species belonging to 14 genera of thermophilic and thermotolerant fungi from temperate soils of Northern India. Among them, ten species were thermophilic and the rest were thermotolerant. *Chaetomium senegalense* and *Myceliophthora fergusii* had been reported for the first time from

India (Salar and Aneja 2006). The significant role of thermophilic fungi in compost preparation had been reported. Salar and Aneja (1999) described the temperature relationship of 15 thermophilic and thermotolerant fungi isolated from the soils of Northern India. Among them, 2, 11 and 3 displayed optimum growth temperatures of 35, 45 and 55 °C, respectively. Salar (2018), who is now at the Department of Biotechnology (CDL University, Sirsa), recently published a book entitled 'Thermophilic Fungi: Basic Concepts and Biotechnological Applications'.

Bijender Singh and his students at the Department of Microbiology (Maharishi Dayanand University, Rohtak, presently at Haryana Central University, Mahendragarh, Haryana) have been working on phytase, xylanase and cellulase production by the thermophilic mould *Sporotrichum thermophile* (Singh 2016; Singh et al. 2017; Singh et al. 2018; Dahiya and Singh 2019; Dahiya et al. 2020).

Subrahmanyam et al. (1977) reported thermophilic fungi from the dust accumulated on books while working at the Department of Botany (Kakatiya University, Warangal). He described a new genus of Mucorales, *Thermomucor* in 1977 as *T. indicae-seudaticae* (Subrahmanyam et al. 1977). This mould was later shown to be a source of thermostable and neutral glucoamylase (Kumar and Satyanarayana 2003, 2007; Kaur and Satyanarayana 2004a; b). Subrahmanyam (1999) elaborately described the ecology and distribution of thermophilic fungi in a book chapter. S. Girisham, S. M. Reddy and their students (Kakatiya University, Warangal) reported coprophilous thermophilic fungi, and the effect of nutritional factors on the production of L-asparaginase by three thermophilic moulds (Shantipriya et al. 2015).

V.S. Bisaria, his colleagues and students at the Department of Biochemical Engineering and Biotechnology (IIT Delhi, New Delhi) have carried out investigations on the production and applications of xylanases of *Melanocarpus albomyces* (Biswas et al. 2010; Gupta et al. 2014).

Production of xylanase by thermophilic fungi was investigated by S.K. Khare and his students at the Department of Chemistry (Indian Institute of Technology Delhi, New Delhi) (Sadaf and Khare 2014; Sadaf et al. 2016).

Indian mycologists have made sincere efforts in understanding the diversity of culturable thermophilic fungi in a variety of soils, composts, decomposing plant materials, nesting materials of birds and several others. The isolated strains have been characterized and identified based on conventional and molecular methods. The diversity of non-culturable thermophilic fungi from various environmental niches by culture-independent metagenomic approach has not yet been attempted in India. Various aspects of extracellular enzymes of thermophilic moulds have been extensively investigated. The intracellular β-glucosidase and β-xylosidase of a few thermophilic moulds have been studied in view of their utility in saccharification of lignocellulosics in bioethanol production. Innovative approaches need to be attempted in order to improve enzyme titres and characteristics.

19.3 Ecology and Distribution

Thermophilic moulds are ubiquitous in nature and have been isolated from a wide variety of natural and man-made habitats. Most significant natural habitats reported for these fungi are decomposing organic materials where thermogenic conditions prevail as a result of microbial activity (Gregory et al. 1963; Chang and Hudson 1967; Mills and Eggins 1974; Johri and Satyanarayana 1984). Thermophilic fungi have also been reported from hot springs (Hedger 1975; Chen et al. 2003) and geothermal sites (Pan et al. 2010). By employing internal transcribed spacer (ITS) sequencing combined with morphological analysis, Pan et al. (2010) identified thermophilic fungi from geothermal sites to the species level. In total, 102 strains were isolated and identified such as *Rhizomucor miehei*, *Chaetomium* sp., *Talaromyces thermophilus*, *Talaromyces byssochlamydoides*, *Thermoascus aurantiacus* var. *levisporus*, *Thermomyces lanuginosus*, *Scytalidium thermophilum*, *Malbranchea flava*, *Myceliophthora* sp. 1, *Myceliophthora* sp. 2, *Myceliophthora* sp. 3 and *Coprinopsis* sp. Two species, *Thermomyces lanuginosus* and *Scytalidium thermophilum*, were dominant representing 34.78% and 28.26% of the sample, respectively. Most of these species thrive in alkaline conditions. Elevated temperature conditions also develop due to solar heat in the tropics. Moisture content is, however, an important factor that affects the development of thermogenic conditions.

A large number of thermophilic fungal strains have been reported from man-made habitats like hay and manure (Crisan 1969), stored peat (Kuster and Locci 1964), retting guayule (Cooney and Emerson 1964), stored oil palm kernels (Eggins and Coursey 1968), mushroom composts (Fergus 1964; Wiegant 1992; Weigant et al. 1992; Salar and Anejs 2007), naturally heated geothermal soils (Loginova et al. 1962), birds' nests (Tansey 1973; Satyanarayana et al. 1977; Korniłłowicz-Kowalska and Kitowski 2013) and nuclear reactor effluents (Tansey and Fliermans 1978).

The ubiquity of thermophilic fungal occurrence is because of their unusual ability to occupy a high-temperature niche that may exclude other forms. Because of their unique adaptability to sustain in high-temperature habitats, they are worldwide in their distribution. The presence of self-heating piles of organic debris all around the globe provides sustainability to thermophiles. Based on geographical studies, Maheshwari et al. (1987) reported that less than 50% of the known thermophilic fungi occur in India. As compared to the UK and the USA, Indian soils do not appear to have higher proportion of thermophilic moulds.

The major taxonomic divisions or phyla of fungi have been continuously evolving on the basis of molecular characteristics. The current classification of fungi uses recent molecular, multigene, phylogenetic studies and their sexual and asexual reproductive structures and spores to separate or delimit the groups. At high temperatures (55–60 °C), a limited number of fungal species are able to survive and grow. Several thermophilic fungi have been isolated and identified during the last six decades. The thermophilic fungi mostly belong to Zygomycetes, Ascomycetes and Deuteromycetes (anamorphic fungi). In the current system of classification,

anamorphic fungi have been placed in Ascomycetes/Basidiomycetes using molecular criteria such as ITS sequences. At present, more than 50% of the known thermophilic fungi are included in Ascomycetes.

Thermophilic fungi are the source of genes for heterologous expression for the production of biotechnology-relevant enzymes and related products. There is a paucity of information on their genetic diversity as well as genome structure and function. The advent of high-throughput DNA and RNA techniques for sequencing and large-scale proteomic analyses have enhanced new possibilities for exploiting thermophilic mould genomes for biotechnological applications. The data thus generated contribute to elucidating of the adaptations of thermophiles at elevated temperatures, and thus provide background information for fungal ecology. The phylogeny of thermophilic fungi has always been controversial. Morgenstern et al. (2012) analysed 86 fungal genomes and growth curves at different temperatures for 22 thermophilic or thermotolerant fungi for evolving the robust molecular phylogeny. Those which grow faster at 45 °C than at 34 °C were categorized as thermophilic and those that grew better or equally well at 34 °C were considered as thermotolerants. The single-copy marker genes [rpb1(DNA-directed RNA polymerase II subunit RPB1), rpb2(DNA-directed RNA polymerase II subunit RPB2) and mcm7 (minichromosome maintenance protein 7, DNA replication licensing factor (mcm7)] from the genome projects were used in phylogenetic analysis. The nuclear ribosomal small subunit (SSU), the 5.8S gene with internal transcribed spacers 1 and 2 (ITS 1 and 2) and the ribosomal large subunit (LSU) nucleotide sequences had also been used for elucidating the phylogeny of the orders (Sordariales and Eurotiales), which have more number of thermophilic fungal species. The true thermophilic fungi are found only within the Ascomycetes (orders: Sordariales, Eurotiales and Onygenales). It does not appear that true thermophilic fungal species occur in the Basidiomycota.

The amino acid composition of Ile, Val, Tyr, Trp, Arg, Glu and Leu (IVYWREL) has been observed to be related to the optimum growth temperature in prokaryotes (Zeldovich et al. 2007) and also in eukaryotes (van Noort et al. 2013). While analysing the genome sequences of three thermophilic fungi, *Chaetomium thermophilum*, *Thielavia terrestris* and *Thielavia heterothallica*, van Noort et al. (2013) observed that the total frequency of IVYWREL amino acids in *C. thermophilum* is significantly higher than in its mesophilic counterpart *C. globosum.*

19.4 Nutrition and Physiology

It was speculated that thermophily in the kingdom fungi emerged as an adaptation to seasonal changes and high day temperatures rather than an adaptation to colonize new thermal habitats (Powell et al. 2012). Thermophilic fungi have played a significant role in nature ever since they appeared on Earth. Until 1980s, thermophilic fungi were considered to have complex or unusual nutritional requirements (Maheshwari et al. 2000). Based on the information available on the nutritional

aspects of these fungi, it has been thought that they are capable of growth on simple media containing carbon and nitrogen sources and mineral salts, suggesting that they do not have any specific nutritional requirement(s) for growth. Moreover, they are mostly autotrophic for vitamins synthesizing them de novo. The nitrates of sodium and potassium are better sources of nitrogen than ammonium nitrate and ammonium sulphate, and asparagine supports a moderate growth (Satyanarayana and Johri 1984). Many thermophilic fungi are able to grow on starch, cellulose, hemicellulose, lignin and pectin (Deploey 1976; Satyanarayana and Johri 1984), utilizing these as carbon and energy source. Subrahmanyam et al. (1977) reported that *Thermoascus aurantiacus* grows on dulcitol, mannitol, oxalic acid and citric acid, whereas *Scytalidium thermophilum* grows poorly on oxalic and citric acids, with no growth on dulcitol. In another investigation, thermophilic fungi failed to grow on formaldehyde, except *Torula thermophila*, *Sporotrichum thermophile*, *Thermomucor indicae-seudaticae and Thermoascus aurantiacus*, which grew on methanol and formate (C1 compounds) as sources of carbon and energy (Chouhan et al. 1985). Maheshwari and Balasubramanyam (1988) reported simultaneous utilization of sucrose in the presence of glucose due to the invertase insensitivity to catabolite repression by glucose, and repression of the activity of the glucose uptake system by glucose as well as sucrose, since these sugars could be utilized concomitantly at almost equal rates at 30 °C.

An increase in mass of the whole or part of a living organism by synthesis of macromolecules is called as growth, while metabolism refers to the sum total of all biochemical reactions that take place in living cells. The growth processes in filamentous fungi, including thermophilic ones, are complex as compared to those of unicellular fungi like yeast. Filamentous fungal aerial hyphae are nourished through the mycelium which is in contact with the medium. This requires transport of nutrients over considerable distances, particularly in sporangiophores and aerial fruiting bodies that are also routinely produced in thermophilic fungi. Thermophilic fungi are known to grow faster than mesophilic or psychrophilic fungi. A reserve of essential nutrients has to be maintained in the medium for their continuous supply, particularly in fermentation processes. In mushroom composts, thermophilic fungi account for the major components of the total microflora. Of the 21 species of thermophilic fungi isolated from mushroom compost, *Scytalidium thermophilum*, *Humicola lanuginosa*, *Thermoascus aurantiacus*, *Chaetomium thermophile*, *Absidia corymbifera* and *Talaromyces emersonii* are fast growing at high growth rates (Straatsma et al. 1994). Three distinct patterns of growth of thermophilic fungi, isolated from coal mine soils, have been reported (Johri 1980).

Thermophilic fungi possess an amazingly high-temperature optima for their growth and metabolism. Their temperature dependence for cardinal temperatures (minimum, optimum and maximum) makes them an interesting group of organisms for biotechnological applications. Thermophilic fungi are of widespread distribution in tropical and temperate regions of the world. Eukaryotes fail to develop stable and functional organellar membranes at temperatures above 60 °C, which is considered as the upper temperature limit of thermophilic fungi. The optimum temperature for thermophilic fungi varies between 35 and 55 °C (Crisan 1973; Rosenberg 1975;

Satyanarayana and Johri 1984; Kumar and Aneja 1999). Thermotolerant fungi, such as *Aspergillus fumigatus*, *Rhizopus microsporus* and *Emericella nidulans*, grow at or below 20 °C, while thermophilic fungi such as *Thermomyces lanuginosus*, *Thermoascus aurantiacus* and *Talaromyces emersonii* exhibit a little or no growth at 20–30 °C (Tansey 1973; Rosenberg 1975). The length of the lag phase in thermophilic fungi at different temperatures is influenced by the incubation temperature. A short lag phase is expected at growth temperatures under optimal growth conditions. Based on the growth rates, thermophilic fungi are grouped as slow, moderate and fast growing.

Thermophilic fungi are tolerant to a broad range of pH in the range of 4.0–8.0. Rosenberg (1975) recorded the pH optima for 21 thermophilic and thermotolerant fungi isolated from various habitats. The pH optima (pH 6.5) of *Rhizomucor pusillus* and *R. miehei* is close to the pH optima of hay at baling from where these had been isolated. *Thermomyces lanuginosus*, *Malbranchea cinnamomea* and *Talaromyces thermophilus* grow optimally near neutral (pH 7.0). *Talaromyces emersonii* and *Allescheria terrestris* have been reported to grow in the acidic environment of sugarcane bagasse (pH 3.4–6.0). Metal ions like magnesium, iron, calcium and zinc will be available for fungi at low (acidic) pH, and would get precipitated at higher (alkaline) pH.

Oxygen is essential as the terminal electron acceptor, critical for the functioning of the electron transport chain (ETC) in aerobic respiration. Studies are limited on the requirement of oxygen in thermophilic fungi. Most of the fungi need at least 0.2% oxygen for trace growth, 0.7–1.05% for moderate growth and 1.0% for sporulation. The first detailed study was carried out by Noack (1920) while studying the physiology of *Thermoascus aurantiacus*. He observed decline in the rate of respiration due to reduced oxygen supply, but the respiratory quotient (CO_2/O_2) remained almost constant, even at very low concentrations of oxygen. Prasad and Maheshwari (1978) recorded a correlation in the cessation of growth in *Thermomyces lanuginosus* and depleting oxygen concentration in static cultures. Prasad et al. (1979) compared the metabolic rates of mesophilic and thermophilic fungi, and reported the average Q/O_2 (μL of O_2 uptake/mg of dry weight/h) of *Aspergillus niger* and *Thermomyces lanuginosus* to be 53.1 and 42.4, respectively, which suggested that thermophilic fungi are adapted to growth at elevated temperatures, but do not display much higher metabolic rates than mesophilic fungi.

The activities of microorganisms are influenced by the changes in the osmotic concentration in their surroundings. In order to be metabolically active, microbes make a balance between inflow and outflow of the dissolved salts of the external environment. The degree of availability of water to a microbe is indicated by the term water activity (a_w). The water activity of a solution is the ratio of the vapour pressure of its water to the vapour pressure of pure water under identical conditions of temperature and pressure. Like all other organisms, thermophilic and thermotolerant fungi are influenced by alterations in the water activity to which they must adjust for enabling them to grow. A change in temperature proportionately affects the vapour pressure over the substance and water; thus the value of a_w does not alter drastically (Johri et al. 1999). Water activity has a significant influence on

the rate of release of energy and heat that affects their activities. A xerophilic strain of a thermophilic *Humicola* sp., isolated from the sand dunes of Thar Desert, was able to grow in a medium with 50% sucrose (Mahajan et al. 1986). An increase in proline and sterol contents was recorded, suggesting their possible role in desiccation stress.

The light of visible spectrum impacts fungal processes such as mycelial growth, sporulation and spore germination. Kumar (1996) reported alternating zones of sporulating and non-sporulating patterns in thermophilic *Chaetomium thermophile* var. *coprophile* on Emerson YpSs agar. The zones of spores are usually stimulated by light, but may actually form during the subsequent dark periods. Light is known to influence the formation of pigments. Some thermophilic fungal species exhibit bright pigmentation that varies from yellow to red (Cooney and Emerson 1964). The pigmentation depends on the growth temperature, age and substratum. Based on electronic and infrared spectra and chemical analysis, the pigments in *T. dupontii*, *T. aurantiacus*, *M. cinnamomea* and *T. lanuginosus* were similar to aphins, the hydroxylated quinoid pigments of aphids. The pigments in thermophilic moulds are likely to be polyphenolic, polycyclic quinones similar to aphins. Moisture and fungal growth are known to cause deterioration of stored agricultural produce. Fungi require 95–100% relative humidity for their optimum growth, while a relative humidity below 80–85% inhibits the growth of most fungi. The maintenance of a sufficient moisture level in the culture media or composts is required for the growth of thermophilic fungi at their optimal temperatures, as water tends to evaporate quickly. The petri plates of thermophilic fungi on solid media are generally incubated in humidified chambers.

A wide distribution of putrescine, spermidine and spermine in thermophilic moulds had been reported by Singhania et al. (1991). The level of free polyamines is high in growing mycelium of *T. lanuginosus* than in the old stationary-phase mycelium. Polyamine levels declined at temperatures above and below the optimum. Difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC), strongly inhibited mycelial growth of *T. lanuginosus*, *T. emersonii* and *R. pusillus*; this suggested that ODC pathway is present in these moulds. Difluoromethylarginine (DFMA), an inhibitor of arginine decarboxylase (ADC), did not inhibit thermophilic fungi except *R. pusillus*, but mycelial growth was considerably reduced. This could possibly be due to either conversion of DFMA to DFMO by arginase or presence of ADC. Based on the observations, Singhania et al. (1991) concluded that thermophilic fungi require polyamines for normal growth and development.

A unique feature of thermophilic fungi to grow at elevated temperatures, where the majority of fungi perish, may be based partly on the thermostability and functional permeability of membranes. The lipid component of thermophilic moulds, therefore, plays an important role in thermophily, as many cellular functions are membrane linked. Low lipid content was observed in *A. alabamensis* (3.4%) [Satyanarayana et al. 1987], whereas a very high lipid content was recorded in *Remersonia thermophila* (syn. *Stilbella thermophila*) [38.1%], *M. cinnamomea* (24.8%) and *R. pusillus* (16.1–26.2%). The neutral lipid fraction varied between 35.9 and 88.3%, while the polar lipids ranged between 11.7 and 17.1% in

thermophilic moulds (Satyanarayana and Johri 1992). A change in growth temperature from 45 to 29 °C led to a marked variation in the proportion of phosphatidylinositol (1–9 to 11.9%), phosphatidylcholine (15.9–29.8%) and phosphatidylethanolamine (67.2–40.2%) in *Absidia ramosa* (Raju et al. 1976). Thermophilic fungal lipids contain predominantly palmitic (16:0), oleic (18:1) and linoleic (18:2) acids, with low levels of lauric (12:0), palmitoleic (16:1) and stearic (18:0) acids; the fatty acids 15:0, 16:2, 17:0, 19:0 and 20:0 are present in only a few thermophilic fungal species (Satyanarayana and Johri 1992).

19.5 Structure and Function of Thermophilic Fungal Genomes

Thermophilic microbial genomes are generally smaller than those of non-thermophiles. The genomes of thermophilic fungi are small as compared to those of their mesophilic counterparts (van Noort et al. 2013). The reduction in genome may be due to loss of genes coding for specific proteins, loss of transposable elements and reduction in the size of introns and intergenic regions. Such a genomic feature aids in a short division time for rapid reproduction and also reduction in the energy consumption for nucleotide synthesis. On the contrary, the duplication of genes, such as those that play a role in hyphal melanization and pigmentation of reproductive structures (ascocarp, peridia, ascospores and conidia), may provide an insight into the evolution of thermophily in fungi. Such a phenomenon has also been observed in mesophilic fungi for protecting their cells from high temperatures, ultraviolet radiation and desiccation during unfavourable conditions of atmospheric humidity.

A few hundred fungal genomes have been sequenced; this includes important human pathogens, plant pathogens and model organisms (Zhou et al. 2014). The knowledge of fungal genome sequences gives an opportunity for reconstructing evolutionary events. The genomes of several industrially useful fungi like *Aspergillus niger* (Pel et al. 2007) and *Trichoderma reesei* (Martinez et al. 2008) have also been sequenced. As thermophilic fungi represent a potential reservoir of thermostable enzymes, their genome sequencing is advantageous from a biotechnological perspective, besides that their genomes are amenable to manipulation using classic and molecular genetics (Berka et al. 2011). Genomes of some thermophilic fungi have been sequenced in the recent past. *Aspergillus fumigatus* acts as both primary and opportunistic pathogen, causing aspergillosis in human beings. The human respiratory tract is constantly exposed to its conidia because of its prolific nature of producing conidia. Increasing instances of asthma and sinusitis are often linked to the interaction of *A. fumigatus* and other airborne fungi with the immune system. Because of the significant burden of invasive disease caused by *A. fumigatus*, Nierman et al. (2005) sequenced the genome of its clinical isolate Af293. The genome of the organism comprises a 29.4 megabase (mb) genome in eight chromosomes with 9926 predicted genes.

Berka et al. (2011) sequenced the whole genome of the thermophilic Ascomycetes, *Myceliophthora thermophila* and *Thielavia terrestris*. The genome

of *M. thermophila* (38.7 Mb) is in seven telomere-to-telomere chromosomes with 9110 protein-coding genes, while *T. terrestris* genome is 36.9 Mb long in six chromosomes with 9813 genes. More than 200 genes encoding carbohydrate-active proteins (CAZymes) such as glycoside hydrolases (GHs), polysaccharide lyases (PLs) and carbohydrate-binding modules are recorded in both species. *M. thermophila* secretome comprises 683 proteins, whereas that of *T. terrestris* has 789; roughly one-third of these proteins account for CAZymes. The enzyme mixtures from both thermophilic fungi hydrolysed alfalfa straw leading to yield of higher levels of reducing sugars than the mesophiles, *Trichoderma reesei* and *Chaetomium globosum*. As compared to the mesophilic species, *M. thermophila* and *T. terrestris* genomes possess higher GC content of the coding regions; G:C pairs are more resilient to thermal denaturation. The high G:C content may represent an adaptation of the protein-encoding genes to elevated temperatures (Berka et al. 2011).

The genomics and proteomics of three more species of the genus *Myceliophthora* (*M. heterothallica*, *M. hinnulea* and *M. fergusii*) have been reported (van den Brink et al. 2013). The plant biomass degradation potential of *M. heterothallica* was much higher than the other two *Myceliophthora* spp. *M. hinnulea* isolates grew slowly on plant biomass. When plant biomass xylan content was high, most isolates grew better. *M. thermophila* isolates CBS 866.85 and CBS 669.85 grew rapidly on cellulose-rich and xylan-poor spruce. The enzyme activities were optimal at 50 °C for the mesophiles and at 70 °C for the thermophilic *Myceliophthora* isolates. *Chaetomium thermophilum* had been isolated from soil, dung or decomposing plant biomass; Amlacher et al. (2011) sequenced the genome which is of 28.3 Mb, divided into eight chromosomes with 7227 predicted protein-encoding genes.

The draft genome of the *Thermomyces lanuginosus* strain SSBP had been reported by Mchunu et al. (2013), an isolate from South African soil with a high potent xylanase production. The genome contains 23.3 Mb with 5105 genes. The total GC content was 52.14%, a value which increases to 55.6% in the coding regions. The predicted CAZymes summed up to 224 proteins. Considering the gene pathways which could be related to the thermophilic mode of life, *T. lanuginosus* genome possesses the ubiquitin degradation system and the epigenetic machinery that involves histone acetylation/deacetylation, histone methylation and ADP-ribosylation.

Rhizomucor miehei can grow at 50 °C or above and this is a source of industrial lipases and proteases. Zhou et al. (2014) investigated the genome and transcriptome of the *R. miehei* CAU432 strain which was isolated from self-heating hay in China. The genome has a size of 27.6 Mb with 10,345 predicted protein-encoding genes, which corresponds to 47.1% of the genome, in 10 chromosomes. The coding regions present on an average 4.5 introns per gene with an intron mean size of 90 base pairs. The average GC content (43.8%) was lower than that of *T. lanuginosus* (52.14%) (Mchunu et al. 2013), *T. terrestris* (54.7%) (van Noort et al. 2013) and *M. thermophila* (51.4%) (Berka et al. 2011), but higher than the average value for mesophilic zygomycetes (35.3%). Fujii et al. (2015) sequenced the genome of *Talaromyces cellulolyticus* Y-94 (formerly *Acremonium cellulolyticus*), a promising

cellulase producer. The genome is of 36.4 Mb containing genes for several enzymes involved in the degradation of lignocellulosic biomass such as cellulases, hemicellulases, pectinases and amylases.

The Genozymes Research Project (http://fungalgenomics.ca/wiki/Fungal_Genomes) has been sequencing the genomes of thermophiles, *Acremonium thermophilum* ATCC 24622, *Chaetomium olivicolor* CBS 102434, *Dactylomyces thermophilus* ATCC 26413, *Humicola hyalothermophila* CBS 454.80, *Myceliophthora hinnulea* ATCC 52474, *R. miehei* CBS 182.67, *Remersonia thermophila* ATCC 22073 (syn. *Stilbella thermophila*) and *Talaromyces emersonii* NRRL 3221. The project also plans to sequence *Melanocarpus albomyces* ATCC 16460, *Thermoascus aegyptiacus* ATCC56490 and *Thermophymatospora fibuligera* ATCC 62942. The publication of such data is anticipated to make an unprecedented contribution for understanding thermophily in the fungal kingdom and their industrial applications.

19.6 Potential Biotechnologies

Biotechnological applications of thermophilic fungi spring from their ability in decomposition of organic materials/residues, composting and mushroom production, comprising a great variety of thermostable intracellular and extracellular enzymes, single-cell protein (SCP), bioactive compounds (antibiotics, organic acids and nanoparticles), environmental management, soil amendment and biotransformations. All these aspects have been reviewed from time to time in detail (Cooney and Emerson 1964; Satyanarayana et al. 1992; Johri et al. 1999; Singh et al. 2016; Salar 2018); these aspects will be discussed briefly hereunder.

19.6.1 Role in Composting and Mushroom Technology

Organic materials are produced in large amounts annually in nature, which are degraded by the action of microbes. The organic matter decomposes slowly on the surface of the ground at ambient temperatures; this can be speeded up by gathering organic materials into heaps for conserving heat. The accelerated process of decomposition of organic matter by a mixed population of microorganisms in a warm, moist and aerobic environment is referred to as composting. A wide range of agricultural and forest residues (wood chips, bagasse, wool, hemp straw and others) are colonized and decomposed by thermophilic moulds. Self-heating of organic matter takes place by the rapid growth of thermophilic moulds which depends upon the presence of soluble organic components in the substrate. Available components such as starch, cellulose, hemicellulose and lignin are degraded by these moulds for obtaining nutrients for their growth (Sharma and Johri 1992). Wheat straw loses over half the dry weight in 60 days of composting; the loss in dry weight could be accounted for by the loss in hemicellulose and cellulose, while lignin is resistant for fungal and bacterial action (Chang 1967). Nearly similar

observations had been reported for paddy straw compost system (Satyanarayana 1978). In composts, a massive growth of thermophilic fungi contributes significantly to the quality of compost (Wiegant 1992; Weigant et al. 1992). In the field of composting, a new driving force has made entry which is related to the growth stimulation of *Agaricus bisporus* mycelium by thermophilic fungi, in particular *Scytalidium thermophilum* (Straatsma et al. 1995). The inoculation of thermophilic fungi showed that compost colonization by selected isolates is successful; thus the microbial manipulation of phase II composting is feasible (Straatsma et al. 1994).

19.6.1.1 SCP Production

Several attempts have been made for producing protein-enriched upgraded feeds and single-cell protein (SCP) by solid-state fermentation (SSF) by the use of thermophilic moulds (Johri et al. 1999). By employing biological treatment of cellulosic feedstuffs, digestibility, nutritional value, protein content and intake could be improved. The cellulolytic thermophilic fungi offer certain advantages over mesophiles, such as high rates of cellulose breakdown, good sources of protein, activity over a wide range of temperatures between 20 and 55 °C and higher growth rates. *Chaetomium cellulolyticum* (Chahal et al. 1981) had been used for upgrading animal feeds in order to produce SCP. Moo-young et al. (1979) reported *C. cellulolyticum* to be a good mould for the bioconversion of hemicellulose and cellulose to SCP.

19.6.1.2 Environmental Management

Environmental pollution is one of the terrible dangers that the mankind faces today. The increased human activity is leading to the expansion of industries at an accelerated rate that in turn leads to the deterioration of environment. Biological cleaning procedures utilize the fact that most organic chemicals are degraded by microbial action. Thermophilic moulds have been employed in disposing organic materials and toxic chemicals from domestic and industrial wastes (Singh and Satyanarayana 2009).

A significant environmental hazard is due to the existence of heavy metals and radionuclides. The major source of such pollutants is the industry, and the quantities contained in waste materials from both agricultural and domestic sources cannot be overlooked. The biomass of *T. emersonii* CBS814.70 had been reported to have a high biosorption capacity for uranium (Bengtsson et al. 1995). Likewise other organisms have also been confirmed to have biosorption capacity for various heavy metals. Some species of *Mucor* and *Rhizopus* had been known to be useful in the accumulation and removal of heavy metals and radionuclides from wastewater as well as mining operations. Similarly, thermophilic fungal biomass can be used for the biosorption of heavy metals; this area has not yet been adequately explored.

Toxic synthetic dyes and industrial effluents are at present treated at low temperatures because of the lack of suitable thermophilic microbial strains. Effluents from dye industries often contain an array of azo and other synthetic dyes which enhance biological and chemical oxygen demand. Biological treatment involving living and non-living microbial biomass is an effective and eco-friendly process in

comparison with physical and chemical routes. The thermo- and alkali-stable xylanases, cellulases and pectinases of thermophilic moulds could be useful for reducing organic residues in the paper and pulp industry effluents; this makes the process more economical besides being eco-friendly (Singh 2014). The addition of thermophilic fungal phytase to these enzymes reduces the phytic acid content of the pulp that leads to improvement in the quality of paper (Singh and Satyanarayana 2011; Singh 2014).

The effluents from oil industries are rich in fatty materials and, therefore, thermophilic fungi that lipases have found application in the treatment of such effluents. *Rhizopus arrhizus* (Kumar et al. 1993), *T. lanuginosus strain Y-38* and *R. miehei* and *T. lanuginosus* (Noel and Combes 2003) are capable of producing lipases in submerged fermentation, and thus are ideal in the treatment of the effluent from oil industries. Taha et al. (2014) employed the thermophilic fungus *T. indicae-seudaticae* for decolourizing at different temperatures and dye concentrations of azure B, congo red, trypan blue and Remazol Brilliant Blue R. Inactivated biomass was found to be more effective in decolourization than live biomass of *T. indicae-seudaticae*.

19.6.1.3 Soil Amendment

Microbes contribute to the formation of humus by the transformation of plant and animal constituents into humic compounds or by the synthesis of humic substances within the cells. There is a general increase in humic substances in the final degraded product by thermophilic fungi; some like *A. fumigatus* and *Scytalidium thermophilum*, however, form more humic substances than others. This humus contributes to improving the soil structure as reflected in increased aeration, reduction in power for ploughing heavy soils, rapid germination of seeds and reduced volume/weight. Compost application to soil causes a positive effect on the microbial population and the rhizosphere microorganisms, besides contributing towards the reduction of nematode population.

The use of fungal biomass as a soil conditioner is needed because several species are a good source of growth promontory substances, making the habitat ideal for growth of plants and soil fertility. Phosphorus deficiency in soils is a major constraint in agricultural production worldwide. Plants fail to use insoluble phytates directly; thus phytates have to be dephosphorylated by phytases/acid phosphatases before assimilation. Phytase of a thermophilic fungus *S. thermophile* had been shown to hydrolyse various insoluble phytates efficiently (Singh and Satyanarayana 2010). In hydroponics as well as pot experiments, wheat straw degraded by *S. thermophile* displayed growth promotion of wheat seedlings in pot experiments. The growth of wheat seedlings had been promoted by the fungus and the phytase; phosphorus uptake and growth had been enhanced by the activity of the phytase (Singh and Satyanarayana 2010).

19.6.1.4 Biotransformation

The development of novel biocatalytic methods is a continuously evolving area of chemistry, microbiology and genetic engineering; such biocatalysts are selective,

easy to handle and eco-friendly (Bo'dai et al. 2003). Thermophilic fungi have been recognized as potential sources of thermostable enzymes of scientific and commercial interest in synthetic chemistry (Bo'dai et al. 2003). Fourteen thermophilic fungi (*C. thermophilum* TUB-F-69, *S. thermophilum* CBS-183.64, *S. thermophilum* CBS-147.64, *M. thermophila* TUB-F-39, *Paecilomyces* sp. TUB-F-70, *T. emersonii* NRRL-3221, *T. thermophilus* NRRL-2155, *T. aurantiacus* TUB-F-43, *T. thermophilus* NRRL-5208, *T. indicae-seudaticae* NRRL-6429, *T. lanuginosus* ATCC-38.905 and *T. lanuginosus* CBS-224.63) had been cultivated in shake flasks which display lipase/carboxylesterase activities on olive oil, p-nitrophenyl palmitate and p-nitrophenyl butyrate (Bo'dai et al. 2003). Acetone precipitates of the enzymes of these moulds exhibited a wide range of enantiotopic selectivity, i.e. acetylation compared to the most common commercial enzymes. The transformation of steroids, progesterone, androst-4-en-3,17-dione, testosterone, pregnenolone and dehydroepiandrosterone by thermophilic mould *Rhizomucor tauricus* had been reported to be oxidative with allylic hydroxylation of the predominant route of attack that functionalized the stero-anthelmintic drug albendazole for producing novel and active metabolites of commercial interest such as albendazole sulfoxide, albendazole sulfone, N-methyl metabolite of albendazole sulfoxide and a novel product (Prasad et al. 2011). When steroids, progesterone, testosterone acetate, 17-acetoxy-5-androstan-3-one, testosterone and androst-4-en-3,17-dione were on incubation with *M. thermophila* CBS 117.65, a wide range of biocatalytic activities had been observed with the modification at all four rings of the steroid nucleus and the C-17 side chain (Hunter et al. 2009). This is the first thermophilic mould that brings about the side-chain cleavage of progesterone. Transformation of the saturated steroid 17-acetoxy-5-androstan-3-one resulted in the generation of 4-hydroxy-3,4-seco-pregn-20-one-3-oic acid. This mould has also been observed to carry out reversible acetylation and oxidation of the 17-alcohol of testosterone. *Acremonium alabamensis* and *T. emersonii* transformed cholesterol to cholestenone, while the former converted stigmasterol and sitosterol to stigmostadienone (Satyanarayana and Chavant 1987).

19.6.1.5 Antimicrobials and Bioactive Compounds

Among 61 strains of filamentous moulds, Svahn et al. (2012) reported *A. fumigatus* to display antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase-producing *Escherichia coli*, vancomycin-resistant *Enterococcus faecalis* and *Candida albicans*. Among the characterized molecules, gliotoxin was the secondary metabolite identified from *A. fumigatus*. Six indole alkaloids have been isolated from the thermophilic fungus *T. thermophilus* strain YM3–4 (Guo et al. 2011). Among the compounds, 1 and 2 were found to be new analogues of precursor notoamide E; the compound 3 was a novel analogue of prechinulin and compound 998. Compound 4 was found to be a naturally occurring cyclo(glycyl-tryptophyl) for the first time. This thermophilic fungus possesses a unique biosynthetic pathway for these talathermophilins. Two such alkaloids, talathermophilins A and B (1 and 2), were isolated from a thermophilic fungus *T. thermophilus* strain YM1–3 which were identified by NMR and MS spectroscopic

analyses (Chu et al. 2010). Macrocyclic PKS-NRPS hybrid metabolites, representing a unique family of natural products, have been found from *T. thermophilus* (Guo et al. 2012). These PKS-NRPS (polyketide synthase-nonribosomal peptide synthetase) hybrid metabolites contain a 13-membered lactam-bearing macrolactone, thermolides A–F (1–6). Among the thermolides, molecules 1 and 2 display a potent inhibitory activity against three nematodes with LC50 values of 0.5–1 mg/mL which is equally active like commercial nematocidal products avermectins. Yaginuma et al. (1989) isolated thiol protease inhibitors, estatin A and B, from *M. thermophila* M4323 culture filtrate. The basic water-soluble inhibitors were characterized as possessing an agmatine, trans-epoxysuccinic acid and L-phenylalanine or L-tyrosine moieties in the structure. Estatins are specific inhibitors of thiol proteases such as papain, ficin and bromelain. They suppress IgE antibody production in mice, but not IgG. These inhibitors were found to be useful in medicine and as reagents for research.

Xylooligosaccharides (XOS) and fructooligosaccharides (FOS) are well known to exhibit prebiotic effect and health benefits in supporting the growth of lactic acid bacteria like Bifidobacteria and *Lactobacillus* in the gut (Christakopoulos et al. 2003; Katapodis et al. 2003; Sadaf and Khare 2014; Jain et al. 2015). Besides as prebiotics, they are useful in food, feed and pharmaceutical industries. Biosynthesis of FOS was observed during the growth of *M. thermophila* on media which contained high concentrations of sucrose (Katapodis et al. 2003); submerged fermentation with sucrose (250 g/l) led to the production of 12.5 g FOS/L. The FOS mixture was characterized by acid hydrolysis and HPLC as 1-kestose, 6-kestose and neokestose. By using compatible solutes like sugars and amino acids, the osmotic adaptation of *M. thermophila* was maintained. Fatty acid analysis of the membrane lipids displayed a relatively high percentage of unsaturated components which are known to be associated with high membrane fluidity (Katapodis et al. 2003). Acidic XOS were obtained from birch wood xylan by the action of family 11 endoxylanase of *M. thermophila* (Christakopoulos et al. 2003); this xylanase liberated an aldopentauronic acid. Acidic XOS were found to be active against several Gram-positive and Gram-negative aerobically grown bacteria as well as *Helicobacter pylori*. Endoxylanase of *M. thermophila* had been used in generating XOS from xylan hydrolysis at pH 7.0 and 45 °C (Sadaf and Khare 2014). HPLC analysis revealed the presence of xylobiose, xylotriose and xylotetraose; xylotetraose level increased to 80–82% in 6 h. The absence of xylose in XOS is highly desirable for applications in prebiotics and food additives.

Biological synthesis of nanoparticles has received attention due to their eco-friendly and cost-effective nature. Syed et al. (2013) reported biosynthesis of silver nanoparticles by a *Humicola* sp. The TEM analysis of nanoparticles showed them to be spherical with good dispersity. Cell viability assays on NIH3T3 mouse embryonic fibroblast cell line and MDA-MB-231 human breast carcinoma cell line recorded positive results. Khan et al. (2014) also observed the synthesis of extracellular gadolinium oxide nanoparticles by a thermophilic *Humicola* sp. These nanoparticles were bioconjugated with chemically modified anticancer drug taxol

Table 19.1 Bioactive compounds produced by thermophilic/thermotolerant fungi

Thermophilic/ thermotolerant fungus	Bioactive compound	Bioactivity
Malbranchea pulchella var, *sulfurea*	Penicillin G, 6-aminopenicillanic acid, Malbranchins A and B	Antibacterial antibiotic
Malbranchea cinnamomea	Malbranchin A and B	Antibacterial and cytotoxic
Myriococcum albomyces	Myriocin	Antifungal antibiotic
Thermoascus aurantiacus	Thermozymocidin	Antifungal
Aspergillus fumigatus	Gliotoxin and others	Active against antibiotic-resistant bacterial strains and *Candida albicans*
Talaromyces thermophilus YM3-4	Talathermophilins (indole alkaloids), thermolides	Nematicidal activity
Myceliophthora thermophila	Estatins A and B	Thiol protease inhibition, suppression of IgE in mice
Humicola sp.	Metal nanoparticles (silver nanoparticles, gadolinium oxide nanoparticles)	Anticancer effect by nanoparticles conjugated with taxol
Various thermophilic fungi	Sillucin, miehein and vioxanthin	Active against Gram-positive and Gram-negative bacteria

for the treatment of cancer. The biomolecules produced by thermophilic fungi are presented in Table 19.1.

19.6.1.6 Bioethanol from Lignocellulosic Materials

Ethanol is the most common renewable fuel today produced from sugar or grain (starch); this raw material base will, however, be inadequate. Therefore, large-scale use of ethanol will most certainly be based on the production from lignocellulosic biomass in future. The major challenges in this context are optimizing the integration of process engineering, fermentation technology, enzyme engineering and metabolic engineering. To meet the growing demand for energy for transportation, heating and industrial process is one of the big challenges in the twenty-first century. The availability of raw material for the industry in a sustainable way is another challenge. An increasing concern of the sustainable oil supply has been the constant increase in oil prices. On a large scale, ethanol has already been introduced in Brazil, the USA and some European countries. Ethanol can be blended with petrol or used neat in dedicated engines, to take the advantage of higher octane number and high heat of vaporization with low emissions of unburnt hydrocarbons, CO and particulate matter (Hahn-Hägerdal et al. 2006).

Lignocellulose-degrading enzymes of thermophilic fungi are expected to play an important role in the saccharification of lignocellulosic biomass into sugars which can be fermented to ethanol by yeasts or bacteria. *Thermoascus aurantiacus* and *Thielavia terrestris* have been cultivated on various biomass substrates for glycoside hydrolases (McClendon et al. 2012). Crude culture filtrates utilized in the saccharification of ionic liquid-pretreated switch grass (*Panicum virgatum*) indicated that *T. aurantiacus* enzymes released more sugars (glucose) than those of *T. terrestris*. Enzyme preparation from *T. aurantiacus* has been shown to retain higher level of activity at elevated temperatures than a commercial enzyme mixture of Novozyme (Cellic CTec2). Switch grass pretreated with dilute acid, ammonia fibre expansion or ionic liquid was efficiently hydrolysed by *T. aurantiacus* enzymes with simultaneous liberation of sugars. Proteomic analysis of the *T. aurantiacus* culture supernatant revealed the secretion of dominant glycoside hydrolases which belong to different GH families. Despite substantial research carried out in the past on cellulolytic enzymes, there are still several issues/concerns that require concerted efforts in order to come up with novel/innovative/improved solutions (Chandel et al. 2018; Chandel et al. 2019).

19.6.1.7 Intracellular and Extracellular Thermostable Biocatalysts

Enzymes are biomolecules synthesized in living beings for accelerating metabolic processes for carrying out a large number of biochemical interconversions. These are like chemical catalysts in a chemical reaction, which aid in accelerating the pace of biochemical reactions inside and outside cells that are known as biocatalysts. Since the time immemorial, enzymes have been used by Egyptians in preserving foods and beverages. The first enzyme to be discovered was diastase by the French chemist Anselme Payen in 1833; this had catalytic property of hydrolysing starch (Payen and Persoz 1833). Since then, a large number of enzymes have been discovered from living beings, and a major proportion of these has come from microbes. A majority of industrial enzymes produced using thermophilic fungi are hydrolytic and used in breaking down various natural substances.

The thermophilic fungal enzymes have been receiving due attention mainly because of their potential utility in catalysing reactions at elevated temperatures in various industrial processes. The array of thermostable enzymes produced by these fungi like cellulases, amylases, proteases, phytases, xylanases, lipases and several others are useful in industries like food and feed, textiles, detergents, leather, dairy and pharmaceuticals. Depending on their applications, the industrial enzyme market has been classified into three sections: (1) technical enzymes, (2) food enzymes and (3) animal feed enzymes. The largest section is that of technical enzymes, where enzymes used for detergents and the pulp and paper industry constitute 20 and 25% of the total global market, respectively.

Several extracellular and intracellular (cell-bound) enzymes (Satyanarayana et al. 1992, Johri et al. 1999; Singh et al. 2016; Salar 2018) have been sourced from thermophilic fungi, which are listed in Table 19.2. The details of the various aspects of production, characteristics and applications of all thermophilic fungal enzymes have been reviewed by Johri et al. (1999) and Salar (2018).

Table 19.2 Extracellular and intracellular enzymes produced from thermophilic and thermotolerant fungi

Source	Names of enzymes
Various thermophilic/thermotolerant fungi	**Extracellular** Amylases Cellulases Xylanases Lipases Proteases Pectinases Phytases Phosphatases Laccases α-D-Glucuronidase Cellobiohydrolase D-Glucosyltransferase DNase
Various thermophilic/thermotolerant fungi	**Intracellular (cell bound)** Trehalase Invertase β-D-glucosidase β-D-xylosidase ATP sulfurylase Protein disulphide isomerase Lipoamide dehydrogenase Leucine aminopeptidase Cystine aminopeptidase Phosphoamidase β-Glucuronidase

19.7 Future Perspectives

There is a definite scope for using bioinformatics tools for improving the catalytic efficiency of enzymes. The research efforts are also needed to understand the capabilities of thermophilic moulds in carrying out environmental bioremediation, and bioconversions/biotransformations in steroid and non-steroid compounds, and in unravelling inter- and intra-species similarities and differences in steroid metabolism. Metagenomics tools can aid in unravelling their diversity and role in various ecosystems like soil, compost and others. Moreover, concerted efforts will be needed in strain improvement and scaling up of the production processes of thermostable enzymes with ameliorated catalytic efficiencies and their large-scale applications.

19.8 Conclusions

Thermophilic fungi are ubiquitous in their distribution; they efficiently degrade plant organic materials, and thus have immense potential in biotechnology and environmental management. These have not yet been explored adequately, although the new

genomics programmes focus on their enzymes with a potential application in biofuel production which opens up new avenues for their applications. The ability of these moulds to grow and secrete several extracellular thermostable enzymes (cellulases, laccases, xylanases, lytic polysaccharide monooxygenases, pectinases, lipases, phytases and several other enzymes) makes them efficient in decomposing organic residues. The enzymes produced extracellularly by the moulds are thermostable with higher catalytic efficiency than their mesophilic counterparts. The enzymes also possess a high specific activity for degrading lignocellulosic biomass, and thus would be useful in the production of biofuels and generation of biomolecules of pharmaceutical and therapeutic interest. The production levels of enzymes of these fungi are generally low as compared to those of mesophiles. The heterologous expression in mesophilic microbes gives an opportunity to overcome this barrier in a cost-effective manner. The availability of thermophilic fungal whole-genome sequences now provides an added opportunity for genome mining. Nevertheless, concerted efforts are called for explaining the presence of multiple copies of genes for several enzymes in thermophilic moulds and their role in physiology and growth. Furthermore, the scale-up studies and synthetic biology approaches are needed for attaining improvement in the fungal enzyme titres.

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Yeast Research in India: A Perspective on Taxonomy and Applications

20

Reshma Jadhav and Abhishek Baghela

Abstract

Yeasts have remained one of the important microbes because they represent excellent scientific model systems of eukaryotic origin and find numerous biotechnological applications. Yeasts can be considered as one of the earliest domesticated microorganisms involved in ancient fermentation processes without even human's understanding. For centuries, mankind has exploited their potential to produce fermented food and alcoholic beverages and for various other applications. Research in the areas of yeast diversity and applications has shown an unprecedent growth in recent times all across the globe. Exploration of yeasts in the natural habitats has resulted in the description of novel yeast species from India, while many researchers have used them for various biotechnological applications. This chapter is thus intended to give a brief account on the progress of yeast research in the field of diversity, taxonomy, and their applications in the production of ethanol, biodiesel or yeast lipids, enzymes, traditional fermented food and beverages, probiotics, xylitol, pullulans, and biosurfactants to mention a few. Their application in bioremediation, biosorption, and plant growth promotion has also been briefly discussed. Considering a rich history of yeast research in India, which is still the tip of the iceberg, the field offers a vast potential for future research. This chapter would also be helpful in understanding the established strong yeast research grounds and as to how these interesting organisms can further be utilized for future collaborative research in India.

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Yeasts · India · Taxonomy · Biotechnology · Applications · Fermentation · Biofuel

20.1 Introduction

Yeasts are among the smallest eukaryotes, which grow predominantly as unicellular organisms and multiply through budding/fission. Since their first discovery as the fermentative agent in wine and beer, mankind has exploited the metabolic activities of yeasts for the production of beverages, food, organic acids, enzymes, proteins, lipids, and pigments. Yeasts have existed on earth as old as 400 million years ago (Shen et al. 2018). However, the first microscopic observation of yeast cells was made by A. van Leeuwenhoek in 1680. During 1836 and 1838, Charles Cagniard Latour, Friedrich Kützing, and Theodor Schwann showed that "yeast" is a living organism and recognized it as a fungus. The first yeast genus *Saccharomyces* was described by Julius Meyen in 1838 (Barnett 2004). Though the formal description of yeast genera and species started in the early nineteenth century, they have however been extensively used for many thousands of years for traditional fermentation applications worldwide.

The primary application of yeasts in ancient India had been in traditional fermentations, which are about more than 3000 years old. Even the Harappans (c.3200–1500 BC) appear to have known the process of not only alcoholic fermentation but even distillation. The Rig Veda (c.1500 BC) mentions a sweet substance known as *Soma-rasa*, which is probably the first product of fermentation in India. The Rig Veda also mentions the use of kinva or nagnahu (ferment or yeast) for the preparation of *Soma-rasa* (Singh et al. 2010). Arishtas and asavas, the self-generated herbal fermentations of traditional Ayurvedic system, were also prepared by fermentation of sugar with dhataki (*Woodfordia fruticosa* Kurz) dried flower buds, wherein the wild yeasts present in the *W. fruticosa* flowers acted as the inoculum for fermentation (Sekar and Mariappan 2008). Though the formal description of yeast research in India started quite late, the history of yeasts in Indian civilization is quite old.

The field of yeast research is very vast which includes diversity, taxonomy, alcoholic beverages, bakery products, cheese, sausages, fermented foods, single-cell protein (SCP), feeds, polymers like pullulan, industrial enzymes, and metabolites like xylitol. Several yeast species have been used for heterologous production of enzymes and proteins. *S. cerevisiae* has been used extensively as a model organism to understand the basic molecular and cellular processes. Yeasts have also played important roles in agriculture as biocontrol agents and in bioremediation. The most important application of yeast has been in the field of biofuel research that includes bioethanol and biodiesel production.

In this chapter, we have compiled literature on yeast research in India emphasizing on yeast taxonomy, diversity, and some selected applications, e.g., bioethanol, biodiesel or yeast lipids, enzymes, traditional fermented food and

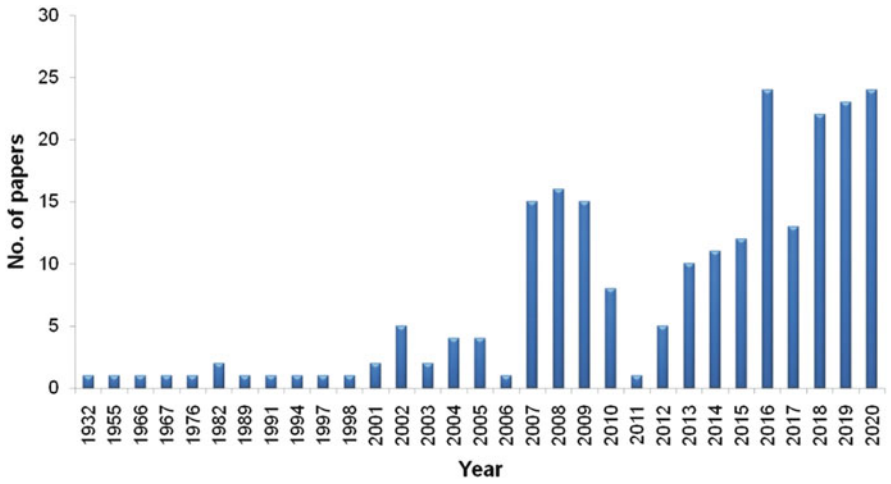

Fig. 20.1 Number of yeast research articles published since the twentieth century in India under the selected research areas

beverages, probiotics, xylitol, pullulan, and biosurfactants. Further applications included are bioremediation, biosorption, biocontrol, and plant growth promotion. Due to limited space, a few other important aspects, viz. medically important yeasts in India, yeast as model organism and their applications in nanotechnology, heterologous expression systems, organic acid production, etc., have not been dealt with. While tracing the yeast research in India, among the selected topics, the earliest record dates back to 1932, after which the progress in yeast research was a little slow and steady till 1998. Thereafter, there has been an increase in the number of yeast research papers in India. There has been a sharp increase in the number of yeast research papers published in India during 2016–2020. Considering the ascending trend, a significant contribution of Indian yeast researchers is expected in the coming years. Among various selected applications being reviewed, the highest number of research papers were published in the field of bioethanol-based biofuels, followed by enzyme and lipid (biodiesel) production. The contribution of Indian yeast researchers in few selected fields is discussed in the subsequent sections (Figs. 20.1 and 20.2).

20.2 Yeast Taxonomy Research in India

Earlier based on phenotype, yeasts were considered as primitive fungi; however, the advent of DNA-based phylogenetic analyses has brought a paradigm shift in the yeast taxonomy. It was initially believed that all yeasts are ascomycetes, but identification of some yeasts producing ballistoconidia and basidiospores changed the general perception and basidiomycete yeasts were also recognized (Kurtzman et al. 2015). As per the last official record in 2011, there are about 149 genera and

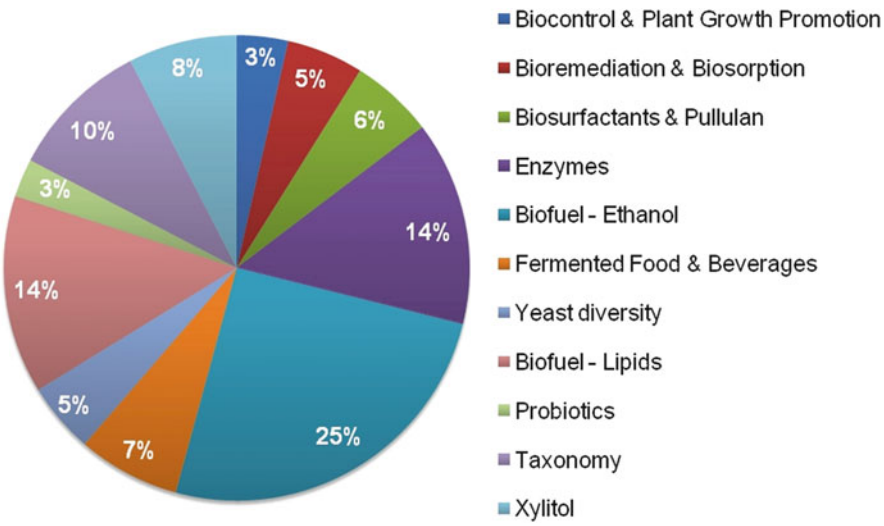

Fig. 20.2 Distribution of research papers published in different selected domains of yeast research in India

nearly 1500 species, reported across the world (Kurtzman et al. 2011). The numbers of novel yeast taxa have increased significantly during the last 10 years, and this 1500 species count must have increased significantly. Nevertheless, it is estimated that about 99% of the potential biodiversity of this group of fungi is still unknown and therefore not accessible for biotechnology or basic research (Amann et al. 1995). Total numbers of 22 novel yeast species have been described from India till now, which are discussed below (Table 20.1).

After the report of first novel species discovered in the twentieth century, *Sporobolomyces salmonicolor* var. *fischerii* was isolated from the cerebrospinal fluid of a meningitis patient. Dr. G.S. Prasad and his colleagues have done a pioneering work on yeast taxonomy by discovering a number of novel yeast species from different sources like *Candida digboiensis* from acidic-tar sludge-contaminated soil of Digboi Refinery; *Geotrichum silvicola* from *Drosophila* flies and from oak-tasar silkworm larvae (*Antheraea proylei*); *Cryptococcus rajasthanensis* from the inflorescences of *Digera* sp.; *Andrographis echioides, Debaryomyces singareniensis*, and *Torulaspora indica* from coal mine soil; *Candida ruelliae* from *Ruellia* sp. flower; and *Rhodotorula svalbardensis* from glacier cryoconite holes. Another piece of scholarly work on yeast taxonomy was done by Dr. S. Shivaji and colleagues, who discovered *Candida hyderabadensis*, *Blastobotrys serpentis*, *Rhodotorula himalayensis*, and two species of the genus *Pichia* (*Pichia cecembensis* and *Pichia garciniae*) from decaying green wine grapes, intestine of a dead trinket snake, Roopkund Lake soil, decaying papaya, and mangosteen fruit (*Garcinia mangostana* L., *Clusiaceae*), respectively. *Coniochaeta dendrobiicola, Leucosporidium himalayensis, and Aureobasidium tremulum* were reported by Dr. Rohit Sharma and team. Recent studies on yeasts associated with

Table 20.1 List of novel yeast species described from India

Sr. no.	Novel taxa	Host/place	Authors and reference
1	*Sporobolomyces salmonicolor var. fischerii var. nov.*	CSF of meningitis patient	Misra and Randhawa (1976)
2	*Candida digboiensis* sp. nov.	Acidic tar sludge-contaminated soil Digboi Refinery	Prasad et al. (2005)
3	*Geotrichum silvicola* sp. nov.	*Drosophila* flies, Atlantic rainforest sites (Brazil), and oak tasar silkworm larvae (*Antheraea proylei*) (India)	Pimenta et al. (2005)
4	*Candida hyderabadensis* sp. nov.	Decaying green wine grapes	Rao et al. (2007a, b)
5	*Cryptococcus rajasthanensis* sp. nov.	Inflorescences *Digera* sp. and *Andrographis echioides*	Saluja and Prasad (2007a, b)
6	*Debaryomyces singareniensis* sp. nov.	Singareni coal mines' soil	Saluja and Prasad (2007a, b)
7	*Pichia cecembensis* sp. nov.	Decaying papaya fruit	Bhadra et al. (2007)
8	*Blastobotrys serpentis* sp. nov.	Intestine of a dead trinket snake	Bhadra et al. (2008a, b, c)
9	*Candida ruelliae* sp. nov.	Flowers of the *Ruellia* species of the *Acanthaceae* family	Saluja and Prasad (2008)
10	*Pichia garciniae* sp. nov.	Decaying mangosteen fruit (*Garcinia mangostana* L., *Clusiaceae*)	Bhadra et al. (2008a, b, c)
11	*Rhodotorula himalayensis* sp. nov.	Roopkund Lake Soil	Shivaji et al. (2008)
12	*Cryptococcus randhawai* sp. nov.	Tree trunk's decaying wood *Ficus religiosa*	Khan et al. (2010)
13	*Candida stigmatis* sp. nov.	Stigmas of ant-visited Magnolia flowers	Sipiczki (2010)
14	*Torulaspora indica* sp. nov.	Coal mine soils, Singareni	Saluja et al. (2012)
15	*Rhodotorula svalbardensis* sp. nov.	Glacier cryoconite holes	Singh et al. (2014)
16	*Malassezia arunalokei* sp. nov.	Seborrheic dermatitis patients	Honnavar et al. (2016)
17	*Blastobotrys bombycis* sp. nov.	Larval gut of *Bombyx mori*	Barretto et al. (2018)
18	*Wickerhamiella shivajii* sp. nov.	Spent wash of distillery unit of sugar factory	Avchar et al. (2019)
19	*Aureobasidium tremulum* sp. nov.	Culture contaminant of laboratory	Inamdar et al. (2019)
20	*Coniochaeta dendrobiicola* sp. nov.	*Dendrobium longicornu*	Shah et al. (2019)

(continued)

Table 20.1 (continued)

Sr. no.	Novel taxa	Host/place	Authors and reference
21	*Leucosporidium himalayensis* sp. nov.	Chhota Shigri Glacier, Gramphu-Batal-Kaza Rd	Singh et al. (2019)
22	*Suhomyces drosophilae* sp. nov.	*Drosophila* gut, feeding on gleba of stinkhorn mushroom *Phallaceae*	Jadhav et al. (2020)

fungus-feeding insects were done by Dr. Abhishek Baghela and team with the discovery of *Blastobotrys bombycis* and *Suhomyces drosophilae* from silkworm and *Drosophila* gut, respectively. They also discovered *Wickerhamiella shivajii* from spent wash of distillery unit of sugar factory. *Malassezia arunalokei, Candida stigmatis,* and *Cryptococcus randhawai* were reported from decaying wood of *Ficus religiosa*, stigmas of ant-visited *Magnolia* flowers, and seborrheic dermatitis patients (Table 20.1).

20.3 Yeast Diversity Research in India

Yeasts are ubiquitous in nature and are found in various environmental habitats. Though they are present in various habitats, they are not evenly distributed. Some species occur in wide geographic ranges and are, therefore, known as ubiquitous generalists, while some are restricted to certain habitats or geographical locations only. The study of yeast diversity and biogeography is a vast area of research, which has not been explored adequately. In India, the majority of yeast diversity studies were conducted on marine habitats. The very first report on yeast diversity was published in 1955 by Dr. J. V. Bhat, who intrigued to identify yeasts from marine environment, in contrast to other researchers who focused mainly on terrestrial unicellular fungi. Dr. Mukund Deshpande's group reported a number of yeast species from grapes, which are used for wine making. Dr. J. W. Fell explored the yeast diversity from Indian Ocean and found multiple yeast species like *Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Sterigmatomyces, Debaromyces, Hanseniaspora, Pichia,* and *Saccharomyces*. Two other groups of researchers led by Dr. S. Shivaji and Dr. R. Dave studied and reported the presence of yeasts in Schirmacher Oasis, Antarctica, and extreme acid mine drainage of the Lignite Mine. A few researchers have also reported the diversity of yeasts from different parts of Indian coasts; for example, Dr. R. Gupta and Dr. N. Prabhakaran investigated the water samples of EEZ and South Coast of India and reported several yeasts like *Rhodotorula, Candida, Debaromyces, Saccharomyces, Geotrichum,* and others. Dr. S. N. Kutty reported the presence of *Candida* spp. from slope sediments of Arabian Sea and Bay of Bengal. Recently, a less explored niche, i.e., termite gut yeast diversity, has been investigated by Abhishek Baghela's group. They revealed that diverse yeasts belonging to both Ascomycota and Basidiomycota were found in

Table 20.2 List of yeast diversity studies reported from India

Sr. no.	Yeast sp.	Location/habitats	Authors and references
1	*Debaryomyces*, *Candida*, *Torulopsis*, *Rhodotorula*, *Cryptococcus*, and *Trichosporon*	The Indian Coast	Bhat And Kachwalla (1955)
2	*Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Sterigmatomyces, Debaromyces, Hanseniaspora, Pichia,* and *Saccharomyces*	The Indian Ocean	Fell (1967)
	Candida	Fish	
	Rhodotorula, Candida, and *Hanseniaspora*	Mauritius Island	
3	*Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Sterigmatomyces, Debaromyces, Saccharomyces,* and *Trichosporon*	The EEZ of Indian Coast	Gupta and Prabhakaran (1989)
4	*Rhodotorula, Candida, Debaromyces, Saccharomyces,* and *Geotrichum*	The South Coast of India	Prabhakaran and Gupta (1991)
5	*Rhodotorula, Bullera,* and *Candida*	Schirmacher Oasis, Antarctica	Shivaji et al. (1994)
6	*Cryptococcus neoformans* var. *gattii*	*Eucalyptus camaldulensis*	Chakrabarti et al. (1997)
7.	*Pichia anomala*	Dried flower buds of *Woodfordia fruticosa*	Vohra and Satyanarayna (2001)
7	*Candida digboiensis*	Extreme acid mine drainage of the Lignite Mine	Patel et al. (2009)
8	*Candida azyma, C. quercitrusa, D. hansenii, H. guilliermondii, H. viniae, H. uvarum, I. orientalis, I. terricola, P. membranifaciens, S. cerevisiae,* and *Zygoascus steatolyticus*	Grapes used for wine making	Chavan et al. (2009)
9	*Candida*	Slope sediments of Arabian Sea and Bay of Bengal	Kutty et al. (2013)
10	*Candida*	Gut of *Perionyx excavatus*	Samanta and Das (2016)
11	Multiple yeast species	Gut of wood-feeding termites	Tiwari et al. (2020)

the gut of wood-feeding termites. A list of studies on the yeast diversity reported by Indian researchers is presented in Table 20.2.

20.4 Yeast from Fermented Foods and Beverages of India

Traditional fermented foods and beverages are preferred in certain communities due to their characteristic flavor, color, and texture (Mondal et al. 2016). The fermented foods and beverages are associated with various health benefits as they contain beneficial nutraceuticals, bioactive components, and good microbes (Tamang et al. 2016). Such traditional food and beverages have been tried and tested since ages and because of their community-proven safety and beneficial experiences, researchers have been focusing to identify the wild microbial resources present in them. Thus these microbial resources can be exploited for the welfare of mankind. Most commonly *Saccharomyces cerevisiae*, also known as baker's yeast, is associated with the preparation of various fermented foods. Many researchers have identified the yeasts involved in various fermentation foods and drinks all over India to study their potential applications. The pioneering research in this field was carried out by Prof. Jyoti P. Tamang, who has worked on various traditional northeast Indian food and beverages, e.g., Marcha and Thiat, the traditionally prepared amylolytic starters, and a few other dried starters of Indian alcoholic beverages of India (Table 20.3). Baker's yeasts' versatile abilities are proven by its applications in the production of different types of beverages like fermenting tropical fruits into wine. A variety of wines have been prepared using traditional starters containing different yeast species, viz. rice, apple, and mango wines (Table 20.3). Here, a group of yeasts, namely *Candida glabrata, Debaryomyces hansenii, Ogataea parapolymorpha, Dekkera bruxellensis*, *Wickerhamomyces anomalus, Saccharomycopsis malanga, Saccharomycopsis fibuligera,* and *Saccharomycopsis malanga,* were found in Xaj-pitha, which is a traditional starter used for rice wine production. For apple and mango wine, *S. cerevisiae* itself is enough to achieve the desired product (Table 20.3). A tea preparation called as Kombucha tea also requires yeast species belonging to *Candida* genus for the fermentation. It has been reported that yeasts are also involved in a consortia of starter cultures for fermentation of the cereal-based alcoholic beverages for conversion of sugar into ethanol (Table 20.3).

20.5 Yeast as Probiotics

Probiotics are living microorganisms that are beneficial to the host organisms when administered at the correct dosage. The most well-characterized probiotic microbes have been bacteria such as Bifidobacteria and *Lactobacillus*; however, certain yeasts have also been shown to exert health benefits across various studies (Czerucka et al. 2007). The most common probiotic yeast is *Saccharomyces boulardii*, which is recommended for the treatment of acute gastrointestinal diseases, bacterial diarrhea, and inflammatory bowel disease (Kelesidis and Pothoulakis 2012). The use of *Saccharomyces cerevisiae* for probiotic applications is generally regarded as safe (GRAS) by the US Food and Drug Administration (FDA). Further, yeast-based probiotics are desirable because of their natural resistance to most antibiotics allowing them to persist in the gastrointestinal (GI) tract during an antibiotic regimen

Table 20.3 List of yeast species identified in various fermented food and beverages in India

Sr. no.	Yeasts	Isolation source/used for drink	Authors and references
1	*Saccharomyces, C. glabrata, P. anomala, P. burtonii Saccharomycopsis fibuligera, Sm. capsularis*	Marcha	Tsuyoshi et al. (2005)
2	*S. cerevisiae, P. anomala, Trichosporon* sp., *C. tropicalis, P. guilliermondii, C. parapsilosis, T. delbrueckii, P. fabianii,* and *C. montana*	Hamei—traditional starter used for rice wine production	Jeyaram et al. (2008)
3	*P. kudriavzevii* (RS-3)	Cabernet Sauvignon	Sharma et al. (2012)
4	*Candida* sp.	Kombucha tea	Chakravorty et al. (2016)
5	*S. cerevisiae* (MTCC-170)	Conversion of tropical fruits to wine	Baidya et al. (2016)
6	*K. marxianus*, *I. orientalis, T. asahii, S. cerevisiae*, and *E. dermatitidis*	Soft chhurpi	Rai et al. (2016)
7	*M. guilliermondii, W. ciferrii, S. cerevisiae, C. glabrata, D. hansenii, D. bruxellensis,* and *O. polymorpha*	*Xaj-pitha*	Bora et al. (2016)
8	*S. cerevisiae, Sm. fibuligera,* and *Sm. malanga*	Balma starter of Chhang	Bhardwaj et al. (2016)
9	*S. cerevisiae*	Fermented foods of Western Himalayas	Kanwar and Keshani (2016)
10	*C. glabrata, C. tropicalis, C. lusitaniae, Issatchenkia* sp., *P. anomala, P. ranongensis, P. burtonii, Sm. fibuligera, Sm. capsularis, S. cerevisiae,* and *Sacch. bayanus*	Marcha and Thiat, traditionally prepared amylolytic starters of India	Sha et al. (2018)
11	*W. anomalus, P. anomala, Sm. fibuligera, Pichia terricola, P. kudriavzevii, C. glabrata*	Dried starters of Indian alcoholic beverages	Sha et al. (2018)
13	*K. marxianus, S. cerevisiae, C. parapsilosis,* and *Sagenomella keratitidis*	Indigenous dairy products from Republics of Benin and Niger	Sessou et al. (2019)
14	*S. cerevisiae* KY069279	Development of apple wine	Sukhvir and Kocher (2019)
15	*S. cerevisiae*	Starter consortia for on-farm cocoa fermentation	Saunshi et al. (2019)
16	*S. cerevisiae* MTCC 178	Wine production from *Mangifera indica* L.	Patel et al. (2020a, b)
17	*S. cerevisiae, W. anomalus, Saccharomycopsis malanga, Saccharomycopsis fibuligera,* and *Sm. malanga*	Rice wine starter *Xaj-pitha*	Keot et al. (2020)

Table 20.4 Summary of probiotic yeasts from India

Sr. no.	Yeasts	Isolation source	Authors and references
1	*S. cerevisiae*	Fermented nectar of toddy	Srinivas et al. (2017)
2	*Y. lipolytica*, *K. lactis*, *Lipomyces starkeyi*, *Sm. fibuligera,* and *Brettanomyces custersianus*	Fruits, vegetables, plants, meats, insects	Ragavan and Das (2017)
3	*P. barkeri, Y. lipolytica, W. anomalus,* and *S. cerevisiae*	Avocado, curd, mosambi, sweet lime, and pineapple fruit	Suvarna et al. (2018)
4	*S. cerevisiae, I. occidentalis*	Toddy and fermented apple juice	Kunyeit et al. (2019)
5	*S. cerevisiae*	Rhizospheric soil	Puppala et al. (2019)
6	*Y. lipolytica*, *K. lactis*, *L. starkeyi*, *Sm. fibuligera*, and *B. custersianus*	Fruits, vegetables, plants, meats, insects	Ragavan and Das (2020)

as compared to bacterial microflora which may be compromised (Takayama et al. 2006). In India, researchers have reported probiotic potential of yeasts isolated from various natural food sources like fruits, vegetables, plants, meats, insects, and industries (Table 20.4). Various yeasts present in fermented nectar of toddy, fermented apple juice, and rhizospheric soil have also been evaluated for their probiotic potential (Table 20.4).

20.6 Yeasts in Plant Growth Promotion

In the current agricultural system, the use of commercial chemical fertilizers and pesticides has replaced the native microbial flora and fauna. The imbalance of beneficial microbial diversity and natural competitors increases the severity of plant diseases. Different metabolic activities of microorganisms directly, e.g., production of plant hormones (indole-3-acetic acid), siderophore, nutrient solubilization, and ammonium, or indirectly like production of, e.g., hydrogen cyanide, chitinase, protease, and antibiotics have effect on plant growth promotion. However, the plant growth-promoting microbes like bacteria, fungi, mycorrhizal fungi, and algae are widely explored although there are very few reports on yeasts which have the ability to produce a group of plant growth-promoting activities and biocontrolling activity (Mukherjee et al. 2020). A very interesting study was conducted to study the interaction between the mycorrhizal fungus, *Glomus mosseae*, and six soil yeasts (*Rhodotorula mucilaginosa, Metschnikowia*

pulcherrima, Trichosporon cutaneum var. *cutaneum, Saccharomyces cerevisiae, Cryptococcus laurentii, Debaryomyces occidentalis* var. *occidentalis*), and their effect on growth and nutrition of cowpea. Surprisingly, all the yeasts had a synergistic interaction with the mycorrhizal fungus and dual inoculation improved plant growth compared to single inoculation with *G. mosseae* alone (Boby et al. 2008). Another report highlights the potential of novel rhizospheric yeast *Candida tropicalis* as a soil-fertilizing inoculant for improving the growth of maize (Mukherjee and Sen 2015).

20.7 Yeasts in Biosorption and Bioremediation

Biosorption is a mechanism for removing heavy metals and rare earth elements from aqueous solutions. Yeast cells represent inexpensive, readily available sources of biomass that exhibit removal ability for a broad range of heavy metals to varying degrees due to their ability to adapt to extreme conditions such as temperature, pH, and high levels of organic and inorganic contaminants (Bahafid et al. 2017). The yeasts have, therefore, been used in the biosorption and bioremediation processes. In this field, the pioneering work was done by Dr. Smita Zinjarde and Dr. Amita Ravi Kumar, who have vastly studied *Yarrowia lipolytica* for its various properties like production of emulsifier in the presence of alkanes or crude oil, hydrolytic dehydrogenation, COD reduction in palm oil mill effluent, removal of chromium (VI) ions from wastewater, and transformation of 2,4,6-trinitrotoluene into aminodinitrotoluene which is widely used as an explosive in military shells, bombs, and grenades. They have also demonstrated high tolerance to heavy metals (Pb(II), Cr (III), Zn(II), Cu(II), As(V), and Ni(II) ions) in marine strains of *Y. lipolytica*. A nonconventional yeast *K. marxianus* has also been shown to absorb lead, mercury, arsenic, cobalt, and cadmium ions. *Candida digboiensis* TERI ASN6 has been found to have the ability to degrade petroleum hydrocarbons in acidic conditions as reported by Dr. Banwari Lal and team. A few more scientists have contributed to this research field by investigating on yeasts like *K. marxianus*, *Pichia sydowiorum* MCM Y-3, and *S. cerevisiae* for their use in lead (PbII ion) biosorption, remediation of high-melting explosive (HMX) wastewater in fixed-film bioreactor (FFBR), phenol degradation, and cadmium (II) biosorption. Apart from yeast taxonomic work, Dr. G.S. Prasad has also reported the potential of *Kluyveromyces lactis* to reduce purine content in foods (Table 20.5).

20.8 Biosurfactant and Pullulan Production

Biosurfactants are surface-active compounds derived from living organisms, mainly microorganisms. Mostly, all the surfactants in current use are chemically derived from petroleum but nowadays chemical surfactants are being substituted by an increased use of microbial surfactants. The advantages provided by microbial surfactants are lower toxicity, higher biodegradability, and better environmental

Table 20.5 List of bioremediations and biosorption-associated yeast research in India

Sr. no.	Yeasts	Bioremediation/biosorption	Authors and references
1	*Yarrowia lipolytica,* NCIM 3589	Produce emulsifier in the presence of alkanes or crude oil	Zinjarde and Pant (2002)
2	*Y. lipolytica* NCIM 3589	COD reduction of palm oil mill effluent	Oswal et al. (2002)
3	Baker's yeast	Biosorption of cadmium (II)	Vasudevan et al. (2003)
4	*Y. lipolytica* NCIM 3589	Transformation of 2,4,6-trinitrotoluene into amino-dinitrotoluene	Jain et al. (2004)
5	*S. cerevisiae*	Phenol degradation	Patel and Rajkumar (2009)
6	*Candida digboiensis* TERI ASN6	Degradation of petroleum hydrocarbons	Sood and Lal (2009)
7	*Pichia sydowiorum* MCM Y-3	Remediation of high-melting explosive (HMX) wastewater	Kanekar et al. (2009)
8	*Y. lipolytica* (NCIM 3589 and 3590)	Removal of chromium (VI) ions	Bankar et al. (2009)
9	*K. marxianus*	Metal sorption (lead, mercury, arsenic, cobalt, and cadmium)	Pal et al. (2009)
10	*C. digboiensis* TERI ASN6	Degrades acidic petroleum hydrocarbons	Sood et al. (2010)
11	*Saccharomyces*, *Hansenula*, *Klockera*, *Rhodotorula*, and *Debaryomyces*	Rock phosphate solubilization	Narsian et al. (2010)
12	*K. marxianus*	Biosorption of lead (PbII ion)	Subhashini et al. (2012)
13	*Y. lipolytica* (NCIM 3589)	Hydrolytic dehalogenation	Vatsal et al. (2015)
14	*K. lactis*	Reduces purine content in food	Mahor et al. (2016)
15	*Y. lipolytica*	Tolerance to Pb(II), Cr(III), Zn (II), Cu(II), As(V), and Ni (II) ions	Bankar et al. (2018)

compatibility. Most known biosurfactants are of bacterial origin, and only a few biosurfactants come from yeasts and molds (Katemai 2011). Another important microbial product is pullulan. Pullulan has been considered as one of the important polysaccharides for production of biodegradable plastics. *Aureobasidium pullulans* is the only yeast-like fungus which produces higher amount of pullulan and has been exploited all over the world for its excellent genetic makeup to produce various important metabolites at a commercial scale (Gaur et al. 2010). In India various yeasts capable of producing pullulan and biosurfactants are presented in Table 20.5. The pioneering work on pullulan production was credited to Prof. R. S. Singh and

Table 20.6 Summary of biosurfactant and pullulan-producing yeast reported from India

Sr. no.	Yeasts	Source	Biosurfactant/ pullulan	Authors and references
1	*A. pullulans* FB-1 and FG-1	*Ficus benjamina* and *Ficus glometa*, respectively	Pullulan	Singh and Saini (2008)
2	*Rhodococcus* sp. MTCC 2574	MTCC, IMTECH, Chandigarh	Biosurfactant	Mutalik et al. (2008)
3	*A. pullulans* FB-1	*Ficus benjamina*	Pullulan	Singh et al. (2009)
4	*A. pullulans* (thermotolerant)	Flowers and leaves	Pullulan	Singh et al. (2012)
5	*C. tropicalis* (BPU1)	Rumen of the Malabari goat	Biosurfactant and polyhydroxybutyrate	Priji et al. (2013)
6	*A. pullulans*	Asian palm kernel	Pullulan	Sugumaran et al. (2013)
7	*A. pullulans* MTCC 2195	MTCC, IMTECH, Chandigarh	Exopolysaccharide	Padmanaban et al. (2015)
8	*Candida* sp., *Saccharomycopsis* sp., and *Brettanomyces* sp.	Hydrocarbon-polluted soil samples (Hisar)	Biosurfactant	Kaur et al. (2017)
9	*M. guilliermondii* YK32	Hydrocarbon-polluted environments	Biosurfactant	Sharma et al. (2019)
10	*A. pullulans* FB-1	*Ficus benjamina*	Pullulan	Singh and Kaur (2019)
12	*C. albicans* SC5314and *C. glabrata* CBS138	–	Sophorolipid biosurfactant	Gaur et al. (2019)
13	*A. pullulans* MTCC 2013	MTCC, IMTECH, Chandigarh	Pullulan-biodegradable plastic	Rishi et al. (2020)

colleagues. They had isolated *A. pullulans* from *Ficus benjamina* and *Ficus glometa* and found them to be efficient pullulan producers. Biosurfactant properties of many yeasts like *C. albicans*, *C. glabrata*, *M. guilliermondii*, *C. tropicalis*, *Saccharomycopsis* sp., *Brettanomyces* sp., and *Rhodococcus* sp. were studied by various groups of researchers in India (Table 20.6). Most of the biosurfactant-producing yeasts have been isolated from hydrocarbon-polluted soil and rumen of the Malabari goat (Table 20.6).

20.9 Yeasts in Xylitol Production

Xylitol, a polyalcohol, is an alternative sweetener with a lower calorific value (one-third fewer calories) but the same level of sweetness as sucrose. It is among the 12 most commercially valuable products that can be obtained from low-cost feedstocks. It has a plethora of applications in the pharmaceutical, nutraceutical,

food, and beverage industries. It is used in the manufacture of dietary food products, chewing gums, bakery products, and chocolates due to its anticariogenic properties. It is also used in medicinal syrups, vitamin supplements, and tonics as a sugar substitute. The use of microorganisms for xylitol production is gaining popularity in recent times as the chemical method requires highly pure substrate and intense reaction parameters making this a costly and energy-consuming process (Tiwari and Baghela 2020). Yeasts are regarded as the most efficient xylitol producers among the microorganisms (Guo et al. 2006).

In India a good number of researchers have worked on xylitol production from various substrates using yeasts. Prof. L. Venkateswar Rao and colleagues have shown the potential of *Candida tropicalis* strains in xylitol production from pure xylose as well as sugarcane bagasse hydrolysates. Similarly, Dr. R. K. Saxena and Dr. Vijayanand S. Moholkar also reported xylitol production using *Candida tropicalis* strains from pure xylose and sugarcane bagasse as substrates. Dr. Anand Ghosalkar from Praj Industries Limited, Pune, has extensively used corncob hemicellulosic hydrolysate and sugarcane bagasse hydrolysates as substrates from xylitol production using *S. cerevisiae* strains. Lignocellulosic biomass has also been used by Dr. Debashish Ghosh and colleagues for xylitol production by a nonconventional yeast *K. marxianus* with 0.315 ± 0.01 g/g yield. Few other groups of researchers have exploited the xylitol production potential of *Yarrowia lipolytica*, *Hansenula anomala*, *Candida* magnolia, *Pichia caribbica*, and *P. manchurica* from xylose in association with pure as well as crude glycerol, sucrose, and glucose (Table 20.7).

20.10 Yeasts as Source of Valuable Enzymes

All living organisms require enzymes for synthesis as well as breakdown reactions. Many harmful chemical reactions have been replaced by eco-friendly biological processes due to these biocatalysts. In recent times, industrial enzyme production technology has attained a huge success due to major advancements in bioprocess technology (Patel et al. 2017). Novel enzymes are continuously being discovered from various microorganisms, which provide new opportunities for enzyme technology to flourish. Among different microorganisms, yeasts have also been shown to be good sources of valuable enzymes.

Exceptional amount of work has been carried out by Indian researchers on enzyme production using yeasts. The scholarly work on yeast phytase was done by Prof. T. Satyanarayana and few other researchers, wherein they have used *Pichia anomala* and *P. kudriavzevii* for enzyme production. One of the most studied enzyme lipases was produced using *Rhodotorula mucilaginosa*, *Pichia pastoris, Candida antarctica*, *Candida rugosa*, and *Candida parapsilosis* by many researchers. *Kluyveromyces marxianus* strains have been utilized for the production of inulinase in multiple studies. An interesting piece of work was done by Dr. S. M. Singh on cold-tolerant lipase and endoglucanase production using *Rhodotorula* sp. Y-23 and *Mrakia robertii*, respectively. Xylanase is another enzyme on which

Table 20.7 List of research papers published by Indian researchers on xylitol production by yeasts

Sr. no.	Yeast name	Material used and xylitol yield	Authors and references
1	*H. anomala*	Sucrose, 76.43%	Patil et al. (2002)
2	*C. tropicalis*	Xylose, 0.87 g/g	Rao et al. (2006a, b)
3	*P. caribbica*	Xylose, 0.58 g/g	Rao et al. (2007a, b)
4	*C. magnoliae*	Glycerol, 20 g/L (mannitol)	Khan et al. (2009)
5	*C. tropicalis*	Xylose, 0.5 g/g	Misra et al. (2012)
6	*D. hansenii*	Xylose, 83.82%	Pal et al. (2013)
7	*C. tropicalis*	Xylose, 0.59 g/g	Misra et al. (2013)
8	*Cyberlindnera saturnus*	Xylose and corncob, 0.54 g/g	Kamat et al. (2013)
9	*S. cerevisiae*	Corncob hemicellulosic hydrolysates, 34 mg/g/h	Kogje and Ghosalkar (2016)
10	*C. tropicalis*	Corn fiber and sugarcane bagasse hydrolysates, 0.58 g/g and 0.65 g/g	Rao et al. (2006a, b)
11	*S. cerevisiae*	Corncob hemicellulosic hydrolysate, 318.6 mg/L/h	Kogje and Ghosalkar (2017)
12	*K. marxianus*	Lignocellulosic biomass, 0.315 ± 0.01 g/g	Dasgupta et al. (2017)
13	*P. manchurica*	Glucose, 27.6 g/L (arabitol)	Sundaramoorthy and Gummadi (2019)
14	*C. tropicalis*	Sugarcane bagasse, 0.65 g/g	Tizazu et al. (2018a)
15	*C. tropicalis*	Sonicated sugarcane bagasse, 0.61 g/g	Tizazu et al. (2018b)
16	*K. marxianus*	Lignocellulosic pentosans, 6.89 g/L	Dasgupta et al. (2019)
17	*Y. lipolytica*	Xylose + glycerol, 0.97 g/g and 0.92 g/g, sugarcane bagasse, 0.54 g/g	Prabhu et al. (2020)

several investigations have been carried out by Indian researchers. Dr. D. V. Gokhale has reported purification and characterization of two distinct xylanases from *Pseudozyma hubeiensis* NCIM 3574 and he further worked on the production of novel β-xylosidase. Termite gut-associated yeasts such as *P. hubeiensis* and *Hannaella pagnoccae* also showed xylanase activities in recent times (Table 20.8). List of valuable enzymes produced by different yeast species in India is given in Table 20.8.

20.11 Biodiesel or Lipid Production by Yeasts

The global demand for transportation fuels poses a threat to fossil fuel reserves and environmental and economic security of the world. Therefore, there is a need of renewable energy from sustainable feedstocks, which can reduce the load on fossil fuels. Biofuels, specifically biodiesel, are potential fuels because of their renewability, reduced carbon emissions, unburned hydrocarbons, and particulate emissions than petro-diesel engines (Arora et al. 2019). Microbial oils could be seen as an

Table 20.8 List of valuable enzymes produced by different yeast species in India

Sr. no.	Yeasts	Enzymes	Authors and references
1	*S. marxianus*, *S. bayanus, S. cerevisiae var. ellipsoideus*, and *Schizosaccharomyces* sp.	Pectinolytic enzyme	Agate and Bhat (1966)
2	*Pichia etchellsii* JFG-2201	β-Glucosidases	Waghmare et al. (2003)
3	*P. anomala*	Phytase	Vohra and Satyanarayana (2004)
4	*C. rugosa* NCIM 3462	Lipase	Rajendran and Thangavelu (2007)
5	*A. pullulans* DBS66	Tannase	Banerjee and Pati (2007)
6	*K. marxianus* YS-1	Inulinase	Singh et al. (2007)
7	*Debaryomyces nepalensis* NCYC 3413	Pectinase	Gummadi et al. (2007)
8	*C. viswanathii*	Carbonyl reductase	Soni et al. (2008)
9	Ascomycetous and Basidiomycetous yeasts and yeast-like fungi	Extracellular endoxylanases	Bhadra et al. (Bhadra et al. 2008a, b)
10	*S. cerevisiae*	Glucanase with acid trehalase–invertase	Basu et al. (2008)
11	*R. mucilaginosa* MTCC 8737	Lipase	Potumarthi et al. (2008)
12	*C. rugosa*	Lipase	Rajendran et al. (2008)
13	*Z. rouxii*	Glutaminase	Iyer and Singhal (2008)
14	*D. nepalensis* (novel strain)	Pectin lyase and pectate lyase	Gummadi and Kumar (2008)
15	*P. hubeiensis*	Xylanases	Adsul et al. (2009)
16	*Cryptococcus gastricus, Cryptococcus terricolus, R. muscorum, M. psychrophila, M. gelida,* and *R. glacialis*	Lipase, protease, pectinase, cellulase, and amylase	Pathan et al. (2010)
17	*P. pastoris*	Lipase B	Vadhana et al. (2013)
18	*K. marxianus* var. *marxianus*	Inulinase	Dilipkumar et al. (2014)
19	*P. pastoris*	β-Glucosidase	Batra et al. (2014)
20	*P. anomala*	Phytase	Joshi and Satyanarayana (2015)

(continued)

Table 20.8 (continued)

Sr. no.	Yeasts	Enzymes	Authors and references
21	*P. pastoris*	Cal A and Cal B lipase	Bharathiraja et al. (2016)
22	*C. parapsilosis*	Lipase and phytase	Balakrishna et al. (2017)
23	*P. hubeiensis* NCIM 3574	Extracellular β-xylosidase	Mhetras et al. (2016)
24	*K. marxianus* (MTCC 5933 and MTCC 5934)	Xylosidase activity	Behera et al. (2016)
25	*P. pastoris*	Streptokinase	Adivitiya et al. (2016)
26	*Rhodotorula* sp. Y-23	Cold and organic solvent-tolerant lipase	Maharana and Singh (2018)
27	*Y. lipolytica* NCIM 3589	Ylehd, an epoxide hydrolase	Bendigiri et al. (2017)
28	*C. antarctica*	B lipase	Yadav et al. (2018)
29	*M. robertii* A2-3	Cold-tolerant endoglucanase	Dhume et al. (2019)
30	*R. mucilaginosa*	Laccase isozymes	Kumar et al. (2019)
31	*K. marxianus*	Inulinase	Santharam et al. (2019)
32	*P. kudriavzevii* OG32	Phytase	Ogunremi et al. (2020)
33	*P. hubeiensis* and *H. pagnoccae*	Xylanase	Tiwari et al. (2020)

alternative for lipid production because these oleaginous (lipid producing) microorganisms such as bacteria, fungi, yeast, and algae can accumulate up to 60–70% lipids of their dry cell weight (Thliveros et al. 2014). Among different microorganisms, oleaginous yeasts belonging to *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon*, and *Lipomyces* have been shown to have a great potential for lipid production from various industrial and agricultural residues, therefore making them promising sustainable sources for biodiesel production (Arora et al. 2019).

Indian researchers have contributed significantly to the area of oleaginous yeasts and lipid production. Initial studies were done by Prof. Kannan Pakshirajan, who evaluated the oleaginous potential of *Candida bombicola* using sugarcane molasses and mixed hydrophilic substrates. Prof. Ameeta Ravikumar's group reported lipids and biodiesel production by *Lipomyces starkeyi* using glucose and waste cooking oil in two separate studies. Dr. Parul A Pruthi has extensively used *Rhodosporidium kratochvilovae* for triacylglyceride production from nonedible lignocellulosic biomass of *Cassia fistula* L. fruit pulp and mixed carbon sources. Prof. Yuvraj S. Negi

and team have reported *Cryptococcus vishniaccii* and *Cryptococcus psychrotolerans* for lipid production using paper mill sludge extract and aromatic hydrocarbons, respectively. A good amount of scholarly work was carried out by Dr. Debashish Ghosh and colleagues, wherein they have extensively used *Rhodotorula mucilaginosa* for lipid production from xylose, corncob, and crude glycerol as substrates. Another interesting piece of work was done by Prof. P. Sivashanmugam and colleagues; they studied two different yeasts *Lipomyces starkeyi* and *Naganishia liquefaciens* for lipid production from municipal waste-activated sludge as a low-cost feedstock. Prof. Mrinal Kumar Maiti's group has tested the lipid-producing potential of *Candida tropicalis.* A list of research articles published by Indian researchers in oleaginous yeasts and lipid (biodiesel) production is presented in Table 20.9.

20.12 Yeasts in Bioethanol Production

At present fossil fuel is a major nonrenewable source of energy which contributes more than 80% of total resources being used for generating energy for global transport. Recently, research has been focused on the identification of low carbon-emitting alternative renewable fuel that can be eco-friendly as well as can maintain economic sustainability. Ethanol is one of the most popular sources of alternative energy because it can be blended with petrol to increase the heat of vaporization and octane number (Bušić et al. 2018).

Amon all the selected applications, maximum number of research papers have been published by Indian researchers in the field of bioethanol production by yeasts. The very first report for bioethanol production from molasses was reported by Bandyopadhyay in 1982 using *S. cerevisiae*. After this report a number of researchers have exploited ethanol fermentation potential of many yeasts using various substrates like molasses, *Prosopis juliflora*, glucose, rice straw, wheat straw, paddy straw, lignocellulosic material, kinnow waste, banana peels, cane molasses, cauliflower waste, and sugarcane bagasse for ethanol production. One of the major contributions in bioethanol production studies was done by Prof. L. Venkateswar Rao, who has primarily exploited *S. cerevisiae* for ethanol production using various substrates. He also worked on *Pichia stipitis* and *Candida shehatae* and used wheat straw hemicellulose as an ethanol-producing substrate. Dr. H. S. Oberoi tried to optimize and improvise the ethanol production potential of *S. cerevisiae* using a combination of cane molasses and household wastes as starting material for bioethanol production. R. C. Kuhad and J. N. Nigam also contributed to this field with their studies on *P. stipitis* and *S. cerevisiae*. Dr. Naseem A. Gaur has done a comparative evaluation of ethanol production from various substrates like glucose, rice, wheat straw, and lignocellulosic mass. A recent study by Dr. Anju Arora reported high ethanol yield while working on *Kodamaea ohmeri* and using paddy straw hydrolysate as substrate. A very interesting work on consolidated bioprocessing of biogenic municipal waste for production of bioethanol was done by Dr. Avanthi Althuri, who has improvised ethanol productivity with the help of

Table 20.9 List of research papers published by Indian researchers on biofuel-lipid production by yeasts

Sr. no.	Yeast	Material used	Lipid produced	Authors and reference
1	*C. bombicola*	Sugarcane molasses, yeast extract, urea, and soybean oil	Sophorolipid—63.7 g/L	Daverey and Pakshirajan (2009)
2	*C. bombicola*	Mixed hydrophilic substrate	33.32 ± 0.83 g/L	Daverey and Pakshirajan (2010)
3	*L. starkeyi, R. toruloides*	Glucose	4.14–6.44 g/L	Khot et al. (2012)
4	*L. starkeyi*	Basic medium containing glucose	Long chain polyunsaturated fatty acids—7.29 g/L	Salunke et al. (2015)
5	*R. kratochvilovae* HIMPA1	Mixed carbon sources	TAG accumulation—9.2 6g/L	Patel et al. (2015a, b)
6	*Rhodosporidium kratochvilovae* HIMPA1	*Cassia fistula* L. fruit pulp	Triacylglycerides—4.86 ± 0.54 g/L	Patel et al. (2015a, b)
7	*R. mucilaginosa* IIPL32	Xylose	Single-cell oil—0.17 g/g	Khot and Ghosh (2017)
8	*C. vishniaccii* (MTCC 232)	Paper mill sludge extract	7.8 ± 0.57 g/L	Deeba et al. (2016)
9	*Y. lipolytica* (YlB6, YlC7, and YlE1)	Waste cooking oil	0.062 g/L/h	Katre et al. (2017)
10	*Trichosporon* sp. (RW)	Glucose, glycerol, sugarcane bagasse	21.45, 18.41, 10.25 g/L	Brar et al. (2017)
11	*L. starkeyi* (MTCC-1400)	Municipal waste-activated sludge	Lipids—64.3% dwt	Selvakumar and Sivashanmugam (2017)
12	*Rhodotorula kratochvilovae* HIMPA1	Wet oleaginous yeast biomass	5.51 g/L	Patel et al. (2018)
13	*Cryptococcus psychrotolerans* IITRFD	Phenol, naphthalene, anthracene, and pyrene	0.0444, 0.0441, 0.0394, and 0.0383 g/L/h	Deeba et al. (2018)
14	*R. toruloides* strain (ATCC20409)	–	76% of dry cell weight biomass	Singh et al. (2018)
15	*P. guilliermondii*	Nitrogen limitation	10.8 ± 0.5 g/L	Chopra and Sen (2018)
16	*N. liquefaciens* NITTS2	Municipal waste-activated sludge	88.34 ± 1.2%	Selvakumar and Sivashanmugam (2018)
17	*C. tropicalis* and *P. kudriavzevii*	Glucose	1.1 and 3 g/L	Diwan and Gupta (2018)

(continued)

Table 20.9 (continued)

Sr. no.	Yeast	Material used	Lipid produced	Authors and reference
18	*Cryptococcus curvatus* MTCC 2698	Vegetable waste (VW)	28.3 ± 0.5% and 26 ± 0.5%	Chatterjee and Mohana (2018)
19	*S. pastorianus, R. mucilaginosa,* and *R. glutinis*	Starchy wastes	Single-cell oil production—0.022 g/g and 0.025 g/g	Chaturvedi et al. (2018)
20	*M. pulcherrima* (MTCC 632)	Waste wood chips	–	Tamilalagan et al. (2019)
21	*Y. lipolytica* (NCIM 3590)	Glucose	0.33 g/g	Pawar et al. (2019)
22	*C. oligophagum* (JRC1)	Dairy waste cheese whey	0.0335 ± 0.0004 g/L/h with treated cheese whey	Vyas and Chhabra (2019)
23	*R. minuta* (MTCC 2518)	Cane molasses, diammonium phosphate (DAP), and deoiled cake (DOC)	0.46 ± 0.04 g/g	Ghosh and Roy (2019)
24	*C. tropicalis* (SY005)	Mixed glucose and xylose	0.792 g/L/day	Chattopadhyay and Maiti (2020)
25	*C. tropicalis* (SY005)	Basal medium with glucose	Lipid droplet protein CtLDP1 ~2.67-fold	Chattopadhyay et al. (2020a, b)
26	*R. mucilaginosa* IIPL32	Corncob	1.83 g/L for C/N ratio 60	Banerjeea et al. (2020)
27	*C. tropicalis* (SY005)	Industrial lipid feedstock	0.37 g/g dry cell weight	Chattopadhyay et al. (2020a, b)
28	*Geotrichum candidum*	Glucose, sucrose, and maltose	19.4% CDW	Diwan and Gupta (2020)
29	*C. tropicalis* (ASY2)	Sago-processing wastewater	0.010 g/L/h	Thangavelu et al. (2020)
30	*M. caribbica*	MGYP media	Emulsification activity (*E*24: 70–80%)	Bhaumik et al. (2020)
31	*R. mucilaginosa* IIPL32	Crude glycerol	5.6 g/L	Bansal et al. (2020)

thermophilic anaerobes and yeasts. Another latest study in this field was carried out by Abhishek Baghela and team, who have reported ethanologenic potential of yeasts, which were associated with wood-feeding termite gut. A list of research articles published by Indian researchers on bioethanol production by yeasts is given in Table 20.10.

Table 20.10 List of research papers published by Indian researchers on biofuel-ethanol production by yeasts

Sr. no.	Yeasts	Material used	Ethanol yield	Authors and references
1	*S. cerevisiae*	Molasses fermentation	–	Ghose and Bandyopadhyay (1982)
2	*S. cerevisiae*	Cane molasses	28.6 g/L/h	Tyagi and Ghose (1982)
3	*Saccharomyces diastaticus*	Liquefied cassava starch	53.5 g/L	Amutha and Gunasekaran (2001)
4	*P. stipitis*	Hardwood spent sulfite liquor	20.2 g/L	Nigam (2001)
5	*P. stipitis* NRRL Y-7124	Water hyacinth hemicellulose	0.35 g/g	Nigam (2002)
6	*S. diastaticus* SM-10	Starch and dextrose	3.50 and 6.5 (% v/v)	Sharma et al. (2002)
7	*S. cerevisiae*	Rice husk, straw, wood shavings, plastic pieces, or silica gel	5.5% (v/v)	Sankh and Arvindekar (2004)
8	*C. tropicalis*	Agricultural wastes	29–32 g/L 11–54 g/L	Patle and Lal (2007)
9	*S. cerevisiae*	Kinnow waste and banana peels	0.426 g/g	Sharma et al. (2007)
10	*S. cerevisiae*	Cane molasses, cauliflower waste	0.358 g/g	Dhillon et al. (2007)
11	*S. cerevisiae*	*Lantana camara*	0.431 ± 0.018 g/g	Pasha et al. (2007a, b)
12	*S. cerevisiae*	Lignocellulosic substrate	0.459 ± 0.012 g/g	Pasha et al. (2007a, b)
13	*S. cerevisiae* (CTCRI)	Mahula (*Madhuca latifolia* L.)	152 g/kg	Swain et al. (2007)
14	*C. shehatae* NCIM 3501	Sugarcane bagasse	0.48 g/g	Chandel et al. (2007)
15	Multiple yeasts	Fruits and tree barks	0.12–0.38 g/g	Rao et al. (2008)
16	*Kluyveromyces* sp. IIPE453	Glucose + xylose	38 ± 0.5 g/L	Kumar et al. (2009a, b)
17	*S. cerevisiae* and *P. stipites*	*Prosopis juliflora*	18.52 g/L	Gupta et al. (2009)
18	*P. stipitis*	Water hyacinth hemicellulose acid hydrolysate	0.425 g/g	Kumar et al. (2009a, b)
19	*Debaromyces hansenii*	Oat spelt xylan and wheat bran hemicellulose	9.1 g/L and 9.5 g/L	Menon et al. (2010a, b)
20	*D. hansenii*	Tamarind kernel powder	0.43 g/g	Menon et al. (2010a, b)
21	*S. cerevisiae*	Water hyacinth biomass	4.4 g/L	Aswathy et al. (2010)

(continued)

Table 20.10 (continued)

Sr. no.	Yeasts	Material used	Ethanol yield	Authors and references
22	*C. shehatae*	Leafy biomass of mango, poplar, neem, and asoka	1.44 g/L	Das et al. (2012)
23	*Sm. fibuligera*	Jaggery medium	69.57 g/L	Manwar et al. (2013)
24	*T. estonicum* and *S. cerevisiae*	Lignocellulosic waste sawdust	55.2 g/L	Saravanakumar and Kathiresan (2013)
25	*S. cerevisiae*	Ipomoea carnea biomass	0.414 g/g	Kumari and Pramanik (2013)
26	*Kluyveromyces* sp. IIPE453	Sugarcane molasses B	0.688 g/g/h	Dasgupta et al. (2014)
27	*S. cerevisiae*	*Lantana camara*	6.01% (v/v)	Kuila and Banerjee (2014)
28	*Kluyveromyces* sp. IIPE453	Bagasse hydrolysates	0.43 g/g	Kumar et al. (2015)
29	*S. cerevisiae* NCIM 3570	Banana pseudo stem	17.1 g/L	Ingale et al. (2014)
30	*Candida albicans* OMC3E6	Insoluble starch and potato starch	437 g/kg	Aruna et al. (2014)
31	*Kluyveromyces* sp.	Rice straw, wheat straw, sugarcane bagasse	Rice straw—23.23 mg/mL	Narra et al. (2015)
32	*K. marxianus*	Glucose + xylose	0.39 ± 0.37g/g and 0.43 ± 0.05 g/g	Arora et al. (2015)
33	*S. cerevisiae*	Cotton stalk	0.44 g/g	Keshav et al. (2016)
34	*S. cerevisiae*	Cheese whey	28.9 g/L	Kokkiligadda et al. (2016)
35	*S. cerevisiae*	Water hyacinth (*Eichhornia crassipes*)	13.6 mg/ml	Das et al. (2016)
36	*P. stipitis* and *C. shehatae*	Wheat straw hemicellulose	0.450 ± 0.009 g/g	Koti et al. (2016)
37	*S. cerevisiae*	Glucose, rice, and wheat straw	Glucose - 0.48 g/g	Dubey et al. (2016)
38	*K. marxianus*	Xylose/glucose	2.88 g/L	Sharma et al. (2016)
39	*S. cerevisiae*	Rice straw	0.44 g/g	Banoth et al. (2017)
40	*S. cerevisiae* JRC6	Paddy straw	3.8 g/L	Choudhary et al. (2017)
41	*Kodamaea ohmeri*	Paddy straw hydrolysates	0.25 g/g	Sharma et al. (2018a, b)
42	*P. stipitis*	Corncob hydrolysate	0.43 g/g	Kashid and Ghosalkar (2018)

(continued)

Table 20.10 (continued)

Sr. no.	Yeasts	Material used	Ethanol yield	Authors and references
43	*S. cerevisiae*	Lignocellulosic biomass	34–43 g/L	Mithra et al. (2018)
44	*S. cerevisiae*	*Pogonatherum crinitum*	42.2 and 39.4 g/L	Waghmare et al. (2018)
45	*S. cerevisiae* LN	Xylose	0.30 ± 0.01 g/g	Sharma et al. (2018a, b)
46	*S. cerevisiae* and *P. stipitis*	Biogenic municipal solid waste (BMSW)	25% (w/v)	Althuri and Mohan (2019)
47	*S. stipites* hybrid SP2-18	Glucose-xylose mixture	0.447 g/g	Jetti et al. (2012)
48	*S. cerevisiae* NGY10	Lignocellulosic material	46.81 ± 21.98 g/L at 40 °C	Pandey et al. (2019)
49	*S. cerevisiae*	Sugarcane molasses	0.471 ± 0.002 g/g	Jagtap et al. (2019)
50	*K. marxianus*	Woody stem *Prosopis juliflora*	21.45 g/L	Sivarathnakumara et al. (2019)
51	*P. stipitis* NCIM-3498	Biogenic municipal solid waste (BMSW)	0.26 g/g	Althuri and Mohan (2020)
52	*S. cerevisiae* WTS1A	Wheat straws	0.46 g/g and 0.43 g/g	Patel et al. (2020a, b)
53	*S. cerevisiae*	*Sesamum indicum* L. residue	1.90 g/L	Kumar et al. (2020)
54	*S. cerevisiae*	Sugarcane bagasse	80% Ethanol conversion efficiencies	Baral et al. (2020)
55	*S. cerevisiae* RPP-03O	Rice straw	3.3% (w/v)	Ashoor and Sukumaran (2020)
56	*S. cerevisiae*	Food and kitchen waste	0.316 g	Sindhu et al. (2020)
57	*S. cerevisiae*	Nitrosative stress on media	34.4 g/L	Sengupta et al. (2020)
58	*C. tropicalis*	5% Glucose and rice straw hydrolysate	22.92 g/L and 8.95 g/l	Tiwari et al. (2020)

20.13 Conclusions and Future Prospects

India has a long history of research and outstanding discoveries in various scientific disciplines including microbiology. The yeast research in India is at a very exciting juncture, where the Indian researchers are not only contributing to understanding the yeast diversity but also utilizing them for various applications. India has some of the world's most biodiverse regions, viz. the Himalayas, the Western Ghats, the Indo-Burma region, and the Sundaland. These biodiversity hot spots offer pristine habitats

to explore yeast diversity, which will enrich the yeast inventory in India. Such exploration will also eventually help in describing a greater number of novel yeast taxa from India, thereby marking India's presence in global yeast taxonomy research. Although *S. cerevisiae* is the most domesticated and widely used industrial yeast, many other yeast species called nonconventional yeasts may also have a great potential in biotechnology. Indian researchers have also contributed significantly to nonconventional yeast research; however, these yeasts still hold a great potential for future research. Indian researchers have contributed exceptionally to the field of biofuels, enzymes, and xylitol production using yeasts. Considering the potential of lignocellulosic biomass-based production of biofuel, the Indian researchers can provide sustainable solutions for biofuel production. There is a need to develop a strong, active, network of yeast researchers in India to work in collaboration on various aspects of this fascinating group of organisms in future.

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History and Development of Myxomycetes Research in India

21

T. N. Lakhanpal, Vaneet Jishtu, and R. Sharma

Abstract

Myxomycetes, acellular slime moulds or plasmodial slime moulds are interesting organisms exhibiting characteristics of both plants and animals. Herein is given a historical account of the systematics, ecology and life cycle studies on Indian Myxomycetes. The history of research of Indian slime moulds can be broadly divided into three periods. Period 1 (up to 1950) started with Drake's maiden collections (1911–1927), followed by the first published record (Lister, *A monograph of the Mycetozoa. Eds. 1, 2, and 3, the latter two rev. by G. Lister*, Brit. Mus. Nat. Hist., London, 1924) and culminated with the first monographic treatment (Lodhi (1934), Indian slime molds (Myxomycetes) (Being Descriptions of the species collected by Late Mrs. A. Drake). From 1931 to 1951, there was virtually no work on Myxomycetes. Period II extended from 1952 to 1980, and witnessed great research activity in the North (Thind and associates, 1952–1969), South [Agnihothrudu and co-workers (1954–1969)] and North-East [Agnihothrudu and collaborators (1958–1965)]. During this period six doctoral theses were produced on Myxomycetes of their respective regions: P.U. Indira (Studies in Myxomycetes. Unpubl. Thesis, Univ. of Madras, 1966) from the University of Madras; T.N. Lakhanpal (1975) from the University of Delhi; S.S. Dhillon (1976) from Punjab University, Chandigarh; V.D. Ranade (1978), Poona University; S.P. Nanir (Myxomycetes of Marathwada. Ph.D. Thesis,

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Dr. Babasaheb Ambedkar Marathwada Univ. Aurangabad, 1979) from Marathwada University, Aurangabad; and R. Venkatramani from the University of Madras. The first comprehensive monograph on 'Myxomycetes of India' was also published during this period (Thind, Indian Council, Agric Res. New Delhi), describing 183 species from India. Period III extends from 1980 onwards till date. In 1981, another monograph on 'Indian Myxomycetes' was published (Lakhanpal and Mukerji, Bibliotheca Mycol (J. Cramer, Vaduz) 1981;78:1–531) describing 293 species of Myxomycetes. During this period four individuals received doctorates on different aspects of Myxomycetes: R.K. Chopra (Biology of corticolous Myxomycetes. Thesis, ined., Himachal Pradesh Univ., Shimla, India, 1984) from H.P. University, Shimla; Rajesh Sharma (Studies on the Myxomycetes of Eastern Himalayas and adjoining hills. Ph.D. Thesis Panjab University, Chandigarh, 1986) from Punjab University, Chandigarh; B.G. Rokade (Taxonomic studies in Myxomycetes of Jalgaon and Dhulia, Ph.D. Thesis, Marathwada Univ. Aurangabad, 1989); and V.B. Salunkhe (1995) from B.A. Marathwada University, Aurangabad; they worked on corticolous Myxomycetes; Myxomycetes of N.E. India and Royal Kingdom of Bhutan; Myxomycetes of Jalgaon, Maharashtra; and Myxomycetes of Dang forest, Western Ghats, respectively. Ranade et al. (Mycosphere 2012:3 (3):358–390) published a checklist of Indian Myxomycetes listing 373 species. There are more than 200 species of Myxomycetes published by Nanir's group (Aurangabad), some of which are recorded for the first time for India and Maharashtra; several new species have also been proposed but most of the work remained unpublished. With Chopra's work and that of Sharma, the total number of species in Myxomycetes is estimated to be around 450 or so now. The life cycle and ecological studies have also been carried out, the details of which are serialised in the text.

Keywords

Myxomycetes · Slime moulds · Systematics · Monograph · Corticolous · Life cycle

21.1 Introduction

Myxomycetes, slime moulds or slime fungi are an interesting group of curious organisms which are akin to both plants and animals. The plant-like features are expressed in their reproductive phase which is comprised of a spore mass within or without a spore case, simple or complex, membranous or tough and non-cellular in organisation, within which usually is intermixed a network of free or netted threads, called capillitium or pseudocapillitium, depending upon their origin. Some of them also have characteristic calcareous accretions within or without spore case or both (Martin et al. 1983). Their assimilative or somatic or vegetative phase consists of a free-living, mobile, acellular multinucleate mass of protoplasm termed as

plasmodium which absorbs nutrients in solution and can also engulf solid particles including bacteria, algal cells or fungal spores. Right from the time of discovery they have been thus treated and classified with plants or animals, as Myxomycetes or Mycetozoa. The molecular data on their phylogeny now treats them as neither fungi nor plants; they are treated as protozoan fungal analogues (Hibbett et al. 2007; Kirk et al. 2008). Whatever be their placement, they are not organisms belonging to 'no man's land' but mycologists own them and still study them; hence they are in the domain of mycology and mycologists, irrespective of their placement riddle.

They have a peculiar life pattern: The spores issue on germination naked cells or myxamoebae, which multiply mitotically, increase the haploid population and then behave as gametes, eventually fusing in pairs, to produce diploid zygote. The zygote undergoes free nuclear division; the nuclei divide further synchronously resulting into a multinucleate acellular mobile mass of protoplasm, the plasmodium. This animal-like phase culminates with the plasmodium. The plasmodium then metamorphoses and sets in the plant-like phase and produces fructifications bearing spores, with the meiosis taking place during the delimitation of spores. Though they have been called as dual organisms, in reality they integrate the duality into the unity, crossing man-made artificial boundaries, which are ever-changing in nature. In addition to true slime moulds or Myxomycetes, there are three more groups of slime moulds: the net slime moulds (Labyrinthula), endoparasitic slime moulds (Plasmodiophorales) and cellular or communal slime moulds (Acrasiales). Initially, the limits and relationship among the four groups were not well defined and certain, but now all the four groups stand well established and well defined, though many people are not happy with the use of term slime moulds for all of them and even with their systematic position (Jishtu et al. 2018).

The name of slime moulds given to this group of organisms by Link in 1833 is derived from a Greek word '*myxa*' meaning slime and '*mykes*' meaning fungus. Link considered Myxomycetes to be fungi; de Bary, however, considered Myxomycetes to be closer to protozoans and proposed the name Mycetozoa, which in Greek means '*mycet*' (fungi) and '*zoon*' (animal). Later workers followed either Link in the treatment or de Bary depending upon what one considered them to be closer to: Macbride (1899), Macbride and Martin (1934), Alexopoulos (1973), Martin and Alexopoulos (1969), Farr (1976), Thind (1977), Lakhanpal and Mukerji (1981), Lakhanpal et al. (1994), Nannenga-Bremekamp (1991) and Stephenson and Stempen (1994) followed Link's treatment, whereas Lister (1894), Lister and Lister (1925), Hagelstein (1944) and Olive (1975) followed de Bary's treatment.

In the past few years, enormous data has been generated on morphology, molecular biology and phylogenetic relationships of fungi. Therefore, there has been a sea change in the classification of fungi (Hibbett et al. 2007; Kirk et al. 2008). Traditionally, the fungi and fungus-like eukaryotes were classified as a subkingdom in the Kingdom Plantae (Bessey 1950a, b), a practice which was discontinued after the introduction of the concept of Kingdom Fungi (Jahn and Jahn 1949: Whittaker 1969). Hawksworth et al. (1983) presented a summary of various schemes of classification for fungi proposed till 1983. They also proposed a classification system, in which the Kingdom was studied under two divisions: Myxomycota (for

plasmodial forms) and Eumycota (for non-plasmodial, frequently mycelial form) (Sharma & Lakhanpal, in Press, 2021).

21.2 History of Research on Indian Myxomycetes

It is imperative and also essential to trace a brief history of Myxomycetes research at the global level before undertaking the history of Indian Myxomycetes, as the foundation of Myxomycetes was laid outside India. The pioneers in the exploration of Indian slime fungi were the amateur Britishers. In the pre-Linnaean era the first ever collection on record of Myxomycetes was of *Lycogala epidendrum*, under the name 'fungi cito crescentes' which was validly published by Micheli (1729) who erected the genus *Lycogala* for it and also described and illustrated several other genera and species. Haller (1742) also described several species of the Myxomycetes with illustrations in his 'Enumeratio Methodica Stirpium Helvetiae Indigenarum'. These pre-Linnaean publications did not use binomial names consistently.

The 'Species Plantarum' by Linnaeus (1753) is considered as the starting point of nomenclature for Myxomycetes. In the subsequent years several scientists developed interest in Myxomycetes. The prominent among them were Batsch (1783–1789), Bulliard (1791), Gmelin (1791), Schrader (1797), Persoon (1801), Fries (1829) Link (1833), de Bary (1858–1864), Cienkowski (1863), Rostafinski (1875) and Schroeter (1885–1886). In the Linnaean and post-Linnaean era, Myxomycetes received the due recognition and binomial names as per the International Code of Botanical Nomenclature (ICBN).

By this time enough data on the systematics of Myxomycetes was gathered which led to the attempts on monographic treatments. Lister (1894) produced a beautifully illustrated world monograph 'A Monograph of the Mycetozoa'. The second revised edition of this monograph appeared in 1911 by Lister's daughter G. Lister and the third edition followed in 1925 again by G. Lister. This edition is a masterpiece in science and art providing detailed description of the Myxomycetes species till 1925 along with impressive oil paintings of many Myxomycetes. Hagelstein (1944) following A & G Lister's concept monographed North American Myxomycetes as 'Mycetozoa of North America'. Most of the subsequent American workers treated Myxomycetes as plants except Olive (1970).

Macbride and Martin (1934) monographed the North American species of Myxomycetes as the 'The Myxomycetes'. Martin (1949) and Martin and Alexopoulos (1969) treated them again as such in the compilation of North American Myxomycetes and The Myxomycetes, respectively. Farr (1976) published the 'Flora Neotropica' which has been recently updated by Lado and Wrigley de Basanta (2008). Martin et al. (1983) updated the generic treatment of the Myxomycetes. Now all treatments after Hibbett et al. (2007) and Kirk et al. (2008) are in jeopardy.

Martin and Alexopoulos (1969), who produced a world monograph of Myxomycetes, placed the fungi in the division 'Mycota' with two subdivisions, viz., Myxomycotina comprising a single class Myxomycetes and Eumycotina

comprising the classes of the true fungi. The Myxomycetes further had two subclasses Ceratiomyxomycetidae and Myxogastromycetidae.

Ross (1973) created another subclass Stemonitomycetidae on the basis of the development and structure of the sporangium in the order Stemonitales, equivalent to Ceratiomyxomycetidae and Myxogastromycetidae. These were duly recognised by Alexopoulos (1973) and Martin et al. (1983).

Alexopoulos and Brooks (1971) erected a family Clastodermataceae under Echinosteliales to accommodate the genera *Clastoderma* and *Barbeyella* (which were earlier included under the order Stemonitales family Stemonitaceae), on the basis of the sub-hypothallic type of development as is also true for the genus *Echinostelium*, thereby bringing the three genera together for the first time under the order Echinosteliales.

21.3 Biodiversity Explorations of Indian Myxomycetes

For expositional convenience, the history of Indian Myxomycetes can be divided into three periods: Period I (up to 1951), Period II (1952–1980) and Period III (1980 till date).

21.3.1 Period I (up to 1951)

The first myxomycetes collection from India is presumably of Dr. Wight before 1830, who collected *Physarum cinereum* (Batsch) Pers. on grass in Madras (fide Thind 1977). Hooker in 1849 or 1850 is reported to have collected three species, viz., *Physarum conglomeratum* (Fries) Rost., *Dictydiaethalium plumbeum* (Schum). Rost. and *Lycogala epidendrum* (L.) Fries, from Sikkim and Darjeeling, respectively (Ranade et al. 2012). Subsequently, some other species of Myxomycetes were reported by Berkeley (1854, 1882): *Tubifera ferruginosa* (Batsch) J.F. Gmel., *Lycogala epidendrum* (L.) Fries, *Fuligo septica* (L.) Wiggers, *Physarum pusillum* (Berk. & Curt.) G. Lister, *Diachea leucopodia* (Bull.) Rost., *Diderma hemisphaericum* (Bull.) Hornem., *Didymium difforme* (Pers.) S.G. Gray, *Didymium melanospermum* (Pers.) Macbr., *Didymium squamulosum* (Alb. & Schw.) Fries, *Stemonitis herbatica* Peck and *Comatricha typhoides* (Bull.) Rost. (Lakhanpal and Mukerji 1981a).

In Lister's (1894) 'A Monograph of the Mycetozoa' under the 'habitat' is referred ten Indian collections circumscribed to ten different species. In the third edition of A. Lister's monograph revised by Lister and Lister (1925), 18 species of Myxomycetes are listed. But unfortunately there is no information about the collector, the substratum, the date of collection, etc.

Historically the first attempt to collect Indian Myxomycetes was by Mrs. Darke[1]* (1911–1927) who is reported to have collected 124 specimens of Myxomycetes from

[1]*There is no publication by Drake and Stewart.

different parts of India, mostly the Himalayas. These were placed in 74 species. Out of these 124 collections, 40 collections belonging to 36 species are known from the Eastern Himalayas (Thind 1977). Four among them were made from Tindharia and 36 from Darjeeling (West Bengal) alone. R.R. Stewart* (1927) is also reported to have collected 1 species of *Lycogala*, viz., *L. epidendrum* (L.) Fries from Pahalgam (J&K) and G. Foreau, and 12 species from Palani Hills, South India, from 1932 to 1934.

Lister (1924) was the first to publish a paper on Indian Myxomycetes 'Mycetozoa from North India', giving an account of 36 collections made by Mrs. Darke from 1912 to 1919. The second paper on 'Indian Slime Fungi (Myxomycetes or Mycetozoa) was published by Bruhl and Gupta (1927) describing 16 species from West Bengal. Lodhi (1934) described and illustrated 43 species (all based on Mrs. Darke's collection) in his monographic compilation 'Indian Slime Moulds (Myxomycetes)'. Out of these 43 species, 5 species are the same as recorded by Bruhl and Gupta (1927). Unfortunately, there were no Myxomycetes records during 1934–1951.

21.3.2 Period II (1952–1980)

Erady (1953) described one new species of Myxomycetes, viz., *Stemonitis travancorensis* Erady, from Southern Travancore which is presently not recognised as a distinct species. A systematic pioneering attempt on Indian Myxomycetes was initiated by Prof. K.S. Thind, at the Department of Botany, Punjab University, Chandigarh, in 1952. With their earnest efforts Thind and his students published a series of 29 research papers between 1955 to 1980, describing 170 species, including 17 new species, 2 new varieties and 4 new forms. Thind (1977) published the first ever monograph on 'Myxomycetes of India', which includes 186 species, 3 varieties and 6 forms (till 1973). Out of these 173 species have been fully described and illustrated.

Agnihothrudu (1954a, b, 1956a, b, c, 1965–1966), Agnihothrudu and Chinnappa (1966–1969) and Indira (1968a, b, 1975) described 78 species from South India, including 1 new species; Agnihothrudu (1958–1968) described 56 species including 2 new species from Northeast India. Ghosh and Dutta (1962a, b, c, 1963) described 2 species from Kolkata and 15 species from Delhi, respectively. Likewise, Pathak and Ghosh (1962) from Uttar Pradesh, Patwardhan and Joshi (1975), Patil and Ranade (1975), Ranade and Mishra (1977), Mishra and Ranade (1979), Chavan and Kulkarni (1974), Thite (1975) from Maharashtra, Rokade (1989) from Maharashtra, Dhillon (1976) and Dhillon and Nannenga-Bremekamp (1977a, b, 1978) reported some more species of Myxomycetes from the North Western Himalayas.

Lakhanpal (1971–1983) and Lakhanpal and Mukerji (1975–1981) reported a large number of Myxomycetes from Delhi and North Western Himalayas. Lakhanpal and Mukerji (1981), in their monograph 'Indian Myxomycetes', described an additional 111 species to those described by Thind (1977). Thus a

total of 293 species, 3 varieties and 1 form were known from India till that time (Lakhanpal and Mukerji 1981a). Recently Ranade et al. (2012) published a revised checklist of Indian Myxomycetes recording 50 genera and 373 species, 17 varieties and 4 forma species.

During this period, the decade 1970–1980 became the most significant as six individuals received doctorates on different aspects of Myxomycetes. P.U. Indira was the first to earn doctorate on Myxomycetes from the CAS in Botany University of Madras. Her work included in addition to taxonomy experimental studies on different slime fungi. This was followed by T.N. Lakhanpal (1975) who received Ph. D. from the Department of Botany, University of Delhi. His work involved systematic studies, life cycle studies, sporophore development and some ecological aspects. The third Ph.D. on slime fungi was Dhillon (1976) from the Botany Department, Punjab University, Chandigarh, who worked on the Myxomycetes of Eastern Himalayas as well as NW Himalayas. The fourth person to be awarded Ph.D. on taxonomic and experimental studies on Myxomycetes was R. Venkatramani from the University of Madras. The fifth candidate to receive Ph.D. from Marathwada University (1979) was S.P. Nanir, who carried out taxonomic studies on the Myxomycetes of Maharashtra. The sixth person to earn Ph.D. on myxomycetes of Maharashtra was V.D. Ranade from Poona University (1978).

21.3.3 Period III (From 1980 Onwards)

The period starting from 1980 onwards witnessed even more explorations on Myxomycetes. Four people earned their doctorates during this period. In 1981, Lakhanpal and Mukerji published another monograph on Indian Myxomycetes describing 293 species. In 1984, R.K. Chopra received his Ph.D. degree from Himachal Pradesh University on corticolous Myxomycetes. This was followed by another Ph.D. degree from PU, Chandigarh (1986), to Rajesh Sharma who worked on the 'Myxomycetes of N.E. India and the Royal Kingdom of Bhutan'. B.G. Rokade (1989) and V.B. Salunkhe (1995) received their doctorates from Marathwada University, Aurangabad, on 'The Myxomycetes of Jalgaon' and 'Myxomycetes of Dang Forest of Western Ghats', respectively. They worked with Dr. S.P. Nanir and this group has contributed more than 200 species of Myxomycetes. Recently, Manoharachary et al. (2012), Manoharachary and Rajithasri (2015) and Manoharachary and Nagaraju (2017) for the first time described Myxomycetes from Andhra Pradesh and Telangana, some of which appear to be interesting.

Most of this work reviewed herein is related to the plains of India and NW Himalayas. Eastern Himalayas has been comparatively less explored (Thind 1977). Systematic studies on the Myxomycetes of Eastern Himalayas were carried out by Sharma (1986) during the years 1978–1985. He has described 139 taxa, 14 species and 3 varieties of which are new records for India/Himalayas and 22 taxa (18 species and 4 varieties) that are new to science. This contribution also provides floristics of Myxomycetes from the Royal Kingdom of Bhutan (Sharma and

Lakhanpal 2003) which includes a detailed account of 80 taxa (76 species and 4 varieties), all new to Bhutan, and 8 taxa new to science (Sharma 1997; Sharma and Lakhanpal, in press 2021).

21.4 Systematic Treatment of Class Myxomycetes

21.4.1 Subclass: Ceratiomyxomycetidae

In the subclass Ceratiomyxomycetidae, the monotypic order, family and genus *Ceratiomyxa* is represented by two species and two varieties from India. Ceratiomyxales was validated in 1977 by Farr and Alexopoulos. *C. sphaerospermum* Boedijin has been reported only from Assam and South India (Agnihothrudu 1959; Agnihothrudu and Chinnappa 1966, 1969; Thind 1977; Lakhanpal and Mukerji 1981a).

21.4.2 Subclass: Myxogastromycetidae

21.4.2.1 Order Liceales

The order is comprised of three families: Liceaceae, Cribrariaceae and Enteridaceae. The order Liceales and the family Liceaceae have a monotypic genus *Licea*. Some workers prefer to divide Liceaceae into three subgenera on the basis of dehiscence (Nannenga-Bremekamp 1965). But others prefer to retain it as a single genus. The same treatment is followed herein. The genus remained unrepresented in India till 1967, when Thind and Dhillon described a new species of *Licea*, *L. erecta*, from Darjeeling. Ranade and Mishra (1977) described a new species of *Licea*, *L. singhadensis* S.D. Patil, Ranade & R.L. Mishra, from Maharashtra. At present, out of the four species collected and described from nature in India, three are from Eastern Himalayas, e.g. *L. erecta* K.S. Thind & Dhillon , *L. biforis* Morgan and *L. variabilis* Schrad. From NW Himalayas, the only species of *Licea* recorded in nature is *Licea minima* Fries. Lakhanpal and Chopra (1994) isolated 18 species of Licea from the bark of different trees in and around Shimla; 12 of these were unknown earlier. Six of these are new records for India and another six are new species, bringing the total of *Licea* species to 23. Moist chamber cultures on different substrates will certainly yield many more and much diverse species. Lakhanpal and Sood (1981a) and Chopra (1984) have isolated many of the interesting species reported above and two additional forms, *L. operculata* (Wingate) Martin and *L. parasitica* (Zukal) Martin, respectively, from the bark of different trees kept in moist chambers. Licea are among the smallest Myxomycetes and are therefore easily overlooked in the field. But they readily come up, if the bark is kept in moist chambers (Gilbert and Martin 1933). This is corroborated amply by the studies carried out at Himachal Pradesh University, Shimla (India), and earlier by Keller and Brooks (1971). In the family Enteridaceae (Reticulariaceae) of Liceales, four genera *Reticularia* Bull., *Tubifera* J.F. Gmel., *Dictydiaethalium* Rost. and *Lycogala* Adans,

known from the world over, are also represented in India. Farr (1976) in accordance with the rules of the International Code of Botanical Nomenclature (ICBN) pleaded convincingly for the replacement of the name *Reticularia* with *Enteridium* Ehrenb. All the genera and species reported so far have been collected and described from nature. Genus *Tubifera* J.F. Gmel. is represented by three species, two of which, *T. ferruginosa* (Batsch) J.F. Gmel. and *T. microsperma* (Berk. and Curt.), are widely distributed whereas *T. papillata* is recorded only from the Eastern Himalayas. In a reappraisal of the genus *Tubifera*, Thind et al. (1991) after a detailed re-examination of specimens at PU, Chandigarh, pointed out that *T. microsperma* recorded from India actually represented a newly described species, *T. dimorphotheca* Nanm.-Brem. & Loerak. The true *T. microsperma* (Berk. & M.A. Curtis) G.W. Martin was recorded by them from Eastern Himalayas along with this species.

In the genus *Enteridium, E. lycoperdon* (Bull.) M.L. Farr is cosmopolitan (reported from Assam, HP and Telengana), whereas *E. juranum* from HP and *E. splendens* from Chandigarh and Uttarakhand. *E. intermedium* (Nann.-Brem.) Farr. var. bhutanensis Sharma & Lakhanpal is reported from Bhutan (Sharma 1986; Sharma and Lakhanpal, in press 2021). Lakhanpal and Mukerji (1976a, b, c, d, e, f, g, h) reported *E. lycoperdon* and Sekhon (1978) recorded *E. splendens* (Morgan) Farr. Kowalski (1975) from a study of type material of the first two species prefers to treat *E. juranum,* a var. of *E. splendens*.

In the genus *Lycogala* out of the five species known from India till date, four are represented in both NW and eastern stretches of the Himalayas whereas *L. flavofuscum* (Ehrenb.) Rost. is so far reported only from NW Himalayas. *Lycogala conica* var. *pustulata* Thind is not a valid variety. However, the most significant contribution from India in this genus has been the solution to the delimitation of three very intriguing species, i.e. *L. conicum* Pers., *L. epidendrum* (L.) Fries and *L. exiguum* Morgan on the basis of the development of their cortical scales (Lakhanpal and Mukerji 1981b). The remaining two species, viz., *L. mysorensis* (Agnihothrudu) Agnihothrudu and *L. flavofuscum*, are easy to identify as both possess smooth peridium; the former has warted spores while the latter has reticulate spores. The other three species and *L. epidendrum* var. *tessellatum* were so far delimited by characteristics which would overlap frequently and complicate identification. Lakhanpal and Mukerji (1979a, b) studied the development of cortical scales in these species and observed that each species exhibits a specific trend of scale development from the basic smooth and circular type. One trend involves the cleavage of basic scale type giving a tessellated appearance, as is exemplified by *L. exiguum* and *L. epidendrum* var. *tessellatum*. The second trend involves swelling, enlargement and flattening of the scales leading to the formation of non-tessellate scales, as exemplified by *L. epidendrum*. In the third course the pustules swell, enlarge, branch and then anastomose (without cleavage) forming an intricate reticulum as is possessed by *L. conicum*.

The genus *Dictydiaethalium* is represented by a single representative, *D. plumbeum*, and it is widely distributed. In the family Cribrariaceae of Liceales, all the three genera (*Cribraria* Pers., *Dictydium* Schrad., *Lindbladia* Fries), known the world over, are also reported from India. The family has representative species

from nature as well as from the moist chambers. The fruiting bodies in some members are minute and in others of fairly discernible size. The genus *Cribraria* has around 29 species including *C. costata* n. sp., described by Dhillon and Nannenga-Bremekamp (Dhillon and Nannenga-Bremekamp 1977a; b, 1978) from India. Before 1970, only 5 species of this genus were reported from India; however, by 1980 the number increased to 16. Three of these (*C. piriformis* Schrad., *C. rubiginosa* Fries and *C. vulgaris* Schrad.) were recorded by Thind and Khara (1969); four by Lankhanpal and Mukerji (1976d) and Lakhanpal and Sood (1981b), viz., *C. aurantiaca* Schrad., *C. personii* Nann.-Brem., *C. pachydictyon* Nann.-Brem. and *C. splendens* (Schrad.) Pers.; and another three, i.e. *C. violacea* Rex, *C. meylanii* Brandza and *C. minutissima* Schw., by Dhillon (1976) and Dhillon and Nannenga-Bremekamp (1978); Lakhanpal and Chopra (1994) recorded *C. atrofusca* var. *nannengae* var nov., and *C. microcarpa* (Schrad) Pers., and Dhillon and Nannenga-Bremekamp (1978) *C. costata*. Out of these, 6 species which occur in the Eastern Himalayas (Sharma 1986) are *C. argillacea* (Pers.) Pers., *C. intricata* Schrad., *C. languescens* Rex, *C. macrocarpa* Schrad., *C. splendens* (Schrad) Pers. and *C. tenella* Schrad. No addition to the already known two species of the genus *Dictydium* has been made. It remains to be represented by the same two species, recorded in the past, *i.e. D. cancellatum* (Batsch.) Macbr and *D. mirabile* (Rost) Meylan. The third genus *Lindbladia* Fries was recently recorded from India by Lakhanpal and Mukerji (1976c). This is a monotypic genus with *L. tubulina* as its only species, an interesting species with an aspect like that of *Cribraria argillacea* (Pers.) Pers., but either without a peridial net or with a scantily developed one.

21.4.2.2 Order Echinosteliales

The order Echinosteliales comprises two families, Clastodermataceae and Echinosteliaceae. The family Clastodermataceae was erected by Alexopoulos and Brooks (1971) for *Clastoderma* Blytt and *Barbeyella* Meylan. In the family Clastodermataceae both genera *Clastoderma* and *Barbeyella* are known from India. In the genus *Barbeyella, B minutissima* Meylan has been reported from India. For *Clastoderma, C. debaryanum* A. Blytt and *C. dictyosporum* (Lakhanpal and Mukerji 1981a) are reported from India.

The monotypic genus *Echinostelium* in the family Echinosteliaceae was for a long time represented by six species from India, out of which five were known from NW Himalayas and one also from Eastern Himalayas (Lakhanpal and Chopra 1994). *E. epitectum* Whitney, *E. coelocephalum* Brooks & Keller and *E. corynophorum* Whitney were first reported from NW Himalayas (Lakhanpal and Chopra 1994). Nannenga-Bremekamp (1991) recorded *E. vanderpoelli* Nann.-Brem. and the total number of species now is ten. The *Echinostelia*, like Licea, are minute members of Myxomycetes and, therefore, rarely collected in nature. But they appear on the bark of living trees in moist chambers quite frequently. Most of the species listed have been obtained by this technique only, and same holds true for most of the Echinostelia reported from different parts of the world.

21.4.2.3 Order Trichiales

The order Trichiales has two families: Dianemaceae and Trichiaceae. The family Dianemaceae is represented by three genera: *Calomyxa, Dianema* and *Listerella*. The fourth genus *Minakatella* G. Lister has been transferred to Trichiaceae on the basis of ultrastructure (Keller et al. 1973). *Calomyxa metallica* (Berk) Nieuwl. and *Dianema nivale* (Meylan) G. Lister are reported from India. Out of these two, *C. metallica* has been obtained in a moist chamber culture on the bark of *Cedrus deodara* and *D. nivale* has been collected in nature (Lakhanpal and Mukerji 1976d; Dhillon 1976).

In the family Trichiaceae, out of the 11 genera reported *Minakatella* and *Prototrichia* are yet to be reported from India. The genus *Arcyria* Wiggers was so far represented by around 24 species from all over the world. Dhillon and Nannenga-Bremekamp (1978) and Lakhanpal and Mukerji (1979a) added one new species each from Uttar Pradesh and Himachal Pradesh, respectively, namely *A. fasciculata* sp. n. and *A. brooksii (Originally published as A. brooksea)* n. sp. From 1970 to 1980, six more species were described by Lakhanpal and Mukerji (1976a, b, c, d, e, f, g, h, 1981) and Chopra (1984) from Himachal Pradesh, viz., *A. affinis* (Rost.) Nann.-Brem., *A. gulielmae* Nann.-Brem., *A. magna* Rex, *A. nigella* Emoto, *A. pomiformis* (Leers) Rost. and *A. virescens* G. Lister, and one by Dhillon (1976), i.e. *A. glauca Lister* from Uttar Pradesh. With these additions the total number of species known from India is now 20. The genus *Arcyria* has now 19 species reported from India. Among these species, only *A. nigella* Emoto has been exclusively isolated in a moist chamber culture on the bark of *C. deodara* whereas others have been collected in nature as well. Sharma (1986) has added two more taxa, *A. magna* var. *rosea* Rex and a new species of *Arcyria*, *A. wangchucki* Sharma & Lakhanpal sp. nov. (Sharma and Lakhanpal in press, 2021). *Cornuvia serpula* (Wigate) Rost., sole member known from India, is also recorded from the Himalayas.

The genus *Hemitrichia* Rost., which comprises 11 species, was represented in India by 5 species only. Lakhanpal and Sood (1981b) and Lakhanpal and Chopra (1982) have recorded two more species, viz., *H. leotricha* (A. Lister) G. Lister and *H. abietina* (Wigand) G. Lister, so that now seven species of this genus are recorded from India. Lakhanpal (2016) recorded two new species, *H. ellae* sp. nov., and *H. thindi* sp. nov., from Himachal Pradesh. These two species were obtained from the bark kept in moist chambers. The remaining six species have been collected in natural habitats.

Chopra (1984) reported the genus *Calonema* for the first time for India. The genus was known by two species *C. aureum* Morgan and *C. luteolum* Kowalski. A new species *C. dissipatum* sp. nov. and a new variety *C. dissipatum* var. *ellae* var. nov. have been added to it from moist chamber culture on the bark of *Pinus wallichiana* and *Cedrus deodara* (Lakhanpal and Chopra 1994).

Metatrichia vesparium (Batsch) Pers. is the single species of *Metatrichia* Ing recorded from India long back. *M. horrida* Ing has not been recorded from India so far. Lakhanpal and Mukerji (Lakhanpal and Mukerji 1976e), however, amended the description of the former species by making a comparative study of about

70 populations procured from Mrs. N.E. Nannenga-Bremekamp, Netherlands, representing collections from various parts of the world. Based on the broadened concept, they have also affected the transfer of *H. paragoga* Farr and *T. arundinariae* Rammeloo to *Metatrichia* by making new combinations. *Oligonema flavidum* (Peck) Peck remains to be represented by *S. flavidum*, the same species still, and it is known only from the eastern part of Himalayas. The genus *Perichaena* has the same species still, five of which are reported from the Himalayas. Four of these were represented by collections from natural habitat and one (*Perichaena* sp.) was isolated from the bark of *Picea*. However, three of the species known earlier from natural habitats have now been recorded in moist chamber cultures. These are *P. chrysosperma* (Curry) Lister, *P. depressa* Libert and *P. vermicularis* (Schw) Rost. Dhillon and Nannenga-Bremekamp (1977a; b) described an unnamed species of *Perichaena*.

Twelve species are known in the genus *Trichia* from India and the Himalayas. Lakhanpal and Chopra (1994) recorded *T. munda* (*A. Lister*) Meylan from Himachal Pradesh bringing the number of species known to 13. Out of these *T. crateriforme* Martin, *T. lutescens* (Lister) Lister, *T. subfusca* Rex and *T. subretispora* Lakhanpal and Mukerji are known only from NW Himalayas. Most of these species except *T. crateriforme* Martin, *T. subfusca, T. munda* (A. Lister) Meylan and *T. erecta* Rex have been collected and described from natural habitats. These four species have been obtained in moist chambers as well.

21.4.2.4 Order Physarales

Three families recognised in Physarales are Elaeomyxaceae, Physaraceae and Didymiaceae. This is the largest order of Myxomycetes and almost half of the species are contained in it. The first one is a monotypic family and *E. miyazakiensis* (Emoto) Hagelst. is yet unknown from India.

Physaraceae is comprised of 19 genera, of which *Cienkowekia* Rost., *Leocarpus* Link, *Erionema* Penzig, *Physarella* Peck, *Protophysarum* Alexopoulos & Blackwell and *Badhamiopsis* Keller & Brooks are all monotypic. The remaining four genera are *Physarum* Pers., *Badhamia* Berk., *Craterium* Trent. and *Fuligo* Hall. Out of the nine genera known in the family Physaraceae, *Protophysarum* is still not recorded from India whereas *Badhamiopsis ainoe* (Yam.) Brooks & Keller has recently been recorded from Himachal Pradesh (Chopra 1984). The species has been isolated in a moist chamber culture.

The genus *Badhamia* was so far known by seven species, five of which were recorded from the Himalayan region as well. Chopra (1984) recorded *B. iowensis* Macbr. and *B. nitens* Berk. for the first time from India and also described *B. evada* sp. nov. Sharma described *B. gracilis* (Macbr.) Macbr. var. echinulatum Sharma & Lakhanpal sp. nov., from the Eastern Himalayas, so that at present the genus *Badhamia* is represented by nine species. Out of these *B. evada* sp. nov., *B. iowensis* Macbr., *B. nitens* Berk. and *B. panicea* (Fries) Rost. have been isolated in moist chambers.

The genus *Wilkommlangea* (Cienkowskia), a monotypic genus, still remains to be unrepresented and same is the case of *Leocarpus fragilis* (Dicks) Rost. and *Erionema aureum* Penzig. The first one is reported from Eastern Himalayas and

the second one from both NW and Eastern Himalayas. All these have been collected in nature only. *Erionema aureum* Penzig was first recorded from India by Thind and Khara (1969) and subsequently by Lakhanpal and Mukerji (1978).

The genus *Physarella* remains to be represented by a single species, *P. oblonga* (Berk. and Curt) Morgan, from India. *Physarella oblonga* f. *alba* Alexop. was not known from India till Lakhanpal (1972) recorded it from Delhi. *P. oblonga* has been, however, known from many parts of India. There is every likelihood that many of the earlier collections represent *F. alba* since all of them have been identified on the basis of morphological characters alone, whereas *F. alba* has been established on the basis of a white plasmodium that it produces in culture whereas the type species produces yellow plasmodium (Alexopoulos 1964).

The genus *Fuligo* so far had three representative species; only *F. cinerea* (Schw) Morgan and *F. septica* (L) Wiggers are represented in India. Sekhon (1978) recorded *F. megaspore* from Chandigarh. The genus *Craterium* is a widely distributed genus and represented in India by seven species. Dhillon and Nannenga-Bremekamp (1978) described the seventh species *C. costatum* from UP.

The genus *Physarum*, perhaps the largest in the family Physaraceae, is reported to be represented by 62 species in India, out of which 50 are also known from the Himalayan region alone. Chopra (1984) described *Physarum decipiens* Curtis and three new taxa, *P. complexum* sp. nov., *P. tubulatum* sp. nov. and *P. leucophaeum* var. *columellatum* var. nov., from HP. Sharma (1986) reported the following five species from the study area; *P. javanicum* Racib., *P. luteolum* Peck, *P. penetrale* Rex, *P. straminipes* A. Lister and *P. urne* Singh & Pushpavathy K.K. Sharma (1986) reported *P. penetrale* Rex for the first time from India, while the other four species are new records for the Himalaya. The other species obtained by moist chamber culture from NW Himalayan region are *P. bivalve* Pers, *P. crateiforme* Petch, *P. echinosporum* A. Lister, *P. javanicum* Racib., *P. superbum* Hagelst., *P. compressum* Alb. and Schw., *P. rigidum* A. Lister and *Physarum* sp. Dhillon & Nann.-Brem. Rest of the species have been collected in nature itself.

The family Didymiaceae so far was represented by six genera, viz., *Wilczekia* Meylan, *Mucilago* Micheli ex Batt., *Physarina* von Hohnel, *Lepidoderma* de Bary, *Diderma* Pers. and *Didymium* Schrad. Alexopoulos and Sáenz-Renauld (1975) transferred *Diachea* Fries to Didymiaceae from Stemonitomycetidae based on the findings of Blackwell (1973). Kowalski (1972) established a new genus *Squamuloderma* for an isolate which possessed crystalline lime like *Didymium* but lacked capillitium. Martin et al. (1983) also recognised six genera in the family Didymiaceae. *Squamuloderma* also seems to be valid, though Farr pleads to the contrary, and so are *Lepidodermopsis* Honn. and *Trabrooksia* Keller. With these three genera, the generic number in Didymiaceae stands at nine in India.

The genus *Wilczekia* Meylan, till recently considered a valid genus, has been transferred to *Diderma*. The genus *Diachea* is represented by six species in India. These are *D. bulbillosa* (Berk. & Br.) Lister, *D. leucopodia* (Bull.) Rost., *D. megalospora* Thind & Manocha and *D. splendens* Peck.

In the genus *Diderma*, 29 species are reported from India. In the 1970s another 11 species were recorded from India. This number increased to 20 by the early 1980s and now it stands at 29. Thind (1977) described two new species, one from Mussoorie (*D. alpino-spumarioides*) and other from West Bengal (*D. badhamoid*). Lakhanpal (1978) recorded *D. alexopoulii* n. sp. from HP and Mishra and Ranade (1979) described four new species from Maharashtra (*D. circumscissilis* n. sp., *D. lohgadense* n. sp., *D. marie* n. sp., *D. punensis* n. sp.). Thind et al. (1971) also recorded *D. globosum* Pers. from HP and Thind (1977) *D. simplex* (Schroer.) G. Lister from West Bengal. Thind et al. (1971) reported *D. cor-rubrum* Macbr. from HP which was again recorded from the same state along with *D. roanense* (Rex.) Macbr. by Lakhanpal (1974) and from Maharashtra by Patil and Ranade (1975) along with very important and rare species, *D. lyalli* (Mass.) Macbr. *D. platycarpum* var. *platycarpum* Nann.-Brem. was described from Delhi by Lakhanpal (1972). This interesting specimen has so far been recorded from the Netherlands (type locality) and Delhi only. Lakhanpal and Mukerji (1978) recorded another rare but very interesting species of *Diderma*, *D. asteroides* (A. & G. Lister) G. Lister from HP. Its name signified the manner of dehiscence into star-shaped lobes. Thind and Dhillon (1979) also reported two more rare *Diderma* from HP, *D. niveum* (Rost.) Macbr. and *D. trevelyani* (Grey.) Fries. In the later years, Lakhanpal and Chopra (unpublished) recorded *D. platycarpum* var. berkeleyanum Nann.-Brem., for the first time from India, and also described two new species, *D. intermediurn* sp. nov. and *D. yamamotii* sp. nov. Sharma recorded *D. effusum* (Schw.) Morgan var. *pachytrichon* Nann.-Brem. and *D. subfloriformis* F. Cand. et. Nann.-Brem., for the first time from the Himalayan region. He also proposed two new species in the genus, *D. echinospora* sp. nov. and *D. lakhanpali* sp. nov. From among these, *D. intermedium, D. platycarpum* var. *berkeleyanum*, *D. yamamotii* and *D. simplex* have been isolated by moist chamber technique.

The genus *Didymium* has been customarily divided into two subgenera: *Didymium* and *Lepidodermopsis*. The latter has so far been monotypic with *D. leoninum* Berk. & Br., as its species. Lakhanpal (1978) described a new species *L. martinii* n. sp. in this subgenus and observed that the distinction between *Didymium* and *Lepidodermopsis* is more clear-cut than it is between *Didymium* and *Diderma* which are treated by all as two distinct genera and are separated from each other by the crystalline nature of lime on the peridium, a character which may not always be absolute. *Didymiu*m and *Lepidodermopsis*, on the other hand, are distinguished on the basis of the nature of its peridium. In fact cartilaginous peridium of *Lepidodermopsis* is unique in *Didymium* and this characteristic supplemented by large stellate lime crystals and yellow lines of dehiscence makes the distinction between *Didymium* and *Lepidodermopsis* more clear-cut and taxonomically valuable. Therefore, Lakhanpal (1978) restored the generic rank accorded to it by Hohnel. *Lepidodermopsis* therefore now has two species, *L. leonina* (Berk. & Br.) Hohn. and *L. martinii* Lakhanpal. Accordingly, the number of genera in Didymiaceae becomes nine. Thirty-two species are so far known in the genus *Didymium*, and 21 out of these are known from the Himalayan region alone. Sharma and Lakhanpal (2021) have proposed three new species in the genus based on the

collection made from Eastern Himalayas: *D. agnihothrudianum* Sharma & Lakhanpal sp. nov, *D. spinulatum* Sharma & Lakhanpal sp. nov. and *D. indirianum* Sharma & Lakhanpal sp. nov.

The genus *Didymium* is comprised of around 37 species in India. Kowalski and Lakhanpal (1973) described *D. disciformis* n. sp. from Delhi. *D. disciformis* is an interesting species which possesses eggshell-like peridium like *D. vaccinum* (Dur. & Mont.) Buchet. Till recently these two were the only species in the genus privileged with such a peridium when Lakhanpal and Mukerji (1978) described *D. haretianum* n. sp. which by virtue of a similar peridium is delimited among these species. Lakhanpal and Mukerji (1976h, 1978, 1979a; b) described *D. muscorum* n. sp. and *D. dehlianum* n. sp. from Delhi and *D. simlensis* n. sp. and *D. projectile* n. sp. from Himachal Pradesh. The last one of these needs special mention as it possesses unique type of capillitial threads having projectile-like ends. Lakhanpal (1973) recorded *D. flexuosum* Yamashira and *D. verrucosporum* Welden from HP and Delhi, respectively. The first one is a rare species which was known from Japan and N. America before its report from India. *D. verrucosporum* is equally rare and it is its first report outside S. America. Lakhanpal and Mukerji (1978) recorded three more rare species of *Didymium*, viz*., D. intermedium* Schroet. *S. karstenii* Nann.-Brem. and *D. saturnus* Keller, from Delhi. Similarly *D. perforatum* Yamashiro was recorded from Chandigarh by Sekhon (1979) and *D. sturgisii* Hagelst. by Indira (1975) from Tamil Nadu and later by Dhillon (1976) from Uttar Pradesh. Thind and Dhillon (1979) also described *D. ovoideum* Nann.-Brem. from HP. *D. floccosum* was first described from India by Martin et al. (1959). It has been recorded from Delhi (Singh et al. 1979) and HP (Lakhanpal 1973). The species remains unknown outside this country.

The genus *Lepidoderma* remains to be represented by the same species *L. tigrinum* (Schrad) Rost., and *Lepidodermopsis* by two species, *L. leonina* (Berk. Br.) Hohn. and *L. martinii* Lakhanpal & Mukerji. The status of *Physarina echinospora* Thind and Manocha is also unchanged. *Wilczekia* and *Mucilago* have not been recorded from India so far whereas *Lepidoderma* remains to be represented by a single species, *L. tigrinum* (Schrad.) Rost. Kowalski (1971) in his monographic treatment of *Lepidoderma* pointed out that most of its species are alpine in distribution. A search in alpine regions or near the melting snow might help in increasing the representation of this genus in India.

21.4.3 Subclass: Stemonitomycetidae

In the subclass Stemonitomycetidae the single order Stemonitales has two families Schenellaceae and Stemonitaceae.

21.4.3.1 Order Stemonitales

Earlier the Stemonitales had 15 genera (Martin and Alexopoulos 1969), of which *Barbeyella* and *Clastoderm*a have been transferred to the Echinosteliales (Alexopoulos and Brooks 1971). Hertel (1956) and Ing and Nannenga-Bremekamp

(1967) segregated some of these into more genera and recognised several subgenera as well. Lakhanpal and Mukerji (1981) recognised two of their genera *Symphytocarpus* Ing & Nann.-Brem. and *Stemonitopsis* Nann.-Brem. in their treatise on Indian Myxomycetes and have pointed out that *Comatricha* and *Collaria* should not be split up into subgenera till enough information accumulates with regard to the sporophore development of as many species and genera as possible.

The family *Stemonitacae* has ten genera. The two genera erected by Ing and Nann.-Brem., cl. c and as recognised by Lakhanpal and Mukerji (1981) are also included herein. Nann.-Brem. et al. also created a new genus *Stemonaria*, which is also recognised herein.

The family *Schenellaceae* is monotypic with genus *Schenella*, not yet recorded from India. The genus *Amaurochaete* is not yet recorded from India. *Colloderma, Diacheopsis* and *Brefeldia* are still known by one species each. *Colloderma* G. Lister has also been reported from India so far. Monotypic *Leptoderma iridescens* G. Lister was recorded from HP by Lakhanpal (1971) and by Indira from Tamil Nadu in 1975. No species has been added to *Diacheopsis*. *Enerthenema papillatum* (Pers.) Rost. was recorded in NW Himalayas in a moist chamber culture and in nature from Eastern Himalayas (Chopra 1984; Sharma 1986).

In the genus *Comatricha* only seven species were known from India before 1970. Lakhanpal and Mukerji (1977) and Dhillon and Nannenga-Bremekamp (1977a, b) described, respectively, two (*C. nannengae* n. sp., *C. kowalskii* n. sp.) and one (*C. parvispora* n. sp.) new species from India. Lakhanpal and Mukerji (1977, 1981) recorded *C. aequalis* Peck and *C. nodulifera* Wollman & Alexop., and Dhillon (1976) described *C. subcaespitosa* Peck for the first time from India. With these species the total number of *Comatricha* species recorded from India has now increased to 17. New additions are as follows: Lakhanpal and Chopra (unpublished) recorded for the first time *C. rigidireta* Nann.-Brem., *C. ellae* Hark. and *C. acanthodes* Alexop., and two new species, *C. laxifila* Chopra & Lakhanpal sp. nov. and *C. variabilis* Chopra & Lakhanpal sp. nov. Sharma also described *C. acanthodes* Alexop. and *C. elegans* (Racib.) G. Lister for the first time from India (Eastern Himalayas). He also proposed a new species, *Comatricha* sp. nov. Out of these *C. laxifila* Chopra & Lakhanpal, *C. confusa, C. ellae Hark., C. rigidireta Nann.-Brem., C. tenerrima* (M.A. Curt.) G. Lister and *C. nodulifera* Wollman & Alexop. have been obtained in moist chambers.

In the genus *Lamproderma*, nine species have been recorded from India. Lakhanpal and Mukerji (1979a, b) published three new species, *L. collinii, L. thindianum* and *L. alexopoulii*, and Dhillon and Nannenga-Bremekamp (1977a, b) published *L.* (*Collaria*) *retispora* sp. nov. Sharma recorded *L. echinatum* (Berk.) Rost. and *Lamproderma* sp. nov. from Eastern Himalayas.

Macbrideola H.C. Gilbert was unknown in this country till Lakhanpal and Mukerji (1977) isolated *M. cornea* (G. Lister & Cran.) Alexop. from the bark of *Pinus excelsa*. In the same year they also described a new species, *M. robusta* n. sp. from Himachal Pradesh. Nannenga-Bremekamp et al. (1979) added another species *M. coprophila* n. sp. to this genus. Earlier Sarbhoy et al. (1975) had published a new species in Mucorales, *Utharomyces indicus*. A critical re-examination of the type of

these two species revealed that both the taxa are the same. Nann.-Brem. et al. rightly treated it in the genus *Macbrideola* but apparently unaware of Sarbhoy et al.'s binomial, they described it as a new species. The correct name *M. indica* for this taxon has been given by Lakhanpal and Mukerji (1981), based on *U. indicus*—the earliest legitimately published name. Lakhanpal and Chopra (1994) also reported *M. decapillata* N Gilbert and *M. bremekempii* sp. nov. These as well as the remaining species in *Macbrideola, M. cornea, M. indica* and *M. robusta* all have been obtained in moist chamber cultures.

The genus *Stemonitis* is represented by 16 species from India; Lakhanpal (1973) recorded *S. inconspicua* Nann.-Brem. and *S. fusca* var. *papillosa* Meylan from Himachal Pradesh. In 1977 Lakhanpal and Mukerji described *S. farrensis*, also collected from HP. So now we have on record 15 species of this genus from India. Lakhanpal and Chopra (1994) have isolated a new species, *S. enerthenemoides* sp. nov. Sharma recorded *S. equalis* (Peck) Massee var. *microspora* Nann.-Brem., and Yamamato *S. microsperma* Ing, *S. hyperopta* Meylan and *S. webberi* Rex for the first time from India. Nannenga-Bremekamp et al. (1979) described a new species *S. rhizoideipes* from the Eastern Himalayas. Species in *Stemonitis* which have been isolated in moist chambers are *S. enerthenemoides, S. farrensis, S. inconspicua, S. nigrescens, S. smithii, S. herbatica* and *S. uvifera* var. *microcarpa*.

The genus *Stemonitopsis* erected by Nannenga-Bremekamp (1974) for those species of *Comatricha* and *Stemonitis*, which possess a fragmentary capillitial net in the upper part of the fructifications, is represented in India by four species, viz., *S. hyperopta* (Meylan) Nann.-Brem., *S. irregularis* (Res.) Nann.-Brem., *S. typhoides* (Bull.) Nann.-Brem. and *S. suksdorfii* (Ellis & Ev.) Nann.-Brem. The genus *Symphytocarpus* Ing & Nann.-Brem. is represented in India by a single species, *S. herbaticus* (Lakhanpal and Mukerji 1977). Ing and Nannenga-Bremekamp (1967) recognised six species in this genus. Though many new genera have been created to include borderline species with passage of time, in practice one has to fall back on the concepts propounded by Martin et al. (1983) who created a new genus in *Stemonitales*, *Stemonaria*, which is being recognised herein.

21.5 Experimental Studies on Indian Myxomycetes

The earliest studies on cultural aspects of Myxomycetes in India seemed to be of Kar (1962, 1963a, 1963b, 1964) who described the plasmodium in *Licea* sp., *Physarum wingatense* and *Physarella oblonga*. Simultaneously result of studies on the genetic behaviour of *Didymium viridis* and *D. squamulosum* and the effect of x-irradiation were reported by Mukherjee and associates during the period from1964 to 1966. More systematic work on cultural studies was initiated by Indira and Kalyanasundaram (1963). They studied the cultural characteristics of *Physarum compressum*, *Stemonitis herbatica* and *Arcyria cinerea*. Indira (1965) described the *in vitro* cultivation of *Diachea splendens* Peck and in 1966 screened some more species of myxomycetes, observed their response and behaviour in culture and conducted detailed studies on the various aspects of the life cycle of *S. herbatica*.

The species which have been cultivated *in vitro* from spore to spore in India are as follows:

Kar (1963a, b–1964)	*Licea* sp. *Physarella oblonga* (Berk. & Curt.) Morgan *Physarum wingatense* Macbr.
Indira and Indira et al. (1965–1974)	*Arcyria cinerea* (Bull.) Pers. *Stemonitis herbatica* Peck *Physarum compressum* Alb. & Schw. *P. cinereum* (Batsch) Pers. *P. gyrosum* Rost. *P. serpula* Morgan *P. vernum Somm*. Ex Fries
Lakhanpal and Mukerji (1976h) Lakhanpal (1983)	*Didymium muscorum* Lakhanpal & Mukerji *D. karstenii* Nann.-Brem. *D. intermedium* Schroet. *D. squamulosum* (Alb. & Schw.) Fries *Physarum nicaraguense* Macbr.

The Myxomycetes spore germination takes place in two ways: either by dissolving a pore in the spore wall or by the formation of a wedge-shaped crack (Gilbert 1928a, b). The former is considered an enzymatic action and the latter an osmotic phenomenon. Indira (1969a; b; c) reported irregular rupturing of the spore wall in *S. herbatica* and Lakhanpal and Mukerji (1976h) in four species of *Didymium* and Lakhanpal (1981) in *P. nicaraguense* also observed the germination of spores by a wedge-shaped crack in the spore wall. Pasricha and Mukerji (1985) studied the growth and sporulation in *D. muscorum.*

The time required for germination as well as percentage of germination varies with the conditions, the age of the spores, the species and the strain and even with particular fruiting body (Collins 1961). Lakhanpal and Mukerji (1976h) observed it to be true in *D. intermedium* as fresh spores germinated within 30 min whereas 1-year-old spores took around 24 h for germination. Indira and Kalyanasundaram (1971) also observed in *S. herbetica* that spore germination is affected to a great extent by the age of spores.

The spore on germination released a single swarmer in *S. herbetica* and *P. nicaraguense* (Indira 1969b; Lakhanpal 1981) whereas it released 1–3 swarm cells in the four species of *Didymium* (Lakhanpal and Mukerji 1976h). The swarmers usually issued within 48 h but they could be maintained as such for several days in a nutrient medium (Indira 1969b). In contrast to the earlier observations (Gilbert 1928a, b) that the swarmers obtain their food in solution and by putting forth pseudopodia, Indira (1969b) recorded a hitherto unrecorded mode of feeding in the swarm cells of *S. herbatica*. She observed that the swarmers ingest bacteria at a definite region of their body bending over it, trapping it and then revolving round and round till it reaches inside the body. She pointed out that this type of nutrition is reminiscent of certain flagellates and may be of phylogenetic significance. She further pointed out that the haploid phase in this slime mould is of completely flagellate type. The swarm cell undergoes several successive divisions presumably

by amitosis and the zygote is formed by their isogametic union. In several species of myxomycetes it has been observed that swarm cells get converted into myxamoebae by a permanent withdrawal of flagella. She observed in *S. herbatica* that the swarm cells retain their flagella until they form zygote or undergo encystment. She observed fusion of swarm cells posteriorly, laterally or end to end and plasmodium formation during 48 h of germination as also coalescence of zygotes in *S. herbatica*.

Lakhanpal (1981) (Lakhanpal and Mukerji 1976h) did not observe fusion of swarm cells but observed plasmodium formation after 3, 4, 6 and 8 days, respectively, in *P. nicaraguense, D. intermedium, D. kastenii, D. muscorum* and *D. squamulosum.* Evidently fusion of swarm cells must have taken place much earlier before the appearance of plasmodia. The plasmodium in all these species is a phaneroplasmodium in conformity with other members of Physarales (Alexopoulos 1969) whereas in *S. herbatica* it is aphanoplasmodium (Alexopoulos 1969). Lakhanpal and Mukerji (1976g) described the plasmodial types in *Licea scyphoides* Brooks & Keller, *Clastoderma debaryanum* Blytt. and *Macbrideola cornea* (G. Lister & Cran) Alexop., and Lakhanpal and Sood in *Echinostelium cribrariodes* Alexop. In the first two and *E. cribrariodes* the plasmodium is a protoplasmodium (Alexopoulos 1960). It has been observed to show at times feeble, irreversible movements in *L. scyphoides*. In *C. debaryanum* protoplasmodium usually multiplies and produces groups of 2–5 plasmodia which become angular due to mutual pressure. The multiplication is most rapid in plasmodia covered by a thin film of water. They migrate for some distance on the agar medium leaving behind tracts of their movement. *M. cornea* possesses aphanoplasmodium which forms a delicate reticulum of veins and veinlets. The veins lack differentiation into ecto- and endoplasm but show reversible streaming of protoplasm. A similar plasmodium has been recorded for *Lamproderma scintillans* (Berk. & Br.) Morgan by Indira (1974). The phaneroplasmodia in the species of *Didymium* and *P. nicaraguense* have been reported to exhibit typical rhythmical, reversible streaming of cytoplasm. The direction of protoplast stream is reversed every 43–47 s following a stationary phase of 3–10 s.

Indira (1969c), in in vitro cultivation of some Myxomycetes, obtained best results with oatmeal agar, out of the 11 media tried. Indira and Venkataramni (1974) introduced a new medium, the coconut milk agar medium, for the cultivation of Myxomycetes but it was found to be less effective than carrot agar. From their trials with *Arcyria denudata*, *Didymium iridis*, *D. clavus, Perichaena chrysosperma*, *Physarum bogoriense*, *P. compressum*, *P. gilkeyanum* and *P. nicaraguense*, they concluded that in order to obtain plasmodia from old collections of spores, direct plating of spores was more effective than plating after germination in liquid media.

21.6 Sporophore Development

The sporophore development has been studied in *Licea scyphoides*, *Clastoderma debaryanum*, *Macbrideola cornea* (Lakhanpal and Mukerji 1976a), *Echinostelium cribrorioides* (Lakhanpal and Sood 1981b), *Physarum nicaraguense* (Lakhanpal 1981) and *Stemonitis herbatica* (Indira 1971).

Alexopoulos (1969) stressed the significance of the type of plasmodia and the type of sporophore development in the taxonomy of myxomycetes. Ross (1957) described subhypothallic and epiphypothallic type. On the basis of protoplasmodia, subhypothallic type of sporophore development and granular stuffed stalk, Alexopoulos and Brooks (1971) removed *Clastoderma* and *Barbeyella* from the Stemonitales to Echinosteliales. Similarly, Ross (1973) transferred Stemonitales to a subclass of its own—Stemonitomycetidae, on the basis of plasmodium and sporophore development. Two major changes were brought in the classification of Myxomycetes with such studies of sporophore development in Myxomycetes.

Lakhanpal and Mukerji studied the sporophore development in *L. scyphoides* and reported it to be a subhypothallic type. This is the first species in Liceales which has been worked out in this regard. This study justifies to some extent the inclusion of this order in Myxogastromycetidae.

Although the spore-to-spore life cycle of *C. debaryanum* had been worked out earlier (McManus 1961) no details of its sporophore development had been provided. Lakhanpal and Mukerji (1976a) described the sequential development of sporangia and differentiation of spores and capillitium for the first time in this species. They observed that the sporangial development is subhypothallic and the stalk though stuffed is granular at base and is composed of tubular threads above, as composed of the capillitium, columella and stalk in the members of Stemonitales. They pointed out that whereas the protoplasmodium, stuffed granular stipe and subhypothallic type of sporophore development justify the placement of *C. debaryanum* in the Echinosteliales (Alexopoulos and Brooks 1971), the presence of tubular threads in the upper region of the stipe and the capillitium relates it to the Stemonitales. Unfortunately at that time sporophore development in any member of the Echinosteliales was not known. Lakhanpal and Sood (1981a) studied the sporophore development in *E. cribrariodes* and established it to be of subhypothallic type. They pointed out the stipe in *E. cribrariodes*, thereby justifying the transfer of the latter to Echinosteliales.

Indira (1971) described the details of sporangial development in *S. herbatica* and pointed out that these are basically similar to those described for other Myxomycetes. She found the methods of capillitial development different from that described by Ross (1957) and similar to other Myxomycetes. However, Lakhanpal and Mukerji (1976a) in *Macbrideola cornea* observed the sporophore development to be epihypothallic type and the capillitium differentiation of Comatricha type (Ross 1957). Likewise, Lakhanpal (1981) recorded observations on the sexuality of *P. nicaraguense* and its life cycle. He made single-spore isolation following Collins (1961) and found the species to be homothallic and sporophore development of subhypothallic type. It is worthwhile to point out here that

diplohaplontic life cycle of Myxomycetes, in which both generations can be manipulated and maintained genetically, is a great research asset. Unfortunately, there have been no recent developmental and cultural studies on Myxomycetes in India.

It appears from the above account that the ecological studies are so fragmentary that any generalisations made will be premature. Presently there is a need for screening as many trees as possible in different regions to find out their specificity.

21.7 Ecological Aspects

21.7.1 General Observations

Myxomycetes are almost cosmopolitan being distributed in almost all regions of the globe. They usually occur in terrestrial habit and predominantly occur on decaying wood, leaf litter, dung, soil and bark. Unfortunately only a few biologists have studied Myxomycetes ecology. Sharma and Mukerji (1973) laid guidelines for the isolation of Myxomycetes from soil.

The ecological data on slime moulds has been deduced only from brief notes appended with each species such as habit and habitat, locality and altitude, and these are not very conclusive ecologically. Hence the studies on ecology of Myxomycetes have been fragmentary. Specific and detailed ecological studies have been carried out by Stephenson (1988), Harkonen (1981), Stephenson et al. (1994) and Lakhanpal and Chopra (1994). Their appearance is governed by moisture and temperature regimens. They flourish well during the rainy season and are primarily seasonal in appearance. Most species appear to be independent of substratum but some species tend to be rather consistently associated with certain type of substrates. Indira (1968) observed that calcareous species were more often found on dead leaves, twigs and debris. Lakhanpal and Mukerji (1981) pointed out that *Lycogala epidendrum* usually colonises exposed surface of freshly cut stumps during July–August. *Tubifera ferruginosa* also appears in the beginning of the rainy season. *Metatrichia vesparium* appears in the beginning of the rainy season and lasts till the end of the season. *Stemonitis axifera* forms beautifully combed colonies. *Diderma* appears when the rainy season recedes. Martin et al. (1981) generalised the observation and pointed out that some *Badhamia* most often fructify on the bark of deciduous trees, and some on coniferous wood (*Cribraria*), dead leaves (*Didymia*) and dead wood (*Trichiales*). Some species have been reported to be exclusively or predominantly fimicolous (Eliasson and Lundqvist 1979). They pointed out that none of these correlations are absolute, but they occur too often to be entirely coincidental. The reasons for substrate specificity in Myxomycetes are not known (Eliasson 1981; Blackwell 1984) but may very well involve the interaction or a number of physical and biotic factors.

Stephenson et al. (1994) reviewed the data on insect association of Myxomycetes and observed beetles to be the most common associates. His observation that presence of beetle is often discovered in a particular fruiting only after the latter

has been collected and brought back to home or laboratory and that beetles can decimate a large fruiting body within a matter of few days is fully corroborated by our experience. Blackwell et al. (1982) and Blackwell (1984) observed that because bodies of beetles are dusted with Myxomycetes spores it indicates that they play some role in the dispersal of spores. Similarly various species of flies belonging to family Mycetophilidae are also commonly associated with Myxomycetes.

A good number of hyphomycetous fungi grow on Myxomycetes fruiting bodies. They have been reported to be restricted to Myxomycetes and are thus obligatory myxomycetous. Common species of *Metatrichia vesparium*, *Comatricha, Stemonitis*, *Hemitrichia* and *Trichia* turn mouldy (Ing 1965, 1974, 1976; Ellis and Ellis 1988; Samuels 1973, 1988; Rogerson and Stephenson 1993).

Bryophytes offer a good substrate for Myxomycetes bryophyte association and harbour common species of *Barbeyella minutissima* and *Lepidoderma tigrinum* (Stephenson and Studlar 1985). Many species of *Diderma* have been observed growing with bryophytes (personal observation).

21.8 Corticolous Myxomycetes

Bark of living trees is a favourable substrate for a distinct Myxomycetes flora. The Myxomycetes that grow and fruit on the bark surface of trees are called corticolous Myxomycetes. This was first discovered by Gilbert and Martin (1933). Most of the studies on Myxomycetes have been on floristics except for Harkonen's work, which included data on various ecological parameters. Similar studies have been conducted by Chopra (1984) and Lakhanpal and Chopra (1994, 1997) on corticolous Myxomycetes.

In these studies, initially, the bark of 17 dominant tree species of HP, both angiospermic and gymnospermic, was used for moist chamber culture. Finally, the choice was placed on the bark of three dominant living trees of Shimla, viz., *Pinus roxburghii* A.B. Jackson, *Cedrus deodara* (Roxb. ex D. Don) G. Don and *Quercus oblongata* D. Don, that were sampled for 12 months during 1980–1981 at monthly intervals and data based on 972 moist chambers was recorded on the following ecological parameters: (1) tree species and their barks as habitat for myxomycetes, (2) estimation of Myxomycetes genera and their incubation period, (3) pH requirements for incubation and fruiting and (4) monthly production and productivity potential of different tree heights. The three selected trees were randomly and arbitrarily divided into three heights, i.e. 0.3 m, 3–6 m and 6–9 m. The bark from these heights was randomly sampled at monthly intervals for 1 year. During these studies 83 taxa belonging to 21 genera and 10 families were isolated; this number of corticolous genera is much larger than any other recorded earlier. Out of these 31 species have been obtained repeatedly, whereas the remaining ones less frequently. Most of these species appeared after 5–10-day incubation, whereas others took 15–26 days.

The bark of *Cedrus deodara* was found to be more favourable as a substrate for the production of Myxomycetes than *Pinus wallichiana* and *Quercus oblongata*. It

yielded 70.07% fertile moist chambers, whereas *P. wallichiana* and *Q. oblongata* yielded 63.58% and 68.51%, respectively. As far as the production of different species of Myxomycetes is concerned, *P. wallichiana* yielded 20 different species and 1 variety, *Q. oblongata*, 19 species and *C. deodara* 13 species. Species of *Echinostelium, Arcyria, Comatricha debaryanum* and *Calomyxa metallica* preferred the bark of *P. wallichiana* and *C. deodara*. Some species exhibited no preferences for any particular bark and were equally abundant on the three barks studied. These species are *Licea parasitica, L. operculata, Arcyria cinerea, Trichia crateriformis, Badhamia nitens* and *Physarum nutans*. Most of the species isolated have been found to favour a pH range between 6 and 7.

The data on the production was analysed statistically following the analysis of variance. The F values for heights at df 2 came out to be 14.92, 1.89 and 0.09, respectively, in *Pinus wallichiana, Cedrus deodara* and *Quercus oblongata*. Among these F values, the F in case of *P. wallichiana* only was significant at 0.01 level, meaning thereby that the total production of Myxomycetes differed significantly among themselves at different heights; CD value was calculated which came out to be 0.68; this shows that the average production of Myxomycetes is maximum at lower heights (i.e. 6–9 m and 3–6 m). Also, the average production at medium heights is minimum and insignificantly lower than the average production at higher heights. Since F value for heights was not significant even at 0.05 levels in case of *C. deodara* and *Q. oblongata*, the heights seem to have no effect on the production of Myxomycetes in these two species.

Similarly, the F values at df 11 for monthly production came out to be 53.44, 3.93 and 5.58, respectively, in *P. wallichiana, C. deodara* and *Q. oblongata*. All these F values are significant at 0.05 levels, meaning thereby that the production of Myxomycetes in different months differed significantly from one another in all the three tree species. To test the significance of mean differences, the CD values were calculated which came out to be 1.38, 3.16 and 3.44, respectively, for *P. wallichiana, C. deodara* and *Q. oblongata*. This shows that the production of Myxomycetes was observed to be maximum in January 1981, for *C. deodara* in November 1980 and for *Q. oblongata* in July 1981, whereas it was found to be minimum in all the three tree species in the months of April and May 1981. But this minimum value was found to be significant compared to the remaining months of minimum productivity. These studies clearly show that some species are specific to certain bark types whereas others are not. The incubation period is also variable and the pH ranges between 6 and 7. The species obtained herein have surpassed all previous data and a large number of species have been observed to be corticolous.

In all 82 species and 3 varieties were isolated which belong to 22 genera with the number of species in parenthesis: *Ceratiomyxa* (1), *Licea* (23), *Cribraria* (3), *Echinostelium* (7), *Clastoderma* (1), *Calomyxa* (1), *Perichaena* (3), *Calonema** (1+1 var.), *Arcyria* (3), *Arcyodes** (1), *Hemitrichia* (2), *Trichia* (3), *Badhamiopsis** (1), *Badhamia* (4), *Physarum* (10), *Diderma* (3), *Didymium* (1), *Enerthenema** (1), *Stemonitis*(6+1 var.), *Macbrideola* (1), *Comatricha* (5) and *Paradiacheopsis* (2). The data presented reveals that bark is a rich habitat, especially the fissured bark for species which otherwise appear to be rare or are so minute that they are missed in

natural collections. As many as five genera (*) are reported for the first time from India. There are 27 species which are new records for India and as many as 20 species and 3 varieties are new to science. These are: *Liceamarginata, L. lilacina, L. morchelloides, L. stephensonii, L. kellerii*, *Cribrearianannengae*, *Calonemadissipatum*, *Hemitrichiathindii*, *Badhamiaevada*, *Physarummehranum*, *P. mohanramii*, *P. natarajanium*, *Diderma intermedia, D. platycarpum var. berkeleyanum, D. lakhanpalii*, *Stemonitisenerthenemoides*, *Comatrichaanastomosa, C. confusa*, *Paradiacheopsisbremekampii*, *Liceascyphoidesvar. reticulata var. nov*., *Calonemadissipatum var. ellae var. nov*. and *Stemonitisuvifera var. microspore var. nov*. It is clear, therefore, that the bark of living trees is a distinctive environment with somewhat distinctive Myxomycetes flora. It appears from the above account that the ecological studies are so fragmentary that any generalisations made will be premature. At present, there is a need for screening as many trees as possible in different regions to find out their specificity.

21.9 Future Perspectives

Myxomycetes or the true slime moulds have been substantially worked out in India as well as far as taxonomic treatment is concerned. But other aspects of Myxomycetes have not yet received due attention. Their life cycle presents excellent experimental opportunities. Recent study shows that they are a good source of novel compounds which exhibit biological activity that functions as antibiotics and antimicrobials and are cytotoxic to cancer cells which are yet to be tested clinically. Remarks of Keller and Everhart (2010) highlight their importance which needs to be recognised and considered for future resources: 'The plasmodial stage of *Physarum polycephalum* has been used as a model research system to study responses to gravity in outer space, solve the shortest pathway through a maze exhibiting "primitive intelligence" develop a biologically controlled robot, discover what controls synchronous nuclear division, and the development of a new drug Polycefin that shows promise in the treatment of breast and brain cancerous tumors'. Therefore Myxomycetes need to be studied more intensely by morphologists, physiologists, biochemists and molecular biologists to utilise their full potential.

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