Fungal Biology

Vijay Rani Rajpal
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Fungal diversity, ecology and control management

Springer

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Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. 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 applications. 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. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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ISSN 2198-7777 ISSN 2198-7785 (electronic)
Fungal Biology
ISBN 978-981-16-8876-8 ISBN 978-981-16-8877-5 (eBook)
https://doi.org/10.1007/978-981-16-8877-5

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Food security is one of the major concerns in the world today. The continuously increasing world population coupled with global climate change poses a grave challenge to feed a billion mouths. Fungi play a significant role in modulating plant's environment, in both rhizosphere and phyllosphere resulting in growth changes that affect agricultural productivity significantly. Fungi have serious consequences on food security in the changing global environmental scenario.

Fungi play key roles in the ecosystem as decomposers, mutualists and pathogens and represent the second most diverse group after insects. Fungal diversity is not only reflected in its morphological characters but also in bioactive molecules produced, their pathogenicity and virulence, and impact on crop production. The increasing number of infectious fungal diseases is regarded as a global threat to agricultural productivity and food security. Therefore, it is important to document the fungal diversity and inventorize it. Further, to sustain agricultural productivity, it is important to mitigate or control the plant diseases. Plant pathogens cause severe losses to crops and significantly reduce the quality and quantity of agricultural products. The global tendencies are shifting towards a preferred use of various biocontrol agents in plant disease management. Fungal antagonists are widely used as biocontrol agents to control plant diseases globally. Biological control mechanism, however, needs an understanding of the complex interactions among plants, pathogens, and the environment.

This book provides a consolidated and comprehensive account of research being conducted by scientists all over the world in the areas of fungal biodiversity, fungal ecological services, fungal biology and ecology, and biological disease control and provides perspectives on crop protection and management and control of various fungal pathogens. The book also serves as an invaluable resource for researchers and educators working in the above fields. It will be useful to students studying mycology, plant pathology, crop protection, agricultural sciences, and plant sciences. Students will find this book handy to clear their concepts and to get an update on the recent research conducted in this area. Also, scientists involved in biological and

agricultural research, crop management and environmental sciences and industries that manufacture agrochemicals as well as small- to large-scale growers and producers will find the book useful.

Delhi, India — Vijay Rani Rajpal
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Acknowledgments

The editors sincerely thank all the authors for agreeing to contribute chapters despite their hectic schedules and other work commitments and also for putting in their sincere efforts for providing up-to-date information. We also thank the authors for providing their revisions on time to avoid any delays in publication of this book. We are thankful to Prof. K.G. Ramawat for inspiring us to take up this assignment. Vijay Rani Rajpal is thankful to the two other co-editors for their active involvement from inception to reviewing, editing, and compilation process through the course of this book. The book would not have been possible without their involvement and whole-hearted support.

The editors gratefully acknowledge their families for their understanding, patience, and emotional support during the course of this book. Our sincere thanks are due to the whole Springer team involved in the production of this book. We especially appreciate Ms. Aakanksha and Ms. Priya for their continued support.

We are sure that this book will attract scientists, undergraduates, graduates, and postdocs who are working on fungal diversity, ecology, and biocontrol mechanisms.

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About the Editors

Vijay Rani Rajpal M.Sc., M.Phil (Botany), Ph.D., is a Professor in Botany Department, Hansraj College, Delhi University, Delhi, India, and has ~25 years of research experience working on population biology, phylogenetics and diversity of plants. She has 45 published research papers, review articles and book chapters and has edited four books on molecular diversity, abiotic stress mitigation and improvement of plants published from Springer Switzerland. She has guided Ph.D. students and successfully completed a number of research projects funded by various agencies of the Government of India. She is a reviewer of several reputed national and international research journals and invited guest editor for a thematic issue of *Frontiers in Genetics*.

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Part I

Biology and Diversity of Fungi

Biology and Management of Spot Blotch Pathogen *Bipolaris sorokiniana* of Wheat

1

Rashmi Aggarwal, Shweta Agrawal, Malkhan Singh Gurjar, Bishnu Maya Bashyal, and M. S. Saharan

Abstract

Bipolaris sorokiniana (Teleomorph: *Cochliobolus sativus*), causal agent of spot blotch of wheat, emerges as a serious concern for yield losses to wheat crop in warm and humid regions of the world. Due to global warming and late sowing of wheat crop, spot blotch becomes a major concern worldwide. Spot blotch mainly occurs in North eastern plain zone in India as well as in other South Asian countries. To meet escalating demand of wheat crop in near future due to increasing global population growth rate and nutritive changes, management of this disease is necessary. This review summarizes the biology of the pathogen (*Bipolaris sorokiniana*) and an overview of distribution, impact, and management of the disease. In addition, it also provides insights into wheat—*B. sorokiniana* pathosystem at histological and molecular level. There are several approaches for the management of spot blotch disease, by way of identifying QTLs (quantitative trait loci) for spot blotch resistance, exercising marker-assisted selection and integrated approaches such as agronomic practices, proper crop rotation, biological control, and seed treatment with fungicides.

Keywords

Bipolaris sorokiniana · Spot blotch · Biology · Host–pathogen interaction · Management · Wheat

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_1

1.1 Introduction

Wheat (*Triticum aestivum* L.) is one of the main cereal crops all around the world. It is grown in an area of about 220 million hectares with 763.06 million tons of production globally. Utmost area covered under wheat cultivation is in India (14%) shadowed by Russia (12.43%). India is the second major producer of wheat (98.5 mt) with the state of Uttar Pradesh being the largest producer (28 million tons) and holding largest share area wise (9.75 million hectare) in the country (Sendhil and Singh 2019). Improving yield and quality of wheat crop is necessary to cope up with the massive challenge of meeting future wheat demands with the same cultivable land and water available as on today.

The limitations in wheat production system include various pathological wheat diseases, among which fungal diseases like spot blotch has arisen as the most devastating disease in warm and humid environments of South Asian countries. Spot blotch/Leaf blight/foliar blight is caused by *Bipolaris sorokiniana* (Teleomorph: *Cochliobolus sativus*). Yield losses through *B. sorokiniana* have been reported to be highly variable from 2.7 to 100% depending on varieties across different countries (Mehta 1993 Duveiller and Gilchrist 1994; Villareal et al. 1995). The average yield loss has been estimated to be 15.5% in India due to spot blotch, which can be worst in severe conditions of infections (Joshi and Chand 2002). In addition to spot blotch, *B. sorokiniana* also causes common root rot in wheat in southern Brazil and Australia (Diehl et al. 1982; Tinline et al. 1988). Spot blotch disease is favored by many conditions like continuously increasing global temperature, rice–wheat cropping system (wheat sowing is delayed), and stress conditions (fertility is reduced) (Duveiller et al. 1998). At present *B. sorokiniana* is defined as the major pathogen in India and its frequency is highest in north eastern plains zone due to warm and humid weather conditions. *B. sorokiniana* infection increases with temperature, bright sunshine hours and in the morning. However, it is also observed that relative humidity (both maximum and minimum) and pathogen infection progression shows negative correlation (Devi et al. 2018).

In wheat cultivars, resistance to spot blotch is generally unsatisfactory due to environmental alteration, application of extreme or little fertilization, development of new races, and lack of effective durable resistance, therefore identification and characterization of new sources of resistance are helpful to overcome this disease (Mahapatra et al. 2020). Studies have been conducted on the biology and management of spot blotch fungal pathogen, over the last few decades which include: its life cycle, reproduction, survival, source of inoculum, symptoms developed on host plant, host range, diagnosis through various methods and management *via* integrated approaches and genetic resistance. A comprehensive information gathered from all these studies in the present genomics era is explicitly presented in this chapter.

1.2 Worldwide Distribution of the Pathogen

Spot blotch is common in the Mega Environment 5 (ME5) where humid and warm weather prevails during the growth of wheat crop. Surveys indicated that spot blotch has become a serious disease of wheat in several parts of the world, particularly in those areas characterized by moderate temperature and high humidity during the late growth stage such as eastern India, Bangladesh, Tarai of Nepal, and Brazil. Therefore, causal organism *B. sorokiniana* is considered as most destructive fungal pathogen in the warmer and humid areas mainly for wheat crop. Wheat grains are rich in carbohydrates, energy, dietary fiber, fat, riboflavin, protein, thiamine, niacin, vitamin B6, folate, pantothenic acid, calcium, magnesium, iron, phosphorus, zinc, potassium, and manganese (Gebhardt et al. 2006). Due to its high nutritive value, wheat grains are consumed in different forms across the world. *B. sorokiniana* also affects wheat in other warmer parts of the world like Asia, Latin America, Africa, Southern Asia, etc. The disease has been under investigation since it was first recorded in 1914 by Mohy in India (Joshi et al. 1986), but recently it has been recognized as a major concern. Severity of spot blotch has increased many fold after green revolution in India and in many countries of tropical and sub-tropical climate where rice–wheat cropping system is commonly practiced. Worldwide distribution of *B. sorokiniana* according to https://www.cabi.org/isc/datasheet/14694 is elaborated in Table 1.1.

Table 1.1 Worldwide distribution of *B. sorokiniana*

Continent	Country
Africa	Nigeria, Angola, Algeria, Cameroon, Ghana, Egypt, Kenya, Uganda, Ethiopia, Morocco, Libya, Malawi, Tunisia, Mauritius, Zambia, South Africa, Zimbabwe, Sudan, Tanzania
Asia	Thailand, Saudi Arabia, Azerbaijan, Afghanistan, Japan, Israel, Bangladesh, China, Bhutan, India, Kazakhstan, Indonesia, Iran, Myanmar, Iraq, Kyrgyzstan, Taiwan, Laos, Lebanon, Malaysia, Nepal, Oman, Uzbekistan, North Korea, Pakistan, Philippines, South Korea, Turkey, Sri Lanka, Syria
Oceania	Solomon Islands, American Samoa, Australia, Kiribati, Tonga, New Zealand, Papua New Guinea, New Caledonia
Europe	Belarus, Czechoslovakia, Belgium, Poland, Bulgaria, Croatia, Greece, Cyprus, Czechia, Federal Republic of Yugoslavia, Union of Soviet Socialist Republics, Serbia, Denmark, Estonia, Finland, France, Austria, Germany, United Kingdom, Italy, Norway, Hungary, Ireland, Lithuania, Moldova, Netherlands, Ukraine, Romania, Montenegro, Slovakia, Latvia, Spain, Russia, Sweden, Switzerland
North America	United States, Nicaragua, Canada, Guatemala, Costa Rica, El Salvador, Jamaica, Cuba, Mexico
South America	Venezuela, Argentina, Brazil, Colombia, Paraguay, Peru, Bolivia, Uruguay

1.3 Naming and Taxonomy of Spot Blotch Pathogen

B. sorokiniana (Teleomorph: *Cochliobolus sativus*) causing spot blotch disease was initially named as *Helmithosporium sorokinianum* Sacc. by Trans. Soc. Nat. Univ. Kazan 22:15 in Sorokin in 1890. In 1934, conidia at ascigereous stage (teleomorph) were first observed in the lab by Ito and Kurib and they named it as *Opliioholus sativus*, which was later renamed as *Cochliobolus sativus* by Drechsler ex Dastur in 1942.

In 1959, Shoemaker suggested the generic name *Bipolaris* by observing fusoid, straight, or curved conidia in helminthosporium species having one germ tube germinated from each end (bipolar germination). He renamed the spot blotch pathogen as *Bipolaris sorokiniana* (sacc.) shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain. The genus *Bipolaris* belongs to Ascomycota, Pleosporales, Dothideomycetes, and Pleosporaceae. This pathogen has many names like *Helminthosporium sorokinianum* Sacc., *Helminthosporium acrothecioides* Lindfons, *Helminthosporium sativum* Pammel, *Helminthosporium californicum* Mackie and Paxton, C.M. King and Bakke, *Drechslera sorokiniana* (Sacc.) Subram. & B.L. Jain but valid name currently is *B. sorokiniana*. On the basis of character of conidial germination, it is named as *Cochliobolus sativus* at teleomorph stage and *B. sorokiniana* at anamorphic stage.

1.4 Reproduction of the Pathogen

B. sorokiniana causing spot blotch disease is the asexual state of the pathogen. *Cochliobolus sativus* (teleomorph) is its sexual stage, which is hardly perceived in nature but forms in pure culture with compatible mating types. Consequently, this pathogen reproduces mainly through asexual state through conidia. In *C. sativus* and some species of *Helminthosporium* sexuality and a parasexual cycle has been reported (Nelson 1960; Tinline 1962; Chand et al. 2003). One such study in eight different species of *Helminthosporium* confirms viable ascospores progenies from nine of the 13 interspecific crosses. In four crosses, perithecia were also observed signifying sexuality presence in some forms, and also presence of some pathogenicity genes, which segregate in these crosses. However, within or between *B. sorokiniana* isolates derived from wheat, sexuality has not been reported. *B. sorokiniana* is heterothallic and exhibits variability (Tinline 1951). Sexual reproduction of this pathogen is still baffling (Rapper 1966). Although, in Zambia, sexual reproduction of *B. sorokiniana* has been observed in the field conditions (Raemaekers 1987). Sexual reproduction leads to genotypic diversity which eventually helps the progeny to better fit into the changing environment (Colegrave 2002; Goddard et al. 2005; Heitman 2006, 2010; De Visser and Elena 2007). Historically, *B. sorokiniana* is a variable fungus (Christensen 1925; Tinline 1962) due to sexual reproduction, heterokaryosis and parasexual recombination (Tinline and Dixon 1958; Burdon and Silk 1997). Inheritance of the variability has been studied using two methodologies: crosses among two different genotypes trailed by an analysis of

segregation pattern (Mendelian approach), and another using molecular markers which are involved in a quantitative genetics approach (Gupta et al. 2017).

1.5 Pathogen Survival and Source of Inoculum

As the pathogen is seed borne, its survival on the host or in the soil as source of inoculum depends on several factors. In nature, the sources of inoculum of this pathogen are collateral hosts, infected seeds, free dormant conidia in the soil, and crop residues (Reis 1991). The presence of *B. sorokiniana* in seeds of wheat correlated with higher disease (Mehta 1981). The pathogen has been generally introduced through infected seeds into new wheat growing areas. In eastern India, seeds are considered as the most important source of inoculum for the reoccurrence of spot blotch in rice–wheat cropping system (Pandey et al. 2005). Sporulation of *B. sorokiniana* occurs on necrotic tissues of leaves and it reaches to the spike and finally moves to seeds. This sporulation continues on the residue till it gets completely decomposed. Pathogen can also survive in soil as free dormant conidia. Ability of fungus to colonize in diseased wheat straw results in pathogen survival in the soil, and the inoculum density of the pathogen in soil is associated to the quantity of sporulation occurring in crop residues (Burgers and Griffin 1968; Reis and Wunsche 1984). Melanin content in pathogen has a direct association with conidiogenesis, signifying that melanin formed by the pathogen neutralizes antimicrobial activity of the host cells, accordingly contributing to the pathogen survival (Henson et al. 1999; Aggarwal et al. 2011a). Large number of plant species act as collateral hosts for the pathogen such as weeds that are ubiquitous in different cropping systems and also present on adjacent uncultivated land (Neupane et al. 2007). *B. sorokiniana* infects a wide host range differing from isolate to isolate. *B. sorokiniana* mainly infects Poaceae members including *Triticum aestivum*, *Bromus erectus*, *Secale cereale*, *Hordeum vulgare*, *Alopecurus pratensis*, *Hordeum murinum*, *Avena sativa*, *Agropyron pectinatum*, *Agropyron repens*, *Beckmannia eruciformis*, *Poa pratensis*, *Pennisetum villosum*, *Bromus inermis*, *Festuca heterophylla*, *Festuca ovina*, *Lolium perenne*, *Setaria virdis*, and *Dactylis glomerata*. However, it is also reported in some dicot crops including Beans, Alfalfa, Red and Yellow clover, and Buck wheat. Chinese isolates have been shown to infect 29 graminaceous hosts. *Zizania caduciflora*, *Saccharum officinarum*, *Paspalum thymbergii*, *Apluda mutica*, *and Ischaemum ciliare* have been reported as new hosts for the pathogen (Naitao and Shenyang 1987). Therefore, the knowledge of inoculum survival of a pathogen in off-season through diverse sources is very vital to develop suitable disease management approach.

1.6 The Pathogen: Primary and Secondary Disease Cycle and its Morphology

B. sorokiniana (Sacc.) can survive in dormant phase on stubbles, seeds, and roots; but once it becomes active, primary infection initiates. Pathogen can survive on stubbles or soil for many months and infects the healthy wheat plants through conidial spore germination followed by germ tube formation (Aggarwal et al. 2008; Acharya et al. 2011), which ultimately produces an appressorium within 8 h, which later on develops hyphae. Within 12 h of hyphae formation, it penetrates into the cuticle of hosts (Sahu et al. 2016). In host, the hyphae multiply rapidly and spread into intercellular space infecting the mesophyll tissue of the wheat leaf. After 48 h of multiplication in the intercellular space, conidiophores are formed which are 100–150 × 6–8 μm long and integrate a new generation of conidia in it. The conidia (60–120 × 15–20 μm in size) are olive brown in color, thick walled, and tapered toward the end (bipolar) with 5–9 septa; which is inclined for secondary infection (Fig. 1.1). Conidia mainly disperse through rain or dew therefore secondary infection of spot blotch is mainly caused by airborne conidia (Duveiller et al. 2005).

B. sorokiniana is demarcated from other *Bipolaris* species, in terms of morphological features of conidiophores and conidia. In axenic culture, growth on PDA (potato dextrose agar) plates or test tubes shows maximum radial growth with loose cottony mass at initial phase of hyphal development, which later on turns to fluffy blackish colonies due to sporulation. The colonies can be white or light to dark gray depending on different isolates (Kumar et al. 2002; Aggarwal et al. 2009). Appressoria development at the initial stages can be clearly seen under light

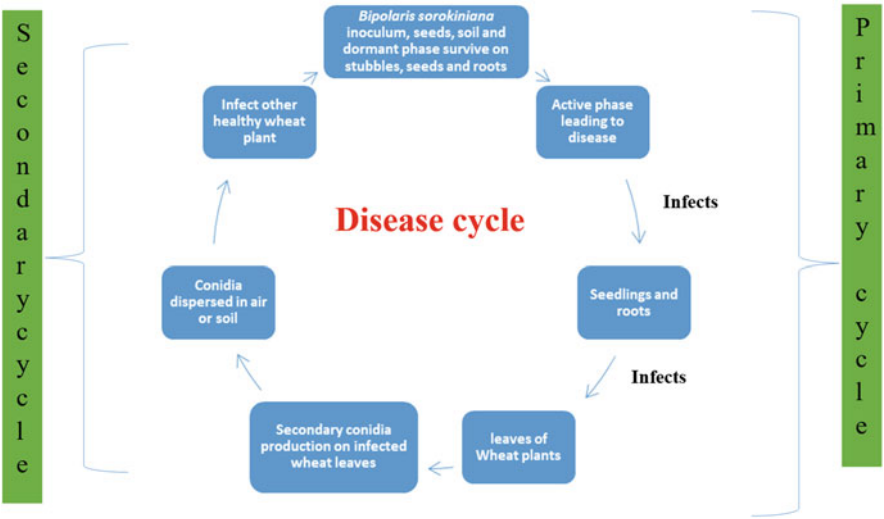

Fig. 1.1 Primary and secondary disease cycle of *Bipolaris sorokiniana*

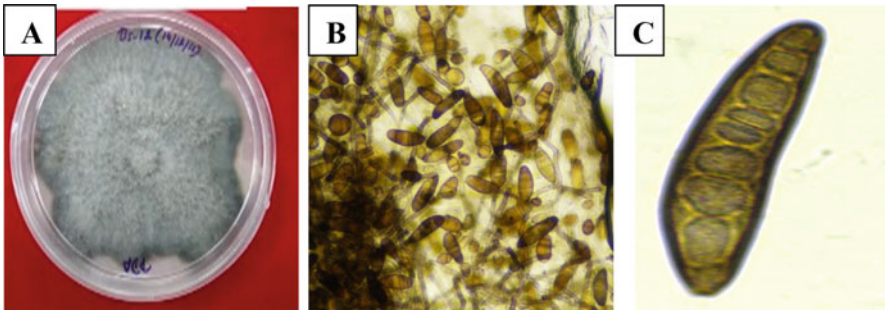

Fig. 1.2 Morphology of *Bipolaris sorokiniana*. (**a**) Fungal growth on PDA. (**b**) Conidia showing polar germination (10×). (**c**) Conidium showing 5–8 thick walled septa (40×)

microscope. Conidia appear black and shiny with 5–8 thick walled septa (Fig. 1.2) under light microscope with bipolar germination (Aggarwal et al. 2002).

1.7 Symptomatology

Symptoms of spot botch of wheat typically appears on leaf, sheath, nodes, and glumes; but in more severe conditions it affects spike and develops dark brown to black discoloration which is called as Black point. Lesion are developed on the leaf, coleoptiles, crowns, storms, and roots starting from few mm and extend up to 1–2 cm which appear as a dark brown spot (Chand et al. 2002). These spots progress and join with other spots forming large blotches that damage the entire part of the plant eventually killing it. Under humid conditions, conidia before coalescing develop on leaves and induce leaf tissue death. Due to copious production of these conidia under humid conditions, chlorotic streaks sometimes diffuse from the border of the lesions, which result in toxin production (Mercado vergnes et al. 2006; Bockus et al. 2010). Heavily infected plants may cause stunting and reduced tillering which ultimately lead to premature death.

1.8 Fungal Isolation and Its Diagnosis

Symptoms developed on various parts of the host plant are used for collection, isolation, diagnosis and to check the severity of the disease. For the isolation of this pathogen, leaves that are infected are surface sterilized and necrotic lesions are cut into smaller fragments. These fragments are washed with sodium hypochlorite ($NaOCl_2$) solution, followed by two times washing with water. To induce sporulation, these fragments are placed on PDA (potato dextrose agar) under a 12-h photoperiod at room temperature. Several single spores from each of the PDA plates are transferred to another PDA plate and are allowed to grow at 25 °C. Meanwhile, these spores are observed under microscope and fungus is identified based on

conidia and mycelial morphology. Conidiophores observed are unbranched, septate with conidia being brown to olivaceous brown in color.

Microscopic identification of the pathogen further needs verification, for which many molecular markers have been developed. RAPD (Random Amplified Polymorphism DNA) markers are very versatile, promising and informative tool to detect pathogen isolates, which are organized into different groups on the basis of their color and shape of colonies (Pandey et al. 2008). A SCAR marker (sequence characterized amplified region) based on diagnostic PCR assay was developed to detect this pathogen in wheat leaves and field soil, which detected the pathogen at very early stage even before the visual symptoms appear (Aggarwal et al. 2011b). More recently, the RPA (recombinase polymorphism amplification) assay was developed which is more rapid and effective method for detection of *B. sorokiniana*. This RPA depends on the sequences of calmodulin gene sequences (Zhao et al. 2021).

1.9 Disease Assessment

Aggressiveness or disease severity of the pathogen can be tested through a continuous scale using two available methods. Firstly, a single digit scoring method called as ADI (average disease index) calculated using 0–5 scale. In this disease severity (%) is calculated as per the scale; 0 = free of spots; 1 = up to 5% area of leaf enclosed with necrotic spots; 2 = 6–20% of the leaf area covered; 3 = 21–40% of the leaf area covered; 4 = 41–60% of the leaf area covered; 5 = spots inclusion more than 60% of the leaf area tangled.

$$\text{ADI} = ((\text{sum of rating of each leave})/(\text{total leaf} * 5)/100)$$

ADI is converted into disease responses *viz.* 0 = No infection; 0–10 = resistant response (R); 11–20 = moderately resistance (MR); 21–30 = moderately susceptible (MS); 31–50 = susceptible (S); and more than 50 = highly susceptible (HS) (Adlakha et al. 1984).

Secondly, a double digit scale (00–99), where the first digit (D1) signposts disease progress in the canopy height from ground level; the second digit (D2) denotes severity based on diseased leaf area. Both D1 and D2 are scored on a scale of 1–9. For each score, the percentage of disease severity is estimated based on the following formula:

$$\text{Severity } (\%) = (\text{D1}/9) \times (\text{D2}/9) \times 100$$

This disease evolves very rapidly around the affected portion, so it is necessary to record disease scores per plot at 3–7 days intervals over 3- to 4-week period between anthesis and the dough stage (Duveiller and Sharma 2009). The area under the disease progress curve (AUDPC) can be calculated using the formula for percentage severity:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_i + X_i + 1)/2](t_i + 1 - t_i)$$

where X_i severity on the ith date, t_i ith day, and n number of dates on which the disease recorded.

AUDPC calculation is categorized into disease responses as: (1) immune (00), (2) *R* resistant (<12), (3) MR moderately resistant (12–34), (4) MS moderately susceptible (56–68), (5) S susceptible (78–89), and (6) HS highly susceptible (89–99).

Recently, imaging technique has also been introduced to sense electromagnetic spectrum external to visible light, which allows enumerating disease symptoms that are not visible by eye (Mukta et al. 2015). More recently, a reliable and accurate disease detection is facilitated, i.e., sensor-based analysis such as RGB imaging, multi- and hyper-spectral sensors, chlorophyll florescence or thermography (Mahlein 2016).

1.10 Toxin Production and Host–Pathogen Interaction

Etiology of spot blotch disease majorly depends on toxins. Toxins are the secondary metabolites produced by a pathogen that penetrate the host and cause disease. Role of toxins in pathogenesis is reported in differentiating resistance and susceptible genotypes of wheat (Aggarwal et al. 2008). Phytopathogenic fungus *B. sorokiniana* produces a series of non-host selective toxins which are sesquiterpenoid toxins that are synthesized from farnesol and belong to eremophilane family like prehelminthosporol, helminthosporic acid, helminthosporol, sorokinianin, victoxinine, and bipolaroxin (Nakajima et al. 1994; Olbe et al. 1995; Apoga et al. 2002; Kumar et al. 2002; Jahani et al. 2006). The most abundant and active phytotoxin formed by the pathogen is Prehelminthosporol ($C_{15}H_{22}O_2$), a hydrophobic sesquiterpene with stumpy water solubility and little thermal stability (Carlson et al. 1991). It interrupts the integrity of cell organelles, plasma membrane and it stimulates enzyme callose synthase in host plants (Olbe et al. 1995; Apoga et al. 2002). Helminthosporol is synthesized from Prehelminthosporol, which on further transformation gives helminthosporic acid. Both helminthosporol and helminthosporic acid are plant growth regulators which inhibit seed germination and shoot growth (Qader et al. 2017). Sorokinianin, a novel phytotoxin is derived biogenetically from a farnesyl pyrophosphate having an inhibitory effect on germination of seeds (Nakajima et al. 1994). Victoxinine ($C_{17}H_{29}N_O$) is a toxic metabolite, a tricyclic base (sometimes called victorin) inhibits root growth (Pringle 1976). A bicyclical sesquiterpene belonging to family Eremophilane compound was identified and named as bipolaroxin. From culture filtrate of virulent isolate BS-75, bipolaroxin was purified using prep TLC, which is characterized further using NMR and GC-MS techniques. It produces necrotic lesions not only on wheat but also on barley, sorghum, maize, *Phalaris minor*, *Cynodon dactylon* and *Avena sativa* as studied using leaf infiltration bioassay (Jahani et al. 2006, 2014).

1.11 Population Differentiation and Molecular Characterization of *B. sorokiniana*

It is observed that like many other plant pathogens, specialization for virulence also arises in *B. sorokiniana* which is apparently more in barley as a host (Christensen 1922). Different isolates of *B. sorokiniana* do not show clear and unique differential virulence patterns on wheat genotypes like rust and it also has a range of aggressiveness in different strains with no specific host–pathogen interactions (Maraite et al. 1998; Duveiller and Garcia 2000; Aggarwal et al. 2019). In earlier study, it was noticed that only 1–2% of the variance is observed between 12 wheat differentials inoculated with 206 *B. sorokiniana* isolates (Hetzler et al. 1991). However, three, four or six pathotypes (0, 1, 2) or eight virulence groups (VIGs) were recognized in barley—*B. sorokiniana* pathosystem (Ghazvini and Tekauz 2007).

B. sorokiniana shows vast physiological and morphological variability with respect to multinucleate mycelium and conidia, with subsequent heterokaryosis (Mitra 1931; Day 1974). It was firstly reported that *B. sorokiniana* isolates varied significantly with respect to their virulence on wheat and barley (Christensen 1926). Many reports on barley—*B. sorokiniana* pathosystem are available, but scarce reports were available for wheat—*B. sorokiniana* pathosystem. *In vitro* morphological variability based on color and morphology of colonies observed on PDA (potato dextrose agar) plates has been reported in the population of wheat—*B. sorokiniana*, which ranges from black to white, and confirms more viability and aggressiveness in black colony (presence of melanin) as compared to white colony (Chand et al. 2002). Fifteen pathotypes of *B. Sorokiniana* monoconidial isolates were observed from diverse parts of the world (Hetzler et al. 1991). Earlier, pathological variability was testified, but it has not been correlated with the morphological or physiological variability. Morphological, molecular, and pathogenic variability has been studied in Brazilian (Oliveira et al. 1998) and Bangladesh isolates (Ahmed et al. 1997). A study was conducted to assess the cultural, pathogenic, and genetic variability in the Indian isolates of *B. sorokiniana* of wheat cultivars collected from different agro climatic zones, which confirmed the existence of five pathotypes in India (Aggarwal et al. 2009). The morphological variability correlated with pathological variability resulted in monitoring the populations of *B. sorokiniana*.

Heterokaryosis and parasexuality are the two major aspects that may be responsible for variability in the pathotypes of *B. sorokiniana*. Less than 10% of the total conidia form heterokaryotic conidium. Heterokaryosis (single hyphal cells comprising 3–6 nuclei) conditions may result from fusion between head-to-head hyphae belonging to the same or different individual mycelia through anastomosis (Glass et al. 2000). Heterokaryons can also be formed from nuclear migration amidst two genetically different hyphae (Tinline 1962). Heterokaryosis play important role in the variation of the pathogen. The isolation of recombinants constituted the genetic evidence of somatic heterozygous diploid and parasexuality in the fungus. Parasexual cycle is led by presence of heterokaryons recombination, during which nuclear and cytoplasmic material is swapped between two anastomosing hyphae (Burdon

and Silk 1997). In *B. sorokiniana*, the parasexual recombination is complex and very rare due to the presence of *Het* genes, which restrain the heterokaryon formation. Individuals differing at one or more of the loci of *Het* gene, the hyphae are aborted and it results in vegetative incompatibility (Glass et al. 2000).

Molecular characterization of the fungal isolates involves several approaches which includes: (1) molecular markers such as SCAR marker (sequence characterized amplified region) which is based on diagnostic PCR assay, RAPD (random amplified polymorphism DNA) markers, RPA (recombinase polymorphism amplification) assay, AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat), RFLP (restriction fragment length polymorphism), etc.; (2) karyotyping using squashing and microscopic examination (Hrushovetz 1956; Huang and Tinline 1974), GTBM (germ tube burst method), and CHEF (contour-clamped homogeneous electric field method) (Zhong and Steffenson 2001; Zhong et al. 2002); and (3) whole genome sequencing using different platforms like Illumina Hiseq, Oxford nanopore sequencing, and ion-torrent platform technologies.

Molecular markers are an excellent tool for genetic analysis of a pathogen genome. PCR-based markers have been the most frequently used molecular markers for molecular characterization and variability study of *B. sorokiniana* isolates. For the study of diversity and variability among *B. sorokiniana* isolates, a number of universal rice primer (URP)-PCR-based markers were studied (Aggarwal et al. 2010). According to this study, from 12 URP markers 10 markers generated polymorphic fingerprint patterns in DNA of *B. sorokiniana* isolates obtained from diverse geographic regions. Ribosomal DNA polymorphism can also be used for characterization of isolates which involves sequence variation in the ITS (Aggarwal et al. 2014). As described earlier, a SCAR marker was developed to detect *B. sorokiniana* at pre-symptomatic stage in wheat tissue and field soil (Aggarwal et al. 2011b). Marker designated as SCRABS600 could clearly differentiate *B. sorokiniana* from other fungal plant pathogens. RAPD (random amplified polymorphism DNA) markers were used to detect *B. sorokiniana* isolates (Pandey et al. 2008). Markers from another species (*Pyrenophora teres* f.sp. *maculate*) were also developed and used for exploring the variation in *B. sorokiniana* isolates (Lu et al. 2010). RPA (recombinase polymorphism amplification) assay has also been developed which is more rapid and effective method for detection of *B sorokiniana* (Zhao et al. 2021). Molecular maps of *B. sorokiniana* whole genome covering all the 15 chromosomes, based on molecular markers (e.g., RFLP, SSR, AFLP, etc.) have also been successfully established (Mann et al. 2014).

1.11.1 Chromosome and Karyotypes of *B. sorokiniana*

Earlier, on the basis of microscopic examination, the haploid chromosome number of *B. sorokiniana* was $n = 7$ or 8. But later on, by GTBM and CHEF analysis it was found to be $n = 15$. To identify chromosome length polymorphisms the barley *B. sorokiniana* collected from varied regions of the world (USA, Japan, Canada,

Brazil, Poland, and Uruguay), were analyzed for the karyotypes using CHEF electrophoresis of 16 isolates comprising the three known pathotypes of *B. sorokiniana*, i.e., 0, 1, and 2. CHEF bands ranging from 8 to 13 were observed with a size range of 0.85–3.80 mega-bases (Mb). A unique banding pattern was observed in each of the 16 isolates, except for two isolates, i.e., ND90Pr and ND91-Bowman (North Dakota isolates). This signifies that large-scale structural changes took place in *B. sorokiniana* isolates at the chromosome level. This study has not been subjected to wheat isolates. However, study on karyotypes from wheat isolates can be rewarding to recognize the causes of variation and also to what extent structural changes can be a part of variation. Karyotypes results concluded that in the virulent isolate ND90Pr, at least five of the fifteen chromosomes were involved in translocations. In *B. sorokiniana* chromosomes, structural rearrangements are very common between the corresponding chromosomes responsible for length polymorphisms of two isolates of opposite mating types with complementary virulence (ND93-1 and ND90Pr). Hybridization through southern blots carrying CHEF-separated chromosomes can be used for single-copy DNA probes, to recognize highly polymorphic chromosomes among isolates. Such a comparison allowed identification of unequal chromosomal rearrangements among dissimilar isolates. For hybridizations with the CHEF blots, DNA markers linked to *VHv1* were also used as probes which suggests that the chromosome carrying *VHv1* in a specific isolate was longer than the corresponding isolates chromosome that lacked this gene. This recommends that the genomic region carrying *VHv1* is exclusive (Gupta et al. 2018).

1.11.2 Whole Genome Sequence Analysis

Using whole genome sequencing (WGS), host–pathogen interactions at the molecular level in several pathosystems, synthesis of secondary metabolites through genes encoding enzymes and other proteins which are involved in virulence can be identified. For wheat, till now whole genome sequences of seven *B. sorokiniana* isolates are available. Using next generation sequencing technology, whole genome sequence of Indian virulent isolate BS 112 of *B. sorokiniana* was generated (Aggarwal et al. 2019). A total sequence assembly size of 35.64 Mb was predicted with GC content of 50.2%, providing coverage of 97.6% on reference ND90Pr genome. A total of 235 scaffolds were obtained using pyScaf assembler with N_{50} of 1,654,800 bp. In addition, 152 transcription factors involved in various biological processes were identified and a total of 682 secretory proteins were predicted using secretome analysis. Total of 10,460 genes were analyzed with an average gene density of 250–300 genes/Mb. The average gene length predicted was 435–545 bp, the maximum gene length was 8506 bp, and the minimum gene length was 50 bp. Gene ontology (GO) annotations resulted in 10,460 annotated genes, which was further characterized into 1024 genes for biological processes, 493 genes for cellular components, and 1274 genes for molecular functions. Single-nucleotide polymorphisms (SNP) were identified as a result of which 93,122 variants

containing 88,672 SNPs and 4450 indels were identified. Further, simple sequence repeat (SSR) identification using the MISA tool version showed 5996 SSRs, and 146 of the 235 SSR-containing sequences were further examined (Aggarwal et al. 2019). Comparative secretomics analysis between available whole genome sequence of *B. sorokiniana* and *B. oryzae* led to the identification of 262 and 247 predicted small secreted proteins (SSPs), respectively, out of which 34 and 28 SSPs respectively were assigned gene ontology terms for putative function (Singh 2016). Functional analysis of several strain unique polyketide synthase and non-ribosomal peptide synthetase revealed a strong correlation with a role in virulence (Condon et al. 2013). Three Australian isolates of *B. sorokiniana* (BRIP27492, BRIP26775 and BRIP10943) were sequenced and screened for identification of the *ToxA* pathogenicity gene (Mcdonald et al. 2018). During Perrenoud (1990), *ToxA* was discovered in *Parastagonospora nodorum* causing Septoria nodorum blotch (SNB) which horizontally got transferred to *Pyrenophora tritici repentis* causing tan spot (Friesen et al. 2006; Stukenbrock and McDonald 2007). *ToxA* element is unique but not conserved in *Pyrenophora tritici repentis* genome (Moolhuijzen et al. 2018). More recently, Friesen et al. (2018) showed that *ToxA* gene is also present in the *B. sorokiniana* population in the winter wheat region of the United States. Necrosis was induced on wheat leaves of *ToxA*-sensitive wheat genotypes (possessing the *Tsn1* susceptibility gene) (Faris et al. 2010). A sensitivity gene *Tsn1* occurrence in wheat generally helps a *ToxA* positive pathogen to cause spot blotch disease. In general, the *ToxA-Tsn1* system is an illustration of an inverse gene-for-gene relationship (Navathe et al. 2019). *ToxA* gene was amplified in different isolates of *B. sorokiniana* collected from different regions of India, confirming the presence of this gene in Indian population (Anonymous 2019).

To understand genetic and molecular interactions of *B. sorokiniana* with its cereal hosts, it is significant to isolate and characterize genes for virulence or pathogenicity in the pathogen and resistance in the host based on genomic information. The gene responsible for infecting wheat was labeled as *VTa1* (Zhong et al. 2002). A dominant gene *Rbs7* conferring resistance to spot blotch was mapped in a genomic interval of 304 kb on barley chromosome 6H (Wang et al. 2019). A new spot blotch resistance gene designated as *Sb4* was identified and mapped in a genomic interval of 1.34 Mb on long arm of wheat chromosome 4B (Zhang et al. 2020). *VHv1*, a virulence gene was also identified in barley—*B. sorokiniana* pathosystem (Zhong et al. 2002). Recently in wheat—*B. sorokiniana* pathosystem, *ToxA* gene (virulence gene) has been mapped to wheat chromosome arm 5BL, which reveals inverse gene to gene relationship with a sensitivity gene called as *Tsn1* in wheat through which fungus invades the host and disease is caused (Liu et al. 2006; McDonald et al. 2018).

1.12 Disease Management

1.12.1 Use of Resistant Genotypes

Spot blotch disease resistance crosses involved moderately resistant cultivars like BH 1146 from Brazil. However, when similar tests were carried out at Poza Rica, Mexico (CIMMYT = International Maize and Wheat Improvement Centre), the level of resistance was insufficient. Resistance in wheat genotypes is mainly controlled by two to three genes, but in some of the Chinese lines such as Longmai 10 and Yangmai 6, polygenic resistance is observed (Sharma et al. 1997).

Spot blotch disease infection may occur at any growth stage of the crop and it is an important factor to decide the extent of losses in grain yield, therefore it is essential to assess resistance at important growth stage. A study was undertaken on character association analysis to assess the nature of magnitude of association between grain yield components and disease severity (Singh et al. 2008). A study conducted in different environmental conditions confirmed that a few genotypes had low disease severity while some showed higher disease severity in commercial cultivars of South Asia (Sharma et al. 2007). Another study reported high resistance in some wheat genotypes under different environmental conditions (Sharma et al. 2004; Kumar et al. 2015). A few genotypes (Chirya 7, Yangmai 6, and Chirya 1) with lower disease severity had relatively low grain yield and weight in South Asia. However, the genotype with the maximum grain yield and weight (Altar-84/*Ae. sq.* (224)//Yaco) also had less disease severity. This displays progress in merging high grain yield and spot blotch resistance, which was not possible earlier. High yielding commercial wheat cultivars in the region with lower resistance still shows 20% of yield loss due to spot blotch disease (Siddique et al. 2006). Wheat genotype K 8027 shows good level of resistance as studied earlier (Dubin et al. 1998), and reported that in early 1990s leading commercial wheat cultivars of South Asia had developed spot blotch disease severity than genotype K 8027. These findings demonstrate that improvement achieved in the eastern gangetic plains of South Asia is due to combined efforts at international level in improving spot blotch resistance of wheat cultivars. This confirms that disease resistant wheat genotypes are available for direct use in breeding programs to develop commercial cultivars.

During the late 1980s, the extensive crossing program at CIMMYT produced resistant sources, which contained *Thinopyrum curvifolium* for spot blotch resistance as an alien donor in their pedigree (Duveiller and Gilchrist 1994). Donors for resistance may include many species of *Aegilops* and *Triticum* species, such as *Aegilops triuncialis*, *A. speltoides*, *A. cylindrical*, *A. triaristata*, *T. dicoccoides*, *T. timopheevii*, *T. araraticum T. boeoticum*, *T. persicum*, *T. urartu*, and *T. sphaerococcum* (Singh and Dhaliwal 1993; Smurova and Mikhailova 2007). Further, extensive studies were conducted in different parts of the world through conventional breeding for selection of genotypes resistant for spot blotch disease in wheat. However, there is still need for further assessment of sources conferring resistance to spot blotch.

1.12.2 QTLs Identification

It is often challenging to achieve the anticipated level of resistance of host through conventional breeding. Genetics of resistance to spot blotch has been classified into two major categories. Mendelian approach as a first category involving crosses between resistant and susceptible genotypes, and quantitative genetics approach as a second category using molecular markers. Earlier, spot blotch resistance on genetic basis has been recognized as eight major QTLs, viz., QSb.bhu-2A, QSb.bhu-2B, QSb.bhu-2D, QSb.bhu-3B, QSb.bhu-5B, QSb.bhu-6D, QSb.bhu-7B, and QSb.bhu-7D (Kumar et al. 2009, 2010). Three microsatellite markers (Xgwm67, Xgwm469, and Xgwm570) allied with spot blotch resistance were reported by Sharma et al. (2007). Of these QTLs, four with major and stable effects have been designated as *Sb1* on chromosome 7DS (Lillemo et al. 2013), *Sb2* on 5BL (Kumar et al. 2015), *Sb3* on 3BS (Lu et al. 2016), and *Sb4* on 4BL (Zhang et al. 2020). Recently, four promising new QTLs on different chromosomes were identified, namely 1A (497.2 Mb), 1D (89.84 Mb), 2B (421.92 Mb), and 6D (6.84 Mb) linked with numerous protein families which show resistance to disease (Tomar et al. 2021). *Lr34* and *Lr46*, broadly described genes linked with leaf rust resistance, have also been reported in spot blotch resistance. *Lr34*, main locus for spot blotch resistance on chromosome 7D explaining up to 55% phenotypic variation across the six environments in the mean disease data, this locus was designated as gene *Sb*1 (Lillemo et al. 2013). Over the last few years, for spot blotch resistance, several QTLs and genetic markers have been recognized in wheat (Gurung et al. 2014; Zhu et al. 2014; Singh et al. 2018). Resistance genes effectiveness can be lost over time, due to evolutionary changes in pathogen populations. Therefore, breeding for disease resistance in wheat is the outcome of identification and mapping of novel resistance. This can be achieved through association mapping of spot blotch resistance QTLs.

1.12.3 Agronomic Practices

Manipulation of agronomic practices from different countries was suggested to manage spot blotch, like use of different mineral nutrients (Singh et al. 1998; Krupinsky and Tanaka 2000). Some reports also suggest the role of potash in reducing spot blotch disease severity (Regmi et al. 2002). Spot blotch disease severity can be reduced to certain level through good crop husbandry and optimum agronomy (Sharma et al. 2006). Potassium helps to reduce disease severity by hindering multiplication and survival of pathogen and it also controls the internal metabolism of the host plant and prevents the establishment and spread of the pathogen within the host species (Perrenoud 1990). It is reported that application of nitrogen can upsurge the severity of spot blotch (Singh et al. 2012). Crop rotation is an integrated management strategy which helps to manage spot blotch in the field. It promotes better plant nutrition and also favors beneficial soil organisms. Crop rotation is an appropriate way to break the cycle of disease by growing unrelated

crops. It is difficult to find out the suitable non-host crop for spot blotch pathogen because of wide host range. This pathogen can also survive on weeds, therefore, plowing the volunteer cereals, stubble, and grass weeds can reduce inoculum of the pathogen in the field (Diehl et al. 1982).

1.12.4 Fungicides, Bioagents, and Botanicals

For the management of spot botch several fungicides have been recommended for use. The fungicides like difenoconazole (Ishikawa et al. 2012), carbendazim (Yadav et al. 2013), propiconazole (Singh and Singh 2009; Gupta et al. 2018), and azoxistrobin (Navathe et al. 2019) were found efficient in managing spot blotch. At particular stage of host plant, i.e., between heading and grain filling stage, application of fungicides such as epoxiconazole, tebuconazole, cyproconazole, flutriafol, epoxiconazole, flusilazole, and metaconazole have been proved to be cost effective. The yield increase in fungicide-treated areas suffering from spot blotch diseases in comparison to untreated areas was 30% in Argentina (Castro et al. 2018) and 10% in Sweden (Djurle et al. 2018). Besides, it is reported that silver nanoparticles also act as fungicide against spot blotch (Mishra et al. 2014). Similarly, the use of silicon was also established to improve resistance of wheat against spot blotch (Domiciano et al. 2010). Seeds are the primary source of infection. Thus, seed treatment with a suitable fungicide can reduce inoculum potential. In Nepal, seed treatment with fungicide vitavax 200 B and bavistin reduced seedling infection and increased seed germination by 43% (Sharma et al. 2005). Seed treatment with vitavax 200B and carbendazim improved early plant establishment in wheat–rice rotation cropping soil areas. The seed treatment with fungicidal formulation vitavax 200 WS (carboxin + thiram 1:1) @ 2.0, 2.5, and 3.0 g/kg seed reduced incidence of foliar diseases and seedling mortality at different locations of India (Singh et al. 2007). Later on, seed treatment with vitavax power at 3 g/kg of seed followed by two sprays of propiconazole at 0.1% at the early disease infection stage, reduced disease intensity by 39.03% (Singh 2017).

The consequences of recommended dose of fungicides (carbendazim, propiconazole, and hexaconazole), bioagents, and botanicals on seed yield of wheat and severity of spot blotch disease have been documented (Yadav et al. 2015). Bio-control of spot blotch is usually restricted by environmental factors and growing conditions. Successful antagonists in suppressing *B. sorokiniana* identified are *Chaetomium* sp., *Gliocladium roseum*, and *Idriella bolleyi* (Knudsen et al. 1995) and recently, *Bacillus subtilis* TE3 strain verified to be effective against seed borne *B. sorokiniana* (Villa-Rodríguez et al. 2019). Furthermore, *Bacillus safensis* and *Ochrobactrum pseudogrignonense* have been described to stimulate spot blotch resistance (Sarkar et al. 2018). *Chaetomium globosum* Kunze is considered as a potential antagonist of numerous soil and seed borne plant pathogens (Vannacci and Harman 1987; Walther and Gindrat 1988). Bioefficacy of *C. globosum* under in vitro, in vivo, and mechanism of antagonism has been studied in detail (Aggarwal et al. 2004; Aggarwal 2015). Culture conditions for resourceful production of

extracellular xylanase from *C. globosum Cg 2* have been standardized and partially purified enzyme has been characterized. Antifungal activity was shown by both purified xylanase (100 μg ml^{-1} concentration caused 100% inhibition of conidia germination) and culture filtrate (inhibit germination up to 67.5%) against *B. sorokiniana* (Ahammed et al. 2008). Further, an extracellular β-1, 3-glucanase produced by *C. globosum* isolate Cg2, inhibited 93.5% conidial germination of spot blotch fungus *B. sorokiniana*, whereas the culture filtrate inhibited conidial germination up to 59.9% (Ahammed et al. 2012). Further, biology, bioefficacy, and mechanism of action of *C. globosum* against plant pathogens have been reviewed (Aggarwal 2015). *C. globosum Cg 2* also detoxifies the toxin Bipolaroxin produced by *B. sorokiniana* (Aggarwal et al. 2011c).

There are reports that application of ethanolic plant extracts, aqueous plant extracts in amalgamation with cow dung, and aqueous plant extracts in amalgamation with cow urine can inhibit conidial germination of *B. sorokiniana* causing spot blotch. Additionally, ethanolic extracts of *Adhatoda vasica* (leaf) and *Zingiber officinale* (rhizome) at 2.5% provided 100% inhibition of conidial germination (Akhter et al. 2006). The leaf extract of *Rauwolfia serpentina* at 10M concentrations inhibited the spore germination up to 93.7% and increased grain yield by 28.9% (McDonald et al. 2018). Garlic extract treatment of wheat seeds reduced the incidence of spot blotch and increased the seed germination (Khalaf et al. 2011). Extracts of garlic clove, eucalyptus leaf, neem cake, and neem leaf at tillering and boot leaf stage resulted in the higher yield of wheat (Yadav et al. 2015). Six plants extracts, namely garlic, clove, eucalyptus leaf, neem leaf, onion bulb, ginger rhizome, and black cumin, could significantly inhibit the growth of *B. sorokiniana* (Tiwari and Singh 2021).

1.13 Conclusions

It may be concluded that this disease will continue to be a major concern worldwide especially in warm and humid regions despite the substantial progress already made in understanding the biology of the pathogen. Therefore, there is a need of further attention from the researchers for the management of this disease, which can be a threat in near future due to climate change and changing agronomy. QTLs have been identified which will help to develop new resistant cultivars. However, resistance is very low in most of the cultivars. Improving resistance through hybrid program by exploring new resistance donors should be done continuously to keep disease at its lowest level, improving grain yield and to develop resistant cultivars for future use. Integrating conventional breeding, molecular approaches, application of fungicides and bioagents will offer eco-friendly and cost-effective control of spot blotch disease worldwide. The information generated through genomics will further aid in better understanding of pathogenesis leading to disease management.

Acknowledgments The authors are grateful to Head, Plant Pathology and Director, ICAR-IARI New Delhi for all kind of support. The research program on pathogenomics under CRP Genomics

(Project code: 12-151) financially supported by Indian Council of Agricultural Research, New Delhi is duly acknowledged.

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Biology and Management of *Ustilaginoidea virens* Causing False Smut Disease of Rice (*Oryza sativa* L.)

2

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Abstract

Rice false smut is emerging as one of the most devastating rice fungal disease. The disease not only causes severe yield loss and grain quality reduction, but also threatens food safety due to production of mycotoxins in rice grains which raises great concerns for food and feed safety. This chapter describes the biology of the false smut pathogen *Ustilaginoidea virens* and the management approaches against the false smut disease of rice.

Keywords

False smut · Rice · *Ustilaginoidea virens* · Biology · Management

2.1 Introduction

Rice (*Oryza sativa* L.) is the second largest produced food grain next to maize. It belongs to the family *Poaceae* and is the most important food crop of India covering about one-fourth of the total cropped area and providing food to about half of the Indian population. Major diseases that occur in rice include rice blast, sheath blight, bacterial blight, bakane and false smut. Many diseases which were considered as minor have now become serious in many rice-growing areas. For example, false

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_2

smut disease of rice which was earlier considered as a sign of bumper yield (Lakshmi disease) has now become a serious threat in many rice ecosystems.

Rice false smut caused by *Ustilaginoidea virens* (Cooke) Takahashi was epidemically reported for the first time in Tamil Nadu, India and later in many countries of the world (Singh and Pophaly 2010). In recent years, rice false smut has become a serious problem in many parts of the world due to climate change and growing high yielding rice varieties, which are more susceptible to diseases. The false smut occurs in more than 56 countries, including India (CABI 2021).

In India, the disease has been observed in severe form since 2001 in major rice-growing states, viz., Haryana, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Jharkhand, Gujarat, Maharashtra, Jammu & Kashmir and Puducherry (Mandhare et al. 2008). Pannu et al. (2010) also reported losses up to 44% in Punjab. In Uttar Pradesh, yield losses up to 44% were observed by Singh and Dubey (1984). In some rice-growing districts of Bihar, 15–50% losses occur due to false smut of rice when it comes in as medium to severe form. The disease causes sterility of the spikelets and increases the chaffy grain percentage. In severe infection, the number of smut balls reaches to more than 50 per panicle (Ladhalakshmi et al. 2012).

False smut balls have been reported to emerge about 20 days after the initial infection of kernels of the rice panicle during the flowering of the rice plant. Infection results in one or more kernels on mature heads of plants being replaced by globose, yellowish-green, velvety smut balls. When smut balls burst open, powdery dark green spores are released (Atia 2004). The symptoms produced by *U. virens* are visible after flowering only, when the fungus transforms individual grains of the panicle into a yellowish smut ball, which changes to yellowish orange, green, olive green and finally to greenish black coloured smut balls. *U. virens* produces both sexual (ascospores) and asexual (chlamydospores) stages in its life cycle (Biswas 2001).

Under bright-field light microscopy, the conidia are found to be round to elliptical and warty on the surface with diameters approximately ranging from 3 to 5 mm. Under scanning electron microscope, the globose to irregularly rounded conidia are ornamented with prominent spines. The spines are pointed at the apex or irregularly curved, and approximately 200–550 nm long. In addition, the chlamydospores produced in culture medium are found prone to germinate in distilled water and produces secondary spores. The conidia are holoblastically and sympodially produced at the apex of each conidiophore cell (Fu et al. 2012).

2.2 Biology of the Pathogen

2.2.1 Taxonomy

The anamorphic state of the false smut pathogen *Ustilaginoidea virens* (Cooke) Takah., (1896) belongs to Division: Ascomycota, class: Sordariomycetes, Order: Hypocreales, and Family: Clavicipitaceae. Other synonyms for the pathogen are

Claviceps oryzae-sativae Hashioka (1971), *Sphacelotheca virens* Omori (1896), *Tilletia oryzae* Patouillard (1887), *Ustilaginoidea oryzae* (Pat) Brefeld (1895), *Ustilago virens* Cooke (1878). The teleomorphic state *Villosiclava virens* was introduced in 1934 and was allied with the family Clavicipitaceae. To date, the teleomorph of rice smut fungus has been reported in Japan (Sakurai 1934; Hashioka et al. 1951), India (Singh and Dubey 1984), Korea (In et al. 1984), and China (Wang et al. 1998).

2.2.2 Life Cycle

U. virens produces both sexual (ascospores) and asexual (chlamydospores) stages in its life cycle (Biswas 2001). Recently, *Villosiclava virens* has been proposed as the new name for the teleomorph of the false smut fungus (Tanaka et al. 2008). The interiors of the false smut balls are intertwined with hyphae at early stage and then chlamydospores are formed (Fig. 2.1). The chlamydospores are almost smooth when young, and become warty when mature (Kim and Park 2007). It has been reported that *U. virens* can potentially infect coleoptiles during the seed germination stage, and also infect the seedling roots (Prakobsub and Ashizawa 2017). However, the infections of the coleoptiles and roots may not spread to the spikelets and cause the characteristic symptom of rice false smut disease as no invasive hyphae have been observed beneath the pedicels or in the stems of naturally severely infected panicles (Tang et al. 2013). The initiation of *U. virens* infection blocks the pollination process, and mimics the fertilization of the rice ovaries to hijack the rice nutrient supply (Song et al. 2016). General infection process observed in India is presented in Fig. 2.2.

Fig. 2.1 False smut chlamydospores and spore ball colour morphs observed in different rice varieties

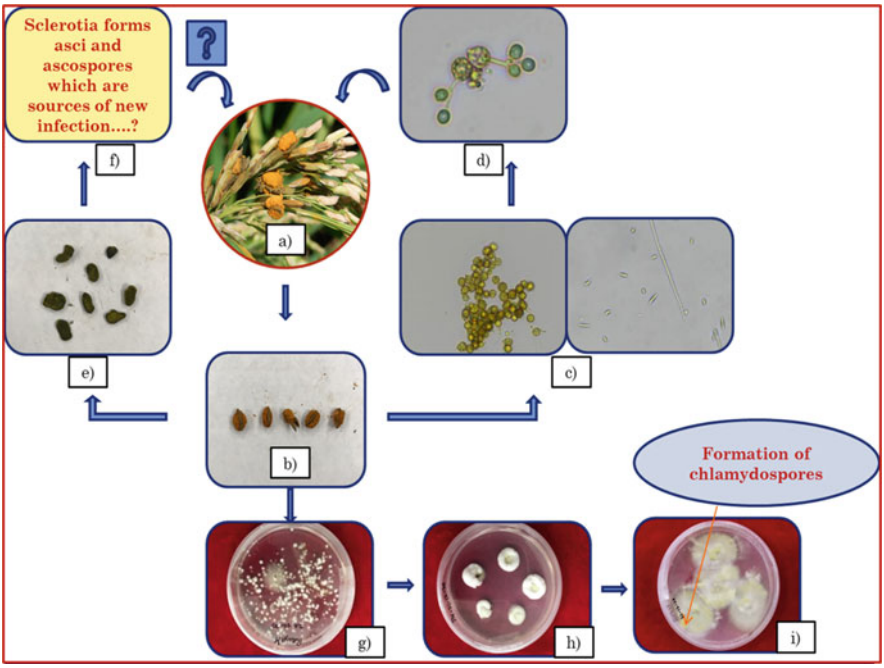

Fig. 2.2 Life cycle of the false smut pathogen. (**a**) Infected panicles. (**b**) Smut balls. (**c**) Chlamydospores and Conidia. (**d**) Germinating chlamydospores. (**e**) Sclerotia. (**f**) Formation of asci. (**g**) Smut powder dusted on Potato Sucrose Agar media. (**h**) Formation of fluffy white colony. (**i**) Production of chlamydospores in the culture with yellow raised structures

2.2.3 Infection Process

Based on the current understanding of the infection process, the conidia of *U. virens* under optimum conditions germinate to produce a huge number of secondary conidia and hyphae on the surfaces of rice spikelets (Fan et al. 2014). Extended hyphae can enter in the inner spaces of spikelets through small gaps between the lemma and palea to infect the stamen filaments, and possibly the stigma orlodicules, without haustorium or appressorium, at approximately 4 days post inoculation (Ashizawa et al. 2012; Li et al. 2013; Tang et al. 2013; Song et al. 2016). It has been reported by transmission electron microscope observation that invasive hyphae of *U. virens* extend along the cell gaps of the filaments without penetration of the host cell walls (Tang et al. 2013). The initial infection of *U. virens* blocks the pollination process, and copies the fertilization of the rice ovaries to hijack the rice nutrient supply (Fan et al. 2015; Song et al. 2016). It is one of the unique features to distinguish infection process of *U. virens* in comparison to other rice fungal pathogens (Talbot 2003). At approximately 10 dpi, hyphae cover the anthers, stigmas, styles of the pistils, and start to grow out of the spikelets. Lastly, ball-like colonies are formed due to infection of one or multiple *U. virens* isolates, at 15 dpi

(Yu et al. 2013). Inside false smut rice balls, the ovaries are present alive, justifying *U. virens* does not kill the host during infection process (Tang et al. 2013).

2.2.4 Mycotoxins

Rice false smut pathogen produces mycotoxins, including ustiloxins and ustilaginoidins. Ustiloxin is a kind of 13-membered cyclic peptide found in mature rice false smut generated by *Ustilaginoidea virens* infecting rice spikelet. So far, six ustiloxins designated as A, B, C, D, F and G have been identified from false smut balls (FSBs) in which ustiloxin A is the main component accounting for approximately 80% of the total ustiloxin content. The ustiloxins of *U. virens* inhibit the microtubule assembly and skeleton formation of eukaryotic cells (Koiso et al. 1994, 1998; Lin et al. 2018). Ustilaginoidins are a class of bis-naphtho-γ-pyrones, which have cytotoxic activities on cancer cells and inhibitory effects on the radical elongation of rice seeds (Lu et al. 2015; Sun et al. 2017). To date, 26 ustilaginoidins, namely ustilaginoidins A, B, C, D, E, E1, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V and W, isochaetochromin B2, and 2,3-dihydroustilaginoidin T have been identified in *U. virens* (Sun et al. 2017).

2.2.5 Disease Epidemiology

Low temperature induces sclerotium formation, therefore, sclerotia are often produced from false smut balls in late autumn. Sclerotia overwinter with 2–5-month dormancy in paddy fields and can maintain a high germination rate for up to 5 years under dry and low-temperature conditions and suitable humidity (Fan et al. 2015). The climatic factors favouring the rice false smut include cloudy weather, high relative humidity (>95%), low temperature (25–30 °C), water stress and rainy days at the time of flowering (Sanghera et al. 2012; Raji et al. 2016) and late sowing and application of higher nitrogen doses favours the development of disease (Ahonsi et al. 2000). Although, high relative humidity (89–95%) and no rainfall were also found to be favourable for development of false smut in rice (Devi and Singh 2007).

2.3 Genomics of *U. virens*

2.3.1 Whole Genome Sequences

In recent years, with the advancement in the sequencing techniques, several isolates of *U. virens* have been fully sequenced. The studies revealed that the genome size varies between 26–38.8 Mb in length encoding 6451–8496 proteins (Zhang et al. 2014a, b; Kumagai et al. 2016; Pramesh et al. 2020). The genome is characterized by presence of ~25% repeat sequences primarily comprising of transposable elements (Zhang et al. 2014a, b). Comparative analyses of genome with transcriptome

revealed role of effectors in pathogenicity, virulence, and host adaptation. Zhang et al. (2014a, b) observed the role of proteins alterations in the infection biology of false smut pathogen. For instance, downregulation of carbohydrateactive enzymes, major facilitator superficial transporters, dehydrogenases, cytochrome P450 and proteins involved in energy metabolism and secondary metabolism. These characters indicated that *U. virens* may have a limited ability to acquire nutrients from plant tissues and organs. Therefore, the presence of large amount of easily accessible nutrients in the male flower parts including pollens may contribute to primary colonization of *U. virens* in the rice florets. Additionally, the secondary metabolites contribute to detoxification of phytoalexin repertoires of the host plants.

The genome of *Uv IPU010* has also been revealed role of virulence effectors and secondary metabolites in disease establishment (Kumagai et al. 2016). The toxicity gene, ustiloxin located in *Uv IPU010* has been found to be homologous with previously sequenced *U. virens*, UV-8b genome (Zhang et al. 2014a, b; Tsukui et al. 2015). Further, Pramesh et al. (2020) compared the UvGvt genome with the genomes available in NCBI database (Uv 8b and Uv IPU010) to understand the evolutionary relationship and genetic diversity amongst different isolates. The genomic study also supported the fact that the predicted proteins are involved in host penetration, effectors transportation, synthesis, modification of secondary metabolites, transcription factors and other essential cellular processes thus validated biotrophic way of pathogenesis. Additionally, the variant analysis revealed low genetic diversity (0.073–0.088%) among *U. virens* strains (Pramesh et al. 2020).

Recently, a chromosome-based analysis of the genome has been published. This study divided the entire genome of *U. virens* in seven different chromosomes ranging in size from 2.4 to 7.5 Mb (Zhang et al. 2021). The study also supported the incidence of low intraspecific divergence as evident through previous phylogenetic studies. Further, the comparative study of eight mitochondrial genomes revealed size variations from 94 to 102 kb. Consistently, *U. virens* contains conserved lengths of exons and highly dynamic mobile introns, which contribute to intraspecific size variations due to gain/loss of homing endonuclease genes (Zhang et al. 2021). Hence, the genomic studies play an important role in evolutionary studies and infection biology of *Ustilaginoidea virens*.

2.3.2 Genetic Diversity of *U. virens*

Genetic diversity and population analysis studies aid in understanding the evolution of the pathogen and hence prove beneficial in understanding host resistance mechanism. The studies related to genetic diversity were carried out in China where the disease seems to have more destructive effects. Zhou et al. (2008) studied population of two different zones, North China and Beijing. It was concluded that the population is mostly genetically similar (70%). Further, Sun et al. (2013) identified three important SNP markers, which were used for population study of 162 isolates of *U. virens* purified from 15 major rice-growing provinces of China. The study suggested that the genetic variability is high among various isolates of *U. virens*. It

was further found that the genetic variability is associated with their geographical locations, i.e. isolates from a particular region are similar and variation exists between the isolates obtained from geographically distinct regions. Further research found that the genetic variability in *U. virens* is linked to the geographical location (Wang et al. 2014). SSR-based research by Jia et al. (2015) divided the entire observed population of *U. virens* in two groups on the basis of origin. The higher diversity in one group has been associated with rice evolution from the particular place directing towards host pathogen co-evolution.

2.3.3 Functional Characterization Studies of Pathogenic Genes

The sequencing studies have unravelled many genes playing important role in virulence and pathogenicity. However, a few have been characterized due to limitation in efficacy of homologous recombination, and stability of artificial inoculations (Zheng et al. 2016). For instance, some genes related to sporulation, hyphal growth and pathogenesis have been identified (Huang et al. 2013; Yu et al. 2013, 2015; Wang et al. 2015; Yin et al. 2017). The genes functionally characterized in *U. virens* pathogenicity and virulence are Uvt3277, UvSUN2 and SCRE2 (Yu et al. 2015; Zheng et al. 2017). UvSUN2 role has been known for proper fungal growth, cell wall construction and stress responses in *U. virens* (Yu et al. 2015). Further a novel promoter has also been identified for its role in gene expression of conidiation and mycelia growth genes (Hu et al. 2013). The SCRE2 factor has been characterized for full virulence, which is found to be identical among isolates from different regions of world such as Japan, China, India and United states (Fang et al. 2019). Besides, some other genes such as Uvslt2, UvHog1 and UvCom1 have also been identified to maintain cell wall and membrane stresses (Zheng et al. 2016). In fact, it was proposed that UvSLT2 deletion may lead to hyperactivation of UvHog1 that in turn increased the tolerance to hyperosmotic and oxidative stresses. These findings suggest role of the aforementioned proteins in the growth of fungi and modulation of host defence mechanisms in establishment of infection. However, the genomic studies should be used efficiently for finding out involvement of more virulence and pathogenicity genes followed by functional characterization.

2.4 Disease Management

Minimization of disease severity depends upon overall integrated approach of disease management. It should start with selection of seeds involving proper cultural methods and selection of efficient methods to control and prevent the disease. Proper consideration of preventive and control measures with respect to time will be beneficial in improving yield.

2.4.1 Host Plant Resistance

Singh and Sunder (2015) reported rice genotypes HKR 05-10, HKR 05-22, HKR 07-95, HKR 07-239, HKR 08-12, HKR 08-17, HKR 08-71, HKR 08-110 and HKR 08-118 are promising against false smut disease. Kumar et al. (2017) evaluated 21 rice genotypes and four rice genotypes and reported Swarna Shreya, IR96321-1447-521-B-2-1-2, IR96321-1447-651-B-1-1-2 and IR 83294-66-2-2-3-2 were immune or highly resistant against false smut. Dodan et al. (2013) identified rice genotypes HKR 98-418, HKR 2K-645, IR 48725-B-B-86-2-2, IR 65907-191-1-B, IRGA 317-56-1-1F-1-2, OR 1509-9-VE, P1121-92-81-3-3, RR 373-21-1 and UPR 2472-18-1-2 as highly resistant to false smut. Li et al. (2011) evaluated 18 hybrid rice varieties and observed strong resistance to rice false smut in varieties R18/2348, CGY2348, GY66, YLY973 and N5Y828. Banasode and Hosagoudar (2020) screened 102 genotypes against false smut disease of rice under natural epiphytotic conditions and reported 11 genotypes/varieties, viz., (IET 24956, IET 25530, IET 26273, IET 26218, IET 26275, IET 25798, IET 24995, IET 25523, Varshadhan, IET 27274 and IET 27277) as highly resistant. Kumar and Kumar (2020) evaluated forty-four elite germplasm at Bihar Agricultural University research farm, Sabour and identified seven germplasms, *viz.* RVK-04, RVK-06, RVK-16, BRR-0057, BRR-0060, BRR-0078 and Rajendra Swasini as immune or highly resistant (HR) to false smut disease. Rashmi et al. (2016) identified Harsha, Vaishak, Makom, Thekkancheera, Pavizham and Karthika as resistant to the false smut in Kerla, India.

Kumari et al. (2021) conducted mapping of QTLs using a recombinant inbred line (RIL) population derived from a cross between resistant line, RYT2668, and a highly susceptible variety, PR116. A total of seven QTLs were mapped on rice chromosomes 2, 4, 5, 7 and 9 of rice using 2326 single nucleotide polymorphism (SNP) markers. Among them, a novel QTL *qRFSr9.1* affecting total smut ball (TSB)/panicle on chromosome 9 exhibited the largest phenotypic effect. Sheng et al. (2008) used recombinant inbred line (RIL) population with 157 lines derived from an inter-subspecific cross of Daguandao/IR28, where 5QTLs controlling false smut resistance were detected on chromosomes 1, 4, 10, 11 and 12 respectively, with the phenotypic variance explained from 9.8 to 22.5%. The directions of the additive effects at the four loci *qFsr1*, *qFsr10*, *qFsr11* and *qFsr12* coincided with those predicted according to phenotypes of the parents.

Hiremath et al. (2021) screened 125 diverse lines from global rice diversity panel under natural hot spot for 3 years and identified 21 germplasm lines showing high level of resistance in all the years under natural hot spot as well as artificial inoculation conditions which were HEI CHIAO CHUI LI HSIANG KENG::IRGC 1112-1 (admixed), ITA 235::IRGC 64854-1 (tropical-japonica), BARAN BORO:: IRGC 27509-1 (Aus), KEN CHIAO JU HSIAO LI::IRGC 1217-1 (temperate-japonica), PADI KOMPAI::IRGC 25510-1(tropical-japonica), REXORO::IRGC 1715-1 (tropical-japonica), TAINUNG 29::IRGC 65309-1 (temperate-japonica), MUT IAC25-44-807::IRGC68799-1 (tropical-japonica), HAWM OM::IRGC 23729-1 (admixed-japonica), GEMJYA JYANAM::IRGC 32411-C1

(admixed-japonica), GAO GAN DA NUO::IRGC 73974-1 (temperate-japonica), ARC 11294::IRGC 21296-1 (tropical-japonica), KHAO DO NGOI::IRGC 29772-1 (admixed-japonica), TAK SIAH::IRGC 73126-1 (admixed), KAKANI 2::IRGC 13373-C1 (admixed), INDANE::IRGC 33130-1 (admixed-japonica), NENG NAH::IRGC 78275-1 (admixed-japonica), PAE URA::IRGC 27321-1 (tropical-japonica), OIRAN::IRGC 8257-1 (admixed-japonica), KHAU MEO::IRGC 78330-1 (admixed-japonica), DAVAO::IRGC 8244-C1 (tropical-japonica) as highly resistant with disease score zero. Genome-wide association study for false smut related traits identified significant associations at chromosomes 2, 3, 6, 9 and 11. Using near-isogenic introgression lines of "Lemont/Teqing", two quantitative trait loci (QTLs) for false smut resistance (*qFsr10* and *qFsr12*) were detected and mapped in Lemont (Zhou et al. 2014) and further ten QTLs affecting percentages of diseased hills, panicles, and spikelets have been subsequently detected and mapped (Xu et al. 2002).

2.4.2 Cultural Methods

In the United States, research has showed that a combination of crop rotation, soil tillage, fertility rate, and several other alternative crop management practices were developed to provide effective control of smuts in susceptible rice cultivars (Brooks et al. 2010). Early transplanted rice showed higher disease incidence when compared to late planting. To avoid severe damage, sowing date and heading period could be planned in such a way that flowering should not coincide with rainy period. Initial occurrence of the disease can also be minimized by using sclerotic free seeds for sowing and cleaning of bunds. Cultivation practices including specific crop rotation, furrow irrigation and conservation tillage are also effective in minimizing the RFS disease index (Brooks et al. 2010). Furrow irrigated rice cultivation system recorded less disease severity as compared to flooded fields.

2.4.3 Chemical and Biological Management

Numbers of fungicides and biocontrol agents were identified effective against the false smut disease of rice. However, efficacies of fungicides were observed to be varied according to the growth stage and time of application. Chemical biocontrol agents effective against the false smut disease are listed in Table 2.1. Use of biocontrol agents is an environment friendly method to manage plant disease. Some plant extracts observed promising against the *Ustilaginoidea virens* under in vitro conditions are also listed in Table 2.1.

Table 2.1 Chemical management of false smut disease

S. No.	Chemical	Reference
1	Copper fungicides, viz., copper oxychloride, copper hydroxide	Dodan and Singh (1997)
2	Spraying 2.5% Wenquning, suspension of *Bacillus subtilis* in solution of validamycin with 4.5 L/ha at 6 days before heading	Liang et al. (2014)
3	Fujione 40 EC (0.1, 0.2 and 0.3%) and carbendazim 50% WP (0.1%) at the booting stage	Bagga and Kaur (2006)
4	Copper oxychloride 50 WP (0.3%) and Propiconazole 25 EC (0.1%)	Bagga and Kaur (2006)
5	Application of fungicide Simeconazole under submerged condition at 3 weeks before rice heading	Tsuda et al. (2006)
6	Trifloxystrobin 25% + tebuconazole 50%	Raji et al. (2016)
7	Propiconazole 25 EC	Raji et al. (2016)
8	Prochloraz + carbendazim followed by chlorothalonil	Mohiddin et al. (2012)
9	Azoxystrobin (18.2%) SC + difenoconazole (11.4%) SC	Muniraju et al. (2017)
10	Metiram (55%) WG + pyraclostrobin (5%) WG	Muniraju et al. (2017)
11	Trifloxystrobin 25% + Tebuconazole 50% 75 WG @ 0.4 gm/lt at 100% panicle emergence	Hosagoudar (2018)
12	Simeconazole	Tsuda et al. (2006)
13	*Antennariellaplacitae*	Andargie et al. (2017)
14	*Trichoderma viride, Trichoderma virens, Trichoderma harzianum* and *Trichoderma reesei*	Kannahi et al. (2006)
15	*Bacillus subtilis*	El-Naggar et al. (2015)
16	Garlic bulb extract, rhizome extract of turmeric, bael, extracts of lemon grass, cinnamon, palmarosa	Raji et al. (2016)

2.5 Conclusions

False smut of rice is emerging as a serious disease of rice. Although, the disease was reported more than 100 years back still information on disease cycle, role of mycotoxins in disease development, molecular mechanism of mycotoxin synthesis in *U. virens* and new types of mycotoxins besides ustiloxins and ustilaginoidins needs to be explored. Further, efforts are needed for the resistance evaluation under artificially inoculated conditions and pathogen diagnostics.

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Diversity of Some of the Major Fungal Pathogens of Soybean and Potential Management Options

3

Shrishail S. Navi and Steven Harris

Abstract

In this review, we have examined the diversity of some of the major pathogens of soybeans in various countries of the world. We tried to summarize observations as concisely as possible and as informatively as possible so that students, scientists, and academicians can extend their innovations to reach growers. We selected four economically important and significant diseases of soybean (sudden death syndrome, root rot, damping-off, and charcoal rot) based on the impacts of these diseases in causing yield losses as well as the worldwide diversity of the causative pathogens. To date, six major *Fusarium* spp. are associated with sudden death syndrome, 25 *Fusarium* spp. associated with *Fusarium* root rot, and up to 54 *Pythium* spp. associated with damping-off. Although there do not appear to be diverse species of the genus *Macrophomina* that causes charcoal rot, we were able to note the cultural, morphological, and genetic diversity of *M. phaseolina*. In this review, we have compiled management options for each of the diseases mentioned above.

Keywords

Diversity · Soybean · Sudden death syndrome · *Fusarium* root rot · *Pythium* damping-off · Charcoal rot · Disease management

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_3

3.1 Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most economically important crops due to its potential as an oilseed crop and major plant protein source used for livestock and human consumption. The top producers of soybean are Brazil (37% of the world's total), the USA (31%), Argentina (14%), China (5%), Paraguay (3%), India (3%), and Canada (2%) (https://apps.fas.usda.gov/psdonline/circulars/production.pdf). Hartman (2015) and Hartman et al. (2015) compiled a complete account of soybean diseases in major soybean growing countries. Yield losses due to *Rhizoctonia* root rot, *Pythium* damping-off, Phytophthora root rot, sudden death syndrome (SDS), and white mold are up to 35% (Wrather and Koenning 2009). In a recent review, Navi and Yang (2016b) have compiled the global occurrence of SDS, economic significance, symptoms, factors affecting SDS, and various management options. In their review, they have also given an estimated economic loss due to SDS ranging from US$15.7 million in 1988 to US$669.2 million in 2010. Total estimated yield loss (million mt) due to white mold (2.8 MMT), SDS (5.7 MMT), charcoal rot (6.0 MMT), and seedling diseases caused by *Rhizoctonia*, *Pythium*, *Fusarium* and *Phomopsis* (6.6 MMT) in 28 US states and Ontario, Canada from 2010 to 2014 (Allen et al. 2017; Navi et al. 2019). Estimated economic loss due to seedling diseases compiled from various sources ranges from $67.60 million in 1999 to $779.92 million in 2008 (Navi et al. 2019).

3.2 Diversity of *Fusarium* spp. Associated with Soybean Sudden Death Syndrome

Sudden death syndrome (SDS) of soybean is one of the significant yields reducing diseases in soybean. Diverse species of *Fusarium* spp. cause this disease. In North America, *F. virguliforme* (Aoki et al. 2003) and *F. brasiliense* (Wang et al. 2019); in South America, *F. tucumaniae*, *F. brasiliense*, *F. cuneirostrum*, *F. virguliforme,* and *F. crassistipitatum*, (Aoki et al. 2003, 2005, 2012); in Africa, *F. virguliforme* and *F. brasiliense* (Tewoldemedhin et al. 2014, 2017); and in Asia, *F. virguliforme* (Chehri et al. 2014), and in Hokkaido, Japan, *F. azukicola* sp. nov., an exotic azuki bean (Aoki et al. 2012a).

After SDS was first noticed in Iowa in 1993 (Yang and Rizvi 1994), an extensive investigation confirmed SDS in 28 counties by 1996 (Yang et al. 1998), and by 2010, SDS had spread to 96 of the 99 counties in Iowa. This suggests that *F. virguliforme* may have been present in most Iowa counties' soils in previous years but had not been detected in several of them because of low inoculum densities and unfavorable weather (Leandro et al. 2013). The occurrence of *F. virguliforme* infection early in the season results from colonization in xylem tissue, a process essential for foliar symptom expression (Navi and Yang 2008).

In the off-season, the SDS fungus survives in soil and crop residue either in conidial or chlamydospore form (Navi and Yang 2016a). Infected plants show symptoms both in seedling and reproductive growth stages. Characteristic symptoms

Fig. 3.1 Characteristic field symptoms of soybean sudden death syndrome (SDS), (**a**) severe symptoms of SDS observed in commercial productions during 2010 growing season in Iowa, (**b**) chlorotic spots and interveinal chlorosis, (**c**) interveinal necrosis, (**d**) severe interveinal necrosis and puckering, (**e**) loss of leaf lamina, (**f**) defoliation with leaf petioles intact on stems, and (**g**) fungus growth on the taproot

are chlorotic spots, interveinal chlorosis, interveinal necrosis, puckering, mottling of leaves, loss of leaf lamina, defoliation, and fungus on taproot (Fig. 3.1).

The first reports of a diversity of *Fusarium* spp. associated with soybean SDS reported in North America (Canada and United States), South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay), Africa (South Africa), and Asia (Japan and Malaysia) is given in Fig. 3.2, and the first reports of *F. virguliforme* and *F. brasiliense* associated with soybean SDS within the United States in Fig. 3.3. In a recent review, Navi and Yang (2016b) have compiled the global occurrence of SDS, economic significance, factors affecting SDS, and various management options. Mbofung et al. (2008, 2012) have reported diversity in genetic structure and aggressiveness of *F. virguliforme* in the Midwest United States.

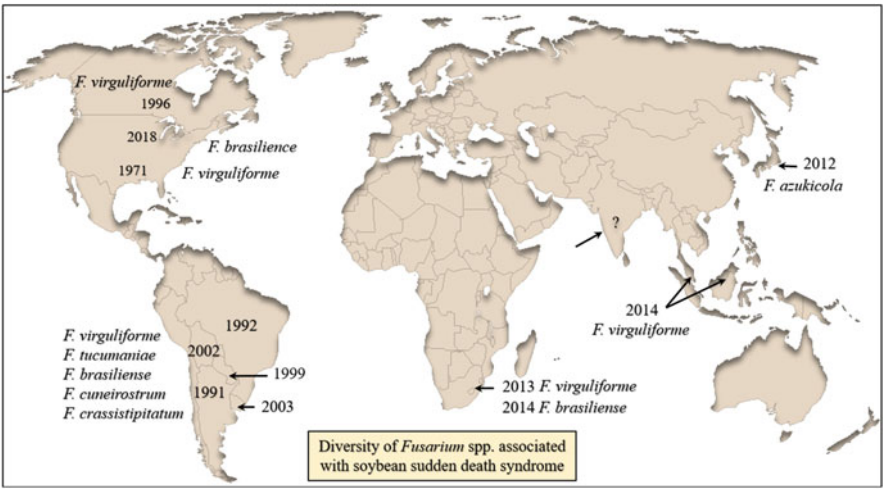

Fig. 3.2 Diversity of *Fusarium* spp. associated with soybean sudden death syndrome in different continents. Note: Whether the pathogens have been introduced into new regions or if they have been present in the soil of these countries for some time without being detected is unknown

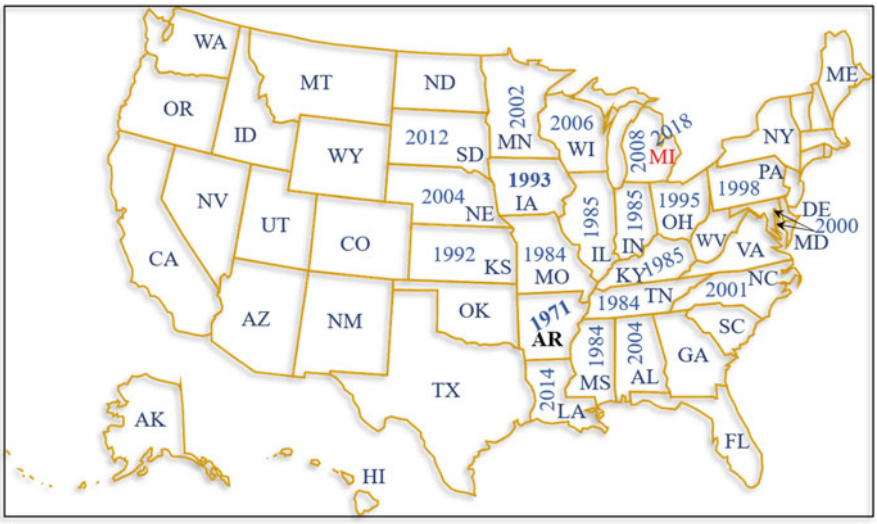

Fig. 3.3 Diversity of *Fusarium* spp. associated with soybean sudden death syndrome (SDS) in the United States (mainly *F. virguliforme*, but in Michigan, SDS is caused by *F. virguliforme* and *F. brasiliense*)

3.3 Diversity of *Fusarium* spp. Associated with Soybean Root Rot

Similar to SDS, soybean root rot caused by species of diverse *Fusarium* spp. *Fusarium* root rot is an important disease in many soybean production areas in the USA and worldwide. This disease may be difficult to diagnose because the causal agent(s) may either act as primary pathogens or colonize root systems and other soilborne pathogens. *Fusarium* species are often isolated from soybean roots infected by other pathogens such as *Pythium*, *Phytophthora*, and *Rhizoctonia*. Many *Fusarium* species have been found associated with root rot of soybean. Most frequently associated are *F. solani* and *F. oxysporum* (French and Kennedy 1963; Killebrew et al. 1993; Nelson et al. 1997; Pant and Munkhopadhyvay 2002). Other *Fusarium* species include *F. acuminatum*, *F. chlamydosporum*, *F. compactum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. merismoides*, *F. proliferatum*, *F. pseudograminearum*, *F. semitectum*, *F. subglutinans*, and *F. verticilliodes*.

The diversity of *Fusarium* spp. associated with soybean root rot reported in major soybean-producing areas of the world are discussed here. In Canada, plant samples collected from fields in southern Alberta and Manitoba identified four species of *Fusarium* (Table 3.1) causing root rot, of which *F. proliferatum* was the first report (Chang et al. 2015). Whereas in the samples collected from central and southern Alberta, ten species of *Fusarium* were identified (Table 3.1); these include the first report of *F. commune*, *F. redolens*, and *F. torulosum* causing root rot (Zhou et al. 2018). In eastern Ontario, seven *Fusarium* spp. causing root rot have been isolated (Zhang et al. 2013; Table 3.1). In Manitoba, *F. sporotrichioides* (Abdelmagid et al. 2021) and *F. cerealis* (Abdelmagid et al. 2018) were the first reports of causing root rot in soybean.

In the United States, nine species of *Fusarium* in South Dakota (Okello et al. 2020; Table 3.1), and ten species in Minnesota (Bienapfl 2011, Table 3.1) causing root rot were reported. Out of the ten, *F. redolens* was the first report from Minnesota (Bienapfl et al. 2010). Fifteen *Fusarium* species have been reported to cause root rot in Iowa (Díaz Arias et al. 2013; Table 3.1), of which *F. proliferatum* (Díaz Arias et al. 2011) and *F. armeniacum* (Ellis et al. 2013) are the first report from Iowa. Recently, Detranaltes et al. (2021) reported the association of *F. fujikuroi* causing root rot and seedling elongation in Indiana (Table 3.1). Seven *Fusarium* spp. were recovered from soybean in 2015 and 2016 in Nebraska (Parikh et al. 2018; Table 3.1, Fig. 3.4).

In samples collected from soybean fields in southern and tropical central Brazil, *F. paranaense* was reported as causing root rot (Costa et al. 2016; Table 3.1), and the *F. graminearum* complex was similarly implicated in Argentina (Barros et al. 2014; Table 3.1), *F. graminearum* and *F. meridionale* in Argentina (Chiotta et al. 2016). In China, nine species of *Fusarium* causing root rot were reported in Sichuan province (Chang et al. 2018; Table 3.1). In addition, there are reports of other species of *Fusarium* causing root rot in the Altay region of Xinjiang, China (Baili et al. 2009), in northeast China (Wang and Wen 2011), as well as *F. oxysporum* in northeast

Table 3.1 Reports of *Fusarium* spp. associated with soybean root rot in North America (USA, Canada), South America (Brazil and Argentina), and Asia (China and India)

Fusarium spp.	USA	Canada	Brazil	Argentina	China	India
F. acuminatum	IA[a], MN[b], SD[c], NE[d]	Central/ Southern AB[e]				–
F. armeniacum	IA, SD	–				–
F. avenaceum	IA,	Southern AB, MB[f], Central/ Southern AB, Eastern ON[g]			Sichuan[h]	–
F. bulbicola	SD,	–				–
F. cerealis	–	MB[i]				–
F. commune	SD,	Central/ Southern AB			Sichuan	–
F. culmorum		Southern AB, MB; Central/ Southern AB				–
F. equiseti	IA, MN, NE	Central/ Southern AB, Eastern ON			Sichuan	–
F. fujikuroi	IN[j]				Sichuan	–
F. graminearum	IA, MN, SD, NE	Eastern ON		[k]	Sichuan	–
F. meridionale				[l]		–
F. nanum	SD,	–	–	–	–	–
F. oxysporum	IA, MN, SD, NE	Southern AB, MB, Central/ Southern AB, Eastern ON			Sichuan, Northeast	–
F. paranaense	–	–	South/ Central[m]			–
F. poae	IA,	Eastern ON				–
F. proliferatum	IA, MN, SD	Southern AB, MB; Central/ Southern AB			Sichuan	–

(continued)

Table 3.1 (continued)

Fusarium spp.	USA	Canada	Brazil	Argentina	China	India
F. redolens	MN,	Central/ Southern AB				–
F. semitectum	IA,					–
F. solani	IA, MN, SD, NE	Central/ Southern AB, Eastern ON			Sichuan	Udaipur[n]
F. sporotrichioides	IA, MN, NE	Central/ Southern AB, MB[o], Eastern ON				–
F. subglutinans	IA, MN	Central/ Southern AB				–
F. torulosum		Central/ Southern AB				–
F. tricinctum	IA,	Eastern ON				–
F. verticillioides	IA, MN	Central/ Southern AB			Sichuan	–
F. virguliforme	IA, SD	–				–

IA Iowa, *MN* Minnesota, *SD* South Dakota, *IN* Indiana, *NE* Nebraska, *ALB* Alberta, *MB* Manitoba, *ON* Ontario
[a] Díaz Arias et al. (2013)
[b] Bienapfl (2011)
[c] Okello et al. (2020)
[d] Parikh et al. (2018)
[e] Zhou et al. (2018)
[f] Chang et al. (2015)
[g] Zhang et al. (2013)
[h] Chang et al. (2018)
[i] Abdelmagid et al. (2018)
[j] Detranaltes et al. (2021)
[k] Barros et al. (2014)
[l] Chiotta et al. (2016)
[m] Costa et al. (2016)
[n] Tetarwal et al. (2013)
[o] Abdelmagid et al. (2021)

China (Li et al. 2018; Table 3.1). *Fusarium* root rot was a new record in India (Agarwal 1976), though Tetarwal et al. (2013) later reported soybean root rot caused by *F. solani* and *Rhizoctonia solani* in Udaipur, India and *Fusarium* spp. causing wilt and root rot in Assam, India (Borah 2019). Lastly, *F. graminearum* has been implicated in causing root rot in South Korea (Kang et al. 2019).

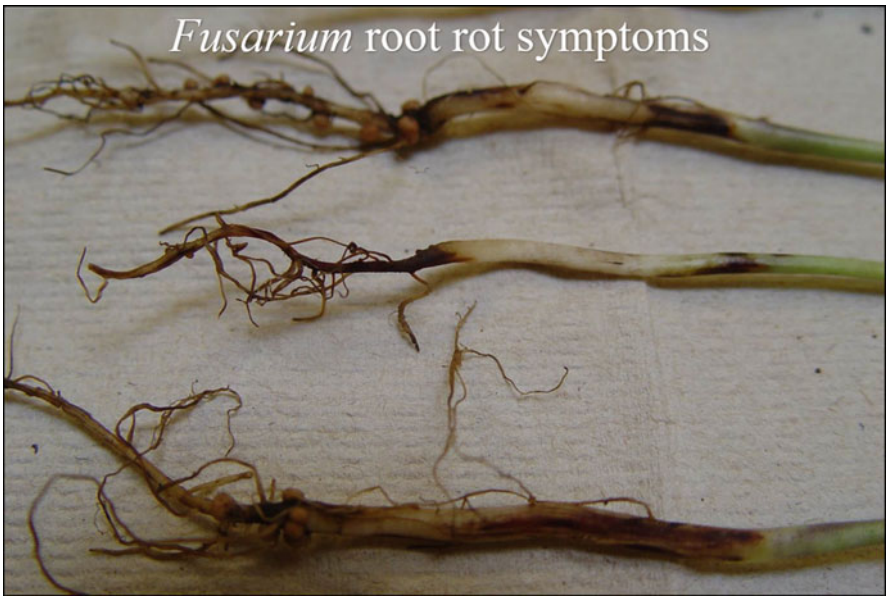

Fig. 3.4 Characteristic symptoms of *Fusarium* root rot of soybean. (Courtesy: Dr. Loren J. Giesler, Professor and Department Head, Department of Plant Pathology, 406 Plant Science Hall, University of Nebraska, Lincoln, NE 68583, USA)

3.4 Diversity of *Pythium* spp. Associated with Damping-Off of Soybean

Damping-off and root rot caused by the soilborne Oomycete pathogen *Pythium* spp. is an important seedling disease in soybean. Diverse species of *Pythium* have a wide host range, including grasses and a wide variety of dicotyledonous plants (Waterhouse and Waterston 1964). *Pythium* spp. cause reduced stand establishment, seedling emergence, kill emerged seedlings and reduce plant vigor (Kirkpatrick et al. 2006; Broders et al. 2009b). One of the biggest threats for US soybean growers is the rapid speed by which *Pythium* attacks soybean seed. The dormant propagules (oospores) germinate in response to seed and root exudates and infect seeds within 90 min of planting (Stranghellini and Hancock 1971). The infection leads to seed rot and the premature weakening and death of developing seedlings (Fig. 3.5), referred to as damping-off (Kirkpatrick et al. 2006). An estimated economic loss due to seedling diseases ranges from $67.60 million in 1999 to $779.92 million in 2008 (Navi et al. 2019).

Diversity of *Pythium* spp. associated with soybean in the United States, Ontario, Canada, Brazil, China, and India are compiled in this chapter. Rizvi and Yang (1996) reported the existence of the *Pythium* complex in Iowa soybean fields (*P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. sylcaticum*, *P. ultimum* var.

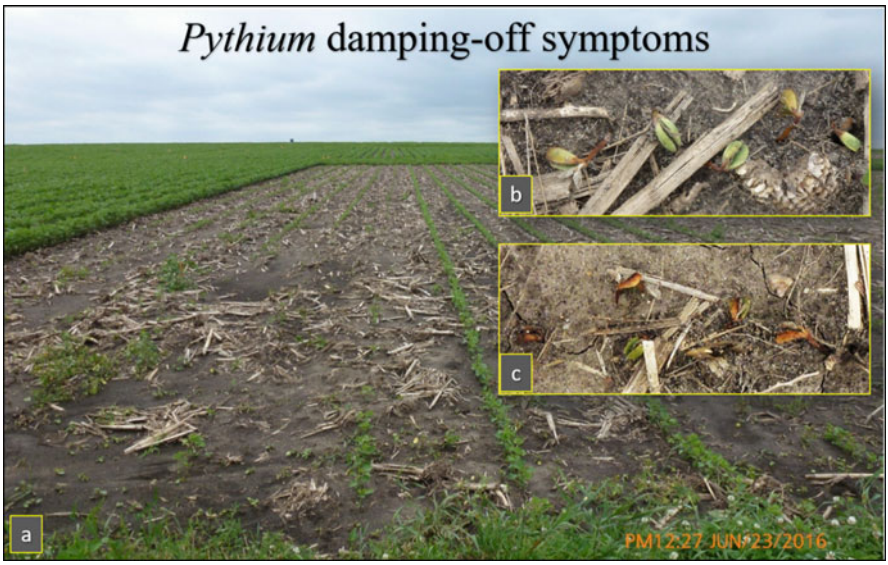

Fig. 3.5 Characteristic field symptoms of soybean *Pythium* damping-off observed during June 2016, Northeast Research Farm, Nashua, Iowa, USA ((**a**) gaps within rows, (**b**) damping-off symptomatic seedlings with green cotyledons, (**c**) damping-off symptomatic seedlings with dead cotyledons)

sporangiiferum, and *P. ultimum* var. *ultimum*). Surveys conducted across 11 major soybean-producing states in the United States and Ontario, Canada, reported 51 *Pythium* spp. in 2011 and 54 in 2012 (Rojas et al. 2017a, b). Navi et al. (2019) reported nine isolates of *Pythium* spp. (one isolate each of *P. orthogonon* and *P. torulosum*; three of *P. inflatum*; two of *P. ultimum* var. *ultimum*; and two isolates of *P. ultimum* var. *ultimum* or *P. ultimum* var. *sporangiferum*) from a single field in northeast Iowa. Elsewhere, in southeastern Pennsylvania, 25 *Pythium* species were recovered from ten-soybean-corn rotation fields (Coffua et al. 2016), and in North Dakota, a total of 26 known *Pythium* spp. were recorded in 125 fields surveyed (Zitnick-Anderson and Nelson Jr 2015). These include the first reports of *P. kashmirense, P. minus, P. periilum, P. rostratifingens, P. terrestris, P. viniferum*, and *P. violae* as pathogens of soybean seedlings and the first reports of *P. kashmirense, P. viniferum*, and *P. terrestris* in the United States (Zitnick-Anderson and Nelson Jr 2015). In Ohio, from three locations where soybean and corn stand establishment was a concern, *P. catenulatum, P. irregulare, P. paroecandrum, P. splendens*, and *P. torulosum* were reported (Dorrance et al. 2004). *P. delawarii*, a new species of *Pythium*, was also isolated from soybean in Ohio (Broders et al. 2009a). In Illinois, 27 species of *Pythium* were identified, and *P. cryptoirregulare*, *P. irregulare*, *P. sylvaticum*, *P. ultimum* var. *sporangiiferum*, and *P. ultimum* var. *ultimum* were highly pathogenic on soybean seedlings (Jiang et al. 2012). Four *Pythium* spp. (*P. lutarium, P. oopapillum, P. sylvaticum*, and

P. torulosum) from 11 states in the United States (A.R., IA, IL, IN, KS, MI, MN, ND, NE, SD, and W.I.) have been reported by Matthiesen and Robertson (2021).

In Ontario, Canada, six species of *Pythium* (*P. ultimum* var. *ultimum, P. sylvaticum, P. dissotocum, P. ultimum* var. *sporangiiferum*, *P. irregulare*, and *P. hypogynum*) associated with soybean damping-off have been recorded (Marchand et al. 2014). In southern Brazil, *P. conidiophorum, P. acanthicum, P. deliense*, *P. inflatum*, and *P. torulosum* associated with soybean damping-off has been reported (Molin et al. 2021). In Indore, India, the first report of root rot and damping-off in soybean caused by *P. deliense* was reported (Kumar et al. 2021). In China, from a survey conducted in the Huang-Huai region, one of the main areas of soybean–wheat rotation farming area 26 *Pythium* isolates were identified, of which 12 *Pythium* spp. were highly pathogenic. Some major species were *P. spinosum*, *P. ultimum*, *P.* species 1 (tentatively designated as "Candidatus *Pythium huanghuaiense*"), *P. aphanidermatum*, and *P. myriotylum* (Feng et al. 2020).

3.5 Diversity of *Macrophomina* spp. Associated with Soybean Charcoal Rot

Macrophomina phaseolina (Tassi) Goid. an ascomycete fungus is a soilborne fungus present worldwide that infects, over 500 plant species of economic significance, including sorghum and corn (Adeyanju et al. 2015). In our literature search, we did not find diverse species of *Macrophomina* causing charcoal rot. However, there are several reports of diversity in colony morphology, genetics (Mahdizadeh et al. 2011), simple sequence repeats in the US isolates (Baird et al. 2010) and our recent selective isolations in 2021 (Fig. 3.6). There are several first reports of charcoal rot of soybean in the USA; the first report in Iowa was in 2003 (Yang and Navi 2005).

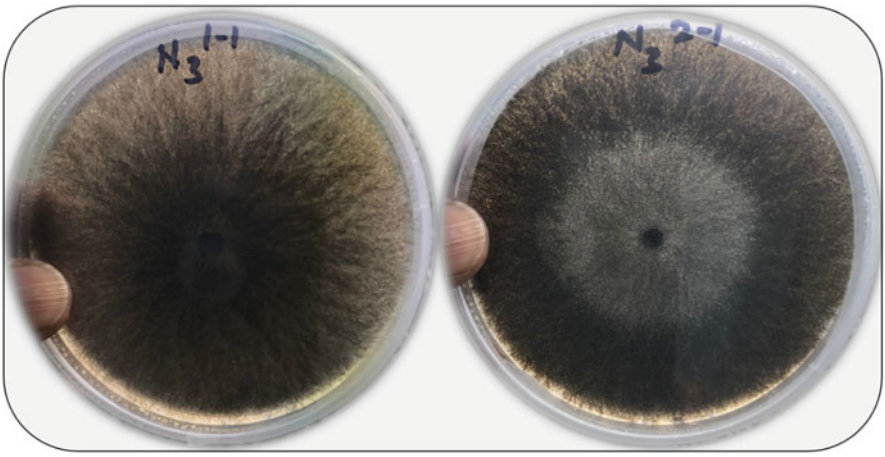

Fig. 3.6 Diversity in colony morphology of *Macrophomina phaseolina*, isolated from infected plants collected from Northeast ISU Research Farm, Nashua, Iowa, USA

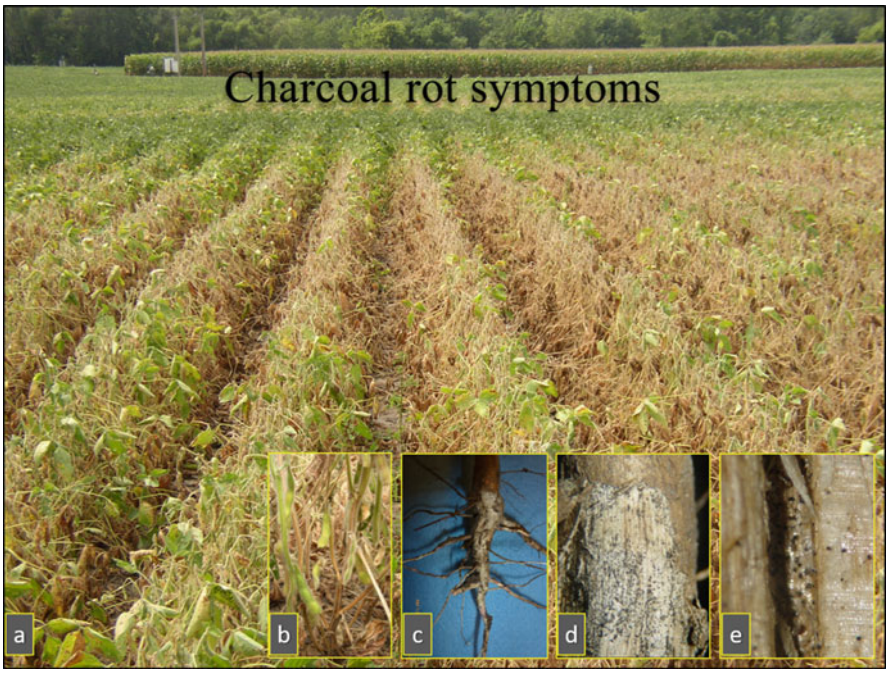

Fig. 3.7 Symptoms of soybean charcoal rot (*Macrophomina phaseolina*) observed during 2003 growing season ((**a**) field with severe charcoal rot symptoms, (**b**) unfilled upper pods, (**c**) microsclerotia beneath the epidermis of the taproot, (**d**) microsclerotia beneath the epidermis of basal stem, and (**e**) microsclerotia inside taproot and basal stems), Hinds Research Farm, Ames, Iowa, USA

Charcoal rot infected plants show wilting, premature yellowing of top leaves and premature leaf drop, premature plants seen in patches, unfilled upper pods, and in some cases, the upper 1/3 of a plant may have only the flat pods without seed production. Microsclerotia can be found beneath the epidermis of basal stem and taproot and inside roots and basal stems (Fig. 3.7). Also, *M. phaseolina* causes stem and root rot, charcoal rot, and seedling blight (Ghosh et al. 2018). Under high temperatures (30–35 °C) and low soil moisture (below 60%), this fungus can cause substantial yield losses in crops like soybean and sorghum, impacting the incomes of farmers (Kaur et al. 2012). In the worst-case scenario, 100% yield losses have been recorded in groundnut/peanut at the pre-emergence stage (Sharma and Bhowmik 1986). Some of the major symptoms of charcoal rot (Fig. 3.7) were captured in 2003 at the ISU Research Farm, Ames, Iowa.

3.6 Management Options to Minimize Yield Losses Due to Some of the Major Diseases of Soybean

3.6.1 Sudden Death Syndrome (*Fusarium* spp.)

Current management options of SDS to break the life cycle of the pathogen include (1) planting resistant varieties (Rupe et al. 1991), (2) delayed planting (Hershman et al. 1990), adjusting planting dates (Kandel et al. 2016b), (3) fall tillage (Wrather et al. 1995), (4) crop rotation (Wrather et al. 1995; Rupe et al. 1997; Xing and Westphal 2009), (5) modifying row spacing and seeding rate (Swoboda 2010), (6) fungicide seed treatments (Navi and Yang 2010; Weems et al. 2015; Kandel et al. 2016a, b; Sjarpe et al. 2019), (7) manganese phosphite seed treatment (Carmona et al. 2013), (8) use of biocontrol agents (Huynh et al. 2016; Navi et al. 2016a, b, 2018; Navi and Yang 2020), and (9) clean harvest of corn and soybean (Navi and Yang 2016). Growers have not fully adopted planting resistant varieties and adjusted planting dates, and the effects of crop rotation, tillage practices, and row spacing are inconclusive (Yang and Navi 2006; Navi and Yang 2016a, b).

3.6.2 *Fusarium* Root Rot

Various management approaches reported in the literature include:

1. Biochar amendment with soil reduced severity of root rot in greenhouse and field studies (Rogovska et al. 2017).
2. Integration of bio-agent (*Trichoderma harzianum* isolates) with several fungicides (Hexaconazole 5%EC, Pyroclostribin 5% + Metiram 55% WP, Carboxin 37.5% + Thiram 37.5% W.P., Carbendazim 50% W.P., Mancozeb 80% W.P.) and organic amendment (oil cakes of mustard, sesame, soybean, coconut, and tea waste) (Rahman et al. 2020).
3. Seed and soil treatments with biocontrol agents, such as *Rhizobium japonicum* (Al-Ani et al. 2012), *Bacillus subtilis* (Zhang et al. 2009; Li et al. 2013; Gao et al. 2015), *B. velezensis* (Huang et al. 2017), bacterial strains RSA-1 and RSA-13 (Rodovikov et al. 2021), *T. viride*, and *Bacillus* spp. (Tetarwal et al. 2013), and *Trichoderma* spp. (Pimentel et al. 2020).
4. Seed treatments with biocontrol agents (*T. viride* and *Bacillus* spp.) or a combination of biocontrol agents with fungicides (carbendazim, thiram, vitavax, bavistin, hexaconazole, tebuconazole, dithane M-45, and copper oxychloride 50) reduced *Fusarium* root rot compared with untreated controls (Tetarwal et al. 2013).
5. Fungicide seed treatments either alone or in combination (Apron Maxx RTA, EverGol Energy, Trilex EverGol, Rancona Summit + Apron XL, Rancona Summit + Maxim 480FS, Rancona Summit + Maxim 480FS + Vibrance, Vibrance + Apron XL, Vitaflo 280 and Vitaflo 280 + Apron XL) improved

seedling emergence and reduced *Fusarium* root rot severity (Nyandoro et al. 2018).

6. Corn-soybean intercropping significantly reduced *Fusarium* root rot (Chang et al. 2020).

3.6.3 *Pythium* Damping-Off

Cooler soil temperature and wet soil (high soil moisture or saturated level) play a key role in infection. Fungicide (Metalaxyl/Mefenoxam) seed treatment is the most widely used method to reduce losses due to this disease in interfering life cycle of the pathogen. Mixed results have been observed from fungicide seed treatments (Bradley et al. 2001; Bradley 2008). Most of the available fungicides are labeled for use against specific pathogens like *Rhizoctonia*, *Fusarium*, and *Macrophomina*, but some oomicides are used against oomycetes such as *Phytophthora* and *Pythium* (Sweets 2006). Bradley (2010) suggested combining fungicides effective against oomycetes (mefenoxam or metalaxyl) with at least one other fungicide (e.g., fludioxonil, trifloxystrobin, pyraclostrobin, or ipconazole) to provide additional control of *Fusarium*, *Rhizoctonia*, or other fungal pathogens. Seed treatments with potassium and manganese phosphites to control *Pythium* damping-off represent a feasible alternative to fungicides proposed by Carmona et al. (2018). The use of biocontrol agents has also been proposed (Navi et al. 2016a, b). Recently, the efficacy of quinone outside inhibitors (QoI), demethylation inhibitors (DMI), and succinate dehydrogenase inhibitors (SDHI) were tested for seed treatments in control of *Pythium* damping-off and yields of soybean (Navi et al. 2019).

3.6.4 Charcoal Rot (*Macrophomina phaseolina*)

Management of *M. phaseolina* remains a challenge. This pathogen results from interactions between the host plant, the pathogen, and the biotic and abiotic components of the environment. Several effective strategies have been reported. Although crop rotation is not a reliable management approach due to its wide host range and the persistence of microsclerotia in soil, avoidance of continuous soybean (Short et al. 1980; Almeida et al. 2008), tillage (Baird et al. 2003), no-tillage (Mengistu et al. 2009), application of phosphorus and potassium (Mengistu et al. 2016), irrigation (Kendig et al. 2000), biological control (Gacitua et al. 2009; Senthilkumar et al. 2009; Choudhary 2011; Al-Ani et al. 2012; Elham et al. 2016; Khalili et al. 2016; Navi et al. 2016b, 2018; Navi and Yang 2020), manganese application (Gettier et al. 1984), fungicide application (Hooda and Grover 1989; Yang et al. 2003, 2004; Lokesh et al. 2020), lower plant densities, and planting of resistant varieties (Coser et al. 2017) have all been proposed as potential management strategies. In a recent review, Marquez et al. (2021) have compiled various management strategies such as promotion of plant defense response using biocontrol agents, chemical elicitors via systemic acquired resistance, breeding for resistance,

agronomic practices (biosolarization and irrigation), biological control using arbuscular mycorrhizal fungi, and use of plant metabolites. Similarly, Romero Luna et al. (2017) compiled disease diagnosis and management approaches (crop rotation, tillage, irrigation, fertility and plant nutrition, wed control, cyst nematode management, fungicides, biocontrol, cultivar selection, and screening methods).

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Fungi and Mycotoxin in Rice: Concerns, Causes, and Prevention Strategies

4

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Abstract

Rice is an important staple food for more than 50% of the population worldwide. However, contamination of rice grains with microorganisms, particularly fungi, and their associated toxins causes deteriorated rice quality. The pathogenic fungi can attack rice crops before or after harvest, causing colossal damage and diseases such as rice blast, the most challenging disease of rice grains. Microbial contamination of the grains can lead to loss of germination ability due to the consumption of carbohydrate and oil reserve of the grain by microorganisms. Fungal infection can cause other issues, including off-flavor, color changes, the disintegration of the structure, mycotoxin generation, and the production of allergic compounds. The consumption of fungal and mycotoxin contaminated rice has been negatively associated with humans' health disorders and is thus considered a food safety issue. Therefore, there is a desperate need to control and combat fungal contamination in rice. This chapter provides a consolidated review on the occurrence of fungi and their associated toxins in rice grains, their effects on rice quality, and

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_4

other related issues. Preventive methods for fungal and toxin occurrence in rice grains are also summarized.

Keywords

Rice · Fungi · Toxins · Preventive strategies · Quality · Pre- and post-harvest

4.1 Introduction

4.1.1 Fungal Contamination in Rice Grain

Rice is an important staple food comprising 75% of the food composition of the world population. Also, it provides 60% of the food intake in South Asia (Al-Zoreky and Saleh 2019). Rice grains infected by microorganisms and their associated toxins are named diseased rice grains (Christensen and Kaufmann 1965). Microbial contamination of the grains can lead to loss of germination ability and carbohydrate and oil reserves consumption by microorganisms (Mohapatra et al. 2017). In addition, fungal infection can cause flavor deterioration, discoloration, the disintegration of the structure, production of mycotoxins, and allergenic compounds (Filtenborg et al. 1996; Atungulu et al. 2018).

Microorganisms may infect rice grains in the field or after harvest during storage (Christensen and Kaufmann 1965). Fungi can contaminate different forms of rice, as shown in Table 4.1. Grains are usually stored at the moderate moisture content (MC) after harvest (MC $<$ 15%). At this condition, bacterial spoilage is seldom but fungal, and pests are the primary cause of spoilage (Magan and Aldred 2007). Spores of fungi can contaminate rice grains in the field. They grow very fast in favorite conditions and cause damage to rice grains. *Cladosporium*, *Alternaria*, *Fusarium*, *Helminthosporium*, and *Pullularia* are some fungi found on rice grains in the field (Anthony et al. 2009; Mohapatra et al. 2017).

On the other hand, *Penicillium* and *Aspergillus* are the fungal species that contaminate the grains during storage (Anthony et al. 2009; Kushiro 2015). *Aspergillus flavus* is the most prevalent source of fungal contamination in rice all over the world. It is tolerant to a wide temperature range and low relative humidity (RH). Also, it can colonize on the surface of stored rice grains (Mannaa and Kim 2016). Studies are also available which reported the presence of other fungi species. Commercially available rice samples in Thailand were tested to investigate the presence of *Penicillium* species and mycotoxin contamination. Ten samples were collected, of which seven were contaminated with *Penicillium* species. *Penicillium citreonigrum* and seven *P. brocae* isolates were identified in the rice samples (Shiratori et al. 2017). In a study in Korea, *Fusarium* spp. were the most dominant fungi in rice samples and by-products generated by rice milling. Other fungal contamination included *Penicillium*, *Aspergillus*, and *Alternaria* spp. (Lee et al. 2011).

Table 4.1 Fungal contamination in a different type of rice reported by various studies

Source	Region	Fungal species	Reference
Rough rice	Arkansas, Louisiana	*Alternaria*, *Helminthosporium*, *Curvularia*, *Penicillium*, *A. flavus*, *Cladosporium*, *Scopulariopsis*, *Paecilomyces*, *Mucor*, *Rhizopus*, *Fusarium*	DeLucca et al. (1978)
Rice bran from raw rice and parboiled rice	Madras/India	*A. flavus*, *A. candidus*	Jayaraman and Kalyanasundaram (1990)
Discolored rice grains in the field	Philippines	*Drechslera oryzae*, *Curvularia lunata*, *Trichoconiella padwickii*, *Sarocladium oryzae*, *Alternaria tenuis*, *Fusarium solani*	Lee et al. (1986)
Discolored paddy seeds	India	*Drechslera oryzae*, *Curvularia lunata*, *Phoma* sp., *Alternaria alternata*, *Nigrospora oryzae*	Misra and Vir (1988)
Stored rice seeds	Philippines	*Alternaria padwickii (Ganguly) MB Ellis*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Rhizopus* sp., *Tilletia barclayana*	Misra et al. (1995)
Stored unhulled and white rice	Korea	*A. flavus*, *A. fumigatus*, *A. candidus*, Various species of *Penicillium*	Oh et al. (2010)
Polished rice	Korea	*P. citrinum*, *A. candidus*, *A. versicolor*, *A. niger*, *A. flavus*, *A. ochraceus*, *F. proliferatum*	Park et al. (2005)
Rough rice	Indonesia	*Curvularia* sp., *Fusarium* sp., *A. flavus*, *Rhizopus* sp., *P. purpurogenum*, *P. miczynskii*, *A candidus*	Phillips et al. (1988)
Commercially available rice grains	Thailand	*P. citreonigrum*, *P. brocae*	Shiratori et al. (2017)

4.2 Fungi and Mycotoxin Generation in Rice

Mycotoxins are secondary metabolites of fungi that are produced on food and feed ingredients (Table 4.2). Mycotoxins can pose serious health effects on humans and animals. About 25% of agricultural products are contaminated with mycotoxins each year. About 4.5 billion people in developing countries are exposed to aflatoxins (Atungulu and Mohammadi-Shad 2019). Aflatoxins are the most toxic and prevalent group of mycotoxins (Kabak et al. 2006). Not all species of fungi can produce mycotoxins (Fleurat-Lessard 2017). Mycotoxin contamination is limited to a particular food. Corn and related products such as corn silage and corn distillers dried grains are frequently contaminated with fungi and their associated mycotoxins (Mohammadi Shad et al. 2019b, 2021). Knowing the specific type of fungi that infect individual foods is essential for acting against mycotoxin contamination

Table 4.2 Mycotoxin contamination in different agricultural products

Source	Region	Mycotoxin	Reference
Rice (white, brown, red, black, basmati, Jasmine)	Canada	Aflatoxin B1 & B2, Ochratoxin A, Fumonisin B1 & B2 & B3	Bansal et al. (2011)
Paddy and brown rice	UK	Aflatoxin B1	Castaño et al. (2017)
Corn and rice samples from wholesale, retail and household	Vietnam	Aflatoxins, Fumonisin B1	Huong et al. (2016)
Five varieties rice samples from vendors, superstores and processing mills	Pakistan	Aflatoxin B1 & B2 & G1 & G2	Iqbal et al. (2014)
Rice bran from raw rice and parboiled rice	India	Aflatoxins	Jayaraman and Kalyanasundaram (1990)
Rice	China	Aflatoxin B1 & B2, Ochratoxin A	Lai et al. (2015)
Brown rice, blue-tinged rice, discolored rice, polished rice	Korea	Deoxynivalenol (DON), Nivalenol (NIV), Zearalenone (ZEA)	Lee et al. (2011)
Imported rice samples	Iran	Aflatoxin B1 & B2 & G1 & G2	Mazaheri (2009)
Imported rice samples	Saudi Arabia	Aflatoxin B1 & B2 & G1 & G2	Al-Zoreky and Saleh (2019)
Unhulled and brown rice	Korea	Aflatoxin B1 & B2 & G1 & G2	Oh et al. (2010)
Polished rice	Korea	Aflatoxin B1, Fumonisin B1, Ochratoxin A, Deoxynivalenol, Invalenol, Zearalenone	Park et al. (2005)
Rice	Thailand	Aflatoxin B1	Shiratori et al. (2017)

(Filtenborg et al. 1996). Six major types of mycotoxins happened in food ingredients, including aflatoxins, ochratoxins, patulins, fumonisins, trichothecenes (Deoxynivalenol (DON)), T2 toxins and HT-2), and zearalenone (Atungulu et al. 2018). *Penicillium* and *Aspergillus* are the two significant fungi that produce mycotoxin in stored grains in poor storage conditions (Filtenborg et al. 1996; Fleurat-Lessard 2017).

Aspergillus is more resistant to low water activity (aw) compared to other species. Several toxin-inducing species of *Aspergillus* have been recognized on stored rice, such as *A. candidus*, *A. flavus*, and *A. niger* (Filtenborg et al. 1996).

Aflatoxin contamination in rice has been reported all over the world. There are aflatoxin prevalence reported in rice from Tunisia and Japan. However, the aflatoxin contamination in rice reported in the Philippines, India, Spain, and Nigeria was high (Elzupir et al. 2017). Aflatoxins are produced by *A. flavus*, *A. parasiticus*, and *A. nomius* (Filtenborg et al. 1996). Aflatoxin B1 (AFB1) production by *A. flavus* was reported in rice, while aflatoxin B2 and G2 were not detected in rice samples (Reddy et al. 2009; Elzupir et al. 2015). In other studies, prominent aflatoxin families

B1, B2, G1, and G2 were produced by *A. parasiticus* in rice (Elzupir et al. 2015). AFB1 is reported as a group 1 carcinogen and fumonisin B1 and B2 as group 2 carcinogens by International Agency for Research on Cancer (IARC) (Reddy et al. 2008). Not all *Aspergillus* strains can produce mycotoxins. Non-aflatoxigenic species of *Aspergillus* include *A. oryzae* and *A. sojae*. These fungi are used in industries for fermentation and production of food-grade amylase (Reddy et al. 2009).

Generally, the level of aflatoxin contamination in rice has been reported less than in other grains. For example, the aflatoxin contamination in stored rice was minor than peanuts and other agricultural products. A number of rice samples (71) imported to Iran were investigated to measure AFB1, AFB2, AFG1, and AFG2 contamination. It was reported that 59 samples out of 71 samples were contaminated with aflatoxins; only two rice samples had aflatoxin levels more than the allowable limit considering local regulation (5 μg/kg) (Mazaheri 2009). AFB1 and total aflatoxin concentration in different rice cultivars collected in the local retail markets in Saudi Arabia were investigated. It was reported that total aflatoxin and AFB1 complied with the European (EU) limit for aflatoxin (Al-Zoreky and Saleh 2019).

Other groups of mycotoxins such as ochratoxin (OTA), patulin, and citrinin that are produced by *Penicillium* also occur in rice samples. *Penicillium verrucosum* and *P. nurdicum* are identified as species that produce ochratoxin A (OTA) in rice grains. It was reported that OTA is the most commonly found mycotoxin in rice in North Korea (Park et al. 2005). In another study in Canada, imported rice samples were investigated for aflatoxin, OTA, and fumonisins levels; the AFB1 and B2 and OTA and fumonisin B1 were found in different percentages. For example, fumonisin B1 was the highest level of contamination in all the investigated rice samples related to contamination of the grains in the field. It was also suggested that AFB1 contamination of the grains mainly happened during storage (Bansal et al. 2011). OTA is produced by limited species such as *A. alliaceus*, *A. ochraceus*, and *P. verrucosum* (Filtenborg et al. 1996). Fungi can also produce citreoviridin, luteoskyrin, and cyclochlorotine mycotoxins in rice grains (Kushiro 2015).

4.3 Mycotoxin Contamination and Health Issues

Penicillium species, *P. fellutanum*, can produce ergot alkaloids (agroclavine 1 and epoxyagroclavine 1) in rice (Mannaa and Kim 2016). The ergotoxins cause a disease named Holy fire or Saint Anthony's fire and, more recently, called ergotism. These alkaloids can produce symptoms like a painful spasm, diarrhea, nausea, headache, or gangrenous symptoms in the fingers and toes (Mannaa and Kim 2016).

Mycotoxin produced by the comparatively rare fungal species, *P. citreonigrum*, is called citreoviridin (CTV). It can lead to beriberi disease, and the first recorded historical data related to an outbreak of this disease was in 1937 in Japan; it was associated with the consumption of contaminated rice imported from Thailand. A recent outbreak of this disease was reported in Brazil in which 1207 people were infected, and 40 of them died. The investigation showed that not all the rice samples

were contaminated with citreoviridin, and only one rice sample was contaminated with AFB1 (Shiratori et al. 2017). A study of mycotoxin contamination in rice in Northern Vietnam revealed that aflatoxin and fumonisin contamination are actual health hazards for ethnic minority groups consuming these staple foods (Huong et al. 2016). The contamination level of aflatoxins has been reported comparably low in rice grains. However, considering the high consumption rate of rice, this contamination is significant (Elzupir et al. 2015). For instance, rice, a staple food in the Korean diet, is the main contributor to the daily intake of AFB1 in Korea (Park et al. 2005).

4.4 Mycotoxin Contamination and Regulations

Mycotoxin contamination is a food safety concern, and it is regulated in many countries. Non-complying with these regulations will lead to the destruction of rice grains. This will lead to economic losses for producers (Fleurat-Lessard 2017). Due to health concerns, the maximum residue limit (MRL) of this contaminant is regulated by the European Commission (EC). The maximum allowable aflatoxin in raw grain used for human consumption is 2–5 μg/kg. However, for the cereals used in infant formula and young children's food, MRL is 0.1 μg/kg for aflatoxin and 0.5 μg/kg for ochratoxin. AFB1 limit in rice as per EC regulation is 2 μg/kg, and that of total aflatoxin is 4 μg/kg (Al-Zoreky and Saleh 2019).

This MRL for the cereal used as animal feed is higher compared to human food. The maximum contamination limit of ochratoxin in complete feedstock used for feeding pigs and poultry (older than 4 months) is 50–100 μg/kg and for complementary feed is 250 μg/kg. MRL for aflatoxin in all the feedstuff is 5 μg/kg for dairy calves below 4 months and 20 μg/kg for less sensitive animals like poultry and pigs. According to World Health Organization (WHO), the regulation of maximum aflatoxin contamination in grains is 30 μg/kg. Ergot alkaloid is a less toxic mycotoxin. So, MRL for them is 0.05% in the grains used for human consumption. United Kingdom (UK) has more stringent regulations in this regard. This limit is ten times less, 50 mg/kg (Fleurat-Lessard 2017).

4.5 Contributing Factors to Fungal and Mycotoxin Generation

Environmental conditions and storage practices are two important factors contributing to fungal contamination of grains and mycotoxin generation (Filtenborg et al. 1996; Iqbal et al. 2014). Critical factors that imply fungal contamination of grains are listed as grain MC, temperature, initial contamination rate, type of storage practices, the physical structure of grains, and pest infestation.

4.5.1 Moisture Content (MC)

Safe storage MC recommended for rice grains is 120 g/kg or 12% (Pardo et al. 2004; Atungulu et al. 2016; Mohapatra et al. 2017). For every 10 g (1%) reduction of moisture, 1 year is added to the shelf life of the rice grains. Restricted moisture availability in the grains leads to reduced respiration rate, microbial contamination, and enzymatic activity in the grains (Pardo et al. 2004; Mohapatra et al. 2017). MC and aw are directly linked together. At water activities below 0.65, most of the fungal growth and germination of the spore would be stopped (Fleurat-Lessard 2017). To prevent rice quality loss and microbiological contamination, freshly harvested rice with a high MC should be dried fast (in 24–48 h) to a moisture level of about 14% and stored at 5 °C (Atungulu et al. 2015; Zhao et al. 2021).

Commercial rice drying facilities may not fully dry the grains during the short harvesting season immediately. Storage of rice at MCs more than 14% led to quality deterioration such as temperature increase, loss of germination, and milled-rice discoloration (Atungulu et al. 2019). Physiochemical changes in rice grains stored in different MCs were reported to be related to fungal contamination. Upon increasing storage duration, normal rice seedling was reduced. This change was related to the reduction of dehydrogenase activity during storage. The number of dead seeds was very high after 12 months of storage. Also, during storage, the whiteness of the grain was reduced, which was related to increasing the brown and white rice (Misra et al. 1995). Finally, accidental rewetting of the grain or generation of the hot spots may lead to fungal contamination (Fleurat-Lessard 2017).

4.5.2 Storage Temperature

The investigation related to the impact of delayed drying or wet holding of the grain on the quality deterioration of rice grains revealed that the initial mold count, MC of the grain, storage duration, and temperature affected microbial contamination of the rice. At low MC, temperature and storage duration did not show significant effects on total mold count. Also, the total microbial count was stable and did not increase when storage temperature was maintained low (10 and 15 °C). Finally, it was concluded that the optimum condition for rice storage was moisture level below 17%, temperature below 27 °C, and storage duration for a maximum of 6 months. At this condition, the microbial load did not increase. Even microbial count reduction was reported during storage at low MC (Atungulu et al. 2016).

The optimum temperature for fungal growth was suggested from 20 to 40 °C. In case of delay in rice drying, cooling of the grains is recommended during a couple of storage months to prevent fungal infection (Atungulu et al. 2016; Fleurat-Lessard 2017; Mohapatra et al. 2017). The lower limit of MC for fungal growth is less than the moisture requirement for mycotoxin generation (Fleurat-Lessard 2017). In a study, *A. parasiticus* contamination and aflatoxin production in Basmati rice was investigated. This study was conducted at 31% MC and temperatures 25 and 37 °C. After 1–3 weeks of storage, there was more fungal contamination at 25 °C, but more

aflatoxin was detected at 37 °C. At 25 °C, only AFB1 and AFB2 were detected, but at 37°, AFB1, AFB2, AFG1, and AFG2 were detected (Al-Zoreky and Saleh 2019). Safe MC and temperature should be provided to store rice grains for the expected duration without any quality deterioration (Fleurat-Lessard 2017).

4.5.3 Grain Morphology

Along with weather conditions, morphological differences between rice cultivars are essential to determine the microbial contamination of rice grains. Different rice cultivars (long-grain hybrid, long-grain pureline, and medium grain) harvested in 2013 and 2014 in Arkansas were investigated for bacterial and fungal contamination. It was reported that long-grain hybrid and pureline cultivars were less contaminated compared to medium-grain cultivars in both consecutive years. Medium-grain rice cultivars are typically harvested at higher MC, so the aw of the grain is higher, making them more susceptible to fungal contamination (Atungulu et al. 2015). Similarly, Mohammadi Shad and Atungulu (2019) reported more fungal contamination on long-grain hybrid rice (XL753) than long-grain pureline (Roy j) and medium grain (Titan).

4.6 Storage Condition and Type of Fungal and Mycotoxin Contamination

The MC of rice grains defines fungal contamination on the grain (Trigo-Stockli and Pedersen 1994). Regarding weather conditions, different fungal contaminants were found in cereal grains stored at different MCs and temperatures. *Aspergillus* and *Penicillium* are the most tolerant fungi in a dry environment. *Aspergillus*, *Penicillium*, and *Fusarium* are the three predominant fungi responsible for mycotoxin contamination in agricultural commodities (Mannaa and Kim 2016).

Even at the safe storage condition after harvest, rice grains and microbial contaminants continue to have slow respiration (Magan and Aldred 2007). With the increasing storage duration, biochemical deterioration of rice grains was reported. These changes included increased soluble sugar and fat acidity value. Different fungal species were isolated from rice samples collected in the Philippines at different MCs and storage duration. The majority of these fungal species were weak parasites or saprophytes in nature, and the rest were rice pathogens. Pathogenic fungal infection during storage decreased, and saprophytic species increased (Misra et al. 1995). This change was related to the different moisture requirements of these fungal species.

Field fungi that mainly infect rice grains in the field require 24–25% MC for their growth. Because usually MC of rice grains after harvest is less than this rate, these fungi typically do not grow on rice grains after harvest. Meanwhile, storage fungi can tolerate MC less than 16%; they do not contaminate rice grains before harvest.

The storage fungi mainly include several species of *Aspergillus* (Christensen and Kaufmann 1965).

The combined effect of aw and temperature and carbon dioxide (CO_2) was investigated on *A. flavus* growth and aflatoxin production on paddy rice during storage before drying. It was reported that aflatoxin generation was positively linked to aw values. A negative correlation was noted between fungal growth and mycotoxin generation with increasing CO_2 concentrations in the atmosphere. No fungal growth was reported in 80% CO_2 concentration and 0.92 aw during storage. No aflatoxin production was reported in this condition, enriched with CO_2 and oxygen levels below 1%. Fungal infection and aflatoxin production were optimal at 30 °C and 0.98 aw (Mousa et al. 2016).

AFB1 contamination in two varieties of paddy and brown rice forms was investigated at different aw and temperatures. It was reported that paddy rice was less contaminated. It was proposed that it is due to the less accessibility of rice-endosperm starch by fungal contamination. Also, higher MC and temperature, increased respiration rate, dry matter loss (DML), and AFB1 contamination were observed. The DML and respiration rate can be used as indicators for fungal contamination and AFB1 generation (Castaño et al. 2017).

In a study, 370 rice samples were collected in six different provinces of China and the AFB1 and OTA levels were measured. It was reported that 63.5% (235 out of 370) of the rice samples were contaminated by AFB1, and 4.9% (18 out of 370) were contaminated by OTA (Lai et al. 2015).

Early rice cultivation may lead to early harvest before changing weather during the filling stage of the grain, resulting in increased MC and susceptibility of rice grains to microbial deterioration (Atungulu et al. 2015).

4.7 Mitigation Strategies for Fungal Contamination in the Grains

There are three strategies to control mycotoxin contamination; the first is to prevent mycotoxin generation, the second is detoxification, and the third is to prevent adsorption of mycotoxins (Kabak et al. 2006). Mycotoxins are resistant to heat treatment and processing, and they can transfer to the final product (Filtenborg et al. 1996). So, prevention is the best strategy to control mycotoxin contamination. There are different methods to prevent fungal contamination, as discussed below:

4.7.1 Chemical Fungicide

Several chemicals have been used to prevent fungal contamination. Phosphine is a toxic gas that is useful to control insects and molds. It had been used in a concentration from 1000 to 2000 ppm to control *A. flavus* and *A. parasiticus*. Other effective fungicides to control AFB1 are dichlorvos, landrin, malathion, and diazinon (Kabak et al. 2006). Dithane M45 and Benlate (Benomyl) are chemical fungicides that can

effectively control fungal contamination in rice grain without any harmful effect on the plant cells and protoplast. A study was conducted to investigate fungal growth on culture media after using benomyl fungicide for rice plants. It was reported that this fungicide could discourage the *Penicillium* growth (Hauptmann et al. 1985).

Natamycin is another preservative used against molds and yeast. It is mainly used for surface treatment in cheese and sausage. Due to low solubility, it does not migrate from the surface into the food. If taken orally, it is reported least toxic, and after 7 days of feeding more than 500 mg/day, no natamycin was absorbed from the human intestine (Thomas and Broughton 2003). In a study by Mohammadi Shad and Atungulu (2019), natamycin was applied to rice grains during storage. It was reported that this fungicide was able to limit mold growth and milled-rice discoloration slightly. However, the same study reported a significant reduction of fungi and rice discoloration in rice samples treated with sodium chloride.

Chemical treatments with propionic, acetic, sorbic acid, hydrogen peroxide, and ammonium hydroxide are other methods to control fungal contamination (Naseer et al. 2014). Propionic acid is reported to have an antifungal effect on grains stored in an open area. The combination of acetic acid and formaldehyde was used to preserve grains targeted for animal feed (Bothast 1978). Injection of gaseous ammonia into the silo was recommended to control fungal contamination in high moisture grains (Bothast 1978).

4.7.2 Non-chemical Treatments

Chemical residuals resulted from the use of fungicides can lead to health side effects. Because of consumers' concerns, several non-chemical strategies have been introduced to control fungal infection. These strategies include drying and aeration, hermetic storage, dielectric heating, cold plasma treatment, ozonation, irradiation, ultra-superheated steam treatment, and application of vegetable oils and plant derivatives (Mannaa and Kim 2016; Mohapatra et al. 2017). Grain drying before storage and the use of clean and insect-free grains for storage are some of the strategies to prevent fungal contamination of the grains. Removal of rice bran with the highest volume of fungal contamination was also recommended to reduce fungal contamination (Jayaraman and Kalyanasundaram 1990). Interestingly, the unhulled rice samples were less contaminated with fungi than white or milled rice in Korea. However, aflatoxin contamination in unhulled rice was 2.45 and 3.43 ng/g and in white rice was 1.29–2.09 ng/g (Oh et al. 2010).

The parboiling process is another effective technique to reduce fungal contamination and aflatoxin generation in rice grains and the bran. The parboiling process before rice milling reduces fungal and mycotoxin contamination on rice grains (Jayaraman and Kalyanasundaram 1990). Prevention of aflatoxin contamination is possible through several approaches like aeration, cooling, and rice storage at low moisture of less than 100 g/kg (Bothast 1978). Solar drying used by small and medium-scaled farmers is too slow, and during prolonged drying duration, mold infection can happen. On the other hand, condensation at night can cause moisture to

increase. One technology for rice drying is the in-bin drying system. It is forecasted that in the United States, 20% of the rice is dried in the farms using an in-bin drying system (Atungulu et al. 2015).

Rice dried using natural air in-bin drying is also prone to fungal growth and mycotoxin synthesis (Atungulu et al. 2016). One of the concerns about using this system for rice drying is slow drying from the bottom layers to the upper layers. This delayed drying may lead to fungal contamination and rice quality losses (Atungulu et al. 2015). It was also reported that mold infection was higher at the upper layer than bottom layers during the storage of the grains in the bin. At the start of this period, field fungi like *Fusarium* and *Curvularia* were predominant. However, later on, as drying continued, *A. flavus* and *Fusarium*, and *Penicillium* increased during 8 weeks of storage (Phillips et al. 1988).

During storage of the grains in metal storage bins, condensation is one of the most critical environmental issues that will lead to the MC increase in grain and fungal contamination. This is especially important in the grains in contact with the cold metal surface in the bins. They get a load in MC due to the moisture condensation. This will exceed the safe moisture limit for the storage of the grains. It will increase fungal contamination and create hot spots in rice grains. This will result in grain deterioration and pest infestation in the bulk rice grains (Fleurat-Lessard 2017).

Ozone technology was also recommended as an effective method to maintain rice quality by retarding the fungal and insect contamination. Ozone can eradicate different microorganisms, including bacteria, fungi, viruses, protozoa, and fungal spores. It also rapidly breaks down to oxygen without leaving any residues in rice. When ozone decomposes, it binds water and reduces water content in rice. It should be considered that excessive use of ozone can lead to the degradation of protein, carbohydrates, and fat (Nur et al. 2015).

A combination of preventive methods has also been recommended, such as reduced temperature coupled with low MC, reduced O_2 and increased CO_2 of the atmosphere, and chilling combined with treating with chemicals (Naseer et al. 2014). For example, rice grains should be dried to a safe storage MC and stored in hermetic conditions (Mohapatra et al. 2017). When corn and wheat grains were stored in silo bags, the CO_2 increased, and O_2 decreased in the bag. This is related to the respiration of the grains during storage. This increase in CO_2 was more in corn than wheat grains. Two reasons were proposed for this difference: it can be related to the higher respiration rate in corn, and smaller grains in wheat lead to a more intact mass of the grains, thereby less respiration activity due to less oxygen availability. Thus, the hermetic environments are recommended to store the grains; because no fungal contamination and mycotoxin infection were reported in the grains (Gregori et al. 2013).

CO_2 becomes liquid that is named supercritical carbon dioxide (SC-CO_2) in high pressure and temperature. The SC-CO_2 can destroy microorganisms by extracting some components from the inside (Mohapatra et al. 2017).

4.7.3 New Sophisticated Technologies

Several studies investigated the effectiveness of new technologies to prevent fungal contamination. A combination of microwave heating and tempering treatment was found to be very effective in controlling fungal contamination. Microwave and radiofrequency heating have some disadvantages, including non-homogeneous heating of the whole bulk and loss of quality and germination ability, making these grains unsuitable for sowing. Another method to preserve rice grains is irradiation which uses Co-60 and Selenium as the source of radiation. Electron beam irradiation (EBI), laser beam radiation, and ultraviolet radiation are three methods used to eradicate fungi and bacteria in rice grains. Rice grains irradiated with nonthermal EBI (dose of 14 kGy) could deactivate more than 99% of fungi present on the grains (Mohammadi Shad et al. 2019a). Another non-chemical treatment to decontaminate grains is cold plasma or corona discharge treatment based on ionized gases, radicals, and electrons. The advantages of these methods are:

1. uniform exposure to the whole surface of the grain,
2. can be active at low temperature,
3. have good diffusivity even into complicated structures,
4. short processing time,
5. non-destructive in nature,
6. do not leave any toxic material and are safe for consumers and processors.

Pulsed light (PL) irradiation is another technology that uses high-intensity light in few seconds to remove contaminating microbes on food. This technology is suitable to decontaminate the grains' surface, but more research is needed for using this technology for bulk stored grains (Mohapatra et al. 2017). PL is an FDA-approved nonthermal method, which is proposed for detoxification of mycotoxin contamination. Research was conducted in China in which contaminated rough rice and husk with AFB1, AFB2, and OTA were exposed to PL irradiation. It was found that the PL method reduced the mycotoxin level of both samples, but it was more effective in the detoxification of husk. It was suggested that since the husk is a thin layer, it was more exposed to irradiation. However, the rough rice thickness and the cracks prevented total exposure of the rice grains to irradiation; this is called the shadow effect, affecting the effectiveness of this technology. The PL technology was more effective in eliminating AFB1 as compared to AFB2. The temperature was kept below 49 °C because the temperature increase due to PL may affect starch gelatinization (Wang et al. 2016).

4.7.4 Biological Control

The antifungal activity of some microorganisms is another alternative for chemical approaches. Sangmanee and Hongpattarakere (2014) investigated the antifungal activity of *Lactobacillus plantarum* to control growth and aflatoxin production of

A. flavus and *A. parasiticus*. This antifungal activity is related to the damage of the cell wall that causes cytoplasmic destruction. The use of these bio-preservatives could be adequate to control fungal contamination in animal feeds (Sangmanee and Hongpattarakere 2014). Knowing specific molds contaminating individual foods is essential to prevent mycotoxin contamination (Filtenborg et al. 1996).

Biocontrol of fungal contamination is through the hindrance of fungal growth, mycotoxin production, or degradation of mycotoxins. *Trichoderma* was a microbial species that can control *Aspergillus* and AFB1 production (Reddy et al. 2009). *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* bacteria were effective against *Rhizopus Solani*. Microbial degradation of mycotoxins and also prevention of aflatoxin biosynthesis pathway in mycotoxin-producing fungi are two ways to reduce mycotoxin contamination. Two examples of mycotoxin interfering bacteria are the use of *Bacillus megaterium* and *Streptomyces* spp. (Mannaa and Kim 2016). Also, it was reported that botanic agents like *Rhodococcus erythropolis* could protect grains against *A. flavus* contamination and subsequent aflatoxin generation because of extracellular metabolites produced by this microorganism (Reddy et al. 2009).

4.7.5 Plant Extracts as Fungicides

Plant extracts and microorganisms have been reported to control fungal and mycotoxin contamination as an alternative to fungicides. The antifungal activity of some herbal ingredients has been investigated (Naseer et al. 2014). Plant extracts are recommended to control the grains' fungal contamination (Aldred et al. 2008).

Complete prevention of AFB1 production due to the antifungal ingredients in a plant extract was reported using *Syzygium aromaticum*. Clove and its extract eugenol were also reported as botanical ingredients to control *A. flavus* and stop the biosynthesis of aflatoxins (Naseer et al. 2014). In another study, three essential oils, including bay, clove, and cinnamon oils, were reported to control the growth of *P. verrucosum* and *A. westerdijkiae*. These fungi produced OTA in wheat grain. Resveratrol, an antioxidant, was more effective in controlling OTA than essential oils (Aldred et al. 2008).

The use of vegetable oils is a method to control fungal contamination in rice grains. It works as a barrier for oxygen. It also prevents moisture adsorption in the grain, preventing both fungal and insect growth in grains. Essential oils of plants have a lipophilic activity that destroys fungal plasma membrane activity and acts as a fungicide (Mohapatra et al. 2017).

Antifungal activity of lemon and pomegranate was also reported in rice, which was reported as natural preservatives against fungal activity (Naseer et al. 2014). An experiment was designed to measure mycotoxin contamination and vitamin E content in five rice varieties in India. HPLC equipped with a fluorescence detector was used in this study. Vitamin E can prevent mycotoxin contamination in rice (Iqbal et al. 2014). It was also reported that *Michelia alba* leaf essential oil in the vapor phase at a concentration of more than 300 μl/L in the air could prevent both spore and mycelium of *A. flavus* in cooked brown rice grain. Antifungal components

of this oil included Linalool and caryophyllene, at 10:1 concentration ratio, were also adequate to control *A. flavus* (Songsamoe et al. 2017).

4.7.6 Integrated Management Strategies to Control Fungal Contamination

According to Fig. 4.1, several parameters should be considered when thinking about fungal and mycotoxin contamination. A preventive approach to avoid fungal infection and mycotoxin contamination in stored grains is integrated management for fungal and mycotoxin contamination. This strategy consists of four steps (Fleurat-Lessard 2017) as follows:

1. Identification of critical controls that affect mycotoxin contamination of grains.
2. Monitoring early fungal infection signs in grains.
3. Preventive control to avoid fungal infection and mycotoxin contamination.
4. Actions to remove fungal and mycotoxin contamination in grains.

Fig. 4.1 Key factors involved in germination and growth of mycotoxigenic fungi developing in stored cereal grain during long-term storage. (Modified from Pardo et al. 2006; Magan and Aldred 2007)

4.7.7 Prevent Fungal Adsorption

It was reported that mineral clays like diosmectite (SMECTA), montmorillonite (green montmorillonite argile), and illite (little green algae) had protective effects on aflatoxin toxicity (Romero et al. 2016). Clay additives are recommended to be used in animal feed to avoid aflatoxicosis. One of these additives is sodium bentonite which was used to control AFB1 contamination in animal feed in Argentina (Magnoli et al. 2011).

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Bioactive Profile of the Wild Mushroom *Trogia cantharelloides*

5

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Abstract

Wild mushrooms have become an integral part of the human diet, health, and industrial applications worldwide. However, many of them will not serve as food due to their unpalatable taste or poisonous or gastrointestinal problems. *Trogia cantharelloides* is one such mushroom not preferred as food by the tribals in the Western Ghats of India. This study provides baseline data on the *T. cantharelloides* obtained from the foothills of the southwest region of the Western Ghats of India. Biochemical components like organic acids, sugars, polyphenols, flavonoids, phytic acid, vitamins, trypsin inhibition activity, hemagglutinin activity, and antimicrobial potential of *T. cantharelloides* are addressed. The therapeutic potential of the bioactive compounds of *T. cantharelloides* was documented using Duke's phytochemical and ethnobotanical database (www.ars-grin.gov/cgi-bin/duke). Accordingly, a total of 15 compounds compiled along with their characteristics, biological activity, and applications. This study provides scope to explore the bioactive potential of non-edible mushrooms for their use in future health, therapeutic and industrial applications.

Keywords

Bioactive principles · Therapeutics · Antimicrobial potential · The Western Ghats

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5.1 Introduction

The current global estimate of macrofungi is between 2.2 and 3.8 million species (Hawksworth and Lücking 2017). About 2000 species are considered safe for human consumption, and 700 species possess therapeutic potential (Lima et al. 2012). Many mushrooms are inedible owing to their tough texture, tastelessness, and poisonous qualities (Krupodorova et al. 2012; Ivanova et al. 2014; Sevindik 2020). Although many mushrooms are inedible, they are an essential source of biologically active metabolites of health, pharmaceutical, and industrial value (Bal et al. 2017, 2019; Özaltun and Sevindik 2020). There are many reports on mushroom poisoning owing to misidentification in different parts of the world (Lima et al. 2012; Jo et al. 2014; Özaltun and Sevindik 2020).

Mushrooms of the genus *Trogia* are glass funnel mushrooms distributed in tropical and subtropical regions (Mortimer et al. 2014). *Trogia infundibuliformis* exits in Thailand and Laos on rotten woody materials. This species has been reported from Maharashtra (India) on twigs (Senthilarasu 2014). The occurrence of toxic amino acids in *Trogia venenata* has been reported by many researchers (Shi et al. 2012; Zhou et al. 2012; Xu et al. 2018; Yin et al. 2019). The *Trogia cantharelloides* is a widespread species recorded in Puerto Rico, Cuba, Thailand, and China. Sevindik (2020) has documented the antioxidant potential of 13 species of poisonous mushrooms belonging to 10 genera. Index Fungorum has recorded 108 species and 156 records of the genus *Trogia* (accessed on November 23, 2020). It is also one of the wild mushrooms in the foothills of the Western Ghats of India. However, it is not acceptable for edibility by the tribals and local dwellers for unknown reasons (Ravikrishnan 2019).

If a mushroom is inedible or toxic, or poisonous, it is likely not to follow its biochemical composition and bioactive components. This chapter aims to document biochemical composition (organic acids, sugars, polyphenols flavonoids, and phytic acid), vitamins (vitamin C and b-carotene), pigment (lycopene), nutritional (proximal, mineral, amino acids, and fatty acids), antinutritional activity (trypsin inhibition and hemagglutinin) and antimicrobial potential of *T. cantharelloides* as baseline data for future exploitation.

5.2 Biology of *Trogia*

The genus *Trogia* (*Marasmiaceae*, *Basidiomycota*) has been named after Swiss mycologist Jacob Gabriel Trog. This genus circumscribed by Fries (1835) and erected the type species *Trogia montagnei*. Another species, *Cantharellus aplorutis* has been described by the French mycologist Camille Montagne in 1834 (Bélanger 1846). Subsequently, British botanist Corner (1966) emended the genus to include 56 species. Currently, the Index Fungorum has 156 records consisting of 108 species.

Trogia spp. commonly grow on woody litter, possess clitocyboid gills devoid of partial veils with white, yellowish, or pinkish spores. They have cartilage-like stipe,

broad to depressed pileus without partial veil and volva. On drying, their fruit bodies become tough and could be rejuvenated during moist conditions. So far, eight species of *Trogia* are reported from the Indian Subcontinent (*T. benghalensis*, *T. cantharelloides*, *T. cyanea, T. grisea, T. infundibuliformis, T. liaceogrisea*, *T. montagnei,* and *T. subviridis*) (Graham 1915; Uppal et al. 1935; Manjula 1983; Natarajan et al. 2005; Kumar and Manimohan 2009; Senthilarasu 2014; Dutta et al. 2017; Ravikrishnan et al. 2017).

Trogia buccinalis can produce enzymes to degrade pollutants like anthracene, pentachlorophenol, and polyvinyl chloride (Martins-Franchetti et al. 2010). *Trogia venenata* is a poisonous mushroom responsible for the death of 400 people in Yunnan Province of China (Zhou et al. 2012). This mushroom possesses cardiotoxic amino acids, which leads to arrhythmia. *Trogia cantharelloides* is also a widespread neotropics (Halling and Mueller 2002). However, the tribals residing in the foothill of the Western Ghats of India are not consuming this mushroom. *Trogia infundibuliformis* possess small- to medium-size basidiomes with infundibuliform, perforated, membranous, pileus split, decurrent, distant lamellae, stipe central to excentric arising from the white discoid base, while *T. cantharelloides* although it resembles *T. infundibuliformis* distinctly differs in possessing crowded lamellae with small spore size (Pegler 1983).

5.3 Harvest and Process

We sampled mushrooms from Someshwara wildlife sanctuary in Udupi District (13°29′N, 74°50′E) of Agumbe Ghat in the Western Ghats of Karnataka. The average temperature ranges from 23 to 24 °C with 97% humidity. This sanctuary is composed of semi-evergreen as well as moist mixed deciduous forests. The climatic conditions and secondary products of forests are the primary sources for the growth of mushrooms. The whole fruit bodies of *Trogia cantharelloides* (Mont.) Pat. [synonym: *Panus cantharelloides* Mont.; *Pocillaria cantherelloides* (Mont.) Kuntze] were harvested from three locations of the forests (Fig. 5.1) as replicates, stored in a cool pack, and transferred to the laboratory within 4–5 h for processing. The transported fruit bodies were spread on blotting papers to remove the debris. Later, we processed part of the fruit bodies (about 3–5) from triplicate samples for moisture content gravimetrically. The rest of the fruit bodies were rinsed in distilled water and blot dry, followed by drying (58 ± 2 °C) in a hot-air oven by spreading on aluminum foils until attaining the constant weight. Triplicate dried fruit bodies blend into coarse to a fine powder. The flour of fruit bodies was transferred into air-tight glass containers and preserved in a refrigerator for further analysis.

Fig. 5.1 Profuse growth of *Trogia cantharelloides* on the woody litter buried in humus in Someshwara wildlife sanctuary of the Western Ghats of India

5.4 Biochemical Composition

5.4.1 Bioactive Components

Among the biochemical components of *T. cantharelloides* assessed, the flavonoids were highest (14.7 mg/g), followed by total phenolics (11.1 mg/g) and tannins (3.2 mg/g) (Table 5.1). Total phenolics content was assessed according to the procedure by Rosset et al. (1982). Gallic acid served as standard to express total phenolics as mg of gallic acid equivalents per gram dry mass of the mushroom (mg GAEs/g). Total phenolics of the wild mushrooms are an essential component in defense against herbivores. It varies between geographic conditions (Okoro 2012;

Table 5.1 Biochemical constituents of *Trogia cantharelloides* (dry mass basis) ($n = 3$, mean ± SD)

Constituent	Quantity
Total phenolics (mg GAEs/g)	11.1 ± 0.23
Tannins (mg CEs/g)	3.2 ± 0.05
Phytic acid (mg/g)	0.7 ± 0.06
Flavonoids (mg QEs/g)	14.7 ± 0.13
Vitamin C (mg AAEs/g)	1.8 ± 0.12
β-carotene (μg/g)	1.96 ± 0.02
Lycopene (μg/g)	1.40 ± 0.03

Attarat and Phermthai 2015). Total phenolics is the major constituent in *T. cantharelloides* along with flavonoids. The phenolics can combat cardiovascular diseases (Visioli et al. 2000; Meng et al. 2002). The vanillin-HCl method was adapted to determine tannin content (Burns 1971). It was denoted as catechin equivalents in mg per gram dry mass of the sample (mg CEs/g). In addition to phenolics, the presence of tannins and phytic acid provides additional strength for antioxidant activity.

The procedures with KH_2PO_4 performed extraction and estimation of phytic acid as standard to determine phytate (Deshpande et al. 1982; Sathe et al. 1983). In addition to antioxidant activity, phytic acid helps preventing kidney stone formation and calcium deposition in arteries (Knekt et al. 2004; Ye and Song 2008). The content of flavonoids is detected by the standard curve of quercetin dihydrate. The flavonoid content is expressed as quercetin equivalents in mg per gram dry mass (mg QEs/g) (Jia et al. 1999). Flavonoids are known for health-promoting attributes like cardioprotective, hepatoprotective, anti-inflammatory, and anti-diabetes (Champ 2002; Tapas et al. 2008).

Estimated the vitamin C content according to Roe (1954) with ascorbic acid as standard, and its content was noted as ascorbic acid equivalents in mg per gram of the dry mass (mg AAEs/g) (Table 5.1). Vitamin C is present in substantial quantity in *T. cantharelloides*, and it is a potent antioxidant as well as radical-scavenger; however, it will be vulnerable to increased temperature (Gregory III 1996; Podmore et al. 1998). The β-carotene and lycopene contents are assessed by the method outlined by Barros et al. (2007) (Table 5.1). Carotenoids in mushrooms are also known for their antioxidant activity (Barros et al. 2007). A substantial amount of vitamin C is found in *T. cantharelloides* compared to β-carotene and lycopene. All these components are known as potential antioxidants and radical scavengers.

5.4.2 Sugars, Organic Acids, and Polyphenols

Soluble sugars of *T. cantharelloides* are assessed using an amino column with acetonitrile and water (3:1) as the mobile phase (Reis et al. 2012). Three soluble sugars found with the highest quantity of arabinose in methanol extract (2.5 mg/g) below the detectable level in aqueous extract (Table 5.2). The second highest soluble sugar was glucose in aqueous extract (1.7 mg/g), and it was 0.9 mg/g in methanol extract. Trehalose was higher in methanol extract than aqueous extract (0.4 vs. 0.3 mg/g). Turfan et al. (2018) opine that the soluble sugar composition of wild mushrooms is controlled by many factors (e.g., genetic, stage of growth, and conditions of harvest).

Organic acids were evaluated based on the protocol by Pereira et al. (2013). Four organic acids were detected with the highest quantity of succinic acid in aqueous extract (24.4 mg/g), while it was below the detectable level in methanol extract (Table 5.2). Like succinic acid, acetic acid (2.3 mg/g) and tartaric acid (0.9 mg/g) were found only in methanol extract. Pyruvic acid is detected in aqueous and methanol extracts (2.3 and 1.3 mg/g, respectively). Organic acids in mushrooms

Table 5.2 Soluble sugars, organic acids, and polyphenols of *Trogia cantharelloides* (dry mass basis) (*A* aqueous, *M* methanol, *BDL* below detectable level) ($n = 3$, mean ± SD)

Soluble sugar	mg/g
Arabinose (A)	BDL
Arabinose (M)	2.5 ± 0.00
Glucose (A)	1.7 ± 0.14
Glucose (M)	0.9 ± 0.02
Trehalose (A)	0.3 ± 0.02
Trehalose (M)	0.4 ± 0.00
Organic acid	
Acetic acid (A)	BDL
Acetic acid (M)	2.3 ± 0.09
Pyruvic acid (A)	1.3 ± 0.10
Pyruvic acid (M)	0.4 ± 0.01
Succinic acid (A)	24.4 ± 0.80
Succinic acid (M)	BDL
Tartaric acid (A)	BDL
Tartaric acid (M)	0.9 ± 0.03
Polyphenols	
Methyl catechol	0.5 ± 0.01
Ethyl catechol	3.2 ± 0.00

principally serve as flavoring agents and antioxidants (Vaughan and Geissler 1997; Silva et al. 2004). These components are not susceptible to changes depending on the mushroom processing and storage conditions (Cámara et al. 1994).

Polyphenols were estimated based on the method by Dasgupta et al. (2015). Among polyphenols, the ethyl catechol was highest (3.2 mg/g), followed by methyl catechol (0.5 mg/g) (Table 5.2). Like fruits and vegetables, mushrooms are also a source of many polyphenols that possess considerable therapeutic value and correlated with antioxidant activities (Barros et al. 2009; Ren et al. 2014; Lin et al. 2015; Smolskaité et al. 2015).

5.4.3 Bioactive Compounds

The GC-MS/MS assessed the chemical composition of mushroom with the Scion 436-GC Bruker model coupled with a triple quadrupole mass spectrophotometer. The relative percentage of each component estimated by comparison of average peak area to the total areas with software MS Work Station 8. The National Institute of Standard and Technology (NIST) Version # 2.0 library database employed to identify the chemical components. The spectrum of the unknown component compared with the spectrum of the known component stored in the NIST library. Particulars of each compound ascertained by Srinivasan and Kumaravel (2016). Therapeutic potential of each compound (NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library Version # 2.0; Gaithersburg, MD, USA)

established based on the Dr. Duke's phytochemical and ethnobotanical databases (www.ars-grin.gov/cgi-bin/duke).

The GC-MS/MS analysis of a crude extract of *T. cantharelloides* showed up to 16 bioactive compounds of applied value (Table 5.3). Those compounds include purine nucleobase, cyclic purine nucleotide, indonel galactonic acid derivative, fatty acid methyl esters, saturated fatty acids, triterpene, g-lactam, and phytosterol. Details of bioactive compounds present in *T. cantharelloides* include: purine derivatives (adenosine 3,5-cyclic monophosphate and 6H-purin-6-one,1,7-dihydro-); growth hormone (indole); squalene; 2-pyrrolidinone; 2-acetamide-2-deoxygalactono-1,4-lactone; ergosterol; saturated fatty acids (tetradecanoic and n-hexadecanoic acids); ethyl ester (9,12-octadecadienoic acid); fatty acid methyl esters (hexadecanoic, 9,12-octadecadienoic, stearic, dodecanoic and 9-octadecenoic acids). Many are useful in health protection: anti-inflammatory, immunostimulant, cytoprotective, anticarcinogenic, antitumor, hypercholesterolemic, antioxidant, antiandrogenic, antiangiogenic, and diuretic. Several compounds possess antibacterial, antifungal, nematicidal, and antiviral properties. Some of them are industrially valued potential, such as nutraceuticals, flavors, and lubricants.

5.5 Nutritional and Antinutritional Attributes

5.5.1 Nutritional Components

The nutritional profile of *T. cantharelloides* (proximal qualities, minerals, amino acids, and fatty acids) was evaluated by Ravikrishnan et al. (2017). The carbohydrate content was highest (86.7%), followed by crude fiber (11.1%), crude protein (9.5%), and total lipids (2.3%) with a calorific value of 1720 kJ/100 g. Among the minerals, phosphorus was the highest (260 mg/100 g), followed by potassium (12.6 mg/100 g), calcium (1.3 mg/100 g), and magnesium 1.2 mg/100 g). The rest of the minerals (iron, copper, sodium, selenium, and zinc) were <1 mg/100 g. The Na/K ratio of *T. cantharelloides* (<1) obeys the NRC-NAS standard (NRC-NAS 1989; USDA 1999), and it is a favorable ratio to combat the blood pressure (Yusuf et al. 2007).

Among dispensable and indispensable amino acids of *T. cantharelloides* (mg/100 g protein) similar to other edible mushrooms, the glutamic acid was highest (12.9 mg) followed by glycine (9.1 mg), serine (8.6 mg), alanine (7.8 mg), arginine (7 mg), lysine (6.9 mg), leucine (6.6 mg), proline (6.3 mg), valine (5.7 mg), arginine, threonine (4.9 mg each), and isoleucine (4.5 mg). The rest of the amino acids were: <4 mg/100 g protein (histidine, tyrosine, methionine, cystine, and phenylalanine).

The fatty acid profile of *T. cantharelloides* consists of ten saturated and six unsaturated fatty acids. Among saturated fatty acids (g/100 g total lipids), palmitic acid content was the highest (10.2 g), followed by stearic acid (2.7 g) and lignoceric acid (1.7 g). Rest of the saturated fatty acids (capric, lauric, myristic, pentadecanoic, heptadecanoic, behenic, and tricosanoic acids) were <1 g. Among the unsaturated fatty acids (g/100 g total lipids), the linoleic acid content was highest (34.6 g),

Table 5.3 Bioactive compounds, medicinal properties, and applications of *Trogia cantharelloides* (www.ars-grin.gov/cgi-bin/duke)

Compound number	Retention time	Compound	Nature of the compound	Biological activity/uses/applications	Peak area (%)
1	13.87	Adenosine 3,5-cyclic monophosphate	Cyclic purine nucleotide	Second messenger	1.44
2	3.74	6H-Purin-6-one,1,7-dihydro-	Purine nucleobase	Anti-inflammatory and cytoprotective	1.29
3	7.07	Indole	Indole	Anticariogenic, cancer preventive, anti-acne, antibacterial, anti-*Salmonella*, anti-streptococci	0.44
4	27.79	Squalene	Triterpene	Cancer preventive, diuretic, chemopreventive, and immunostimulant	0.69
5	3.61	2-Pyrrolidinone	γ-lactam	Serve as intermediate/precursor in pharmaceutical drugs and used in inkjet cartridges	3.12
6	7.66	2-Acetamide-2-deoxygalactono-1,4-lactone	Galactonic acid derivative	N-acetylglucosaminidase inhibitor	0.16
7	34.49	Ergosterol	Phytosterol	Antitumor, antiangiogenic, anti-flu, and antiviral	3.75
8	12.64	Tetradecanoic acid	Saturated fatty acid	Cancer preventive, nematicidal, hypercholesterolemic, antioxidant, lubricant, and antifungal	8.58
9	16.03	*n*-Hexadecanoic acid	Saturated fatty acid	Flavoring agent, antiandrogenic, hypocholesterolemic, and antioxidant	0.38
10	18.40	9,12-Octadecadienoic acid, ethyl ester	Fatty acid ethyl ester	Anti-inflammatory agent	55.57
11	15.11	Hexadecanoic acid methyl ester	Fatty acid methyl ester	Nutrient, energy storage and membrane stabilizer	4.84
12	17.43	9, 12-Octadecadienoic acid, methyl ester	Fatty acid methyl ester	Personal care products	0.75
13	17.89	Stearic acid, methyl ester	Fatty acid methyl ester	Flavoring agent and industrial value	1.67
14	10.18	Dodecanoic acid, methyl ester	Fatty acid methyl ester	Flavoring agent	2.92
15	17.53	9-octadecenoic acid, methyl ester, (E)-	Fatty acid methyl ester	Industrial value	1.75

followed by oleic acid (18.5 g), docosahexaenoic acid (9.8 g), and linolelaidic acid (1.2 g). Palmitoleic and linolenic acids were <1 g. The ratio of total unsaturated and saturated fatty acids was as high as 4.

5.5.2 Antinutritional Qualities

Trypsin inhibition activity is measured based on Kakade et al. (1974). The control consists of all reagents without the mushroom extract—calculated trypsin inhibition units (TIu) per mg of dry mass. The TIu was very low in *T. cantharelloides* (0.08/mg). Many mushrooms are devoid of trypsin inhibition activity (e.g., Ghate and Sridhar 2017). Hemagglutination activity is determined based on Occenã et al. (2007) by heparinized human erythrocytes (A+, B+, AB+, and O+) to express hemagglutination unit per gram (Hu/g). There was no hemagglutination activity against B+ erythrocytes, while it was up to 200 Hu/g in the other three erythrocytes. Like trypsin inhibition activity, many mushrooms are devoid of hemagglutinin activity (Ghate and Sridhar 2017). It is likely the antinutritional factors diminished or eliminated in mushrooms on cooking or by heat treatment.

5.6 Antimicrobial Potential

Four Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*), five Gram-negative bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Haemophilus influenzae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*), and one yeast (*Candida albicans*) were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Bacterial strains were maintained on Muller–Hinton agar medium, while the *C. albicans* were on Sabouraud dextrose agar medium. Dispensed overnight grown culture of each strain (200 ml) into sterile Muller–Hinton broth (20 ml for bacteria), Sabouraud dextrose broth (20 ml for yeast), and incubated at 37 °C to obtain 105 CFU/ml. Powder of (5 g) *T. cantharelloides* (from triplicate samples) extracted in 25 ml of methanol on a rotary shaker (120 rpm) up to 24 h followed by oven drying (50 ± 2 °C). The dried methanol extract dissolved in 20% dimethyl sulfoxide (DMSO) to get a stock solution (2 mg/ml), and the DMSO was used as solvent control to test its inhibitory effect.

The antibacterial and antifungal activity of the mushroom extracts were evaluated using the well-diffusion method (Bauer et al. 1966). Bacterial culture (0.1 ml; 105 CFU/ml) of 24 h old was inoculated on Muller–Hinton agar and spread out. Similarly, spread the yeast culture on the Sabouraud dextrose agar. Agar well was cut, and 200 μg of the mushroom extract loaded into each well, and the DMSO served as control. Each plate comprised of three wells (experimental) with a standard antibiotic disc (Vancomycin, 30 μg/disc for bacteria; Fluconazole, 25 μg/disc for yeast). The plates incubated at 37 ± 1 °C (18–24 h) with bacteria and for yeast at

Table 5.4 Antimicrobial activity of *Trogia cantharelloides* and standard antibiotics ($n = 3$, mean) (*NI* no inhibition, – not tested)

	Diameter of inhibition (mm)		
	T. cantharelloides (200 µg/well)	Vancomycin (30 µg/disc)	Fluconazole (25 µg/disc)
Gram-positive bacteria			–
Bacillus cereus MTCC430	11.3	19.7	–
Bacillus subtilis MTCC441	17.0	25.7	–
Staphylococcus aureus MTCC96	12.0	22.0	–
Streptococcus pneumoniae MTCC655	15.7	26.0	–
Gram-negative bacteria			
Enterobacter aerogenes MTCC424	NI	20.0	–
Escherichia coli MTCC443	09.3	22.0	–
Haemophilus influenzae MTCC3826	16.0	21.7	–
Proteus vulgaris MTCC1771	09.0	18.7	–
Pseudomonas aeruginosa MTCC424	07.7	31.0	–
Yeast			
Candida albicans MTCC183	08.7	–	26.0

26 ± 1 °C (48–72 h). The plates were examined for inhibition to measure the inhibition diameter using a dial caliper.

The standard DMSO did not inhibit tested bacteria and yeast. The crude extract of *T. cantharelloides* showed antimicrobial activity against Gram-positive and Gram-negative bacteria and *C. albicans* (Table 5.4). The zone of inhibition was highest against *H. influenzae* (16 mm), followed by *B. subtilis* (17 mm) and *S. pneumoniae* (15.7 mm). Although the extract of *T. cantharelloides* used for the test was about seven-fold higher than the standard antibiotic vancomycin, none showed higher activity than the vancomycin. The *C. albicans* showed an 8.7 mm inhibition diameter at 200 mg/well, lower than the standard antibiotic fluconazole (25 mg/disc, 26 mm).

Lindequist et al. (2005) opined that wild mushrooms possess antimicrobial compounds to survive in their natural habitats. Managing Gram-negative bacteria is more complex than Gram-positive bacteria (e.g., cell wall inhibiting antibiotics). Inhibition of Gram-positive bacteria, Gram-negative bacteria, and *C. albicans* by the extracts of *T. cantharelloides* indicated its broad-spectrum antibiosis. However, the inhibition ability of *T. cantharelloides* against bacteria and yeast was below the standard antibiotics. It is interesting to note that *T. cantharelloides* can inhibit a broad range of pathogenic microorganisms.

5.7 Conclusions

Several wild mushrooms are not edible owing to their unpalatable or toxic, or poisonous properties. However, many of them have value-added bioactive components of health, pharmaceutical, and industrial importance. The

T. cantharelloides, although not consumed by the tribals and local dwellers of the foothills of the Western Ghats of India, possesses many biochemical, nutritional, and bioactive components in substantial quantities. The carbohydrate content was highest in the fruit bodies, followed by crude fiber and crude protein. The Na/K ratio (<1) obeys the NRC-NAS standards and is favorable to combat blood pressure. It possesses many essential amino acids (e.g., threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) as well as essential fatty acids (e.g., linoleic, linoleic, and docosahexaenoic acids). It possesses reasonable quantities of flavonoids, phenolics, vitamin C, carotenoids, and succinic acid. Numerous bioactive components of health combating potential found in this neglected mushroom (e.g., anti-inflammatory, immunostimulant, cytoprotective, anticarcinogenic, antitumor, hypercholesterolemic, antioxidant, antiandrogenic, antiangiogenic, and diuretic). It is endowed with broad-spectrum antibiosis against pathogenic bacteria, fungi, nematodes, and viruses. Owing to the potential bioprospective qualities, *T. cantharelloides* may be a future bioresource of health, pharmaceutical, and industrial values.

Acknowledgments The first author (VR) is grateful for the facilities provided by the Department of Biosciences, Mangalore University. He is indebted to the Board of Research in Nuclear Sciences (BRNS), Bhabha Atomic Research Centre, Mumbai, India, for funding this research.

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Prospects of Cordycepin and Polysaccharides Produced by *Cordyceps*

6

Mahesh Borde and Sanjay K. Singh

Abstract

Cordyceps generally known as caterpillar fungus infects various insects in nature. These fungi have unique mechanism to infect the host insects and are generally hostile to unique ecological niches. The well-recognized caterpillar fungus is usually found at high altitudes on the Himalayan plateau and is used as a traditional medicinal mushroom producing a number of active substances used in medicine and naturopathy. Cordycepin is one of them having nutraceutical potential and helps in maintaining good health. Polysaccharides having health promoting activity are also reported under in vivo and in vitro conditions. High demand of these bioactive compounds requires a suitable strategy to meet out the demands. In addition, anticancer, antidiabetic, anti-hyperlipidaemia, antifungal, immunomodulatory, antioxidant, antiaging, antiviral, hepato-protective, hypo-sexuality, cardiovascular diseases and anti-inflammatory activities are also reported. In this review, we have compiled the information on potential of cordycepin and other polysaccharides of *Cordyceps* and discuss about optimization of culture conditions for enhanced recovery of these bioactive compounds.

Keywords

Biopotential · *Cordyceps* · Cordycepin · Polysaccharides · Fungi

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_6

6.1 Introduction

Cordyceps genus is an entomopathogenic fungi belonging to the class *Ascomycetes*. *Cordyceps* originates from two Latin words that is c*ord* meaning club and *ceps* meaning head. Two main commercially important species of *Cordyceps*, *C. militaris* and *C. sinensis*, parasitize within the larvae or pupae body of lepidopteran insects, mummify the whole body of larva and fruit body growing outside the insect hosts. *C. sinensis* (Berk.) Sacc. is known as the caterpillar fungus in China or Tochukaso in Japan and is a useful and widely used medicine in china. *Cordyceps* have many bioactive metabolites like cordycepin, polysaccharides, ergosterol, mannitol, etc. (Das et al. 2010a; De Silva et al. 2013).

In 2003, during the severe acute respiratory syndrome (SARS) outbreak, there was an increasing demand of *C. sinensis* in China. So that, over the last 10 years, the use and price of *C. sinensis* has increasing in many countries (Winkler 2009; Au et al. 2012; Jeffrey 2012). The main bioactive compounds from *C militaris viz.* cordycepin, adenosine, polysaccharides, ergosterol, and cordycepic acid have shown to be effective against various disorders and diseases (Song et al. 2010; Ding et al. 2011; Marchbank et al. 2011; Zhang et al. 2011; Yue et al. 2013). In China, *C. militaris* is used in treatment of a number of diseases such as lung disease, kidney dysfunction, immunomodulatory disorder, etc. Polysaccharide and cordycepin are the most important bioactive compounds in *C. militaris* that have shown various biological activities as detailed in Table 6.1.

6.2 Biological Activities of Compounds Especially Cordycepin and Polysaccharides from Cordyceps Species

6.2.1 Anticancer

Cordycepin controls the cell cycle by enhancing sub-G1 accumulation in various cancer cells such as cancer of lung (Wang et al. 2016; Cho and Kang 2018), oral cancer (Hsu et al. 2017), and testicular cancer (Pan et al. 2015; Chang et al. 2019) and stimulates G2/M arrest in bladder cancer (Lee et al. 2009a, b, 2010a, b) and colon cancer (Lee et al. 2010c).

Cordycepin inhibits cancer cell progression via proposed number of signaling pathways like activation of the death receptor signaling pathway (Lee et al. 2013a, b, c), stimulation of adenosine receptors, poly adenosine-diphosphate-ribose polymerase pathway (Chen et al. 2010, 2014a; Lee et al. 2010a, b). Cordycepin reduces the epidermal growth factor receptor (EGFR) phosphorylation and its downstream interaction of AKT and ERK1/2 signaling pathway in H1657 cells of lung cancer by leading to inhibition of the cancer progression (Wang et al. 2016). Cordycepin changing the expressions of p-ERK1/2, p-Rb, E2F1, proteins which play role in cell cycle and fibroblast growth factor receptors (FGFR) 1–4 proteins, resulted into inhibition of FGF9-induced testicular tumor progression (Huang et al. 2020). Cordycepin from *C. sinensis* combined with radiotherapy controlling oral

Table 6.1 Various bioactivities of cordycepin and polysaccharides from *Cordyceps* sp.

Species	Compound	Bioactivities	Reference
C. militaris	Polysaccharide	Immunomodulatory	Lee and Hong (2011)
C. militaris	Polysaccharide	Immunomodulatory	Lee et al. (2010a, b)
C. militaris	Polysaccharide	Immunomodulatory, antioxidant	Wang et al. (2012)
C. militaris	Cordycepin	Anti-inflammatory and neuroprotective	Zhang et al. (2021)
C. militaris	Cordycepin	Anti-inflammatory, anticancer	Rao et al. (2010)
C. militaris	Cordycepin	Antidiabetic	Ma et al. 2015
C. militaris	Polysaccharide	Antioxidant	Chen et al. (2013a, b)
C. militaris	Cordycepin	Prostate cancer	Hwang et al. (2016)
C. militaris	Cordycepin	Breast cancer	Choi et al. (2011)
C. militaris	Polysaccharide	Antihyperlipidemic	Wang et al. (2015)
C. militaris	Cordycepin	Hepatoprotective	Lan et al. (2021)
C. militaris	Cordycepin	Liver cancer	Guo et al. (2020)
C. militaris	Cordycepin	Ovarian cancer and apoptosis	Jo et al. (2020)
C. sinensis	Cordysinin B	Anti-inflammatory	Yang et al. (2011)
C. sinensis	Cordycepin	Anticancer, antimetastatic	Nakamura et al. (2015)
C. sinensis	Cordycepin and Polysaccharide	Immunomodulatory	Kuo et al. (2007)
C. sinensis	Cordycepin	Antiaging	Lee et al. (2009a, b)
C. sinensis	Cordycepin	Antifungal	Sugar and Mccaffrey (1998)
C. sinensis	Cordycepin	Anticancer (Colon cancer)	Lee et al. (2010b)
C. sinensis	Cordycepin	Lung cancer	Tao et al. (2016)
C. sinensis	Ophicordin	Antifungal	Kneifel et al. (1977)
C. militaris	Polysaccharide	Antioxidant	Yu et al. (2006)
C. sinensis	Polysaccharide	Effect on human leukemia	Chen et al. (1997)
C. sinensis	Cordycepin	Antitumor	Kuo et al. (1994)
C. sinensis	Polysaccharide	Immunomodulatory	Cheong et al. (2016)
C. sinensis	Exopolysaccharide	Immunomodulatory	Zhang et al. (2005)
C. sinensis	Exopolysaccharide	Immunomodulatory	Wang et al. (2011)
C. sinensis	Exopolysaccharide	Anti-inflammatory	
C. sinensis	Exopolysaccharide	Anti-inflammatory	Li et al. (2020)
C. sinensis	Intracellular polysaccharides	Immunomodulatory	Li et al. (2021)
C. sinensis	Cordycepin	Anticancer	Wang et al. (2020)

squamous cell carcinoma prolong toxicity by modulation of DNA damage repair (Su et al. 2019). Cordycepin from *C. sinensis* showed anticancer activity by stimulation of adenosine A3 receptor followed by GSK-3b initiation and cyclin D1 and antimetastatic effect by inhibiting the aggregation platelet initiated by ADP released

and preventing the activity of MMP-2 and MMP-9 and inducing the TIMP-1 and TIMP-2 pathways inhibiting the production of cancer cells (Nakamura et al. 2015). A peptide Cordymin isolated from *C. militaris* showed antiproliferative activity against breast cancer cells (MCF-7) (Wong et al. 2011).

Cordycepin acid lowered lung cancer proliferation by regulating the pathways Nrf-2/HO-1/NLRP3/NF-κB signal, which reduced inflammation, induction of tumor angiogenesis and promote apoptosis (Wang et al. 2021).

6.2.2 Antidiabetic

Cordyceps fruit bodies oral administration in nicotinamide and streptozotocin-induced diabetic rat may have potential of functional food for preventing diabetes (Lo et al. 2004). Ma et al. (2015) stated that Cordycepin from *C militaris* administration results in improvement of metabolic syndrome by regulating the absorption of glucose in diabetes. The *C. sinensis* compound ameliorated the complications caused due to diabetes such as administration of nucleosides and nucleobases from C. sinensis alleviation to diabetic renal fibrosis by EMT and the subsequent ECM deposition (Dong et al. 2019).

6.2.3 Anti-hyperlipidemia

In developed and developing countries, rise in lipid level causes cardiovascular diseases that have become one of the major causes of death due to various factors such as sedentary lifestyle, consumption of tobacco, and alcoholism (Le 2008). Hyperlipidemia, rise in cholesterol, trans fats and triglycerides in tissues cause heart disease (Rosenson 2006; Le and Walter 2007; Das et al. 2010a, b). Cordycepin plays a role in lowering the lipid level by activating the AMP-activated protein kinase (AMPK). Activated AMPK causes phosphorylation and inhibition of acetyl-CoA carboxylase resulting into reduction of the level of cholesterol and triglycerides. This can therefore be used as an anti-hyperlipidemic nutraceutical agent (Aymerich et al. 2006; Guo et al. 2010). Cordycepin also lowers the lipid activity by synthesis of palmate through induction of autophagy and PKA/mTOR pathway, thus reducing the intracellular levels of total lipids and total cholesterol (Gao et al. 2011; Wu et al. 2014).

6.2.4 Antifungal

Wong et al. (2011) reported a novel antifungal peptide Cordymin, isolated from *C. militaris* showing antifungal activities against pathogenic fungi. One of the antibiotics Ophiocordin extracted and purified from cultured *Cordyceps ophioglossoides* by using n-butanol showed antifungal activity antagonized by ammonia and nitrate ions and some amino acids (Kneifel et al. 1977). The mycelial

extract of *O. sobolifera* showed antifungal activity against *C. albicans*, a human pathogenic fungus (Sangdee et al. 2018).

6.2.5 Immunomodulatory

The immunomodulators are the molecules which boost the immune response of the body. *Cordyceps* spp. possess a number of bioactive compounds which show immunomodulatory activity. Kuo et al. (2007) reported the immunomodulatory activity in the pathogenicity of Group A *Streptococcus* infection by *C. sinensis* that leads to phagocytosis. Polysaccharide from *C. militaris* (*L.*) administration studies showed that the immunosuppressed mice induced immune activation (Wang et al. 2012).

6.2.6 Antioxidant

Polysaccharide from mycelia of *Cordyceps* spp. reported strong anti-oxidative activity (Gu et al. 2003). Polysaccharide derived from *C. sinensis* prevents tumor through alteration of hosts antioxidative enzymes such as SOD, GPx activity of brain and liver respectively, and also decreases the MDA level in liver and brain.

Polysaccharides isolated from artificially grown *C. militaris* studied in vitro antioxidant activities showed major antioxidative potential (Chen et al. 2013b). Fractions of polysaccharide derived from *C. militaris* (L.) culture Fr. reportedly produces good antioxidative defense response (Chen and Huang 2014). Cordycepin was also reported to prevent the neurotoxicity in adrenal pheochromocytoma cells treated with 6-OHDA by increasing the antioxidant enzymes activities (Olatunji et al. 2016). In addition, protein-bound polysaccharide causes increase in the antioxidative enzymes and reduction in lipid peroxidation in liver (Shin et al. 2001).

6.2.7 Antiaging

Aging is a process where degeneration of organs and body takes place after growth and development (Li et al. 2011; Xue et al. 2017). Excess generation of reactive oxygen species (ROS) in the body causes oxidative damage leading to aging (Bonomini et al. 2015). Repairing the oxidative damage requires antioxidants. Polysaccharides from *Cordyceps cicadae* showed significant antioxidant and antiaging properties and could be explored as a new dietary antiaging supplement (Zhu et al. 2020).

6.2.8 Antiviral

C. militaris acidic polysaccharide increased the survival rate of virus influenza A infected rats (Ohta et al. 2007). Pany et al. (2021) reported that the cordycepin inhibit DENV RNA replication in vitro cells. In silico study showed that cordycepin binds to DENV protein 5, which indicates that cordycepin is potentially a bioactive compound for dengue treatment.

6.2.9 Hepato-protective

Exopolysaccharides isolated from cultivated *Ophiocordyceps sinensis* administered to CCl4 induced rats possess moderate activity of ABTS and hydroxyl radical scavenging leading to increase in the GSH and reduced MAD, AST, and ALT activities, thereby providing hepatoprotective activity against hepatotoxicity (Nguyen et al. 2021). The mycelial liquid extract of *C. militaris* directed to bile duct ligation and scission rats decreased the levels of enzymes which are important in amino acid metabolism, total bilirubin in serum, malondialdehyde contents and hydroxyproline content in liver. This results in reduced antifibrotic effects on fibrotic rats (Nan et al. 2001).

Choi et al. (2014) studied that the consumption of *C. militaris* extracted in water by the obese mouse significantly decreased serum glucose, insulin, serum free fatty acid, hepatic total lipids and triglyceride contents resulting in improving antioxidant levels in obese mouse.

6.2.10 Hypo-sexuality

Erectile dysfunction in males of all ages is one of the disorders associated with *Diabetes mellitus* (Nguyen et al. 2017). Administration of *C. militaris* culture in diabetic rats affects the mating behavior, increased the sperm count, intracavernosal pressure responses, increased the levels of penile NOS, testicular SOD activities and testosterone (Pohsa et al. 2020).

Kopalli et al. (2019) reported that the administration of Cordycepin improved sperm motility and progressiveness in young rats. Also, a significant increase in spermatogenesis-related proteins and mRNA expression level was observed in cordycepin administered rat at 20 mg/kg dose. Thus, cordycepin is a nutritional and therapeutic product for alleviating the age-related sexual dysfunction in males.

6.2.11 Cardiovascular Diseases

Extract and cordycepin isolated from *C. sinensis* has anti-proliferatory and vasorelaxant effect in the human pulmonary artery smooth muscle cell (Luitel et al. 2020). Cordycepin (10 mg/kg) from *Cordyceps militaris* induced the Akt/

GSK-3b/p70S6K signaling and reduced the Bax and cleaved caspase-3 expression resulting in cardioprotective effects against ischemic/reperfusion rat heart injury (Park et al. 2014).

6.2.12 Anti-inflammatory Activities

Cordycepin plays an important role in anti-inflammatory activities by reducing the phosphorylation of protein kinase B (Akt), IκBα, and p38 and suppression of TNF-α, cyclooxygenase-2 (COX-2), iNOS, and translocation of NF-κB in macrophages (Kim et al. 2006). *C. sinensis* culture extracted heteropolysaccharide reduced the oxidative injury and modulation of cytokines (IL-4, -5, and -17) (Zhang et al. 2011). Various compounds such as cordycepin, polysaccharides, and adenosine induce NK-cell activity and activation of macrophage leads to reaction of phagocyte resulting in immunomodulation. Another compound, cerebrosides from *C. militaris* also showed anti-inflammatory activity (Chiu et al. 2016). Cordycepin has a greater ability in anti-inflammatory activity in neurological diseases by hindering the excessive production of NO, prostaglandin E2, and pro-inflammatory cytokines (Jeong et al. 2010).

Because of its bioactive potential, the fruiting bodies are in high demand and are sold in market at very high price. However, due to limited growth in particular areas and harvesting from natural habitat for use as drug, the Cordyceps has become rare. So, to overcome the scarcity of cordycepin and other chemicals, large quantities are harvested by using cultivation of *Cordyceps* by solid state or submerged cultivation method.

For artificial cultivation of cordyceps, two main fermentation methods are used, solid state fermentation and liquid state fermentation for enhancement of cordycepin synthesis from *C. militaris* (Das et al. 2010a, b, c; Zhang et al. 2016). Xia et al. (2017) reported that the cordycepin production is higher in artificial cultivation of *C. militaris* than growing on insect host. Further, liquid fermentation has reduced the time of cultivation and increased the productivity of cordycepin and reduced the cost of production than solid state fermentation (Wen et al. 2017).

6.3 Liquid State Fermentation

In liquid culture, Cordyceps is grown in liquid media which forms mycelium and not the fruit bodies. This is one of the alternative methods for industrial scale production of higher cordycepin in liquid culture in short period of time (Fan et al. 2012; Kang et al. 2014; Tang et al. 2015; Wen et al. 2017). The liquid state fermentation is carried out by three different ways: submerged culture, static culture, and two-step shaking static culture.

Submerged culture: In this method, the media inoculated with *Cordyceps* is grown under continuous shaking at 150 rpm. Carbon and nitrogen source are the important ingredient for enhancing the cordycepin in liquid culture. Another factor is

dissolved oxygen that is quite important for promoting the cordycepin production (Mao and Zhong 2004).

Surface/Static culture: In this liquid culture, the *Cordyceps* grows in stable condition. After 40 days, the mycelial mat of Cordyceps is developed on the surface of the medium. The modification of carbon and nitrogen source in a ratio of 2:1 in the culture medium improves cordycepin production (Masuda et al. 2006).

Two-step shaking static culture: The culture is grown first in shaking incubator and then after a few days, it is shifted to under surface/static stage. Two different conditions such as under shaking condition, promoting the biomass production in short period of time and under static condition induces the cordycepin accumulation.

6.4 Solid State Fermentation

In this type, the *Cordyceps* are fully grown on different solid media using grains such as rice, wheat, and cereals (Kim et al. 2010; Lim et al. 2012; Gregori 2014; Liang et al. 2014; Wen et al. 2014, 2016; Adnan et al. 2017; Chiang et al. 2017; Kang et al. 2017). There are three main steps involved in solid substrate fermentation. First step is the growing of mycelium all over the grain media, second is the formation of fruiting body on solid media and after that in the fruit bodies the cordycepin get accumulated. After inoculation of mother culture on solid substrate, the substrate is incubated at 19–21 °C for 7–10 days in dark for white mycelium to grow and cover the entire surface of solid substrate. After that the container having solid substrate is grown in 8–12 h light/dark per day, 20–23 °C temperature, and 70–95% relative humidity condition for initiating the fruiting body.

In general, there are a number of ways through which the polysaccharides are extracted from fruit bodies and mycelium of *C. militaris* like extraction in water, acidic/alkaline solution, by using boiling buffer solution (Yu et al. 2004a, b, 2009; Chen et al. 2013a, b; Smiderle et al. 2013; Lee et al. 2015; Zhu et al. 2016). Hot water extraction of polysaccharides is the most common method for most of the fungal extraction, but this results in lower yield. To overcome the low yield disadvantage, some innovative methods of extraction have been developed to enhance the extraction efficiency, including subcritical water extraction (Luo et al. 2017; Zhang et al. 2018), ultra-high-pressure extraction (Chen et al. 2014b), and ultrasonic extraction (Jing et al. 2014). Out of these extraction methods, ultrasonic assisted extraction has been more employed method for polysaccharide extraction (Wen et al. 2018a, b) because it increases the yield of polysaccharide due to ultrasound effect and mechanical disruption of the cells to release more polysaccharides (Wen et al. 2018a, b, 2019).

6.5 Conclusion and Future Prospectus

Over the past few years, people are using natural *Cordyceps* for consumption and for medicinal purposes in traditional Chinese medicine because of bioactive compounds such as cordycepin and polysaccharides. Based on several research findings, these two compounds have shown pharmacological interaction against various diseases by modulating various cellular signaling pathways. In future, it is necessary to increase the production of cordycepin and polysaccharides through artificial growing of *Cordyceps* sp. by optimizing the culture media of liquid and solid-state fermentation techniques and isolation of these bioactive compounds for further application. Also, there is need to study the clinical studies to check the exact mechanism of cordycepin and polysaccharides in cure and amelioration of various diseases along with toxicity studies.

Acknowledgments We acknowledge SERB, New Delhi for financial support under the SERB-TARE (TARE/2019/000051, dt:4/12/2019) and authorities of Savitribai Phule Pune University and Director, MACS-Agharkar Research Institute, Pune for providing all laboratory facilities.

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7 Genome-Mediated Methods to Unravel the Native Biogeographical Diversity and Biosynthetic Potential of *Trichoderma* for Plant Health

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Abstract

Trichoderma is a soil-borne fungal pathogen distributed in diverse climatic zones of India. They are economically most important fungi which are widely used in commercial and agricultural sector. They produce some enzymes, antibiotics and secondary metabolites and therefore with the help of biotechnological applications these are applied against several fungal pathogens. These fungi are used as a good alternative to chemically produced pesticides and insecticides and are useful for sustainable agriculture. Till date, 375 *Trichoderma* species have been identified and among them some species including *T. harzianum*, *T. viride*, *T. atroviride*, *T. reesei*, *T. hamatum*, *T. Longibrachiatum* and *T. asperellum* are widely used as biocontrol, biofertilizer and growth-promoting agents. Among these species, a few *viz. T. reesei*, *T. atroviride*, *T. virens*, *T. harzianum* and *T. Asperellum* are the most studied and their whole genome is sequenced. *T. reesei* with a genome size of 33 Mb is the first *Trichoderma* species whose genome is completely sequenced. The aim of the present chapter is to give current status of the diversity of *Trichoderma* species in India and their uses on different agricultural crops to protect from pathogens.

Keywords

Biological activity · Diversity · Management · *Trichoderma* species · Whole genome

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_7

7.1 Introduction

Trichoderma belongs to family *Hypocreaceae* and was first described in 1794 by Persoon. Weindling (1934) found that this fungus can control other plant disease or kill other fungi. These are asexual fungi that produce conidia and chlamydospores, and ascospores under wild conditions. *Trichoderma* species are among the most significant microbes because they produce economically important industrial enzymes, antibiotics and secondary metabolites and therefore can be used for many biotechnological applications (Poovendran et al. 2011; Mukherjee 2015) as biocontrol agents to protect various crops from soil-borne fungal pathogens, to develop and increase root growth thereby increasing crop production and productivity, resistance to biotic and abiotic stresses, and to enhance the uptake and use of nutrients (Prameeladevi et al. 2012, 2017). However, the most significance usage of the fungus is as a biological control agent.

During the Second World War, various synthetic pesticides were discovered, and simultaneously, biological control of several diseases was also emphasized. The biocontrol activity of the fungus follows mechanisms like mycoparasitism (fungus emits some enzymes that degrade cell wall to facilitate pathogenic infection of the pest); antibiosis (fungus releases some antimicrobial secondary metabolites which adversely affect pathogens); and competition (in which pathogen growth is reduced for nutrients and space) (Kumari and Srividhya 2020). In recent times, the species of *Trichoderma* are not only used for biocontrol as biofungicide and biopesticides, but also *Trichoderma* is used as biofertilizers because these biofertilizers enhance the stress tolerance and plant growth, and develop the ability of plants for systemic resistance against the pathogenic fungi (Prameeladevi et al. 2012, 2017). Although several members of this genus are being used and sold for decades as biocontrol agents, the identification of potential biocontrol *Trichoderma* species is still going on by using conventional and recently developed methods. Taxonomy of *Trichoderma* in the past 40 years has gone through a remarkable transformation.

Today, approximately 375 species are recognized in the genus (Cai and Druzhinina 2021), and most of them were described after 2000, many as anamorphs of *Hypocrea* species (Samuels et al. 2012; Prameeladevi et al. 2021). In India, the identification was first given by Thakur and Norris (1928) by isolating *Trichoderma* from the soil. Thirteen Indian *Trichoderma* species were reported in the 'Monographic contribution of *Trichoderma*' (Nagamani et al. 2002). To realize the importance of new *Trichoderma* species, there is an urgent need to document the total number of commonly available species of *Trichoderma* from India.

Due to the homoplasy of characters, morphology-based identification is very and proper identification is possible only by combined approach using morpho-molecular characterization (Prameeladevi et al. 2021). In India, genome-mediated methods are established and being used to unravel the native biogeographical diversity and biosynthetic potential of *Trichoderma* spp. for plant health.

7.2 Diversity of *Trichoderma* Species in India

Trichoderma is a cosmopolitan fungus available in all environmental habitats and climatic conditions. The taxonomical characterization of *Trichoderma* will be helpful to explore the diversity of this fungus. Persoon was the researcher who first identified the genus *Trichoderma* in 1794. *Trichoderma* is soil-borne and globally available plant pathogenic fungi which are commonly found in rhizosphere and phyllo-sphere zone of crop plants. All type of soils, cropping systems and ecozones have *Trichoderma* spp. under various climatic conditions.

Generally, every species in the genus has specific environmental condition for survival. For example, *T. harzianum* can be found in plenty where the moisture is poor and weather is hot, while *T. koningii* is mostly isolated from diverse climatic conditions (Denielson and Davey 1973). Under long dry soil conditions, the population of *Trichoderma* may decline. As compared to natural conditions, *Trichoderma* develops faster in artificial culture media containing carbon, nitrogen and growth factors.

It is well known that *Trichoderma* species are widely present throughout the world but North Asia and India have great abundance and diversity of *Trichoderma* species. These areas have been thoroughly investigated to examine the effect of climatic factors (temperature, humidity and rain) on the prevalence of *Trichoderma* species (Wuczkowski et al. 2003). As per the survey study of Indian region, l482 isolates of *Hypocrea/Trichoderma* were identified or isolated and it was recognized that the majority of the strains were Asian (Singh et al. 2020).

As *Trichoderma* species are widely used in antagonistic activities against plant pathogens and decomposer of woody and herbaceous plant residue, therefore, the biodiversity of the fungus is useful for human beings. In India, several research workers are working on different aspects including diversity, taxonomy, ecology and biocontrol applications of *Trichoderma* species (Prameeladevi et al. 2012, 2017, 2021; Prabhakaran et al. 2017; Sharma et al. 2020; Sood et al. 2020). Throughout India, several researchers from different agricultural and central universities and research institutes are working on several *Trichoderma* species and nearly 500 research articles have already been published (Sharma et al. 2014). Two *Trichoderma* species viz., *Trichoderma harzianum* and *Trichoderma viride* are broadly used and exploited on overall agricultural crops and soil-borne and foliar pathogens, respectively. Earlier, few *Trichoderma* species were known for biocontrol efficiency due to poor identification but with the advent of modern molecular techniques, it has become easy to resolve the taxonomic confusion in *Trichoderma* species. Meyer et al. (1992) used DNA fingerprinting technique and reclassified several species of *Trichoderma*. By the use of PCR-based techniques, it became easy to characterize and identify different strains of *Trichoderma* species (Lieckfeldt and Seifert 2000). In India, several isolates of different *Trichoderma* species have been isolated and characterized for their biocontrol efficiency against different plant pathogens (Prameeladevi et al. 2012, 2017). Many recent publications indicate the application of modern tools, namely RAPD, RFLP, Sequence-based identification, DNA barcoding and fingerprinting and whole genome sequencing in the

Trichoderma systematics worldwide (Cai and Druzhinina 2021) and in India (Atanasova et al. 2013a, b; Prameeladevi et al. 2021). In a recent study, Prameeladevi et al. (2021) isolated more than 100 isolates of *Trichoderma* spp. from various geographical locations of India and subjected to phenotypic evaluation. The isolates were segregated into 20 different species viz., *T. aggressivum*, *T. asperellum*, *T. atroviride*, *T. brevicompactum*, *T. citrinoviride*, *T. crassum*, *T. erinaceum*, *T. ghanense*, *T. hamatum*, *T. harzianum*, *T. koningiopsis*, *T. longibrachiatum*, *T. longipile*, *T. minutisporum*, *T. pubscenes*, *T. reesei*, *T. saturnisporum*, *T. spirale*, *T. tomentosum* and *T. virens* based on the integrated approach of both morphological and molecular characterization (Fig. 7.1).

7.3 Taxonomy of Some Important *Trichoderma* Species

7.3.1 *Trichoderma aggressivum*

Conidiophores arise mainly singly from main axis with the branches in 2–3 whorls at less than 90° to the axis with long 'internodes'. Phialides are flask-shaped, enlarged in the middle, sharply constricted below the tip to form a narrow neck and slightly constricted at the base. Cylindrical, ampulliform and laginiform phialides are also seen. Solitary phialides are common. Conidia are often ovoidal and smooth 4.0–6.0 × 2.5–3.0 µm, green.

7.3.2 *Trichoderma asperellum*

Conidiophores have a symmetric aspect terminating in two or more phialides, and primary branches arising below the tip frequently paired and projecting at nearly 90° to the main axis. Phialides are typically produced at the tips of primary, secondary and tertiary branches, typically in whorls of two to four phialides, straight, ampulliform, only slightly enlarged in the middle. Conidia are globose to subglobose or ovoidal, finely spinulose (ornamentation could be seen in SEM and TEM only), dark green, 3.5–6.0 × 3.0–5.0 µm. Chlamydospores are abundant, terminal or infrequently intercalary, on immersed hyphae, subglobose to ovoidal, smooth, pale green, 5.0–15.0 µm diameter.

7.3.3 *Trichoderma atroviride*

Conidiophores are fertile and paired branches that typically arise from 90° or less with respect to the branch above the point of branching. Phialides are flask-shaped, enlarged in the middle, sharply constricted below the tip to form a narrow neck and slightly constricted at the base. Cylindrical, ampulliform and laginiform phialides are also seen. Solitary phialides are common. Conidia are globose to ovoidal and

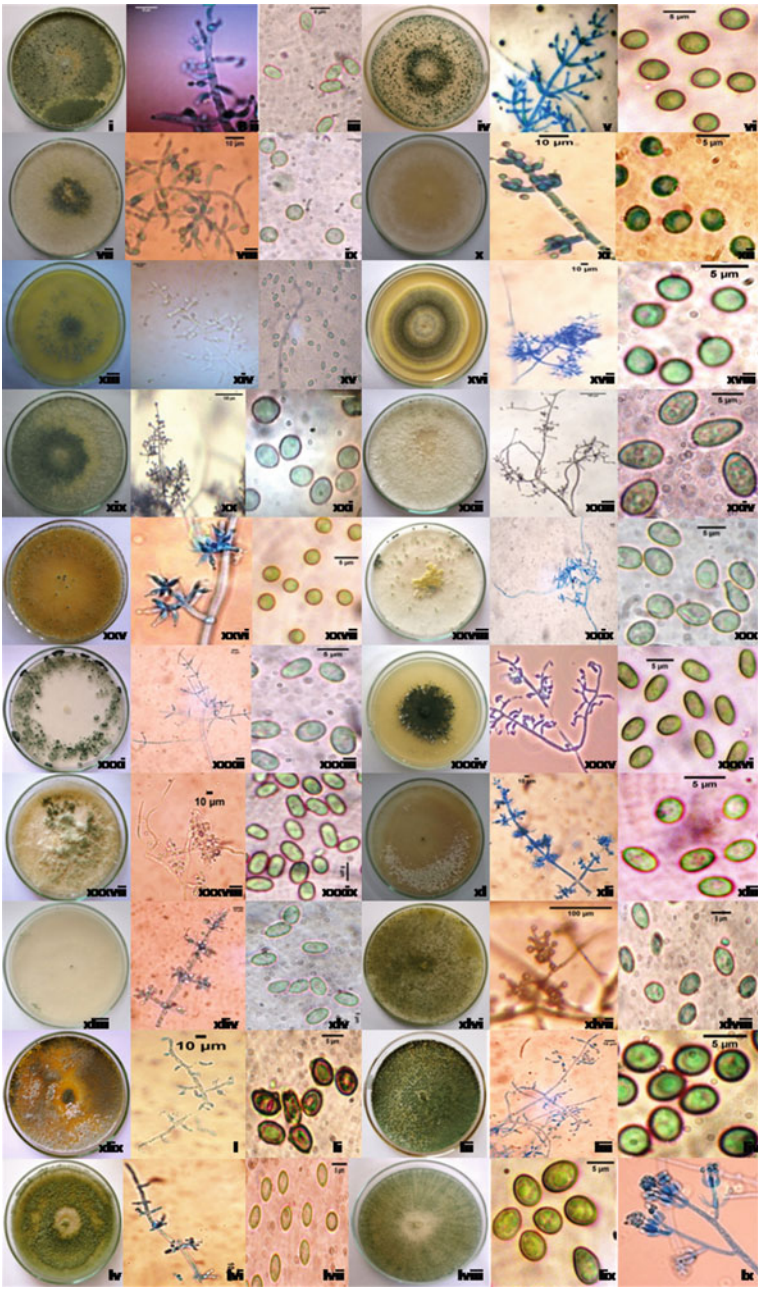

Fig. 7.1 Colony, phialides and conidia of *Trichoderma* species. (**i–iii**) *T. aggressivum*, (**iv–vi**) *T. asperellum*, (**vii–ix**) *T. atroviride*, (**x–xii**) *T. brevicompactum*, (**xiii–xiv**) *T. citrinoviride*, (**xvi–xviii**) *T. crassum*, (**xix–xxi**) *T. erinaceum*, (**xxii–xiv**) *T. ghanense*, (**xv–xvii**) *T. harzianum*, (**xviii–xxx**) *T. hamatum*, (**xxxi–xxxiii**) *T. koningiopsis*, (**xxxiv–xxxvi**) *T. longibrachiatum*, (**xxxvii–xxxix**) *T. longipile*, (**xl–xlii**) *T. minutisporum*, (**xliii–xlv**) *T. pubescens*, (**xlvi–xlviii**) *T. reesei*, (**xlix–li**)

smooth 3.00–3.50 × 3.80–4.00 μm, green. Chlamydospores are globose and smooth.

7.3.4 *Trichoderma brevicompactum*

Conidiophores are thick, branching along the entire length, often at base of conidiophores or at interior of pustules. Branches are loosely paired at a node when arising towards the tip of the conidiophores. Phialides arise in verticals of three to six– from the tip of long conidiophores and are ampulliform, globose to subglobose. The broad and short phialides and branches give a compact and compressed appearance to the conidiogenous structures. Conidia are subglobose or short ellipsoidal, mostly 3.00–5.00 × 2.00–3.50 μm, smooth-walled, appearing pale grey-green. Chlamydospores are subhyaline, intercalary or terminal, solitary, globose and pyriform, 5.00–7.00 μm.

7.3.5 *Trichoderma citrinoviride*

Conidiophores are fertile with less secondary branches. Mostly the phialides arise singly on the main axis. Phialides are langeniform to ampuliform, long and slender, horn shaped, their base is little narrower than the middle part which projects into conical or sub-cylindrical neck. Conidia are cylindrical to ellipsoidal, smooth, 4.00–5.25 × 2.45–2.75 μm, green.

7.3.6 *Trichoderma harzianum*

Conidiophores branching and phialides are in the non-pigment producers. Phialides are ampulliform in pigment producers and laginiform to subulate in non-producers. They are usually 3–4, verticillate. Conidia are subglobose to obovoid, smooth, 2.5–3.0 × 2.0–2.5 μm, and pale green. Chlamydospores are terminal and intercalary, globose, 4.0–8.0 μm in diameter.

7.3.7 *Trichoderma hamatum*

Conidiophores in pustules are broad, comprising a regular, undulate and humate sterile elongation with the phialides arising near the base. Phialides are short, broadly ellipsoidal to ovoidal, pyriform and ampuliform, formed on small branches, smooth

Fig. 7.1 (continued) T. saturnisporum, (**lii–liv**) *T. spirale*, (**lv–lvii**) *T. tomentosum*, (**lviii–lx**) *T. virens*. (Source: Prameeladevi et al. 2021)

walled, hyaline or pale green colour. Conidia are oblong to ellipsoidal and smooth, 5.0–6.0 × 3.0–4.0 μm, green. Chlamydospores are terminal and intercalary, globose, 4.0–8.0 μm in diameter.

7.3.8 *Trichoderma koningiopsis*

Fertile branches arise along the length of the main axis, with longer or shorter internodes; terminal part of conidiophore sparingly branched and with long internodes between branches; branches sometimes formed pustules with short, pachybasium-like crowded phialides. Phialides are straight, sometimes hooked or sinuous, lageniform or sometimes conspicuously swollen in the middle, in whorls of two to five, sometimes several phialides arising from the same point. Conidia are dark green, ellipsoidal and smooth, 4.50–6.50 × 3.00–4.00 μm. Chlamydospores are abundant, terminal and intercalary, globose to subglobose and pear shaped, 10.00–15.00 μm in diameter.

7.3.9 *Trichoderma longibrachiatum*

Conidiophores typically consist of a strongly developed central axis, sparingly branched, primary branches long, secondary branches usually short and rarely re-branched, and have solitary phialides. Phialides arise directly, mostly solitary, occasionally in verticils of two to three. Phialides are ampuliform to laginiform or cylindrical but, when in whorls, enlarged in the middle or squat, straight or hooked to sinuous, Intercalary phialides are common and conspicuous. Conidia are oblong to ellipsoidal and smooth, 3.5–8.0 × 3.0–5.0 μm, green in colour. Chlamydospores are sometimes present, terminal or intercalary, subglobose to globose 8–10 μm in diameter.

7.3.10 *Trichoderma reesei*

Conidiophore comprises of a well-defined main axis that is rarely re-branched. Phialides are typically solitary, straight or sinuous or hooked; some phialides are cylindrical and ampulliform and sometimes flask-shaped, constricted to the tip and slightly at the base. Intercalary phialides are common. Conidia are green, oblong to ellipsoidal, smooth, 4.50–6.00 × 3.50–4.00 μm. Chlamydospores become abundant within 7 days, globose to subglobose, terminal or intercalary, 8.00–10.00 μm in diameter.

7.3.11 *Trichoderma saturnisporum*

Conidiophores arise from the aerial mycelium of developed pustules, asymmetrically branched, the branches producing phialides directly or rebranching, the secondary branches producing phialides along the length and ending in a single phialide; sometimes the main axis of a conidiophore terminates in a sterile, septate, hypha-like elongation. Phialides mainly arise singly, less frequently in appressed to divergent whorls of two to three, typically curved, ampulliform to broadly lageniform, sometimes hooked or sinuous. Conidia are green, smooth walled but with conspicuous sinuate, bullate or wing like inflations of the outer wall, ellipsoidal, 4.5–7.5 × 3.5–4.5 μm. Chlamydospores are present, globose, 6.0–12.0 μm in diameter.

7.3.12 *Trichoderma virens*

Conidiophores arise in clusters in lateral branches from undifferentiated aerial mycelium, sterile at the base and is unbranched, but the upper part is fertile towards the apex, and each branch terminating in a penicillus of (2–)3–6 closely appressed phialides. Phialides mainly arise in closely appressed whorls of two to five on terminal branches, less frequently in pairs or singly, straight, lageniform to ampulliform and sometimes laginiform to subulate, base constricted, swollen in the middle, attenuate at the tip. Conidia are broadly ellipsoidal to obvoidal and minutely warted at high magnifications (SEM), 3.5–8.0 × 3.0–5.0 μm, and dark green in colour. Chlamydospores are abundant, terminal and intercalary, globose to subglobose, 6.0–12 μm in diameter.

7.4 Genome-Mediated Methods for Characterizing the *Trichoderma* Species and Their Antagonistic Activities: Conventional to Recent

The illustrative morphological characteristics of the genus *Trichoderma* were first identified and given by Bissett (1991a, b). However, the taxonomical and molecular information of *Trichoderma* was further investigated or reviewed by several research workers throughout the world (Druzhinina et al. 2005; Samuels 2006; Samuels et al. 2012; Prameeladevi et al. 2012, 2017; Sandoval-Denis et al. 2013) to gain accurate, deep and recent knowledge of species in *Trichoderma*. So far, the taxonomy in *Trichoderma* is largely based on morphological characters but due to species complexity and their pleiomorphism, it is very difficult to name *Trichoderma* species. Based on sexual stage (teleomorph) the species is known as Hypocrea, its generic name, while the name *Trichoderma* is given due to its anamorphic or mitosporic stage.

A combined approach of phenotypic and genetic identification is important to validate *Trichoderma* species. Using advanced molecular techniques for

investigations encouraged researchers to resolve the confusion related to taxonomy in *Trichoderma*. In recent years, phylogenetic approach is widely accepted for accurate identification, differentiation and characterization of *Trichoderma* species. In taxonomic study, molecular markers are used to unambiguously identify a large number of species. However, it has been noticed that some techniques are moderately ambiguous, for example NCBI BLAST (GenBank) sequence deposition at times does not decisively allocate the correct genus (Kopchinskiy et al. 2005). Also, ITS sequencing when performed singly was not found to be satisfactory and explanatory to identify phylogenetically close *Trichoderma* species and also misled the appropriation of *Trichoderma* diversity (Lieckfeldt and Seifert 2000; Chaverri et al. 2003; Hoyos-Carvajal et al. 2009).

Recently, it has been suggested that taxonomic identification should be based on poly-phasic approach, where morphological characteristics (mycelium, micro- and macrospores, etc.) and molecular identification (using multigene DNA sequence) are applied together (Kubicek et al. 2001). With the help of phylogenetic species concept (PSC), Chaverri et al. (2003) developed seven phylogenetic lineages in *H. lixii*/*T. harzianum* using multigene viz., ITS1 and ITS2, transcription elongation factor 1-(tef1α), and short fragments of the actin (act1) and calmodulin (cal1) exon sequences.

The genus *Trichoderma* is so widely researched due to release of cell wall degrading enzymes, production of bioenergy-related and mainly serve as biocontrol agents against several plant pathogens. This genus is top ranked in the list of fungal biocontrol agents (BCA). Due to the ability of *Trichoderma* species to enhance plant growth, stress tolerance and induce systemic resistance of the plant to other fungi, it has been used not only as biopesticides, but also as biofertilizers. Many *Trichoderma* spp. are reported as good BCA in India and abroad. List of some important and potential biological control species are given in Table 7.1.

7.5 Whole Genome Sequencing and Their Interpretation for Biocontrol Activity

In the genus *Trichoderma*, *T. reesei* is the first species whose genome is completely sequenced. Recently, genome sequencing of *Trichoderma harzianum* has also been attempted. Some *Trichoderma* species including *T. reesei*, *T. atroviride*, *T. virens*, *T. harzianum* and *T. asperellum* are most studied with respect to genome sequencing. Usually, filamentous fungus has small genome size. They have a haploid nucleus. *Trichoderma* species have average genome size range of 31–39 Mb and chromosome range of three to seven. The chromosome size may differ depending on the species. *T. Reesei* genome is sequenced because of its commercial significance. This strain is very important as it produces industrially important cellulose and hemicelluloses enzymes. The genome size of *T. reesei* is 33 Mb and number of chromosomes is 7. The specific characters of the sequenced genomes are given in Tables 7.2 and 7.3.

Table 7.1 Important biocontrol species of *Trichoderma* and their application in management of plant diseases

S. No.	Species name	Crop	Disease/Pathogen	Treatment type
1	*T. harzianum*	Rice, Pepper plants, Eggplant, Cucumber, Cotton	*Bipolaris oryzae*, *R. solani*, *Macrophomina phaseolina*, *Pythium sp.*	Soil and seed
2	*T. viride*	Brinjal, Jute, Mushroom, Soybean, Corn	*Fusarium solani*, *Rhizopus*, *Rhizopus*, *Pythium*, *F. oxysporum* f. sp. *Adzuki*	Soil and Seed
3	*T. reesei*	Chickpea, Mango	*F. oxysporum*, *Pestalotia* sp.	Soil, Seed and foliar
4	*T. pseudokoningii*	Cassava, Chilli	*Chaetomium*, *Sclerotium rolfsii*,	Soil and seed
5	*T. longibrachiatum*	Groundnut, Tomato	*Aspergillus niger*, *Pythium aphanidermatum*, *S. rolfsii*	Seed and soil
6	*T. hamatum*	Wheat, Lentil	*Biopolaris*, *F. oxysporum*	Seed and soil
7	*T. koningii*	Rice, Sapodilla	*Fusarium* spp., R. solani	Seed
8	*T. virens*	Tomato, Mung bean	*Rhizoctonia solani*, *R. bataticola*	Soil and seed
9	*T. lignorum*	Bamboo	*Polyporus*	Soil
10	*T. asperellum*	Cocoyam, Tomato	*Pythium myriotylum*, *Fusarium oxysporum*	Seed and soil
14	*T. atroviride*	Cotton	*S. delphinii*	Soil and seed
19	*T. tomentosum*	Soybean	*R. solani*	Soil and seed

Source: Sharma et al. (2014) and Zin and Badaluddin (2020)

The sequenced genomes of several *Trichoderma* species are openly accessible on U.S. department of energy (DOE) Joint Genome Institute (JGI). The *Trichoderma reesei* parental strain (QM6a-v2.0 draft genome; 33.4 Mb) contains 87 scaffolds and 9129 predicted genes (Martinez et al. 2008a, b). *Trichoderma reesei* hyper-cellulolytic strain (RUT-C30-v1.0 draft genome; 32.7 Mb) has 182 scaffolds and 9852 predicted genes (Koike et al. 2013). The genome size of QM6a-v2.0 is small comparatively to the genomes of *T. atroviride* and *T. virens* (Schmoll et al. 2016). These genomes are very important because they have some key genes which are responsible for transcriptional factors expressing CAZymes and cell wall degradation enzymes.

Trichoderma harzianum, *Trichoderma atroviride* and *Trichoderma virens* are known to enhance plant resistance against many pathogens (Kubicek and Druzhinina 2013), whereas *T. reesei* is known to promote plant growth by producing enzymes that facilitate the use of renewable nutrients (Kubicek 2013). In addition, *Trichoderma* spp. also can remediate heavy metal-contaminated environments. For example, *T. asperellum* protects crop health by reducing the bioavailability of heavy

Table 7.2 Features of the sequenced genomes of some important *Trichoderma* species

S. No.	Features	*T. reesei*	*T. virens*	*T. atroviride*	*T. harzianum*	*T. asperellum*	*T. longibrachiatum*	*T. citrinoviride*
1	Genome size (Mb)	34.1	39	36.1	40.98	37.36	32.34	33.48
2	No of predicted genes	9129	12,427	11,863	14,095	12,566	10,792	9397
3	Glycosyl hydrolases	23	41	37	Nil	Nil	Nil	Nil
4	Secondary metabolites biosynthesis, transport and catabolism (KOG)	262	440	349	438	358	253	285
5	PKS	11	18	18	Nil	Nil	Nil	Nil
6	NRPS	10	28	16	Nil	Nil	Nil	Nil
7	PKS-NRPS	2	4	1	Nil	Nil	Nil	Nil
8	SSCPs	260	319	301	Nil	Nil	Nil	Nil
9	Xenobiotics bidegradation and metabolism (KEGG)	327	519	453	610	432	232	359
10	Mating types	MAT1–2	MAT1–2	MAT1–2	MAT1–2	MAT1	MAT1–1	MAT1–2

Table 7.3 Genes isolated from *Trichoderma* species and their role in biocontrol mechanisms

S. No.	Gene	*Trichoderma* species	Function
1	Th-Chit	*Trichoderma harzianum*	It has antifungal activity in transgenic tobacco plant
2	tri5	*Trichoderma harzianum*	This gene synthesizes trichothecene enzyme that inhibits the protein and DNA synthesis in the cells of the pathogens and inhibits their growth
3	ThPG1	*Trichoderma harzianum*	It effects endopoly-galacturonase enzyme which helps in degradation of pathogens cell wall of *R. solani* and *P. ultimum*
4	erg1	*Trichoderma harzianum*	This gene is responsible for squalene epoxidase enzyme, which helps in the synthesis of ergosterol and silencing of this gene provides resistance to terbinafine, an antifungal compound
5	Thkel1	*Trichoderma harzianum*	This gene encodes for putative kelch-repeat protein which helps in regulating the glucosidase activity and enhances salt tolerance and osmotic stresses in *Arabidopsis thaliana* plants
6	qid74	*Trichoderma harzianum*	This gene plays a significant role in cell protection and provides adherence to hydrophobic surfaces that help the fungus in mycoparasitic activity against *R. solani*
7	TgaA and TgaB	*Trichoderma virens*	This gene shows its antagonist activity against *R. solani* and *Sclerotium rolfsii*
8	Tvsp1	*Trichoderma virens*	It affects production of serine protease enzyme against *Rhizoctonia solani* which affects the cotton seedlings.
9	tac1	*Trichoderma virens*	This gene shows mycoparasitic activity against *R. solani* and *P. ultimum*
10	egl1.	*Trichoderma longibrachiatum*	This gene shows biocontrol activity against *P. ultimum* in damping-off of cucumber
11	TvGST	*Trichoderma virens*	This gene provides cadmium tolerance
12	Taabc2	*Trichoderma atroviride*	This gene has a significant role in ATP Binding Cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitic activity
13	tri5	*Trichoderma brevicompactum*	This gene is responsible for the production of *Trichodermin* which shows antifungal activity against *S. cerevisiae*, *Kluyveromyces marxianus*, *Candida albicans* and *Aspergillus fumigatus*
14	TrCCD1	*Trichoderma reesei*	This gene is involved in carotenoid metabolism that helps in the development of conidiospores and hyphal growth in *T. reesei*

Source: Srivastava et al. (2014)

metals, such as cadmium (Cd) and lead (Pb), through detoxification and sequestration (Zhang et al. 2018a). *T. asperellum* has been successfully produced as chlamydospores to remediate arsenic (As) contaminated soils via bio-volatilization (Wang et al. 2015). Their first function is growth promotion. *T. asperellum* SM-12F1

can improve the activities of soil enzymes associated with nutrient activation and antioxidant stress (Zhang et al. 2018b) and induce plant growth-promoting attributes of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, auxin and siderophore production (Qi and Zhao 2013). This strain can also induce systemic resistance (ISR) in plants by regulating ethylene pathways and signalling jasmonic acid release (Guo et al. 2019). The second important function is biocontrol where *T. asperellum* resists pathogenic fungi and nematodes by competing for nutrients and growing space, destroying the hyphae of pathogenic fungi, mycoparasitic activity, antibiotic production, and inducing disease resistance. The third important function is bioremediation where *T. asperellum* SM-12F1 can remediate As-contaminated environments by triggering the transformation of species by reduction, oxidation, methylation, demethylation and cellular sequestration. *Trichoderma asperellum* SM-12F1 can also remediate environments contaminated by lead (Pb) and cadmium (Cd) (Zhang et al. 2018a).

The genome of biocontrol strain ITEM 908 has also sequenced which is known as *Trichoderma harzianum*. This strain has both capacities to enhance the plants growth and antagonistic activity against several plant pathogens including *Rhizoctonia solani*, *Fusarium graminearum*, and root-knot nematode *Meloidogyne incognita*. This strain can develop root system, enhance the growth of tomato plant and help it to be rhizosphere competent (Kubicek and Druzhinina 2013). This strain also reduces the inoculum of *Stemphylium vesicarium* and application of this strain in soil and leaf litter and ground-cover litter in pear orchards controls brown spot of pear (Arvas et al. 2011). This strain also has antagonistic against *Fusarium* head blight of wheat and other grains under in-vitro conditions and inhibits the formation and development of perithecia (Hakkinen et al. 2014). Some secondary metabolites are also produced by this strain which are active against aphid pests. This strain is also used in soil treatment of tomato crop to reduce the growth of the *Meloidogyne incognita* (root-knot nematode) (Druzhinina et al. 2016). ITEM 908 also triggers the plant defence mechanism against *Meloidogyne incognita* (root-knot nematode) by changing gene expression associated to disease (Kim et al. 2014). The isolate ITEM 908 is currently being registered under the European Union regulation as an active ingredient for the production of commercial biopesticides.

7.6 Conclusion

In the conclusion, it was observed that *Trichoderma* species produced several types of enzymes and secondary metabolites which have potential biocontrol activity like cell wall degradation, biotic and abiotic stress tolerance, and antagonistic activity against plant pathogens. In past, due to the lack of technologies and *Trichoderma* species complex it was very difficult to identify or characterize the genus at species level. But recently, several PCR-based molecular technologies are available and now it is easy to identify the genus and its species. Species of *Trichoderma* have now become potential biocontrol agent at the commercial level for production of environmentally safe enzymes and secondary metabolites biotechnology industry. With the

development of the molecular markers the progress has been made for genetic diversity analysis in the genus and new *Trichoderma* species beneficial for industrial and agricultural research have been identified in this versatile genus.

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8 The Diversity and Taxonomy of Phytopathogenic Fungi in the Genus *Cladosporium* from India

Haridas Bhartiya, Paras Nath Singh, and Nisha Kumari

Abstract

We report three new phytopathogenic fungi *Cladosporium almondae, C. annonacinum,* and *C. astericolum* isolated from active leaves of *Prunus dulcis* (*Rosaceae*), *Annona squamosa* L. (*Annonaceae*), and *Eupatorium* sp. (*Asteraceae*), respectively. This report is a partial outcome of survey conducted in natural deciduous forests of Bahraich and Gorakhpur situated in North-Eastern part of Uttar Pradesh, India during January 1997–November 1999. These are described, illustrated and compared with allied and type species *C. herbarum*. A key to species of *Cladosporium* is provided. Descriptions and nomenclatural details were deposited in MycoBank.

Keywords

Cladosporium fungi · *Hyphomycetes* · Taxonomy · Uttar Pradesh

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_8

8.1 Introduction

The anamorphic hyphomycetes genus *Cladosporium* was established by Link (1816). It is a largest and most heterogenous class of Hyphomycetes. Recently encompassing more than 800 species have been reported (Dugan et al. 2004). The genus *Cladosporium* is distinguished from other *Mycosphaerella* asexual morphs by its unique type of thickened scars and conidial hila. The species of this genus causes leaf spots on forest flora of tropical and subtropical region. *Cladosporium* is cosmopolitan in their distribution and commonly encounters in every habitat. Many of the species are plant pathogenic, i.e., they are casual organism of leaf spots and other lesions (Schubert and Braun 2005). They occur as hyperparasites on other fungi (Heuchert et al. 2005). Monographic studies of *Cladosporium* S. lat. Were presented by Bensch et al. (2012). This genus is characterized by causing leaf spot mostly amphigenous, colonies discrete, hyphae immersed or superficial, well-developed stromata, producing fasciculated conidiophores, macro- to mononematous, septate, smooth-walled, branched, straight to curved, flexuous. Detailed historical accounts of *Cladosporium* and allied genera with its ecology, systematics, and phylogeny were described by Bensch et al. (2012).

Further additions to the genus were done by Indian Mycologists (Butler and Bisby 1931; Bilgrami et al. 1979, 1981, 1991; Sarbhoy et al. 1986). Recently some new *Cladosporium* species were added to the Indian Mycota from Uttar Pradesh (Sharma et al. 1998; Kumar et al. 2006, 2007; Singh et al. 2008; Bhartiya et al. 2015, 2016). This chapter included with illustration and description of three new species of *Cladosporium* such as *C. almondae, C. annonacinum*, and *C. astericolum* from active leaves of *Prunus dulcis* (Badam), *Annona squamosa* (Custard apple), and *Eupatorium* sp. (Banmar).

8.2 Materials and Methods

8.2.1 Survey Conducted

More than 200 different types of infected leaf samples were collected from diverse host plants in paper bags and the specimens were brought to the laboratory. The samples were pressed in a plant press machine. To remove the moisture from samples, gradual changes were made using fresh blotting paper sheets and finally the specimens were enveloped using paper wax sheets. The passport data for each specimen like scientific name of the fungus, host name and family, place of collection/date, etc. were marked on envelop.

8.2.2 Identification

With the help of fine forceps and sharp blades the scrape was taken from infected leaf and the semi-permanent microscopic slides were prepared in lactophenol cotton blue

mounts. To observe the fungal fructifications on infected leaves, Nikon Binocular stereo microscope (Model SMZ-1500 with Digi CAM was used. The morphological details were observed and microphotographs were taken using an Olympus CX-41 compound microscope and the Camera Lucida drawings were made of characteristic morphological features. The conidiophores and conidial measurements were taken using an ocular micrometer. Taxonomic determinations were done with the help of pertinent literature including monographs, flora, and research paper. The holotype specimens were deposited in HCIO, Indian Agricultural Research Institute, New Delhi, India and an isotype is kept in the Departmental Herbarium of DDU Gorakhpur University, Gorakhpur for further references. The taxonomic concept is based on the following sources: Cannon and Kirk (2007), Kirk et al. (2008), and Index Fungorum (2015) (www.indexfungorum.org). Detailed description and nomenclatural novelties were deposited in MycoBank (www.MycoBank.org).

8.3 Results and Discussions

8.3.1 Taxonomic Description

1. *Cladosporium almondae* Bhartiya, H. D, Singh, P.N., Kumari Nisha sp. nov. Fig. 8.1
   MycoBank MB 835157, Holotype-HCIO 43150

Description

Leaf spots amphigenous, subcircular to irregular, dark blackish. Colonies amphiphyllous, discrete, whitish gray. Mycelium of hyphae partially superficial, immersed in the substratum, smooth-walled, branched, septate, dark olivaceous, 19 μm wide. Stromata well-developed, irregular, pseudoparenchymatous, dark brown, 15–23 μm wide, 16–26.5 μm in long. Conidiophores arising in fascicles of 2–8 from stromata, terminal lateral branches from mycelia hyphae, macronematous, mononematous, 2–5 septate, smooth-walled, branched, erect to sub-erect, straight to curved, flexuous, dark olivaceous, 13–18 × 3.5–6.5 μm. Conidiogenous cells integrated, terminal to intercalary, polyblastic, sympodial, cicatrized, bearing distinctly thickened conidial scars. Conidia holoblastic, dry, acropleurogenous, ramocatenate (in branched chains), cylindrical to variable in shape (ellipsoidal, fusiform, oval), spherical conidia bearing protuberant, 0–3 transverse only septate, apex obtuse, base truncate to rounded, hila distinctly thickened, light olivaceous, 7.6 × 3.5–5 μm.

Collection examined: Type. India, Uttar Pradesh, Gorakhpur, 17 January 1997, on living leaves of *Prunus dulcis* (Mill.) D.A.Webb (Rosaceae); Coll. Nisha Kumari, HCIO 43150 (Holotype), GPU Herb No. 8530 Isotype.

Etymology: The species epithet "*almondae*" is derived from the host genus (Table 8.1).

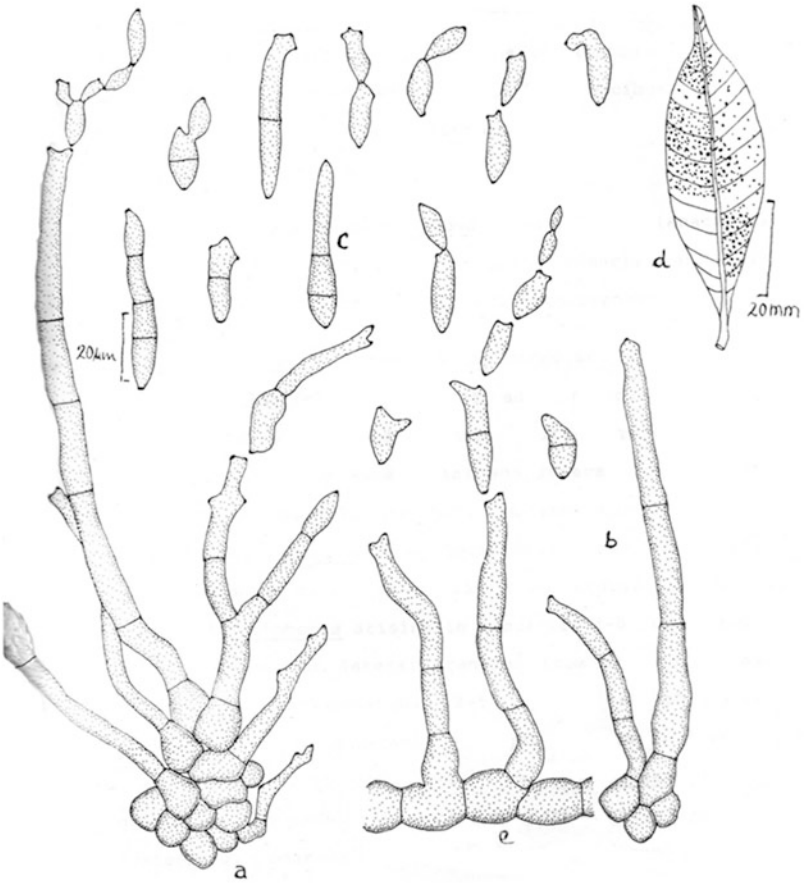

Fig. 8.1 *Cladosporium almondae* Bhartiya HD, Singh PN, Kumari Nisha sp. nov., MycoBank MB 835157; Holotype-HCIO 43150, (**a**) stomatal cells and conidiophores, (**b**) conidiophores, (**c**) conidia, (**d**) infection spots on leaf surfaces, scale bars—20 μm

Remarks

It is evident from the fungal database that no species of this genus was reported earlier from this host of the family, thus it appears worthwhile to compare the morphotaxonomic feature of the present collection with the type species *Cladosporium herbarum* Link (1816).

The comparative analysis shows that *Cladosporium almondae* sp. nov. has very different size and symptoms on infection spots and stroma. The conidiophores are unbranched, longer (250 μm) flexuous and vesicular swelling in *C. herbarum* in contrast to *C. valmondae* which has fasciculate shorter (13–180 μm), branched, septate, smooth and dark brown conidiophores. Conidia of *C. almondae* is very larger (7–66 × 3.5–5 μm) and entirely different in septation (multiseptate), smooth and always simple in contrast to *C. herbarum* which bears shorter (5–23 × 3–8 μm)

Table 8.1 Morphotaxonomic comparison of *Cladosporium almondae* sp. nov. reported on host species *Prunus dulcis* with allied species

Characters	*C. herbarum* Link	*C. almondae* sp. nov.
Stromata	Well-developed	Well-developed, pseudoparenchymatous, dark brown, 15–23 × 16–26.5 μm
Conidiophore	Macronematous, flexuous, geniculate, thick terminal and intercalary, vesicular swelling, pale to mid olivaceous brown, 250 μm long, 3–6 μm thick	In fascicles 2–8, macronematous, mononematous, 2–5 septate, branched, flexuous, dark olivaceous, 13–180 × 3.5–6.5 μm
Conidia	Branched chains, ellipsoidal, oblong, thick walled, verruculose, rounded at the nodes, 0–1 septate, 5–23 × 3–8 μm, olivaceous brown, hila protuberant	Ramocatenate (in branched chains), ellipsoidal, fusiform, oval, protuberant, 0–3 transversely septate, apex obtuse, base truncate to rounded, hila distinctly thickened, light olivaceous, 7.66 × 3.5–5 μm

verruculose and rounded at the nodes. Therefore, the description of fungus is treated here as new species of the fungal genus in question.

2. *Cladosporium annonacinum* Bhartiya HD, Singh PN, Kumari Nisha sp. nov. Fig. 8.2
   IF 558254
   Holotype-HCIO 43089

Description

Infection spots amphigenous, circular to irregular, later spreading on entire leaf surfaces, discrete, dark blackish. Colonies amphiphyllous, scattered, dark brown. Mycelium of hyphae external, superficial, septate, branched, narrow, smooth-walled, light olivaceous to pale olivaceous, 4.5–11.5 μm wide. Stromata absent. Conidiophores arising in single or in fascicles of 2–7, terminal from lateral branches from mycelia hyphae, macronematous, mononematous, 3–5 transversely septate, smooth-walled, branched, cylindrical, erect to sub-erect, straight to flexuous, light olivaceous to light brown, 21–133 × 3.5–6 μm. Conidiogenous cells integrated, terminal to intercalary, polyblastic, sympodial, cicatrized, bearing thickened conidial scars. Conidia simple, holoblastic, dry, acropleurogenous, ramocatenate, in branched chains, variable in shape and size (ellipsoidal, fusiform, oval, subspherical) cylindrical, 0–3 septate, protuberant, smooth-walled, subacute to obtuse, bases subtruncate or obconicotruncate to rounded, hila dark, thickened, light olivaceous, 5–27 × 4.5–6.5 μm.

Collection examined: Type. India, Uttar Pradesh, Gorakhpur, 20 Feb. 1997; On living leaves of *Annona squamosa* L. (*Annonaceae*) Gorakhpur, Coll. Nisha Kumari, HCIO 43089 Holotype, GPU Herb No. 8503 isotype.

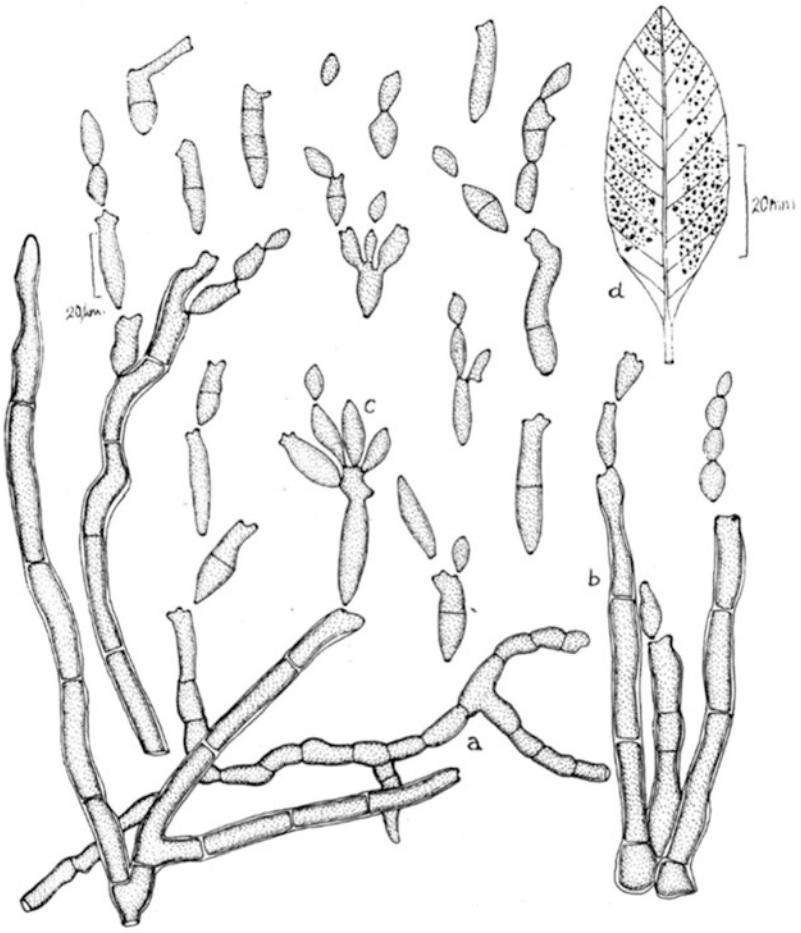

Fig. 8.2 *Cladosporium annonacinum* Bhartiya HD, Singh PN, Kumari Nisha sp. nov. IF 558254, Holotype-HCIO 43089, (**a**) hyphae and conidiophores, (**b**) conidiophores with catenate conidia, (**c**) ramocatenate conidia, (**d**) infection spots on leaf surfaces, scale bars—20 µm

Etymology: The species epithet "*annonacinum*" is derived from host genus (Table 8.2).

Remarks

A perusal of fungal database indicates that only one *Cladosporium* species has earlier been described on same host species. Therefore, it is compared with this in above table to justify its distinct identity. Through comparative analysis shows that the conidiophores are multiseptate (2–5), fasciculate (2–7) and are longer (133 µm) in proposed species as against *C. oxysporum*. In *C. annonacinum* hila darkly thickened, catenate, obtuse, subtruncate or obconicotruncate, and broader than *C. oxysporum* conidia are also multiseptate, in branched chains, cicatrized and

Table 8.2 Morphotaxonomic comparative features of *Cladosporium annonacinum* sp. nov. reported on the host species *Annona squamosa* with allied species

Characters	*C. oxysporum*	*C. annonacinum* sp. nov.
Stromata	Absent	Absent
Conidiophore	Macronematous, straight to slightly flexuous, distinctly node, smooth, 500 µm long or sometimes even longer in culture, 3–5 µm thick, terminal and intercalary, pale to mid olivaceous brown	Single or fascicles, macronematous, mononematous, 3–5 septate, branched, cylindrical, erect to sub-erect, straight to flexuous, light brown, 21–133 × 3.5–6 µm
Conidia	Terminal swelling, simple or branched chains, cylindrical, rounded at the end, ellipsoidal, limoniform or subspherical, subhyaline or olivaceous brown, smooth, 5–23 × 3–8 µm	Simple, holoblastic, ramocatenate, ellipsoidal, fusiform, oval, spherical, cylindrical, 0–3 septate, protuberant, apex subacute to obtuse, base subtruncate or obconicotruncate to rounded, hila thickened, light olivaceous, 5.5–27 × 4.5–6.5 µm

protuberant in *C. annonacinum* as contrast to straight, slightly flexuous, smooth, 500 µm long and pale brown in *C. oxysporum*. The proposed collection is also entirely different in overall symptomatology. Therefore, this is sufficiently distinct to be recognized as new taxon of the species rank.

3. *Cladosporium astericolum* Bhartiya HD, Singh PN, Kumari Nisha sp. nov. Fig. 8.3
   Holotype-HCIO 8521

Description

Infection spots amphigenous, circular to irregular, later coalescing to form large patches, light brown. Colonies amphiphyllous, scattered, dark brown. Mycelium of hyphae external and internal, branched, immersed to superficial, smooth-walled, septate, dark brown, 4.5 µm wide. Stromata well-developed, irregular, pseudoparenchymatous from which producing conidiophores, dark brown, 8–15 µm height, 11–19 µm. width. Conidiophores arising singly or in loose fascicles from stromata and terminal or lateral branches. Mycelial hyphae, macronematous, mononematous, 1–8 transversely septate. Smooth-walled, erect to sub-erect, cylindrical, swollen at the apex, dark brown, 23–326 × 3.5–6.5 µm. Conidiogenous cells integrated, terminal to intercalary, polyblastic, cicatrized, bearing thickened and distinctly conidial scars. Conidia holoblastic, dry, acropleurogenous, cylindrical, variable in shape (ellipsoidal, fusiform, oval), spherical to subspherical, protuberant, septate. Smooth-walled, apex obtuse, base obconicotruncate to rounded, hila thickened, mid olivaceous, 5.5–28 × 2.5–4.5 µm.

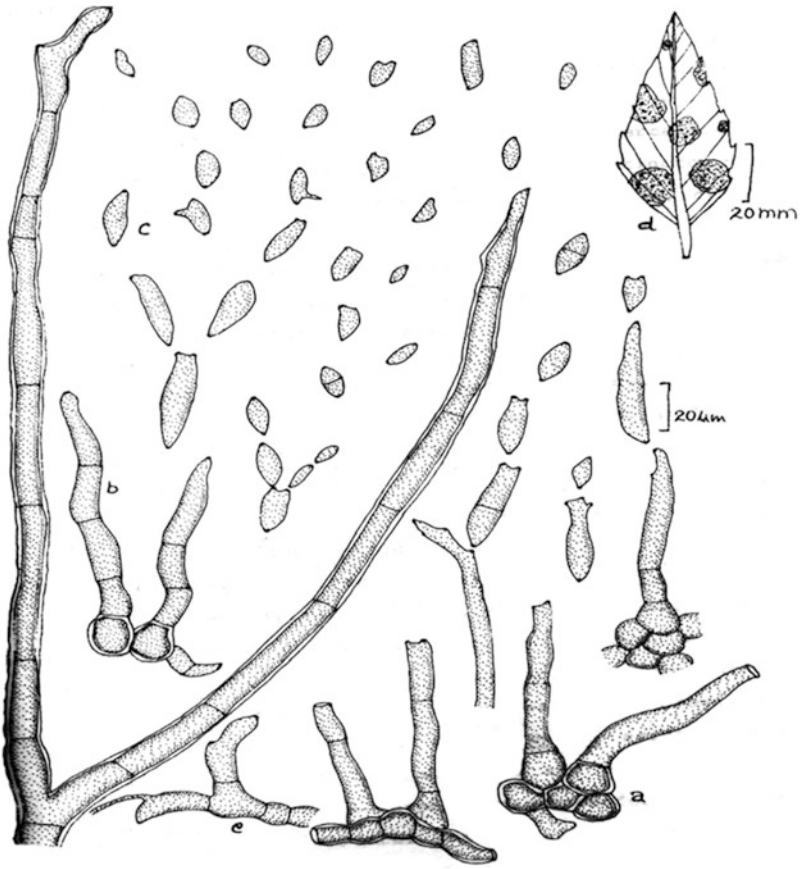

Fig. 8.3 *Cladosporium astericolum* Bhartiya HD, Singh PN, Kumari Nisha sp. nov. Holotype-HCIO 8521, (**a**), (**b**) stroma and conidiophores, (**c**) conidia, (**d**) infection spots on leaf surfaces, (**e**) superficial hyphae bearing lateral conidiophores, scale bars—20 µm

Collection examined: Type, India, Uttar Pradesh, Bahraich, 20 Nov. 1999, on living leaves of Eupatorium sp. (*Asteraceae*), Gorakhpur, Coll. Nisha Kumari, HCIO 43107 holotype, GPU Herb No. 8521 isotype.

Etymology: The species epithet "*astericolum*" is derived from the host family (Table 8.3).

Remarks

Fungal database indicates that no *Cladosporium* sp. on *Eupatorium* sp. has earlier been recorded on host species, host genus even on host family from world. The present taxon is compared with type species *C. herbarum*. *Cladosporium astericolum* differs from *C. herbarum* in having dark brown and well-developed stromata as against less developed in type species. *C. herbarum* Link, 1816. Conidiophores are septate (1–8), swollen at the apex, dark brown and longer

Table 8.3 Morphotaxonomic comparison of *Cladosporium astericolum* sp. nov. reported on host genus *Eupatorium* sp. with allied species

Characters	*C. herbarum* Link (1821)	*C. astericolum* sp. nov.
Stromata	Well-developed	Well-developed, pseudoparenchymatous, dark brown, 8–15 × 11–19 μm
Conidiophore	Macronematous, flexuous, geniculate, thick terminal and intercalary, vesicular swelling, pale to mid olivaceous brown, 250 μm long, 3–6 μm thick	Loose fascicles, macronematous, mononematous, 1–8 septate, swollen at the apex, dark brown, 23–326 × 3.5–6.5 μm
Conidia	Branched chains, ellipsoidal, oblong, thick walled, verruculose, rounded at the nodes, 0–1 septate, 5–23 × 3–8 μm, olivaceous brown, hila protuberant	Holoblastic, ellipsoidal, fusiform, oval, 0–1 septate, protuberant, septate, smooth-walled, obtuse, base obconicotruncate to rounded, hila thickened, mid olivaceous, 5.5–28 × 2.5–4.5 μm

23–326 × 3.5–5.6 μm in *C. astericolum* while smaller, less septate, olivaceous brown and vesicular swelling in *C. herbarum*. Conidia are septate, obtuse, base obconicotruncate with thickened hila and longer 5.5–28 × 3–8 μm in length in proposed collection while septate, thick walled, verruculose, protuberant rounded nodes and smaller in size (5–23 × 3–8 μm) in type species *C. herbarum*. Therefore, illustration and description of *C. astericolum* treated as new fungal species.

Acknowledgments The authors are thankful to Head, Department of Botany, DDU Gorakhpur University, Gorakhpur for providing library and laboratory facilities. Special thanks to the Curator of Indian Agricultural Research Institute, (IARI) New Delhi accepted the holotype specimen and provided an accession number thereof.

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Hidden Earthstar Diversity in the Jharkhand State of India

9

Vineet Vishal, Somnath Singh Munda, Geetanjali Singh, and Shalini Lal

Abstract

Some of the most peculiar and charming fruiting bodies of gasteroid fungi are found in the deciduous or semi-deciduous Sal forest of Jharkhand state of India. The gasteroid fungi such as *Astraeus* (*Diplocystidiaceae*) and *Geastrum* (*Geastraceae*) typically develop just below the soil surface and at maturity the fruiting bodies split into star-like structures. These have always been subject of great interest. These are widely known as "Earthstar Fungi" and considered to be a culinary delicacy, and have a high market value. The state of Jharkhand is blessed with high diversity of macrofungi due to diverse physiographic and agroclimatic conditions but there is a dearth of information on earthstar. The vast gaps in our knowledge about earthstar diversity and how these organisms are affected by trade, land management practices, deforestation, forest fire, and upsurge global warming causes severe threats and therefore demand special attention for restoration and conservation. The work is aimed to contribute to the knowledge of earthstar diversity and distribution in Jharkhand mycoflora which opens up new possibilities regarding the exploitation and utilization of wild mushrooms in India.

Keywords

Diversity · Earthstar · Mycoflora · Diversity and physiographic

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_9

9.1 Introduction

Macrofungi biodiversity is critical for ecosystem functioning and stability. Many Agaricales are becoming extinct or threatened with extinction, so studies on taxonomy and biodiversity are becoming more important. The preservation of fungal biodiversity has received much less consideration than that of other living species. After several years of research on plants and fungi, scientists estimated 1.5 million fungal species but, the actual range could increase to 2.2 to 3.8 million (Hawksworth 1991). There are now 120,000 fungal species known. According to estimates only 8–10% of fungal species have been identified (Hawksworth and Lücking 2017; Martins 2017). From the Indian tropical region only 2% of the 41,000 mushroom species have been reported though India has one-third of the world's fungal diversity (Manoharachary et al. 2005). A large number of wild mushrooms are available in Jharkhand alone, but only a fraction of total fungal wealth has been subjected to scientific scrutiny to explore the richness, abundance and ecology of the fungi. The gasteroid or the "stomach fungi" are the affiliates of basidiomycetous fungi, which includes, puffballs, stinkhorns, bird's nest fungi, and earthstars. Majority of affiliates are terrestrial either parasites or saprophytes (Bessey 1951).

The earthstar mushrooms such as *Geastrum* Pers. (*Geastraceae*) and *Astraeus* (Pers.) Morgan (*Diplocystidiaceae*) have great variety of form, structure, physiology and promote ectomycorrhizal synthesis (Chang and Miles 1989). They can grow below ground (hypogeous) or above ground (epigeous) and can be handpicked. They are cosmopolitan to sub-cosmopolitan in distribution and recorded from all part of the world except Antarctica. However, they are more abundant in tropical, subtropical and temperate regions (Ponce de León 1968; Phosri et al. 2007; Zamora et al. 2015). The peridium of these stipeless mushrooms is complex and anatomically divided into three layers: exoperidium, endoperidium and mesoperidium. The exoperidium splits into a star-like form as it matures hence, named as Earthstars (*Geastrum*) (Zamora et al. 2015) and false or barometer earthstar (*Astraeus*) (Phosri et al. 2007; Arpha et al. 2012). The endoperidium enclosed hymenophores-fertile glebal mass in most of the species; the mesoperidium is connected to both the inner exoperidium and outer endoperidium. The fertile gleba discharges spore using a bellow mechanism (Sunhede 1989; Calonge 2001). Most of these species are terrestrial, lignicolous and coprophilous in habit, whereas some are reported form leaf litters, termite mounds, humus, decomposing twigs or barks (*Pongamia* and *Acacia* sp.) decaying twigs (*Sapium insigne*) (Karun and Sridhar 2014; Verma et al. 2018).

According to Index Fungorum database (Fungorum 2021, http://www.indexfungorum.org/) genus *Geastrum* comprises more than 330–350 species while the genus *Astraeus* consists of 15–19 species only (Fungorum 2021). *Astraeus hygrometricus, A. odoratus* (in Thailand and India); *A. asiaticus, A. thailandicus* (in Thailand) are edible (Petcharat 2003; Phosri et al. 2007; Pavithra et al. 2015). Similarly, *Geastrum fimbriatum* (in India); *G. triplex* (in India); *G. saccatum* are edible species (Boa 2004; Karun and Sridhar 2014; Verma et al. 2018). In India, about 25 distinct species of *Geastrum* (Karun and Sridhar 2014; Verma et al. 2018)

and two species of *Astraeus* (Hembrom et al. 2014; Pavithra et al. 2015) are reported from moist deciduous forest, semi-evergreen forest, scared groves, Western Ghats and West-coast, Karnataka, Kerala and Gujarat, of which only the genera *Geastrum* aff. *albonigrum* (Vishal et al. 2021b), *Astraeus hygrometricus* (Khan and Chandra 2019) and *A. odoratus* (Hembrom et al. 2014) are reported from Jharkhand, India (Table 9.1). These indigenous edible agaricomycetes (mushrooms) locally known as "rugra/putka/putu" inhabit the rhizosphere of *Shorea robusta* Gaertn. The tribal population collect and consume these wild edible mushrooms, since a long time. The state of Jharkhand is blessed with high diversity of macrofungi due to diverse physiographic and agroclimatic conditions but there is a scarcity of information on earthstar fungi (Kumar and Saikia 2020a). The vast gaps in our knowledge about macrofungal biodiversity along with trade, land management practices, deforestation, forest fire, global warming and climate changes on biodiversity often result in rapid decrease in the yield of earthstar. Hence, a special attention for restoration, mitigation and conservation of earthstar is the need of the time. This chapter aims to gather information on diversity and distributions of earthstar in Jharkhand and use them for exploring new opportunities for the exploitation and utilization of wild mushrooms.

9.2 Diverse Physiographic and Climatic Condition of Jharkhand

9.2.1 Vegetation and Forest Types

The state of Jharkhand is densely covered with forest and called "land of forest" with a very rich biodiversity. It comprises of Chota-Nagpur plateau surrounded by hills and valleys. The state of Jharkhand has a forest area of 23,605 km^2 of which is 81.28% belongs to Protected Forests, 18.58% are Reserved Forests, and 0.14% are Unclassed Forests (Fig. 9.1) (ISFR 2019). According to Indian State of Forest Report (ISFR) of 2019, the forests of Jharkhand are classified into nine different types as: (1) Dry Peninsular Sal Forest (53.77%); (2) Northern Dry Mixed Deciduous Forest (35.01%); (3) Plantation/Trees out of Forest (5.65%); (4) Dry Deciduous Scrub (2.36%); (5) Moist Peninsular Low Level Sal (2.34%); (6) Dry Bamboo Brake (0.55%); (7) Moist Peninsular Valley Sal (0.28%); (8) *Boswellia* Forest (0.04%); and (9) *Butea* Forest (~0.00%). Deciduous forest is the indigenous vegetation of Jharkhand, which consists of trees like Sal (*Shorea robusta* Gaertn.), Asan (*Terminalia tomentosa* Willd.), Mahua tree (*Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr), Palash (*Butea monosperma* (Lam.) Taub.), Chironji, Char or Achar (*Buchanania lanzan* Spreng.), Kendu (*Diospyros melanoxylon* Roxb.), bamboo, etc.

Around two-fifth of the Jharkhand's population consists of people belonging to the Scheduled Tribes and Schedules Castes which form the group of indigenous people and are actively engaged in forest related activities such as collection of wild mushrooms, timber, leaves, etc. (Kumar and Saikia 2020a).

Table 9.1 *Geastrum* and *Astraeus* species reported from India

Name of Macrofungi	Habitat	Distribution	Reference
G. archeri Berk.	Soil	Nainital (UK)	Khare (1977)
G. aff *albonigrum*	On underground Shorea roots	Ranchi, Jharkhand	Vishal et al. (2021b)
G. arenarium Lloyd	Soil	Solan (H.P)	Gupta et al. (1974)
G. clelandii Lloyd	Soil	Jalori pass (H.P)	Cunningham (1944)
G. congolense Dissing & M. Lange	On decaying leaves of teak and humicolous soil	Meghalaya	Thind (1989)
G. coronatum Pers.	Dead bamboo stump	Jorhat (Assam)	Gogoi and Vipin (2015)
G. fimbriatum Fr.	Soil	H.P, Kodagu (Karnataka), Jabalpur (M.P)	Cunningham (1944), Karun and Sridhar (2014), and Verma et al. (2018)
G. floriforme Vittad.	Sandy soil	Gopalpur forest (H.P)	Sohi et al. (1965)
G. indicum (Klotzsch)	Ground	India	Klotzsch (1832)
G. lageniforme Vittad.	Decaying twigs and bark of *Pongamia pinnata*	Mangalore, Karnataka	Karun and Sridhar (2014)
G. limbatum Fr.	Soil	Nichar (H.P)	Cunningham (1944)
G. lloydianum Rick	Bamboo leaf litter	Jorhat (Assam)	Gogoi and Vipin (2015)
G. mammosum Chevall.	Soil	Jalori pass (H.P)	Bottomley (1948)
G. minimum Schwein.	Moist humicolous soil and sand	Srinagar (J & K)	Thind (1989)
G. minus (Pers.) G. Cunn.	Soil	Gurdaspur Punjab	Cunningham (1944)
G. morganii Lloyd	Soil	Mashobra (H.P.)	Sohi et al. (1965)
G. pectinatum Pers.	Soil	Chennai	Bottomley (1948)
G. pseudostriatum Hollós	On soil with litter of *Canarium strictum* and *Dysoxylum malabaricum*	Kodagu (Karnataka)	Karun and Sridhar (2014)
G. quadrifidum DC. ex Pers.	Soil	India	Bottomley (1948)
G. rufescens Pers.	On soil along with mixed leaf litter of *Madhuca longifolia*	Panchmahal (Gujarat)	Patel et al. (2020)
G. saccatum Fr.	Soil; *Canarium strictum,Dysoxylum malabaricum*and *Holigarna nigra*; bamboo leaf litter	Ahmedabad, Gujarat, Varanasi, Uttar Pradesh, Karnataka, Assam, Gujarat	Rao (1964), Khare (1977), Karun and Sridhar (2014), Gogoi and Vipin (2015), and Patel et al. (2020)

(continued)

Table 9.1 (continued)

Name of Macrofungi	Habitat	Distribution	Reference
G. schweinitzii (Berk. & M.A. Curtis) Zeller	On soil; on debris of *Acacia auriculiformis,Sapium insigne* and cashew; bamboo leaf litter	Ahmedabad, Gujarat, Varanasi, Uttar Pradesh, Karnataka, Jorhat Assam	Khare (1977), Karun and Sridhar (2014), and Gogoi and Vipin (2015)
G. simulans Lloyd	Soil	Rohtang pass, Himalayas (H.P)	Cunningham (1944)
G. striatum DC.	Soil	Gondia, Maharashtra	Bhuskute et al. (2018)
G. subiculosum Cooke & Massee	On ground under *Casuarina* tree	West Bengal	Cunningham (1944)
G. triplex Jungh.	On the ground; *Terminalia paniculata,Artocarpus heterophyllus, Canarium strictum* and *Mangifera indica;* on soil along with mixed leaf of *Madhuca longifolia*	Mussoorie (UK), Kerala, Amarkantak (M. P), Gujarat	Butler and Bisby (1931), Mohanan (2011), Karun and Sridhar (2014), Verma et al. (2018), and Patel et al. (2020)
Astraeus hygrometricus (Pers.) Morgan	On underground Shorea roots	Himalaya (H.P.) West Bengal, Karnataka, Madhya Pradesh, Ranchi	Ahmad (1950), Biswas et al. (2011), Karun and Sridhar (2014), Verma et al. (2018) and Khan and Chandra (2019)
A. odoratus Phosri Watling M.P. Martín & Whalley	Underground Shorea on roots	Rajmahal (Jharkhand), Konaje (Karnataka)	Hembrom et al. (2014) and Pavithra et al. (2015)

9.2.2 Overview of Wild Mushrooms of Jharkhand

Jharkhand has a well-defined framework for the agricultural industry owing to enough surface and ground water, fertile terrain, and a temperate climate. Jharkhand's terrain is rich in mineral and ore resources. In fact, the Chota-Nagpur Plateau is India's richest mineral belt (Jain 1989; Kumar and Saikia 2020b). Apart from ore and minerals, Jharkhand is home to a rare mushroom known as Rugra/Putka/Putu in local dialect. Some common wild edible mushrooms found in the forests of Jharkhand include *Macrolepiota procera* (Scop.) Singer (Bada Khukhri), *Termitomyces clypeatus* Heim. (Namak Khukhri), *Lentinula* spp. (Bansh Khukhri), *Volvariella* spp. (Pagla Khukhri), *T. heimii* Natarajan (Chirko, Bada Khukhri, Patiyari), *Lycoperdon* spp. (puffball), *Calvatia, Boletus edulis* Bull. (Jamun Khukhri), *Agaricus* spp. (Button mushroom), *Calocybe* (Milky mushroom), *Ganoderma* (Medicinal mushroom), *Geastrum* and *Astraeus* (Rugra/Putka/Putu), etc. (Kumar and Saikia 2020a; Vishal et al. 2021a, b). Government of Jharkhand

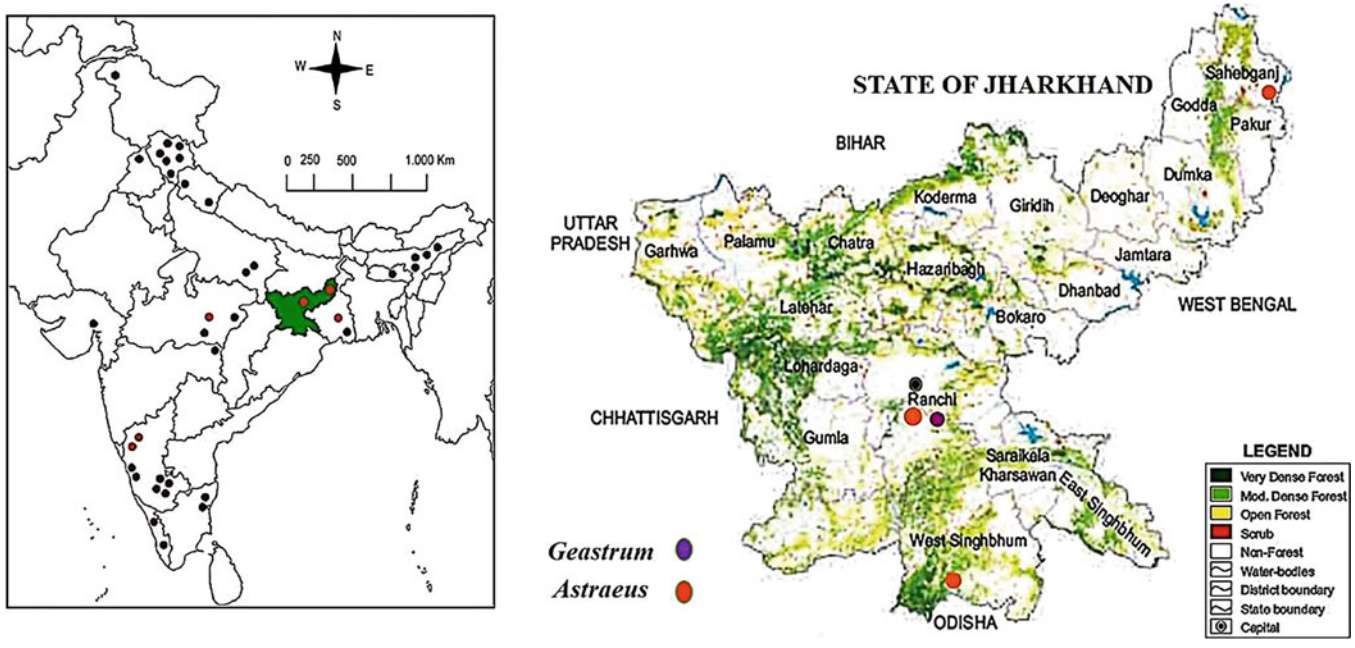

Fig. 9.1 Map of India and State of Jharkhand showing distribution of *Geastrum* spp. and *Astraeus* spp. (indicated by purple and orange colour)

through its Rural Development Department implemented Jharkhand State Watershed Mission (JSWM)—mushroom cultivation under Society Registration Central Act of 1860 on 17/07/2009 (https://jswmridf.jharkhand.gov.in/). Under this programme, local tribals or women were trained by experts to setup cultivation and production unit of edible mushrooms such as *Pleurotus* spp., *Calocybe indica* and *Volvariella* spp. However, these mushrooms are not as well known as Rugra.

9.2.3 Earthstar—The Wild Edible Mushrooms

Rugra the wild edible mushroom is indigenous to Jharkhand and, unlike other species of wild mushrooms that grow above the soil, it grows solitary or in cluster either fully or partially buried in lateritic soil specifically under Sal tree. Mostly found in Bundu, Tamar, Pithoria, Chaibasa, Lohardaga, Simdega, Rajmahal, Dhanbad, Netarhat, Jamtara, Dumka and, Hazaribagh districts of Jharkhand in dense Sal forests. Sal trees exclusively provides ambient moist and humid condition, dense canopy shade, sandy lateritic soil and decomposing organic material such as leaf litter that favour the germination and growth of Rugra (Hembrom et al. 2014; Vishal et al. 2021a, b). All these places receive an average heavy rainfall between 320 and 350 cm in monsoon seasons (June to August) with a good sunlight and temperature around 30–35 °C, which set a perfect condition for the growth and germination of Rugra. It is believed that a lightning stimulates the growth of Rugra. They are dug out by tribals after thunderstorms. Early in the morning, the local ladies or tribal girls go out in quest of Rugra near the Sal tree. In just few hours of exploration, the wooden basket is loaded with a kilogramme of Rugra (Srivastava et al. 2013). These wild mushrooms are sold at a high price between June and August due to their high nutritional and mineral content. In Jharkhand, only three species of earthstar have been reported so far, viz., *Astraeus hygrometricus* (Khan and Chandra 2019); *Geastrum* aff. *albonigrum* from Ranchi by (Vishal et al. 2021b) and *Astraeus odoratus* described from Rajmahal hills of Santhal Pargana by Hembrom et al. (2014). All three mushrooms are edible and form ectomycorrhizae with Sal tree (Table 9.1).

9.3 Taxonomy of Earthstar

Persoon (1801) placed both *Geastrum* and *Astraeus* in *Gasteromycetes* family. *G. coronatum* Pers. was the typified species of *Geastrum*, while the genus *Astraeus* was previously known as *Geastrum hygrometricum* Pers. Later, Morgan (1889) pointed out several differences which seemed to justify placing the fungus in a distinct genus—*Astraeus* followed by comprehensive description. At present, the genus *Astraeus* is placed under the family, *Diplocystidiaceae*. Kreisel (1976) placed family *Diplocystidiaceae* under the order *Boletales*. The taxonomy of earthstars (*Geastrum* and *Astraeus*) is fascinating and challenging. Traditionally, taxonomy of these fungi mainly rely on the morphological traits like basidiomata size and

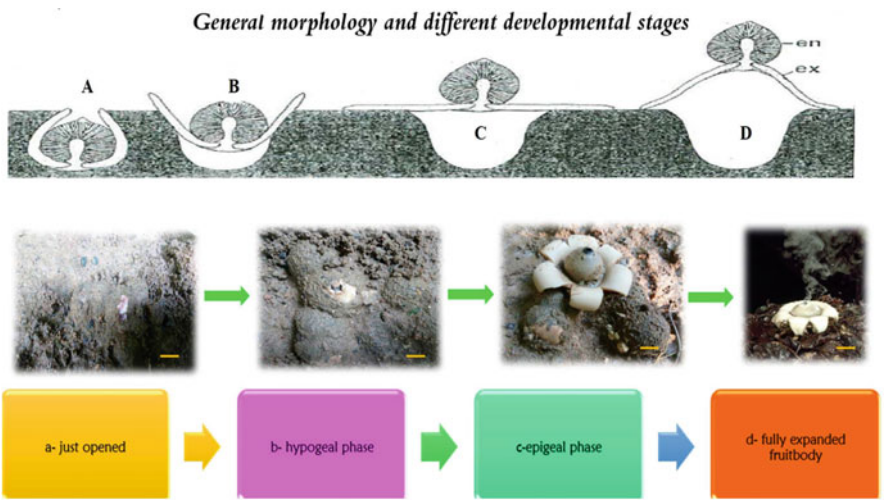

Fig. 9.2 Schematic diagram showing general morphology and different developmental stages of earthstar mushrooms

colour, exoperidium layers, endoperidium surface, hygroscopic behaviour of rays, rhizomorph, structure of hyphae from mycelial layers, peristome type (fribrillos or plicate), apophysis presence or absence, and stipe between exoperidium and endoperidium. Species in this genus are hypogaeic, sub-hypogaeic and epigaeic in origin (Fig. 9.2). The immature fruitbodies resembles a small, pointed, globular, subglobular or dome-shaped body lacking stalk and cap like other common fungi.

The peridium consists of several layers. The fruitbodies of earthstars and its allies have persistent endoperidium, which covers the hymenophore. Spores are released passively by bellows mechanism through one or more apical mouth (Bessey 1951; Sunhede 1989; Calonge 2001; Phosri et al. 2007; Karun and Sridhar 2014; Pavithra et al. 2015; Zamora et al. 2015). The Genus *Geastrum*, including *Geasteroides, Myriostoma, Nidulariopsis, Phialastrum* and *Radiigera* belongs to the order *Geastrales* which is the represented by subclass *Phallomycetidae* (Hosaka et al. 2006). *Astraeus*, including *Diplocyctis, Diploderma, Endogonpsis* and *Tremellogaster* belongs to order *Boletales* which is represented by the subclass *Agaricomycetidae* (Locquin 1984) (Table 9. 2).

9.3.1 Morpho-anatomy of Earthstar

Geastrum and *Astraeus* have a striking resemblance on soil surface, making them difficult to distinguish in the field. The cross morpho-anatomical studies revealed that, the genus *Astraeus* of *Diplocystidiaceae* is taxonomically very complex and superficially resemble other gasteroid fungus *Geastrum* (*Geastraceae*). But it is distinct due to characters such as features of Peridium (mycelial layer, fibrous

Table 9.2 Ectomycorrhizal mushrooms—*Astraeus* archived at Index Fungorum database

Name of the species	Year
Astraeus hygrometricus (Pers.) Morgan	1889
A. hygrometricus f. hygrometricus (Pers.) Morgan	1889
A. hygrometricus var. hygrometricus (Pers.) Morgan	1889
A. stellatus (Scop.) E.	1898
A. hygrometricus f. decaryi (Pat.) Pat.	1928
A. pteridis (Shear) Zeller	1948
A. hygrometricus f. ferrugineus V.J. Stanek	1958
A. hygrometricus var. koreanus V.J. Stanek	1958
A. koreanus (V.J. Staněk) Kreisel	1976
A. odoratus Phosri Watling M.P. Martín & Whalley	2004
A. thailandicus Petcharat	2005
A. asiaticus Phosri M.P. Martín & Watling	2007
A. morganii Phosri M.P. Martín & Watling	2013
A. smithii Watling M.P. Martín & Phosri	2013
A. telleriae M.P. Martín Phosri & Watling	2013
A. sirindhorniae Watling Phosri Sihan. A.W. Wilson & M.P.	2014
A. ryoocheoninii Ryoo	2017
A. sapidus (Massee) P.-A. Moreau	2017
A. macedonicus Rusevska Karadelev Tellería & M.P. Martín	2019

layer, pseudoparenchymatous layer), peristome, columella, glebal colour, varied spore ornamentation and size and capillitial hyphae, (Table 9.3) (Sunhede 1989; Fangfuk et al. 2010; Braga-Neto and Baseia 2014; Zamora et al. 2014). A short morpho-anatomical description along with ecological information, distribution and habitat is provided in Fig. 9.3.

9.3.1.1 *Astraeus odoratus* Watling M.P. Martín & Whalley

Common Name

Water measure or Barometer Earthstar.

Local Name

Rugra/Putu/Putka.

Description

Unexpanded basidiome are epigenous**,** whitish to brownish, particolous, mosaic pattern, globose to subglobose, 15–25 × 20–23 mm, apex rounded, surface not encrusted with debris, coriaceous, rhizomorph, brownish persistent, encrusted. Expanded basidiome saccate 25–35 × 20–25 mm. Exoperidium composed of several layer (≤1 mm), split into 5–6 non-hygroscopic rays which expand or recurved when moist and roll again inward when dry. Endoperidium subglobose, brownish to violaceous black, sessile, apophysis absent, irregular apical mouth, peristome absent, thin papery. Gleba pulverulent, dark brown to coffee colour at maturity.

Table 9.3 Comparison of represented morphological characters of earthstar mushrooms

Feature	*A. odoratus* Hembrom et al. (2014)	*G.* aff. *albonigrum* Vishal et al. (2021b)
Basidiomes Size in diam.	• Immature = 10–25 mm • Mature = 35–50 mm	• Immature = 12–17 mm • Mature = 34–40 mm
Exoperidium	• Immature = 13–23 mm • Surface = smooth and mosaic like pattern	• Immature = 34–40 mm. • Surface = hairy, up to 120–130 μm long
Number of rays	• 5–6; non-hygroscopic	• 5–7; non-hygroscopic
Endoperidium	• Sessile • 8–18 mm in diam. (immature) • Opening by irregular pore	• Sessile • 12–16 mm in diam. (immature) • Opening by irregular pore
Peristome	• Absent	• Fibrillose and not-delimited
Gleba	• Raw umber to coffee colour	• Greyish brown
Basidiospore	• 7.9–11 μm diam • Globose • Colour golden yellow to yellowish brown	• 3.2–5.2 μm diam • Globose • brownish
Fresh Odour when fresh	Distinctive	–
Fruiting Seasons	June–August	June–August
Habitat	Sandy or lateritic red soil in dipterocarp Forest and on surface *Hopea ponga*	Sandy or lateritic red soil in dipterocarp Forest
Distribution	Rajmahal (Jharkhand), Konaje (Karnataka)	Ranchi (Jharkhand)
		

Basidiospore globose to subglobose, 6.4–12.8 × 6.2–12.9 μm, golden yellow to yellowish brown, thick-walled, ornamentation includes coalesce to spinoid; lack columella, capillitium 2.9–4 μm diam.

Substrate

Sandy lateritic soil covered with litter, fruiting frequently in monsoon season (June–October). It is found on the ground under *Shorea robusta* as solitary or scattered in small groups.

Distribution

Thailand (Phosri et al. 2007), North-eastern part of Jharkhand, India (Hembrom et al. 2014), Southern India (Pavithra et al. 2015), Southern part of Jharkhand, India.

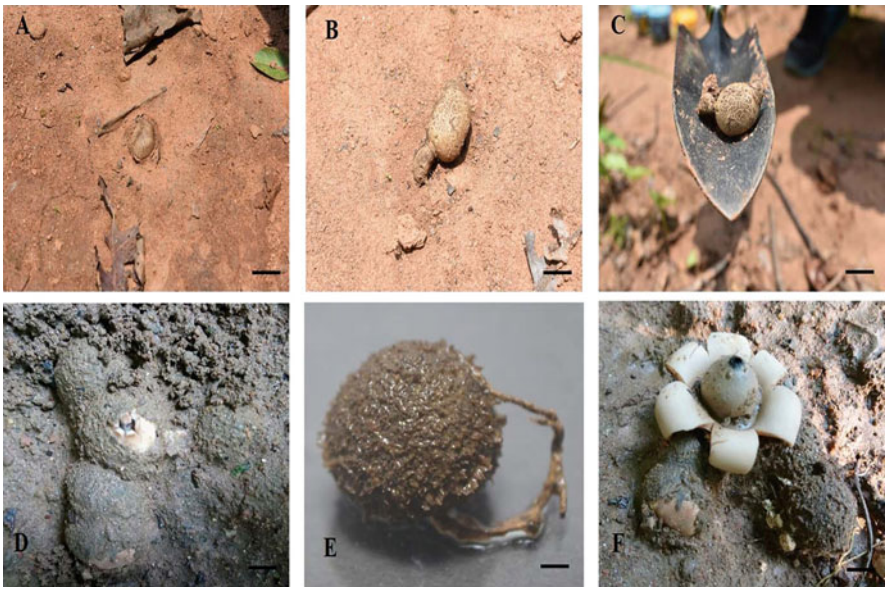

Fig. 9.3 (**a–c**) Edible fruit bodies of *Astraeus odoratus*; (**d–f**) Edible fruit bodies of *Geastrum* aff. *albonigrum*

9.3.1.2 *G. albonigrum* Calonge and M. Mata

Common Name

Earthstar

Local Name

Rugra/Putu/Putka

Description

Unexpanded Basidiomata semi-hypogeous, globose to subglobose, whitish 12–17 mm in diam, hirsute surface, brownish, single large robust rhizomorph attached at the base, persistent rhizomorph, 25–28 mm long, encrusted with debris. Expanded Basidiomata saccate, 34–40 mm in diam, 18–20 mm tall. Exoperidium non-hygroscopic, split into 5–7 revolute rays, Endoperidium globose to subglobose, 12–16 mm in diam, 14–18 mm tall, sessile, dark brown, glabrous without apophysis, peristome fibrillose, not-delimited, mammiform, circular, slightly darker than endoperidium. Gleba greyish brown (7F2) and dusty at maturity. Basidiospore globose to subglobose, 3.2–5.1 × 3.1–5.1 µm, brownish, verrucose, short cylindrical warts 0.3–0.5 µm long with rounded tips; apiculus conspicuous. Capillitial hyphae 2.9–5.9 µm diam, straight, thick-walled. Crystalline matter: Rhizomorph white, thick-walled, 1.015 × 1.086 µm diam, surface encrusted, branched, clamped present and rhizomorph with distinct calcium oxalate pyramidal crystal.

Substrate

Sandy lateritic soil covered with litter, fruiting frequently in monsoon season (June–October). It is found on the ground under *Shorea robusta* as solitary or scattered in small groups.

Distribution

Central America: Costa Rica (Calonge and Mata 2004); North America: Mexico (Calonge and Mata 2004); South America: Brazil, Mato Grosso State (Trierveiler-Pereira et al. 2012), Rio Grande do Norte State (Sousa et al. 2014). India, Jharkhand.

9.3.2 Molecular and Phylogenetical Characterization

DNA-based phylogenetic analyses have been employed to infer closely related biological specimens that are complicated or even impossible to differentiate based on morphological traits and to provide species delimitation more precisely. Phylogenetic analyses reveal the placement of earthstars *Astraeus* and *Geastrum* into two different clades—former is placed under *Agaricomycetidae* clade while the later represent *Phallomycetidae* clade (Hosaka and Castellano 2008). It is because *Geastrum* superficially resemble to *Astraeus*, but dissimilar in morphological, anatomical and phylogenetic traits. *Astraeus* has large pedicellate spores and lacks peristome and columella (Vishal et al. 2021b). These characteristics should be enough to distinguish *Geastrum* from *Astraeus*. The puffball *Lycoperdon* spp. of family *Agaricaceae* were used as an outgroup (Fig. 9.4). Cannon and Kirk (2007) suggested convergent evolution might have occurred among *Astraeus* and *Geastrum*.

The clade *Agaricomycetidae* represent *Diplocystaceae, Gyroporaceae* and *Boletaceae* families, whereas, *Phallomycetidae* clade had only one family—*Geastraceae*. Many attempted to establish the phylogenetic relationship of gasteroid fungi *Geastrum* and *Astraeus* by targeting large subunit of rDNA region. Kasuya et al. (2012) revealed the polyphyly of previously known *Geastrum* species by targeting ITS, nrLSU and atp6 amplified DNA region. Jeppson et al. (2013) used ITS, nrLSU, tef-1α to demonstrate the phylogeny of European earthstars along with morphological and ecological traits. Zamora et al. (2014, 2015) used integrative (morphological, chemical and molecular phylogenetic) taxonomic approach to reveal the unexpected diversity of *Geastrum.* They used molecular sequence data (ITS1, 5.8S, ITS2, nrLSU, rpb1, atp6) to study phylogenetic relationships of *Geastrum* spp. They also re-described the forgotten species of *G. argentinum.* Cabral et al. (2017) and Sousa et al. (2019) analysed a combination of nrLSU and atp6, to justify the recognition of new species *G. verrucoramulosum* section Exareolata, *G. caatingense* and *G. parvistellum* from Neotropical region. The molecular phylogenetic study of the genus *Astraeus*, based on globally collected samples, was carried out using ITS, nrLSU, 5.8S, rbp1, rbp2 and tef1α to unravel the hidden taxonomy and systematic of the genus *Astraeus* viz., *A. odoratus, A. asiaticus, A. morganii, A. smithii* and *A. telleriae* (Phosri et al. 2007, 2014). Moreover, *A. asiaticus*, which was previously known in Southeast Asia as *A. hygrometricus*, has currently been classified as a new species based on

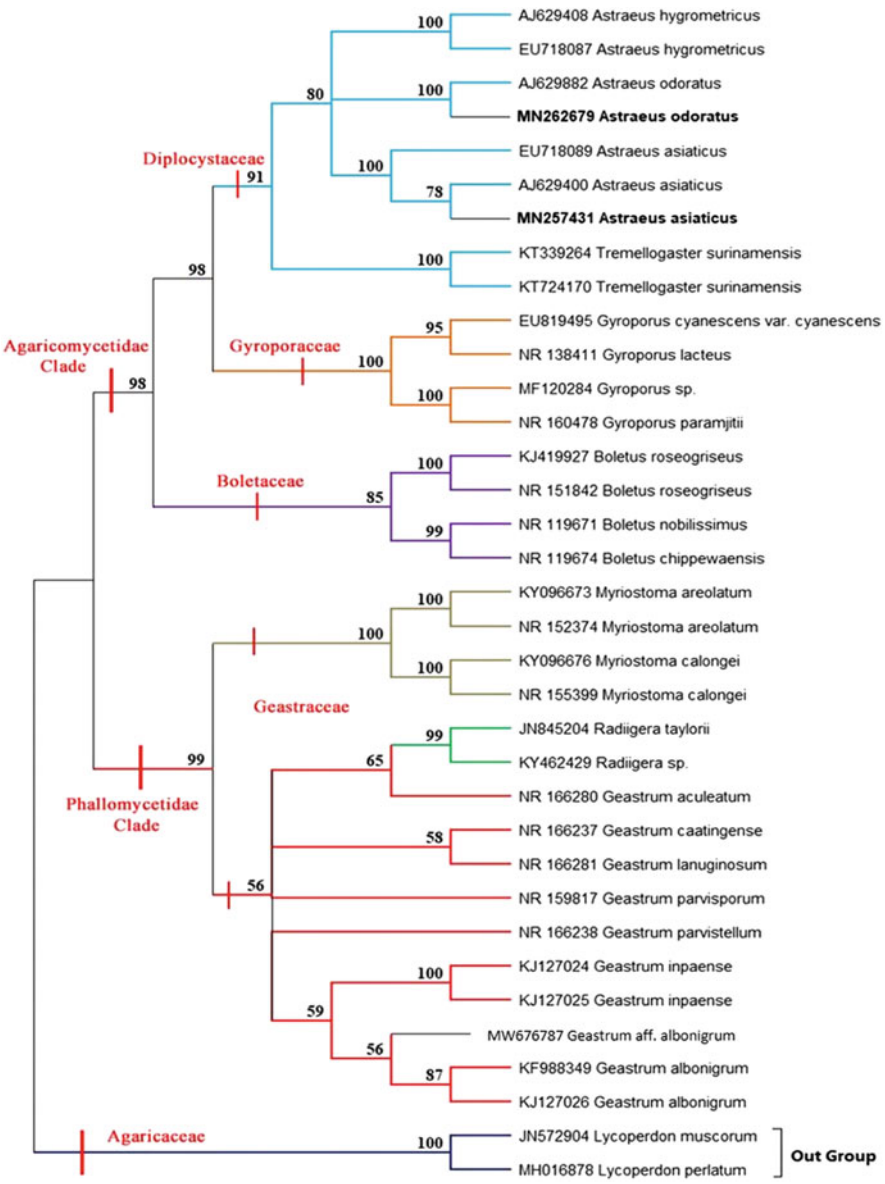

Fig. 9.4 The Maximum likelihood tree from nrDNA ITS (Internal Transcribe Spacer) dataset reveals the placement of earthstars i.e., species of *Astraeus* and *Geastrum* placed into two different clades—former is in *Agaricomycetidae* and *Phallomycetidae* clade. The puffball *Lycoperdon* spp. was used as an outgroup

morphological traits and phylogenetic positioning (Phosri et al. 2007). Fangfuk et al. (2010), described Japanese *Astraeus* species, formerly identified as *A. hygrometricus* and *A. hygrometricus var. koreanus.* Ryoo et al. (2017) reviewed *A. hygrometricus* var. *koreanus* and *A. koreanus* from Korea and Japan and proposed a new species *A. ryoocheoninii* based on morphological and molecular data (ITS region). At the species level, molecular analysis is useful for identifying a complex group of fungi but taxonomy of *A. hygrometricus* specimens from other geographic regions need clarity thus, making the genus much more complicated (Lucking et al. 2021). It may due to the diverse distribution of biodiversity and geo-climatic conditions, which could be the leading factor for the variability of the *Astraeus* and its allied taxa (Phosri et al. 2007, 2014). Previous phylogenetic studies substantiate monophyletic origin of *Astraeus*.

9.3.3 Global Molecular Data Repositories of Earthstar

Index Fungorum database (http://www.indexfungorum.org) is a maintained and supported by The Royal Botanic Gardens, Kew (represented by the Mycology Section), Landcare Research-NZ (represented by the Mycology Group), and Institute of Microbiology (Chinese Academy of Science). The Index Fungorum database now has information on 5,88,186 fungi (including yeasts, lichens, chromistan fungal analogues, protozoan fungal analogues, and fossil forms) at all ranks as of May 2021. Based on an integrated taxonomic approach, approximately 330 species of *Geastrum* and 19 species of *Astraeus* have been archived at the database worldwide (Table 9.2; Fig. 9.5).

9.4 Earthstar Ecology

9.4.1 Interaction with Host and their Symbiosis

Unfortunately, very little attention has been paid to the host association of earthstar. Wild edible Mushrooms, a non-wood forest product radiated into most niches during monsoon seasons growing as underground vegetative mycelium (Singha et al. 2017). They enter into mycorrhizal symbiosis with forest plants belonging to families, *Dipterocarpaceae*, *Fagaceae* and *Pinaceae* and support forest ecology by performing multitude of roles in the ecosystem like decomposing dead organic matters especially those containing lignin and cellulose and providing protection against drought (Zeller 1948; Ahmad 1950; Malajczuk et al. 1982). Apart from that, they remarkably fix nitrogen and phosphorus for the host plant and are the major component of the earth's biogeochemical cycles. In return they get photosynthetically assimilated carbon-derived compounds like glucose and sucrose which make them to act as "ecosystem engineers" (Zotti et al. 2020). The earthstar mycelium, found running through the surface layer of soil rich in decomposed plant wood and leaf litter, have short lateral branches i.e., rhizomorph or network of mycelium. The

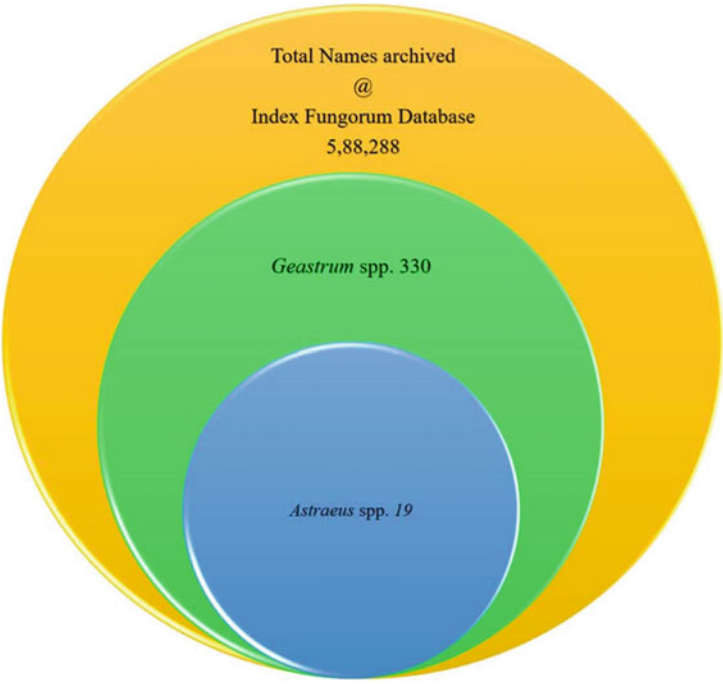

Fig. 9.5 Stacked Venn showing total number of fungal names archived at Index Fungorum database

rhizomorphs allow translocation of water, nutrients and minerals from distal parts of the extraradical mycelial network to the *Shorea* root tip via an extracellular interface within the root cortex resulting in the modification of root exudation, qualitatively and quantitatively (Brundrett 2002; French 2017). These fungi also alter the physical, chemical and microbiological features of the surrounding soil, resulting in the formation of a unique ecosystem known as the ectomycorrhizosphere (EcM rhizosphere), in which microbial populations differ from those found in the rhizosphere soil. (Gaiero et al. 2013).

9.4.2 Interaction with Microbes

Plant microbiome is one of the most studied domains of microbial biodiversity, little is known about the microbial communities that thrive in the ectomycorrhizosphere of mushrooms. Mycorrhizal symbioses are prevalent in most boreal, temperate and dry deciduous tropical forest ecosystems, accounting for a major percentage of the microbiota (Uroz et al. 2016, 2019). They contribute massive quantities of organic carbon fluxes from leaf litter, wood and plant biomass, resulting in microbial population enrichment and associated functions. The ectomycorrhizosphere is a

zone where plant roots and soil microorganisms (ectomycorrhizal fungus and bacteria) interact actively, inhibiting and stimulating one another. The ectomycorrhizosphere also acts as a food source for microbes that support forest trees in a variety of ways (Dighton 2018). Furthermore, the microbiome of the ectomycorrhizosphere is the driving force behind the processes such as quorum sensing, regulation of microbial gene expression, symbiosis, biofilm formation, antibiotic production, motility, conjugation, virulence, etc. (Vishal et al. 2021a). According to Bonfante and Anca (2009), bacteria may promote mycorrhizal development, aid in the fungal-plant identification system and increase the host root's receptivity to the mycorrhizal fungus. Recent rhizospheric metagenome analysis of ectomycorrhizosphere (EcM rhizosphere) reveals presence of bacterial phyla such as Gram-negative Proteobacteria (*Agrobacterium, Azospirillum, Azotobacter, Burkholderia, Bradyrhizobium, Enterobacter, Pseudomonas* and *Rhizobium*), *Gram-positive* Firmicutes (*Bacillus, Brevibacillus* and *Paenibacillus*) and Gram-positive Actinomycetes (*Rhodococcus, Streptomyces* and *Arthrobacter*) (Rigamonte et al. 2010; Uroz et al. 2012; Vik et al. 2013; Uroz et al. 2016; Uroz et al. 2019). These associations are expressively imperative in agro-ecosystems where the sustainability of soil fertility is of prime importance (Garbaye 1994; Boddy et al. 2007; Frey-Klett et al. 2007; Smith and Read 2010). Vishal et al. (2021b) reported first ectomycorrhizosphere microbiome associated with *Astraeus*. The analysis reveals that the ectomycorrhizosphere of *Astraeus* is enriched with phosphate solubilizing (PSB) Gammaproteobacteria in the active ectomycorrhizal zone from a dry deciduous forest of *Shorea* (Fig. 9.6). They are involved in mineral weathering, phosphorus and iron mobilization thus playing an important role plant nutrition control.

9.4.3 Mycorrhization and Fruit Body Formation in Earthstar

The mycorrhizal morphology, host specificity and fruiting ecology of the ectomycorrhizal fungus *Geastrum* and *Astraeus* are poorly understood. *Astraeus* form putative ectomycorrhizal associations with variety of host tree species including *Shorea siamensis* Miq., *S. roxburghii* Don., *S. farinose* Fischer., *D. alatus* Roxb. ex G. Don, *D. intricatus* Dyer., *D. Obtusifolius* Teijsm. ex Miq. *and Hopea odorata* Roxb., (Fig. 9.7). This strongly advises that members of this genus are very important ectomycorrhizal components. Fangfuk et al. (2010) tested the ability of *Astraeus* to form ectomycorrhizae with seedling of *Pinus densiflora* Siebold & Zucc., in vitro. Basidiospore of *A. hygrometricus* and *A. odoratus* were inoculated onto Pine seedlings. After several months of inoculation, *A. hygrometricus* ectomycorrhizae developed Harting net in Pine root, a sheath and rhizomorph while no ectomycorrhizal formation was found in samples inoculated with *A. odoratus*. Further, they obtained only mycelium and no fruiting bodies were formed. Kaewgrajang et al. (2013) were first to demonstrate the effect of ectomycorrhizal fungus *A. odoratus* on *Dipterocarpus alatus* seedling of *Dipterocarpaceae* family. After 7 months of inoculation, not only sporocarp of

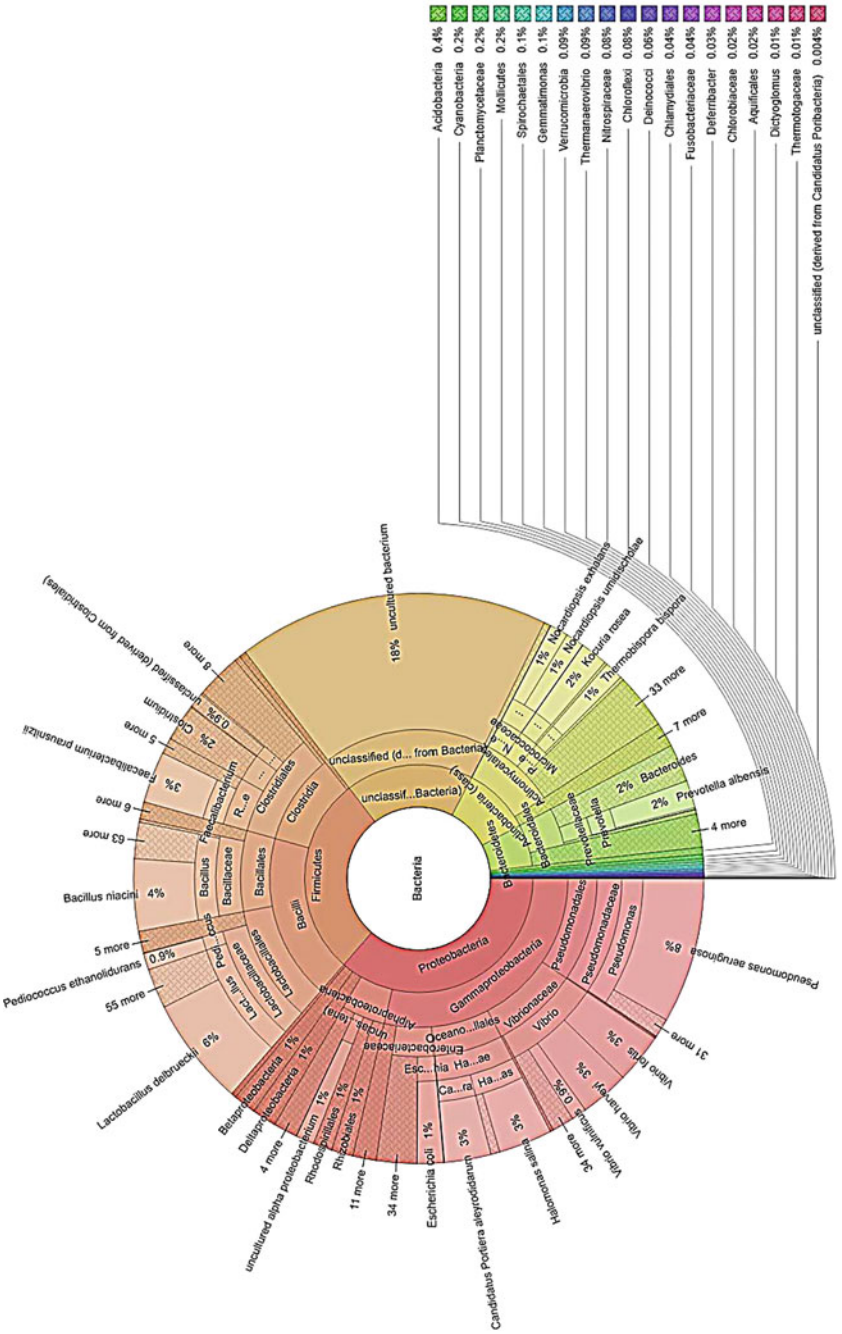

Fig. 9.6 Ectomycorrhizosphere taxonomic distribution and abundance shown in a krona chart. Inner circle represents higher taxonomic rank and outer circle represents lower taxonomic rank up to species level (Source: image taken Vishal et al. 2021b)

Astraeus spp.

Shorea siamensis
S. roxburghii
S. farinosa
Depterocarpus alatus
D. intricatus,
D. Obtusifolius
Hopea odorata
H. parviflora
Phyllanthus emblica
Syzygium cumini
Pinus roxburghii
Artocarpus hirsutus
Holigarna arnottia
Acacia auriculiformis

Geastrum spp.

Terminalia paniculate
Artocarpus heterophyllus
Canarium strictum
Mangifera indica
Shorea robusta

Fig. 9.7 Ectomycorrhizal host tree species of *Astraeus* and *Geastrum* spp.

A. odoratus was developed, but also it enhanced the growth of *Dipterocarpus alatus* under pot culture condition. They used spore suspension and cultivated mycelium inoculum, and Potato Dextrose Agar (PDA) medium to established ectomycorrhizal formation. Additionally, using the previous study protocol, Kaewgrajang et al. (2019) inoculated *A. odoratus* on two mature dipterocarp species *Dipterocarpus tuberculantus* Roxb. and *Shorea roxburghii* G. Don seedling.

A high growth rate was observed in *S. roxburghii* seedlings as compared to *D. tuberculantus* seedlings; this could be due to genetic variations in the seeds. Both the morphotypes had developed mantle, sclerotia, numerous brownish rhizomorphs but no sporocarp formation. Suwannasai et al. (2020), reported the mycorrhization of *A. sirindhorniae* and *Dipterocarpus alatus* using modified Norkrans's "C" agar medium (MNC), modified Melin-Norkrans (MMN), potato

dextrose agar (PDA) and Murashige and Skoog (MS) culture media. *Geastrum* species are mostly non-mycorrhizal and have variety of inhabitation ranging from rotten wood to subiculum, while few species such as *G. saccatum* and *G. triplex, G. fimbriatum* form ectomycorrhizal associations with *Terminalia paniculata* Roth., *Artocarpus heterophyllus* Lam., *Canarium strictum* Roxb., *Mangifera indica* L., and *Shorea robusta*, (Fig. 9.7) (Karun and Sridhar 2014). Agerer (2006) demonstrated a mycorrhizal connection between *Geastrum fimbriatum* with *Fagus sylvatica* L. exhibiting thin mantle and Hartig net structure. However, no studies have been done to establish ectomycorrhizal association between *Geastrum* with variety of host species including in vitro or in vivo cultivation till date.

9.5 Ethnomycological Prospective

Ethnomycology is the study of traditional mushroom uses and consumption patterns. Since green plants have benefitted humans for many years as a source of food, medicine and herbal treatments, but mushrooms are just beginning to earn much-deserved attention for their very real health-giving abilities. Wild edible ectomycorrhizal mushrooms *Astraeus* and a few *Geastrum* spp. are a traditional delicacy during the monsoon season in Southeast Asia, as well as in Bihar, Jharkhand, South-West India, and the South-Western region of West Bengal, while underutilized for rest of the world (Petcharat 2003; Phosri et al. 2007; Fangfuk et al. 2010; Kaewgrajang et al. 2013; Karun and Sridhar 2014; Pavithra et al. 2015; Verma et al. 2018; Vishal et al. 2021a).

9.5.1 Nutritional and Nutraceuticals Profiling

The fruiting bodies of wild edible mushrooms are rich in proteins, carbohydrates, fibre, vitamins (water-soluble vitamins, ascorbic acid and thiamine) and minerals such as calcium, phosphorus, magnesium, sulphur, potassium, iron, zinc, manganese, copper and boron (Varghese et al. 2019; Thakur 2020). Among all the *Astraeus* species, *A. hygrometricus* and *A. odoratus* have received the most attention in terms of nutritional and nutraceutical potential (Sanmee et al. 2003; Gunjan et al. 2010; Singh 2011; Srikram and Supapvanich 2016; Pavithra et al. 2018) whereas, the edibility of *Geastrum* is yet to be established.

The most important factor that influences the nutritive value of mushrooms is their moisture content, which has a direct impact on the nutrient content of mushrooms. The moisture content of *A. hygrometricus* and *A. odoratus* was estimated to be 83.8% and 84.15%, respectively. Ash content provides the idea about the mineral's configuration of mushrooms (Sanmee et al. 2003). The ash content *A. hygrometricus* fruitbody was found to be two-fold higher than that of *A. odoratus.* Studies revealed that basidiocarp (on the basis of dry weight) of *A. hygrometricus* (35.4–64.3%) contain high amount of total carbohydrates compared to *A. odoratus* (20.6%), (Table 9.4). Apart from total carbohydrates content,

Table 9.4 Nutritional qualities of the *Astraeus* species

	A. hygrometricus				*A. odoratus*
	Sanmee et al. (2003)	Singh (2011)	Gunjan et al. (2010)	Pavithra et al. (2018)	Srikram and Supapvanich (2016)
Parameters analysed	Quantity (%)	Quantity (%)	Quantity (g/100 g)	Quantity (% and g/100 g)	Quantity (%)
Moisture	–	83.87 (full body)	–	2.71 ± 0.31 uc 2.93 ± 0.22 co	84.15 ± 0.50
Ash	14.2 ± 0.45 m 27.6 ± 0.29 y	2.5 (full body)	–	18.43 ± 0.17 un 15.52 ± 0.46 co	0.98 ± 0.93 f. w 10.17 ± 7.08 d. w
Carbohydrate	54.4 m 44.9 y	29.48 O. P 35.41 I. P	64.33 + 3.23	46.17 ± 0.76 uc 48.42 ± 0.52 co	3.9 f. w 20.68 d. w
Starch	–	0.11 O. P	–	–	–
Fats	4.4 ± 0.18 m 2.7 ± 0.05 y	1.05 O. P 0.24 I. N	3.20 + 0.85	3.55 ± 0.02 uc 2.49 ± 0.06 co	1.16 ± 0.33 f. w 7.32 ± 2.08 d. w
Protein	14.7 ± 0.2 m 14.0 ± 0.28 y	11.71 O. P 4.66 I. N	16.47 + 1.35	16.80 ± 0.06 uc 17.30 ± 0.46 co	4.18 ± 0.12 f. w 26.37 ± 0.76 d. w
Free amino acid	–	–	6.48 + 0.90	–	–
Fibre	12.3 ± 0.28 m 10.8 ± 0.16 y	0.02 O. P 0.13 I. N	10.80 + 1.02	14.58 ± 0.38 uc 15.91 ± 0.22 co	5.62 ± 2.88 f. w 35.46 ± 18 d.w
Total energy	–	336.74 g calories	–	1185.4 ± 4.18 kJ uc 1191.3 ± 6.75 kJ co	–

O. P outer part, *I. N* inner part, *m* mature, *y* young, *uc* uncooked, *co* cooked, *f. w* fresh weight, *d. w* dry weight

edible ectomycorrhizal fungi usually contain sugars and sugar alcohol, which varies within the species. D-Glucose (0.88 mg/g) was the most abundant sugars in *A. hygrometricus* followed by D-Fructose (0.85 g), Trehalose (0.50 g), D-Mannose (0.26 mg/g), D-Ribose (0.12 mg/g), D-Arabinose (0.21 mg/g), D-Xylose, (0.03 mg/g) and D-Fucose (0.10 mg/g). While, polyol compound-Mannitol (6.52 mg/g) mostly found in the sporocarps of basidiomycetes and ascomycetes was most abundant sugar alcohol followed by Glycerol (0.12 mg/g), Myo-Inositol (0.14 mg/g) and Meso-Erythritol (0.02 mg/g) concentrations in in term of dry weight. High content of Mannitol is useful for diabetic foods. Researches estimated sugar components from extracts with the help of high-performance liquid chromatography (HPLC) (Sanmee et al. 2003).

Mushroom nutritional value is primarily related to their high protein, fibre and low-fat content and are most important constituent of the basidiocarps (Biswas et al. 2017). Findings have revealed that, *A. odoratus* contains high amount of crude protein (26.3%), fibre (35.4%) and fats (7.32%) as compared to *A. hygrometricus.* Both the species of *Astraeus* contain somewhat equivalent amount of protein as that of edible legumes (16.8–17.3%) and higher than the edible mushroom Jelly Ear Fungus (*Auricularia auricula-judae* (Bull.) Wettst.) (Pavithra et al. 2018). A diet with high crude fibre is beneficial for improving digestibility, bowel health and combat cardiovascular disease (Balogun and Fetuga 1986). The overall energy value can be calculated based on crude protein, carbohydrate and lipids. Wild edible mushroom fruitbodies may have a higher nutritive and energy value than cultivated species. The total energy content of *A. hygrometricus* has been assessed to be high (336.74 g calories) after cook the energy content is about 1191.3 $\pm$ 6.75 kJ/100 g making it one of the most nutritionally exceptional mushrooms. The *Astraeus* species has been linked to a number of beneficial nutraceutical components. The concentration of Ca, P, S, K, Mg and Fe constitute about 60–70% of the total ash content (Biswas et al. 2017). Potassium is particularly most abundant mineral in *A. hygrometricus*, followed by Iron, Phosphorus, Sulphur, Cupper, Calcium, Zinc and Manganese, and it met the National Academy of Sciences-National Research Council (NAS-NRC1989) recommended criteria for newborns, children and adults (Table 9.5). The fruitbody of *A. hygrometricus* has nine times more Potassium (3216 mg/100 g) than a banana (358 mg/100 g). Low Na/K (<1) ratio of uncooked and cooked *A. hygrometricus*, as demonstrated by Pavithra et al. (2018), is beneficial for people with high blood pressure or hypertension. Among heavy metals, Copper (Cu) content is highest 329 mg/100 g. Cu helps the body to absorb oxygen and participate in formation of RBC (red blood cell) cells (Varghese et al. 2019). In additions to minerals, important vitamins such as vitamin B1 (ascorbic acid–a water-soluble vitamin), vitamin C (thiamine) and vitamin D2 (ergosterol) were present in the fruitbodies of *A. hygrometricus* (Singh 2011; Singh and Varshney 2020). Furthermore, Singh and Varshney (2020), reported that nonvolatile taste compounds such as soluble sugars and polyols, monosodium glutamate (MSG) such as aspartic and glutamic acid, and umami 5′-nucleotide were responsible for the mushrooms' sweet and meaty flavour.

Table 9.5 Nutraceutical qualities of the *Astraeus hygrometricus*

	Sanmee et al. (2003)	Singh (Singh 2011)	Pavithra et al. (2018)	Infants/ children[a]	Adults[a]
Mineral Name	Quantity (mg/g) dry wt.	Quantity (mg/100 mg)	Quantity (mg/100 g)	Quantity (mg/100 g)	Quantity (mg/100 g)
Ca	2.5 m; 0.8 y	29.5 O. P 25.8 I. P	249.43 ± 1.92 uc 240.46 ± 7.06 co	600–800	800
p	2.2 m 5.7 y	935 O. P 405 I. P	539.41 ± 5 uc 480.93 ± 14.11 co	500–800	800
Mg	1.6 m 1.2 y	242 O. P 11 I. P	169.82 ± 1.57 uc 150.29 ± 4.41 co	60–170	280–350
S	1.7 m 5.0 y	–	509.45 ± 4.72 uc 440.9 ± 12.94 co	–	–
K	12.8 m 26.1 y	–	3216.51 ± 29.8 uc 40.08 ± 1.18 co	500–1600	1600–2000
Na	–	–	69.9 ± 0.65 uc 3045.88 ± 89.38 co	120–400	500
Fe	3254 m 2059 y	2.78 (ppm) O. P 2.35 (ppm) I. P	439.52 ± 4.08 uc 170.33 ± 5.00 co	10	10–15
Zn	203 m 105 y	0.89 (ppm) O. P 0.44 (ppm) I. P	219.76 ± 2.04 uc 140.27 ± 4.12 co	5–10	12–15
Mn	329 m 81.7 y	0.74 (ppm) O. P 0.17 (ppm) I. P	–	1.5–3.5	2–6
Cu	16.5 m 25.2 y	–	329.64 ± 3.06 uc	0.6–2	1.5–3
B	2.4 m 2.4 y	–	–	0.75–0.96	0.87–1.35

O. P outer part, *I. N* inner part, *m* mature, *y* young, *uc* uncooked, *co* cooked
[a]NRC-NAS (1989) recommended pattern

9.5.2 Phytochemical Profiling and Therapeutic Importance

The basidiocarps of earthstar has numerous bioactive compounds of enormous pharmaceutical value. The bioactive compounds derived *from A. asiaticus, A. hygrometricus, A. odoratus* and *A. pteridis* includes a wide range of lanostane triterpenoids, e.g. astrahydrone, astracurol, astrakurcurol and astracurone, astraoric acids a-d astraodorol, astraeusins a-l, lanostane triterpenoids, astrapteridone, astrapteridiol and 3 epiastrapteridiol, astrasiaone and astradiate, (Table 9.6). Their bioactivities include antileishmania, anticandidal, antituberculosis, antimalarial,

Table 9.6 Bioactive phytochemicals of the *Astraeus* and *Geastrum* species of pharmaceutical interest

Mushroom name	Source	Types of phytochemicals	Bioactivity	References
A. hygrometricus	Fruit body	Astrahygrol, 3-epi-Astrahygrol Astrahygrone	–	Takaishi et al. (1987)
A. hygrometricus	Basidiocarps	–	Antibacterial	Badshah et al. (2012)
A. hygrometricus	Basidiocarps	Astrakurkurol Astrakurkurone	Antileishmania and Anticandidal	Lai et al. (2012)
A. hygrometricus	Basidiocarps	Astrakurkurone	Antileishmanial	Mallick et al. (2015)
A. odoratus	Basidiocarps	Astraororic acids A-D	Antituberculosis	Arpha et al. (2012)
A. odoratus	Basidiocarps	Astraodorol	Antimalarial	Nasomjai et al. (2014)
A. odoratus	Basidiocarps	Astraeusins A-L	Antibacterial	Isaka et al. (2016)
A. pteridis	Fruit body	Astrapteridone Astrapteridiol 3-piastrapteridiol	Antituberculosis	Stanikunaite et al. (2008)
A. asiaticus	Fruit body	Astrasiaone Astradiate	Cytotoxicity	Pimjuk et al. (2015)
Geastrum triplex	Fruit body	–	Antibacterial	Chittaragi et al. (2013)
Geastrum spp.	Fruit body	–	Antimicrobial	Panda and Tayung (2015)
G. fornicatum	Fruit body	–	Antimicrobial	Gurgen et al. (2018)

antibacterial, antimicrobial, imunomodulation, cardioprotective and cytotoxicity activities. These false earthstars are also used as hemostatic agent in Chinese folk medicine for ameliorating burns and lesions (Takaishi et al. 1987; Chakraborty et al. 2007; Stanikunaite et al. 2008; Lai et al. 2012; Nasomjai et al. 2014; Mallick et al. 2015; Isaka et al. 2016; Srisurichan et al. 2017; Phadannok et al. 2020). In addition to these, the species of *Geastrum* such as *G. triplex* and *G. fornicatum* have antibacterial and antimicrobial activity (Dore et al. 2007; Chittaragi et al. 2013; Panda and Tayung 2015; Sevindik et al. 2017; Gurgen et al. 2018).

Evidence based research has shown that the extracts from *A. hygrometricus* basidiocarp with a potential antioxidant, antiinflammatory, antidiabetic, anticarcinoma, immunomodulation properties has cardioprotective and apoptogenic activity, (Table 9.7). Recently, Nandi et al. (2019) have characterized a low molecular weight triterpenoid astrakurkurol from the basidiocarps of *A. hygrometricus*, that showed promising therapeutic potential as a cytotoxic molecule on human

Table 9.7 Antimicrobial and therapeutic possibilities of the *Astraeus* and *Geastrum* species

Mushroom name	Therapeutic potential	References
A. hygrometricus	Antioxidant Antiinflammatory Antidiabetic Anticarcinoma Hepatoprotective Immunomodulation Cardioprotective Apoptogenic	Mandal et al. (2015) Pavithra et al. (2018) Biswas and Acharya (2013) Biswas and Acharya (2013) Chakraborty et al. (2007) and Biswas et al. (2012) Nandi et al. (2019) Mallick et al. (2015) Biswas et al. (2011) Biswas et al. (2012)
A. odoratus	Cytotoxicity Antioxidant Anticancer Antidiabetic	Arpha et al. (2012) Srikram and Supapvanich (2016) Arpha et al. (2012) Phadannok et al. (2020)
A. asiaticus	Anticancer Antidiabetic	Pimjuk et al. (2015) Phadannok et al. (2020)
G. arinarius	Antioxidant	Puttaraju et al. (2006)
G. pectinatum	Antioxidant	Sevindik et al. (2017)
G. saccatum	Antioxidant and Antiinflammatory	Dore et al. (2007)
G. fornicatum	Antioxidant and Antiquorum sensing	Gurgen et al. (2018)
G. fimbriatum	Anticoagulant and Antiinflammatory	Sarac et al. (2019)

hepatocellular carcinoma cells (Hep3B). Astraeusins A-L, lanostane triterpenoids isolated from *A. odoratus* displayed anticancer activity against NCI-H187 (human small-cell lung cancer), MCF-7 (human breast cancer) and KB (oral human epidermoid carcinoma) (Isaka et al. 2016). Spiro-astraodoric acid, and astraodoric acids E and F was tested against six human cancer cell lines (Srisurichan et al. 2017). *A. odoratus* also demonstrates cytotoxicity, antioxidant and antidiabetic activity. The extracts of *A. odoratus* and *A. asiaticus* enhanced glucose uptake through insulin dependent pathway that involved the function of glucose transport protein (GLUT1 and GLUT4) by intrinsic activities via p38 mitogen activated protein kinase and therefore has potential to be used for hypoglycemic purpose (Phadannok et al. 2020). Some species of *Geastrum* displayed antioxidant (*Geastrum arinarius*), antioxidant and antiinflammatory (*G. saccatum*), antioxidant and antiquorum sensing (*G. fornicatum*) activities. Sarac et al. (2019) reported the anticoagulant and antiinflammatory activities of *G. fimbriatum* using ethanolic extracts, in vitro (Table 9.7). The wild edible mushroom earthstar is known for much therapeutic intervention. Further exploration of the bioactive metabolites of these species will contribute to the development of new therapeutic agents to combat several human diseases.

Every human action has unintended consequences on the environment. Issues like food access, poverty, deforestation, forest fire, global climate change, population explosion, and unemployment are a major concern for environmentalists,

ecologists, and scientists around the world including, India. Children and women, still suffer from various forms of malnutrition, including stunting, wasting, overweight, obesity, anaemia. Systematic profiling of bioactive metabolites from earthstar mushrooms can be utilized in developing new food, nutraceutical and pharmaceutical products, which, in turn, can help in solving some these problems.

9.6 Conclusions

The gasteroid earthstar mushrooms are epigeous and hypogeous fruiting bodies that serve as natural reservoir of potent nutraceutical and pharmaceutical compounds and are now serve as the new drug discovery interface. They are one of the most significant families of mushrooms due to their edibility, nutraceutical value, medicinal properties and ectomycorrhizal association. Among 19 species of *Astraeus* and 330 species of *Geastrum*, *Astraeus hygrometricus, A. odoratus,* and *Geastrum* aff. *albonigrum* are consumed as popular food in Jharkhand. Commercial production of these mushrooms is difficult thus making it more expensive. Mushroom diversity also forms an integral part of the floristic diversity, but most of the researches are focused on high floristic diversity than mycoflora. Hence, an integrated approach has been applied to study mushroom diversity through the expedition, collection, documentation, mapping, identification, DNA barcoding, phylogenetics analysis, morphological and molecular characterization, nutraceuticals property. The coexistence of this fungus with other microorganisms (particularly bacteria) is critical for optimal mushroom production and higher yields.

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Diversity of Corticioid Fungi Belonging to the Family *Meruliaceae* in Chamba District of Himachal Pradesh

10

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Abstract

This chapter describes 17 taxa belonging to four genera of the family *Meruliaceae* based on the specimens collected from various localities of Chamba district (Himachal Pradesh). Of the recorded taxa, *Hyphoderma clavatum* Sheng H. Wu, *H. lapponicum* (Litsch.) Ryvarden and *H. nemorale* K.H. Larss. are the new additions to the fungal diversity of Himachal Pradesh. *H. sibiricum* (Parmasto) J. Erikss. & Å. Strid. is described for the first time from Chamba district.

Keywords

Basidiomycota · *Agaricomycetes* · Western Himalaya · Wood decaying fungi

10.1 Introduction

Family *Meruliaceae*, an assemblage of corticioid fungi, is characterised by the members with resupinate, adnate sporophores that are crustose to membranous to ceraceous to subceraceous to corneous. The hymenophore varies from smooth to hypochnoid to tuberculate to grandinoid to odontoid to phlebioid to porulose to

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merulioid. The sporophores consist of only generative hyphae which may be simple-septate or clamped and usually possess some kind of ancillary elements. The basidia vary from clavate to subclavate to cylindrical to suburniform and may or may not have a clamp at the base. The basidiospores range from ellipsoid to broadly ellipsoid to cylindrical to subcylindrical to globose to subglobose to allantoid, smooth or ornamented, thin- to thick-walled and their wall is usually not stained in Melzer's reagent (MR) but may or may not be stained in cotton blue (CB). Corticioid fungi play a significant role in the decay of lignin and cellulose as these are responsible mainly for the white and brown rot of coniferous and broad leaved tree wood. The studies on the diversity of these fungi from India were initiated in the second half of the nineteenth century with the work of Berkeley (1850) from eastern Himalaya. Since then different regions of north west India including Himachal Pradesh, eastern Himalaya and adjoining areas, central India and western ghats have been explored for studying the diversity of these fungi (Banerjee 1935a, b; Rattan 1977; Dhingra 1983; Sharma 1995; Bhosle et al. 2005; Dhingra et al. 2011, 2014; Ranadive et al. 2011; Sharma 2012; Prasher and Ashok 2013; Prasher and Lalita 2013; Prasher 2015). The review of literature revealed an account of 22 taxa belonging to presently described four genera i.e., *Crustoderma* (1), *Gyrophanopsis* (1), *Hyphoderma* (16) *and Hypochinicum* (4) of family *Meruliaceae* from Chamba district of Himachal Pradesh (Sharma 2012; Prasher and Ashok 2013; Dhingra et al. 2014; Poonam and Dhingra 2017; Kaur 2018; Devi 2019).

Taking into account of the unexplored localities of Chamba district, the present study on the diversity of corticioid fungi was planned. During this study, specimens of the corticioid fungi were collected. These specimens were identified and described as 17 taxa belonging to four genera of family *Meruliaceae*. The present study describes and illustrates these 17 taxa namely, *Crustoderma dryinum*, *Hyphoderma argillaceum*, *H. clavatum*, *H. guttuliferum*, *H. lapponicum*, *H. nemorale*, *H. pallidum*, *H. praetermissum*, *H. roseocremeum* var. *minutisporum*, *H. setigerum*, *H. setigerum* var. *bicystidium*, *H. sibiricum*, *H. tsugae*, *Hypochnicium erikssonii*, *H. lundelli*, *H. punctulatum and Gyrophanopsis polonensis*. Of these taxa, *Hyphoderma clavatum*, *H. lapponicum and H. nemorale* are the new additions to the mycoflora of Himachal Pradesh. Additionally, *H. sibiricum* is described for the first time from Chamba district.

10.2 Material and Methods

The present study is based on the collections made from different localities of district Chamba (Himachal Pradesh, India) during fungal forays conducted in the years 2013–2018. The sporophores were collected along with a portion of the substrate with the help of a hammer and a chisel. The details pertaining to type and colour of hymenial surface and margins were noted carefully with the help of a hand lens. The colour standards used were as per Kornerup and Wanscher (1978). A moist piece of the sporophore was used to get the spore print on a glass slide. These specimens were dried either in sun or using an electric drier. The micromorphological details of the

collected specimens were observed by making crush mounts/vertical sections of the sporophores in water, 3% KOH solution, 1% phloxine, 1% Congo red, 1% cotton blue and Melzer's reagent (0.5gm Iodine +1.5gm KI + 20gm Chloral hydrate +20 ml Distilled water). The outline of the microscopic structures was drawn with the help of a Camera Lucida mounted on compound microscope at 100×, 400× and 1000× magnifications. The macro and micromorphological details were compiled in the form of a description and were compared with the literature (Thind and Rattan 1970; Rattan 1977; Thind and Dhingra 1985; Dhingra 1989; Dhingra and Singla 1993; Dhingra 1997; Bhosle et al. 2005; Dhingra et al. 2011; Ranadive et al. 2011; Priyanka 2012; Sharma 2012; Prasher and Ashok 2013; Prasher and Lalita 2013; Ranadive 2013; Dhingra et al. 2014; Kaur et al. 2014; Samita 2014; Sanyal 2014; Kaur 2017; Poonam et al. 2017; Sharma 2017; Kaur 2018; Devi 2019) for identification. The identified specimens have been deposited at the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

10.3 Key to the Genera

1. Basidiospore wall bluish black in (amyloid) Melzer's reagent	2
1. No reaction of basidiospore wall in Melzer's reagent (inamyloid)	3
2. Ancillary elements hyphoid, cylindrical	*Gyrophanopsis*
2. Ancillary elements not as above	*Hypochinicum*
3. Hymenial surface smooth to tuberculate to grandinoid to odontoid, ancillary elements of various types, basidiospores usually with oily contents	*Hyphoderma*
3. Hymenial surface smooth, cysidia cylindrical to subcylindrical, with retraction septa, basidiospores usually without oily contents	*Crustoderma*

10.4 Taxonomic Descriptions

10.4.1 *Crustoderma* Parmasto, Conspectus Systematis Corticiacearum. (Tartu): 87 (1968)

Sporophores resupinate, crustaceous to ceraceous, closely adnate, effused, hymenial surface smooth. Hyphal system monomitic. Generative hyphae with clamps, yellowish, thin- to somewhat thick-walled, densely agglutinated. Ancillary elements present, sometimes with retraction septa. Basidia narrowly clavate to subclavate, sinuous, 4–sterigmate, with clamp at the base. Basidiospores narrowly ellipsoid to subcylindrical, smooth, thin-walled, negative to both to MR and CB. *Crustoderma* is distributed worldwide with 14 known species (Mycobank 2021). Of these, three species i.e., *C. corneum*, *C. dryinum and C. testatum* are described earlier from India.

10.4.1.1 *Crustoderma dryinum* (Berk. & M.A. Curtis) Parmasto (Berkeley and Curtis 1873; Parmasto 1968) (Fig. 10.1)

Sporophore annual, resupinate, closely adnate, effused, up to 400 μm thick in section; hymenial surface tuberculate in fresh and dry states; pale orange to light greyish orange when collected; margins thinner than rest of the hymenial surface, paler concolorous to abrupt. Generative hyphae yellowish, septate, clamped, smooth; ≤6.4 μm wide, horizontal, less branched, thin- to thick-walled in the subicular zone; ≤3.2 μm wide, vertical, richly branched, thin-walled in the subhymenial zone. Cystidia cylindrical, clamped at the base, 90–110 × 9–12 μm,

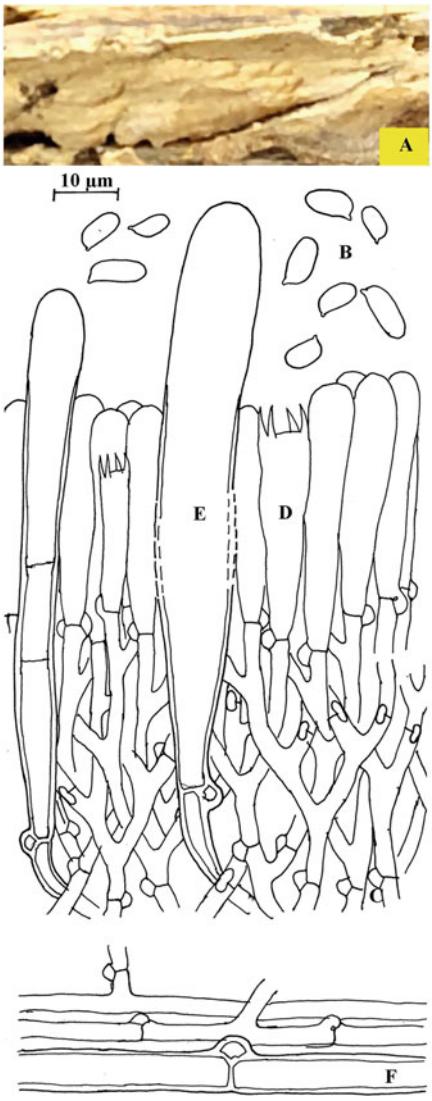

Fig. 10.1 *Crustoderma dryinum*: **A** Sporophore showing hymenial surface, **B**–**F** Line diagrams [**B** Basidiospores, **C** Reconstruction showing a portion of hymenium and subhymenium (**D** Basidium, **E** Cystidium); **F** Generative hyphae] Bar = 10 μm

thick-walled, with retraction septa, smooth; projecting up to 40 μm out of the hymenium. Basidia clavate to subclavate, 23–33 × 4–5.8 μm; sterigma ≤5 μm long. Basidiospores 5.5–9.6 × 3.2–4 μm, ellipsoid to subcylindrical, thin-walled, smooth, acyanophilous, amyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Khajjiar, Lakkar Mandi, on decaying stump of *Cedrus deodara*, Poonam 10766 (PUN), August 15, 2018.

Remarks

C. dryinum is a rereported taxon from the study area. Previously it has been reported by Dhingra et al., (2014) from districts Chamba, Shimla and Solan of Himachal Pradesh, respectively.

10.4.2 *Gyrophanopsis* Jülich, Pers. 10 (3): 329 (1979)

Sporophores resupinate, adnate, effused, hypochnoid to ceraceous; hymenial surface smooth to tuberculate. Hyphal system monomitic. Generative hyphae with clamped septa, thin- to thick-walled, smooth. Ancillary elements (Septocystidia) present. Basidia clavate to subclavate, somewhat suburniform, with clamped septa at the base, 4–sterigmate. Basidiospores ellipsoid to subcylindrical, smooth, thick-walled, positive to CB and negative to MR, with or without oily contents. This genus is represented by 2 known species worldwide (Mycobank 2021) and from India only *G. polonensis* is described.

10.4.2.1 *Gyrophanopsis polonensis* (Bres.) Stalpers & P.K. Buchanan (Bresadola 1903; Stalpers and Buchanan 1991) (Fig. 10.2)

Sporophore annual, resupinate, closely adnate, effused, up to 280 μm thick in section; hymenial surface smooth both in fresh and dry states; yellowish white to pale yellow to greyish yellow both in fresh and dry states; margins pruinose, paler concolorous when determinate. Generative hyphae subhyaline, smooth, septate, clamped; horizontal, ≤5.4 μm wide, less branched, thick-walled in the subicular zone; vertical, ≤3 μm wide, richly branched, thin-walled in the subhymenial zone. Septocystidia cylindrical hyphoid with obtuse apex, septate, clamped, 143–153 × 6.6–8.8 μm, thick-walled, encrusted; projecting up to 70 μm out of the hymenium. Basidia clavate, sinuous, 25–34 × 6–8 μm; sterigma ≤5.5 μm long. Basidiospores 7.2–9.9 × 3.6–5.4 μm, ellipsoid to subcylindrical, thick-walled, smooth, cyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Bharmour, Manimahesh, Tosh ka got, on the burnt stump of *Cedrus deodara*, Poonam 10433 (PUN), September 4, 2016.

Remarks

It is being described for the first time from tehsil Bharmour in the study area. Previously, it has been reported from tehsil Dalhousie of district Chamba by Thind and Rattan (1970), Rattan (1977) and Dhingra et al., (2014).

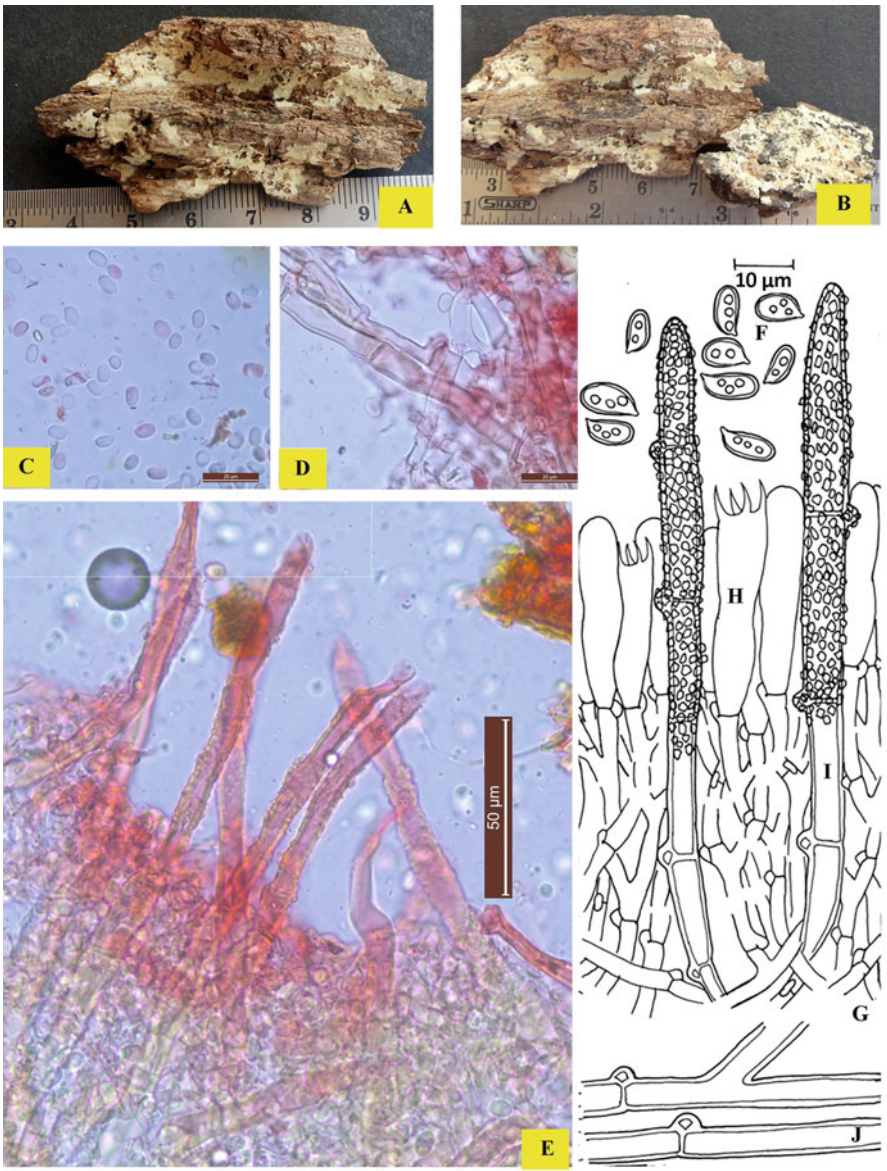

Fig. 10.2 Gyro*phanopsis polonensis*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**E** Photomicrographs (**C** Basidiospores, **D** Generative hyphae, **E** Encrusted cystidia); **F**–**J** Line diagrams [**F** Basidiospores, **G** Reconstruction showing a portion of hymenium and subhymenium (**H** Basidium, **I** Cystidium), **J** Generative hyphae] Bar = 10 µm

10.4.3 *Hyphoderma* Wallr. Wallr Flora Cryptogamica Germaniae 2: 576 (1833)

Sporophores resupinate, adnate, effused, ceraceous; hymenial surface hypochnoid to smooth to tuberculate to grandinoid to hydnoid. Hyphal system monomitic. Generative hyphae usually with clamped septa, thin- to thick-walled. Ancillary elements present or absent. Basidia clavate to subclavate, sometimes with suburniform constriction to sinuous, 4–sterigmate, usually with clamped septa at the base. Basidiospores ellipsoid to broadly ellipsoid to subglobose to globose to cylindrical to allantoid to suballantoid, smooth, thin-walled, negative to both MR and CB, with or without oily contents. It is a large genus represented by 121 species worldwide (Mycobank 2021). Among these 45 species have been described earlier from different parts of India (Rattan 1977; Dhingra et al. 2011, 2014; Ranadive et al. 2011; Sharma 2012; Ranadive 2013).

10.4.3.1 Key to the Species of Genus *Hyphoderma*

1. Clamps absent on generative hypae	*H. parvispora*[a]
1. Clamps present on generative hypae	2
2. Ancillary elements present	3
2. Ancillary elements absent	*H. sibiricum*
3. Septocystidia present	4
3. Septocystidia absent	5
4. Only septocystidia present	*H. setigerum*
4. Septocystidia and capitate leptocystidia present	*H. setigerum* var. *bicystidium*
5. Stephanocysts present	*H. praetermissum*
5. Stephanocysts absent	6
6. Ancillary elements moniliform	*H. nemorale*
6. Moniliform Ancillary elements absent	7
7. Ancillary elements forked at base	*H. luridum*[a]
7. Ancillary elements not forked at base	8
8. Patches of reddish resinous matter present at the hyphal ends or cystidia	9
8. Patches of such reddish resinous matter absent at the hyphal ends or cystidia	13
9. Basidiospores allantoid to suballantoid	*H. pallidum*
9. Basidiospores subcylindrial to ellipsoid to broadly ellipsoid	11
11. Basidiospores, 7.2–12 × 3.8–6.1 μm	*H. magnargillaceum*
11. Basidiospores smaller	12
12. Ancillary elements fusiform	*H. tsugae*
12. Ancillary elements basally widened	*H. argillaceum*
13. Basidia with unilateral outgrowth arising from the middle	*H. singularibasidium*[a]
13. Basidia without such outgrowth	14
14. Encrusted cystidia with globule of non-crystalline matter at apex	*H. guttuliferum*

(continued)

14. Ancillary elements not as above	15
15. Ancillary elements clavate to subclavate	16
15. Ancillary elements otherwise	17
16. Basidiospores ellipsoid to subcylindrical, hymenial surface light orange to greyish orange	*H. clavatum*
16. Basidiospores ellipsoid to suballantoid, hymenial surface greyish orange to brownish orange to light brown	*H. clavigerum*[a]
17. Basidiospores 9–11 × 6–7.8 µm	*H. lapponicum*
17. Basidiospores smaller, 5–6 × 3–3.6 µm	*H. roseocremeum* var. *Minutisporum*

[a]Corticioid taxa reported/rereported/listed by earlier workers from district Chamba but not encountered during the present study

10.4.3.2 *Hyphoderma argillaceum* (Bres.) Donk (Bresadola 1898; Donk 1957) (Fig. 10.3)

Sporophore annual, resupinate, adnate, effused, up to 200 µm thick in section; hymenial surface smooth both in fresh and dry states; yellowish white to pale yellow both in fresh and dry states; margins pruinose, paler concolorous when determinate. Generative hyphae ≤2.3 µm wide subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Cystidia subfusiform, basally widened, narrowing towards apex, with basal clamp, 53–92 × 6.7–9.4 µm, thin-walled, with brown reddish resinous matter in patches, that dissolves in 3% KOH solution; projecting up to 40 µm out of the hymenium. Basidia clavate to subclavate, with suburniform constriction, 20–31 × 7.2–8.3 µm; sterigma ≤4.4 µm long. Basidiospores 6.5–7.1 × 3.5–4.2 µm, broadly ellipsoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Churah, Bhandal, on the stump of *Cedrus deodara*, Poonam 10127 & 10681 (PUN), August 15, 2014; on the sticks of *C. deodara*, Poonam 10682 (PUN), August16, 2014; on the sticks of *Pinus wallichiana*, Poonam 10683 (PUN), August 16, 2014; Pangi, Saichu, on the sticks of *P roxburghii*, Poonam 10684 (PUN), September, 11, 2016; on the stump of *P. roxburghii*, Poonam 10685 (PUN), September, 11, 2016; Chamba, Sihunta, on the sticks of *P. persica*, Poonam 10686 (PUN), September, 11, 2016.

Remarks

It is being described for the first time from tehsils Churah and Pangi in the study area. The previous reports of *H. argillaceum* from Chamba district include Thind and Rattan (1970), Rattan (1977) and Dhingra et al. (2014).

10.4.3.3 *Hyphoderma clavatum* Sheng H. Wu (Wu 1997) (Fig. 10.4)

Sporophore annual, resupinate, adnate, effused, up to 250 µm thick in section; hymenial surface smooth to somewhat tuberculate both in fresh and dry states; light orange to greyish orange both in fresh and dry states; margins pruinose to

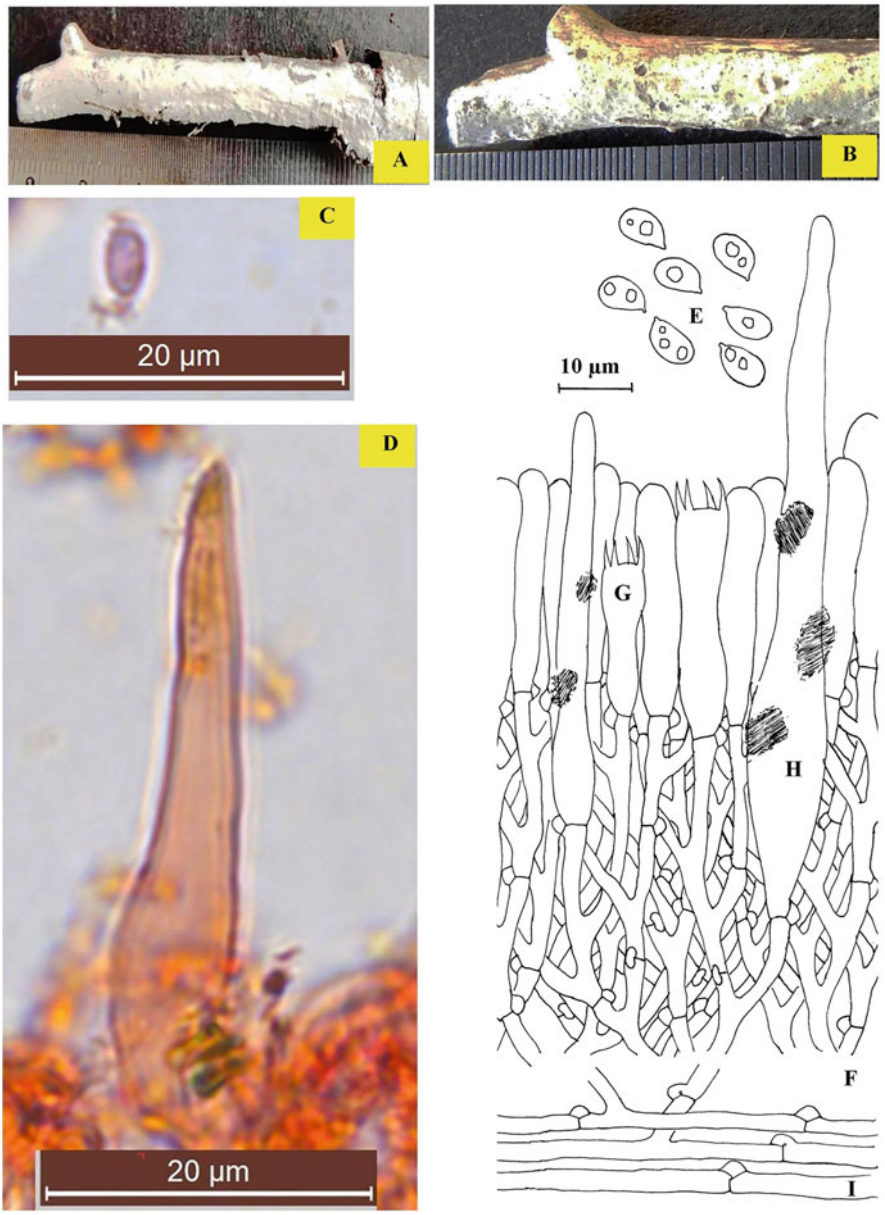

Fig. 10.3 *Hyphoderma argillaceum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Basidiospore, **D** Cystidium); **E**–**I** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Cystidium), **I** Generative hyphae] Bar = 10 µm

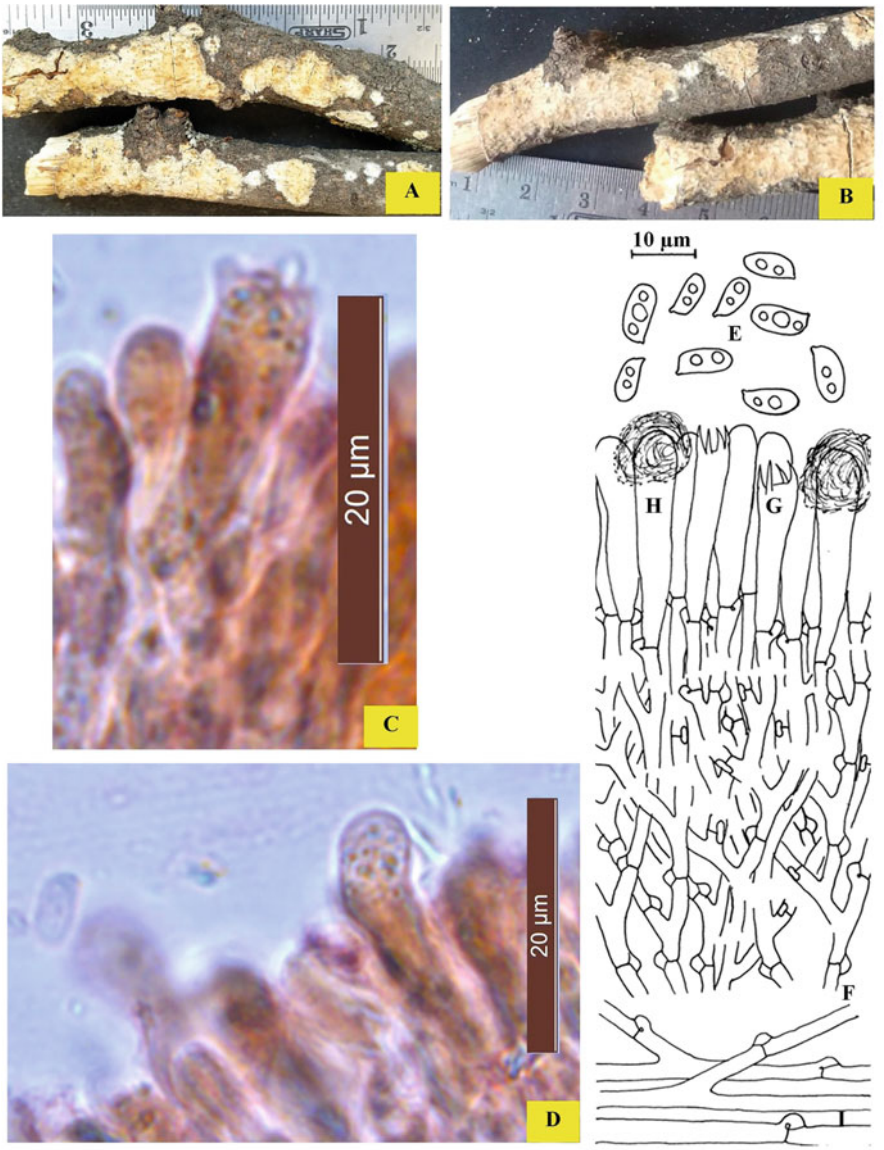

Fig. 10.4 *Hyphoderma clavatum*: **A–B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C–D** Photomicrographs (**C** Basidium, **D** Cystidium); **E–I** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Cystidium), **I** Generative hyphae] Bar = 10 µm

fibrillose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, smooth, thin-walled; horizontal, ≤4 μm wide, less branched in the subicular zone; vertical, up to ≤3.2 μm wide, richly branched in the subhymenial zone. Cystidia clavate, capitate, with basal clamp, 27–32 × 6–7 μm, thin-walled, apically encrusted; embedded in the hymenium. Basidia clavate to subclavate, with suburniform constriction, 22–26 × 4.4–5.5 μm; sterigma ≤4 μm long. Basidiospores 7.2–8.8 × 3.2–4 μm, ellipsoid to subcylndrical, thin-walled, smooth, acyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Pangi, Tilmili Pani, on stick of *Corylus avellana*, Poonam 10187 (PUN), September 12, 2016.

Remarks

H. clavatum is being described for the first time from Himachal Pradesh. It is the second report of this species from India. Earlier, it has only been reported by Sanyal (2014) from Uttarakhand.

10.4.3.4 *Hyphoderma guttuliferum* (P. Karst.) Donk (Karsten 1889; Donk 1962) (Fig. 10.5)

Sporophore annual, resupinate, adnate, effused, up to 220 μm thick in section; hymenial surface smooth to somewhat tuberculate both in fresh and dry states; orange white to greyish orange both in fresh and dry states; margins pruinose, paler concolorous when determinate. Generative hyphae ≤2.4 μm wide subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Cystidia subcylindrical, with basal clamp, 70–80 × 6–7.2 μm, thick-walled, encrusted with crystalline matter, some cystidia having globule of non-crystalline matter at apex; projecting up to 30 μm out of the hymenium. Basidia clavate to subcylindrical, sinuous, 25–30 × 6.4–7.2 μm; sterigma ≤5.5 μm long. Basidiospores 8.3–14 × 4–5 μm, subcylindrical to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Kalatop, on way to Khajjiar, on stump of *Cedrus deodara*, Poonam 10128 (PUN), November 4, 2013.

Remarks

This species is being redescribed from tehsil Dalhousie of district Chamba of Himachal Pradesh as Dhingra et al., (2014) also recorded it from tehsil Dalhousie.

10.4.3.5 *Hyphoderma lapponicum* (Litsch.) Ryvarden (Litschauer 1941; Ryvarden 1971) (Fig. 10.6)

Sporophore annual, resupinate, adnate, effused, up to 160 μm thick in section; hymenial surface smooth both in fresh and dry states; yellowish white when fresh yellowish white to greyish yellow on drying; margins fibrillose, paler concolorous when determinate. Generative hyphae ≤2.6 μm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Cystidia subcylindrical, with basal clamp, 37–76 × 7.2–8.3 μm, thin-walled; projecting up to 10 μm out of the hymenium.

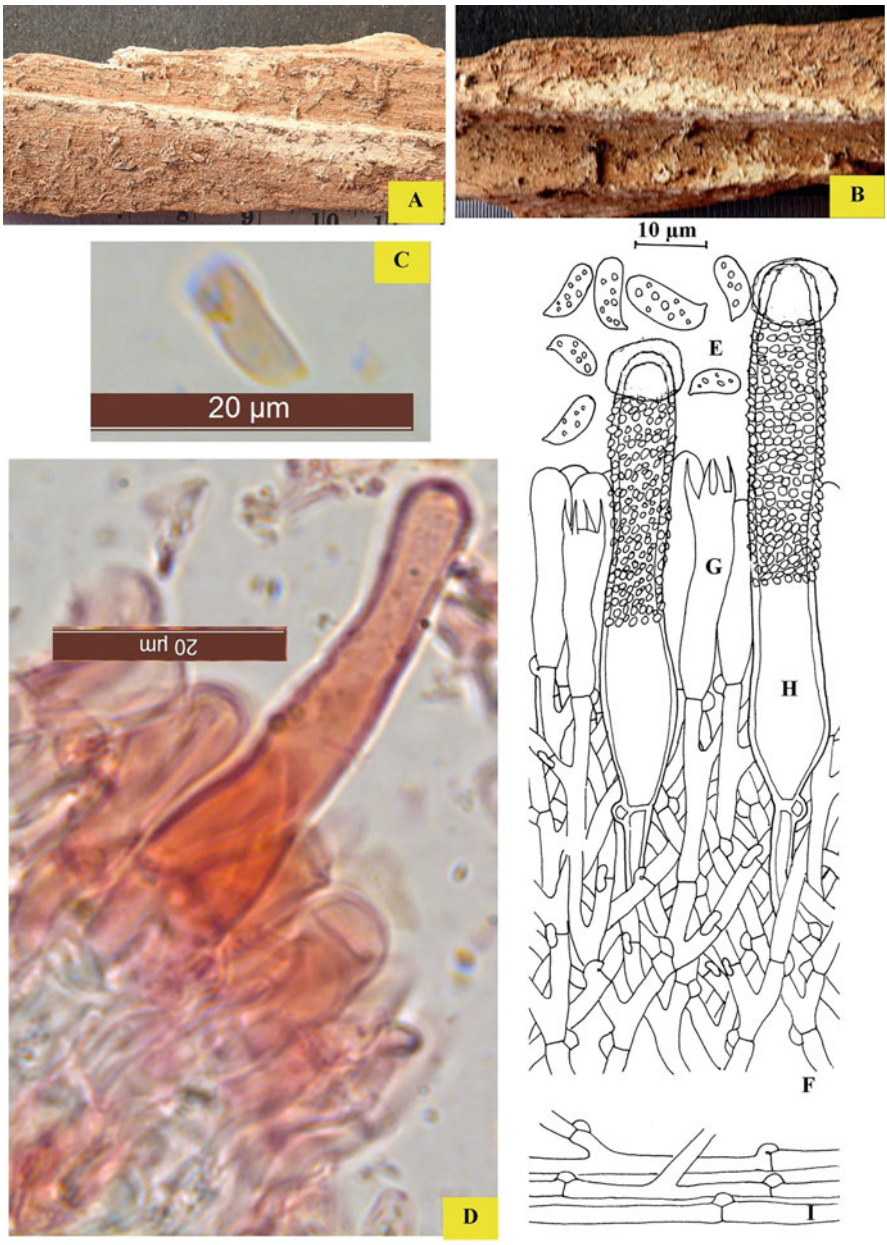

Fig. 10.5 *Hyphoderma guttuliferum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Basidiospore, **D** Cystidium); **E**–**I** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Cystidium), **I** Generative hyphae] Bar = 10 µm

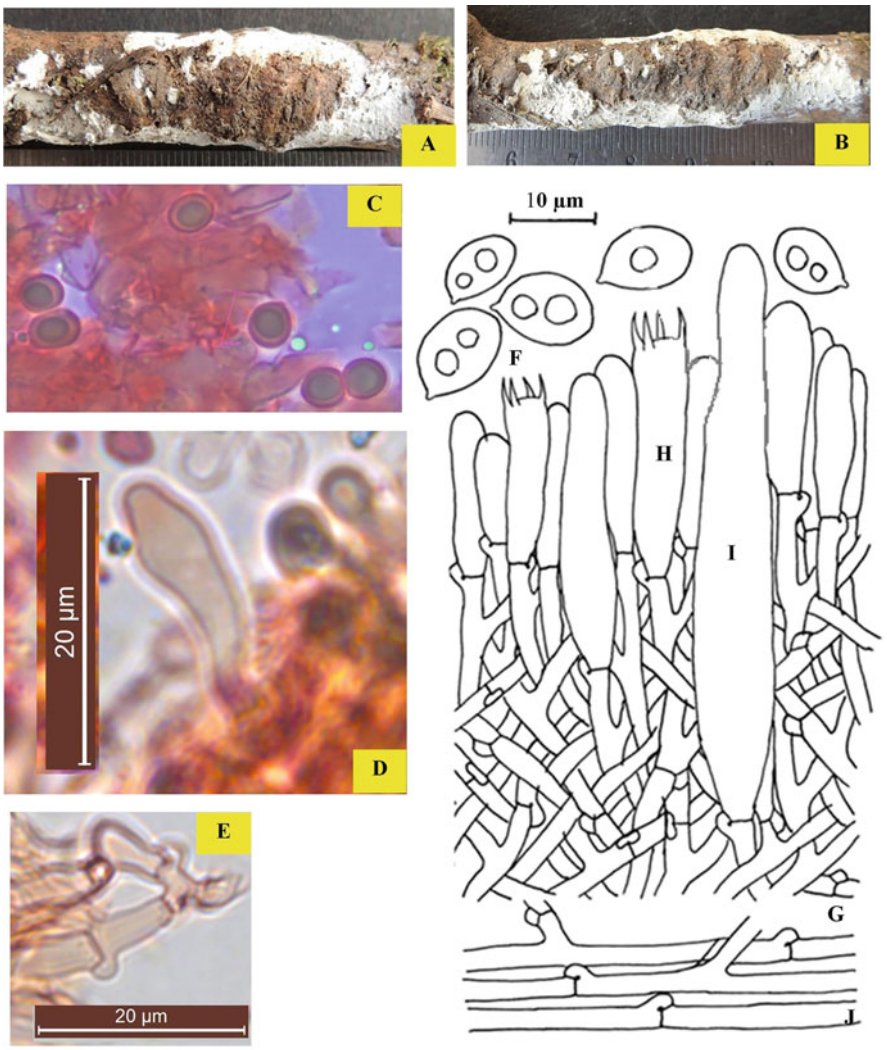

Fig. 10.6 *Hyphoderma lapponicum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**E** Photomicrographs (**C** Basidiospores, **D** Cystidium, **E** Generative hyphae); **F**–**J** Line diagrams [**F** Basidiospores, **G** Reconstruction showing a portion of hymenium and subhymenium (**H** Basidium, **I** Cystidium), **J** Generative hyphae] Bar = 10 µm

Basidia clavate to subclavate, 16–37 × 4.4–6.1 μm; sterigma ≤4.4 μm long. Basidiospores 9–11 × 6–7.8 μm, broadly ellipsoid, thin-walled, smooth, acyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Churah, Bhandal, on stick of *Cedrus deodara*, Poonam 10129 (PUN), August 15, 2014.

Remarks

H. lapponicum is being reported for the first time from the state of Himachal Pradesh. Earlier, it was reported from Maharashtra (Bhosle et al. 2005; Ranadive et al. 2011; Ranadive 2013), Uttarakhand (Sharma 2012) and Punjab (Kaur et al. 2014).

10.4.3.6 *Hyphoderma nemorale* K.H. Larss. (Larsson 1998) (Fig.10.7)

Sporophore annual, resupinate, adnate, effused, up to 120 μm thick in section; hymenial surface smooth both in fresh and dry states; greyish white to yellowish white; margins pruinose, paler concolorous when determinate. Generative hyphae ≤2.4 μm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Ancillary elements of two kinds (1) Leptocystidia up to 30 × 5.5 μm, subcylindrical, sinuous, with basal clamp, thin-walled, generally encrusted in the apical region, (2) Moniliform cystidia up to 46 × 6 μm, often moniliform thin-walled. Basidia clavate to subclavate, somewhat sinuous, 31–48 × 7.2–8.8 μm; sterigma ≤6.7 μm long. Basidiospores 11–15 × 3.9–4.4 μm, subcylindrical to suballantoid to allantoid, thin-walled, smooth, acyanophilous, inamyloid.

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Collections Examined

India, Himachal Pradesh: Chamba, Holi, on *Pinus wallichiana* sticks, Poonam 7651, 10559 & 10560 (PUN), August 23, 2015.

Remarks

This species is being reported for the first time from Himachal Pradesh. Earlier from India, it has only been described by Sanyal (2014) from Uttarakhand.

10.4.3.7 *Hyphoderma pallidum* (Bres.) Donk (Bresadola 1898; Donk 1957) (Fig. 10.8)

Sporophore annual, resupinate, adnate, effused, up to 210 μm thick in section; hymenial surface smooth both in fresh and dry states; greyish white to orange white both in fresh and dry states; margins pruinose, paler concolorous when determinate. Generative hyphae ≤2.7 μm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Cystidia fusiform to subfusiform, with basal clamp, 52–85 × 7.7–9.4 μm, thin-walled; projecting up to 10 μm out of the hymenium. Basidia clavate to subclavate, with suburniform constriction, 17–26 × 5–6.1 μm; sterigma ≤5.5 μm long. Basidiospores 7.2–10 × 2.7–3.3 μm, suballantoid to allantoid, thin-walled, smooth, acyanophilous, inamyloid.

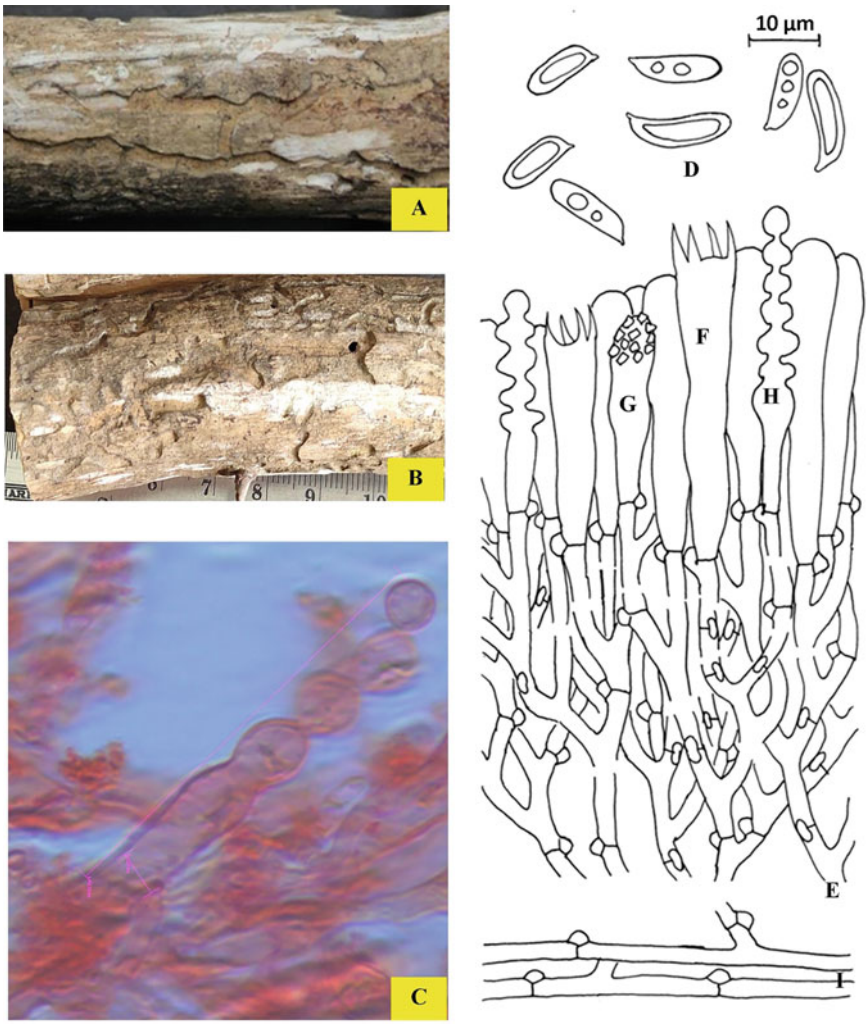

Fig. 10.7 *Hyphoderma nemorale*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C** Photomicrograph showing Moniliform cystidium; **D-I** Line diagrams [**D** Basidiospores, **E** Reconstruction showing a portion of hymenium and subhymenium, (**F** Basidium, **G** Leptocystidium, **H** Moniliform cystidium), **I** Generative hyphae] Bar = 10 µm

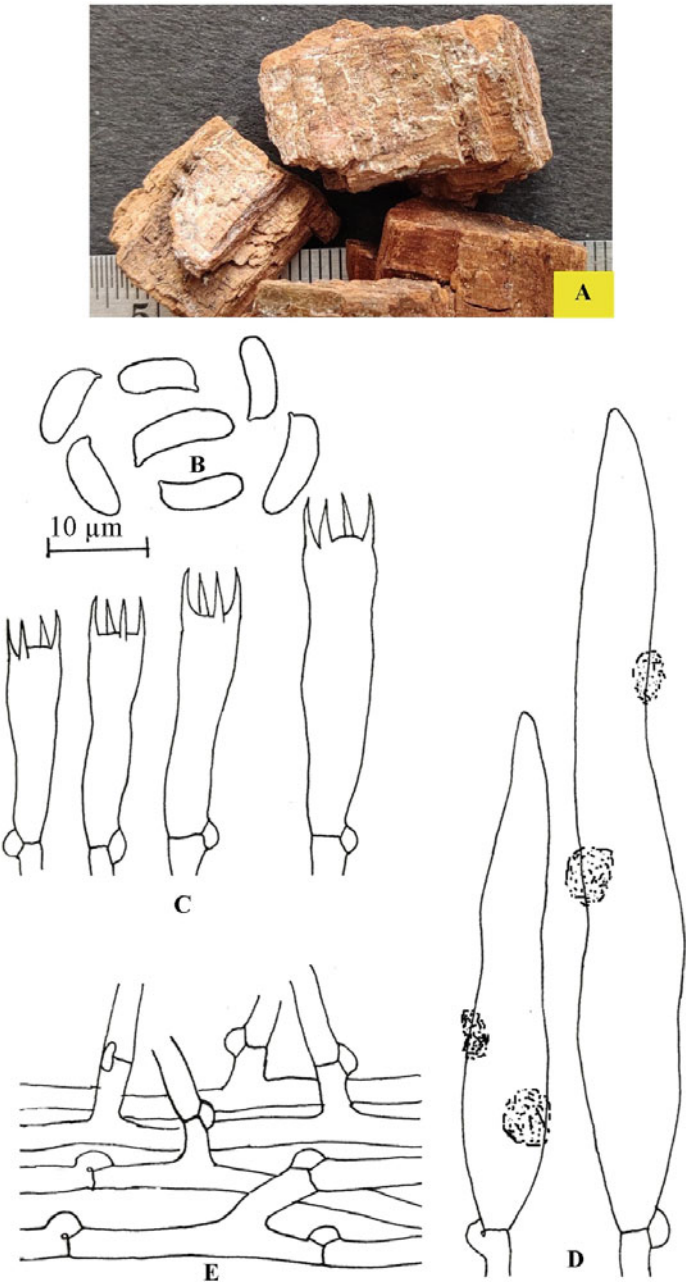

Fig. 10.8 *Hyphoderma pallidum*: **A** Sporophore showing hymenial surface; **B–E** Line diagrams (**B** Basidiospores, **C** Basidia, **D** Cystidia, **E** Generative hyphae) Bar = 10 µm

Collection Examined

India, Himachal Pradesh: Chamba, Bhandal, on *Cedrus deodara* stump, Poonam 10556 (PUN), August 23, 2015.

Remarks

H. pallidum is a rereported taxon from the study area. Previously it has been reported from Chamba district by Rattan (1977).

10.4.3.8 *Hyphoderma praetermissum* (P. Karst.) J. Eriksss. & Å. Strid (Karsten 1889; Eriksson and Strid 1975) (Fig. 10.9)

Sporophore annual, resupinate, adnate, effused, up to 160 μm thick in section; hymenial surface smooth both in fresh and dry states; yellowish white to pale yellow when fresh pale yellow to greyish yellow on drying; margins pruinose, paler concolorous when determinate. Generative hyphae ≤2.7 μm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Ancillary elements of three kinds: (1) Cystidia subcylindrical to subcapitate, with basal clamp, 32–45 × 6.1–6.6 μm, thin- to somewhat thick-walled, with apical encrustation; projecting up to 30 μm out of the hymenium (2) Gloeocystidia fusoid to subcylindrical, with basal clamp, 42–70 × 8.3–9.4 μm, thin- walled, tapering towards apex, with oily contents negative to sulfovanallin; slightly projecting out of the hymenial surface (3) Stephanocysts bladder shaped, having small teeth in whorl, with basal clamp, 9.9–15.5 × 6.1–9.4 μm. Basidia clavate, sinuous, with oily contents, 20–34 × 6–7 μm; sterigma ≤5.5 μm long. Basidiospores 6.4–9.6 × 3.3–4.4 μm, ellipsoid to subcylindrical, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, on way to Banikhet, on stump of *Quercus leucotrichophora*, Poonam 10717 (PUN), October 13, 2012; Udaipur on stump of *Populus ciliata*, Poonam 10718 & 10719 (PUN), October 13, 2015.

Remarks

This species is being described for the first time from tehsil Chamba of district Chamba. The former reports of *H. praetermissum* from Chamba district were by Rattan (1977) and Dhingra et al. (2014).

10.4.3.9 *Hyphoderma roseocremeum* var. *minutisporum* Priyanka & Dhingra (Priyanka and Dhingra 2014) (Fig. 10.10)

Sporophore annual, resupinate, adnate, effused, up to 200 μm thick in section; hymenial surface smooth both in fresh and dry states; orange white to pale orange to greyish orange when fresh pale orange to greyish orange on drying; margins fibrillose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤5 μm wide, less branched, thick-walled in the subicular zone; vertical, up to ≤2.8 μm wide, thin-walled, richly branched in the subhymenial zone. Cystidia subcylindrical, with basal clamp, 44–52 × 5.5–6.1 μm, thin-walled, smooth; projecting 20 μm out of the hymenium. Basidia clavate to subclavate, sinuous, with oily contents, 24–28 × 4.9–5.5 μm; sterigma ≤5 μm long.

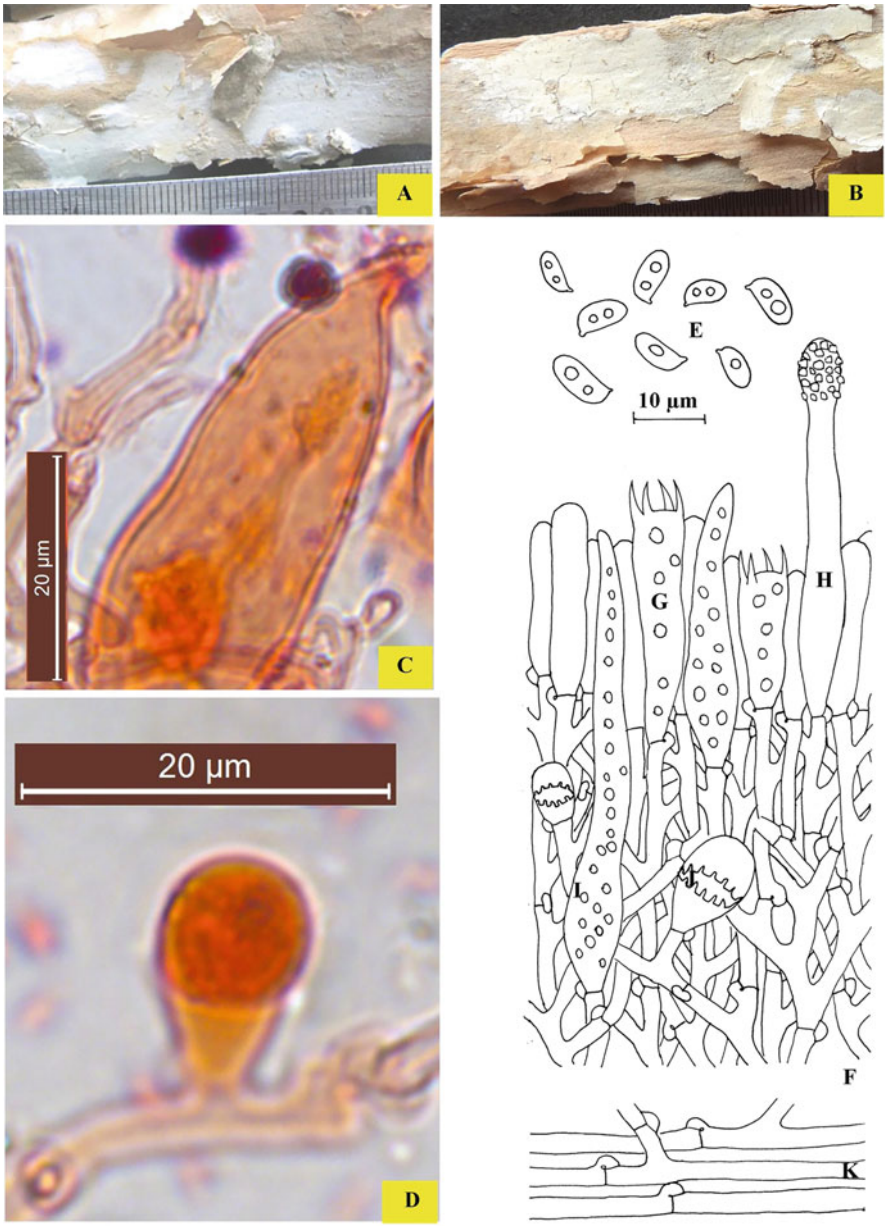

Fig. 10.9 *Hyphoderma praetermissum*: **A–B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C–D** Photomicrographs (**C** Gloeocystidium, **D** Stephanocyst); **E–J** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Cystidium, **I** Gloeocystidium, **J** Stephanocyst), **k** Generative hyphae] Bar = 10 µm

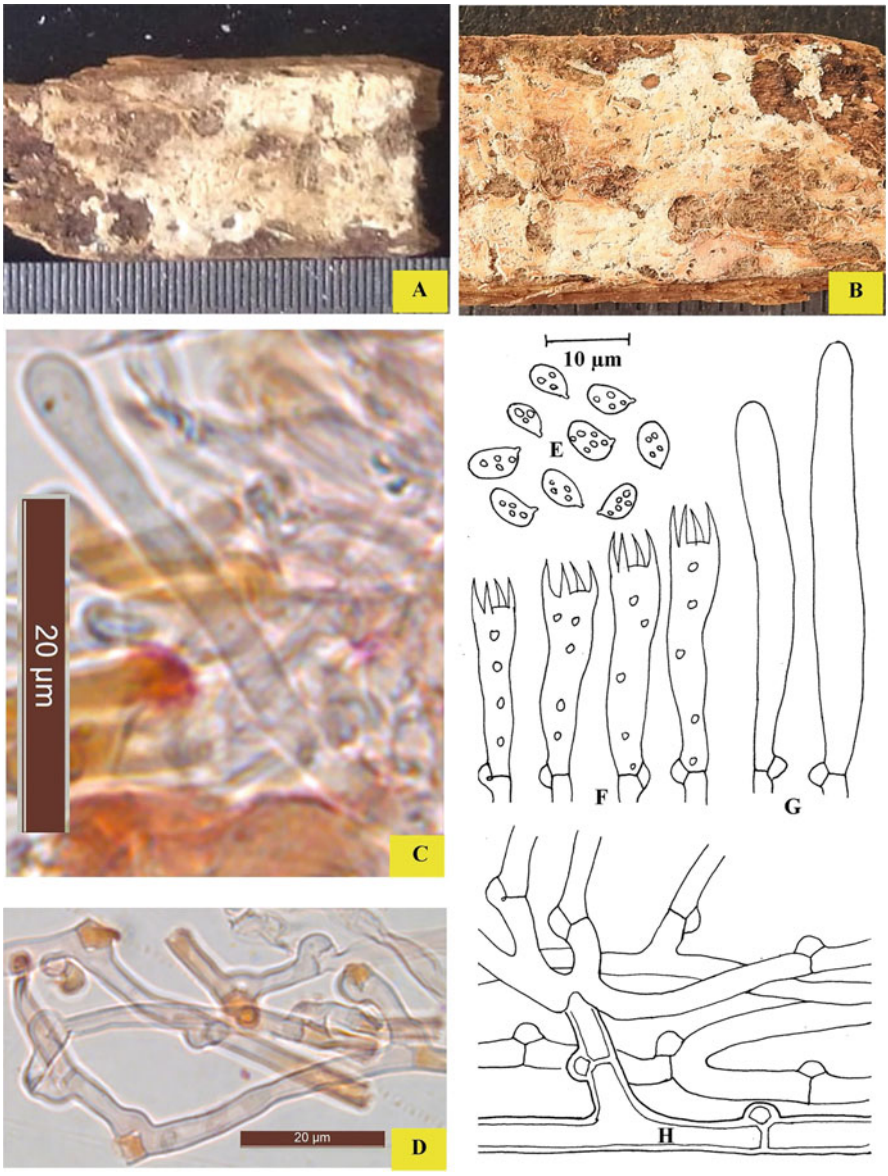

Fig. 10.10 *Hyphoderma roseocremeum* var. *minutisporum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Cystidium, **D** Generative hyphae); **E-H** Line diagrams (**E** Basidiospores, **F** Basidia, **G** Cystidia, **H** Generative hyphae) Bar = 10 µm

Basidiospores 5–6 × 3–3.6 μm, ellipsoid to broadly ellipsoid, thin-walled, smooth, acyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Dalhousie, Lakkar Mandi, on the stump of *Cedrus deodara*, Poonam 10180 (PUN), October 13, 2013.

Remarks

This variety is being described for the second time from district Chamba. The only previous report is by Dhingra et al. (2014).

10.4.3.10 *Hyphoderma setigerum* (Fr.) Donk (Fries 1828; Donk 1957) (Fig. 10.11)

Sporophore annual, resupinate, adnate, effused, up to 160 μm thick in section; hymenial surface smooth both in fresh and dry states; greyish white to yellowish white when fresh light yellow to greyish yellow on drying; margins fibrillose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤5 μm wide, less branched, thick-walled in the subicular zone; vertical, up to ≤2.8 μm wide, thin-walled, richly branched in the subhymenial zone. Septocystidia hyphoid, septate, clamped, 87–136 × 6–7.2 μm, thick-walled, except at the apical portion, encrusted; projecting upto 35 um out of the hymenium. Basidia subclavate to clavate, with oily contents, 22–30 × 6.6–7.7 μm; sterigma ≤5.5 μm long. Basidiospores 7.2–11.2 × 4–5, ellipsoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Churah, Bhandal, on the sticks of *Rosa macrophylla*, Poonam 10130, 10463, 10722 & 10723 (PUN), August 15, 2014; Kohlari, on the stump of *Cedrus deodara*, Poonam 10720 (PUN), August 29, 2014; Doreda Nala, on stump of *C. deodara*, Poonam 10721 (PUN), August 29, 2014; Hardaspura, on the stick of *Adhatoda vasica*, Poonam 10724 (PUN), November 04, 2015; on the stick of *Ficus carica*, Poonam 10725 (PUN), November 04, 2015.

Remarks

Hyphoderma setigerum is being described for the first time from tehsils Chamba and Churah in the study area. It was previously reported from district Chamba by Dhingra and Singla (1993), Sharma (2012) and Dhingra et al. (2014).

10.4.3.11 *Hyphoderma setigerum* var. *bicystidium* Dhingra & Singla (Dhingra and Singla 1993) (Fig. 10.12)

Sporophore annual, resupinate, adnate, effused, up to 200 μm thick in section; hymenial surface hypchnoid to smooth both in fresh and dry states; greyish white to orange white both in fresh and dry states; margins fibrillose, paler concolorous when determinate. Generative hyphae ≤3.3 μm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Ancillary elements of two kinds: (1) Septocystidia cylindrical, septate, clamped, 128–145 × 6–6.6 μm, thick-walled (2) Leptocystidia capitate, with basal clamp, 50–64 × 6–6.6 μm, thin-walled, smooth. Basidia clavate to subclavate, sinuous, with oily contents,

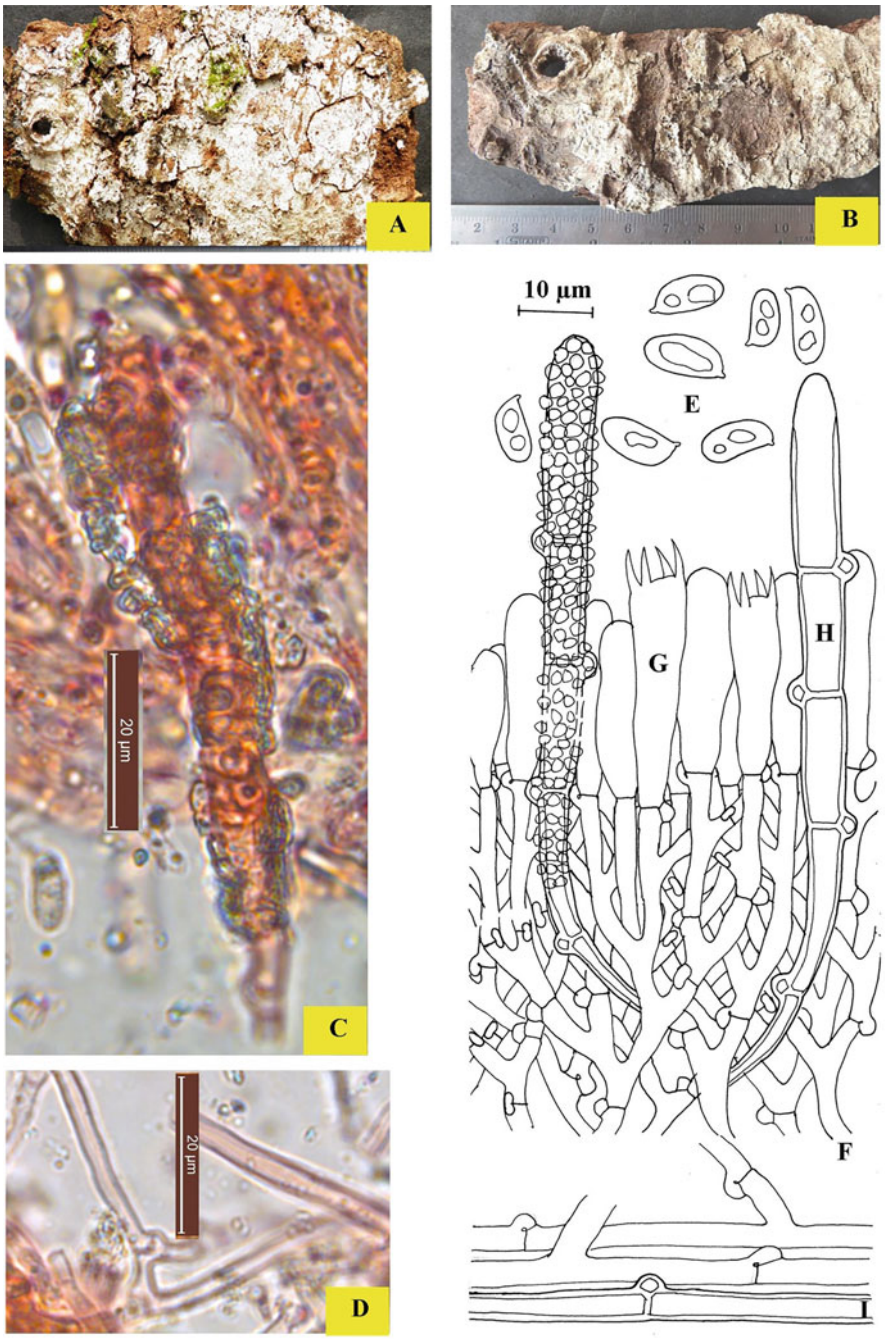

Fig. 10.11 *Hyphoderma setigerum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Septocystidium, **D** Generative hyphae); **E**–**I** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Septocystidium), **I** Generative hyphae] Bar = 10 µm

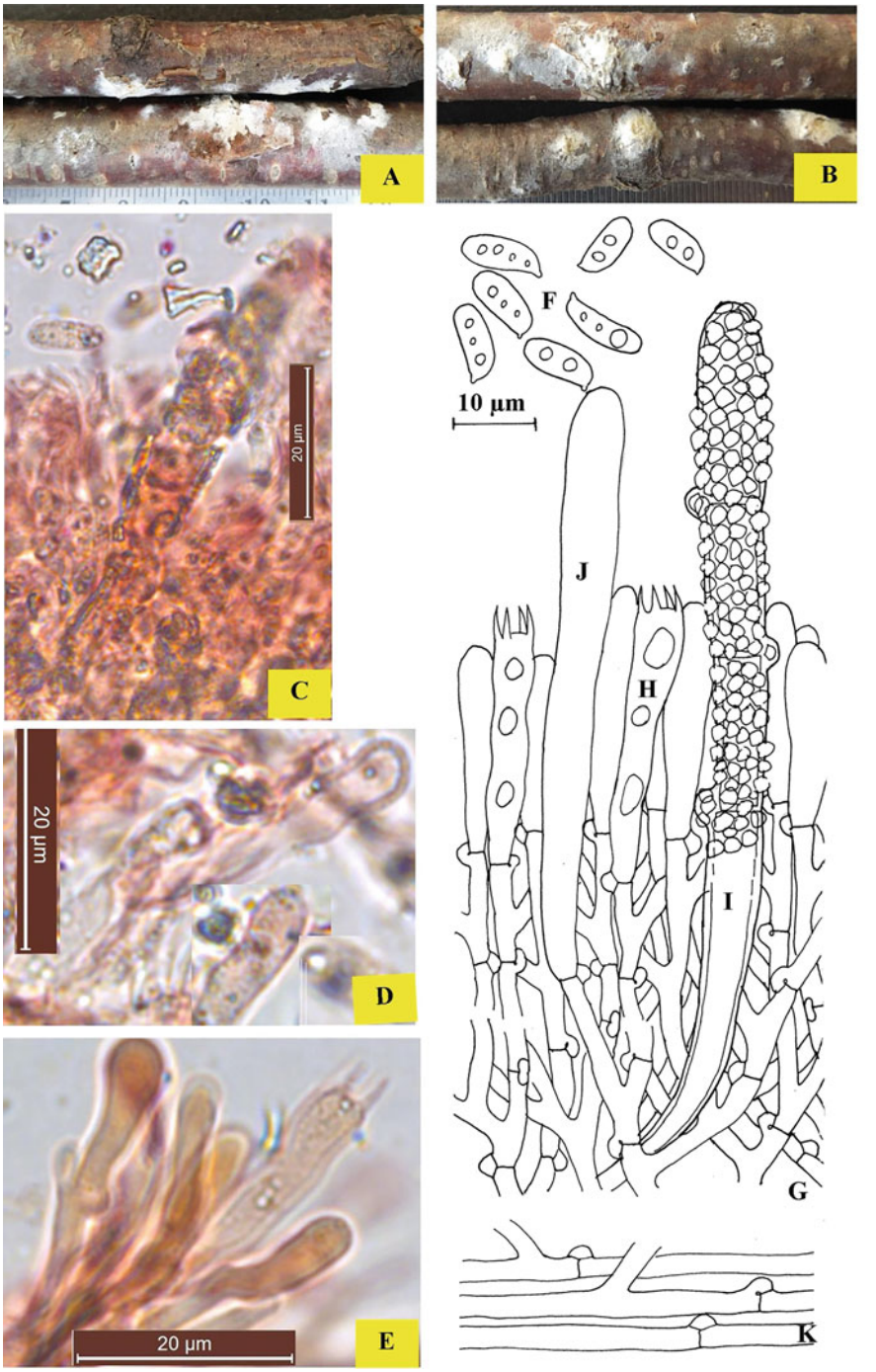

Fig. 10.12 *Hyphoderma setigerum* var. *bicystidium*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**E** Photomicrographs (**C** Septocystidium, **D** Leptocytidium, **E** Basidium); **F**–**K**

30–34 × 4.4–6.6 μm; sterigma ≤4 μm long. Basidiospores 8.9–12 × 3.2–4 μm, subcylindrical to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Khajjiar, on the sticks of *Pinus roxburghii*, Poonam 10470 (PUN), October 13, 2013; Pangi, Sural, on the sticks of *Betula utilis*, Poonam 10131 (PUN), September 12, 2016; Chamba.

Remarks

H. setigerm var. *bicystidium* is being described for the first time from tehsil Pangi in the study area. Earlier it was reported from Dalhousie tehsil by Dhingra and Singla (1993) and Dhingra et al. (2014).

10.4.3.12 *Hyphoderma sibiricum* (Parmasto) J. Erikss. & Å. Strid (Parmasto 1968; Eriksson and Strid 1975) (Fig. 10.13)

Sporophore annual, resupinate, adnate, effused, up to 200 μm thick in section; hymenial surface smooth to somewhat tuberculate both in fresh and dry states; pale orange to greyish orange both in fresh and dry states; margins fibrillose, paler concolorous when determinate. Generative hyphae ≤3 μm wide, subhyaline, septate, clamped, thin-walled, smooth, horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Basidia subclavate to cylindrical, sinuous, with oily contents, 31–62 × 7.2–9.4 μm; sterigma ≤6.1 μm long. Basidiospores 7.2–10 × 3.8–5.5 μm, subcylindrical to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Dalhousie cantonment area, on the sticks of *Quercus leucotricophora*, Poonam 10132 (PUN), August 2, 2014; Pangi, Saichu, on angispermous sticks, Poonam 10726 and 10727 (PUN), September, 11, 2016.

Remarks

A new record for the study area, *H. sibiricum* is peculiar in lacking ancillary elements. Previously, it has been reported from district Kullu and Solan of Himachal Pradesh (Dhingra et al. 2014).

10.4.3.13 *Hyphoderma tsugae* (Burt) J. Erikss. & Å. Strid (Burt 1926; Eriksson and Strid 1975) (Fig. 10.14)

Sporophore annual, resupinate, adnate, effused, up to 220 μm thick in section; hymenial surface smooth both in fresh and dry states; yellowish white to greyish yellow when fresh greyish yellow to yellowish grey on drying; margins fibrillose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤3.3 μm wide, less branched, thin- to thick-walled in the subicular zone; vertical, ≤2.7 μm wide, richly branched, thin-walled in the

Fig. 10.12 (continued) Line diagrams [**F** Basidiospores, **G** Reconstruction showing a portion of hymenium and subhymenium (**H** Basidium, **I** Septocystidium, **J** Leptocytidium), **K** Generative hyphae] Bar = 10 μm

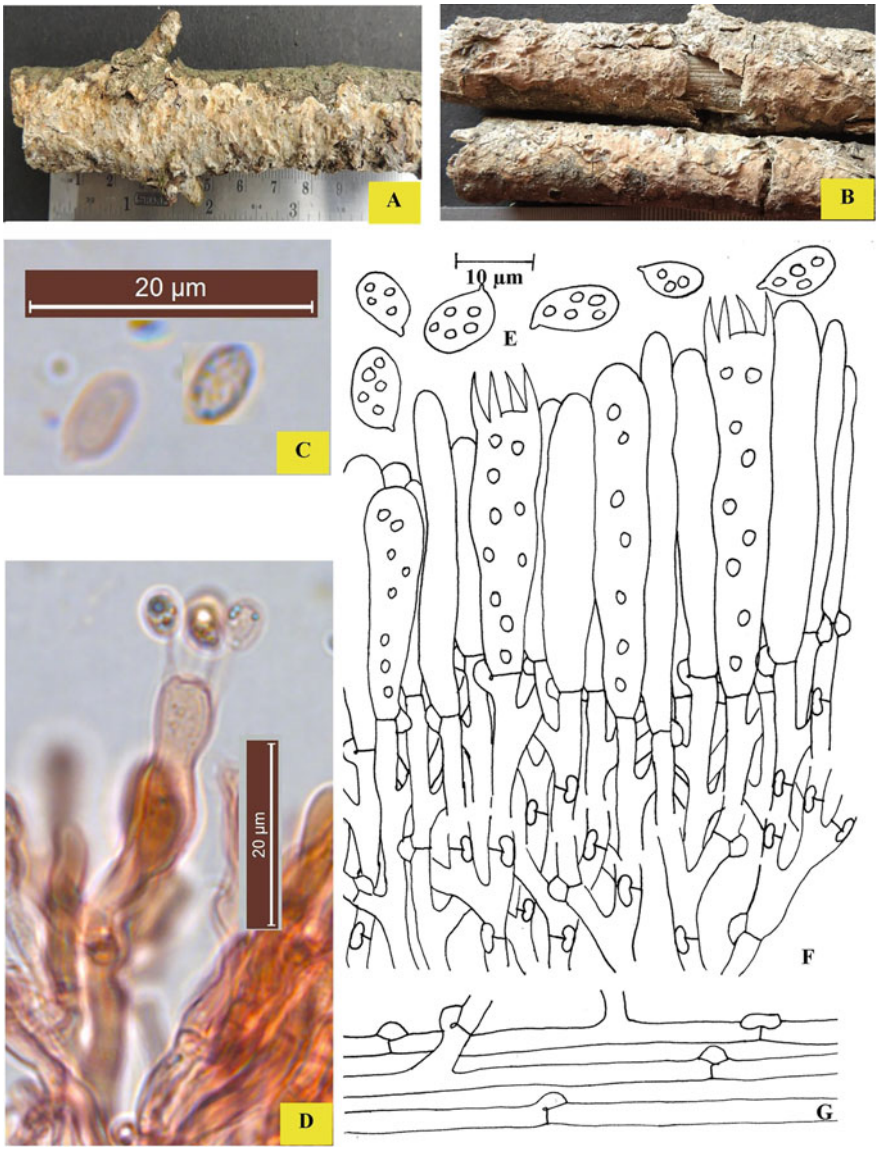

Fig. 10.13 *Hyphoderma sibiricum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Basidiospores, **D** Basidium), **E**–**G** Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium, **G** Generative hyphae] Bar = 10 µm

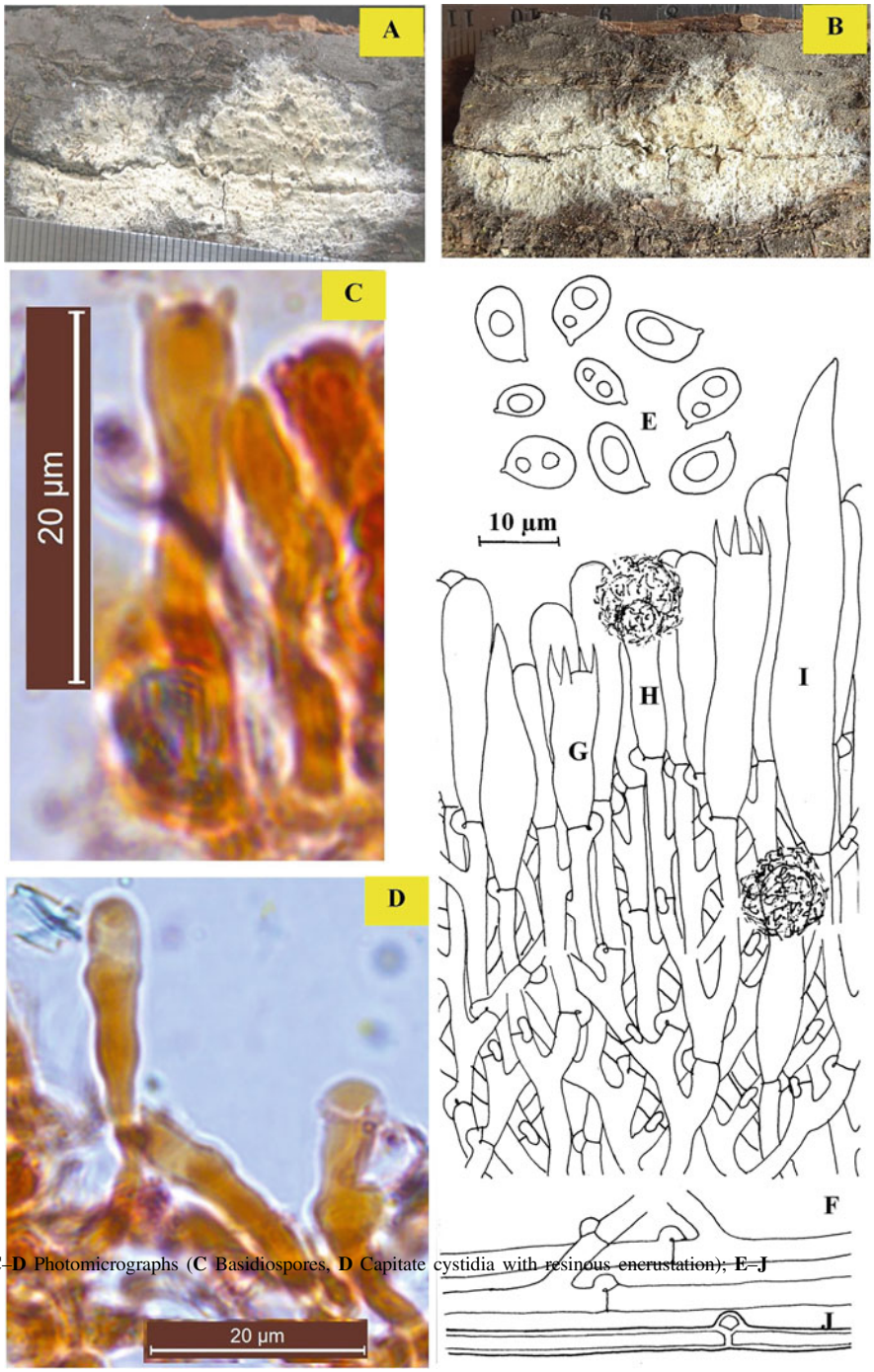

Fig. 10.14 *Hyphoderma tsugae*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C**–**D** Photomicrographs (**C** Basidiospores, **D** Capitate cystidia with resinous encrustation); **E**–**J**

subhymenial zone. Ancillary elements of two kinds: (1) Leptocystidia fusiform, with basal clamp, 35–64 × 6.1–6.6 µm, thin-walled; projecting up to 30 µm out of the hymenium (2) Capitate hyphoid, with basal clamp, 26–28 × 2.7–3.3 µm, thin-walled, with encrustation at the apex. Basidia narrowly clavate, 20–34 × 3.3–6.1 µm; sterigma ≤5 µm long. Basidiospores 5.6–8.8 × 4.8–6.4 µm, ellipsoid to broadly ellipsoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Dalhousie, Jandrighat, on the stump of *Cedrus deodara*, Poonam 10133 (PUN), November 4, 2013; Hardaspura, on the stump of *Populus ciliata*, Poonam 10749 (PUN), November 5, 2018.

Remarks

Hyphoderma tsugae is being described for the first time from tehsil Chamba in the study area. The earlier record of this species is from Dalhousie tehsil (Dhingra and Singla 1993; Dhingra et al. 2014).

10.4.4 *Hypochnicium* J. Erikss., Symbolae Botanicae Upsalienses 16 (1): 100 (1958)

Sporophores resupinate, adnate, effused; hymenial surface smooth to hypochnoid to somewhat tuberculate. Hyphal system monomitic. Generative hyphae with clamped septa, thin- to thick-walled, smooth. Ancillary elements present or absent. Basidia clavate to subclavate, somewhat sinuous, with clamped septa at the base, 4–sterigmate. Basidiospores broadly ellipsoid to subglobose to ovoid, smooth to warted, thick-walled, positive to CB and negative to MR, with or without oily contents. Genus *Hypochnicium* is represented by 32 taxa worldwide (Mycobank, 2021). Of these, 15 taxa are recorded from different parts of India (Rattan 1977; Dhingra et al. 2011, 2014; Ranadive et al. 2011, Sharma, 2012; Prasher and Ashok 2013; Ranadive 2013).

10.4.4.1 Key to the Species of Genus *Hypochnicium*

1. Ancillary elements present	2
1. Ancillary elements absent	*H. lundelli*
2. Basidiospores smooth	3
2. Basidiospores verrucose	4
3. Basidiospores broadly ellipsoid to subglobose, 5.1–6 × 4–4.5 µm	*H. subrigescens*[a]
3. Basidiospores subglobose to globose, 6–7 × 4–5.5 µm	*H. eriksonii*

(continued)

Fig. 10.14 (continued) Line diagrams [**E** Basidiospores, **F** Reconstruction showing a portion of hymenium and subhymenium (**G** Basidium, **H** Capitate cystidium, **I** Leptocystidium), **J** Generative hyphae] Bar = 10 µm

4. Ancillary elements subcylindrical, 53–100 × 6.1–6.6 μm	*H. punctulatum*
4. Ancillary elements tubular with tapering and obtuse apex, 80–150 × 6–10 μm	*H. cremicolor*[a]

[a]Corticioid taxa reported/rereported/listed by earlier workers from district Chamba but not encountered during the present study

10.4.4.2 *Hypochnicium erikssonii* Hallenb. and Hjortstam (Hallenberg and Hjortstam 1990) (Fig. 10.15)

Sporophore annual, resupinate, adnate, effused, up to 220 μm thick in section; hymenial surface smooth to hypochnoid when collected both in fresh and dry states; yellowish white to greyish yellow when fresh greyish yellow to yellowish grey on drying; margins pruinose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, thin-walled, smooth; horizontal, ≤6.2 μm wide, less branched in the subicular zone; vertical, ≤3 μm wide, richly branched in the subhymenial zone. Cystidia subcylindrical, with basal clamp, 60–135 × 8–11 μm, thin-walled, smooth; projecting up to 60 μm out of the hymenium. Basidia clavate to subclavate, 15–30 × 5–8.4 μm; sterigma ≤6 μm long. Basidiospores 6–7 × 4–5.5 μm, subglobose to globose, thick-walled, smooth, cyanophilous, inamyloid.

Collection Examined

India, Himachal Pradesh: Chamba, Khajjiar, Lakkar Mandi towards Kalatop, on the decaying branches of *Cedrus deodara*, Poonam 10553 (PUN), September 5, 2017.

Remarks

H. erikssonii is being redescribed from the study area. It was previously described/listed from Chamba district by Priyanka (2012) and Dhingra et al. (2014).

10.4.4.3 *Hypochnicium lundelli* (Bourdot) J. Erikss. (Bourdot 1949; Eriksson 1958) (Fig. 10.16)

Sporophore annual, resupinate, adnate, effused, up to 280 μm thick in section; hymenial surface smooth when collected; yellowish white to yellowish grey when fresh yellowish white to yellowish grey on drying; margins pruinose, paler concolorous when determinate. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤3.3 μm wide, less branched, thin- to thick-walled in the subicular zone; vertical, ≤2.2 μm wide, branched, thin-walled in the subhymenial zone. Basidia clavate to subclavate, sinuous, with oily contents, 33–39 × 6–6.6 μm; sterigma ≤5.5 μm long. Basidia clavate to subclavate, sinuous, with oily contents, 33–39 × 6–6.6 μm; sterigma ≤5.5 μm long. Basidiospores 5.5–7.7 × 4.4–4.9 μm, broadly ellipsoid to ovoid, thick-walled, smooth, cyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Hardaspura, on the sticks of *Adhatoda vasica*, Poonam 10438 & 10439 (PUN), September 01, 2014; Bharmour, Manimahesh, Tosh ka got, on the log of *Cedrus deodara*, Poonam 8848 (PUN), September

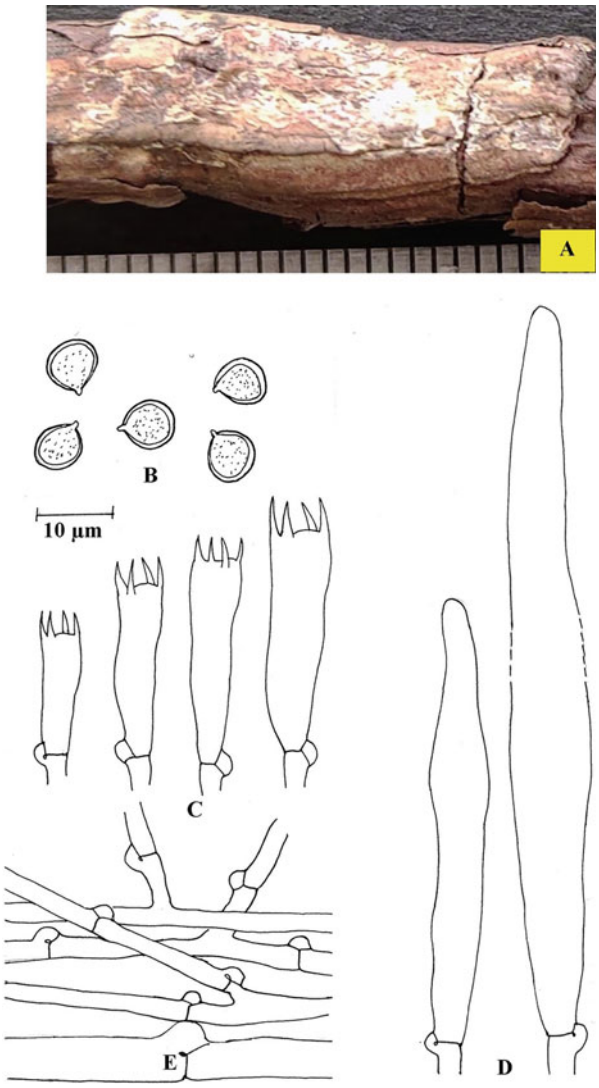

Fig. 10.15 *Hypochnicium erikssonii*: **A** Sporophore showing hymenial surface; **B**–**E** Line diagrams (**B** Basidiospores, **C** Basidia, **D** Cystidia, **E** Generative hyphae) Bar = 10 µm

04, 2016; Churah, Bhandal, on the log of *C. deodara*, Poonam (PUN), September 04, 2016.

Remarks

This species is being described for the first time from district Chamba. Previously it was reported from district Shimla (Rattan 1977; Prasher and Ashok 2013; Dhingra et al. 2014) and Kullu (Sharma 2012).

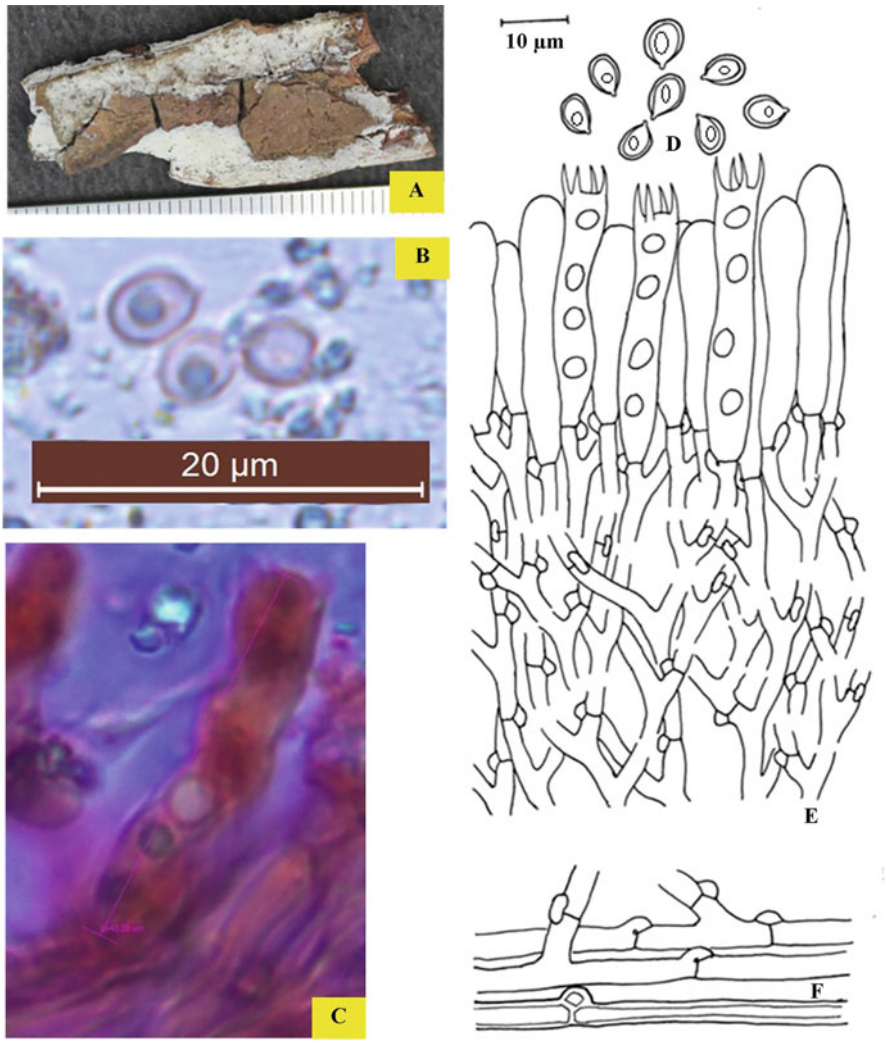

Fig. 10.16 *Hypochnicium lundelli*: **A** Sporophore showing hymenial surface; **B–C** Photomicrographs (**B** Basidiospores, **C** Basidium); **D–F** Line diagrams (**D** Basidiospores, **E** Reconstruction showing a portion of hymenium and subhymenium, **F** Generative hyphae) Bar = 10 µm

10.4.4.4 *Hypochnicium punctulatum* (Cooke) J. Erikss. (Cooke 1878; Eriksson 1958) (Fig. 10.17)

Sporophore annual, resupinate, adnate, effused, up to 320 μm thick in section; hymenial surface smooth to tuberculate when collected; pale yellow to yellowish grey both in fresh and dry states; margins pruinose, paler concolorous when

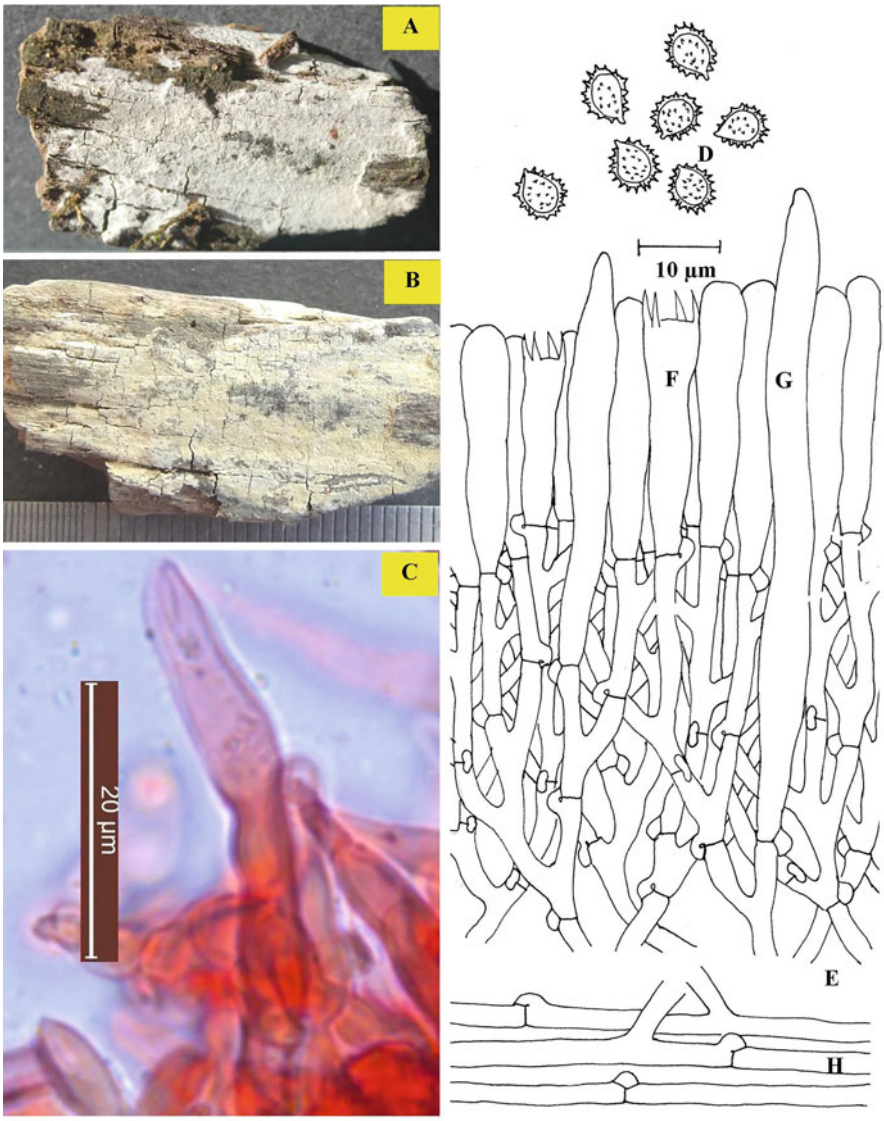

Fig. 10.17 *Hypochnicium punctulatum*: **A**–**B** Sporophore showing hymenial surface (**A** Fresh, **B** Dry); **C** Photomicrograph showing cystidium; **D**–**H** Line diagrams [**D** Basidiospores, **E** Reconstruction showing a portion of hymenium and subhymenium (**F** Basidium, **G** Cystidium), **H** Generative hyphae] Bar = 10 μm

determinate. Generative hyphae subhyaline, septate, clamped, thin-walled, smooth; horizontal, ≤3.5 μm wide, less branched in the subicular zone; vertical, ≤3 μm wide, richly branched in the subhymenial zone. Cystidia subcylindrical to subfusiform, somewhat sinuous, with basal clamp, 53–100 × 6.1–6.6 μm, thin-walled, smooth; projecting up to 15 μm out of the hymenium. Basidia clavate to subclavate, 22–31 × 4.4–6.1 μm; sterigma ≤5.5 μm long. Basidiospores 6.4–7.2 × 4–4.9 μm, broadly ellipsoid to subglobose, thick-walled, smooth, cyanophilous, inamyloid.

Collections Examined

India, Himachal Pradesh: Chamba, Kalatop, on the log of *Pinus roxburghii*, Poonam 10434 & 10435 (PUN), October 13, 2012; Churah, Bhandal, on angiospermous sticks, Poonam 10436 (PUN), October 15, 2014; Nayagran, Holi, on angiospermous sticks, Poonam 10437 (PUN), September 4, 2016.

Remarks

H. punctulatum is being described for the first time from tehsil Churah. Previously, it was reported from different localities of Chamba district by Rattan (1977), Prasher and Ashok (2013) and Dhingra et al. (2014).

10.5 Discussion

Corticioid fungi are an important component of the forest ecosystem. These fungi play an important role on the recycling of fixed carbon by virtue of their ability to secrete enzymes responsible for the breakdown to lignin, cellulose and other macro molecules. These fungi are more frequently distributed in the temperate climates. Chamba district of Himachal Pradesh that offers a wide range of variation in climate and vegetation has always attracted the mycologists. The previous explorations of the Chamba district recorded 22 species of the corticioid fungi belonging to the family *Meruliaceae*. The present study on the diversity of family *Meruliaceae* added to new records for Himachal Pradesh and one new report for the district. The consolidated account of the corticioid fungi forms a base for further studies on the ecology and distribution, host preference, frequency of occurrence and biochemical studies with particular reference to enzymes involved in white and brown rot.

Acknowledgements The authors are grateful to the Head, Department of Botany, Punjabi University, Patiala for providing necessary laboratory facilities; University Grants Commission, New Delhi and DST FIST for financial assistance under DRS-SAP DSA level-I and FIST level-I programme respectively and Prof. Nils Hallenberg, Gothenburg for expert comments.

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Diversity of Some Colourful Poroid and Non-poroid Agaricomycetous Fungi

11

Ramandeep Kaur, Maninder Kaur, Avneet Pal Singh, Navpreet Kaur Ghuman, and Gurpaul Singh Dhingra

Abstract

Twenty nine fungal species spread over 15 genera, (***Antrodia leucaena***, ***A. malicola, A. pulvinascens,*** *Coltricia cinnamomea*, ***C. focicola***, *C. perennis*, *Fomitiporia rosmarini,* ***Ganoderma australe***, ***G. brownii, G. carnosum***, ***Gloeophyllum abietinum***, ***G. carbonarium***, *Hymenochaete leonina*, *H. rheicolor*, *H. semistupposa*, ***Junghuhnia collabens***, ***J. nitida, Phanerochaete chrysosporium***, ***Phlebiopsis crassa***, *Phellinus gilvus,* ***P. nigricans****, P. senex*, ***Pycnoporus cinnabarinus***, ***Radulodon acaciae***, ***Rigidoporus ulmarius***, *Trametes ljubarskyi, T. versicolor*, *Trichaptum abietinum* and *T. biforme*) have been described from Sirmaur district of Himachal Pradesh, India. Seventeen species, highlighted in bold are new to the mycoflora of Sirmaur. Among the described species, four species (*Gloeophyllum carbonarium*, *Phanerochaete chrysosporium*, *Pycnoporus cinnabarinus and Radulodon acaciae*) are new records for Himachal Pradesh and three species (*Antrodia leucaena*, *A. pulvinascens and Coltricia focicola*) are new reports for India. This chapter provides information about the diversity, taxonomy and economic importance of the twenty nine species of poroid and non-poroid Agaricomyceteous fungi.

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_11

Keywords

Himachal Pradesh · Agaricomycetous · Sirmaur · *Basidiomycota* · Fungi

11.1 Introduction

The poroid and non-poroid agaricomyceteous fungi are a unique group of wood inhabiting organisms. In these fungi the carpophores vary from resupinate to reflexed to pileate (sessile/subsessile/stipitate), with hypochnoid, smooth, grandinioid, warted, tuberculate, merulioid, odontoid (in case of non-poroid members) hymenial side and poroid, irpicoid or lamellate hymenial side (in the case of poroid members). Further, the hymenium is unilateral, organized in the form of pores/lamellae in the case of poroid members and aculei, tubercles, warts, ridges/grooves or smooth surface in case of non-poroid members. The carpophores are variously coloured ranging from shades of yellow, grey, orange and brown and rarely the shades of violet, blue, and red. Earlier, these fungi were placed under the order *Aphyllophorales* of *Basidiomycota* as per the traditional morphotaxonomic approach. However, the phylogenetic studies based on molecular techniques have changed their systematic position and at present, these fungi have been placed mainly in the orders, namely *Gloeophyllales, Hymenochaetales* and *Polyporales* of the class *Agaricomycetes* of *Basidiomycota* (Wijayawardene et al. 2020; Index Fungorum 2021; Mycobank 2021).

Sirmaur, a southern district of Himachal Pradesh is bestowed with a diverse vegetation ranging from tropical to alpine type. Geographically it spans from 77°01′12″ to 77°49′40″ east longitude and 30°22′30″ to 31°01′20″ north latitude with an altitudinal range of 900 m to 3994 m above mean sea level. The district has an area of 2825 km^2, of which about 1387 km^2 (49%) is under forest cover. Of the total forrest cover, 131 km^2 is categorized as very dense forest, 568 km^2 as moderate dense forest, and the remaining 688 km^2 as open forest (HPFRD 2021). There are three major climatic seasons i.e., summer (April to June), rainy season (July to October) and winter (November to March). The area receives an annual average rainfall of 1405 mm. The average temperature ranges from −2 °C to 30 °C and the relative humidity remains around 80%. There are six administrative subdivisions, namely Nahan, Paonta Sahib, Pachhad, Rajgarh, Sangrah (Renuka Ji) and Shillai (District Sirmaur 2021).

Previously, several taxonomic studies related to poroid and resupinate non-poroid Agaricomycetous fungi from the Sirmaur district have been conducted over a period of time (Singh 2007; Dhingra and Singh 2009; Priyanka 2012; Sharma 2012; Prasher and Ashok 2013; Dhingra et al. 2014; Kaur 2020). The previous studies were confined to few localities of the district and have described only 92 taxa of the poroid and non-poroid agaricomycetous fungi. Keeping in view the diverse climatic conditions and rich vegetation of the area, the present studies were planned and executed. The present chapter, based on the fungal forays conducted in Sirmaur district, provides taxonomic account of twenty nine species of poroid and non-poroid agaricomycetous fungi that possess colourful carpophores (Table 11.1).

Table 11.1 Agaricomycetous fungi recorded in the district Sirmaur, Himachal Pradesh (India)

Sr. No.	Genus	Species	Remarks (NRS[a]/NRHP[b]/ NRI[c]/RRS[d])	References
1	*Antrodia*	*A. leucaena*	NRI	Dai and Niemelä (2002)
2.		*A. malicola*	NRS	Donk (1966)
3.		*A. pulvinascens*	NRI	Niemelä (1985)
4.	*Coltricia*	*C. cinnamomea*	RRS	Murrill (1908)
5.		*C. focicola*	NRI	Murrill (1908)
6.		*C. perennis*	RRS	Murrill (1903)
7.	*Fomitiporia*	*F. rosmarini*	RRS	Ghobad-Nejhad and Dai (2007)
8.	*Ganoderma*	*G. australe*	NRS	Patouillard (1889)
9.		*G. brownii*	NRS	Lowe and Gilbertson (1961)
10.		*G. carnosum*	NRS	Patouillard (1889)
11.	*Gloeophyllum*	*G. abietinum*	NRS	Karsten (1879)
12.		*G. carbonarium*	NRHP	Ryvarden (1984)
13.	*Hymenochaete*	*H. leonina*	RRS	Berkeley and Curtis (1869)
14.		*H. rheicolor*	RRS	Léveillé (1846)
15.		*H. semistupposa*	RRS	Petch (1925)
16.	*Junghuhnia*	*J. collabens*	NRS	Ryvarden (1972a)
17.		*J. nitida*	NRS	Ryvarden (1972a)
18.	*Phanerochaete*	*P. chrysosporium*	NRHP	Burdsall and Eslyn (1974)
19.	*Phlebiopsis*	*P. crassa*	NRS	Floudas and Hibbett (2015)
20.	*Phellinus*	*P. gilvus*	RRS	Patouillard (1900)
21.		*P. nigricans*	NRS	Karsten (1899)
22.		*P. senex*	RRS	Imazeki (1952)
23.	*Pycnoporus*	*P. cinnabarinus*	NRHP	Karsten (1881)
24	*Radulodon*	*R. acaciae*	NRHP	Imazeki (1952)
25.	*Rigidoporus*	*R. ulmarius*	NRS	Kaur et al. (2014)
26.	*Trametes*	*T. ljubarskyi*	RRS	Pilát (1936)
27.		*T. versicolor*	RRS	Lloyd (1921)
28.	*Trichaptum*	*T. abietinum*	RRS	Ryvarden (1972b)
29.		*T. biforme*	RRS	Ryvarden (1972b)

[a]New Record for Sirmaur
[b]New Record for Himachal Pradesh
[c]New Record for India
[d]Rereport for Sirmaur

11.2 Material and Methods

The specimens of poroid and non-poroid agaricomycetous fungi were collected during mycofloristic surveys conducted in the rainy season of the years 2015 to 2019 in different localities of the district Sirmaur in Himachal Pradesh (India). The carpophores were carefully removed from their substrate using a chisel and a hammer. Data pertaining to host/substrate, carpophore texture, colour, and type of hymenial/abhymenial surface and margins were noted carefully for each collected specimen. The specimens were dried in either the sun or using a portable electric drier. The dried specimens were packed following the standard protocols and were preserved with 1,4-dichlorobenzene. The macroscopic features of the carpophores were studied using a hand-lens. Whereas, the microscopic characters were based on crush mounts and freehand cut-sections in water and KOH solution (3%, 5% and 10%), cotton blue (1% in lactophenol)/Congo red (1% in distilled water)/Phloxine (1% in distilled water)/Melzer's reagent (containing iodine (0.5 g), KI (1.5 g), and chloral hydrate (20 g) in 20 ml of distilled water). The outline of the microscopic structures was drawn using Camera Lucida at different magnifications (100×, 400×, and 1000×) of the compound microscope. The macro- and microscopic characters were compiled in the form of descriptions, which were compared with monographic compilations (Ryvarden 2002; Bernicchia and Gorjón 2010; Sharma 2012; Ryvarden and Melo 2014) for identification. The colour citation of the hymenial/abhymenial surface is according to Kornerup and Wanscher (1978). All the samples studied and described have been deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

11.3 Taxonomic Descriptions

11.3.1 *Antrodia leucaena* Y.C. Dai and Niemelä (Dai and Niemelä 2002) (Fig. 11.1)

Carpophores annual, resupinate, adnate, effused, up to 5 mm thick in cross section; hymenial side poroid, reddish-white to pale-red to pastel-red when collected, no prominent change on drying; pores angular, 3–5 per mm; dissepiments up to 80 μm wide, lacerate; pore tubes up to 350 μm deep, pale-orange; subiculum up to 150 μm thick, pale-orange; margins somewhat fibrillose, paler than the colour of the hymenial side, occasionally indeterminate. Hyphal system dimitic. Generative hyphae septate, with clamps, up to 5 μm wide, branched, thin-walled. Skeletal hyphae aseptate, up to 7 μm wide, occasionally branched, thick-walled. Cystidia subcylindrical, sinuous, 30–42 × 4–5 μm, smooth, thin-walled, with basal clamp, usually crystalline encrustation present in the apical part. Basidia clavate, sometimes constricted, 15–17 × 6–7 μm, four sterigmate, with basal clamp; sterigmata up to 3 μm long.

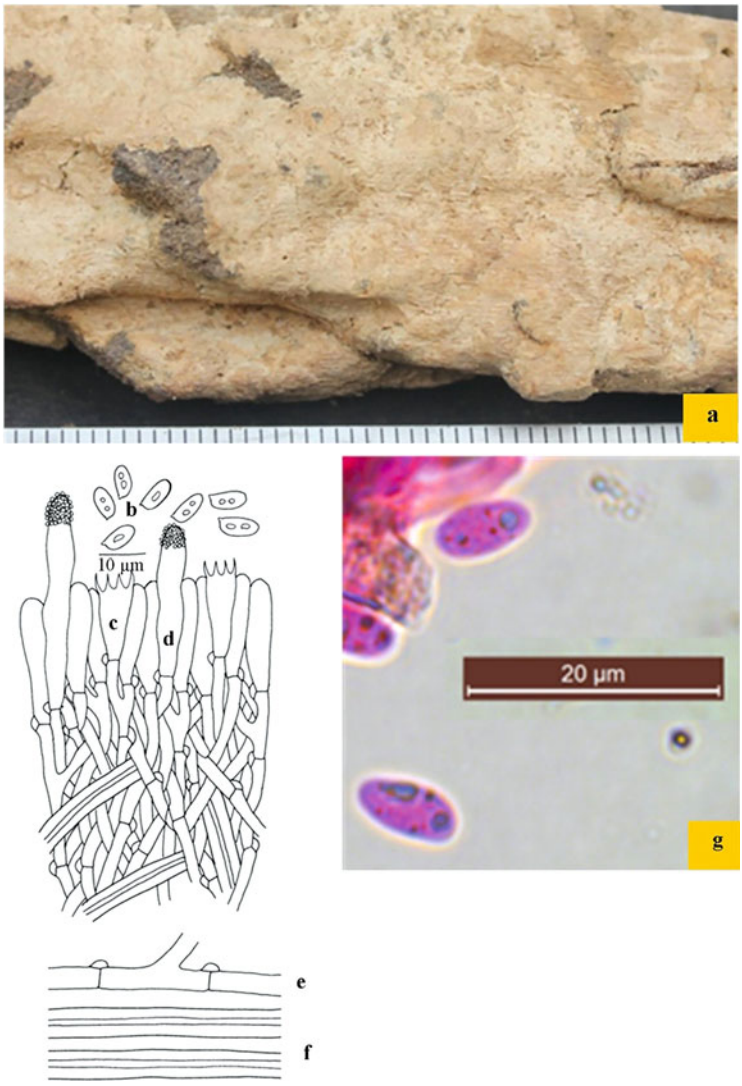


Fig. 11.1 *Antrodia leucaena*: **a** Carpophore showing hymenial side, **b**–**e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidia, **e** Generative hyphae, and **f** Skeletal hyphae, **g** Photomicrograph showing basidiospores

Basidiospores ellipsoid, 7–9 × 4–5 µm, smooth, thin-walled, usually with oily contents, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Ambwala, on the trunk of *Lagerstroemia speciosa*, Ramandeep and Dhingra 10795 (PUN), August 23, 2015.

Remarks

Kaur et al. (2020) have already published the first report of this species from India. Earlier, this fungus has been reported from China, Russia, and Finland (Ryvarden and Melo; 2014; Mycobank 2021).

11.3.2 *Antrodia malicola* (Berkeley and Curtis) Donk (Berkeley and Curtis 1856; Donk 1966) (Fig. 11.2)

Basidiocarps annual, resupinate, effused-reflexed to pileate; pilei up to 2.5 × 0.6 × 0.5 cm (length× width×thickness), laterally fused; abhymenial side tomentose, light orange to greyish-orange to brownish-orange when collected, no prominent change on drying; hymenial side poroid, pale-orange to greyish-orange when collected, no prominent change on drying; pores round to angular, 2–3 per mm; dissepiments up to 120 μm wide, entire; context homogeneous, brownish-orange, up to 3 mm thick; pore tubes up to 2 mm deep, concolorous with hymenial side; margins acute, irregularly wavy, paler concolorous on both sides, sterile up to 1 mm on hymenial side. Hyphal system dimitic. Generative hyphae septate, with clamps, up to 5.5 μm wide, branched, thin- to thick-walled. Skeletal hyphae aseptate, up to 6 μm wide, rarely branched, thick-walled. Cystidial elements absent. Basidia clavate, somewhat sinuous, 22–35 × 5.2–7.6 μm, four sterigmate, with basal clamp; sterigmata up to 4 μm long. Basidiospores ellipsoid, 6–8.5 × 3.3–4.4 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Ambwala, on an angiospermous log, Ramandeep and Dhingra 10895 (PUN), August 23, 2015.

Remarks

This species differs from the previous one in lacking cystidia and being described for the first time from the study area. Formerly in India, it has been reported from the district Bilaspur (Himachal Pradesh) and Punjab by Priyanka (2012) and Kaur (2017), respectively.

11.3.3 *Antrodia pulvinascens* (Pilát) Niemelä (Pilát 1953; Niemelä 1985) (Fig. 11.3)

Carpophores annual, resupinate, effused, adnate, up to 2.5 mm thick in cross section; hymenial side poroid, pale-orange to greyish-orange when fresh, no prominent change on drying; pores angular, 2–7 per mm; dissepiments 80 μm thick, entire; context up to 0.5 mm thick, reddish- white; pore tubes up to 2 mm long, greyish-orange; margins somewhat fibrillose, concolorous with the hymenial side, occasionally indeterminate. Hyphal system dimitic. Generative hyphae septate, with clamps, up to 4.6 μm wide, branched, thin-walled. Skeletal hyphae aseptate, up to 5.9 μm wide, occasionally branched, thick-walled. Cystidia absent. Basidia clavate, sinuous, 17–25 × 5.2–7.2 μm, four sterigmate, with basal clamp; sterigmata up to 3.3 μm

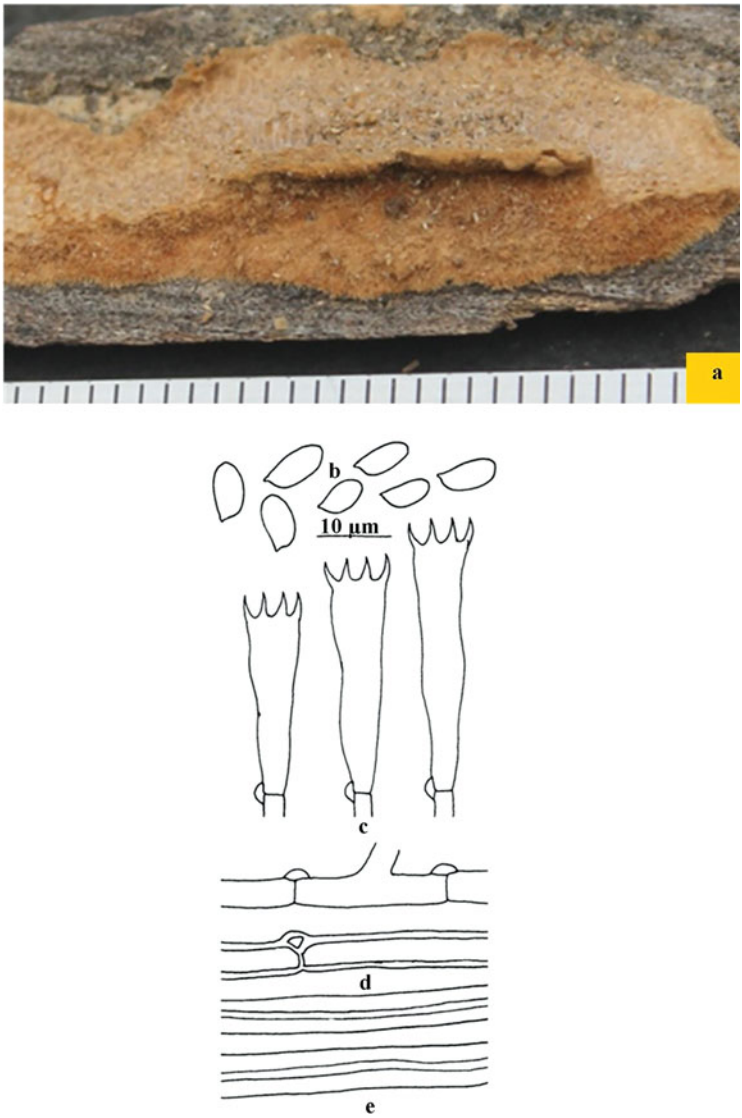


Fig. 11.2 *Antrodia malicola*: **a** Carpophore showing abhymenial side and hymenial side, **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Generative hyphae and **e** Skeletal hyphae

long. Basidiospores ellipsoid to subfusiform, 7.2–9.2 × 3.9–4.6 µm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Sainwala, on the log of angiospermous tree, Ramandeep and Dhingra 10796 (PUN), August 23, 2015.

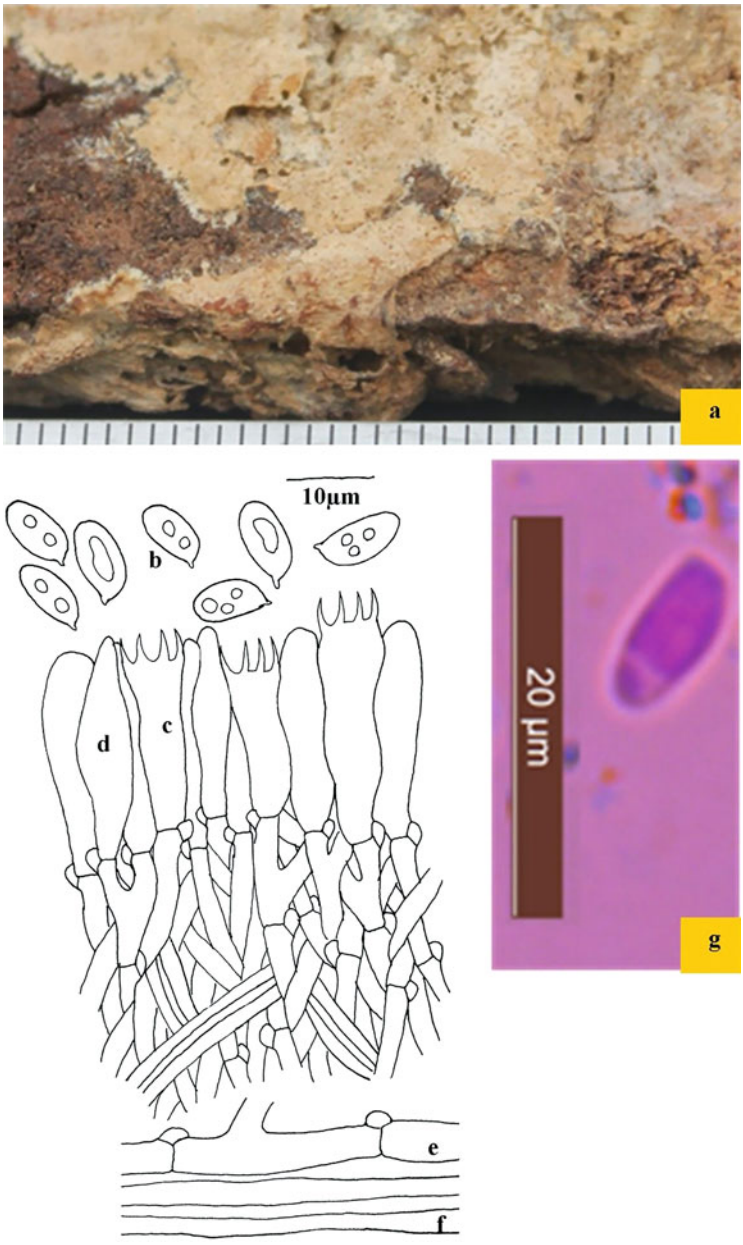


Fig. 11.3 *Antrodia pulvinascens*: **a** Carpophore showing hymenial side **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidioles, **e** Generative hyphae and **f** Skeletal hypha, **g** Photomicrograph showing basidiospore

Remarks

The first report of this species has been published from India by Kaur et al. (2020). Other former reports in the world are from Russia, Switzerland, Poland, Austria, Czechoslovakia, Germany, Yugoslavia, Italy, and Spain (Mycobank 2021).

11.3.4 *Coltricia cinnamomea* (Jacquin) Murrill (Jacquin 1786; Murrill 1904) (Fig.11.4)

Carpophores annual, pileate; pilei up to 5 cm in diameter, up to 0.5 cm thick in cross section; stipitate, solitary or in clusters, infundibuliform, soft, corky when collected, brittle on drying; abhymenial side tomentose to velutinate, concentrically zonate, shiny, light brown to reddish-brown when collected, no prominent change on drying; hymenial side poroid, brown to dark brown when collected, no prominent change on drying; pores round to angular, 3–4 per mm; dissepiments up to 100 μm wide, entire; context homogeneous, brown to dark brown, up to 2 mm thick; pore tubes up to 3 mm deep, yellowish-brown to light brown; margins acute, wavy to lobed, brownish-orange to brown on abhymenial side; paler concolorous on hymenial side, sterile up to 2 mm on hymenial side. Stipe usually centric, cylindrical, up to 3 cm in length, up to 0.5 cm thick in cross section, velutinate, solid, brownish-orange to reddish-brown when collected, reddish-brown to dark brown on drying. Hyphal system monomitic. Generative hyphae subhyaline to yellowish-brown, septate, without clamps, up to 6.8 μm wide, branched, thin- to thick-walled. Cystidial elements absent. Basidia clavate to subclavate, 14–24 × 8–8.5 μm, four sterigmate, without basal clamp; sterigmata up to 4.7 μm long, with oily contents. Basidiospores ellipsoid to broadly ellipsoid, yellowish-brown, 5.5–9 × 4.6–5.2 μm, smooth, thick-walled, with oily contents, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, associated with needles of *Quercus leucotrichophora*, Ramandeep and Avneet 9989 (PUN), September 11, 2016.

Remarks

It is the second report of this species from the study area after the only previous report by Kaur (2018a). Other former reports in India are by Berkeley (1851, 1854) from Sikkim; Bose (1946) from Meghalaya; Banerjee (1947) from West Bengal; Bakshi (1971) from Uttarakhand; Dhanda (1977) from Himachal Pradesh (Chamba, Kullu, and Shimla), Jammu and Kashmir and Uttarakhand; Singh (1987) from Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Tripura and West Bengal; Sharma and Ghosh (1989) from West Bengal and Sharma (1995, 2012) from Uttarakhand and Meghalaya.

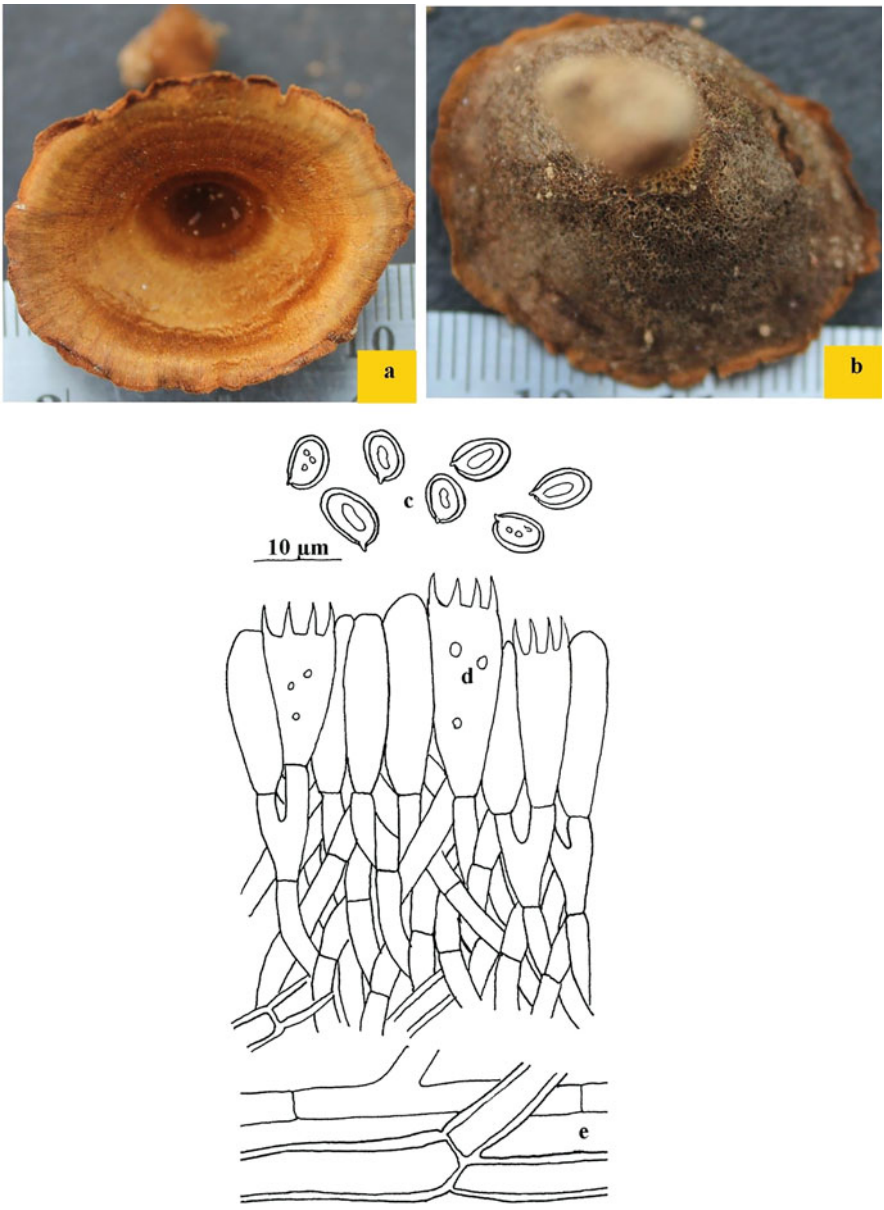
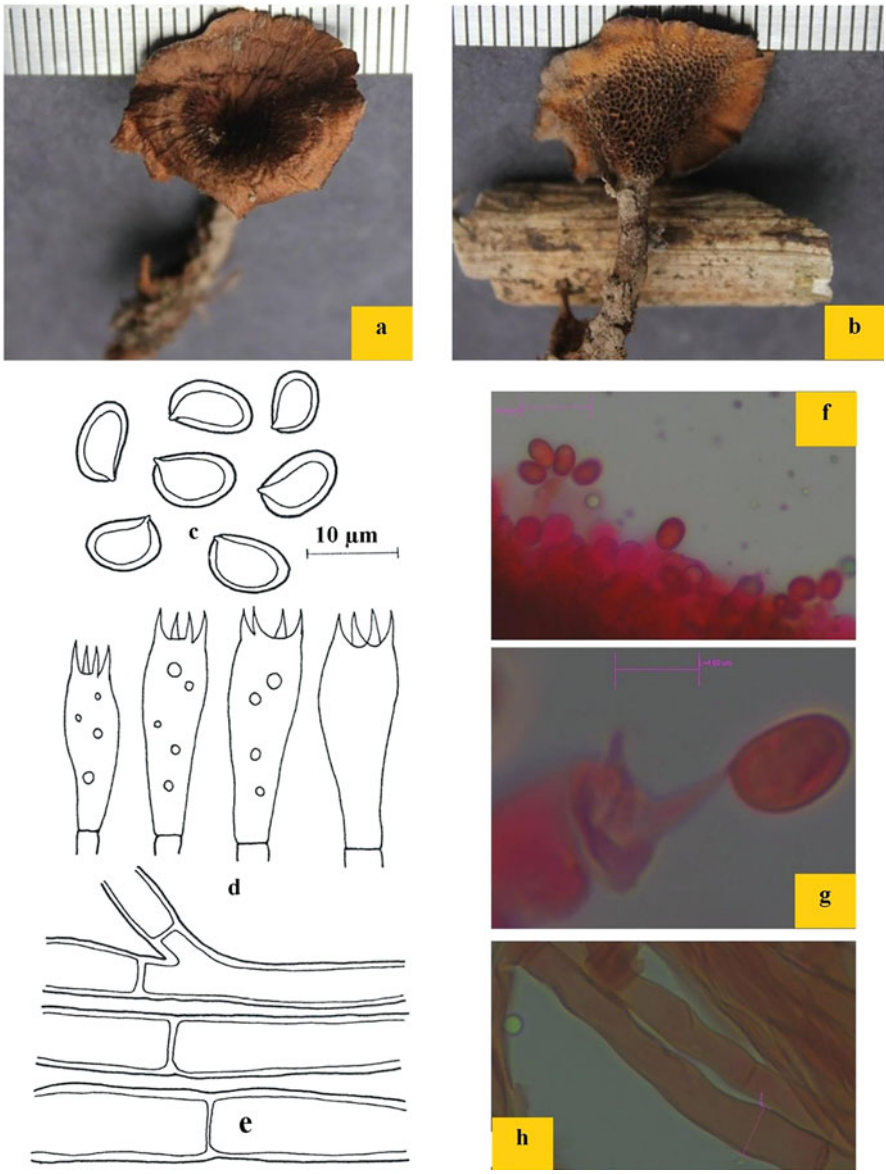


Fig. 11.4 *Coltricia cinnamomea*: **a–b** Carpophore showing **a** Abhymenial side, **b** Hymenial side, **c–e** Line diagrams showing **c** Basidiospores, **d** Basidia and **e** Generative hyphae



Figs. 11.5 *Coltricia focicola*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–e** Line diagrams showing **c** Basidiospores, **d** Basidia and **e** Generative hyphae, **f–h** Photomicrographs showing **f** Basidiospores, **g** Basidia and **h** Generative hyphae

11.3.5 *Coltricia focicola* (Berk. and M.A. Curtis) Murrill (Berkeley and Curtis 1869; Murrill 1908) (Fig. 11.5)

Carpophores annual, stipitate, solitary or in groups, fused laterally; pilei 5 cm in diameter, 3 mm thick in the centre, circular, infundibuliform, coriaceous when fresh, brittle on drying; abhymenial surface velutinate to silky fibrillose, shiny to glossy, with concentric zones, brown to reddish-brown when fresh, not changing much on drying; hymenial surface poroid, brown to dark brown when fresh, not changing much on drying; pores circular to angular, 3-4/mm; dissepiments thin, entire to lacerate; context 1 mm thick, brown to dark brown; pore tubes 2 mm long, concolorous with hymenial surface; margins thin, entire to incised, fertile, concolorous with abhymenial surface; stipe centric to eccentric, cylindrical to flattened, expanded towards the base, finely velutinate, reddish-brown, solid, 3 × 0.5 cm. Hyphal system monomitic. Generative hyphae septate, thin- to thick-walled, subhyaline to yellowish-brown to reddish-brown. Basidia 13.5–22.3 × 6.8–9.3. Basidiospores 4–7 × 3–5.3 broadly ellipsoid, smooth, thin- to slightly thick-walled, yellowish-brown, with oily contents, inamyloid, acyanophilous.

Sample Studied

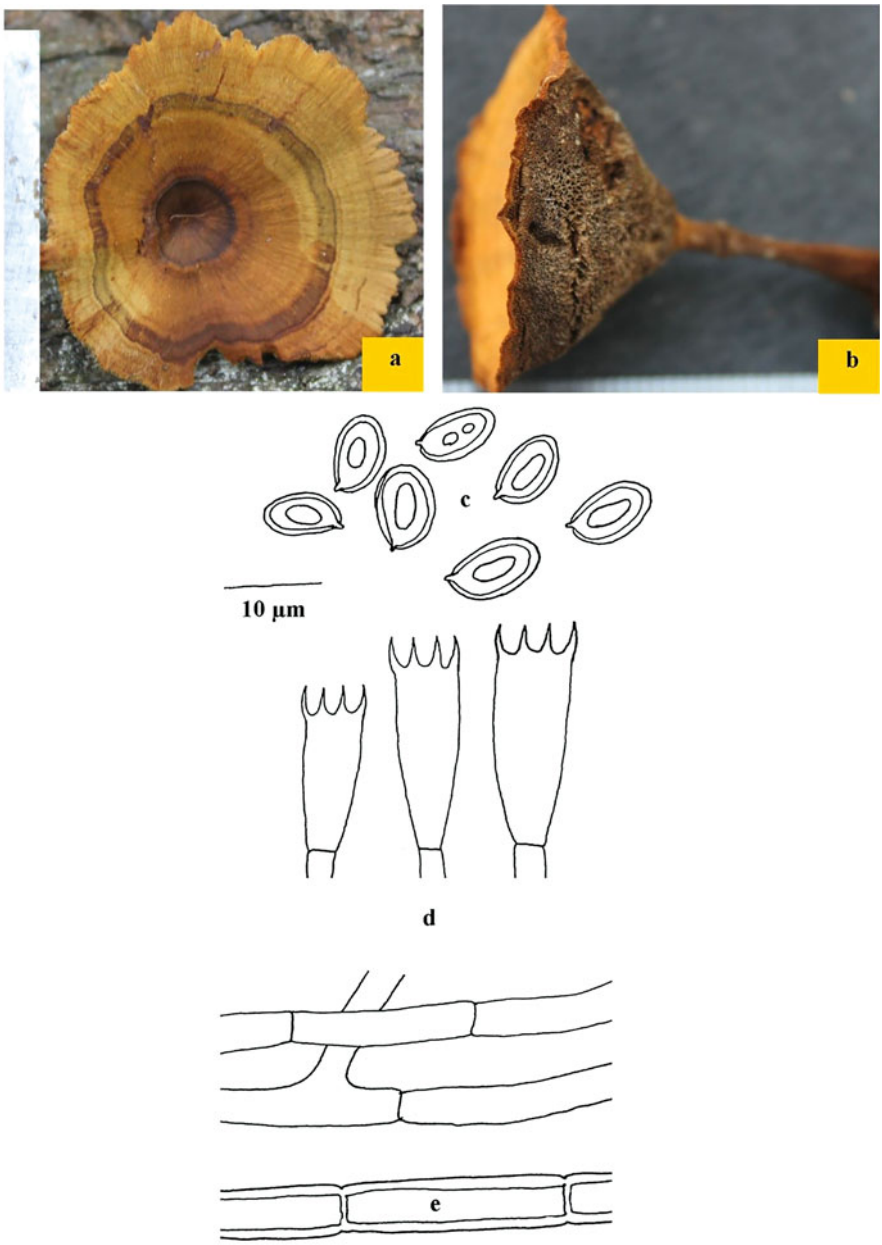
Himachal Pradesh: Nahan, on soil in a mixed forest, Navpreet and Ramandeep 7933 (PUN), August 23, 2015.

Remarks

It differs from other species in the genus by radially wrinkled abhymenial surface in dried carpophores and slightly bigger pores. Ghumman et al. (2016) have published the first report of this species from India. Other reports are from Europe, China, Mongolia, and Eastern North America (Mycobank 2021).

11.3.6 *Coltricia perennis* (Linnaeus) Murrill (Linnaeus 1753; Murrill 1903) (Fig. 11.6)

Carpophores annual, pileate; pilei up to 4 cm in diameter, up to 0.4 cm thick in cross section; stipitate, solitary or in clusters, infundibuliform, soft, corky when collected, brittle on drying; abhymenial side velutinate, concentrically zonate, dull, orange-white to greyish-orange to light brown when collected, no prominent change on drying; hymenial side poroid, brown when collected, no prominent change on drying; pores round to angular, 3–4 per mm; dissepiments up to 95 μm wide, entire; context homogeneous, brownish-orange, up to 2 mm thick; pore tubes up to 2 mm deep, brown; margins acute, irregularly lobed, dentate; concolorous on abhymenial side; paler concolorous on hymenial side, sterile up to 2 mm on hymenial side. Stipe usually centric, cylindrical, up to 2 cm in length, 0.5 cm thick in cross section, velutinate, solid, brownish-orange to reddish-brown when collected, reddish-brown to dark brown on drying. Hyphal system monomitic. Generative hyphae subhyaline to yellowish-brown to brown to dark brown, septate, without clamps, up to 6.2 μm wide, branched, thin- to thick-walled. Cystidial elements absent. Basidia clavate to



Figs. 11.6 *Coltricia perennis*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side. **c–e** Line diagrams showing **c** Basidiospores, **d** Basidia and **e** Generative hyphae

subclavate, 14–22 × 6–8 µm, four sterigmate, without basal clamp; sterigmata up to 3 µm long, with oily contents. Basidiospores ellipsoid to broadly ellipsoid, yellowish-brown, 8.6–10 × 5–6.5 µm, smooth, thick-walled, with oily contents, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Shimogu, associated with the roots of *Pinus roxburghii*, Ramandeep and Avneet 10818 (PUN), September 13, 2016.

Remarks

It is the second report of this species from the study area after the only previous report by Kaur (2018b). Besides Himachal Pradesh, it has also been reported from Sikkim (Berkeley 1851), Shillong (Bose 1927), Uttarakhand (Mitter and Tondon 1932; Thind et al. 1957; Dhanda 1977), West Bengal (Banerjee 1947), Meghalaya and Uttarakhand (Bakshi 1971), Himachal Pradesh (Chamba, Kullu, and Shimla), Arunachal Pradesh, Meghalaya, Manipur, Nagaland, and West Bengal (Singh 1987) and Sikkim, Meghalaya and Uttarakhand (Sharma 1995, 1997, 2012). Here it is being reported for the first time from district Solan in Himachal Pradesh.

11.3.7 *Fomitiporia rosmarini* (Bernicchia) Ghobad-Nejhad and Dai (Bernicchia 1990; Ghobad-Nejhad and Dai 2007) (Fig. 11.7)

Carpophores annual, pileate, sessile; pilei up to 8.5 × 3.5 × 0.8 cm, imbricate; applanate, concave, hard, woody; abhymenial side tomentose, concentrically zonate, sulcate, brownish-orange to reddish-brown when collected, no prominent change on drying; hymenial side poroid, light brown to brown when collected, no prominent change on drying; pores round to angular, 6–7 per mm; dissepiments up to 45 µm wide, entire; context homogeneous, light brown, up to 2 mm thick; pore tubes yellowish-brown to light brown, up to 6 mm deep, indistinctly stratified (3 layered), up to 2 mm long in each layer, separated by very thin layer of context; margins obtuse, irregular, wavy; brownish-orange on abhymenial side; paler concolorous on hymenial side, sterile up to 2 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline to yellowish-brown, septate, without clamps, up to 5 µm wide, branched, thin- to thick-walled. Skeletal hyphae brown, up to 5.5 µm wide, unbranched, thick-walled. Setal hyphae absent. Setae subulate, dark brown 30–49 × 8–12.5 µm, thick-walled, arising from hymenium and subhymenium; projecting up to 13 out of the hymenium. Cystidial elements absent. Basidia clavate, 16–25 × 6–7.5 µm, four sterigmate, without basal clamp; sterigmata up to 5 µm long. Basidiospores broadly ellipsoid to subglobose, subhyaline, 6–9 × 5.5–6 µm, smooth, thick-walled, dextrinoid, cyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Deedag, on trunk of *Phoenix dactylifera*, Ramandeep and Avneet 10822 (PUN), September 12, 2016.

Remarks

It is the second report of this species from the study area. The previous two reports are from the Sirmaur and Solan districts of Himachal Pradesh by Kaur (2018a, b).

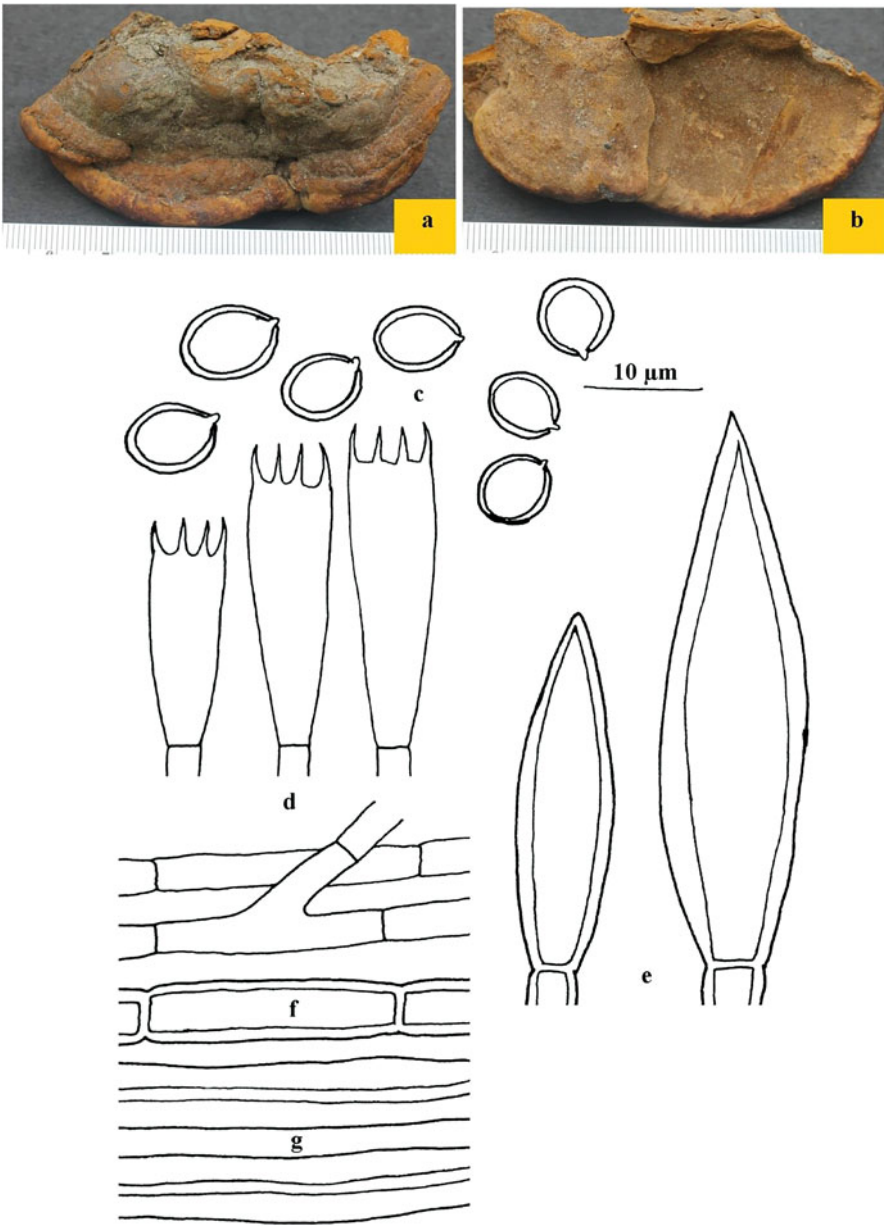


Fig. 11.7 *Fomitiporia rosmarini*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–g** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Setae, **f** Generative hyphae and **g** Skeletal hyphae

11.3.8 *Ganoderma australe* (Fries) Patouillard (Fries 1828; Patouillard 1889) (Fig. 11.8)

Carpophores perennial, pileate; pilei up to 10 × 4 × 4.5 cm, sessile, solitary, dimidiate, woody; abhymenial side non-laccate, sulcate, zonate, greyish-orange to greyish-brown to brown, when collected, no prominent change on drying; hymenial side poroid, yellowish-brown to greyish-brown to reddish-brown when collected, no

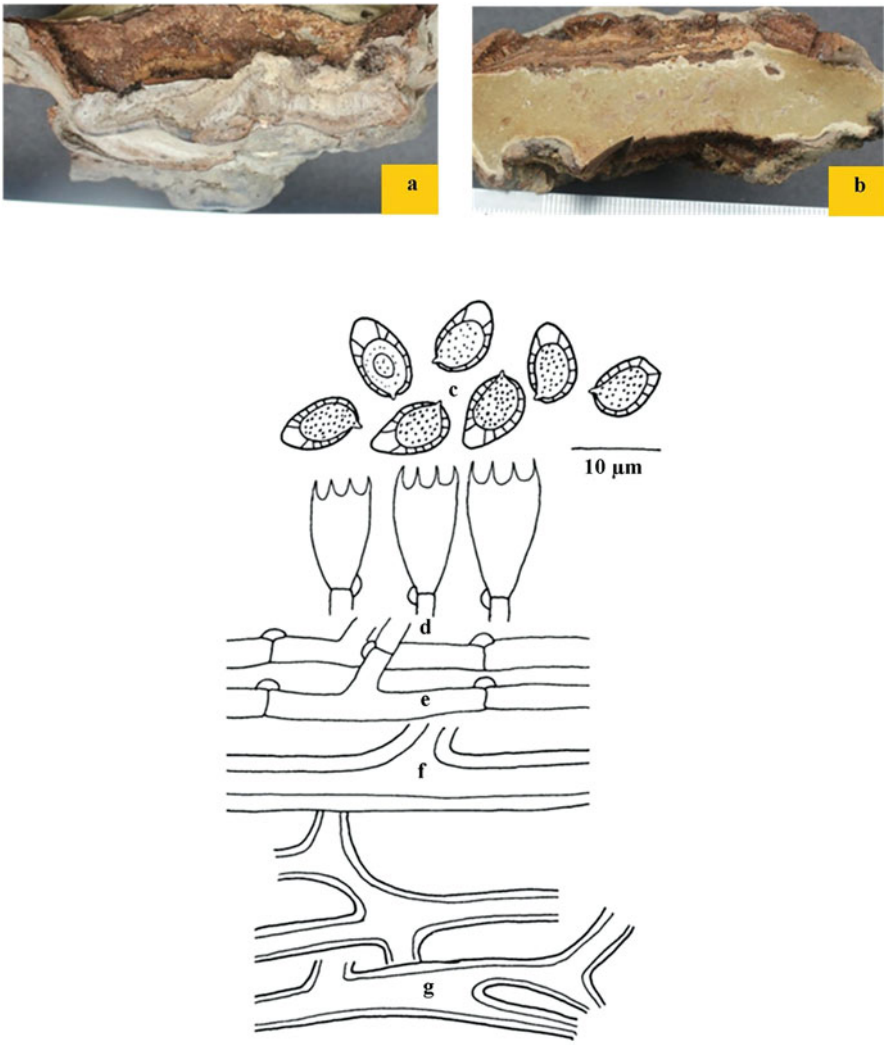


Fig. 11.8 *Ganoderma australe*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–g** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Generative hyphae, **f** Skeleto-binding hyphae and **g** Binding hyphae

prominent change on drying; pores round to angular, 4–5 per mm; dissepiments up to 220 μm thick, entire; basal context homogeneous, soft, brown, up to 0.5 mm thick; pore tubes up to 3 cm long, stratified, each tube layer up to 1 cm long, separated by up to 0.5 mm thick, brown context layer; margins obtuse, irregular, concolorous on abhymenial side, reddish-grey on hymenial side, sterile up to 5 mm on hymenial side. Pilear crust hard, dark reddish-brown anamixoderm, no cracks on pressing with nail. Hyphal system trimitic. Generative hyphae subhyaline, up to 4 μm wide. Skeleto-binding hyphae brown, up to 7 μm wide. Binding hyphae light brown, up to 6.3 μm wide. Basidia clavate, 11–13 × 6–8 μm, four sterigmate, with basal clamp; sterigmata up to 3 μm long. Basidiospores ellipsoid to broadly ellipsoid, truncate, 8–10 × 5.5–7 μm, exine thin, yellowish, smooth; intine thick, brown; the two walls connected by inter-wall pillars; inter-wall pillars up to 1.7 μm long, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Paonta Sahib, Shamsherpur, on the trunk of *Melia azedarach*, Ramandeep 10906 (PUN), September 4, 2017.

Remarks

It is being described for the first time from the study area. Formerly in India, it has been reported from Bilaspur, Kangra, and Shimla districts of Himachal Pradesh, Chandigarh, Jammu and Kashmir, Kerala, Meghalaya, Maharashtra, South India, Uttarakhand and West Bengal as *G. tornatum* (Dhanda 1977), *G. australe* (Bakshi 1971; Leelavathy and Ganesh 2000; Sharma 2000; Kaur 2013, 2017, 2020; Ranadive 2013; Sharma et al. 2013), *G. adspermum* (Sharma and Ghosh 1989) and *G. annulare* (Singh 2016).

11.3.9 *Ganoderma brownii* (Murrill) Lowe and Gilbertson (Murrill 1915; Lowe and Gilbertson 1961) (Fig. 11.9)

Carpophores perennial, pileate; pilei up to 11 × 11 × 2 cm, sessile, solitary, dimidiate to applanate, hard, woody; abhymenial side non-laccate, sulcate, zonate, greyish-brown to brown when collected, no prominent change on drying; hymenial side poroid, greyish-brown to brownish-grey to light brown when collected, no prominent change on drying; pores round to angular, 4–5 per mm; dissepiments up to 90 μm thick, entire; context homogeneous, soft, brown, up to 5 mm thick; pore tubes up to 15 mm long, brown; margins obtuse, irregular, concolorous on both sides, sterile up to 1 mm on hymenial side. Pilear crust greyish-brown anamixodermis, cracks on pressing with nail. Hyphal system trimitic. Generative hyphae subhyaline, up to 4.3 μm wide. Skeleto-binding hyphae, brown, up to 5.5 μm wide. Binding hyphae, yellowish-brown, up to 5.2 μm wide. Basidia not observed. Basidiospores ellipsoid, truncate, 8–10 × 5–6.2 μm; exine thin, yellowish, smooth; intine thick, brown; the two walls connected by inter-wall pillars; inter-wall pillars up to 0.7 μm long, inamyloid, acyanophilous.

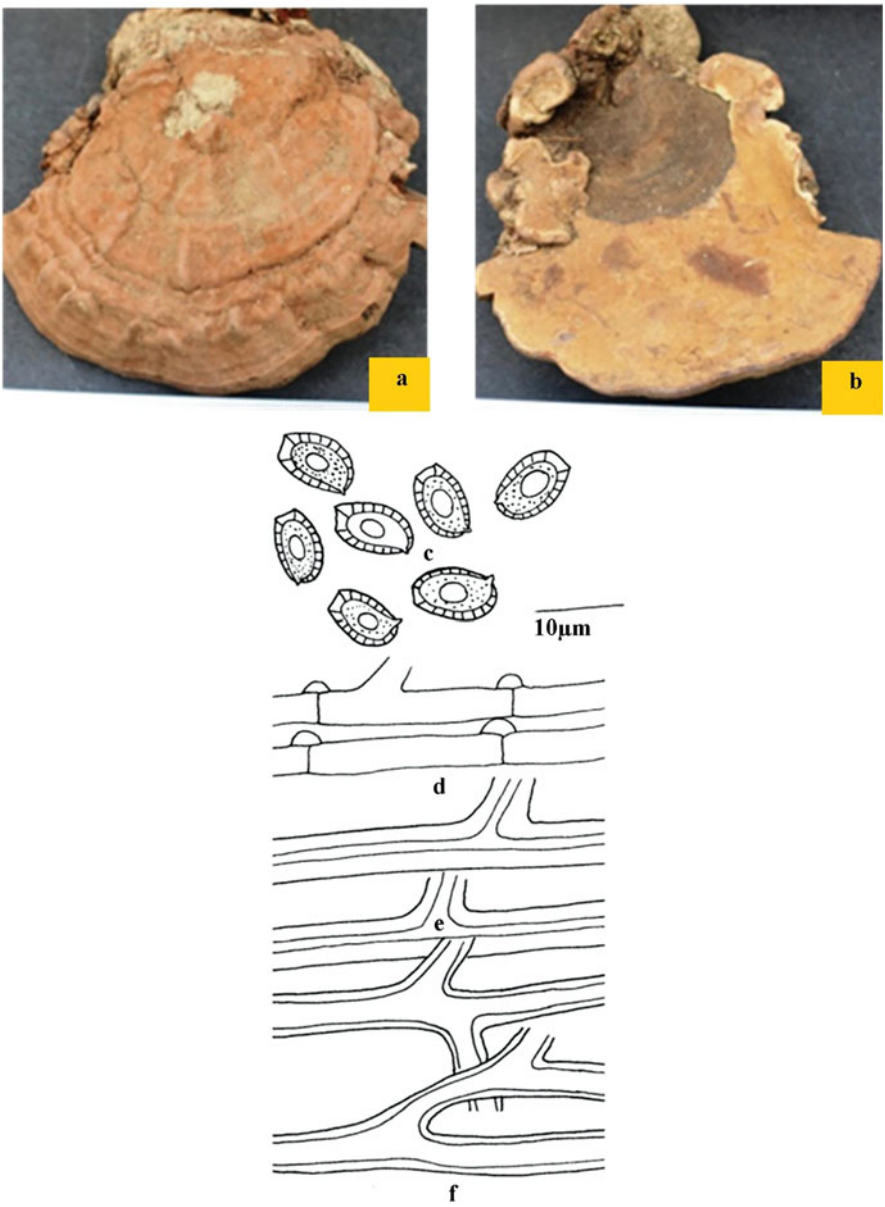


Fig. 11.9 *Ganoderma brownii*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–f** Line diagrams showing **c** Basidiospores, **d** Generative hyphae, **e** Skeleto-binding hyphae and **f** Binding hyphae

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, about 2 km before Nahan from Sainwala, associated with the roots of *Eucalyptus tereticornis*, Ramandeep 10907 (PUN), October 5, 2015.

Remarks

It is being described for the first time from the study area. However, it has been earlier reported from Kangra district of Himachal Pradesh and Uttarakhand by Kaur (2013) and Singh (2016) respectively.

11.3.10 *Ganoderma carnosum* Patouillard (Patouillard 1889) (Fig. 11.10)

Carpophores annual, pileate; pilei up to 9 × 7 × 1.7 cm, stipitate, suborbicular to reniform, corky; abhymenial side laccate, sulcate, radially rugose, brownish-red when fresh, violet-brown on drying; hymenial side poroid, greyish-yellow when fresh, greyish-orange on drying; pores round to angular, 4–5 per mm; dissepiments up to 130 μm thick, entire; context homogeneous, soft, whitish to cream, up to 7 mm thick; pore tubes up to 10 mm deep, light brown to cream; margins obtuse, revolute, irregular, paler concolorous on the abhymenial side, incurved abhymenial portion on the hymenial side up to 5 mm, brownish-red, masking the margins on the hymenial side. Stipe up to 13 × 1.5 cm, lateral, subcylindrical to flattened, solid, laccate, upper exposed portion smooth, brownish-red, lower embedded portion roughly nodulose, somewhat discoloured. Pilear crust reddish-brown hymenioderm, up to 0.7 mm thick, cracks on pressing with a nail. Cuticular elements cylindrical with slightly inflated apex, 35–41 × 7–10 μm, smooth, thick-walled, yellowish-brown, negative to Melzer's reagent. Hyphal system trimitic. Generative hyphae subhyaline, up to 4.2 μm wide. Skeleto-binding hyphae brown, up to 6.6 μm. Binding hyphae subhyaline to yellowish, up to 4.2 μm wide. Basidia clavate, 9–11 × 7–8.3 μm, four sterigmate, with basal clamp; sterigmata up to 3.4 μm long. Basidiospores broadly ellipsoid, truncate, 8.5–11.2 × 6–8 μm; exine thin, subhyaline, smooth; intine thick, brown; connected by inter-wall pillars; inter-wall pillars up to 0.7 μm long, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Batyuri, associated with the roots of *Quercus leucotrichophora,* Ramandeep and Avneet 10908 (PUN), September 12, 2016.

Remarks

It is being described for the first time from the study area. Formerly in India, it has been reported from the Kangra and Shimla districts of Himachal Pradesh by Kaur (2013) and Uttarakhand by Singh (2016).

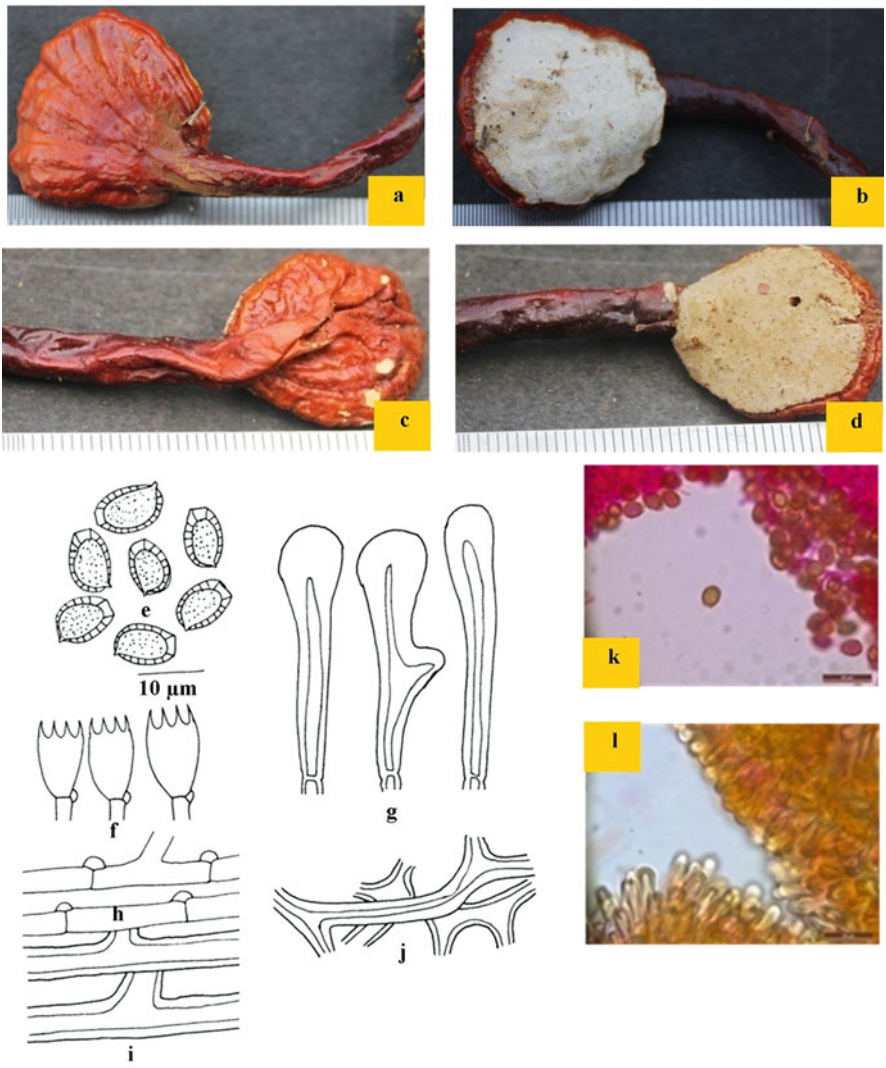
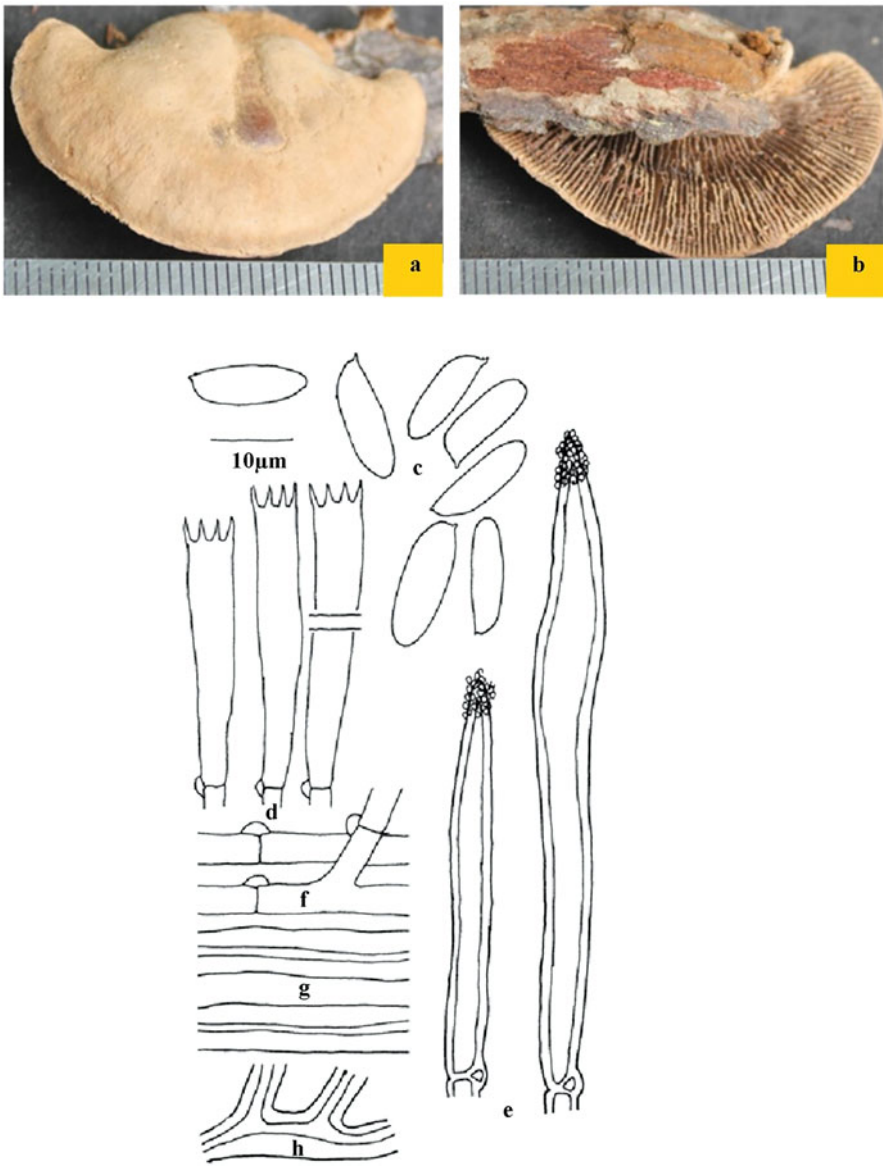


Fig. 11.10 *Ganoderma carnosum*: **a–d** Carpophore showing **a** Abhymenial side-fresh and **b** Hymenial side-fresh, **c** Abhymenial side -dry, and **d** Hymenial side-dry, **e–j** Line diagrams **e** Basidiospores, **f** Basidia, **g** Cuticular elements, **h** Generative hyphae, **i** Skeleto-binding hyphae and **j** Binding hyphae, **k–l** Photomicrographs showing **k** Basidiospores and **l** Cuticular elements

11.3.11 *Gloeophyllum abietinum* (Bulliard) Karsten (Bulliard 1790; Karsten 1879) (Fig. 11.11)

Carpophores annual, effused, reflexed to pileate; pilei up to 6 × 1.3 × 0.5 cm, solitary to imbricate, applanate, fuse laterally, tough to coriaceous; abhymenial side



Figs. 11.11 *Gloeophyllum abietinum*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–d** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Cystidia, **f** Generative hyphae, **g** Skeletal hyphae and **h** Binding hyphae

smooth to somewhat scrupose, azonate, greyish-orange to brownish-orange to light brown to brown when collected, no prominent change on drying; hymenial side poroid to lamellate, greyish-orange to brownish-orange to light brown when

collected, light brown on drying; pores daedaloid, 1–2 per mm, lamellae 8–10 per cm (tangentially), straight to somewhat wavy; dissepiments up to 60 μm wide, entire; context homogeneous, orange to light brown, up to 2 mm thick; pore tubes or lamellae up to 3 mm deep, concolorous with hymenial side; margins acute to obtuse, irregular to wavy, paler concolorous to concolorous on both sides, fertile. Hyphal system trimitic. Generative hyphae subhyaline, septate, with clamps, up to 3.5 μm wide, branched, thin-walled. Skeletal hyphae yellowish-brown to brown, aseptate, up to 6.6 μm wide, rarely branched, thick-walled. Binding hyphae yellowish-brown, aseptate, up to 6 μm wide, highly branched, thick-walled; Context constituted by parallel to substrate, loosely interwoven generative, skeletal and binding hyphae; tramal region constituted by compact generative, skeletal as well as binding hyphae arranged at right angles to substrate, and subhymenial region usually by generative hyphae at right angles to the trama. Cystidia yellowish-brown to brown, fusoid, 46–78 × 5.2–8 μm, thick-walled, scattered in hymenium, apically encrusted; not projecting beyond the hymenium. Basidia narrowly clavate to subcylindrical, 29–33 × 3.3–6 μm, four sterigmate, with basal clamp; sterigmata up to 4 μm long. Basidiospores ellipsoid to subcylindrical to suballantoid, 11.5–15 × 4–5.2 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, on log of *Mallotus philippensis*, Ramandeep 10809 (PUN), August 23, 2015.

Remarks

This species with trimitic hyphal system is being described for the first time from the study area. Earlier in India, it has been reported from the Chamba, Shimla, and Solan districts of Himachal Pradesh and Uttarakhand (Bose 1927; Bagchee and Singh 1960; Bakshi 1971; Dhanda 1977; Thind and Dhanda 1980; Singh 1987; Roy and De 1996; Sharma 2000; Kaur 2013; Ritu 2019).

11.3.12 *Gloeophyllum carbonarium* (Berkeley and Curtis) Ryvarden (Ryvarden 1984) (Fig. 11.12)

Carpophores annual, effused, usually resupinate to rarely reflexed; up to 3 mm thick in cross section, hymenial side poroid brownish-orange to light brown when collected, light brown to dark brown on drying; pores angular to hexagonal, 1–2 per mm; dissepiments up to 80 μm wide; context homogeneous, dark brown, up to 2 mm thick; pore tubes dark brown, up to 3 mm deep; margins acute to obtuse, irregular to wavy, concolorous to indeterminate, fertile. Hyphal system dimitic. Generative hyphae subhyaline, septate, with clamps, up to 4 μm wide, branched, thin-walled. Skeletal hyphae yellowish-brown to brown, up to 5 μm wide, rarely branched, thick-walled. Context constituted by parallel to substrate, loosely interwoven generative and skeletal hyphae; tramal region constituted by compact generative as well as skeletal hyphae arranged at right angles to substrate, and subhymenial region usually by generative hyphae at right angles to the trama. Cystidial elements absent. Basidia narrowly clavate to subcylindrical, 32–45 × 6–7.5 μm, four sterigmate, with basal

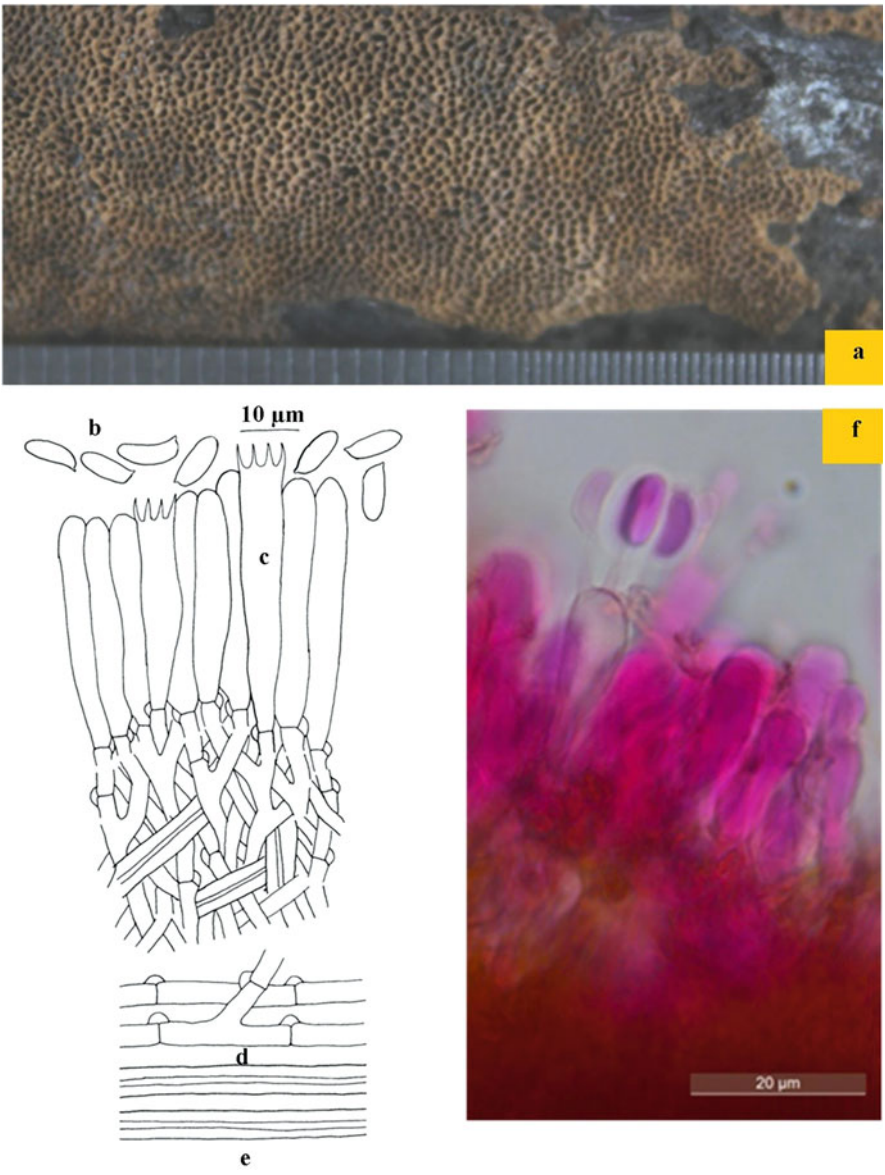


Fig. 11.12 *Gloeophyllum carbonarium*: **a** Carpophore showing hymenial side, **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Generative hyphae and **e** Skeletal hyphae, **f** Photomicrograph showing basidia with basidiospores

clamp; sterigmata up to 4.5 μm long. Basidiospores ellipsoid to subcylindrical to suballantoid, 8–11 × 3–4.5 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Mantali, on stump of *Pinus roxburghii*, Ramandeep and Avneet 10798 (PUN), September 11, 2016.

Remarks

Kaur et al. (2020) have already published the first report of this species from Himachal Pradesh. It has also been reported from Uttarakhand by Sharma (2012).

11.3.13 *Hymenochaete leonina* Berkeley and Curtis (Berkeley and Curtis 1869) (Fig. 11.13)

Carpophores annual, resupinate, adnate, up to 0.5 mm thick in cross section, hard, corky when collected, no prominent change on drying; hymenial side smooth to cracked, greyish- brown to light brown to brown when collected, no prominent change on drying; margins fibrillose, reddish-brown, or occasionally indeterminate. Hyphal system monomitic. Generative hyphae subhyaline to yellowish-brown to brown, septate, without clamps, branched, thin- to thick-walled; brown, up to 6.3 μm wide in subiculum; subhyaline to yellowish-brown, up to 4.2 μm wide in subhymenium. Setae subulate, abundant, dark brown, up to 78 × 7 μm, thick-walled, arising from subiculum and subhymenium, extending through hymenium; projecting up to 30 μm out of the hymenium. Basidia clavate, 17–25 × 4.2–5 μm, four sterigmate, without basal clamp; sterigmata up to 3.5 μm long. Basidiospores ellipsoid to broadly ellipsoid, subhyaline, 5–8.5 × 2.8–3.5 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Paonta Sahib, Sirmaur Tal, on the log of *Syzygium cumini*, Ramandeep 10829 (PUN), September 4, 2017.

Remarks

It is the second report of this species from the study area after the only previous report by Sharma (2012). It has earlier been reported in India from Chamba, Kangra, Kullu, and Shimla districts of Himachal Pradesh and Uttarakhand (Rattan 1977; Sharma 1995, 2012; Lalji 2003; Kaur 2012; Dhingra et al. 2014; Samita 2014; Kaur 2018a, b; Ritu 2019; Poonam 2020).

11.3.14 *Hymenochaete rheicolor* (Montagne) Léveillé (Montagne 1842; Léveillé 1846) (Fig. 11.14)

Carpophores annual, resupinate, effused, reflexed to pileate; pilei up to 360 μm thick in cross section, imbricate, dimidiate, thin when collected, no prominent change on drying; abhymenial side tomentose, concentrically zonate, sulcate, brownish-orange to reddish-brown when collected, no prominent change on drying; hymenial side smooth, brownish-orange to reddish-brown when collected, no prominent change on

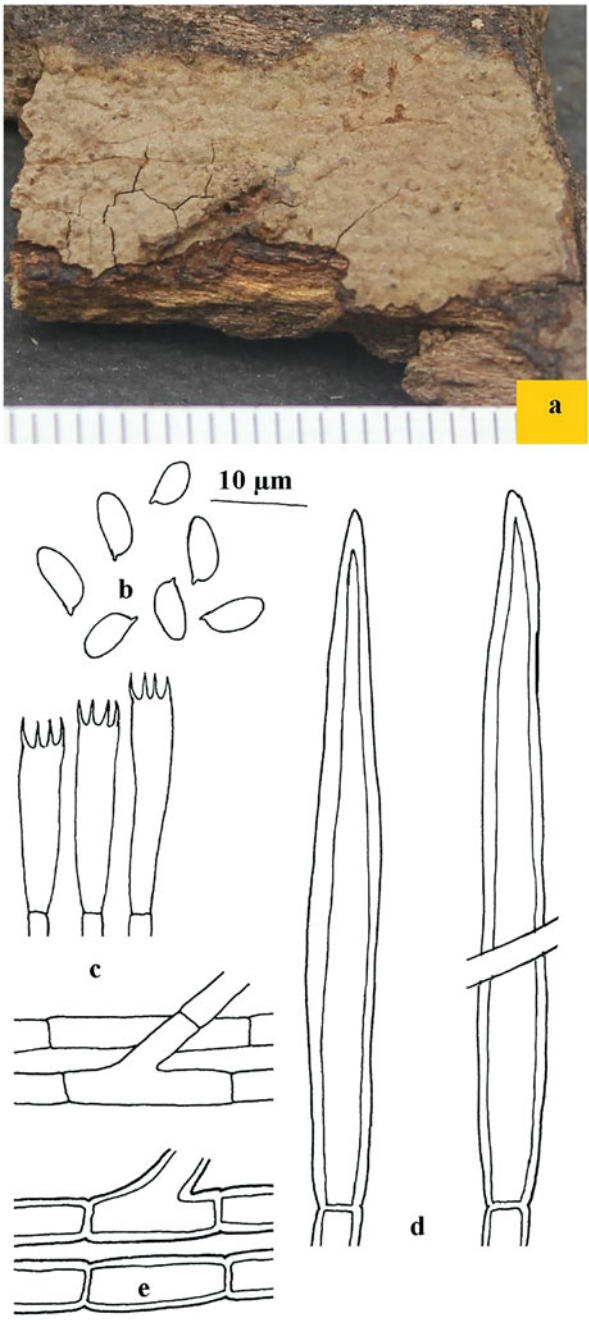


Fig. 11.13 *Hymenochaete leonina*: **a** Carpophore showing hymenial side, **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Setae and **e** Generative hyphae

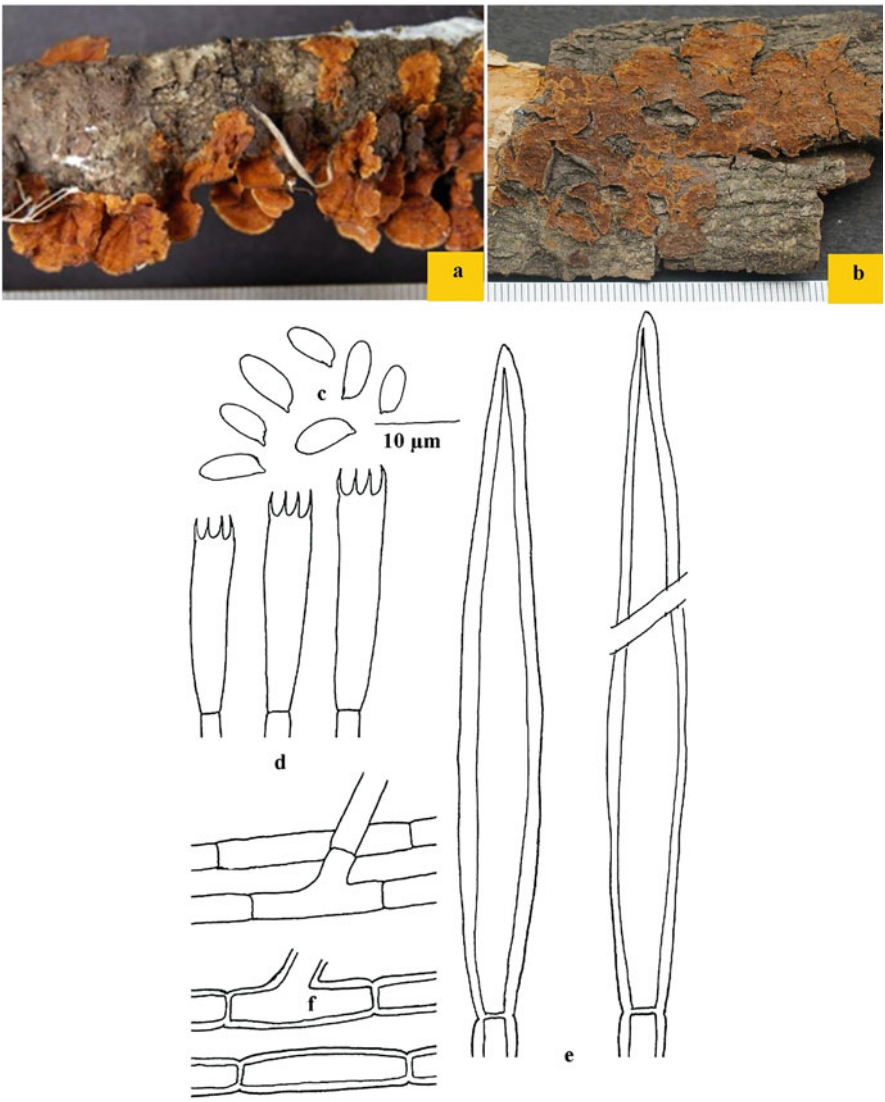


Fig. 11.14 *Hymenochaete rheicolor*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–f** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Setae and **f** Generative hyphae

drying; margins wavy to lobed, concolorous on both sides. Hyphal system monomitic. Generative hyphae subhyaline to yellowish-brown, septate, without clamps, branched, thin- to thick-walled; yellowish-brown, up to 4.3 µm wide in subiculum; subhyaline to yellowish-brown, up to 4 µm wide in subhymenium. Setae subfusiform to subulate, abundant, reddish-brown to brown, 78–87 × 10–11 µm,

thick-walled, arising from subhymenium, extending through hymenium; projecting up to 27 μm out of the hymenium. Basidia narrowly subclavate, 20–27 × 5–5.5 μm, four sterigmate, without basal clamp; sterigmata up to 3 μm long. Basidiospores cylindrical, subhyaline, 5.5–9.3 × 2.5–3.7 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Bhog Batewari, on the log of *Quercus leucotrichophora*, Ramandeep and Avneet 10002 (PUN), September 12, 2016.

Remarks

It is the second report of this species from the study area after Kaur (2018a, b). Other reports in India are from Chamba, Kullu, and Shimla districts of Himachal Pradesh, Andaman, Uttarakhand, and West Bengal as *H. rheicolor* (Montagne 1842; Sharma 1995, 2012; Samita 2014; Sharma and Mishra 2015; Kaur 2018a, b; Poonam 2020) and *H. tenuissima* (Berkeley and Kurtis 1856; Banerjee 1935, 1947).

11.3.15 *Hymenochaete semistupposa* Petch (Petch 1925) (Fig. 11.15)

Carpophore annual, resupinate, adnate, up to 150 μm thick in cross section; hymenial side smooth, brownish-orange to greyish-brown when collected, no prominent change on drying; margins thinning, fibrillose, paler concolorous. Hyphal system monomitic. Generative hyphae subhyaline to pale yellow to yellowish-brown, septate, without clamps, branched, thin- to thick-walled; yellowish-brown, up to 7.6 μm wide in subiculum; subhyaline to pale yellow, up to 4.5 μm wide in subhymenium. Setae subulate, abundant, dark brown, 68–80 × 8–10 μm, thick-walled, arising from subhymenium, extending through the hymenium; projecting up to 30 μm out of the hymenium. Basidia clavate to narrowly clavate, 17–22 × 4.5–5.3 μm, four sterigmate, without basal clamp; sterigmata up to 3.8 μm long. Basidiospores suballantoid, subhyaline, 4.5–6.8 × 2.2–3 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Paonta Sahib, Staun, near bus stand, on the trunk of *Eucalyptus tereticornis*, Ramandeep 10838 (PUN), October 8, 2016.

Remarks

It is a rereport and has been previously published by Kaur (2012) and Dhingra et al. (2014) from Sirmaur district. Other former reports in India are from Chamba, Shimla, and Solan districts of Himachal Pradesh and Uttarakhand by Thind and Adlakha (1956), Rattan (1977), Sharma (1995), Natrajan and Kolandavelu (1998), Bhosle et al. (2005), Ranadive et al. (2011), Kaur (2012), Sharma (2012), Dhingra et al. (2014), Samita (2014), Kaur et al. (2016) and Poonam (2020).

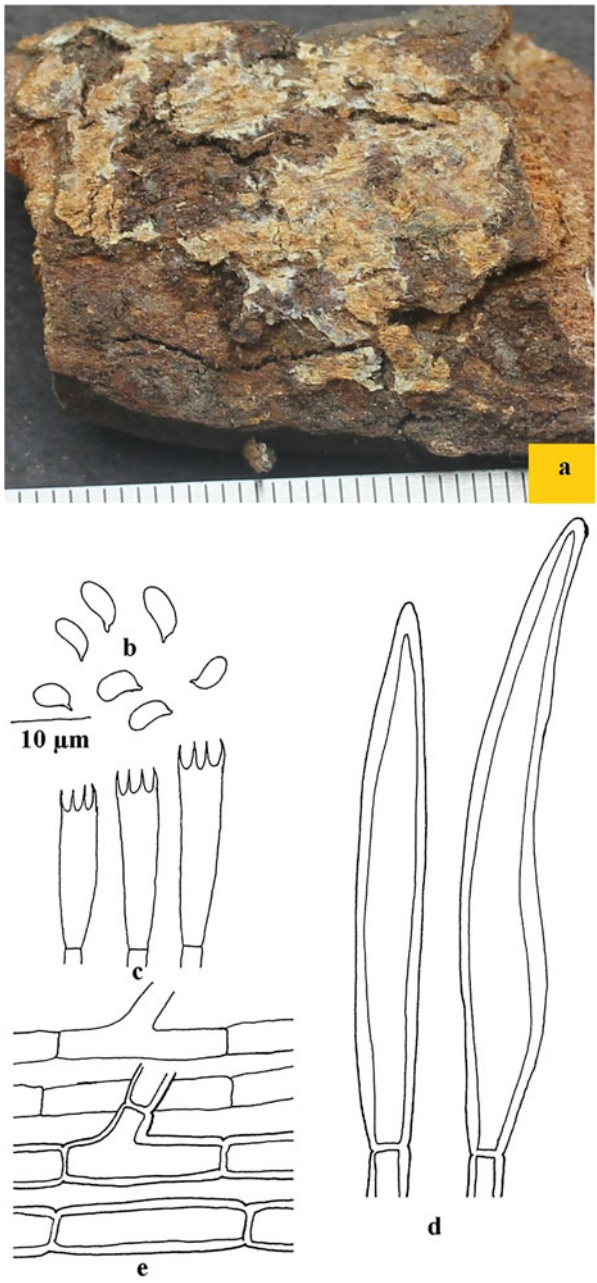


Fig. 11.15 *Hymenochaete semistupposa*: **a** Carpophore showing hymenial side, **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Setae and **e** Generative hyphae

11.3.16 *Junghuhnia collabens* (Fries) Ryvarden (Fries 1874; Ryvarden 1972a) (Fig. 11.16)

Carpophores resupinate, effused, adnate, up to 0.5 mm thick in cross section; hymenial side poroid, greyish-orange to reddish-orange to brownish-orange when collected, pale-orange to greyish-orange on drying; pores angular, split with

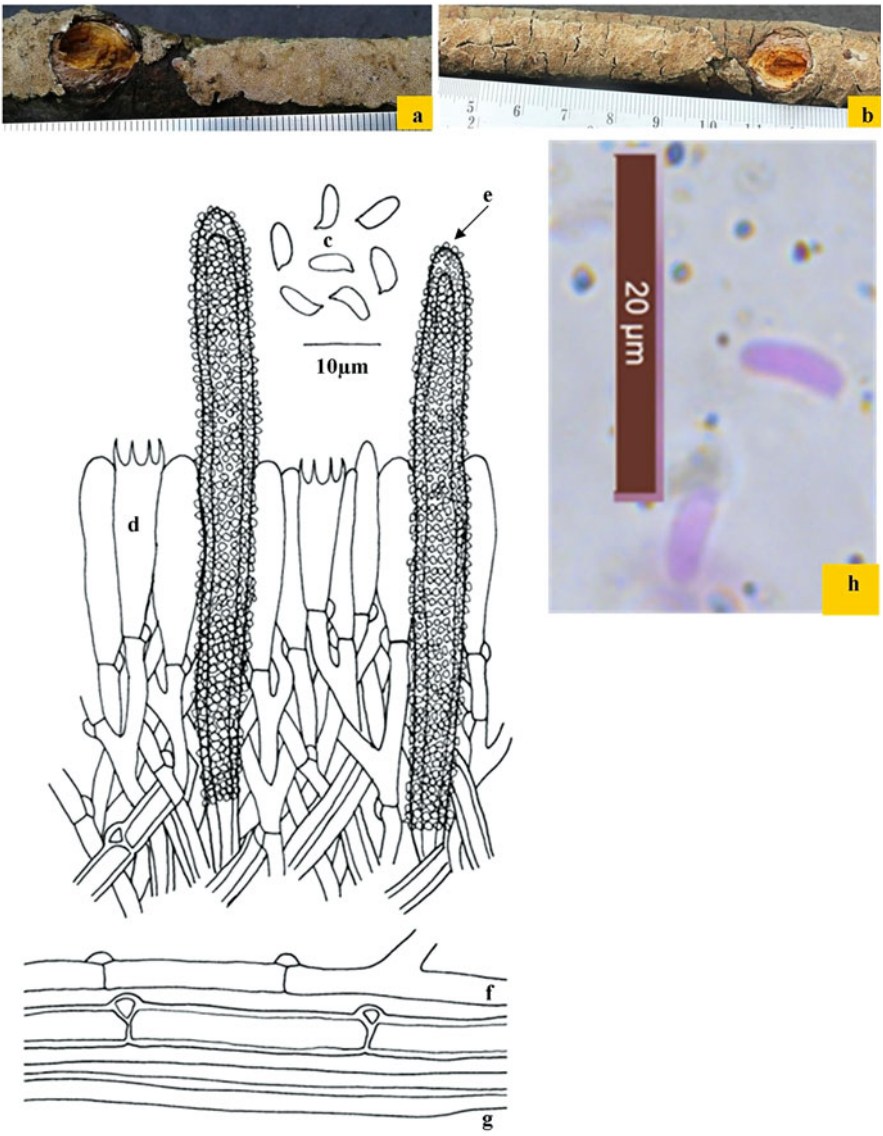


Fig. 11.16 *Junghuhnia collabens*: **a–b** Carpophore showing hymenial side **a** Fresh and **b** Dry, **c–g** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Cystidia, **f** Generative hyphae and **g** Skeletal hypha, **h** Photomicrograph showing basidiospores

maturity, 4–5 per mm; dissepiments up to 180 μm thick, entire; context homogeneous, soft, greyish-orange, up to 0.2 mm thick; pore tubes up to 0. 3 mm deep, greyish-orange; margins sterile up to 1 mm, fibrillose, paler concolorous, occasionally indeterminate. Hyphal system dimitic. Generative hyphae septate, with clamps, up to 5.6 μm wide, branched, thin- to thick-walled. Skeletal hyphae aseptate, up to 6.4 μm wide, unbranched, thick-walled. Cystidia subcylindrical to subfusiform, 81–90 × 6.4–7 μm, thick-walled, densely encrusted; projecting up to 35 μm out of hymenium. Basidia clavate, 18–23 × 4.5–6.5 μm, four sterigmate, with basal clamp; sterigmata up to 4.7 μm long. Basidiospores ellipsoid to suballantoid, 5.5–6.3 × 2–2.8 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nauradhar, on way from Nauradhar to Haripurdhar, on the stick of *Cedrus deodara*, Ramandeep and Avneet 10956 (PUN), September 30, 2019.

Remarks

It is being described for the first time from the study area. Previously, it has been reported from Chamba, Kangra, Kullu, and Shimla districts of Himachal Pradesh, Maharashtra, Tamil Nadu and Uttarakhand in India as *J. collabens* and *Poria rixosa* (Bakshi 1971; Rattan 1977; Roy and De 1996; Natrajan and Kolandavelu 1998; Sharma 2000, 2012; Ranadive 2013; Sanyal 2014; Ritu 2019).

11.3.17 *Junghuhnia nitida* (Persoon) Ryvarden (Persoon 1800; Ryvarden 1972a) (Fig. 11.17)

Carpophores resupinate, effused, adnate, up to 2 mm thick in cross section; hymenial side poroid, yellowish-orange to brownish-orange when collected, no prominent change on drying; pores angular, 4–6 per mm; context homogeneous, soft, yellowish-orange, up to 1 mm wide; pore tubes up to 1 mm deep, yellowish-orange; margins paler concolorous to abrupt. Hyphal system dimitic. Generative hyphae subhyaline, septate, with clamps, up to 3.6 μm wide, less branched, thin-walled. Skeletal hyphae aseptate, up to 6.5 μm wide, thick-walled. Cystidia subcylindrical, 65–78 × 6.6–10 μm, thick-walled, with crystalline encrustation, basal clamp present; projecting up to 30 μm out of the hymenium. Basidia clavate, 11–16 × 4.4–5.5 μm, four sterigmate, with basal clamp; sterigmata up to 2.6 μm long. Basidiospores ellipsoid, 4.4–6 × 2–3.5 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Shillai, Tindi, on the log of *Cedrus deodara*, Ramandeep 10959 (PUN), September 3, 2017.

Remarks

This species differs from *J. collabens* in having smaller cystidia and ellipsoid basidiospores. It is being described for the first time from the study area. Formerly, it has been reported as *Poria euporae* from Chamba and Shimla districts of Himachal Pradesh, Maharashtra and Uttarakhand in India (Bakshi 1971; Rattan 1977; Roy and

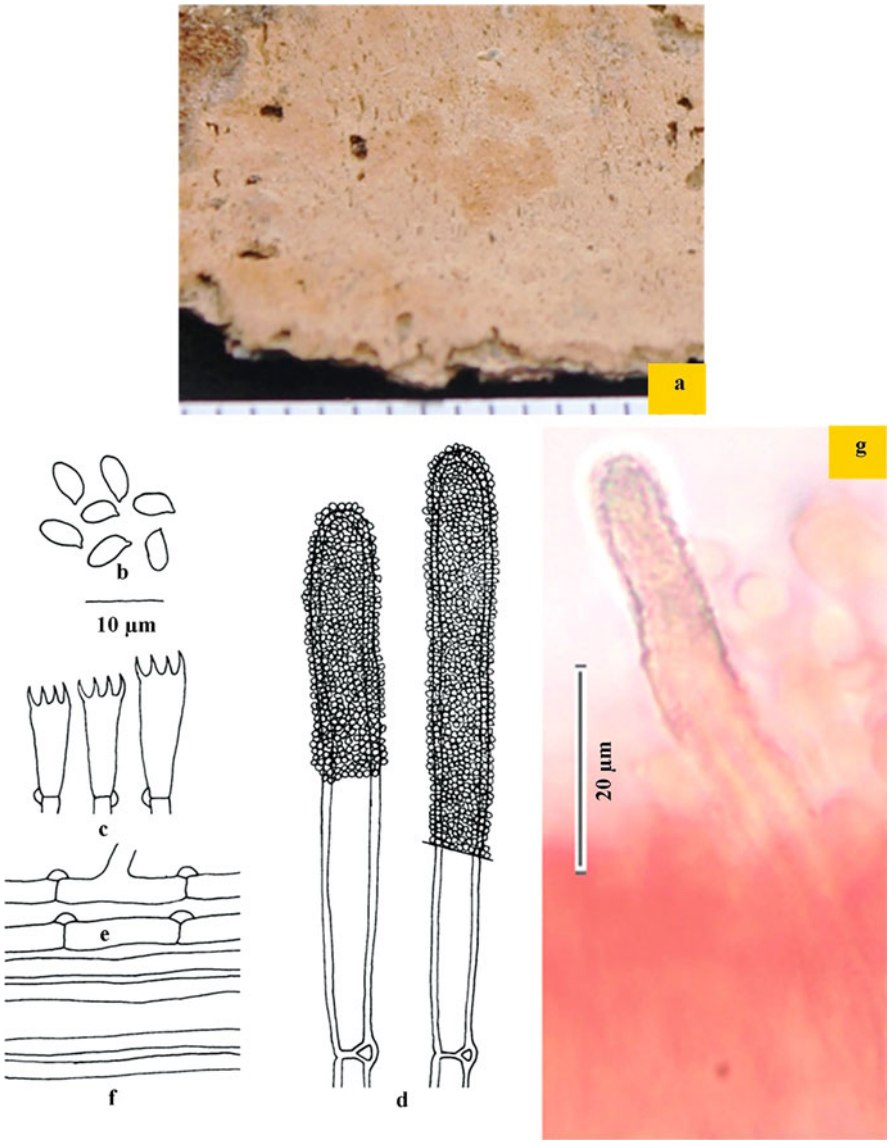


Fig. 11.17 *Junghuhnia nitida*: **a** Carpophore showing hymenial side, **b–f** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidia, **e** Generative hyphae and **f** Skeletal hyphae, and **g** Photomicrograph showing cystidium

De 1996; Sharma 2000, 2012; Ranadive et al. 2011; Priyanka 2012; Ranadive 2013; Sanyal 2014).

11.3.18 *Phanerochaete chrysosporium* Burdsall (Burdsall and Eslyn 1974) (Fig. 11.18)

Carpophores resupinate, effused, adnate, up to 300 μm thick in cross section; hymenial side smooth, orange-white to pale yellow to orange-grey to brownish-orange when collected, no prominent change on drying; margins fibrillose, paler concolorous, occasionally indeterminate. Hyphal system monomitic. Generative hyphae septate, without clamps, up to 5.5 μm wide; thick-walled, less branched, parallel to the substrate, loosely interwoven in subiculum; thin-walled, more branched, gradually at right angles to substrate, compact in subhymenium. Cystidia subcylindrical to cylindrical with obtuse tips, 47–82 × 8–10 μm, thick-walled, without basal clamp; projecting up to 45 μm out of the hymenium. Basidia clavate, 17–30 × 4–5 μm, four sterigmate, without basal clamp; sterigmata up to 4 μm long. Basidiospores ellipsoid, 4.5–6 × 2–3 μm, thin-walled, smooth, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Ambwala, on the trunk of *Ficus religiosa*, Ramandeep and Dhingra 10641 (PUN), August 23, 2015.

Remarks

Previous reports of this species are from Himachal Pradesh and Punjab by Kaur et al. (2019) and Kaur (2017), respectively.

11.3.19 *Phlebiopsis crassa* (Léveillé) Floudas and Hibbett (Léveillé 1844; Floudas and Hibbett 2015) (Fig. 11.19)

Carpophores resupinate, effused, adnate, up to 250 μm thick in cross section; hymenial side smooth, dull red to pale-red to greyish-red when collected, no prominent change on drying; margins irregular, paler concolorous, occasionally indeterminate. Hyphal system dimitic. Generative hyphae septate, usually without clamps, sometimes with single or double clamp/clamps, up to 5.5 μm wide, branched, thin- to thick-walled. Skeletal hyphae aseptate, up to 6.6 μm wide, rarely branched, thick-walled, parallel to substrate, mixed with basal generative hyphae, extend at right angles to substrate as skeletocystidia in the subhymenium/hymenium. Skeletocystidia subfusiform to fusiform, brown, 57–80 × 8–14 μm, thick-walled, apically with crystalline encrustation; projecting up to 40 μm out of the hymenium. Basidia clavate, 23–35 × 5–8.5 μm, four sterigmate, without basal clamp; sterigmata up to 3.3 μm long. Basidiospores ellipsoid to subcylindrical, 6.5–9 × 3–4.2 μm, thin-walled, smooth, inamyloid, acyanophilous.

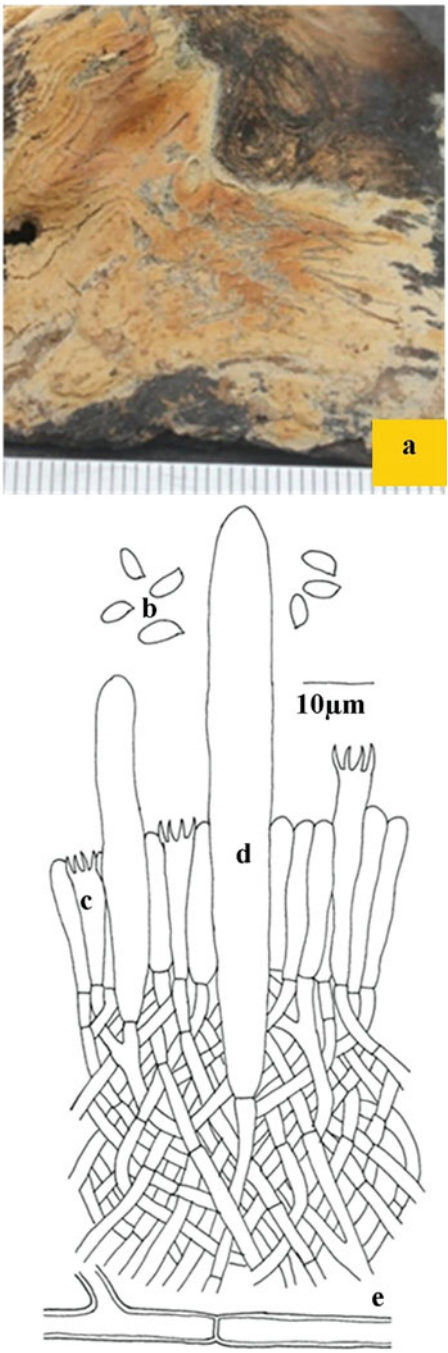


Fig. 11.18 *Phanerochaete chrysosporium*: **a** Carpophore showing hymenial side, **b–e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidia and **e** Generative hyphae

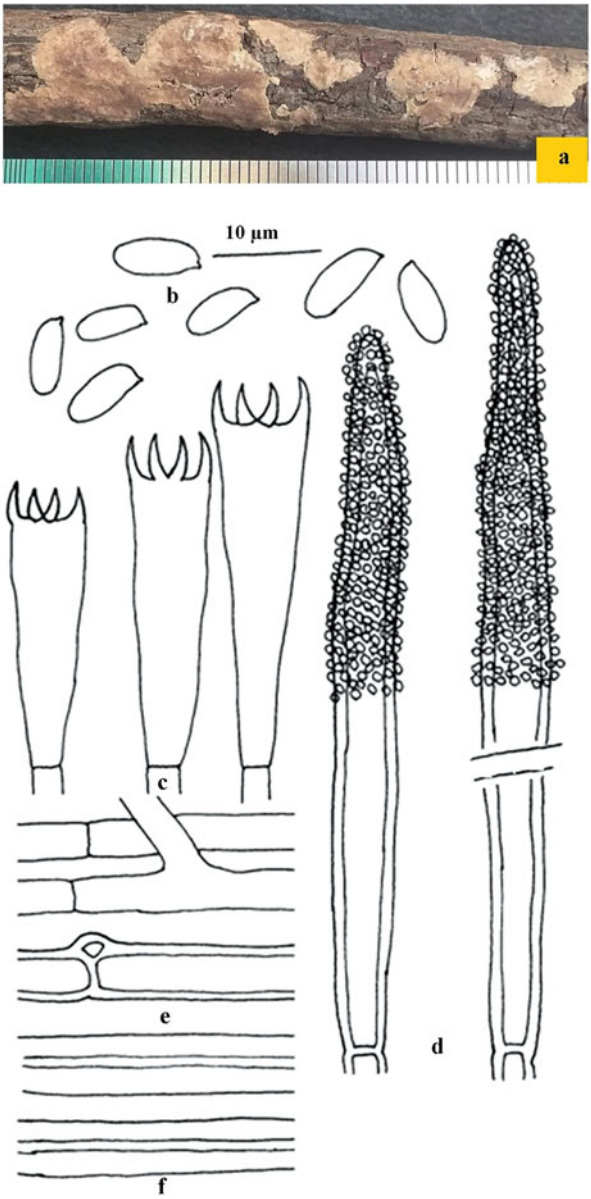


Fig. 11.19 *Phlebiopsis crassa*: **a** Carpophore showing hymenial side, **b–f** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidia, **e** Generative hyphae and **f** Skeletal hyphae

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Ambwala, on the branch of *Ficus religiosa*, Ramandeep and Dhingra 10966 (PUN), August 23, 2015.

Remarks

This species is being described for the first time from the study area. Earlier, it has been reported from Kangra district of Himachal Pradesh, Arunachal Pradesh.,

Assam, Jammu & Kashmir, Manipur, Maharashtra, Punjab, Uttarakhand, and West Bengalin India as *Lopharia crassa* (Banerjee 1935; Bagchee and Bakshi 1954; Dhingra 1983; Sharma 2012; Ranadive 2013), *Hjortstamia crassa* (Sanyal 2014; Kaur 2017; Sharma 2017) and *Phlebiopsis crassa* (Ritu 2019).

11.3.20 *Phellinus gilvus* (Schweinitz) Patouillard (Schweinitz 1822; Patouillard 1900) (Fig. 11.20)

Carpophores annual, effused, reflexed to pileate; pilei up to 6 × 3.5 × 1.4 cm, sessile, imbricate; conchate, dimidiate, soft, corky when collected, hard, brittle on drying; abhymenial side velutinate, azonate, sulcate, light brown to brownish-orange to brown when collected, no prominent change on drying; hymenial side poroid, light brown to brown to dark brown when collected, no prominent change on drying; pores round to angular, 6–9 per mm; dissepiments up to 40 μm wide, entire; context homogeneous, brownish-yellow to brownish-orange, up to 6 mm thick; pore tubes brownish-orange to light brown, up to 8 mm deep; margins acute, regular, lobed; brownish-orange on abhymenial side; paler concolorous on hymenial side, sterile up to 2 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline to yellowish-brown, septate, without clamps, up to 6.8 μm wide, branched, thin- to thick-walled. Skeletal hyphae yellowish- brown, up to 7.5 μm wide, unbranched, thick-walled. Setal hyphae absent. Setae subventricose to ventricose, reddish-brown to dark brown, 34–50 × 7.4–8 μm, thick-walled; arising from the subhymenium and hymenium, extending through the hymenium; projecting up to 12 μm beyond hymenial side. Cystidioles absent. Basidia clavate, 11–16 × 5–6.2 μm, four sterigmate, without basal clamp; sterigmata up to 3.3 μm long. Basidiospores ellipsoid, subhyaline, 4.3–6.2 × 3.2–3.7 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, on the trunk of *Mangifera indica*, Ramandeep and Dhingra 9969 (PUN), August 23, 2015.

Remarks

This species is being described for the second time from the study area; the only previous report is by Kaur (2018a, b). Other reports in India are from Bilaspur, Chamba, Hamirpur, Kangra, Kullu, Shimla, Solan, and Una districts of Himachal Pradesh, Arunachal Pradesh, Andaman, Assam, Chandigarh, Jammu and Kashmir, Kerala, Maharashtra, Meghalaya, Mizoram, South Andaman, temperate regions of Himalaya, Uttarakhand, Uttar Pradesh and West Bengal by Berkeley and Curtis (1856) and Currey (1874) as *Polyporus scruposus*; Hennings (1901) as *P. scruposus* and *P. gilvus*; Bose (1928, 1937) and Banerjee (1947) as *Polyporus hookeri* and *P. gilvus*; Bose (1944, 1946) as *P. gilvus* forma *gilvoides*, *P. gilvus* forma *licnoides* and *P. hookeri*; Bagchee et al. (1954), Bakshi (1958, 1971) as *Fomes scruposus*; Bhargava and Sehgal (1954), Chaudhuri (1959), Singh et al. (1961) and Bakshi (1971) as *Polyporus gilvus*; Thind and Chatrath (1957) as *Fomes gilvus* and *F. scruposus*; Dhanda (1977), Singh (1987), Sharma (1995, 2000, 2012), Leelavathy

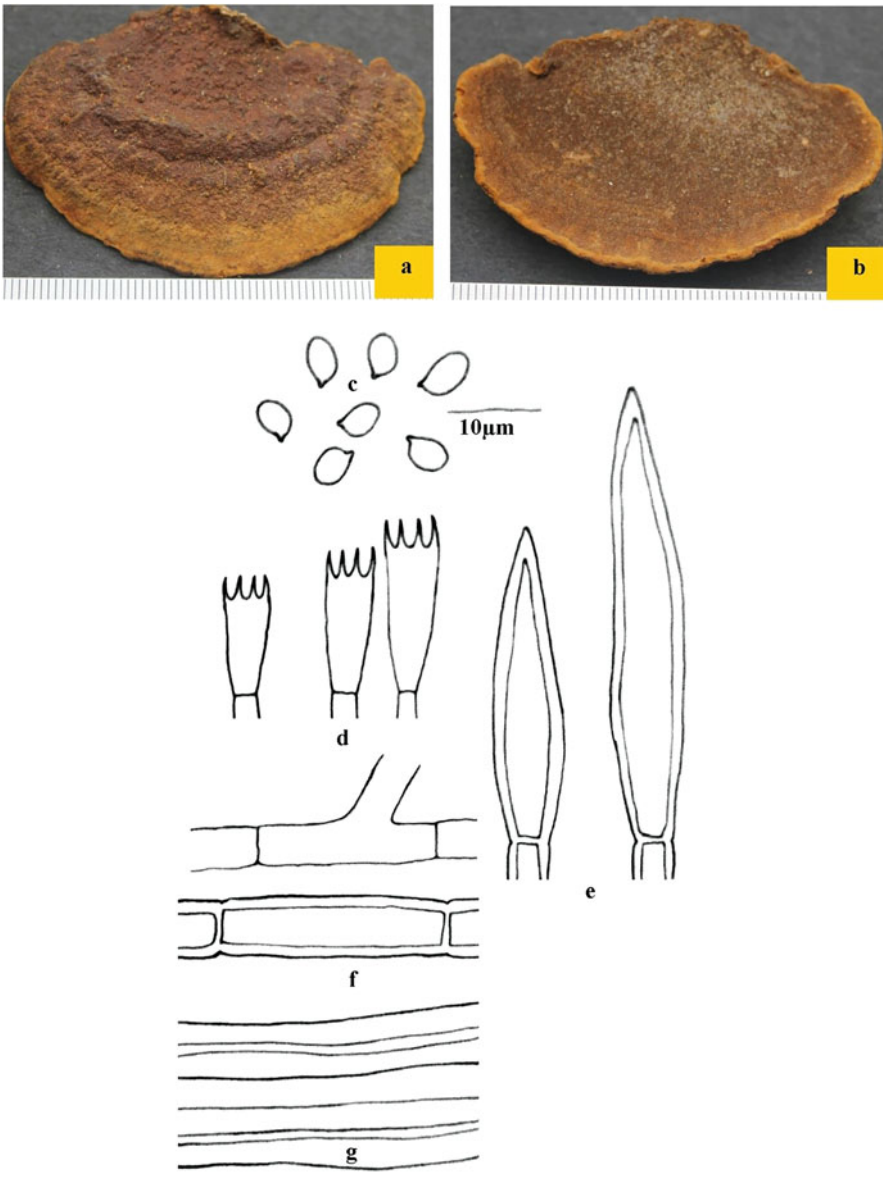


Fig. 11.20 *Phellinus gilvus*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–g** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Setae, **f** Generative hypha, and **g** Skeletal hyphae

and Ganesh (2000), Kaur (2017, 2018a, b), Azeem (2017) and Ritu (2019) as *Phellinus gilvus*, by Dhanda (1977), Singh (1987) and Lalji (2003) as *P. scruposus*; and Kaur (2013) and Sharma and Mishra (2015) as *Fuscoporia gilva*.

11.3.21 *Phellinus nigricans* (Fries) Karsten (Fries 1821; Karsten 1899) (Fig. 11.21)

Carpophores perennial, reflexed to pileate; pilei up to 15 × 7.5 × 3.2 cm, sessile, imbricate; ungulate, heavy when collected, no prominent change on drying; abhymenial side glabrous, zonate, sulcate, reddish-brown to dark brown when collected, no prominent change on drying, crust up to 1 mm thick; hymenial side poroid, light brown to brown when collected, no prominent change on drying; pores round to angular, 5–6 per mm; dissepiments up to 55 μm wide, entire; context homogeneous, light brown, up to 2 mm thick; pore tubes yellowish-brown to light brown, up to 28 mm deep, stratified (4 layers), up to 7 mm in each layer, separated by very thin layers of contextual hyphae; margins obtuse, regular, wavy; reddish-brown on abhymenial side; paler concolorous on hymenial side, sterile up to 2 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline to yellowish-brown, septate, without clamps, up to 5 μm wide, branched, thin- to thick-walled. Skeletal hyphae yellowish-brown to reddish-brown to light brown, up to 5.5 μm wide, unbranched, thick-walled. Setal hyphae absent. Setae subventricose to ventricose, reddish-brown to dark brown, 31–34 × 6.8–8 μm, thick-walled; arising from the subhymenium and hymenium, extending through the hymenium; projecting up to 16 μm beyond hymenial side. Cystidioles absent. Basidia clavate, 11–14 × 4.5–6.2 μm, four sterigmate, without basal clamp; sterigmata up to 2 μm long. Basidiospores broadly ellipsoid, rusty brown, 6.2–8.6 × 4.2–5.2 μm, smooth, thick-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, on the log of *Dalbergia sissoo*, Ramandeep and Dhingra 10865 (PUN), August 23, 2015.

Remarks

This is being described for the first time from the study area. Formerly in India, it has been reported from Chamba district of Himachal Pradesh, Arunachal Pradesh, Sikkim and Uttarakhand by Sharma (1995, 2000, 2012), Azeem (2017), and Kaur (2018a, b).

11.3.22 *Phellinus senex* (Nees and Montagne) Imazeki (Nees and Montagne 1836; Imazeki 1952) (Fig. 11.22)

Carpophores annual, resupinate, effused, reflexed to pileate; pilei up to 6.5 × 2.5 × 0.8 cm, sessile, imbricate; applanate to dimidiate, soft, corky when collected, hard, brittle on drying; abhymenial side velutinate, zonate, sulcate, brownish-orange to light brown to brown when collected, no prominent change on

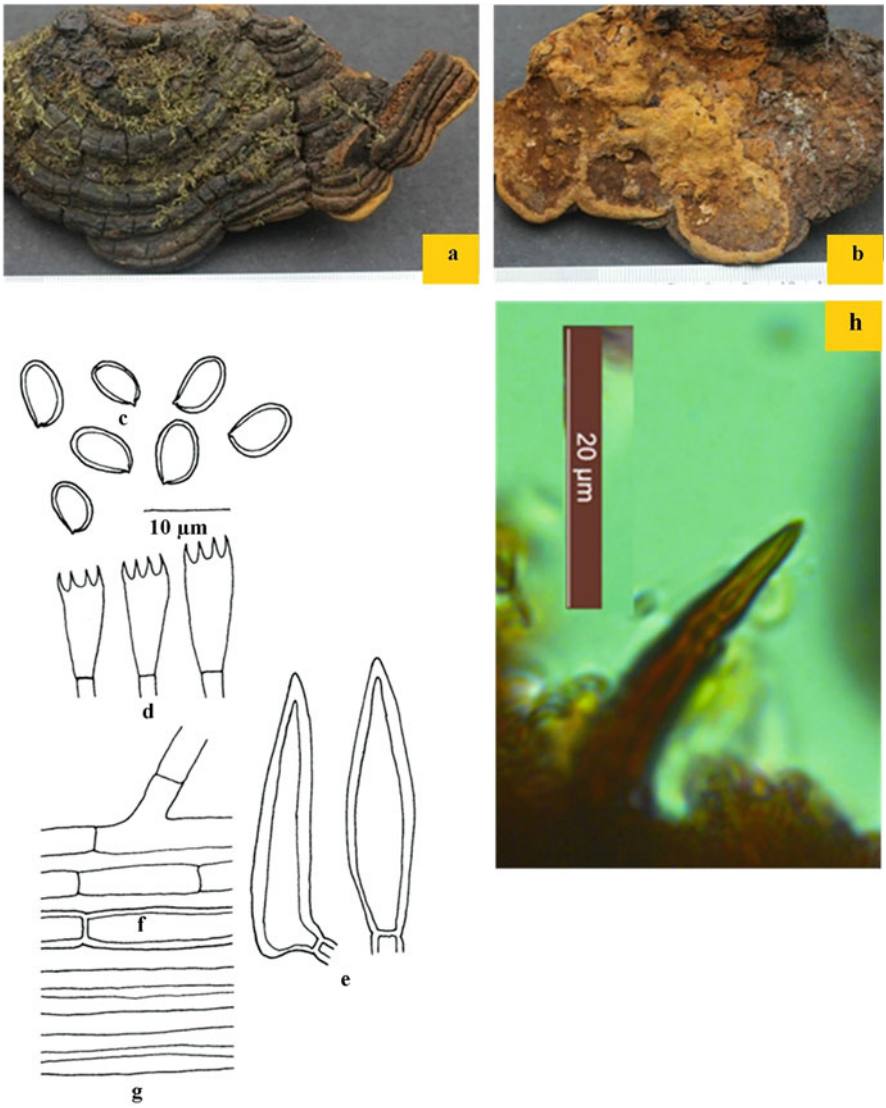


Fig. 11.21 *Phellinus nigricans*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–g** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Setae, **f** Generative hyphae and **g** Skeletal hyphae, **h** Photomicrograph showing seta

drying; hymenial side poroid, reddish-brown when collected, no prominent change on drying; pores round to angular, 7–8 per mm; dissepiments up to 45 µm wide, entire; context homogeneous, brownish-orange, up to 5 mm thick; pore tubes greyish-brown to light brown, up to 3 mm deep; margins acute, regular, wavy; brown to reddish-brown on abhymenial side; paler concolorous on hymenial side,

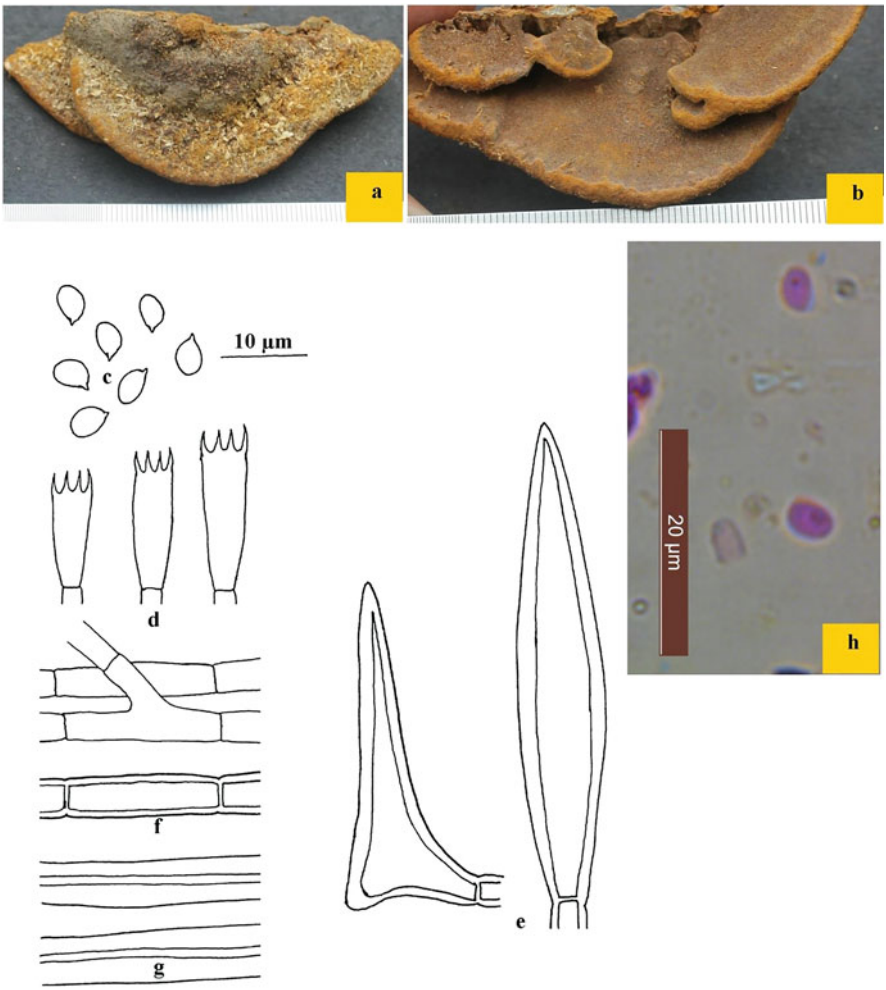


Fig. 11.22 *Phellinus senex*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–g** Line diagrams **c** Basidiospores, **d** Basidia, **e** Setae, **f** Generative hyphae and **G** Skeletal hyphae, **h** Photomicrograph showing basidiospores

sterile up to 2 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline to pale yellowish to light brown, aseptate, up to 5.7 µm wide, branched, thin- to thick-walled. Skeletal hyphae yellowish-brown to light brown, up to 6.6 µm wide, unbranched, thick-walled. Setal hyphae absent. Setae subventricose to ventricose, sometimes rooted, reddish-brown to dark brown, 40–58 × 10–13 µm, thick-walled; arising from subhymenium and hymenium, extending through the hymenium; projecting up to 14 µm beyond hymenial side. Cystidioles absent. Basidia clavate, 11–17 × 4.3–5.5 µm, four sterigmate, without basal clamp;

sterigmata up to 3 μm long. Basidiospores ellipsoid to broadly ellipsoid, subhyaline, 4–5 × 2.5–3.5 μm, smooth, thin-walled, inamyloid, weakly cyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Nahan, Sainwala, on the trunk of *Dalbergia sissoo*, Ramandeep and Dhingra 10873 (PUN), August 23, 2015.

Remarks

This species has been described earlier from the study area by Kaur (2013, 2018a, b). Further, other reports in India are from Bilaspur, Chamba, Hamirpur, Kangra, and Una districts of Himachal Pradesh, Andaman, Arunachal Pradesh, Assam, Kerala, Maharashtra, Meghalaya, Punjab, Tripura, Uttarakhand, Uttar Pradesh and West Bengal by Berkeley (1854, 1856), Theissen (1911, 1913), Bose (1921, 1937, 1944), Bagchee et al. (1954) and Chaudhuri (1959) as *Polyporus senex*; Banerjee (1947) and Bakshi (1971) as *Fomes senex*; Singh (1987), Sharma and Ghosh (1989), Sharma (1995, 2012) and Leelavathy and Ganesh (2000) as *P. senex*; Dargan et al. (2006) and Kaur (2013) as *Fuscoporia senex* and Sharma and Mishra (2015) and Kaur (2018a, b) as *Phellinus senex*.

11.3.23 *Pycnoporus cinnabarinus* (Jacquin) Karsten (Jacquin 1776; Karsten 1881) (Fig. 11.23)

Carpophores annual, pileate; pilei up to 5.2 × 4 × 0.3 cm, solitary to rarely imbricate, dimidiate; abhymenial side smooth to rough, azonate to weakly zonate, greyish-orange to reddish-orange to brownish-red to reddish-brown when collected, no prominent change on drying; hymenial side poroid, greyish-red to brownish-red when collected, no prominent change on drying; pores round to angular, 4–5 per mm; dissepiments up to 100 μm wide, entire; context homogeneous, orange, up to 2 mm thick; pore tubes up to 1 mm deep, light orange; margins acute, round, regular, wavy to somewhat lobed, light orange on both sides, sterile up to 2 mm on hymenial side. Hyphal system trimitic. Generative hyphae, subhyaline, septate, with clamps, up to 4.7 μm wide, branched, thin-walled. Skeletal hyphae yellowish-brown, aseptate, up to 6.2 μm wide, rarely branched, thick-walled. Binding hyphae yellowish-brown, up to 5.3 μm wide, much branched, thick-walled. Context constituted by parallel to substrate, loosely interwoven generative, skeletal and binding hyphae; tramal zone by at right angles to substrate, compact generative, skeletal as well as binding hyphae and subhymenial region usually by generative hyphae at right angles to the trama. Cystidia absent, but fusoidcystidioles present, 22–27 × 4.4–5.7 μm, thin-walled. Basidia clavate to subclavate, subhyaline, 14–23 × 5–6.2 μm, four sterigmate, with basal clamp; sterigmata up to 2.6 μm long. Basidiospores subhyaline, ellipsoid to subcylindrical, 5–7 × 2.5–3.5 μm, smooth, thin-walled, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Paonta Sahib, about 2 km from Rajban towards Staun, on the log of *Shorea robusta*, Ramandeep 8760 (PUN), October 7, 2016.

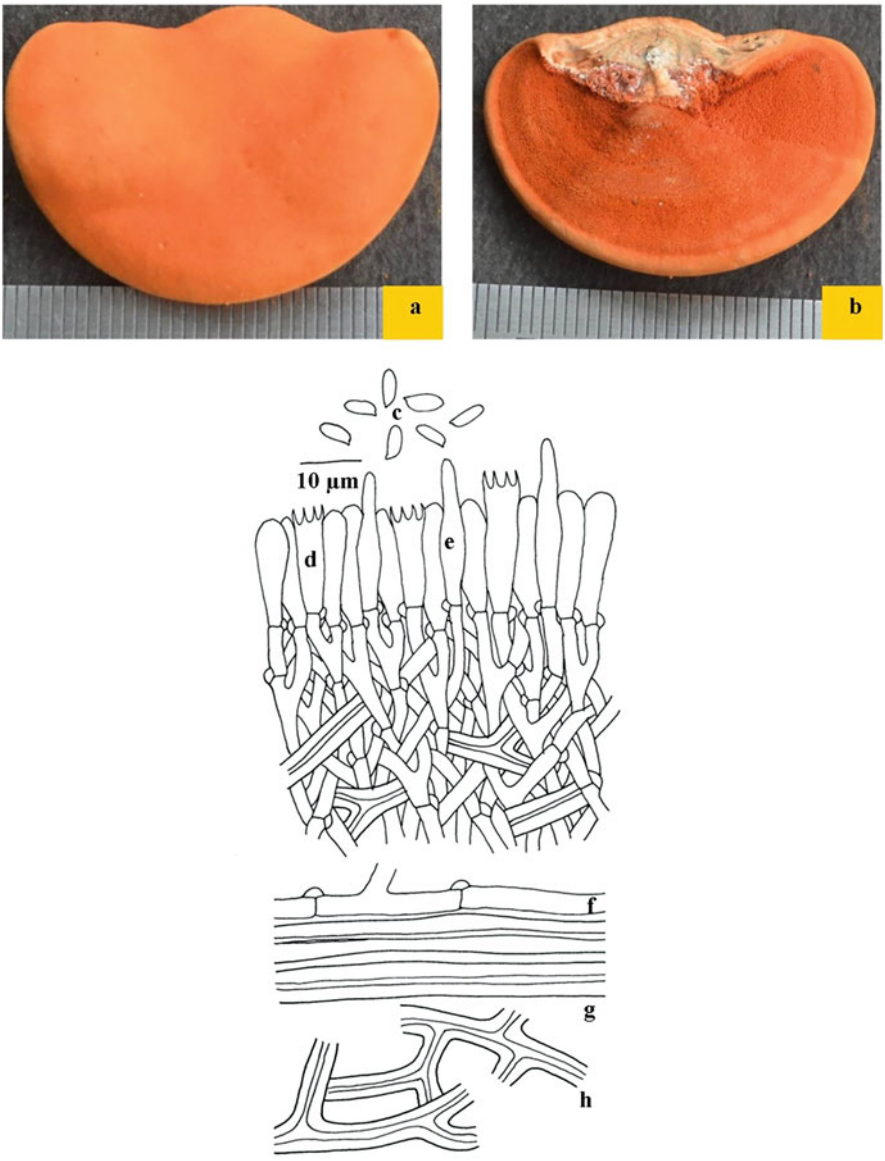


Fig. 11.23 *Pycnoporus cinnabarinus*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–h** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Cystidioles, **f** Generative hyphae and **g** Skeletal hyphae, **h** Binding hyphae

Remarks

The first report of this species from Himachal Pradesh has earlier been published (Kaur et al. 2017). Formerly from India, it has been reported from Jammu and Kashmir, Madhya Pradesh, Uttarakhand, and West Bengal by Currey (1874), Murrill (1924), Banerjee (1947) as *Polystictus cinnabarinus* and by Bakshi (1971), Dhanda (1977), Roy and De (1996) and Sharma (2012) as *Polyporus cinnabarinus*.

11.3.24 *Rigidoporus ulmarius* (Sowerby) Imazeki (Sowerby 1797; Imazeki 1952) (Fig. 11.24)

Carpophores perennial, effused, reflexed to pileate; pilei up to 10 × 7 × 3 cm, solitary to imbricate, dimidiate, coriaceous when collected, woody hard on drying; abhymenial side smooth to tuberculate to rough, glabrous to tomentose, azonate, yellowish-white to orange-white to greenish (because of algal growth) when collected, orange-white to greyish-white to black at base on drying; hymenial side poroid, pale-orange to greyish-orange when collected, brownish-orange to greyish-orange on drying; pores round to angular, 5–6 per mm; dissepiments up to 90 μm wide, entire; context homogeneous, orange-white to pale-orange, basal context up to 3 mm wide; pore tubes orange-white, stratified (3 layers), each tube layer up to 5 mm long, separated by 4 mm thick layer of context; margins thin, obtuse, paler concolorous on hymenial side, sterile up to 2.5 mm on hymenial side. Hyphal system monomitic. Generative hyphae subhyaline, septate, without clamps, up to 5.5 μm wide, branched, thin- to thick-walled. Context constituted by parallel to substrate, less branched, loosely interwoven, thin- to thick-walled hyphae; trama by at right angles, less branched, compact, thin- to thick-walled hyphae and subhymenium usually by more branched, thin-walled hyphae at right angles to the trama. Cystidia absent, but fusoid cystidioles present, 17–27 × 5–11 μm, thin-walled, generally embedded. Basidia ovate to subclavate, 15–20 × 10–11 μm, four sterigmate, without basal clamp; sterigmata up to 5.7 μm long. Basidiospores subglobose to globose, 6.3–7.2 × 5–6.5 μm, smooth, thin-walled, with oily contents, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Shillai, on a gymnospermous log, Ramandeep 10926 (PUN), September 3, 2017.

Remarks

It is the first report of this species from the study area. It has been reported in India from the Chamba, Kullu, and Shimla districts of Himachal Pradesh, Kerala, Maharashtra, Uttarakhand and Western Himalaya by Thind and Chatrath (1957), Bakshi (1971), Leelavathy and Ganesh (2000), Sharma (2000), Ranadive et al. (2011), Sharma (2012) and Ranadive (2013) as *Fomes geotropus* and by Kaur (2013) as *Rigidoporus ulmarius*.

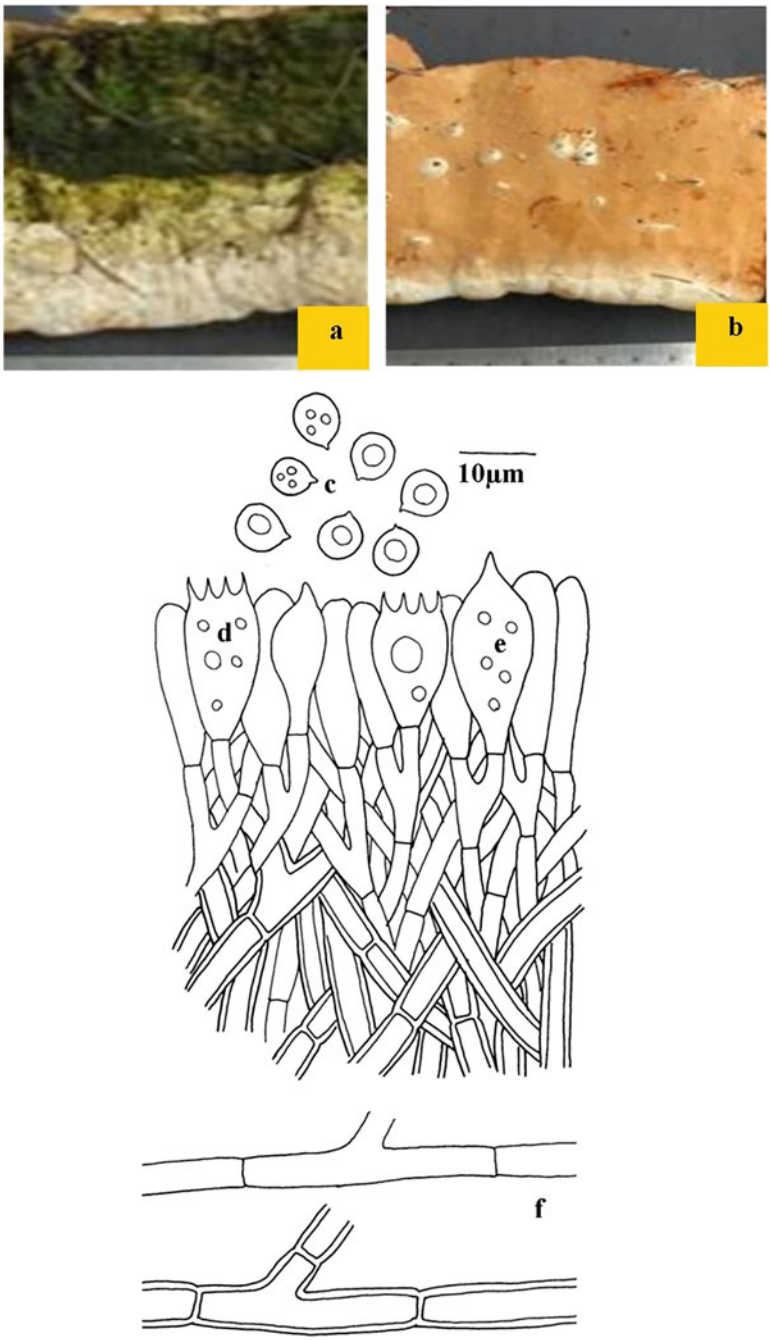


Fig. 11.24 *Rigidoporus ulmarius*: **a–b** Carpophore showing **a** Abhymenial side and **b** Hymenial side, **c–f** Line diagrams showing **c** Basidiospores, **d** Basidia, **e** Cystidia and **f** Generative hyphae

11.3.25 *Radulodon acaciae* G. Kaur, Avneet P. Singh, Dhingra (Kaur et al. 2014) (Fig. 11.25)

Carpophores resupinate, effused, adnate, up to 950 μm thick in cross section; hymenial side aculeate with dense aculei, brownish-orange to brownish-red to violet-brown when collected, dark greyish on drying; margins wavy, very thin and greyish in young carpophores, thinning, paler concolorous, to indeterminate on maturity. Aculei up to 4 mm long, cylindrical, tapering to flattened. Hyphal system monomitic. Generative hyphae branched, septate, with clamps; up to 4.5 μm wide, thick-walled, parallel to substrate, loosely interwoven in subiculum; up to 3.2 μm wide, thin-walled, at right angles to substrate, compact in subhymenium. Cystidia clavate, 35–59 × 4–6.5 μm, thin- to thick-walled, with resinous encrustation at the tip with basal clamp; projecting up to 27 μm out of the hymenium. Basidia clavate, 10–19 × 3.6–5.4 μm, four sterigmate, with basal clamp; sterigmata up to 2.4 μm long. Basidiospores broadly ellipsoid to subglobose, 3.6–4.8 × 2.7–3.6 μm, thin-walled, smooth, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Paonta Sahib, near Gurudwara sahib, on the trunk of *Dalbergia regia*, Ramandeep 8826 (PUN), October 3, 2015.

Remarks

This species has earlier been described and published from the state of Himachal Pradesh by Kaur et al. (2019). Besides, it has also been reported from Union Territory of Chandigarh in India (Kaur 2017).

11.3.26 *Trametes ljubarskyi* Pilát (Pilát 1936–1942) (Fig. 11.26)

Carpophores annual, effused, reflexed to pileate; pileus 5 × 3.5 × 0.6 cm, solitary, dimidiate; abhymenial side glabrous, dull, concentrically zonate, greyish-orange to brownish-orange to light brown when collected, no prominent change on drying; hymenial side poroid, greyish-white when collected, greyish-orange on drying; pores round to angular, 5–6 per mm; dissepiments up to 65 μm wide, entire; context homogeneous, concolorous with abhymenial side, up to 3 mm thick; pore tubes up to 3 mm deep, concolorous with hymenial side; margins acute, somewhat wavy, concolorous on both sides, sterile up to 1 mm on hymenial side. Hyphal system trimitic. Generative hyphae subhyaline, septate, with clamps, up to 4.5 μm wide, branched, thin-walled. Skeletal hyphae subhyaline, aseptate, up to 6 μm wide, occasionally branched, thick-walled. Binding hyphae subhyaline, aseptate, up to 4.5 μm wide, much branched, thick-walled. Basidia broadly clavate to subclavate, 16–21 × 4.7–6 μm, four sterigmate, with basal clamp; sterigmata up to 3.2 μm long. Basidiospores broadly ellipsoid to ovoid, 5–6.8 × 3–3.8 μm, smooth, thin-walled, with oily contents, inamyloid, acyanophilous.

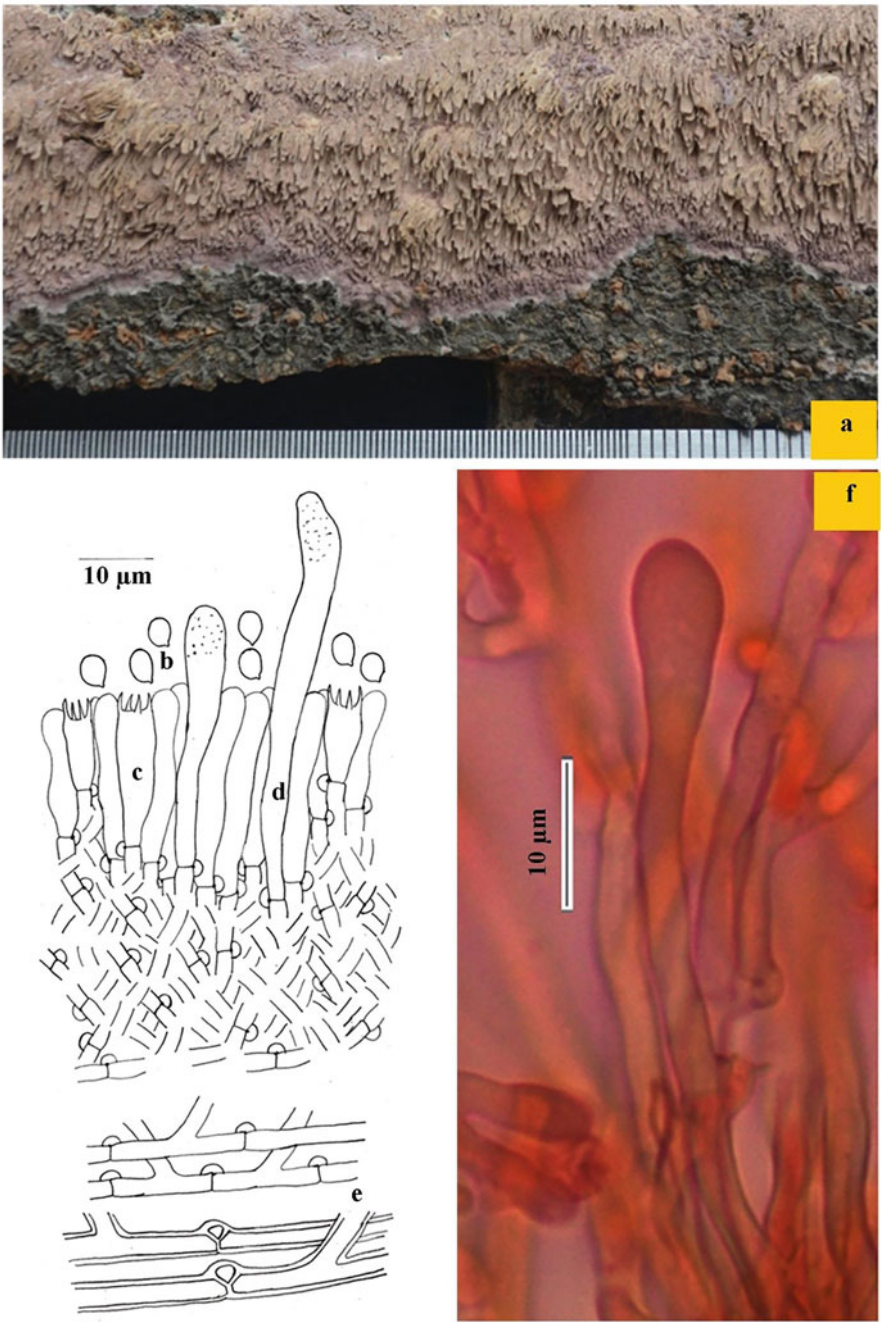


Fig. 11.25 *Radulodon acaciae*: **a** Carpophore showing hymenial side, **a**-**e** Line diagrams showing **b** Basidiospores, **c** Basidia, **d** Cystidia and **e** Generative hyphae, **f** Photomicrograph showing cystidium

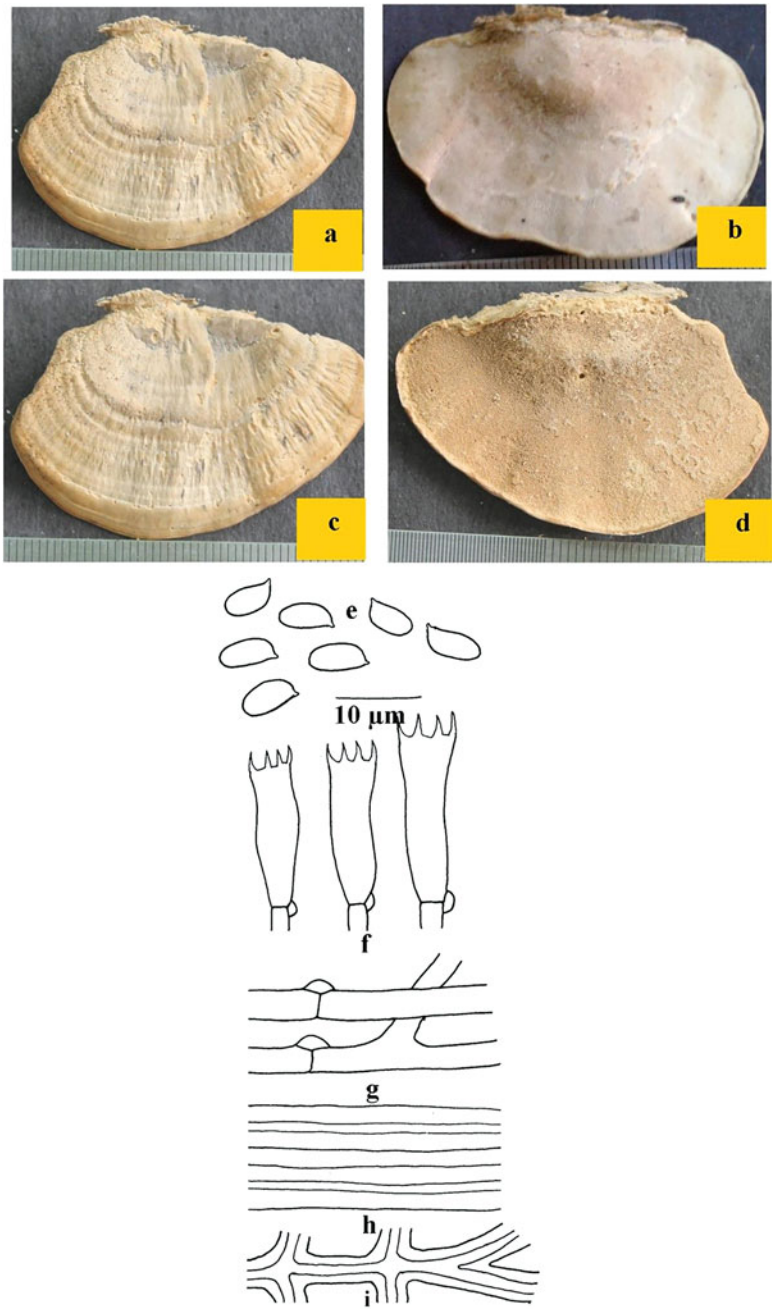


Fig. 11.26 *Trametes ljubarskyi*: **a–d** Carpophore showing abhymenial side **a** Fresh and **c** Dry; Carpophore showing hymenial side **b** Fresh and **d** Dry, **e–i** Line diagrams showing **e** Basidiospores, **f** Basidia, **g** Generative hyphae, **h** Skeletal hyphae and **i** Binding hyphae

Sample Studied

Himachal Pradesh: Sirmaur, Paonta Sahib, Rajban, about 2 km from Rajban towards Staun, on the log of *Shorea robusta*, Ramandeep 11005 (PUN), October 7, 2016.

Remarks

Diagnostic characters of this species are dimidiate carpophore, glabrous abhymenial side and broadly ellipsoid to ovoid basidiospores. It is the second report of this species from India, after the only previous report by Kaur (2013) from the same area.

11.3.27 *Trametes versicolor* (Linnaeus) Lloyd (Linnaeus 1753; Lloyd 1921) (Fig. 11.27)

Carpophores annual, effused, reflexed to pileate; pilei up to 4 × 3.5 × 0.3 cm, imbricate, applanate, dimidiate; abhymenial side hirsute to tomentose, with concentric zones of olive to olive- yellow to greyish-yellow, olive-brown to greyish-orange to dark brown to grey when collected, dull on drying; hymenial side poroid, orange-white to pale-orange when collected, greyish-yellow to brownish-orange on drying; pores round to angular, 3–5 per mm; dissepiments up to 60 μm wide, entire; context homogeneous, whitish to orange-white, with a thin black layer below the tomentum, up to 0.5 mm thick; pore tubes up to 2.5 mm deep, orange-white to greyish-orange; margins acute, regular, wavy, concolorous on both sides, sterile up to 3 mm on hymenial side. Hyphal system trimitic. Generative hyphae subhyaline, septate, with clamps, up to 3.3 μm wide, branched, thin-walled. Skeletal hyphae subhyaline, aseptate, up to 6.8 μm wide, occasionally branched, thick-walled. Binding hyphae subhyaline, aseptate, up to 5 μm wide, much branched, thick-walled. Cystidial elements absent. Basidia clavate to subclavate, 13–22 × 3.8–6 μm, four sterigmate, with basal clamp; sterigmata up to 4 μm long. Basidiospores subcylindrical to allantoid, 4.5–6.5 × 2–2.6 μm, thin-walled, smooth, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, on the log of *Quercus leucotrichophora*, Ramandeep and Avneet 10072 (PUN), September 12, 2016.

Remarks

This species is being described for the second time from the study area; the only previous report is by Kaur (2013). Other reports in India are from Chamba, Kangra, Kinnaur, Kullu, Mandi and Shimla districts of Himachal Pradesh, Arunachal Pradesh, Jammu and Kashmir, Maharashtra, Manipur, Meghalaya, Mizoram, Punjab, Tripura, Tamil Nadu, Uttar Pradesh, Uttarakhand, and West Bengal. It has been described by Thind and Chatrath (1957) as *Polystictus versicolor*; Thind and Dhanda (1978) as *Coriolus azureus*; Dhanda (1977) and Singh (1987) as *Coriolus versicolor* and *C. azureus*; Dulat (1992) as *C. versicolor*; Roy and De (1996) as *Trametes versicolor*; Dargan et al. (2006) as *Coriolus versicolor*; Ranadive et al. (2011) and Ranadive (2013) as *Trametes versicolor*; and Sharma (2000, 2012) and Kaur (2013) as *Trametes versicolor*.

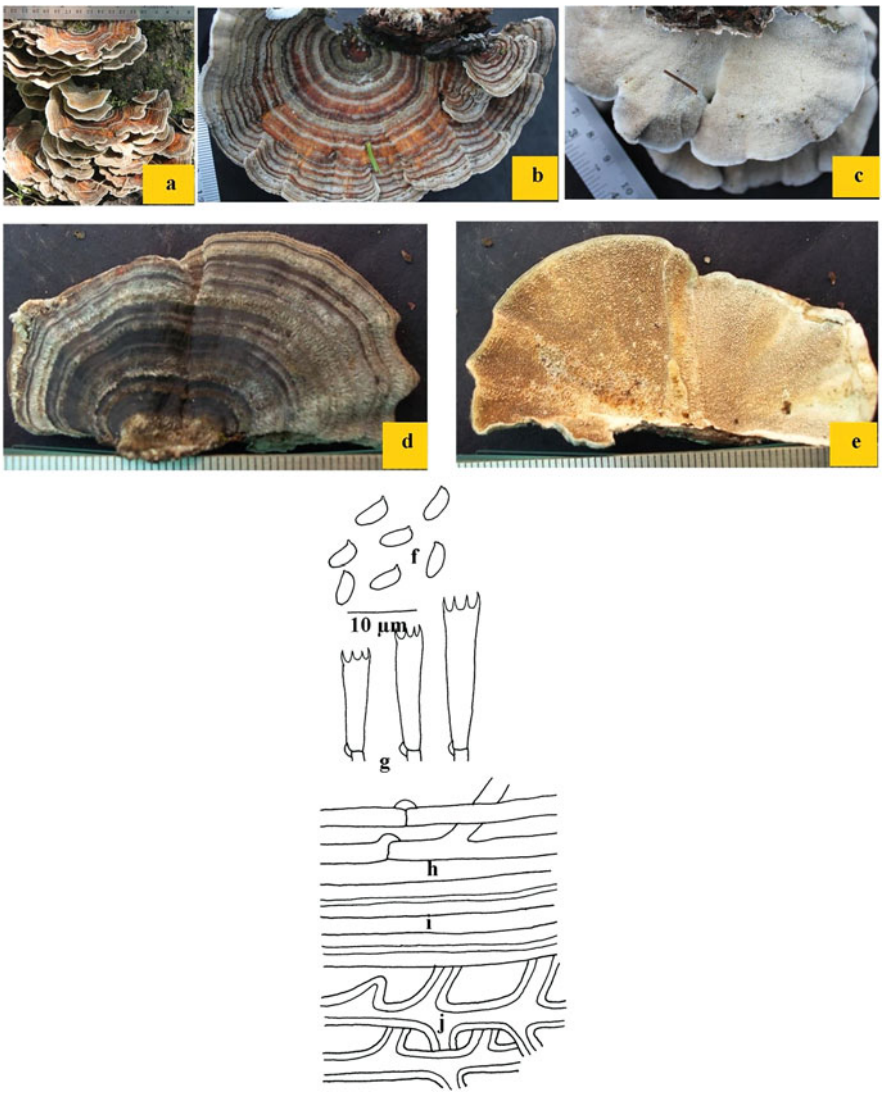


Fig. 11.27 *Trametes versicolor*: **a–e** Carpophore showing **a** Nature and mode of attachment, Abhymenial side **b** Fresh and **d** Dry, Hymenial side **c** Fresh and **e** Dry, **f–j** Line diagrams showing **f** Basidiospores, **g** Basidia, **h** Generative hyphae, **i** Skeletal hyphae and **j** Binding hyphae

11.3.28 *Trichaptum abietinum* (Gmelin) Ryvarden (Gmelin 1792; Ryvarden 1972b) (Fig. 11.28)

Carpophores annual, effused, reflexed to pileate; pilei up to 3 × 2 × 0.4 cm, imbricate, broadly attached, fuse laterally; abhymenial side glabrous to tomentose to velutinate, azonate to zonate, whitish to greyish-orange to greyish-yellow to

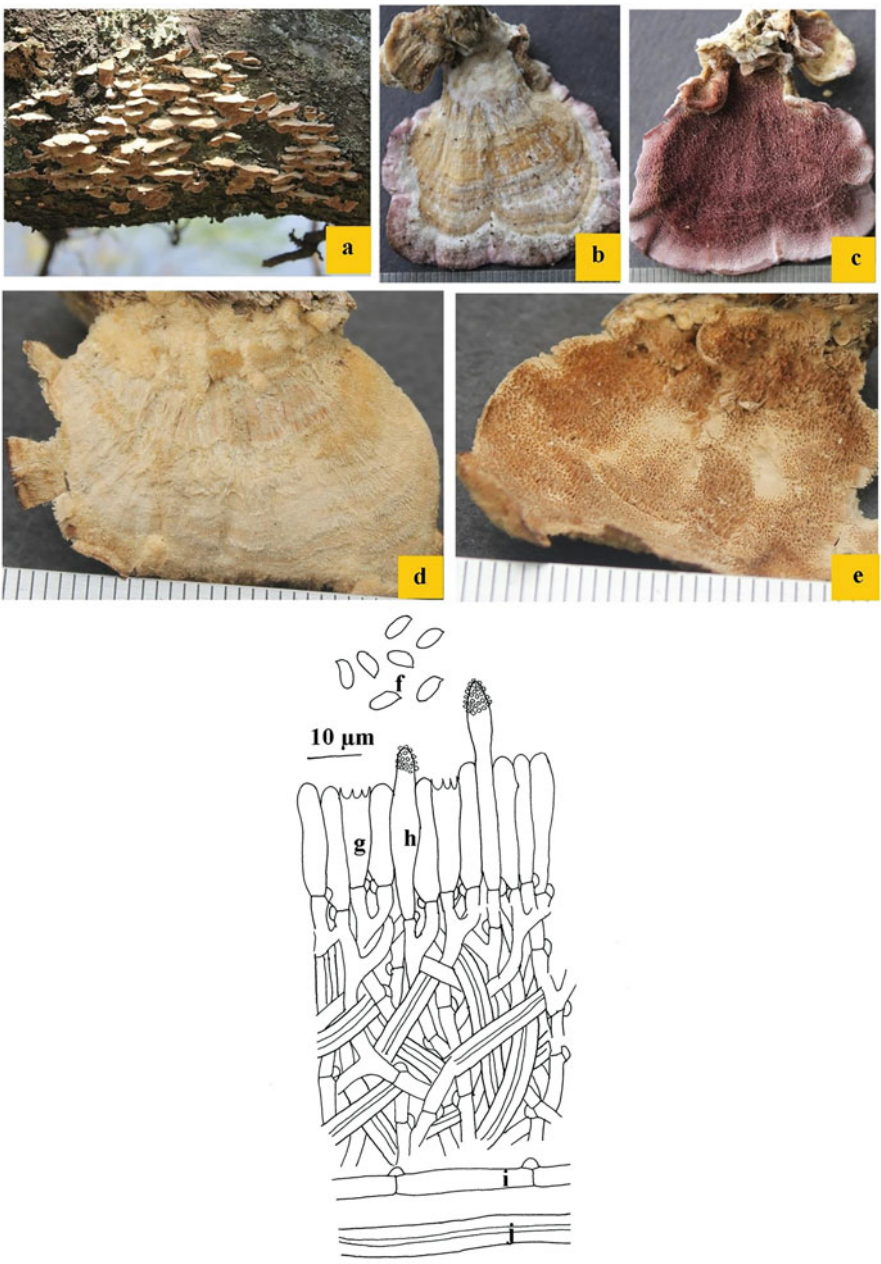


Fig. 11.28 *Trichaptum abietinum*: **a–e** Carpophore showing **a** Attachment, Abhymenial side **b** Fresh and **d** Dry, and Hymenial side **c** Fresh and **e** Dry, **f–j** Line diagrams showing **f** Basidiospores, **g** Basidia, **h** Cystidia, **i** Generative hyphae and **j** Skeletal hypha

brownish-orange to light brown to reddish- grey to brownish-grey when collected, pale-orange to greyish-orange to brownish-orange on drying; hymenial side poroid, greyish-ruby to greyish-violet to greyish-magenta to purplish-grey when collected, light brown to brown to dark brown to reddish-brown on drying; pores round to angular to irpicoid, 5–6 per mm; dissepiments up to 50 μm wide, entire when young, lacerate with maturity; context duplex, upper zone whitish, soft and lower brownish-orange to greyish-orange, tough, up to 2 mm thick; pore tubes up to 2 mm deep, concolorous with hymenial side; tubes and context separated by a thin gelatinous layer; margins acute, regular to wavy to lobed, concolorous both on abhymenial and hymenial sides, sterile up to 1 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline, septate, with clamps, up to 5 μm wide, branched, thin- to thick-walled. Skeletal hyphae subhyaline to yellowish-brown, aseptate, up to 6.7 μm wide, occasionally branched, thick-walled. Context constituted by parallel to substrate, loosely interwoven generative and skeletal hyphae; tramal region by at right angles to substrate, compact generative as well as skeletal hyphae and subhymenial region usually by generative hyphae at right angles to the trama. Cystidia fusoid, 30–38 × 4–6 μm, apically encrusted, thin- to thick-walled, with basal clamp. Basidia clavate to subclavate, 15–19 × 5–6 μm, four sterigmate, with basal clamp; sterigmata up to 2.3 μm long. Basidiospores narrowly ellipsoid to cylindrical, 5–6.8 × 2.5–3.4 μm, thin-walled, smooth, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Nauradhar, on the log of *Quercus leucotrichophora*, Ramandeep and Avneet 8761 (PUN), September 12, 2016.

Remarks

It is the second report of this species from the study area, and has been reported by Kaur (2013), previously. Other reports in India are from Chamba, Kangra, Kinnaur, Kullu, and Shimla districts of Himachal Pradesh, Arunachal Pradesh, Kerala, Maharashtra, Madhya Pradesh, Meghalaya, Uttarakhand, Uttar Pradesh, West Bengal and Western Himalayas by Bose (1934), Bagchee and Singh (1960), Thind and Rattan (1971), and Bakshi (1971) as *Polyporus abietinus*. Dhanda (1977), Sharma (1985, 2000), Singh (1987), Roy and De (1996), Priyanka (2012), Kaur (2013) and Prasher and Ashok (2013) have reported it as *Trichaptum abietinum*.

11.3.29 *Trichaptum biforme* (Fries) Ryvarden (Klotzsch 1833; Ryvarden 1972b) (Fig. 11.29)

Carpophores annual, effused, reflexed to pileate; pilei up to 3.2 × 2 × 0.3 cm, imbricate, dimidiate to flabelliform; abhymenial side smooth to hirsute, concentrically zonate, greyish-orange to brownish-orange when collected, brownish on drying; hymenial side poroid, greyish-ruby to greyish-violet to greyish-magenta to purple-grey when collected, light brown to brown to dark brown to reddish-brown on drying; pores round to angular, 3–5 per mm; dissepiments up to 50 μm wide, entire to lacerate; context homogeneous, orange-white, up to 0.5 mm thick; pore tubes up to 2.5 mm deep, concolorous with hymenial side, tubes and context not

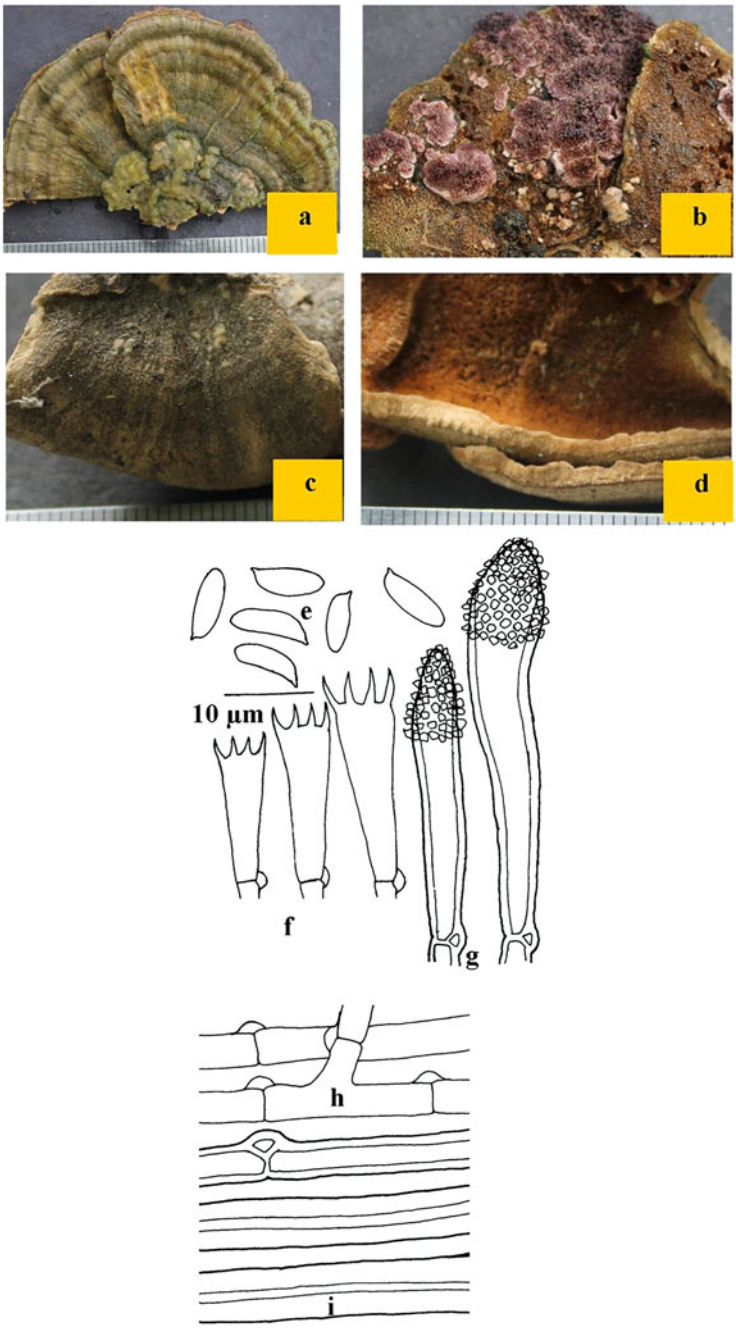


Fig. 11.29 *Trichaptum biforme*: **a–d** Carpophore showing abhymenial side **a** Fresh and **c** Dry, and hymenial side **b** Fresh and **d** Dry, **e–i** Line diagrams showing **e** Basidiospores, **f** Basidia, **g** Cystidia, **h** Generative hyphae and **i** Skeletal hyphae

separated by a thin gelatinous layer; margins acute, regular, wavy to lobed, greyish-magenta, sterile up to 2 mm on hymenial side. Hyphal system dimitic. Generative hyphae subhyaline, septate, with clamps, up to 4.2 µm wide, branched, thin- to thick-walled. Skeletal hyphae yellowish-brown, aseptate, up to 6.8 µm wide, thick-walled. Context constituted by parallel to substrate, loosely interwoven generative and skeletal hyphae; tramal region by at right angles to substrate, compact generative as well as skeletal hyphae and subhymenial region usually by generative hyphae at right angles to the trama. Cystidia fusoid, 32–45 × 5.5–9 µm, apically encrusted, thin- to thick-walled, with basal clamp. Basidia clavate, 14–20 × 3.5–6.3 µm, four sterigmate, with basal clamp; sterigmata up to 4.2 µm long. Basidiospores narrowly ellipsoid to cylindrical, 7–8.5 × 2.4–3.4 µm, thin-walled, smooth, inamyloid, acyanophilous.

Sample Studied

Himachal Pradesh**:** Sirmaur, Rajgarh, Deedag, on the log of *Cedrus deodara*, Ramandeep and Avneet 11021 (PUN), September 12, 2016.

Remarks

This species is being described for the second time from the study area, with the only previous report by Kaur (2013). Other reports in India are from Chamba, Kullu, and Shimla districts of Himachal Pradesh, Arunachal Pradesh, Kerala, Maharashtra, Meghalaya, Uttarakhand, Uttar Pradesh, West Bengal and Western Himalayas by Bagchee et al. (1954) as *Polystictus pargamenus*, Bakshi (1958) as *Polyporus biformis*, Bakshi (1971) as *Polyporus biformis* and *Polyporus pargamenus*, Dhanda (1977) as *Polyporus pargamenum*, Singh (1987) as *T. biformis*, Roy and De (1996), Sharma (2000, 2012), Ranadive (2013), Kaur (2013), Ritu (2019) as *T. biforme* and Leelavathy and Ganesh (2000) as *T. biformis.*

Acknowledgements The authors are grateful to the Head, Department of Botany, Punjabi University, Patiala for providing necessary laboratory facilities and UGC, DSA-I for financial assistance.

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The *Ganoderma*: Biodiversity and Significance

12

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Abstract

The genus *Ganoderma* includes intrinsic wood rotting fungi of economic importance, which are spotted widely across the globe. The various species of *Ganoderma* possess pathogenicity as well as therapeutic and aesthetic qualities. It is commonly referred as 'medicinal mushroom' across the Asia due to the presence of many chemical compounds with significant dietary and curative values. Besides the forementioned utilities, *Ganoderma* is an important phytopathogen that causes basal stem rot in oil palm, coconut, and areca nut trees, as well as many other trees in the forest environment, such as oak and maple. The fungus is a soil-borne facultative parasite that produces chlamydospores and basidiospores while living saprophytically on decaying roots and stumps. This chapter focuses on the *Ganoderma* covering biodiversity, molecular characterisation, detection, pathology including aetiology, epidemiology, mode of dissemination, and management, and economic and ecological implications. Despite the fact that in the diseases caused by *Ganoderma* spp., the primary cause of disease has been well researched, but early detection and management approaches are still in their immature stage. Future research priorities should include gaining a comprehensive understanding of the aetiology and

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_12

epidemiology of diseases on diverse hosts, as well as addressing existing ambiguity in species nomenclature.

Keywords

Biodiversity · Basal stem rot · Characterisation · *Ganoderma* · Integrated disease management · Mushroom

12.1 Introduction

Ganoderma is a genus of the *Ganodermataceae* family, which is the part of the order Polyporales in Basidiomycota. It is well reported to trigger hardwood tree root or butt rot, and is also reported to be a therapeutically significant fungus across Asian continent. The genus *Ganoderma* was initially described in 1881 by Peter Adolf Karsten (Karsten 1881), with *Ganoderma lucidum* as the type species. *Ganoderma* species are important timber-decaying fungi with rough fruiting bodies that are prevalent both in temperate and tropical environments across the world with over 300 species. *Ganoderma* has a broad host range and may invade a variety of perennials, conifers, and palms. *Ganoderma* species are highly varied in the tropical area, impacting plantation crops like coconut, arecanut, and oil palm by inducing basal stem rot, as well as numerous trees in the forest environment like Oak, Maple, and foot rot of betelnut leading to pathogenicity and wood rots (Adaskaveg et al. 1991; Singh 1991; Flood et al. 2000; Pilotti 2005). The oil palm sector loses up to $500 million each year due to this disease (Arif et al. 2011; Ommelna et al. 2012). On the other hand, *Ganoderma* taxonomy generates a variety of biologically active chemicals of commercial value, which are farmed and possibly exploited for their curative and aesthetic qualities. *Ganoderma lucidum* is now regarded as one of the most commonly utilised medicinal mushrooms in the world (Rios et al. 2012). Antitumour, antiviral, antibacterial, anti-inflammatory, antioxidant, anti-platelet aggregation, hepatoprotective, hypotensive, immunomodulating, and immunosuppressive properties have all been documented for *G. lucidum* (Wasser and Weis 1999). The market value of '*G. lucidum'* products reached US$2.5 billion in 2003, making it a globally important commercial and pharmacological medicinal fungus (Chang and Buswell 2008).

The presence of the pathogen is typically confirmed once the fruiting bodies have developed. About 50% of the plant bole tissue gets decayed by this time, leaving the farmer with no means to cure the damaged palms and leading to a major reduction in palm agricultural productivity (Kandan et al. 2010). The *Ganoderma* species is a soil-borne facultative parasite that feeds (saprophytic) on dead, rotting roots and stumps forming chlamydospores and basidiospores. Basidiospores from conks are released for a short period of time (up to 5 months) and are accumulated on soil surfaces or their pruned or wounded fronds on standing palms, where they are passively spread by rain water runoff and air (Pilotti et al. 2018). If favourable substrates are accessible, these spores become pathogenic and may survive for

extended period under adverse circumstances (Rees et al. 2009a, b). Disease incidence was lower in areas with a lot of rain and high relative humidity. The quantity of rain and the number of wet days have a negative association with disease transmission in coconut (Palanna et al. 2012). The disease's severity increases as the soil temperature increases and decreases with the rise in its moisture content. Management control methods now in use, which include cultural and mechanical practices, do not seem to be very beneficial. Chemical therapy (Mohammed et al. 2014), root feeding and soaking (Bhaskaran and Ramanathan 1983), and proper dose of vital plant nutrients (Singh 1990) all help to minimise disease incidence. Biological control agents are used in alternative control approaches to solve the problem, and a number of potential bioagents have been created but have yet to be tested in the field. *Trichoderma* is one among them, and it is considered to be capable of controlling *Ganoderma* (Soepena et al. 2000). The conventional technique of identifying *Ganoderma* spp. based on physical and cultural traits has proven unsuccessful, and the lack of relevant morphological features has resulted in an overabundance of synonyms for the same disease. Therefore, at all levels of *Ganoderma* taxonomy and characterisation, protein- and DNA-based characteristics have become prevalent (Bruns et al. 1991). Numerous monitoring systems, including non-molecular techniques, have been formed based on serology, nucleic acid, secondary metabolites, volatile organic chemicals, remote sensing, and other approaches. Conventional PCR-based techniques have been found to be more hard to manoeuvre, compared to the faster, more accurate, and less cost-effective approach of DNA-based nanosensors and microarrays. While the primary cause of disease has been well researched, early detection and management approaches are still in their development. Research directions target on gaining a comprehensive understanding of the aetiology and epidemiology of diseases on diverse hosts, as well as addressing existing ambiguity in species nomenclature.

12.2 Economic Importance of *Ganoderma* Species

The genus *Ganoderma* integrates far more than 300 species, and is a very common causal agent of root or butt rot on hardwood. They are also familiarly known as medicinal mushroom across different parts of Asia. Having multi-economic value *Ganoderma* species are a rich source of several bioactive compounds, a decomposer of forest wood aiding in its recycling, and also a phytopathogen that targets perennial trees. It is a highly active source of medicine due to several constituent chemicals of high dietetic and therapeutic value (Rios et al. 2012). They have been exploited as an ancient Chinese remedial source dating more than 2000 years as recorded in the Chinese script of 'Classic of Materia Medica' from the Eastern Han dynasty (25–220 AD), and Ben Cao Gang Mu by Li Shin-Zhen during the sixteenth century of Ming Dynasty.

G. lucidum is mostly constituted of polysaccharides, steroids, and triterpenes as well as alkaloids, fatty acids, glycoproteins, inorganic elements, lignins, nucleosides,

nucleotides, peptides, phenols, proteins, sterols, and vitamins (Boh et al. 2007). These bioactive compounds unveil multi-therapeutic properties ranging from antitumour, cancerostatic, antiviral, antibacterial, anti-inflammatory, antioxidant, anti-platelet aggregation, antidiabetic, hepatoprotective, hypotensive, immunomodulating and immunosuppressive effects (Wasser and Weis 1999; Sliva et al. 2003; Gao et al. 2004; Yuen and Gohel 2005; Zhang et al. 2011; Bakshi et al. 2015; Ma et al. 2015; Chiu et al. 2017).

Applications based on the bioactive compounds isolated from the varying *Ganoderma* spp. are enlisted in the Table 12.1. Constituents isolated from different *Ganoderma* spp. exhibit anti-cancerous properties against different cancer cell lines such as of lungs (Loganathan et al. 2014), breasts (Suarez-Arroyo et al. 2013), liver (Lin et al. 1993) etc., they act as antioxidants to prevent oxidative damage of the cells (Bakshi et al. 2015). The immunomodulation is induced through cytokines and by increasing the immunological effectors (Wang et al. 1997). The extracted polysaccharides are beneficial against diabetes (Jung et al. 2005) and cardiovascular diseases (Gao et al. 2004), also. Ganoderol B obtained from *G. lucidum* deter prostate cancer in male due to its anti-androgenic properties (Liu et al. 2007). Many other isolated chemicals exhibit anti-inflammatory and anti-neurodegenerative properties (Sliva et al. 2003; Xu and Beelman 2015). The mushroom is also a part of habitual Chinese and Japanese nutritional supplement (Dong and Han 2015; Zhao 2015). Their derivatives indicate antimicrobial (Sheena et al. 2003; Wang and Ng 2006) and antiviral bioactivities (El-Mekkawy et al. 1998; Eo et al. 1999), also as antimutagenic (Lakshmi et al. 2006) and are used in cosmetics (Hyde et al. 2010; Jiang 2015). Different *Ganoderma* spp. are farmed on a commercial scale for multiple properties such as anticancerous, anti-inflammatory, antioxidants, cosmeceuticals, nutricosmetics, nutraceuticals, etc. (Jeong et al. 2008; Wu et al. 2016; Chaturvedi et al. 2018).

Although the species is of high medicinal and pharmacological value globally, with '*G. lucidum'* based products ensued a market of US$2.5 billion in 2003 (Chang and Buswell 2008). However, *Ganoderma* may be regarded as a commercially significant phytopathogen due to its severity in causing white rot in woody plants by decomposing their polysaccharide content such as the lignin, cellulose, etc. (Hepting 1971; Adaskaveg et al. 1991; Sankaran et al. 2005). It acutely causes root or butt rot of hardwood trees, mostly in coconut, oil palm, and arecanut, and also affects their plantation in the tropical belt by inducing basal stem rot (Singh 1991; Ariffin et al. 2000; Flood et al. 2000; Pilotti 2005). They also infect the ornamental and forest trees of the tropical and temperate region, triggering wood rots and related diseases. They can populate both as saprophytes and parasites on a variety of hosts resulting in an expanded group of white rot fungi.

Table 12.1 Different properties of *Ganoderma* spp. and their bioactive compounds

Properties	Active compounds	*Ganoderma* spp.	References
Medicinal properties			
Antitumour effect	Ganoderic acid X, Lanostanoid triterpenes	*G. amboinense*	Hsu et al. (2008); Li et al. (2017)
	Terpene (Presiccanochromenic acid, Myrocin C, Sphaeropsidin D, Deoxyherqueinone , Xylariacin B, Trichiol C, Comazaphilone D, Zeylasteral, Erinacine H, Applanoxidic acid C, D, E, F, G, H)	*G. applanatum*	Elkhateeb et al. (2018)
	Triterpenoids (Ganoderic acids, *Lucidum*ol, Lucialdehyde, Lucidenic acids), Polysaccharides	*G. lucidum*	Yuen and Gohel (2005), Zhang et al. (2011)
	Crude extract of *G. tsugae*	*G. tsugae*	Hsu et al. (2018)
Antioxidants	Polysaccharides, triterpenoids, polysaccharide-peptide complex and phenolic component	*G. lucidum*	Bakshi et al. (2015), Kan et al. (2015), Rajoriya et al. (2015)
	Polysaccharides	*G. atrum, G. tsugae*	Tseng et al. (2008), Zhu et al. (2016)
	Lingzhilactone B	*G. sichuanense*	Yan et al. (2015a, b)
Liver and gastric injury	Polysaccharides	*G. Lucidum*	Chiu et al. (2017)
Cardiovascular potential	Polysaccharides	*G. lucidum*	Gao et al. (2004)
Diabetes mellitus	Polysaccharides, proteoglycans, Proteins (LZ-8) and Triterpenoids	*G. amboinense, G. atrum* and *G. lucidum*	Jung et al. (2005), Zhu et al. (2013, 2016), Ma et al. (2015)
Anti-inflammatory properties	Ganodermycin, polysaccharide components	*G. applanatum*	Jung et al. (2011), Vazirian et al. (2014)
	Polysaccharide	*G. atrum, G. tsugae*	Ko et al. (2008), Li et al. (2017)
	G. capense glycopeptide (GCGP)	*G. capense*	Zhou et al. (2014)
	Colosolactones	*G. colossus*	El Dine et al. (2009)

(continued)

Table 12.1 (continued)

	Ganoderic acids T-Q and lucideinic acids A, D2, E2, and P	*G. lucidum*	Sliva et al. (2003)
	Lingzhilactone B	*G. sichuanense*	Yan et al. (2015a, b)
	Triterpenoid-enriched lipids	*G. sinense*	Yue et al. (2008)
Anti-androgenic activity	Ganoderol B	*G. lucidum*	Liu et al. (2007)
Immunomodulation	β-D-glucans, the Zhi-8 proteins and triterpenoids	*G. lucidum, G. microsporum*	Wang et al. (1997), Huang et al. (2018)
Neuroprotective effect	*Ganoderma* side A, B, C and D; Ganolucidic acid A; Ganoderic acid S1; Ganodermic acid TQ; *Ganoderma* triol; 7-oxo-ganoderic acid Z; Methyl ganoderic acid A and B	*G. lucidum*	Weng et al. (2011), Zhang et al. (2011), Zhao et al. (2012), Xu and Beelman (2015)
Traditional Chinese Medicine			
Help re-energise, calm the mind, and alleviate cough and asthma	Triterpenoids and Polysaccharides	*G. lucidum*	Wachtel-Galor et al. (2011)
Cures Palpitation, loss of breath, and sleeplessness relief	Triterpenoids and Polysaccharides	Mixture of *G. lucidum* and *Gynostemma pentaphyllum*	Yan et al. (2015a, b)
Help re-energise, calm the mind, and alleviate cough and asthma	Triterpenoids and Polysaccharides	*G. lucidum*	Wachtel-Galor et al. (2011)
Antimutagenic effect			
Inhibits mutagens	Methanolic extract from the fruiting bodies of *Ganoderma lucidum* (Mutagens inhibited: Sodium azide (NaN3), *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) and 4-nitro-O-phenylenediamine (NPD))	*G. lucidum*	Lakshmi et al. (2006)

Antimicrobial properties (compounds isolated from *G. lucidum*)			
Properties	**Active compounds**	**Tested microbe**	**References**
Antibacterial activity	Ganomycin, triterpenoid	*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella sp., Corynebacterium diphtheria, Enterobacter aerogenes* and *Pseudomonas aeruginosa*	Yoon et al. (1994), Sheena et al. (2003), Keypour et al. (2008), Kamble et al. (2011), Heleno et al. (2013), Shah et al. (2014), Singh et al. (2014)
Anti-fungal activity	Ganodermin	*Botrytis cinerea, Physalospora piricola* and *Fusarium oxysporum*	Wang and Ng (2006)
		Trichoderma viride	Heleno et al. (2013)
Antiviral activity	GLhw, GLMe-1, 2, 4 and 7; Acidic protein bound polysaccharides (APBP) and Neutral protein bound polysaccharide (NPBP); Proteoglycan (*G. lucidum* proteoglycan (GLPG))	VSV (vesicular stomatitis virus), HSV-1 and HSV-2 (herpes simplex virus 1 and 2)	Eo et al. (1999), Oh et al. (2000), Liu et al. (2004)
		HSV-1 and HSV-2	Oh et al. (2000)
		Hepatitis B virus	Li and Wang (2006)
		Varicella zoster virus	Hijikata and Yamada (1998)
Anti-HIV activity	Triterpenoids, Lucidenic acid O, Lucidenic lactone, Ganoderiol F, *Ganoderma*nontriol, Ganoderic acid B, ganoderiol B, Ganoderic acid C1, 3β-5α-dihydroxy-6β-methoxyergosta-7, 22-diene, Ganoderic acid-β, Ganoderic acid H , Ganoderiol A, Ganolucidic acid A, *Lucidum*ol B, Ganoderic acid-β, *Ganoderma*nondiol and *Ganoderma*nontriol laccases	Anti-HIV-1 protease activity and inhibits HIV-1 reverse transcriptase	El-Mekkawy (1998), Min et al. (1998), Smith et al. (1998), Gao et al. (2003)

(continued)

Table 12.1 (continued)

Cosmetic products		
Properties	***Ganoderma* spp.**	**References**
Skin lightening	*Ganoderma lucidum*	Hyde et al. (2010), Jiang (2015)
Tyrosinase inhibition activity		Chien et al. (2008)
Stimulate hair growth by lowering Dihydrotestosterone		Meehan (2015)
Skin anti-ageing		Taofiq et al. (2016)
Foods and dietary supplements		
Soup	*G. lucidum* with *Panax ginseng*	Dong and Han (2015), Zhao (2015)
Tea	*G. lucidum* with *Lonicera japonica, Crataegus pinnatifida, Lycium barbarum*	
Wine	*G. lucidum* mixed with *Panax notoginseng*	
Yoghurt	*G. lucidum*	
Role in forest ecosystem		
Cellulose, related polysaccharides and Lignin decomposers and recycling of nutrients	Many *Ganoderma* spp.	Hepting (1971), Adaskaveg et al. (1991), Sankaran et al. (2005)

12.3 Species Diversity and Distribution

First announced in 1881 by Peter Adolf Karsten, the genus *Ganoderma*, with *Ganoderma lucidum* as its type species, comes from the *Ganodermataceae* family and order *polyporales* of *Basidiomycota*. The family incorporates eight different genera classified on the basis of their unique double walled basidiospores. The two subgenera of *Ganoderma* (Moncalvo and Ryvarden 1997) include

- Subgenus *Ganoderma* based on *Ganoderma lucidum* for lactate species.
- Subgenus *Elvingia* based on *Ganoderma applanatum* for with non-lactating fruiting bodies.

Polypore basidiomycetous fungus with a double-walled basidiospore belongs to the *Ganodermataceae* family (Donk 1964). The genus *Ganoderma* has been ascribed to 219 species in the family, with *G. lucidum* (W. Curt.: Fr.) P. Karsten as the type species (Moncalvo 2000). The *Ganoderma* species has a wide variety of possible hosts, infecting more than 44 species from 34 plant genera. In all, 300 species of *Ganoderma* have been identified and are found in tropical and temperate regions of Asia, America, Africa and Europe. They have a large host range and a lot of genetic variation. Different species have different characteristics and pathogenicity. Table 12.2 provides a fully updated list of various species found in different areas of the world. The research reveals that *Ganoderma* has a wide range of host specificity. The two species, *G. applanatum* and *G. lucidum*, have the widest host range.

Turner (1981) identified 15 *Ganoderma* species from Africa, India, Malaysia, America, Papua New Guinea and Thailand as being associated with oil palm basal stem rot, including *G. applanatum, G. boninense, G. chalceum, G. cochlear, G. lucidum, G. miniatocinctum, G. pseudoferreum* (*G. philippi), G. tornatum (G. australe), G. tropicum and G. zonatum. Ganoderma boninense* is the most virulent pathogen that causes oil palm basal stem rot (Wong et al. 2012).

- The comparative abundance of *Ganoderma* diversity reveals that tropical nations have larger range with a total of 23 species recorded from Africa, including *G. cupreum, G. steyaertanum, G. weberianum and G. zonatum* (Kinge et al. 2015).
- China has 13 species, with *G. ellipsoideum* being the most recently discovered species in Hainan Province (Hapuarachchi et al. 2018).
- And a total of 7 species have been identified from Iran (Moradali et al. 2007), 6 from Australia including a new species, *G. steyaertanum* (Smith and Sivasithamparam 2003).
- *Ganoderma* species are extensively dispersed in India, with the exception of a few species such as *G. multicornum, G. sessiliformae and G. perzonatum*, which are confined to certain areas.
- Bakshi (1971) contributed to the study of this genus in India, reporting five species, namely *G. applanatum, G. austral, G. colossum, G. lucidum*, and *G. philippi*.

Table 12.2 Morphological features of different *Ganoderma* spp

Ganoderma species	Morphological features							
	Stipe	Colour of pore surface	Upper surface	Context	Hyphal system	Generative hyphae (width (μm) and colour)	Skeletal hyphae (width (μm) and colour)	Curtis type
G. applanatum	Absent	Whitish	Woody to corky, applanate	Thick, purplish brown, shining	Trimitic	3.3–4.1, pale yellow with clamp connection	5.8 to 6.6, dark brown	Trichodermis
G. chalceum	Absent	Coffee colour	Reddish brown, laccate, highly sulcate, with crust	Coffee	Dimitic	3.5 μm, hyaline, thin walled with clamps	7.5 μm, brown	Claviform29.1–32.8 × 5–5.5 μm
G. curtisii	Subsessile to stipitate	Brownish	Variegated from ochraceous buff to carbo brown	Milky coffee ochraceous buff to ochraeous	Trimitic	2.5 μm, yellowish	5 μm, brown	Claviform 33.3–41.6 × 6.6–8.3 μm
G. lipsiense		Milky coffee	Slightly zonate, pulverulent glabrous	Reddish brown	Trimitic	3.3 μm, yellow	5 μm, brown	Trichodermis
G. lucidum	Subsessile to laterally stipitate	Creamish to milky coffee	Laccate, dark reddish, purplish, yellowish	Brown without horny deposition	Trimitic	3.3 μm, hyaline, with clamp connection	5.8 to 7.5 μm, brown coloured	Claviform type35–42 × 6–8.5 μm
G. multicornum	Subsessile to laterally stipitate	Creamish brown	Slightly sulcate and zonate, reddish black	Cocoa coloured	Dimitic	3–5 μm, hyaline, clamps present	15 μm, yellowish brown	Diverticulatetype50–65 × 4.5–6 μm

G. multiplicatum	Stipitate	Creamy white	Concentrically sulcate, brown of chestnut	Snuff brown, shiny	Dimitic	3.8 µm, hyaline with clamp connection	5.8–7.5 µm, yellowish green colour	Diverticulate28–30.8 × 14 µm
G. orbiformum	Dimidiate to sessile	Creamy white	Flat to concave, sulcate, glabrous, laccate	Umber	Dimitic	3.3–5 µm, pale yellow with clamp	5–6.6 µm, pale brown	Diverticulate40–46 × 9–12 µm
G. perzonatum	Dimidiate	Greyish brown	Glabrous, laccate, sulcate	Ochraceous brown	Dimitic	3.3–4.1 µm hyaline, thin walled, with clamp connection	5–6.6 µm, yellowish	Diverticulate45–51 × 6–10 µm
G. philippi	Sessile, non-laccate	Greyish brown to dull brown	Dull brown, milky coffee	Soft, shiny	Trimitic	5–58 µm, clamps connection	6.6–7.5 µm, brown	Similartoplecodermis
G. praelongum	Rarely umbonate to stipitate		Glaberous, sulcate, laccate, bay to brownish	Soft, without melanoid deposition	Trimitic	3.3 µm	5 µm, thick walled, brown	Diverticulate20–38 × 8.3–9 µm
G. resinaceum	Stipitate, dimidiate	Creamish brown	Bay to wine coloured, slightly zonate, laccate, glabrous when fresh	Ochraceous brown	Dimitic	3.8–4.5 µm, hyaline with clamp connection	7.5 µm, thin brownish yellow	Claviform 37.5–40.6 × 4.1–5 µm
G. sessiliforme	Stipitate	Yellowish white	Sulcate, brittle, rugose, reddish brown	Pale yellow	Trimitic	2.5 µm, by line clamp connection	5 µm, pale yellow	Spheroid pendunculate, 28–30 × 4.1–8.3 µm

(continued)

Table 12.2 (continued)

Ganoderma species	Morphological features							
	Stipe	Colour of pore surface	Upper surface	Context	Hyphal system	Generative hyphae (width (μm) and colour)	Skeletal hyphae (width (μm) and colour)	Curtis type
G. stipitatum	Stipitate	Milky coffee	Flat, sulcate, glabrous, laccate, reddish to chestnut	Bay colour with melanoid	Dimitic	3.3 to 4.1 μm hyaline, clamp connection	5–5.8 μm thick walled, brown	Spheroid pendunculate, 26–40 × 6–8 μm
G. testaceum	Sessile to subsessile	Cream to white yellowish	Brown	Deposits of melanoid substance	Trimitic,	3–4 μm, hyaline with clamp	5–6 μm, yellowish brown	
G. subresinosum	Stipitate	White to creamy	Surface irregular/ wrinkled, blackish blue					

- Additionally, *G. tronatum* was described by Steyaert (1972), and three more species, *G. adspermum, G. annulare, and G. leucophaeum,* were identified by Bilgrami et al. in 1991. In their list, 'Fungi of India,' Bilgrami (1991)) included seven *Ganoderma* species. Considering the taxonomic status of *Ganoderma* in India established 9 genuine species, of which Bhosle et al. found 5 species namely G. *lucidum*, *G. applanatum, G. philippi, G. multiplicatum, and G. resinaceum* (2010). As per Sankaran et al. (2005), the status of majority of the *Ganoderma* species documented from India has been identified solely on the basis of morphological and cultural features. A study of the literatures reveals that there have been a total of 20 species described so far. *G. chalceum, G. curtisii, G. lipsiense, G. multicornum, G. multiplicatum, G. orbiformum, G. perzonatum, G. praelongum, G. sessiliformae, G. stipitatum, and G. testaceum* are among the 11 new species described by Bhosle et al. (2010).

12.4 Cultural and Morphological Characteristics

Cultural features like chlamydospore generation, growth rate, and thermophily have been utilised to distinguish *Ganoderma* species in addition to basidiocarp shape. The culture colony of *Ganoderma* appears white to pale yellow, even felty to floccose, and becomes more yellowish when exposed to light. The cultures develop at various optimal temperatures depending on the species. Various hyphal structures, including generative hyphae with clamp connections, skeletal hyphae, stag-horn hyphae, vesicles, and hyphal rosettes, as well as chlamydospores, are produced by *Ganoderma* species in culture. Chlamydospore generation, growth rate, and thermophily are the main culture-specific features utilised to identify *Ganoderma* species (Seo and Kirk 2000). A significant number of scientists have tried using cultural characteristics to differentiate between *Ganoderma* species. The use of only the cultural morphology in *Ganoderma* taxonomy might lead to erroneous findings and different classification than based on the outcomes of morphological features-based identifications. Individual members of the *Ganoderma* species are distinguished by characteristics such as the shape and colour of the fruit body (red, black, blue/green, white, yellow, and purple), host specialisation, and geographical origin (Zhao and Zhang 1994; Woo et al. 1999; Upton et al. 2000). Murrill (1902, 1903), Atkinson (1908), and Coleman (1927) all applied a combination of taxonomic criteria to identify their subjects. Steyaert (1972, 1980) studied the genus from almost every continent on the planet. Ryvarden (1994) questioned the morphology of *Ganoderma* by examining morphological differences in 53 *G. lucidum* specimens from Norway. By assessing various morphological characteristics it was concluded that for correct basidiocarp shape and size at least 3–5 samples should be investigated. The colour of the pileus and stipe changes with age and should be taken into account. Pore size can be considered as an important taxonomic feature since it remains consistent. Because the colour of the pore surface and surroundings varies with age, specimens of various ages should be studied. The hyphal system was found to be less useful because the majority of G. *lucidum* species have a Trimitic

Fig. 12.1 Fruiting body of Ganoderma spp. on **A** Ficus tree **B** Arecanut **C** Indian rosewood

hyphal system; however Ryvarden (2000) found 15 species with a Dimitic hyphal system from the *lucidum* group. Ryvarden (1994) found that unless there are conspicuous microscopic characteristics combined with unique macromorphological features, low samples ranging from 1 to 2 samples are inadequate to identify a species. *Ganoderma* is the most complicated genus in the *Ganodermataceae* family, and it is split into two subgenera. The genus has been divided into two groups based on the presence of laccase: *G. applanatum* complex and *G. lucidum* complex.

Laccase-positive specimens are classified as G. *lucidum*, whereas laccase-negative specimens are classified as *G. applanatum*. For taxonomical categorisation, basidiocarpic features such as context colour, bracket size, and bracket form were taken into account. Furthermore, basidiocarp size and form, pilear colour, hyphal system and features including generative hypha and clamp connections, shape and size of apical pilear cells, and pore size have all been used to distinguish the species (Ryvarden 1994) (Fig. 12.1). Environmental changes cause morphological variations in basidiocarp development, resulting in substantial variation in size and colour of the basidiocarps across specimens while pore diameters remain unchanged. Sessile, stipitate, imbricate, and non-imbricate morphological features have been seen in naturally occurring G. *lucidum* basidiocarps (Seo and Kirk 2000). The colour of the pileus surface and hymenophore ranges from deep red (non-laccate: laccate) to light yellow to white, and the shape of the isolates varied as well (Shin and Seo 1988). While the typical fruit body is laterally attached to the stipe, eccentric, central, imbricate, and sessile fruiting bodies are seldom formed. Ryvarden (1994) observed considerable differences in the stipe attachment pattern of pileus and the host range. Despite the fact that mycologists who only described fruit bodies as stipitate or sessile had overlooked its significance, stipe characteristics such as attachment type, relative thickness and length were thought to be relevant for species identification. In the taxonomy of this family, the laccate feature of the pileus and stipe has been used in several ways. The colour of the context varies from white to dark brown, and the colour changes with age. Unfortunately, owing to variances in cultivation in different geographical areas under varying climatic circumstances and natural genetic evolution (e.g., mutation, recombination) of particular species, physical features

vary. As a result of the use of macroscopic features, this mushroom now has a huge number of names and a confusing, overlapping, and ambiguous taxonomy. Because of differences in environmental circumstances during growth, the shape of the basidiocarps may change between isolates (Seo and Kirk 2000). The morphological and biological characteristics of *Ganoderma* species are illustrated in Fig. 12.1 and Table 12.2.

12.5 Molecular Characterisation

Across the globe, great diversity is observed in *Ganoderma* species w.r.t. phenotype (shape and colour of the fruit body), host specificity, and geographical origin; that are used to differentiate the individual members of the species (Zhao and Zhang 1994; Woo et al. 1999; Upton et al. 2000). However, morphological features of particular species are prone to change owing to variability in agriculture in different geographical areas under varying climatic regions and natural genetic factors (e.g., mutation, recombination). Due to the use of macroscopic features, this mushroom has a large number of names and a conflicting, overlapping, and imprecise nomenclature. Traditional taxonomic approaches have been ineffective in creating a stable taxonomy for the group, and are unhelpful in defining individual strains. Traditional techniques of identifying wood-decay fungus from decaying trees are challenging due to morphological differences across various populations of this species. In researching these macrofungi, there are taxonomic ambiguities due to a lack of unifying criteria. As a result, protein- and DNA-based markers have become common in *Ganoderma* taxonomy and characterisation at all levels (Bruns et al. 1991). Enzyme gel electrophoresis, Ribosomal DNA (rDNA) sequencing (Moncalvo et al. 1995a; Gottlieb et al. 2000), Internal Transcribed Spacer (ITS) sequencing (Hseu et al. 1996), have been carried out to evaluate the genetic relatedness of *Ganoderma* species complex. For instance, Bonde et al. (1993), Gottlieb et al. (1995, 1998), Mwenje and Ride (1996, 1997) and Gottlieb and Wright (1999) used isozyme analysis to distinguish various *Ganoderma* species.

Smith and Sivasithamparam (2000) used cellulose acetate gel electrophoresis (CAGE) and Polyacrylamide gel electrophoresis (PAGE) to investigate isoenzymes from five Australian species. Pectinase zymograms for 150 *Ganoderma* strains indicated groups that matched the host type from which the strains were acquired. Isolates from palm hosts (*Elaeis guineensis*, *Cocos nucifera*, *Areca catechu, Oncosperma horridum and Ptychosperma macarthurii*) eventually formed a single large cluster (cluster A), wherein palm-derived isolates accounting for 99% of the isolates. Within this functionally defined category, there were no significant differences across isolates obtained from widely different geographic areas such as Colombia, Nigeria, Malaysia, and the Solomon Islands. A second cluster (group B) similarly had a high percentage (85%) of palm-derived isolates (Miller et al. 1995a). *G. lucidum* has been differentiated from several other temperate *Ganoderma* spp. based on intracellular esterase isozymes (Park et al. 1986; Tseng and Lay 1988). *G. applanatum, G. boninense, G. formosanum, G. fornicatum, G. microsporum,*

G. neojaponicum, G. tropicum and G. tsugae isolates can be distinguished by intracellular and extracellular laccase isozymes, according to Hseu et al. (1989), and *Ganoderma* isolates from perennial regions were characterised using intracellular catalase, acid phosphatase, and propionyl esterase profiles. In contrast to pectinase-derived groups, which showed no clear connection to the source host, these isozymes displayed widespread genetic variation in isolates (Miller 1995; Miller et al. 1995b).

PCR-RFLPs, ITS, and rDNA sequences, among other DNA-based methods, have been found to be more useful in *Ganoderma* taxonomy. Among the various molecular DNA markers, Hseu et al. (1996) employed RAPD–Polymerase chain reaction (PCR) and internal transcribed spacer (ITS) sequences to distinguish the isolates of G. *lucidum* complex. Moncalvo et al. (1995a, b) applied ribosomal DNA sequencing to investigate the evolutionary connections of the *G. lucidum* complex. The ITS sequencing has been found to be effective for distinguishing lineages within the *G. lucidum* complex, and RAPDs were useful in distinguishing species with similar ITS sequences at lower taxonomic levels.

Gottlieb et al. (2000) examined ITS sequences to describe South American *Ganoderma* isolates and observed that morphological and molecular data were in accord at the subgeneric level. ITS markers were also applied by Wang and Yao (2005) to identify genetic diversity among *Ganoderma* isolates. ITS sequences were used to identify two biological species of *G. adspersum* and *G. cupreum* from the southern portion of India (Arulpandi and Kalaichelvan 2013). Based on ITS 1 and 2 sequences from Mizoram, six *Ganoderma* species, *G. lingzhi, G. mastoporum, G. mizoramense, G. multipileum, G. subresinosum and G. williamsianum*, were identified to the species level (Zohmangaiha et al. 2019). Park et al. (2012) used the ITS and partial tubulin regions of Korean *Ganoderma lucidum* isolates to differentiate them from isolates of China, Taiwan, and Canada. Haroun et al. (2020) used ITS regions to describe *G. lucidum* isolates from Abuja and Nigeria.

Ganoderma-specific primers (Gan1 and Gan2), as well as a PCR method developed by GanET and ITS3, were used to identify *Ganoderma* infection early (Utomo and Niepold 2000; Mandal et al. 2014; Rajendran et al. 2014). Tang et al. (2005) utilised RAPD and isoenzyme esterase to analyse genetic diversity in a species complex and found that species clustered into three groups based on RAPD analyses: the first group included *G. lucidum, G. resinaceum*, *and G. lucidum* var *xinzhou*; the second group included *G. subamboinense*; and the third group included *G. applanatum*. RAPD and PCR-RFLP techniques were used to examine *Ganoderma* isolates from *G. boninense, G. philipii* and *G. australe*, and it was discovered that species of the same host from different parts of Peninsular Malaysia clustered together. Despite the high degree of similarity among *G. boninense* isolates, RAPD analysis revealed differences (Zakaria et al. 2009). Miller (1999) and Karthikeyan et al. (2007) performed RAPD analyses of *Ganoderma* spp. in a similar fashion (2009). Zakaria et al. (2005) adopted random Amplified Microsatellites (RAMS) markers in addition of RAPD markers to examine genetic diversity among *G. boninense* isolates. Sun et al. (2006) used polymorphic sequence related amplified polymorphism (SRAP) markers to differentiate *G. lucidum* from

G. sinense and discovered *G. lucidum* isolates differed between China and Yugoslavia. Su et al. (2008) created a *G. lucidum* strain 9 unique sequence characterised amplified region (SCAR) marker to identify it from other strains, it has inter simple sequence repeats (ISSR). The ISSR method was used by Praphruet and Peangon (2010) to detect genetic polymorphism among nine *G. lucidum* isolates. The *Ganoderma* taxa were identified using PCR-RFLPs of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) (Bruns et al. 1991; Bunyard et al. 1996; Nicholson et al. 1997). Zheng et al. (2009) used Amplified Fragment Length Polymorphism (AFLP) and ITS-PCR-RFLP to uncover *Ganoderma* spp. taxonomic diversity. Moncalvo et al. (1995a, b), Latiffah (2001), and Utomo and Niepold have all demonstrated that ITS-PCR-RFLP is an effective technique for studying genetic diversity in *Ganoderma* (2000). Multiplex PCR (MPCR) is a method for identifying several *Ganoderma* species in a single test (Wong et al. 2012). DNA microarray, which employs an electrochemical DNA biosensor, is another intriguing approach for detecting *G. boninense* (Dutse et al. 2013).

Two new *Ganoderma* species, *G. angustisporum and G. casuarinicola*, were discovered and described in South-Eastern China based on phylogenetic analyses of sequences of the Internal Transcribed Spacer (ITS) region, the translation Elongation Factor 1-gene (EF1–1), and the second subunit of RNA polymerase II (RPB2) (Xing et al. 2018).

A multilocus phylogenetic approach was established based on the analysis of four separate loci (ITS, tef1a, rpb1, and rpb2), which were further utilised to morphologically compare 13 different species, namely *G. boninense, G. curtisii, G. flexipes, G. lingzhi, G. lucidum, G. multipileum, G. oregonense*, and *G. resinaceum* (Zhou et al. 2015). Using ITS molecular phylogeny, Wang et al. (2009) segregated Asian *G. lucidum* specimens into two clades, each of which was differentiated from European *G. lucidum* (Clade D) and *Ganoderma tropicum* from Taiwan (Clade C). One clade (Clade A), which comprised of tropical specimens was represented as *G. multipileum*, whereas the other clade (Clade B) was unknown (Wang et al. 2009). Later, *G. lingzhi* was the name given to this hitherto unidentified clade (Wang et al. 2012).

The research into the ITS and other conserved gene areas is going to be helpful in providing information on *Ganoderma* species diversity in various ecosystems. It will also serve as a valid tool for phylogenetic analyses and inter- and intraspecific characterisation of the *Ganoderma* species complex. ITS sequencing might also give a starting point for the creation of new molecular markers for the accurate identification of the therapeutic *Ganoderma* spp. complex, as well as for determining host specificity and distribution of virulent *Ganoderma* species. For example, the creation of genetic markers for particular strains, as well as an accurate identification system and phylogeny based categorisation of *Ganoderma* species, would have practical consequences in epidemiological research, the wood industry, and medicine. It would, for illustration, aid in the monitoring of fungal proliferation inside and between fields, as well as bioprospecting for novel genes and metabolites, as well as providing relevant information for genetic engineering and commercial strain breeding.

12.6 Detection Methods of *Ganoderma*

Symptoms such as fading and drooping of mature leaves, stem oozing, or the appearance of pathogen basidiomata on the tree are now the only method to diagnose sickness visually (Lelong et al. 2010). However, by then, it gets too late for any management measures, therefore early diagnosis of disease is critical. For early detection, a colorimetric method, utilising ethlylene di-amine-tetraacetic acid (EDTA) was used (Natarajan et al. 1986). Another approach employed in the early days was semi selective medium for detecting infection by growing fungus (Darus et al. 1993). Antibodies were employed by Reddy and Ananthanarayanan (1984) to detect *Ganoderma* in culture media. Many novel early detection methods were created throughout the PCR and Post-PCR eras. For the identification and detection of *G. boninense*, Utomo and Niepold (2000) employed an enzyme linked immunosorbent test (ELISA) using polyclonal antibodies and PCR, as well as cultural characteristics, but the ELISA findings were found to be false negative. Monoclonal antibodies were created and tested against *G. boninense*, with promising results (Shamala et al. 2006). Madihah et al. (2018) utilised loop mediated isothermal amplification (LAMP) for early detection, and *G. boninense* and nonpathogenic *G. tornatum* were successfully distinguished. The 18 s rDNA gene is viewed as a marker gene for infection detection as well as biodiversity and phylogenetic research (Meyer et al. 2010). The function was served by DNA-based nanosensors (Dutse et al. 2013), Microfocus X-ray fluorescence (MeorYusoff et al. 2009), electronic nose (e-nose) device (Markom et al. 2009; Abdullah et al. 2011), and Terrestrial laser scanner (TLS) device (As'wad et al. 2011; Muniroh et al. 2014; Azuan et al. 2019). Ergosterol content is utilised as a biomarker for primary prevention of *Ganoderma* infection in oil palm. Early diagnosis of diseased trees was done using electrical resistance (Nurnadiah et al. 2014). Secondary metabolites were used by Nusaibah et al. (2016), however no meaningful findings were obtained. A headspace solid-phase microextraction (HS-SPME) method coupled with gas chromatography–mass spectrometry (GC–MS) was utilised to identify infection utilising volatile organic molecules (ZainolHilmi et al. 2019). The method of remote sensing was also used to identify and quantify illness (Khosrokhani et al. 2018).

12.7 *Ganoderma* as a Pathogen

Ganoderma is a natural wood rotting fungus that is encountered all over the world. As an infectious agent, it causes stem and root rots in most ecologically important plantation crops (coconut, arecanut, rubber, coffee, tea, oil palms, and so on) in the tropical regions, as well as wood rots in ornamental and natural forest trees (*Acacia, Macadamia, Populus*, and so on) in tropical and temperate regions (Palanna et al. 2012). Coleman (1911) initially recorded the breakdown of *Ganoderma* species in arecanut in India, while Butler (1913) reported the assault of *Ganoderma lucidum* on coconut palms in Karnataka in 1913, producing basal stem rot (BSR). In different

parts of India, BSR is known as Thanjavur wilt in Tamil Nadu, *Ganoderma* wilt in Andhra Pradesh, Anaberoga, bole rot or foot rot in Karnataka, and so on.

The most destructive disease in oil palms, BSR, causes substantial output losses, particularly in India's southern regions. According to estimates, basal stem rot causes annual economic losses of almost USD 0.5 billion (Jee and Chong 2015; Ahmadi et al. 2017) due to direct stand loss, reduced infected palm output, and increased replant frequency.

12.7.1 Symptomatology

Ganoderma rot is the most destructive disease of cultivated plants and trees. *Ganoderma* may infect plants from seedlings to elderly trees, although palms and forest trees between the ages of 5 and 30 are particularly vulnerable (Kandan 2003). The disease progresses slowly, with affected plants dying eventually. After many years of infection, the disease becomes apparent; nevertheless, outward disease signs are not readily apparent from the outset (Mawar et al. 2020). After several years of incubation, obvious illness signs develop at the late stage of infection, leaving little possibility of healing the afflicted plants (Bhaskaran and Ramanathan 1983). The virus may also infect immature nursery oil palms, demonstrating the disease's potential to spread from old plantations to nursery seedlings (Wong et al. 2012). *Ganoderma*, a silent killer pathogen, may infect all stages of plants and cause a gradual disease progression, although visible signs might develop late in the infection process, resulting in massive crop loss (Naher et al. 2011).

The appearance of unopened spears, yellowing, and shortening of younger fronds in oil palms indicate early disease development (Turner 1981, 1981). *Ganoderma* infects the root system first, and then spreads to the coconut trunk's basal part with reddish-brown exudation. The number of bleeding patches rises as the illness progresses. The crown portion of the coconut starts to wilt, display yellowing, and drooping of the peripheral fronds persists around the trunk at a later stage. Emerging new fronds turn yellow, shrink in size, fronds fail to unfurl correctly, and flower bunches and roots' development is slowed (Kandan et al. 2010). At a mature course of disease in oil palm, the older fronds collapse and die, and the virus spreads to the younger crown areas, resulting in lower yield output (Gorea et al. 2020). The illness causes colouring and rotting of roots, which leads to root disintegration and an alcohol odour in the ultimate stage. At the root of diseased trees, basidiocarps/sporophores/conks are formed (Wong et al. 2012). When one-half of the stem base is infected by the pathogen, foliar symptoms appear; affected trees generally die after 6–12 months of foliar infection (Turner 1981). Internally infected tissues showed a bright yellowish-brown zone linked with host cellular function of vesicular budding onto the outer membrane, signifying the generation of suberisation and lignification (Rees et al. 2009a, b).

The symptomatic manifestation can be visible at many levels, including the stem, crown, and roots.

12.7.1.1 On Stem

- The infected trees leak sticky reddish-brown exudates up to 3 metres from the base section of the stem, which is the first apparent sign.
- Bleeding patches are visible from the bottom up, and as the illness progresses, those bleeding patches expand higher.
- Up to the height of exudation, discoloration (bleeding) and internal rotting of the stem can be seen.

Eventually, in the later phases of infection, the stem's basal part decays entirely. Basal stem rot, often known as the 'silent killer of palms,' is a disease in which the basal sections of the stem get infected and internal rotting may be observed where the leaves of the afflicted plant seem alive (Palanna 2016).

The fructification of the fungus (Basidiocarp) located horizontally connected to the palm stem at the base slightly above the ground level, which might be called a bracket, in the advanced stage of the illness. Bracket is initially a solid white mass that is relatively soft, but as it ages, the basidiocarp protrudes from the tree trunk and creates a shelf-like structure that is firm, semicircular and has a reddish-brown upper surface and a white undersurface (Hennessy and Daly 2007). The most significant diagnostic symptom is the formation of brackets, which can occur individually or in clusters. The illness is also known as anaberoga in palms because of the development of this structure.

12.7.1.2 On Crown

- Wilting signs, such as yellowing and drooping, are seen on the leaflets in the outermost whorls.
- Outer fronds linger for several months before shedding, and in the case of forest trees, leaf shedding is noticeable.
- Newly generated fronds are smaller and chlorotic, with a large number of unopened fronds (spear leaves) visible, flattening the crown.
- In the later stages of infection, the entire crown is blown off, leaving just the decapitated stem left.
- In the infected palm, flower development is disrupted, and button shedding is visible. In certain cases, decomposition of buds might occur, resulting in a foul odour.

12.7.1.3 On Roots

- The most common sign is severe root deterioration and death.
- Discoloration and severe rotting cause cortical tissues to disintegrate readily, and roots become liquid and exude an alcoholic odour.
- The development of new roots is also harmed, resulting in the demise of the afflicted palm. Young infected palms perish between 6 and 24 months, but developed palms might take up to 2–3 years to die (Ariffin et al. 2000).

Ganoderma induces wood rot in woody plants and white rot in hardwoods via delignification, or the breakdown of woody tissues (Peries 1974). As a result,

discoloured zones may be seen in the wood. The wood breaks down entirely, becomes soft or spongy, and eventually loses its tensile strength and dies at the advanced stage of decay.

12.7.2 Epidemiology

The occurrence of *Ganoderma* disease is mostly dependent on soil type, palm age, prior crops, climatic conditions and soil nutrition.

12.7.2.1 Soil Type

The disease is most common around the coastline, where the soil is sandy or sandy loamy in character. In lighter soils, illness incidence was higher than in heavier soils (Satyanarayana et al. 1985). Water stagnation was used by Srinivasulu et al. (2003) to prevent basal stem rot in coconuts. The growth of a hard pan in the subsoil prevents root penetration, making the palms more susceptible to *Ganoderma* infection. Several investigations also showed that oil palms planted in lateritic and inland soils had a high prevalence of BSR (Benjamin and Chee 1995).

12.7.2.2 Age of the Palm

Palms and forest trees that are 5–20 years old have a higher disease incidence than younger plants (Palanna et al. 2012).

12.7.2.3 Previous Crops

Because of the presence of inoculum on trunk tissues and stumps left behind in the field, which acts as a main source of root infection, acute outbreaks of the disease can be seen in regions where oil palms are planted followed by coconut and also when the oil palm is replanted from oil palm (Turner 1981). Oil palms that were 15 years old had a high prevalence of basal rot.

12.7.2.4 Environmental Factors

The illness incidence is seen to be higher mostly during months of March to August (Bhaskaran et al. 1985). In locations with significant rainfall and low relative humidity, the incidence of BSR was lower. The spread of BSR of coconut has a negative relationship with rainfall and the amount of rainy days (Palanna et al. 2012). The severity of the infection rises as the soil temperature rises and falls when the soil moisture rises.

12.7.2.5 Soil Nutrition

Prevalence of disease is also influenced by soil nutrition. Potash muriate and rock phosphate both increase illness incidence, but urea has the opposite effect (Singh 1991). Infected palms have lower amounts of macronutrients such as nitrogen, phosphorus, and potassium, and higher levels of magnesium than healthy palms.

12.7.3 Lifecycle of *Ganoderma* Species

Ganoderma species are soil-borne facultative parasites that thrive saprophytically on decaying roots and stumps before becoming pathogenic when appropriate substrates are available (Rees et al. 2009a, b). *Ganoderma* species have a long life cycle because the pathogen is soil-borne and may persist for a long period in the soil. They generate chlamydospores (asexual spores; *Thermophymatospora* anamorph) to live in harsh environments, which are more resistant than basidiospores and aid in disease transmission.

The major mode of infection is root-to-root contact, but secondary inoculum is found in the soil as basidiospores or chlamydospores, which can be disseminated by rain splash and wind (Wahab and Aswad 2015). The hyphae develop over the palm roots after airborne basidiospores are released from brackets and absorbed into the soil. The roots are not initially harmed; instead, the fungus utilises them to infiltrate the hardwood tissues of the trunk. Monokaryotic hyphae combine to create dikaryons, which infect tertiary roots, lower frond base and bole (causing basal stem rot), and the frond axil (causing upper stem rot). Palms receive both dikaryotic and monokaryotic mycelia from infected neighbours and basidiospores. The fungus colonises and destroys the trunk tissue after the palm is infected, eventually causing the palm to die and collapse. Within the trunk, dikaryotic mycelia continue to develop and generate sporophores on a bracket-like structure (tertiary mycelium). According to Pilotti et al. (2018), basidiospores are released for a longer period of time (5 months) and are deposited on soil surfaces or trimmed or damaged fronds on standing palms, where they are dispersed passively by rain splash and wind. These spores have a resistant structure that allows them to live in adverse conditions for prolonged periods of time, and when favourable conditions return, the spores germinate and the infection begins anew.

12.8 Integrated Disease Management

12.8.1 Cultural Practices

1. Sanitation: Trees that have been infected with the disease must be removed from the plantations. Basidiomata are removed from diseased palms and fungicidal paste is applied. Diseased tree roots should not be allowed to come into touch with healthy palms, and infected trees can be separated from healthy plants by digging trenches around them (Turner 1981; Chung 2011). Fallowing decreases disease incidence by decreasing inoculums in the soil before replanting (Virdiana et al. 2010).
2. Surgery: Because basidiospores are the most common source of infection, removing diseased tissue or basidiocarps from infected palms in plantations may be advantageous (Sanderson et al. 2000; Pilotti et al. 2018).
3. Ploughing practices: Two rounds of deep ploughing of 60 cm and one round of harrowing to break up residual roots before planting new seedlings in disease-

prone locations, provide enough moisture over the summer in plantations, prevent flood irrigation, avoid close planting, and repeat ploughings in infected fields (Rethinam 1987; Flood et al. 2000).

4. Green Manuring, Inter and cover crops: Green manure crops must be grown and ploughed in place before blooming, increasing the nutrient status of the soil and preventing soil erosion. Ailanthus and banana as inter crops are non-hosts for *Ganoderma* (Rethinam 1987). Care should be taken not to introduce legumes that are prone to *Ganoderma* infection, as well as avoid planting collateral hosts such as *Delonix regia* in close proximity.
5. Soil Amendments: By improving soil characteristics using neem cake and farm yard waste, disease incidence is reduced (Naik 2001).
6. Soil Mounding/Heaping: This method of heaping dirt around the trunk to a height of 75 cm may prolong the tree's life, but it is unsuccessful in preventing stem rot (Ho and Khairuddin 1997). However, when used in conjunction with a chemical technique, it provides better control (Mohammed et al. 2014).

12.8.2 Chemical Control

Hexaconazole can be employed to treat *Ganoderma* infection by infusing it into the wood trunk (Mohammed et al. 2014), however it hasn't been proven to be very successful (Chung 2011). Pathogen exhibits resistance to fungicides in later stages of the infection (Susanto et al. 2005). The use of benzoic and salicylic acid to immunise seedlings reduced disease growth (Surendran et al. 2018). After chizeling out the bleeding tissue, a chemical fungicide and then hot coal tar can be applied to protect the bleeding regions in the stem (Ariffin et al. 2000). Hexaconazole root feeding at three intervals of 3 months has also been proven to be beneficial. A common practice is to drench the tree trunk with Bordeaux mixture or copper oxychloride from a distance of 1.5 metres (Bhaskaran and Ramanathan 1983).

12.8.3 Plant Extracts

Neem, banana rhizome extract, and *Tephrosia purpurea* root extract, according to Bhaskaran et al. (1988), exhibited *Ganoderma* suppressive effects. Glyricidia plant extract proved antifungal *in vitro* against *Ganoderma applanatum*, according to Palanna et al. (2013). Several additional plant extracts, such as *Eichhornia crassipes* against *Ganoderma lucidum* (Deepatharshini and Elango 2015), leaf extracts of *Pongamia glabra*, *Azadirachta indica* and *Prosopis julifera* (Karunanithi et al. 2007) and garlic extract (Srinivasulu et al. 2005), were shown to inhibit the fungus to varying degrees.

12.8.4 Plant Nutrition

Plant production can be improved by a proper dosage of major and minor nutrients (Singh 1990; Chung 2011). Calcium nitrate in conjunction with *Trichoderma* has been proven to be beneficial in preventing stem rot (Sariah and Zakaria 2000). Wang et al. (2017) demonstrated the protective effects of silicon in a variety of plant species against a variety of diseases, including *Ganoderma* stem rot. Potassium silicate, silicon oxide, sodium silicate, calcium silicate, and sodium meta-silicate were found to decrease *Ganoderma* incidence in oil palm by Najihah et al. (2015). Application of manganese sulphate, zinc sulphate, sulphur, and lime to the soil decreased disease incidence (Jaganathan and Ramasami 1975; Bhaskaran et al. 1985; Srinivasulu et al. 2002).

12.8.5 Host Resistance

Oil palm from Zaire and Cameroon cross has been found as a moderately resistant source (Idris et al. 2004; Durand-Gasselin et al. 2005). Tisné et al. (2017) discovered four *Ganoderma* resistance loci in oil palm, two of which controlled the onset of *Ganoderma* symptoms while the other two controlled palm tree death.

12.8.6 Biocontrol Management

Many studies have been done on *Ganoderma* biocontrol application (BCA) and several possible biocontrol agents have been discovered to be effective against the disease. Many fungal bioagents have been discovered and proven to be effective during the nursery stage, such as *Hendersonia* isolate (Nurrashyeda et al. 2018), *Scytalidium parasiticum* (Goh et al. 2016) and *Trichoderma harzianum* (Priwiratama and Susanto 2014). In a nursery trial, bacterial bio agents such as *Burkholderia* sp. (Buana et al. 2014) reduced pathogen incidence for up to 3 months. In a nursery study, *Burkholderia cepacia, Pseudomonas aeruginosa*, *and Serratia marcescens* were shown to suppress *G. boninense* by Sapak et al. (2008) and Azadeh et al. (2010). *T. harzianum* and *G. viride* outperformed *Bacillus* sp. in contrast to untreated areas. In that scenario, *T. harzianum and G. viride* had a reduced frequency of disease in treated areas (Susanto et al. 2005). As a potential defence strategy in the oil palm, *Trichoderma* sp. stimulated the synthesis of fungal-cell-wall-degrading enzymes such as glucanases and chitinases (Naher et al. 2011). As a result, as with other plant diseases, these enzymes may damage the invading fungus' cell wall, limiting illness. Arbuscular Mycorrhizal fungi (AMF) are linked with the roots of oil palm, and it has been suggested that they may resist *G. boninense* (Sundram et al. 2015). AMF competes with plant pathogens for nutrients and space, and it can also activate the plant's defence mechanism by activating siderophores, as mentioned in other plant-pathogen systems (Brundrett 2002). There have been biological control experiments using basidiomycetes to prevent stump infections in forest trees (Roy

et al. 2003). But on the other hand, no similar research has yet been done on BSR-infected oil palm trunks. Naidu et al. (2015) identified 25 white rot hymenomycetes from healthy oil palm, and eight of them showed a combative reaction against *G. boninense. G. boninense* was successfully combated by actinomycetes isolated from empty fruit bunches of oil palms. *Streptomyces violaceorubidus, Nocardiopsis* sp., *and Streptomyces* sp. were discovered, with 91.4%, 86.4% and 69.1% inhibition, respectively (Ting and Jioe 2016). Conflicts with non-target organisms, rhizosphere variation decreasing effectiveness, failure to colonise diverse types of soil, susceptibility to climate, difficulty competing with large populations of other microbes, and the target pathogen's genetic diversity can all cause BCA failures (Vidhyasekaran et al. 1997; Meyer and Roberts 2002). Draz-M, a formulation of an arbuscular mycorrhiza that prolongs the productivity of 25-year-old infected oil palms and improves their oil output by 42% and 68%, is one of the products in the market (Sariah and Zakaria 2000). *Trichoderma koningii* has also been developed for commercial usage in Sumatra as a field preventative or curative therapy (Soepena et al. 2000).

Indian origin *Trichoderma* spp. such as *T. hamatum, T. harzianum, T. longibrachiatum, T. viride, T. polysporum,* and *T. virens, Pseudomonas flourescens,* and *Bacillus subtilis* have all been found to be hostile to the pathogen. *T. hamatum, T. longibrachiatum, T. virens, T. polysporum, and T. harzianum* are efficient in suppressing *G. lucidum* (Bhaskaran et al. 1985; Srinivasulu et al. 2002) and *G. applanatum* (Srinivasulu et al. 2005). Naik et al. (2008) developed Talc-based formulations of *Pseudomonas* and *Trichoderma* combined with neem cake that was effective in treating the illness. Surulirajan et al. (2014) found that *T. viride* talc formulation (200 g/palm/year), neem cake, and TNAU (Tamil Nadu Agricultural University) microbial consortia were all highly useful. Depending on the rhizosphere populations of the biocontrol agents, Karthikeyan et al. (2006) advised using antagonists every 3 months.

12.9 Conclusion and Future Prospects

Ganoderma species have become economically important as a source of bioactive chemicals, a decomposer of forest wood, and a perennial tree plant pathogen. *Ganoderma* species are widespread in tropical regions, causing basal stem rot in plantation crops such as coconut, arecanut, and oil palm (Singh 1991; Ariffin et al. 2000; Flood et al. 2000; Pilotti 2005), as well as disease and wood rots in ornamental and forest trees in tropical and temperate areas (Singh 1991; Ariffin et al. 2000; Flood et al. 2000). Remarkably, *Ganoderma lucidum* is now regarded as one of the most commonly utilised medicinal mushrooms in the world (Rios et al. 2012). In the globe, it is an economically and pharmacologically significant restorative fungus. Traditional taxonomic approaches have been ineffective in creating a stable taxonomy for the group, and they are unhelpful in characterising individual strains. Traditional techniques of identifying wood-decay fungus from dying trees are challenging due to morphological differences across various populations of this

species. In researching these macrofungi, there are taxonomic ambiguities due to a lack of unifying criteria. The use of a polyphasic approach to taxonomy and characterisation is urgently needed to resolve nomenclature ambiguities. *Ganoderma* also possesses medical benefits, and the biologically active chemicals responsible for these capabilities can possibly be investigated and confirmed. Because the taxa are so diverse, bioprospecting for various economic properties is critical. Because the majority of these qualities are found in papers, they must be industrially utilised, and tested goods must be appropriately commercialised. Because *Ganoderma* is a slow-growing mushroom, prospective strains of these helpful species that can develop at a quicker pace must be discovered, and the efficient strains or species must be preserved in appropriate Culture collections. Effective management techniques for *Ganoderma* wilt and stem rot are still missing, and these procedures are primarily undertaken in economically significant hosts, despite the fact that the disease is more widespread in forest ecosystems. Since a result, the focus should be on testing effective control strategies, as failing to do so would result in significant economic losses for farmers in the near future, and there is still time to improve efficient early detection systems. There is also a scarcity of data on *Ganoderma* spp. productions of volatile organic compounds (VOCs).

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Exploring Marine Fungal Diversity and Their Applications in Agriculture

13

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Abstract

Marine ecosystem comprises of highly diversified microbial communities, predominantly bacteria, fungi, viruses, and actinomycetes. Fungi are ubiquitous in nature, though certain obligate species inhabitant in both marine flora including plants (notably macroalgae and mangrove plants) and fauna including marine invertebrates (e.g., Sponges, Corals, Ascidians, Holothurians, Bivalves, and Crustaceans), marine vertebrates (mainly Fishes), and inorganic matter (sediments and seawater). Marine fungal communities are the hub of novel bioactive compounds essential for devising and enhancing the study of new technologies to benefit both agriculture and pharmaceutical industries. They exhibit a wide diversity in producing secondary metabolites based on the niches they are attached to. It is noteworthy that only a few studies have been conducted on marine fungi and their agricultural applications. This chapter discusses in detail about marine-derived fungi and their limited applications especially in agriculture management practices, which would be a promising strategy for replenishing the existing environmental crisis by replacing the usage of chemical fertilizers.

Keywords

Marine fungal diversity · Seaweed · Agricultural applications

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_13

13.1 Introduction

Fungi are one of the most important microbial communities in the ecosystems and they occupy a wide variety of environments by virtue of their highly versatile physiology (Gostincar et al. 2010; Tedersoo et al. 2014). There are 1.5 million species of fungi on Earth, approximately; of which, less than 10% have been described (Hawksworth 2001). Our understanding of the fungal communities in marine environments is still extremely limited (Amend et al. 2012). Fungi are found associated with various marine organisms and substrata, including sponges, corals, tunicates, seaweed, seagrasses, mangroves, molluscs, woody substrates, driftwoods, and sediments. Recently, realizing the ecological significance of fungi associated with sessile marine animals focus is on their identification. It is expected that many untapped species, some with unique attributes/ecological roles, within these niches are yet to be isolated/identified. These investigations are driven by both ecological/physiological based research and bioprospecting efforts that are aimed at obtaining novel bioactive compounds (Höller et al. 2000; Raghukumar 2008; Rateb and Ebel 2011). At present, based on cultural and genomic sequence studies, 1112 marine fungal species in 472 genera have been identified. The largest family of marine fungi is Halosphaeriaceae, while the most widespread genera are *Aspergillus*, *Penicillium*, and *Candida* (Le Calvez et al. 2009; Jones et al. 2015; Kumar et al. 2015; Richards et al. 2015). The marine fungi are often termed as "marine-derived fungi" (MDF) as most of the fungi isolated from marine samples are not demonstrably classified as obligate or facultative marine microorganisms (Osterhage 2001). Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat, whereas facultative marine fungi are those of freshwater or terrestrial origin that can grow (and possibly sporulate) in marine environments (Kohlmeyer and Volkmann-Kohlmeyer 2003; Li and Wang 2009).

MDF isolated from various marine habitats and substrata produce an array of novel secondary metabolites belonging to polyketide, shikimate, terpenoid, peptide, and alkaloid groups (Bugni and Ireland 2004; Saleem et al. 2007; Raghukumar 2008; Rateb and Ebel 2011). Apart from playing a key role in the ecosystem, these fungi are also beneficial for industrial and other uses such as pigment, drug production, food industry, and bioremediation. In agriculture, fungi influence the nutrition, growth, and health of plants. The beneficial association of fungi with plants not only provides protection against plant pathogens but also, indirectly promotes plant growth. Fungi as a biofertilizer in agriculture play an important role in plant development by availing several minerals, and growth regulators like auxin and gibberellins (Verma et al. 2017; Kour et al. 2019c).

Currently, there is a need to explore structurally and biologically unique potent metabolites of fungi including MDF because the untapped potential of fungal metabolites and fungi associated with marine organisms such as corals, sponges, seaweeds, and sediments can be utilized for sustainable agriculture. The purpose of this review is to emphasize the importance of MDF.

13.2 Coral Associated Fungi

Coral reefs, oases in the blue deserts of the ocean, are home to colorful, hard and soft corals, sponges, a diverse population of fishes, holothurians, calciferous algae and other myriad communities (Connell 1978).

The phylum *Cnidaria*, containing around 10,000 species, has been the most widely studied taxon in relation to fungal prevalence (Zhang 2011). Scarce literature available on the diversity of both fungi and corals insights to the mechanistic nature of the interactions (Kendrick et al. 1982; Le Campion-Alsumard et al. 1995; Bentis et al. 2000; Golubic et al. 2005). Koh et al. (2000) isolated 16 fungal genera and 51 species, including two yeasts, from 10 species of gorgonian corals in Singapore by culture-dependent technique. The microbial metagenome of *Porites astreoides* collected from Bocal del Toro, Panama, showed fungi to be the dominant community, contributing 38% to the genome of the coral (Wegley et al. 2007). The fungal community associated with the coral *Acropora hyacinthus*, revealed to be the most diverse and metabolically active community (Amend et al. 2012).

Obligate marine fungi, which are called as autochthonous (indigenous or native) fungi, are specific to coral fungi. These fungi belonging to the class *Ascomycetes* were earlier reported from coral reefs at the coast of Belize (Central America) (Kohlmeyer and Volkmann-Kohlmeyer 1992). Examples of autochthonous fungi are *Koralionastes angustus* and *K. giganteus* from Belize and Australia, *K. ellipticus* and *K. ovalis*, and *K. violaceus* from Australia and Fiji (Kohlmeyer and Volkmann-Kohlmeyer 1990). Kendrick et al. (1982) isolated saprotrophic fungi from live hard corals of the Caribbean and the South Pacific and it was demonstrated by inoculating *Aspergillus versicolor* and *Penicillium stoloniferum* (allochthonous (non-native) coral fungi) in the coral *Siderastrea siderea*.

13.3 Ascidians Associated Fungi

Only a few studies describing fungal association with ascidians have been reported as the major focus has remained on prokaryotic symbionts of these animals (Blasiak et al. 2014; Erwin et al. 2014). Perhaps, Menezes et al. (2010) have done the most detailed analysis of fungal diversity in ascidians who isolated over 15 fungal genera, in which the most abundant were *Trichoderma*, *Phoma*, and *Cladosporium*. Wang et al. (2013) revealed that some of the chemicals produced by the defense arsenals produced in sponges are mainly due to the presence of associated fungi. Which include a broad range of compounds (from amino acid derivatives to nucleosides, macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides, and sterols, just to mention a few groups) that have been shown to exhibit biological activity on a variety of cell types (sponges and others). Other reports, mainly related to bioprospecting for novel natural compounds include representatives of known fungal genera such as *Penicillium* in the case of iso-coumarin derivatives (Xin et al. 2007) and *Aspergillus* strains that produce cytotoxic compounds, isolated from *Eudistoma vannamei* (Montenegro et al. 2012).

13.4 Sponge Associated Fungi

In sponges, 40–60% of the biomass is comprised of associated microorganisms that include fungi and other microorganisms (Hentschel et al. 2012). The nature of sponge feeding, which is based on the filtering of vast volumes of water, offers a high potential for the presence of fungi. A number of fungi have been isolated from different sponges of tropical, subtropical, and temperate ocean waters (Höller et al. 2000; Morrison-Gardiner 2002). MDF, such as species of *Aspergillus*, are widespread and have been isolated from sponges, including *Axinella damicornis*, *Cliona chilensis, Mycale* sp., *Petrosia ficiformis*, *Petrosia* sp., *Psammocinia* sp., *Suberites domuncula, Tethya aurantium*, and *Xestospongia exigua*. Similarly, Thirunavukkarasu et al. (2012) studied sponges of southern India and isolated different species of *Aspergillus* from *Biemna fistulosa* Topsent, *Callyspongia diffusa* Ridley, *Cliona quadrata* Hancock, *Cliona viridis* Schmidt, *Fasciospongia cavernosa* Schmidt, *Haliclona madrepora* Dendy, *Lissodendoryx sinensis* Brøndsted, *Pseudosuberites andrewsi* Kirkpatrick, *Sigmadocia pumila* Lendenfeld and *Suberitus carnosus* Johnston. Among them, six were active against Gram positive and negative bacteria; four had insecticidal compounds; three had antialgal metabolites; two were antifungal in nature; five had antioxidant properties; and four were acetylcholinesterase inhibitors. Aspergillusol A, synthesized by *Aspergillus aculeatus* Lizuka isolated from *Xestospongia testudinaria* Lamark, is an inhibitor of α-glucosidase enzyme (Ingavat et al. 2009). Additionally, *Aspergillus* spp. produce many polyketides, isoprenoids, non-ribosomal peptides and lipopeptides of pharmaceutical importance (Lubertozzi and Keasling 2009).

Drechslera hawaiiensis M. B. Ellis elaborates novel spiciferone derivatives (Edrada et al. 2000), *Cladosporium herbarum* synthesizes two novel macrolides (Jadulco et al. 2001), *Aspergillus niger* elaborates seven new diterpenoids (Hiort et al. 2004), *Cladosporium* sp. synthesizes a novel hexaketide (Gesner et al. 2005), *Paecilomyces lilacinus* produces two novel α-pyrones (Elbandy et al. 2009), *Aspergillus versicolor* synthesizes a novel lipopeptide (Lee et al. 2010) and *Arthrinium* sp. produces five novel diterpenoids (Ebada et al. 2011).

Overall, the limited studies on a few sponge species show that certain fungi, such as *Aspergillus* and *Pencillium*, are ubiquitous. Screening of different species of sponges from different locations will help in discovering many more such ubiquitous and sponge-specific fungi, thus increasing the chances of identifying more novel leads in both agriculture and pharmaceuticals.

13.5 Marine Water and Sediment-Associated Fungi

The presence of fungi in deep-sea environments, including deep marine subsurface and their ecological importance, have been recognized with much interest, recently. Many fungi have been isolated from various deep-sea environments, of which, the majority are similar to terrestrial fungi, but some of them are novel species

(Nagahama et al. 2001; Nagahama 2003; RaghuKumar et al. 2004; Damare et al. 2006; Burgaud et al. 2009; Singh et al. 2010; Manohar et al. 2015).

The deep-sea environment is characterized by the absence of sunlight, predominantly low temperature, and high hydrostatic pressure; the fungal communities are diverse in this extreme environment where these major components of microeukaryotes play critical roles (Nagano and Nagahama 2012). In marine sediments and water columns, environmental factors, especially sample depth, oxygen, and nitrate have been found closely related to fungal community composition (Tisthammer et al. 2016). The first report of isolation of deep-sea fungi was from the Atlantic Ocean at a depth of 4450 m (Roth et al. 1964). Since then, an increasing number of fungal species has been reported in several deep-sea environments, such as sediments from the Yap Trench (Xu et al. 2019), the hydrothermal site of South Mid-Atlantic Ridge, the Central Indian Basin (Damare et al. 2006; Singh et al. 2010), the deep-sea coral (Galkievicz et al. 2012), and deep-sea areas of the Pacific Ocean, such as the deep-sea volcano (Akerman et al. 2013), hydrothermal vent (Fortunato and Huber 2016), and water column (Li et al. 2019). However, comparing bacterial and archaebacterial communities it is insignificant, which suggests more attention to be paid for exploring fungal abundance and diversity in deep-sea environments (Wu et al. 2013; Luo et al. 2015; Zhang et al. 2015a, b, 2018; Bienhold et al. 2016; Walsh et al. 2016; Peoples et al. 2019).

A few studies have been conducted to detect fungal assemblages present in bathypelagic and abyssopelagic zones, and other specialized deep environments including hydrothermal systems, methane-dominated regions, and deep subsurface sediments (Bass et al. 2007; Lai et al. 2007; Takeshita et al. 2007; Jebaraj and Raghukumar 2009; Le Calvez et al. 2009; Nagano et al. 2010; Nagahama et al. 2011; Singh et al. 2011, 2012; Thaler et al. 2012; Xu et al. 2014, 2016).

Ascomycota and *Basidiomycota* groups of fungi have been frequently recovered from other deep-sea habitats (Nagano and Nagahama 2012; Xu et al. 2014, 2016, 2018b; Rédou et al. 2015; Zhang et al. 2016; Nagano et al. 2017). Species of genera, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* have been detected in all deep-sea sediment samples around the world and are considered to be ubiquitous in such environment (Nagano et al. 2010; Nagahama et al. 2011; Singh et al. 2012; Zhang et al. 2014; Rédou et al. 2015; Xu et al. 2018a, b, 2019; Vargas-Gastelum et al. 2019). The terrestrial genera, *Penicillium* and *Aspergillus* are the most common and active owing to their physiological versatility in deep-sea ecosystems (Burgaud et al. 2009; Singh et al. 2010; Nagano and Nagahama 2012; Zhang et al. 2013; Raghukumar 2017; Xu et al. 2018b).

The saprotrophic fungi, due to their inordinate capability to produce extracellular enzymes in soil, in the deep-sea environment probably contribute to the maintenance of the sediment structure and nutrient cycling (Treseder and Lennon 2015). Similarly, Pathogenic fungi might accelerate the leaching out of dissolved organic matter from the host that promotes the growth of other microorganisms in the marine environment (Raghukumar 2017). Further, salinity is the most defining feature of the oceanic environment. Physiological and biochemical adaptation of fungi to the salinity can be of relevance to biotechnology.

13.6 Seaweed-Associated Fungi

Marine-derived fungi are more common as endosymbionts of seaweeds than true marine fungi. It is not clear what factors determine the diversity and distribution of this ecological group of organisms. Harvey and Goff (2010) stated that differences among algal host species are less important than geographic isolation in determining genetic covariation of their fungal endosymbionts.

Some fungal species colonize seaweeds to tolerate or detoxify the compounds constantly available in the host tissue, which lead to the evolution of generalist endosymbionts capable of colonizing taxonomically unrelated seaweeds. Such adaptations can possibly explain the constant presence of certain marine-derived fungal genera such as *Aspergillus*, *Cladosporium*, and *Penicillium* as endosymbionts in different seaweeds (Zuccaro et al. 2008). For instance, different species of *Cladosporium* have been isolated from *Caulerpa racemosa*, *C. sertularioides*, *Ulva lactuca*, *Padina gymnospora*, *Sargassum wightii*, *Turbinaria conides*, *Turbinaria* sp., *Portieria hornemannii*, *Grateloupia lithophila*, *Halymenia* spp. and *Fucus serratus* (Zuccaro et al. 2008; Suryanarayanan et al. 2010). *Aspergillus terreus* has been found to be the most frequent endosymbiont of *Caulerpa scalpelliformis*, *Halimeda macroloba*, *Ulva lactuca*, *Ulva fasciata*, *Lobophora variegata*, *Padina gymnospora*, *Stoechospermum marginatum*, *Sargassum ilicifolium*, *Portieria hornemannii* and *Gracilaria edulis* (Suryanarayanan et al. 2010; Noorjahan et al. 2020).

Noorjahan (2019) isolated 189 endophytic fungi exhibiting different activities from seaweeds, namely *Valonia urticularis, Caulerpa scalpeliformis, C. sertularioides, C. racemosa, Centroceras clavulatum, Halimeda opuntia, H. macroloba, Stoechospermum marginatum, Sargassum wightii, Ulva facsiata, Padina gymnospora, Gracilaria edulis Lobophora variegate Turbinaria conoids, Kappaphycus alvarezii, Gelidiella acerosa, Jania rubens, Portiera hornemannii, Sargassum* sp.*, Dictyota dichotoma* of South east coast, Tamilnadu, India. Various species of *Aspergillus* and *Penicillium* were found in abundance. *Penicillium oxallicum, P. purpurogenum, P. citrinum, P. aurantiogriseum, Aspergillus niger, Aspergillus chevalieri, Fomitopsis decrens,* and *Penicilium griseofulvum* showed higher inorganic phosphate solubilizing capability than their terrestrial counterparts (Noorjahan et al. 2019).

13.7 Fungal Enzymes

Microbial communities in marine environments are ecologically relevant as intermediaries of energy and play an important role in nutrient regeneration cycles as decomposers of dead and decaying organic matter. In this sense, MDF can be a source of enzymes of industrial and/or environmental interest. Fungal strains of marine origin isolated from different substrates, such as invertebrates, decaying wood, seawater, sediments, and mangrove detritus, have been reported to be the producers of hydrolytic and/or oxidative enzymes; alginate lyase, amylase, cellulase,

chitinase, glucosidase, inulinase, keratinase, ligninase, lipase, nuclease, phytase, protease, and xylanase.

Marine fungi have been reported to produce cellulases and laccases, and some specific glycoside hydrolases (GHs) related to the marine origin, on the addition of agricultural plant or waste (cottonseed, sugarcane bagasse, rice bran, waste paper, cellulose, sisal waste, molasses spent wash, black liquor, etc.), or algal polysaccharides into the growth medium (Raghukumar 2008; Ravindran et al. 2010; Rodriguez-Jasso et al. 2010; Zhang and Kim 2010; Chen et al. 2011; Faten and Abeer 2013; Bonugli-Santos et al. 2015; Hong et al. 2015; Wang et al. 2016; Balabanova et al. 2018). The capability of metabolic utilization of plant or macroalgae polysaccharides allows for an increase in the production of fungal biomass enriched by mycelium proteins and extracellular enzymes that can be used in animal or fish feeding, or in the bioremediation of soils and water.

Fungi also produce a wide range of carbohydrate active enzymes (CAZymes) and degrade plant complex polymers into digestible and assimilable products for other members of ecosystems. The plant-degrading CAZymes such as cellulases, hemicellulases, ligninases, and pectinases, and the accessory debranching enzymes belong to the following classes: glucoside hydrolase (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), conmitant enzymes [Carbohydrate esterases (CEs), and auxiliary activities (AAs)] that can be linked to carbohydrate-binding modules (CBMs) indicate that marine fungi have developed the metabolic pathways rather related to the breakdown of terrestrial plants than algae or animal residues. (Guillén et al. 2010; van den Brink and de Vries 2011; Arfi et al. 2013; Rytioja et al. 2014; Kumar et al. 2015). The CAZymes of terrestrial fungi are mostly active at pH 4.5–6.0 and low salinity ($\leq$0.05%). In contrast, the CAZymes of the marine fungi remain functional at the high salinity and pH, low water potential, high sodium ion concentrations, extremely low or high temperature, oligotrophic nutrient conditions, and the high hydrostatic pressure. And, therefore have a lot of potentials in biotechnology (Raghukumar 2008; Farinas et al. 2010; Pang et al. 2011; Zilly et al. 2011; Arfi et al. 2013; Del-Cid et al. 2014; Lee et al. 2015; Thirunavukkarasu et al. 2015; Dos Santos et al. 2016; Trincone 2018).

Xylanases are used concurrently with cellulases and pectinases for clarifying juices, the liquefaction of vegetables and fruits as well as in the pretreatment of forage crops to improve the digestibility of ruminant feeds and to facilitate composting (Nadu et al. 2011; Goddard-Borger et al. 2012). In some macroalgae, where cellulose is absent (*Rhodophta*), xylan forms a highly crystalline fiber-like material. The content of xylan (in red/green algae and higher plants) or fuco-glucuronoxylans (in brown algae) may be up to 40% of the total polysaccharide content. The marine bacteria and fungi associated with these macroalgae could have evolved efficient mechanisms for xylan degradation at the genetic and/or molecular levels that may be utilized by their studies (Kraan 2012; Del-Cid et al. 2014; Dos Santos et al. 2016). However, the global significance of mycobionts of seagrasses, particularly associated with the roots of aquatic plants, is not well understood (Kohout et al. 2012; Vohník et al. 2016).

13.8 Fungi as Biocontrol Agent

Fungi such as *Penicillium* sp., *Piriformospora indica*, and *Trichoderma* sp. have been reported to be very efficient as biocontrol agents (Yadav et al. 2018; Sharma et al. 2019). Several reports demonstrate the paramount importance of the interaction between fungi and plants for sustainable plant production (Yadav et al. 2019d, e). Beneficial fungi are chief players in the natural agro-ecosystem as they avail important ecosystem services such as the acquisition of nutrients, organic matter recycling, and protection against plant pests (Pozo et al. 2009; Ramos-Zapata et al. 2012).

Noorjahan et al. (2020) studied the efficacy of seaweed endophytic fungi against *Macrophomina phaseolina*, a root-rot fungal plant pathogen, which is one of the serious economic threats in agriculture. *Penicillium* sp., *Aspergillus* sp., and *Fomitopsis* sp. significantly inhibited the growth of the fungal pathogen indicating the potential of theses marine fungi as biocontrol agents. Further, compounds possessing anti-algal, antifungal, and insecticidal properties are also produced by fungal isolates from green-, red-, and brown-seaweeds (Suryanarayanan et al. 2012).

Motti et al. (2007) screened extracts of 449 MDF for inhibition of pyruvate phosphate dikinase (PPDK), which hinders growth in C4 plants. This enzyme occurs primarily in plants, but not reported so far from vertebrate or invertebrate animals, with the exception of protozoan, *Giardia*, potentially minimizing the risk of PPDK inhibitors exhibiting adverse toxicological effects. They isolated unguinol, a known compound and found it to inhibit PPDK via a novel mechanism of action, which also translates to an herbicidal effect on plants.

13.9 Iron Chelating Fungi

Siderophores are a class of microbial molecules that solubilize iron in the marine environment. Alternative of siderophore production is replacement of iron compounds, as is known for some marine organisms that use flavodoxin in place of ferredoxins (Bauer et al. 1993; Bovy et al. 1993). Siderophores are essential metabolites that respond to oxidative stress in various fungi, including *Tricoderma virens*, *Gibberella zeae*, *Cochliobolus heterostrophus*, *Aspergillus fumigates*, *Aspergillus nidulans* and they also play a major role in conidial germination and sexual development (Verma et al. 2017; Kour et al. 2019b; Rana et al. 2019). Siderophore concentration of *Penicillium funiculosum* strains. Comparison of quantification of siderophore production between marine and terrestrial fungi revealed that four terrestrial isolates (*Aspergillus niger*, *Aspergillus ochraceous*, *Penicillium chrysogenum*, *Penicillium citrinum*) were ahead in siderophore production, while, the other four marine isolates (*Aspergillus versicolor*, *Cunninghahamella elegans*, *Rhizopus* sp., *Syncephalastrum racemosum*) were found to be more potent siderophore producers (Baakza et al. 2004; Noorjahan et al. 2019).

13.10 Fungi as a Source of Secondary Metabolites

The fungal life cycle and mediating interactions between the fungus and host have led to the evolution of biochemical pathways for the synthesis of unusual metabolites that have found many potential applications in anticancer and antimicrobial studies (Yarden 2014; Hasan et al. 2015; Li et al. 2016; Deshmukh et al. 2017). Salinity is another harsh condition poised by seawater where the concentration of sodium chloride remains high. In such conditions, fungi like *Aureobasidium pullulans*, *Hortaea werneckii*, *Phaeotheca triangularis* (Turk et al. 2004; Kogej et al. 2005), *Debaryomyces* sp. (Jiang et al. 2016), *Aspergillus flavus* (Beltagy et al. 2018), *Gymnoascus halophilus, Wallemia* sp. (Chamekh et al. 2019), *Aspergillus sydowii* and *Aspergillus destruens* (González-Abradelo et al. 2019) produce a wide variety of molecules referred to as secondary metabolites (SMs), e.g., polyketides, non-ribosomal peptides and terpenes (Keller et al. 2005). Gibberellic acid produced by *Gibberella fujikuroi* functions as a plant growth regulator. (Adrio and Demain 2003).

The beneficial fungi have been used as plant growth promoters and development for sustainable agriculture. The fungi are potentially useful for improving plant growth and health, nutrients availability, water uptake, stress tolerance and as well as biocontrol (Yadav et al. 2020a, b).

13.11 Biodegradation of Pesticides/Toxic Chemicals and Hydrocarbons

Marine fungi have numerous ecological roles such as degradation of biota, provision of chemical protection, pathogenicity, symbiosis and impact on various holobiont groups (Andreakis et al. 2015). Some marine fungi are key decomposers of organic matter, and others are involved in the denitrification process (Zhang et al. 2015a, b) and hence are considered as a source of industrial enzymes (Bonugli-Santos et al. 2015). Fungi are known to be capable of degrading petroleum hydrocarbons, including heavy hydrocarbons, such as asphalt (Uribe Alvarez et al. 2011; Nasrawi 2012; Xue et al. 2015).

Eight fungal genera (*Aspergillus*, *Penicillium*, *Thielavia*, *Fusarium*, *Emericella*, *Cladosporium*, *Scytalidium*, and *Alternaria*) isolated from Masturah, Saudi Arabia, are potential biodegrader of petroleum products (Alwakeel 2017). The presence of fungi in oil-contaminated marine sediments is often reported, and a dramatic increase of fungal communities in post-oil spill sediments has been observed in the Gulf of Mexico (Sadaba and Sarinas 2010; Bik et al. 2012; Fasanella et al. 2012). Shinde et al. (2018) studied the fungi isolated from tarballs found in Betul Beach, Goa, India. Tarballs are semi-solids formed from crude oil that might have been spilled onto the ocean. Eighteen fungi were identified phylogenetically, including *Aspergillus* sp., *Byssochlamys* sp., *Monascus* sp., *Paecilomyces* sp., *Penicillium* sp., *Talaromyces* sp., *Trichoderma* sp. and Xylogone sp. Some of these fungi (from

genera *Aspergillus*, *Paecilomyces*, *Penicillium*, *Talaromyces*, and *Trichoderma*) have been studied for their hydrocarbon degradation capabilities.

13.12 Conclusions

Fungi are associated with various marine organisms and substrata, including sponges, corals, tunicates, seaweed, seagrasses, mangroves, molluscs, woody substrates, driftwoods and sediments. Environmental influences such as floods and winds carry terrestrial fungi toward marine environments. Thus, marine-derived fungi often routinely exhibit morphological characteristics similar to terrestrial counterparts. Salinity, high pressure, low temperature, oligotrophic conditions, pH extremes, widely ranging mineral content in seawater and sediments, and typical lighting conditions differentiate the unique characteristics and novel metabolite from terrestrial microbial communities. Existing fungal products such as biofertilizers greatly appeal to the agro-industry as they are versatile and environment friendly.

The MDF from various hosts produce diverse, unique, and host-specific metabolites in terms of biogeochemical cycling, biocontrol agents and environmental decomposers. Although only a few reports and research are available on the application of MDF in agriculture. The demand of agricultural inputs is existing to overcome the usage of chemicals. The untapped marine resources should be explored and researchers should be encouraged to discover more novel products from MDF for sustainable agriculture, so that productive agro-ecosystem balance could be achieved in future.

Acknowledgments The authors would like to thank authorities of Annamalai University and our special thanks to Dean and Director, Centre of Advanced study in Marine Biology, Faculty of Marine sciences, Annamalai University, Chidambaram for his support and encouragement.

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Arbuscular Mycorrhizal (AM) Fungal Diversity from Coastal Dunes

14

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Abstract

Coastlands are ecologically sensitive habitats undergoing continuous transformation. They connect the terrestrial and marine ecosystems and impart several ecological services. However, they are susceptible to natural and anthropogenic disturbances that affect the structural stability and vegetation of the dunes. Hence, there is an urgent need to conserve and restore these habitats. Arbuscular mycorrhizal (AM) fungi are essential to soil microorganisms involved in plant community establishment. In coastal dunes, they play a vital role in nutrient cycling and aggregation of sand particles. Therefore, a survey of AM fungal diversity from coastal dunes is necessary to stabilize and conserve dune systems.

Keywords

Dunes · Dune vegetation · Sand aggregation · Disturbances · Arbuscular mycorrhizal fungi · Conservation

14.1 Introduction

Coastlands are highly organized natural dynamic systems undergoing continuous change due to geomorphological processes and varying climatic conditions. Coastal land is characterized by a stressful environment with low fertility, high salinity, intermittent drought, variable temperatures, and an unstable sandy substrate (Yamato et al. 2012; Cui et al. 2016). Dunes are generally of two types viz., an arid interior desert of continental landmasses such as Sahara in Africa or Victoria desert in Australia, and the coastal dunes that occur along the Atlantic and Pacific coast of

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_14

North America and the Australian coast. In Asia, the coastal dunes are found in Japan, India, and several other countries. The coastal dunes have the sand as a byproduct of weathered rocks from inland regions eroded by rain and wind. Additionally, the wave action and sea currents are responsible for shifting sand between the seafloor, beach, and dunes. Such habitats have coarse sand with low levels of inorganic nutrients (Desai and Untawale 2002).

14.2 Coastal Dune Vegetation

Plant species found in coastlands are specifically adapted to the persisting extreme environmental conditions. Characteristic vegetation has adapted to temperate and tropical dunes. Members of *Poaceae* are dominant plant species in temperate dunes, while plant members of *Asteraceae*, *Convolvulaceae*, *Fabaceae*, and *Poaceae* are predominant in tropical dunes (Sridhar 2009). Usually, a transition in coastal plant species is found in the dune ecosystem corresponding to the environmental gradient with distance from the sea, the vegetation closest to the seaside experiencing the most stressful conditions (Yamato et al. 2012). Dune vegetation is usually arranged into three main zones—pioneer zone, foredunes, and hinddunes—that are roughly parallel to the coastline.

Closest to the sea is the pioneer zone, extending landward from the debris line at the top of the beach in the area of the foredune or frontal dune. Only specific pioneer plants such as *Spinifex littoreus* L. (Poaceae), *Ipomoea pes-caprae* (L.) R. Br. (*Convolvulaceae*) and few other herbaceous species that can withstand the harsh conditions colonize areas exposed to salt spray, sandblast, strong winds, and flooding by the sea. These plants have specialized structures such as a waxy coating on stems and leaves; these are prostrate, and have well developed and rapidly spreading root systems. The creeping stems or stolons can interconnect, so if one part is buried in shifting sand or is uprooted, another part continues to grow; and so serve to stabilize the sand, forming and building the dunes (https://www.ehp.qld.gov.au/coastal/ecology/beaches-dunes/coastal_dunes.html).

Plant species on the foredunes, or frontal dunes, are more complex than those in the pioneer zone. Scrub or woodland plants occupy the foredunes as more nutrients support the growth of such plants. Plant species in this zone are generally semi-permanent windswept and include *Spermacoce stricta* L. f. (*Rubiaceae*), *Leucas aspera* (Willd.) Link (*Lamiaceae*), *Vitex negundo* L. (*Lamiaceae*), *Clerodendrum inerme* (L.) Gaertn. (*Lamiaceae*), *Casuarina equisetifolia* L. (*Casuarinaceae*), besides vines and few herbs.

The hind dune is occupied by more complex and developed vegetation such as stunted trees, low shrubs, and forest plants. Protected by the strong winds and salt spray experienced closer to the beach, this area is more protected, making it easier for less hardy and specialized trees to grow and survive. Plants in this zone include *V. negundo* L. (*Lamiaceae*), *C. inerme* (L.) Gaertn. (*Lamiaceae*), *Anacardium occidentale* L. (*Anacadiaceae*), *Pandanus tectorius* Parkinson (*Pandanaceae*), *C. equisetifolia* L. (*Casuarinaceae*), *Cocos nucifera* L. (*Arecaceae*). The occurrence

of these plants in the hind dunes results in more humus and organic matter, thus providing sufficient nutrients for the growth of more plant species. Eventually, plant communities are established in this region, further contributing to the nutrients of the area (Desai 1995; http://www.beachapedia.org/Vegetation; http://www.ozcoasts.gov.au/indicators/beach_dune.jsp).

14.3 Importance of Coastal Dune Systems and Its Vegetation

Dunes serve as natural buffers, protecting the landward side from storm tides, waves, and wind action. Stabilization of large, mobile dunes by the vegetation cover has been recognized as an effective means to decelerate the inland movement of sand (Woodhouse 1982). The dune vegetation traps and holds windblown sand grains on the foredunes. It contains many native plant species and is valued as a habitat of its natural biodiversity. Loss of dune vegetation can trigger dune erosion wherein the exposed, dry sand particles are flown by high-velocity winds resulting in the shifting of large volumes of sand, sometimes forming large depressions in the dunes. This drifting sand can smother the surrounding vegetation, cover roads and properties. Erosion of beaches and foredunes may be a natural process and is often balanced by the supply of sand from the nearshore continental shelf to the beaches by currents and waves.

In some cases, sand from adjacent dunes may replenish beach systems during erosion periods. However, anthropogenic activities can also induce dune erosion. Some of the human activities which can lead to deterioration include grazing, fires, tracks, and foot traffic resulting in loss of dune vegetation; urban development on foredunes; clearance of dunes for agriculture, etc., ultimately result in dune degradation (http://www.ozcoasts.gov.au/indicators/beach_dune.jsp). Therefore it is crucial to protect dune vegetation.

14.4 Arbuscular Mycorrhizal (AM) Fungi

The survival of plants under harsh coastal conditions depends upon their mutualistic associations with soil microorganisms such as AM fungi, rhizobia, and endophytes. AM fungi are ubiquitous obligate soil fungi belonging to phylum *Glomeromycota* (Redecker et al. 2000) that form symbiotic associations with majority of land plants (Smith and Read 2008); developing intra-radical structures (hyphae, arbuscules, vesicles) in the cortical cells and extra-radical structures (hyphae, spores) in soil. The extra-radical mycelial network in the rhizosphere facilitates soil nutrients, especially phosphorus (P) (Bucher 2007). In addition to nutrient acquisition, other benefits provided to the host include plant tolerance to biotic and abiotic stress (Bennett and Bever 2007); improved nutrient cycling (Tiwari and Sati 2008); improved soil stability, binding, and water retention (Rillig and Mummey 2006; Bedini et al. 2009); and bioremediation of soil (Leyval et al. 1997). In return, the fungal partner receives carbon compounds from the host plant (Brundrett 2009).

Thus, under extreme environmental conditions, AM fungi play a significant role in pioneering the vegetation and bringing about ecosystem stability. They also play a vital role in the primary and secondary succession of plant species, including uptake of water and nutrients by plants in coastal dunes (Beena et al. 2000a).

14.5 AM Fungi and Its Benefits to Dune Ecosystems

AM fungi are widespread in coastal dune systems (Stürmer and Bellei 1994). Coastal dunes favor the occurrence of AM fungi mainly because of low phosphorus content (Ranwell 1972). Mycorrhizal diversity in dunes results in an increase in the longevity of feeder roots and improvement in soil texture through the increased aggregation of soil particles (Nasim 2005). AM fungi provide P to plants that enable AM plants to grow better than non-mycorrhizal plants when P is limiting. An increase in yield or biomass of AM plants is often observed compared to non-mycorrhizal plants (Mosse 1972). Increased nutrient supply, salinity tolerance, reduced abiotic stresses, and formation of wind-resistant soil aggregates are the significant benefits derived by the dune vegetation through AM fungal association (Gemma and Koske 1989). Read (1989) showed that plant communities in successional dune chronosequences are governed by an interaction between biotic and physicochemical properties of the sand. Not only does the composition of plant species change with the seasons and age of the dune systems, but also the association with soil microorganisms changes with succession because of an increase in organic matter, improved substrate stability, and nutrient enrichment (Koske and Gemma 1997). The most crucial function of mycorrhizal fungi at the ecosystem scale is their contribution to soil structure. Soil aggregation is also vital in non-agricultural ecosystems, such as in the context of the restoration of disturbed lands, erosion control, global change, or soil carbon storage (Niklaus et al. 2003). Many physical, chemical, and biological factors (and their interactions) contribute to soil aggregation, yet AM fungi are particularly significant among the biological aspects. They create conditions contributing to the formation of microaggregates, and they chemically enmesh and stabilize microaggregates and smaller macroaggregates into macro aggregate structures. Localized drying of soil near the roots promotes binding between root exudates and clay particles, directly facilitating microaggregate formation (Augé et al. 2004). Besides this, other functions of AM in dune ecosystems include increased resistance of plants to root pathogens and increased plant tolerance to salt and drought stress (Koske et al. 1975; Nelson 1987; Newsham et al. 1995; Koske et al. 2004).

An environment is created through close mutualistic associations between plants and soil microorganisms that allow the dune systems to persist. Therefore, understanding AM associations with dune vegetation and their distribution in dunes is required for wise management, restoration, and revegetation of disturbed dunes.

14.6 AM Fungi in Dunes and Their Association with Dune Vegetation

Coastal plant communities are faced with poorly formed soils with shifting sands and nutrient-deficit environments. Dune vegetation is essential for the formation and preservation of dunes and the protection of the coastline. Dune vegetation is highly adapted to salt-laden winds of the coast and maintains the foredunes by holding the sand in the dunes, trapping sand particles blown up from the beach, and aid in repairing the degraded dunes (Desai and Untawale 2002). Coastal dunes face harsh environments, where AM fungi play an essential ecological role in promoting growth, establishment, and survival of plant species that colonize dunes (Dalpe 1989; Tadych and Błaszkowski 2000).

The extra-matrical hyphal network of AM fungi is involved in the transfer of nutrients from the soil nutrient deficiency zones formed around the plant roots. They play a vital role in building and maintaining the structure of dunes and in the stabilization of dune vegetation. Jehne and Thompson (1981) reported a considerable amount of hyphal connections of fungal mycelium in the top 20 cm of mobile sand in Cooloola (Queensland), Australia. These fungi bind loose sand grains into larger aggregates through secretion of hydrophobic 'sticky' glycol-proteinaceous substance known as 'glomalin,' which improves soil stability, binding, and water retention, limiting dune loss or erosion (Bedini et al. 2009). Forster and Nicolson (1981) reported that 1.5% of the aggregate sand grains reach a diameter of 2 mm in Scotland dunes.

AM fungal association is common in dune plants. Stahl (1900) and Asai (1934) initially reported AM associations with roots of dune plants. Since then, several surveys have been carried out in temperate and subtropical regions (Lee and Koske 1994a); a few from the tropical coast of Hawaiian Islands (Koske 1988; Koske and Gemma 1996), India (Mohankumar et al. 1988; Kulkarni et al. 1997; Visalakshi, 1997; Rodrigues and Jaiswal 2001) and Singapore (Louis 1990). AM fungi have also been reported from beaches in Australia (Koske 1975; Jehne and Thompson 1981; Brockhoff 1985), as well as from maritime dunes of other countries (Nicolson and Johnston 1979; Koske and Halvorson 1981; Giovannetti and Nicolson 1983; Bergen and Koske 1984; Sylvia 1986). Studies on AM fungal associations in dune plants in Australia, USA, India, and Europe indicate that dominant dune plants and pioneer grasses are generally associated with AM fungi. These fungi help in dune stabilization through the successful establishment of plant communities by improving their nutrient status. Koske (1988) reported that among all other mycorrhizal types, the AM fungi benefit from plant species in dune ecosystems.

The most common AM fungal genera in coastal dune systems worldwide are *Acaulospora*, *Gigaspora*, *Glomus,* and *Scutellospora*. Variations in AM fungal spore densities per unit volume of soil have been reported, depending upon different factors such as season, host genotype and phenology, and environment, ranging from 1 to >300, 100 g-1 soil (Maun 2009). The greater the number of viable propagules, the more the chances of forming symbiosis and utilizing its benefits by plants. *Scutellospora erythropa* in the Bahamas, *Acaulospora scrobiculata* and

Gigaspora albida in North America (Koske and Walker 1984), *Glomus* spp. in Japanese dunes (Abe et al. 1994) formed the most dominant AM fungal species in dune systems. Koske (1987) reported 14 AM fungal species from the sandy soils of Wisconsin, with *Glomus etunicatum* being the most frequently isolated species. Lee and Koske (1994b) observed *Gigaspora* was the dominant AM genus in dunes of the Atlantic coast of U.S. Kulkarni et al. (1997) recorded 16 AM species in Mangalore coast of Karnataka with *Gigaspora ramisporophora*, *Glomus albidum*, *G. clarum*, and *Scutellospora gregaria* as dominant species. Rodrigues and Jaiswal (2001) recorded AM association in six plant species growing on dune vegetation of Goa and reported the presence of three AM fungal genera viz., *Acaulospora*, *Glomus*, and *Sclerocystis*. Stutz et al. (2000) observed that the taxonomic range of AM fungi was mostly limited to *Glomaceae* and *Acaulosporaceae* at El Socorro, near Ensenada, Baja California.

The roots of dune plants are intimately associated with AM fungi (Koske and Gemma 1997). Most plant species that colonize dunes are known to have AM symbioses with variable degrees of root colonization (Druva-Lusite and Ievinsh 2010). However, on heterogeneous and unpredictable coastal ecosystems, AM colonization might be expected to be more significant in the more stable habitats (Ievinsh 2006). Giovannetti and Nicolson (1983) reported the presence of *Glomus mosseae* and G. fasciculatum in Italian dunes. They observed that plant species of cosmopolitan families were found to be heavily colonized by AM fungi. Mobile to stable dunes of the Gulf of Mexico revealed AM colonization in 97% of plant species (Corkidi and Rincón 1997). El-Giahmi et al. (1976) studied AM fungi from coastal sandy soils of Libya, wherein most of the host plant species recorded colonization levels between 40% and 60%. Giovannetti (1985) reported higher AM fungal root colonization in plant species belonging to families *Asteraceae*, *Papilionaceae*, and *Poaceae* on the Italian dunes.

Koske (1975) reported higher spore density in older, more stabilized dunes than in younger dunes in Australia. The highest AM fungal spore density was recorded in the rhizosphere of *Ammophila breviligulata* dominating the dune vegetation in Rhode Island (Koske and Halvorson 1981). Bergen and Koske (1984) investigated the occurrence of AM fungi from dunes of Capecod-Massachusetts. They recorded five AM species belonging to the genus *Gigaspora* in association with roots of *Ammophila breviligulata*, and reported *Gi. gigantea* as the dominant species. Sylvia (1986) observed spatial and temporal distribution of AM fungi associated with *Uniola paniculata* in the foredunes of Florida. The study also reported that the spore densities in non-vegetated areas adjacent to vegetated dunes averaged less than 6% of the spore densities found in the rhizosphere of sea oats. Beena et al. (2001b) reported the occurrence of 30 AM species from 28 dune plant species belonging to 14 families from the West Coast of India. *Ipomoea pes-caprae* and *Launaea sarmentosa* growing on dunes of the West Coast of Karnataka harbored 41 and 28 AM species, respectively (Beena et al. 1997, 2000a). Ragupaty et al. (1998) reported 14 AM species in the rhizosphere of 31 plant species from dunes in Tamil Nadu. Khan (1971) studied illustrations of six types of AM fungal spores from West Pakistan soils. Mohankumar et al. (1988) studied the distribution of AM fungi in the

sandy beaches of the Madras coast and reported the presence of *Entrophospora* and *Glomus* species. According to them, soil temperature and moisture status influenced the colonization of AM fungi in coastal soils.

Several edapho-climatic factors are known to affect spore germination, root colonization, and efficiency of AM fungi. In the dunes, nutrient input is intermittent by salt spray or precipitation (Kellman and Roulet 1990), and organic matter serves as a significant energy resource (John et al. 1983). The main growth constraints faced by the dune vegetation are the low availability of N, P, K, water, and organic matter (Maun 1994). According to van der Valk (1974), calcium (Ca) and magnesium (Mg) are usually adequate for plant growth, while N, P, and K are limiting in dune systems. Preferential association of AM fungi was observed with decaying organic matter (John et al. 1983). The high organic matter resulted in increased growth of AM fungi in soil (Joner and Jakobson 1995). A significant difference in the edaphic factors or determinants such as moisture, pH, P, Na, K, and N between naturally vegetated and non-vegetated dunes of the West Coast of India is observed in several studies (Beena et al. 1997; Kulkarni et al. 1997; Beena et al. 2000a, b). Organic matter supplies P for acid and alkaline phosphatases of AM fungi. Alkaline phosphatase activity of AM fungi decreased in soil devoid of organic matter (Sridhar 2009). Uptake of Ca by AM fungi plays a vital role in P and water uptake by plants (Pai et al. 1994). Enhancing P nutrition is a significant benefit to the host plant in an AM fungal association. In coastal dunes, the level of available P to the plants is typically deficient. The hardship imposed on plants by such low P levels is compounded by the downward mobility of P in the soil. Available P is removed from near the absorbing surface of roots creating a narrow depletion zone. The AM fungal extra-radical hyphae can cross this zone and provide the plant with P (Koske 1984). AM fungi also play a significant role in N acquisition by plants (Hodge et al. 2010; Smith et al. 2011). However, plant species differ in their mycorrhizal response in complex ways across N and P availability (Hoeksema et al. 2010). It is reported that AM abundance decreases with higher P (Richardson et al. 2011) and N concentration (Treseder 2004) in soil, while soil pH affects fungal community composition (Dumbrell et al. 2010). According to Sieverding (1991), one of the most critical soil physicochemical factors appears to be pH. Many fungi show a wide tolerance to distinct pH ranges, which is reflected in the occurrence of species rather than genera (Koske 1987). While species from different genera can be found in soils covering a broad pH range, others like *Glomus mosseae* have only been reported from soils with pH values greater than 5.5 (Sieverding 1991). According to Stürmer et al. (2018), soil pH appears as the primary factor influencing the dominance of *Gigasporaceae* and *Glomeraceae* in dunes worldwide. Temperature also seemed to be the main factor determining the structure and distribution of AM fungal communities along the latitudinal temperature gradient (Koske 1987; Davison et al. 2015). Although variations in the behavior of AM fungal species are known to exist concerning other soil factors (heavy metals, texture, moisture, nutrient levels, salinity, etc.), the significance of these for AM diversity in native or natural habitats is still poorly understood. The distribution of AM fungal species appears to be more closely related to host plant, soil structure, and environmental conditions than to

competition by other AM species (http://mycorrhizae.ifas.ufl.edu/Files/THESIS.pdf).

Stürmer and Bellei (1994) studied the composition and seasonal variation of AM spore populations in dune soils on the Island of Santa Catarina, Brazil. They observed that spore numbers of *Glomus constrictum*, *G. etunicatum*, and *Acaulospora* species were highest in winter, whereas *Gigaspora albida* peaked in the spring. Beena et al. (1997) reported that AM root colonization peaked during post-monsoon, while AM fungal species richness and spore diversity were highest during monsoon. A two-year seasonal study by Beena et al. (2000a) on the coastal dunes of the West coast of India revealed that the percent AM root colonization was least during monsoon and highest during post-monsoon. Still, the mean spore density was least during post-monsoon and highest during the summer. *Glomus* was the most common genera, with mean species richness being highest in *I. pes-caprae* (Beena et al. 2001a).

Burrows and Pfleger (2002) reported that plant cover could be predictive of spore volume or number. Nicolson (1960) examined AM colonization in dune grass in a more complex dune system and found a dramatic increase in the AM activity from the foredunes to the recently fixed dunes. According to Mayr (1965), disturbed habitats result in a reduced number of AM fungal propagules because of the reduction in host plants. Disturbance of soil leads to elimination or decrease in the number of viable propagules of AM fungi (Reeves et al. 1979). Sylvia and Will (1988) reported changes in AM populations and other soil microorganisms in replenished sand planted with *Uniola paniculata* and *Panicum* species. They observed a shift in dominant AM fungi found in the planted zone concerning those in established dunes. Beena et al. (2000a) reported that the vegetation cover, AM fungal colonization, species richness, and diversity were more significant in moderately disturbed dunes than in severely disturbed dunes of the West Coast of India.

Generally, the AM fungal diversity appears to be greater in more stabilized dunes than in younger or disturbed dunes (Giovannetti and Nicolson 1983). AM associations can be potential determinants of plant diversity in ecosystems. Since plant species differ in their response to AM fungi in the soil, the presence or absence of AM has been linked to the composition of plant communities that grow in the dunes (Francis and Read 1995). AM fungi can probably modify the structure and functioning of a plant community in a complex and unpredictable way (Read 1990). Any shift in the AM fungal population can result in survival, competition, and floristic diversity of plant community composition, causing changes in the ecology of natural habitats (Miller and Allen 1992). Therefore, knowledge of the different factors influencing the population biology of AM fungi is essential for their utilization in conservation of the environment (Allen 1991), biotechnology (Mulongoy et al. 1992), or in sustainable agriculture (Bethlenfalvay and Linderman 1992).

14.7 Conclusions

Being major ecological communities of marine ecosystems, the coastal dunes are of great significance throughout the world. However, they are susceptible to continuous environmental and anthropogenic interferences. The stabilization of dunes depends on the successful establishment of plant communities, and native plant species have a high potential to withstand these disturbances. Hence, there is great potential for rehabilitation of dunes by manipulating dune microbial resources especially AM fungal associations, to accelerate the growth of dune vegetation that will help to conserve and restore the coastal dunes.

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Part II

Ecological Significance of Fungi

Facets of AM Fungi in Sequestering Soil Carbon and Improving Soil Health

15

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Abstract

Soils, particularly agricultural soils, are home to a plethora of microbial communities capable of sequestering soil carbon. In this framework, arbuscular mycorrhizal fungi (AMF) play a pivotal role. This universal group of fungi form an obligate symbiotic relationship with the roots of higher plants leading to improved nutrient uptake and abiotic and biotic stress resistance. In addition, these fungi secrete a group of glycoproteins called glomalin or glomalin-related soil protein (GRSP) that sustain soil health, cement soil aggregates, and sequester soil C in a stable form. AMF symbiosis and GRSP production are however influenced by numerous aspects, including crop and soil management practices. Besides plant and soil type, soil management practices also influence AMF diversity and abundance. The soil carbon sequestration via AMF and GRSP is achievable if AMF supporting agricultural practices are employed. This chapter summarizes the cumulative role of AMF and GRSP in forming and stabilizing soil aggregates for long-term C storage, the influence of AMF-mediated agricultural practices to sequester soil carbon and improve soil quality traits.

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_15

Keywords

Arbuscular mycorrhizal fungi · Glomalin-related soil protein · Soil aggregation · Soil health · C-sequestration

15.1 Introduction

Microorganisms are an essential constituent of soil, driving vital processes such as organic matter flux and soil structure development. The structural framework of soil particles provides a diverse habitat for microbes. Substantial research output elucidated the dynamics of soil carbon pools, soil carbon saturation and stabilization, soil carbon stock, etc. However, the role of soil microbial communities, the primary driver of the terrestrial carbon cycle and the associated biological processes remains inadequately probed. Arbuscular mycorrhizal fungi (AMF) belonging to the phylum *Glomeromycota*, form a significant group in the soil microbial community and are abundantly distributed in the terrestrial ecosystems (Smith and Read 2008; Begum et al. 2019). AMF represents about 20–30% of the soil microbial biomass (Olsson et al. 1995; Olsson et al. 1999; Leake et al. 2004) and significantly impact soil structure and the nutrient cycling, particularly C, N and P (Miller and Jastrow 2000; Smith and Read 2008). They also influence rhizodeposition, root decomposition and root penetration by the virtue of their influence on plant biomass (Rillig and Mummey 2006). Some notable functions of AMF include (1) augmentation of plant-mineral nutrition, (2) vegetation refurbishment and re-establishment, (3) protection from abiotic and biotic stresses (4) plant biodiversity maintenance, (5) formation of soil aggregates through the mechanical action of hyphae (6) mitigation of global climate change (Smith and Read 2008; Begum et al. 2019; Sharma et al. 2020; Chourasiya et al. 2021) and (7) mitigation of heavy metal stress (Wu et al. 2016; Gujre et al. 2021).

AMF produce insoluble, glue-like, hydrophobic and recalcitrant glycoproteins, called glomalin or glomalin related soil protein (GRSP) (Wright and Upadhyaya 1996) which stabilize soil aggregates (Wright and Upadhyaya 1998; Liu et al. 2020), and play an important role in soil carbon sequestration (Rillig et al. 2001). Apart from being soil aggregating substances, GRSP could also be related to AMF metabolism (Purin and Rillig 2008). GRSP production is also influenced by the physical environment. Under stressful environments, AMF improves the survival of both the symbionts by augmenting GRSP production to promote soil aggregation (Rillig and Steinberg 2002), which shows that GRSP production is governed by AMF physiology (Rillig 2004). The co-action of AMF (AMF colonization) and GRSP production play a huge part in improving the soil health and protecting the soil structure (Wright and Upadhyaya 1998; Miller and Jastrow 2000; Rillig et al. 2002).

In this chapter, we have provided a comprehensive overview of AMF mediated soil organic carbon (SOC) sequestration and improvement in soil health and quality through standing hyphal crops and GRSP production. We have provided a detailed insight into the mechanisms of AMF and GRSP-mediated soil aggregation and

stabilization, and consequent SOC sequestration with an emphasis on the influence of agricultural management practices.

15.2 The Concept of Soil C-Sequestration, Health and Quality

Globally, among the chief carbon pools, the terrestrial C pool is ranked third and is approximately fourfold the biotic and thrice the atmospheric pool (Lal 2003; Gougoulias et al. 2014). The pedologic (soil) pool of carbon, which has been estimated to be 2500 Pg, consists of organic (1550 Pg) and inorganic carbon (950 Pg) (Batjes 1996; Lal 2004). Agriculture is ranked as the second-largest emitter of greenhouse gases (GHGs) covering all sorts of emissions i.e., both direct (agriculture induced CH_4 and N_2O) and indirect (CO_2 arising from fossil fuels as well as from changes in land use for agricultural purpose) emissions and represents about 17–32% of all human-induced GHG emissions, globally (Bellarby et al. 2008). Stockmann et al. (2013) described soil carbon sequestration as 'lock up' of C derived from atmospheric CO_2 where the C may be sequestered either by amassing the persistent carbon or it could be achieved via modification of respective magnitude of C-pools differing from each other in terms of residence time. However, the amount of C-stocks is considered less significant than soil quality which is attained through the integration of new carbon i.e., biomass (Stockmann et al. 2013). The three principal mechanisms for soil organic matter (SOM) protection include: (1) recalcitrance of SOM i.e., protection from decomposition (based on molecular characteristics), (2) accessibility (restricted access for degradation) and (3) interaction (association with mineral particles) (Sollins et al. 1996). The combined outcome of the aforementioned aspects is the SOM stability which improves with increased recalcitrance, reduced accessibility, whereas for increasing the interaction, microbial polysaccharides are important (Sollins et al. 1996). Baldock et al. (2004) explained the mechanisms of biological stabilization i.e., recalcitrance, capability and capacity for SOM degradation, physical protection and environmental attributes influencing decomposition. In this context, the physical protection in particular applies to soil aggregation which is a multifaceted process, leading to the formation and stabilization of aggregates and is intermediated by several biotic and abiotic factors (Rillig et al. 2015). During aggregation, the entanglement of particles occurs by means of fungal hyphae and roots (Bronick and Lal 2005; Rillig et al. 2015) and soil aggregates thus formed possess a pore space matrix, providing space for mycorrhizal operation and inhabit microbes (Vadakattu 2011). In this framework, AMF constitute an important biotic factor influencing soil aggregation and stabilization (Rillig et al. 2015). Inside a stable soil structure resides beneficial microorganisms critical in biogeochemical cycling and microbial by-products that further promote soil aggregation, stabilization and C-sequestration. The integration of SOM in aggregates restricts microbial and enzymatic attacks thus, shielding SOM from mineralization (Lützow et al. 2006). Soil aggregate stability is linked to soil physical properties like the ability to sequester soil C, water holding capacity, infiltration frequency and erosion resistance. Water-stable aggregates preserve soil porosity, aeration and

contribute to a stable soil structure to sustain soil biota and plants (Miller and Jastrow 2000; Lützow et al. 2006) thus, improving soil health and quality. The two terms soil quality and soil health are often considered equivalent but, soil quality is a broader term reflecting soil physical, biological and chemical functionality, ultimately influencing the crop yield (Soil Health Partnership 2020). According to the U.-S. Department of Agriculture- Natural Resource Conservation Service (USDA-NRCS), 'soil health, also known as soil quality, is the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals and human'. The most widely accepted definition of soil quality is the soil's capacity to function within the boundaries of the ecosystem and land use, to support biological productivity, environmental quality and promote the health of living beings, i.e., plants and animals (Doran et al. 1994).

15.3 Plant-AMF C-Exchange

AMF exist in two segments, viz., intraradically (inside the root) and extraradically (in the soil) (Fig. 15.1). The intraradical phase comprises hyphae, arbuscules (for the exchange of nutrients and carbon) and vesicles (for lipid storage). The intraradical phase is connected to the extraradical phase, forms spores and increases root surface

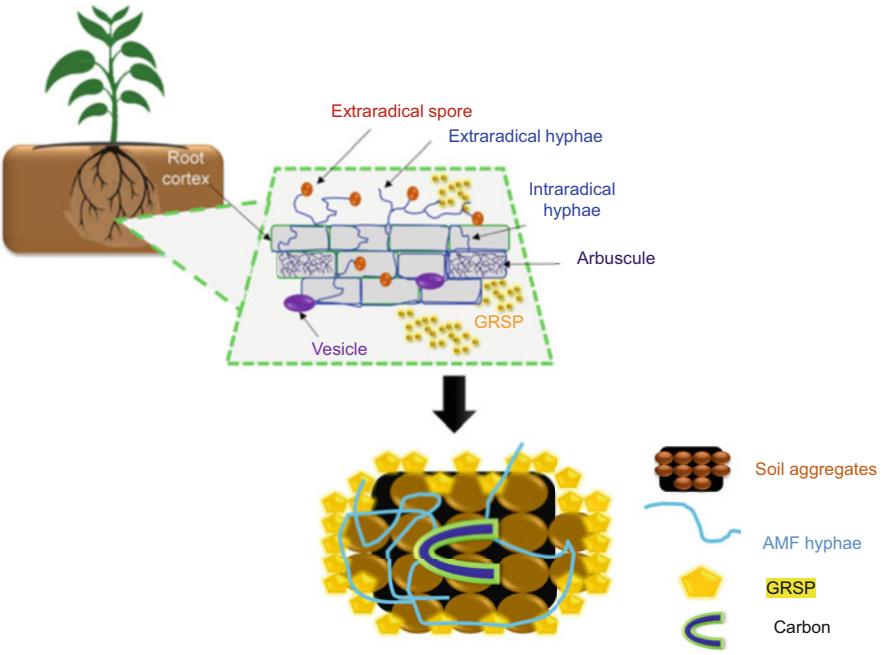

Fig. 15.1 Plant–arbuscular mycorrhizal fungus (AMF) symbiosis, glomalin related soil protein (GRSP) production and soil aggregate formation and stabilization

Table 15.1 Plant-mediated photosynthate allocation to AMF

Plant	AMF	C allocated to AMF (%)	Technique	Reference
C3 and C4 grass (genus *Panicum*)	*Rhizophagus intraradices*, *Claroideoglomus claroideum*, *Funneliformis mosseae*	3.9% higher than non-mycorrhizal counterparts	$^{13}CO_2$ pulse-chase labelling and measurement	Řezáčová et al. (2018)
Allium porrum, *Medicago truncatula* and *Lolium perenne*	*Rhizophagus*, *Funneliformis* (dominant), *Claroideoglomus*, and *Diversispora* (co-dominant)	0.9–10.5%	AMF-specific fatty acids and $^{13}CO_2$ pulse labelling	Konvalinková et al. (2017)
Medicago truncatula	*Rhizophagus irregularis*	2.3–2.9%	$^{13}CO_2$ labelling and measurement	Slavíková et al. (2017)
Sorghum bicolor (L.) Moench	*Glomus clarum*	4%	$^{14}CO_2$ labelling	Calderón et al. (2012)
Lolium perenne	*Glomus hoi*	3–8%	$^{13}CO_2/^{12}CO_2$ labelling and exchange	Grimoldi et al. (2006)
Turfs with *Festuca rubra*, *Agrostis capillaris*, *Poa pratensis* and *Anthoxanthum odoratum* as dominant plants	Native AMF	3.4%	$^{14}CO_2$ labelling	Johnson et al. (2002)
Cucumis sativus L.	*Glomus fasciculatum*	20%	$^{14}CO_2$ labelling	Jakobsen and Rosendahl (1990)
Allium porrum L.	*Glomus mosseae*	7% higher than non-mycorrhizal counterparts	$^{14}CO_2$ labelling	Snellgrove et al. (1982)

area for nutrient and water acquisition (Miller and Jastrow 2000; Smith and Read 2008). AMF are completely dependent on host plants for their C requirements. Recent evidence also suggests that plant could also provide lipids to the AM symbiont (Jiang et al. 2017). Being obligate symbionts, AMF-plant symbiosis could create a C-sink, draining about 0.9–20% of photosynthates manufactured by the host plant, thereby influencing soil C storage (Table 15.1). A sizeable amount of this C could be utilized for producing GRSP as the process requires a significant investment of C (Lovelock et al. 2004a, b).

15.4 AMF and GRSP in Soil Structure Formation and C Storage

The dry weight of AMF hyphae as estimated by phospholipid fatty acid analysis was found to be 0.03–3.5 mg g^{-1} and was determined as the greatest contributor to the soil microbial biomass (Olsson et al. 1999). It has also been observed that the AMF infectious propagules i.e., extra radical hyphae, arbuscules and vesicles represented 15% of SOC (Guo and Tian 2013). About 54–900 kg ha^{-1} of accumulated SOC could be contributed by AMF extraradical hyphae (Zhu and Miller 2003). Adding to this, GRSP production takes place on hyphae and a large portion of GRSP remains attached to hyphae and spores (Driver et al. 2005). GRSP production also occurs inside roots and a significant amount of GRSP is present in a residual form that associates with adjacent soil particles soon after the release (Lovelock et al. 2004a; Driver et al. 2005; Agnihotri et al. 2021). AMF shield SOM, by aggregating soil particles and creating organo-mineral complexes and contribute to aggregate stability through extraradical hyphae and GRSP production (Wright and Upadhyaya 1996; Rillig 2004). The carbonaceous compounds get accumulated and are protected inside the structural framework formed by AMF, plant roots and GRSP (Miller and Jastrow 2000). Inside such stable soil structure, the sequestered C is safeguarded from microbial degradation and respiration (Treseder and Allen 2000; Zhu and Miller 2003; Rillig 2004). Tisdall and Oades (1982) through their aggregate hierarchy concept (Fig. 15.2) pointed out that soil aggregates are formed through the influence of organic matter, hyphae and fine plant roots. Mycorrhizal hyphae physically interweave soil particles and bind microaggregates into macroaggregates. The aggregate hierarchy concept suggested that free primary and silt sized particles (<20 μm) form stable microaggregates (20–250 μm) using persistent binding agents (humified organic matter and polyvalent metal cation complexes) and highly disordered aluminosilicates and oxides. These stable microaggregates in turn form macroaggregates (>250 μm) using temporary (fungal hyphae and roots) and transient linking agents (microbial- and plant-derived polysaccharides). Rillig and Mummey (2006) also discussed the concept of the formation of macroaggregates from microaggregates through AMF. Macroaggregates are the intrinsic elements that form soil structure are formed by a combined action of AMF hyphae and plant roots which are further protected by GRSP (Miller and Jastrow 2000). Nichols and Halvorson (2013) described macroaggregate formation and stabilization as two distinct processes where GRSP are actively involved in the stabilization process. The content of GRSP was positively correlated with the percentage of water-stable aggregates across various soil types (Wright and Upadhyaya 1996, 1998). The positive correlation between GRSP, SOM and water-stable aggregates indicates the role of GRSP and SOM in preserving soil structure (Wright et al. 2007; Fokom et al. 2012). A strong relationship has been reported between AMF colonization, soil aggregation and SOC sequestration in long-term field experiments (Wilson et al. 2009; Singh et al. 2016).

GRSP structures have a significant amount of C (Table 15.2) and are often referred to as 'bulk of SOC' positively influencing soil aggregate stabilization (Rillig et al. 2003b). By producing GRSP, AMF may create an abundant C-sink in

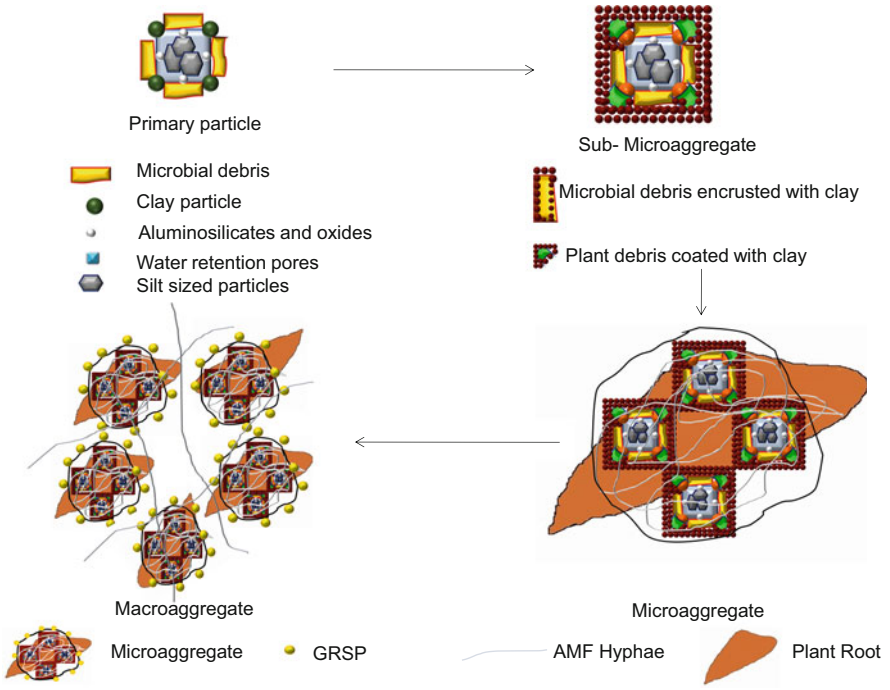

Fig. 15.2 The aggregate hierarchy concept merged with glomalin related soil protein (GRSP) (Tisdall and Oades 1982; Wright and Upadhyaya 1998; Miller and Jastrow 2000)

Table 15.2 Carbon (C) content in GRSP extracted from different ecosystems

Soil type	GRSP-C	Reference
Hawaiian tropical rainforest	10–22%	Rillig et al. (2001)
Bulk soils (acidic loam) (0–10 cm), Maryland, Colorado, Georgia	35–40%	Nichols and Wright (2006)
Mineral soils (0–15 cm), South Dakota, USA and peat soils	41.4–58.6%	Schindler et al. (2007)
Surface soil (0–30 cm) plantation forests, farmland and primary forests, Northeast China	33.4–52.2%	Q. Wang et al. (2015a)
Tropical forests Dinghushan biosphere reserve, southern China	12.95–20.2%	J. Zhang et al. (2017a)
CO_2 enrichment and N addition Guangzhou, China	15.9–30.7%	Zhang et al. (2015)
Songnen plain, Northeast China	43.41%	Z. Zhang et al. (2017c)
Marine sediments (0–10 cm), Old Yellow River delta	10.8–24.3%	Wang et al. (2018)

soil with much higher longevity than the intra or extraradical AMF biomass (Rillig et al. 2001). The positive association between GRSP and SOC has been reported in many ecosystems including croplands, grasslands and forests (Rillig et al. 2003b;

Table 15.3 Contribution of GRSP-C to SOC in different ecosystems

Contribution	Ecosystem	Reference
1–2%	Alaskan boreal forest	Treseder and Turner (2007)
4–5%	Hawaiian tropical rainforest	Rillig et al. (2001)
13%	Bulk soils (acidic loam) (0–10 cm), Maryland, Colorado, Georgia	Nichols and Wright (2006)
3.2%	Tropical rainforest, Costa Rica	Lovelock et al. (2004a)
4–8%	Steppes. SE Spain	Rillig et al. (2003a)
4.6–6.2%	Guangzhou, China	Zhang et al. (2015)

Lovelock et al. 2004a; Wang et al. 2015a; Wang et al. 2017). Further, GRSP have been reported in floodplains, natural deposits (sediments) of seagrass which depicts that GRSP can be deposited in sediments as constituents of SOC (López-Merino et al. 2015). As evinced from the positive correlation observed between GRSP and SOC, GRSP also serve as important C source towards SOC accrual (Rillig et al. 2001) (Table 15.3). Numerous studies have related GRSP to long-term C and N storage (Rillig et al. 2001; Lovelock et al. 2004a). Furthermore, GRSP represent approximately 3.2% of the soil C (Lovelock et al. 2004a) (Table 15.3). In the experiments of Nichols and Wright (2006), GRSP constituted the largest pool of soil C and N (Table 15.3). Overall, GRSP are important components of soil organic matter, constituting a substantial global pool of SOC and N. The C-sequestered by GRSP is long-lived and stays inside the soil matrix for 11–92 years (Preger et al. 2007) due to the tight association with stable and persistent soil organic matter (Rillig 2004) and this complicates the biochemical assessment of GRSP.

15.5 GRSP: AMF Mediated Soil Conditioners

GRSP through sequestering C and increasing its persistence improve soil structure and microbial activity (Bai et al. 2009). The adhesive characteristics of GRSP enable them to bind soil particles, thereby creating aggregates that are critical for soil biological activity, and aeration creating a habitat for microbial growth (Wright and Upadhyaya 1998). Characteristically, GRSP also create a hydrophobic coat on developing hyphae and soil aggregates, averting the disruption of aggregates (Miller and Jastrow 2000) and helps indirectly in soil conditioning. Soluble carbohydrates, soil quality enzymes and proteins associated with GRSP are important in the formation and maintenance of soil structure (Wright and Upadhyaya 1998; Wu et al. 2012; Wang et al. 2015b). The contribution of GRSP to total C has been reported to be significantly higher than microbial biomass carbon (Rillig et al. 2001). Moreover, this association of GRSP with microbial biomass and nutrient cycling enzymes substantiates the role of GRSP in nutrient cycling and soil reclamation (Fokom et al. 2012; Wu et al. 2012; Agnihotri et al. 2021). It has also been suggested that AMF hyphae could stimulate certain microbial populations in the surrounding

Table 15.4 Overview of soil parameters/processes GRSP can provide indication of

Indicator	Reference
SOM quality	Balík et al. (2020)
Soil quality	Vasconcellos et al. (2016)
Soil fertility	Lovelock et al. (2004a), Šarapatka et al. (2019)
Heavy metal contamination	Gujre et al. (2021)
Soil aggregation	Wright and Upadhyaya (1998)
SOC sequestration	Agnihotri et al. (2021)
Soil erosion	Šarapatka et al. (2019)
AMF biomass	Bedini et al. (2007), Agnihotri et al. (2021)
Desertification	Wang et al. (2017), Zhang et al. (2017a, b, c)

area (Miller and Jastrow 2000). Overall, soil aggregate stability is achieved through the co-action of several biotic and abiotic agents. GRSP associate with SOM and minerals clumps soil particles and forms microbial habitat to improve the soil aggregate stability and soil C-sequestration. Owing to their minimal decomposition and low susceptivity to environmental shifts, GRSP have been proposed as bioindicators of soil quality (Rillig et al. 2001; Wang et al. 2015a; Vasconcellos et al. 2016) (Table 15.4).

AMF help in plant establishment and survival under stresses including drought, nutrient deficiency, soil disturbance, etc. AMF have also been considered effective in helping re-vegetation in desertified grasslands (Zhang et al. 2012) and degraded semi-arid lands (Barea et al. 2011). The positive association of GRSP with soil physical, chemical and biological parameters, potentiate their usefulness in soil health monitoring in land restoration processes (Singh et al. 2016; Singh et al. 2017; Kumar et al. 2018; Liu et al. 2020). Both AMF and GRSP help in soil fertility restoration, and also contribute to C-sequestration and soil aggregate formation and stabilization (Miller and Jastrow 2000; Wu et al. 2017; Liu et al. 2020; Agnihotri et al. 2021). Likewise, AMF and GRSP may act as soil quality indicators while studying desertification, soil degradation and degraded soil restoration (Wang et al. 2017; Zhang et al. 2017b). GRSP increase the availability of environmental pollutants such as polycyclic aromatic hydrocarbon (PAH) in soil (Gao et al. 2017) and also possess the potential for accumulating and stabilizing SOC during pedogenesis for the rehabilitation of mining areas (Kumar et al. 2018). Therefore, in planning and implementing bioremediation strategies both AMF and GRSP could be helpful.

15.6 More About GRSP: Structure and Extraction

GRSP are glycoproteins (Wright et al. 1998) that exist as a mixture of thermostable AMF and non-AMF proteins and by the virtue of their sticky nature, they trap plant waxes, lipids, lignin's, metal ions humic and inorganic substances whose varied relative amounts have been reported in various studies (Schindler et al. 2007;

Gillespie et al. 2011; Wang et al. 2015a, b). GRSP are AMF gene product released via AMF hyphae and this explains extreme conditions required to solubilize the GRSP (Driver et al. 2005). The heat-stable humic substances survive the extraction procedure, therefore, GRSP have been more aptly stated as glomalin related soil protein (GRSP) (Rillig 2004; Rosier et al. 2006; Holátko et al. 2021). GRSP are categorized into two pools i.e., easily extractable (EE) and total (T) GRSP denoting the freshly formed and older fraction, respectively (Wright and Upadhyaya 1996; Rillig 2004). GRSP are alkali-soluble substances extracted from soil using sodium citrate as an extractant at neutral pH after autoclaving at 121 °C and 15 lbs. pressure. EE-GRSP require milder extraction conditions than T-GRSP (Wright and Upadhyaya 1996). The protein is quantified by means of monoclonal antibodies raised against AMF spores through enzyme linked immunosorbent assay (ELISA) or spectrophotometrically (Bradford 1976; Wright et al. 1996).

15.7 Aspects Governing GRSP Production

15.7.1 AMF and Plant Species

In general, GRSP have been detected on extra radical hyphae of AMF but their production could vary among AMF species (Lovelock et al. 2004b). In contrast, the diversity of plant species (host and adjoining plants) could shape AMF communities by influencing hyphal growth (Hausmann and Hawkes 2009; Chourasiya et al. 2021). Therefore, both host and AMF species influence GRSP production. Several studies have revealed a reduction in AMF population after monoculture, and fallow, and thereby affecting GRSP production in cropland (Fokom et al. 2012; Burrows 2014) (Fig. 15.3).

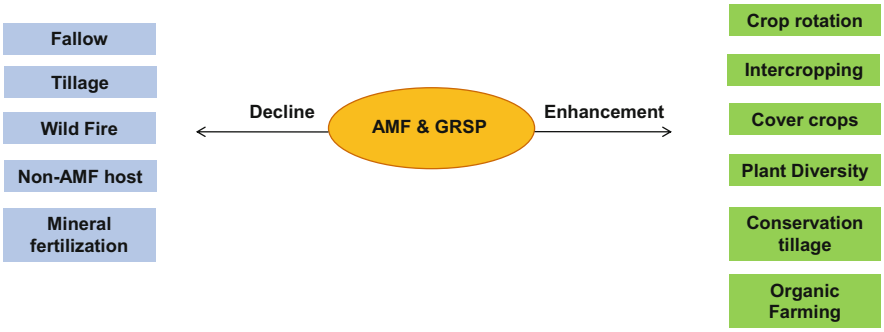

Fig. 15.3 An overview of factors affecting AMF population, diversity and GRSP production

15.7.2 Edaphic Factors

Soil physicochemical properties also have a key role in shaping the AMF communities. Positive correlations have been observed between AMF diversity and SOC and soil available P; whereas, pH was negatively correlated with AMF spore density (Chen et al. 2012). Moreover, SOC and pH strongly regulate GRSP accrual in soil (Wang et al. 2017). Besides, urbanization (Wang et al. 2019), vegetation type and land use (Agnihotri et al. 2021; Gujre et al. 2021) also govern GRSP content in the soil. Temperature (warming) decreases GRSP and their contribution to nutrients and SOC (Wang et al. 2017; Chourasiya et al. 2021).

15.7.3 Agricultural Practices

Soil disturbance reduces the density of AMF spores, species richness and the extraradical hyphal length (Boddington and Dodd 2000). The magnitude of mechanization (tillage) and mineral fertilization reduce AMF biomass and GRSP (Avio et al. 2013; Agnihotri et al. 2021) as tillage physically destroys AMF hyphal networks thereby damaging AMF standing crops and the consequent production of GRSP. Correspondingly, utilizing a natural ecosystem for agricultural purposes decreases AMF spore density and GRSP production (Fokom et al. 2012). It has been observed that tillage regulates the sporulation pattern and *Funneliformis mosseae* were found to be more prevalent in tilled soils, and a prevalence of *Glomus viscosum* and *Glomus intraradices* and a higher GRSP content were observed in no-tilled soils (Avio et al. 2013). Similarly, tillage also influences AMF communities and spore density could be particularly important in determining the changes induced by agricultural practice (Castillo et al. 2006). Besides, N fertilization proved important in structuring AMF communities (Avio et al. 2013) (Fig. 15.3).

15.8 Current Understanding and Knowledge Gaps

Since the discovery of GRSP, the controversies associated with their structure, origin and extraction have never been resolved. Many studies have denied their AMF origin. Recent research, however, have reported a strong association between GRSP and measures of AMF biomass such as spore density (Singh et al. 2016; Kumar et al. 2018; Das et al. 2020; Agnihotri et al. 2021) and an increase in GRSP content upon AMF inoculation (Ghasemi Siani et al. 2017). Taking account of all the humic acid-like compounds co-extracted during GRSP extraction, recent studies referred GRSP to as ‘autoclaved citrate extractable protein’ (ACE) or ‘soil protein’ (Hurisso et al. 2018). Such terminology further expands the applicability of the existing protocol of GRSP extraction in soil biological health assessment without questioning their mycorrhizal origin (Hurisso et al. 2018). Despite the controversies, GRSP remain a reliable indicator of soil health, owing to their ability to accumulate and stabilize across a variety of agro-ecosystems.

15.9 Conclusions

Managing ways to enhance SOC sequestration and sustain the plant productivity under changing climate is one of strategies to mitigate climate change and meeting the UN agenda of 2030 of sustainable developmental goals to protect the planet. AMF and GRSP have a lot of potential for stabilizing soil aggregates and sequestering soil carbon. Appropriate management strategies should be considered for harbouring abundant AMF biomass and increasing GRSP production. Strong relationships between AMF, GRSP and SOC, in both undisturbed (natural) and cultivated lands, have been reported. Understanding the history of soil management, cropping sequences and native AMF species can help us better GRSP production and their distribution. A careful assessment of GRSP in agro-ecosystems would widen their applicability in predicting the C-sequestration, soil health and sustainability of agro-ecosystems. Therefore, studies on the selection of the most adapted AMF species producing higher GRSP under in vitro should be attempted eventually to utilize and exploit their potential in remediating the environmentally vulnerable ecosystems in addition to soil C-sequestration in large-scale field trials.

Acknowledgements This compilation was carried out under DBT-NER (Department of Biotechnology-North Eastern Region) Twinning Programme funded project on AMF-biochar research. The authors are thankful to the Director, ICAR-Indian Institute of Soybean Research, Indore for providing infrastructure facilities.

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Arbuscular Mycorrhizal Fungi Influence Crop Productivity, Plant Diversity, and Ecosystem Services

16

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Abstract

Arbuscular mycorrhizal (AM) fungi living in soil colonize plant roots. The beneficial effect of AM fungi in improving plant growth is well documented. Many of the ecological functions performed by the AM fungi provide essential services to crop productivity, such as nutrient cycling, soil structure improvement, plant growth promotion, resistance against biotic and abiotic stresses, and bioregulation of plant development. These ecological supports are not only essential to ecosystem function but also a critical resource for the sustainable management of agricultural systems. Studies have shown that plant biodiversity, crop productivity, crop protection, and ecosystem stability increase with the increasing number of AM fungi. It appears that the diversity of AM fungi in soil is a major dynamic factor contributing to the maintenance of global plant biodiversity, crop productivity, and ecosystem functioning. The role of AM fungi in plant diversity, crop productivity, and ecosystem services are discussed in this review.

Keywords

AM fungi · Crop productivity · Ecosystem services · Plant diversity · Plant protection

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_16

16.1 Introduction

Soil ecosystems provide shelter and nourishment to a huge variety of organisms. Soil inhabiting organisms are responsible for significant contributions not only toward productivity (e.g., nitrogen fixation, phosphorus solubilization, production of phytohormones, and biocontrol), but also toward ecosystem regulatory functions (e.g., carbon sequestration, soil conservation, regulation of greenhouse gases, and hydrological balance). Diversity of soil organisms is important from the point of inventory, ecological functions and management of agroecosystems (Bagyaraj and Ashwin 2016; Jacoby et al. 2017; Powell and Rillig 2018). Some organisms such as mycorrhizal fungi, rhizobia, plant growth-promoting rhizobacteria, soil-borne pests, and disease-causing organisms may directly benefit or harm the plant growth and performance (Jeffrey and Gardi 2009). Frank (1885), a German botanist, first coined the term "mycorrhiza", which literally means "fungus root" to describe the mutualistic association between roots of higher plants and certain fungi. Although there are different kinds of mycorrhizal association (like ecto, ericoid, orchid, and arbuscular mycorrhizae), arbuscular mycorrhizal (AM) fungi are the most common and widely occurring of all the mycorrhizal associations, and they have great economic significance in agriculture, horticulture, and forestry (Gianinazzi et al. 2010; Rodrigues and Rodrigues 2014). Arbuscular mycorrhizal mutualism is known among >100,000 plant species with several hundreds of morphotypes having asexual propagation with the capability of hyphal fusion to augment genetic diversity without meiosis (Chagnon 2014; Chen et al. 2018). Some plants have both ecto and AM fungal association which probably helps sustenance in harsh environmental conditions (Teste et al. 2020).

16.2 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal (AM) fungi as mutualists with plant species, have a long history of evolution over ~400 million years ago in the early Devonian period, possess perfect genetic and metabolic adaptation between partners (Redecker et al. 2000; Heckman et al. 2001; Schüβler et al. 2001). Such a long-term evolution of AM fungi augmented intimate interaction among soil, plant, and ecosystem, which resulted in gene exchange for AM fungal stability. The AM fungi are known to establish mutualistic relationships with about 80% of vascular plants (Bagyaraj 2011; Berruti et al. 2015). In addition to their widespread distribution throughout the plant kingdom, AM fungi are ubiquitous and occur in plants growing in arctic, temperate, and tropical regions (Bagyaraj 2014a; Vieira et al. 2019). They have been reported to be associated with plants grown in sand dunes, coal mines and aquatic environments (Beena et al. 2000; Sridhar 2006; Bagyaraj 2014a). The fungi live partly inside the root and partly in the surrounding soil. Inside the root they are found in the cortical region producing "fan-like" structures called arbuscules and globular structures known as "vesicles". In soil, they produce extending hyphae as well as large-size chlamydospores. The AM fungi are obligate symbionts and cannot be

cultured on synthetic media. Hence, they are maintained in the roots of living host plants as "pot cultures" for propagation as well as inoculum production.

The AM fungi belong to the phylum *Glomeromycota* has one class *Glomeromycetes* with 4 orders (*Glomerales*, *Diversisporales*, *Paraglomerales*, and *Archaeosporales*), 11 families and 25 genera (Redecker et al. 2013). The commonly occurring genera of AM fungi are *Acaulospora, Entrophospora, Glomus, Gigaspora,* and *Scutellospora*. Improved plant growth due to inoculation of soil with AM fungi has been demonstrated especially under phosphorus-deficient conditions (Mosse 1977; Gianinazzi and Gianinazzi-Pearson 1986; Bagyaraj et al. 2015). The growth improvement is mainly because of enhanced uptake of diffusion-limited nutrients especially P, Zn, and Cu. The AM fungi can also enhance tolerance of plants to root fungal pathogens like *Phytophthora parasitica*, *Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *Chalara* (*Thielaviopsis*) *basicola*, *Rhizoctonia solani*, *Sclerotium rolfsii, Pythium ultimum,* and *Aphanomyces* spp.; bacterial pathogens such as *Pseudomonas syringae* and *P. solanacearum* and nematodes such as *Meloidogyne incognita*, *M. javanica*, *Tylenchulus semipenetrans*, *Pratylenchus dihystera,* and *Radopholus similis* (Bagyaraj and Chawla 2012; Bagyaraj 2016). AM fungi also help in alleviating abiotic stresses such as drought and metal toxicity (Gianinazzi et al. 2010; Bagyaraj 2016; Mathimaran et al. 2017). The AM fungi play a significant role in the formation of stable soil aggregates, building up macroporous structure that allows penetration of water as well as air, and prevents soil erosion. Production of extracellular polysaccharides like β-1,3-glucan by the AM fungi (e.g., *Acaulosporaceae* and *Glomaceae*) leads to soil aggregation (Lemoine et al. 1995). Extracellular hyphae of AM fungi (e.g., *Glomus intraradices* and *G. mosseae*) are capable to produce hydrophobic protein called glomalin, which leads to soil aggregation (1–2 mm , diameter) and it has been correlated with hyphal biomass leading to soil stability (Bedini et al. 2009). The AM fungi enhancing the levels of plant hormones such as cytokinins and gibberellin-like substances has also been reported (Begum et al. 2019). They can tolerate a wide range of soil-water regimes and also improve water relationships of many agriculturally important crop plants (Barrios 2007; Bahadur et al. 2019; Chaitanya et al. 2020).

Improved growth of cultivated plant species is brought about by introduced populations of beneficial soil microorganisms like *Azotobacter*, *Azospirillum*, and phosphate solubilizing bacteria (Pathak et al. 2017; Debnath et al. 2019). Mycorrhizal plants may allow these beneficial species to be maintained in higher numbers than non-mycorrhizal plants (Bagyaraj and Ashwin 2016). Although, AM fungi are not host specific, but have host plant preference. Thus, after screening several AM fungi, the most efficient fungi for inoculating particular crop/tree species have been developed (Ashwin et al. 2018, 2019). Nearly 25–50% of phosphatic fertilizer can be saved through inoculation with efficient AM fungi to a specific plant species (Bagyaraj et al. 2015). Tissue-cultured plantlets also respond very well to AM fungal inoculations. Co-inoculation of AM fungi with nitrogen-fixers and phosphate-solubilisers showed improvement in growth and yields of economically important plants (Desai et al. 2016; Sukeerthi et al. 2020).

The AM fungi constitute the main component of soil microbiota in most of the agroecosystems. Hence, they have a strong influence on the diversity of plant species (Powell and Rillig 2018; Tedersoo et al. 2020). Their existence in soil with genetic and functional diversities is valuable for both plant community and ecosystem productivity (Bidartondo et al. 2002; Gianinazzi et al. 2010). Apart from plant species, various agricultural and soil management practices may also alter the population and diversity of AM fungi (Bagyaraj and Ashwin 2020; Tedersoo et al. 2020). Interest in AM fungal studies has reached a peak in the recent past owing to their ability to produce dramatic responses in plant growth and productivity.

16.3 Significance of AM Fungi

16.3.1 AM Fungi Reduce Application of Phosphate Fertilizer

Nitrogen, phosphorus and potassium are the three major plant nutrients of which phosphorus is non-renewable nutrient. Rock phosphate is a major ingredient for the manufacture of phosphatic fertilizers. Even though India has an estimated amount of 250 metric tons of rock phosphate, much of it is of low-grade having less than 25–30% P_2O_5 and not suitable for manufacture of phosphatic fertilizers. Thus, it is inevitable to India to depend on imports, to an extent of 90%, to meet its domestic requirement of phosphate in the form of rock phosphate, phosphoric acid, and phosphatic fertilizer. Rock phosphate sources, even globally are limited and it is predicted that most of such phosphate mines will be depleted in about 100 years (Geological Survey 2019).

Absorption of nutrients by plants mainly through roots in soil is governed by two major factors: (i) transfer of ions through soil; (ii) absorbing capacity of the root. The transfer of ions through the soil occurs either by mass flow (mobile elements like NO_3, SO_3, and Ca) or by diffusion (immobile or diffusion-limited elements like H_2PO_4, NH_4, Zn, and Cu). The uptake of mobile elements is limited by the absorbing capacity of the roots, while the uptake of immobile elements depends on their movement to the root surface and then the absorbing capacity of the root (Bagyaraj et al. 2015). Phosphorus is a diffusion-limited major nutrient that is essential for plant growth. Plants require adequate phosphorus from the very early stages of growth for optimum production. Root interception to the adsorbed phosphorus on soil particles is enhanced by the AM fungal external hyphal network, which extends beyond root zone and phosphorus-deficient zone as well, thus increasing the effective surface area for uptake of phosphorus. Enhanced plant growth owing to inoculation with AM fungi is well documented (Bidartondo et al. 2002; Bagyaraj 2014b; Ashwin et al. 2019). Many workers have investigated the mechanism of improved plant growth caused by mycorrhizal inoculation. Greater soil exploration by mycorrhizal roots as a means of increasing phosphate uptake is well known. Phosphate ions are fairly immobile and a phosphate depletion zone often develops around roots in phosphate deficient soils. The hyphae are able to reach beyond this zone and directly translocate nutrients from the soil to the root cortex, where transfer to the

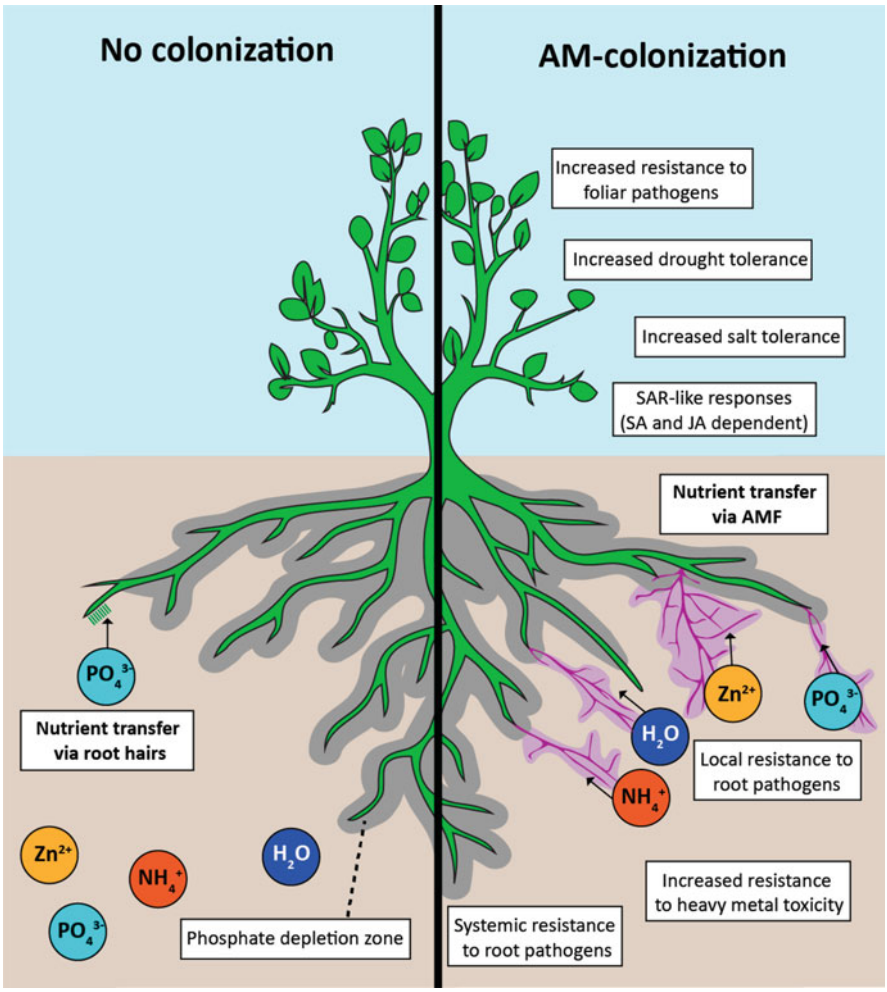

Fig. 16.1 Positive effects of arbuscular mycorrhizal (AM) fungal colonization. The hyphal network of AM fungi extends beyond the depletion zone (gray), accessing a greater area of soil for phosphate uptake. A mycorrhizal-phosphate depletion zone will also eventually form around AM hyphae (purple). Other nutrients that have enhanced assimilation in AM roots include nitrogen (ammonium) and zinc. Benefits from colonization include tolerances to many abiotic and biotic stresses through induction of systemic acquired resistance (SAR). Source: Jacott et al. (2017). [Picture of plant root system listing benefits of AM fungal colonization] [Graphical abstract]. https://www.mdpi.com/238390

plant occurs (Fig. 16.1). Experiments with ^{32}P-labeled phosphate indicate that, AM fungal hyphae obtained their extra phosphate from the labile pool rather than by dissolving insoluble phosphate. The better utilization of sparingly soluble rock phosphate is explained by the hyphae making closer physical contact with the ions disassociating at the particle surface. Significant increases in plant growth and yield

of several plants important in agriculture, horticulture, and forestry is due to AM fungal inoculation in non-sterile soils containing low or insufficient levels of indigenous endophytes or mutualists have been reported by several researchers. These studies also showed that application of phosphatic fertilizer could be reduced by 50% (Thilagar et al. 2015; Jyothi and Bagyaraj 2018). It is evident that such reductions in phosphate application have important economic benefits as well as protection of environment.

16.3.2 AM Fungi Protect Plants against Major Soil-Borne Pathogens

Many soil-borne plant pathogens have been reported to cause serious diseases in several crop plants. Most of the studies on AM fungi vs. root pathogens suggest that the AM fungi reduce or mitigate the disease severity (Bagyaraj and Chawla 2012). Consistent reduction of disease severity has been described by Bagyaraj (2006, 2016) in a wide range of fungal pathogens (e.g., *Gaeumannomyces graminis* var. *tritici*, *Phytophthora parasitica* and *Sclerotium rolfsii*), bacterial pathogens (e.g., *Pseudomonas syringae* and *Ralstonia solanacearum*) and pathogenic nematodes (e.g., *Meloidogyne incognita*, *M. javanica,* and *Tylenchulus semipenetrans*).

Studies performed so far suggest that the mechanisms of disease suppression owing to morphological, physiological, and biological alterations in the host plant species (Fig. 16.1). Thickening of cell walls due to lignification and production of other polysaccharides in mycorrhizal plants has been shown to prevent the penetration of hyphae and growth of pathogens like *Fusarium oxysporum* and *Phoma terrestris* (Santra and Banerjee 2020). The strong vascular system seen in mycorrhizal plants will increase the flow rate of nutrients, impart greater mechanical strength and diminish the impact of vascular pathogens. Study of the interaction of AM fungi vs. *Phytophthora* and many other such associations showed that the pathogen do not penetrate the cortical cells consist of arbuscules, suggesting that localized competition for the infection site occurs between the pathogen and the AM fungus (Veresoglou and Rillig 2012; Bagyaraj 2016).

Colonization of a plant by AM fungi alters the host physiology that in turn leads to decreased root exudation, due to increased membrane phospholipid content, which helps in reducing root pathogens infection (Mada and Bagyaraj 1993; Smith et al. 1994). Higher concentration of ortho-dihydroxy phenols present in mycorrhizal plants compared to non-mycorrhizal plants was also found to be inhibitory to the root-rot fungal pathogen *Sclerotium rolfsii* (Bagyaraj 2016). The activation of specific plant defense mechanisms as a response to AM fungal colonization is an obvious basis for such protective capacity of AM fungi (Bagyaraj 2016; Santra and Banerjee 2020). Among the compounds involved in plant defense studied in relation to AM fungi include production of phytoalexins, chitinases, β-1,3-glucanase, peroxidases, pathogenesis-related (PR) proteins, hydroxyproline-rich glycoproteins (HRGP) and phenolics (Bagyaraj and Chawla 2012). Mycorrhizal plants harbor higher population of microorganisms in the rhizosphere, thus making it difficult for the pathogen to compete successfully and gain access to the root.

Microorganisms producing siderophores (e.g., *Bacillus megaterium*, *Bacillus subtilis,* and *Azotobacter vinelandii*), which are low molecular weight chelating agents that have higher affinity for ferric iron and thus exhibit fungistatic effect between pathogens and mycorrhizal plants than non-mycorrhizal plants. In addition, mycorrhizal plants harbor relatively more actinomycetes, leading to an antagonistic effect to root pathogens (Bagyaraj 2006).

It has been reported that co-inoculation of plant growth-promoting rhizobacteria (PGPR) along with AM fungi protects plants better against root pathogens than inoculation of AM fungus alone. Wilt of the medicinal plant *Coleus forskohlii* (used in Ayurveda medicine) caused by *Fusarium chlamydosporum* is very serious problem in India. Inoculation with AM fungus plus *Trichoderma viride* was found to increase root yield as well as root forskolin (medicinally valued diterpene) concentration, and reduce the severity of the disease significantly under field conditions (Singh et al. 2009). Several studies have proved that combined inoculation of AM fungi and PGPR are very effective in alleviating the severity of the disease caused by soil-borne plant pathogens (Saldajeno and Hyakumachi 2011; Mohamed et al. 2019).

16.3.3 AM Fungi Alleviate Abiotic Stresses

Abiotic stresses are one of the primary causes of crop loss worldwide, which reduced the average yields for most of the major crop plants over 50% (Kang et al. 2014). Abiotic stresses lead to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Mathimaran et al. 2017). Drought, salinity, extreme temperature and oxidative stress are often interconnected and such stresses may induce similar cellular damage in host plant species (Chaitanya et al. 2020). The potential of AM fungi to enhance plant tolerance in abiotic stress conditions has long been well recognized (Smith and Read 2008; Muthukumar et al. 2019). The AM fungal symbiosis enhances osmotic adjustment in roots resulting in higher leaf water potential, less oxidative damage to lipids, enhancement of proline and trehalose content in roots and thus provide tolerance to the host against drought conditions (Chaitanya et al. 2020; Sharma et al. 2020). Mycorrhizal plants may overcome drought through enhanced water uptake at a low soil moisture levels. Available literature also demonstrated that AM fungi often have a substantial impact on water movement into, through, and out of the host plants, with a consequent effect on plant tissue hydration and leaf physiology. Furthermore, the AM fungal symbiosis can allow leaves to fix more carbon during the water stress. Fungal colonization is also known to enhance the water potential of plants, relative water content and chlorophyll concentration in the leaves (Bahadur et al. 2019; Chaitanya et al. 2020).

It is a common belief that antioxidant enzymes play an important role in fungal symbiosis conferring abiotic stress tolerance. Antioxidant enzymes are involved in the removal of reactive oxygen species (ROS) either directly (superoxide dismutases, catalases and ascorbate- or thiol-dependent peroxidases) or indirectly

through the regeneration of the two major redox molecules in the cell, ascorbate and glutathione (glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases) (Begum et al. 2019). Accumulation of trehalose protects the cell by stabilizing cell structures and enables protein to maintain their native conformation under conditions of drought stress. Trehalose production might also improve when plants are colonized by fungi and bacteria, which in turn help imparting stress tolerance to plants (Chaitanya et al. 2020; Sharma et al. 2020).

Among different abiotic stresses, soil salinity is a major challenge to sustainable agriculture. Some of the impacts of soil salinity includes reduction in soil quality, low agricultural production, reduced economic returns, and high costs of reclamation and management (Manchanda and Garg 2008). Although the response of crops to salinity varies with species, in general plant metabolism is affected, resulting in reduced plant growth as well as yield. Soil salinity results in stunted growth in plants due to inhibition of cell elongation. In spite of their sensitivity to salinity, roots are less affected than the shoots (Muthukumar et al. 2019). The beneficial effects of AM fungi on plant growth under saline stress have been demonstrated in many crop plants (Talaat and Shawky 2014). The AM fungal inoculation resulting in increased plant biomass as well as yield than non-mycorrhizal plants under the salinity stress has been reported (e.g., Kadian et al. 2013; Zhang et al. 2018; Begum et al. 2019; Muthukumar et al. 2019). Mangroves, coastal sand dunes, and islands are some of the natural habitats succumbing to the impact of wide range of salinity. Occurrence, diversity, and functions of AM fungi in such ecosystems in association with plants need intense input to adapt the traits of AM fungi those are capable to combat the salinity stress in crops in saline-prone agricultural habitats or to rehabilitate abandoned salterns or aquaculture lands. Salinity stress-tolerant AM fungi have been identified in some of the plants like oats and *Jatropha* (Kumar et al. 2015; Xun et al. 2015; Abdelhamid et al. 2019).

The improved growth and productivity of plants colonized by AM fungi in saline soils primarily has been attributed to AM fungi-mediated increase in the uptake of limiting nutrients (mainly phosphorus), thus increased soil salinity increases the mycorrhizal dependency of plants under salinity stress (Beltrano et al. 2013). Mycorrhizal association can improve the plant's potassium uptake under salt stress resulting in a higher K^+/Na^+ ratio (Kadian et al. 2013). Such increased potassium uptake under salt stress by mycorrhizal plants may modify the negative influence of sodium on plant growth and metabolism (Evelin et al. 2009). Photosynthesis is one of the primary processes to be affected by salinity resulting in reduced crop growth as well as productivity. A few studies have examined the effect of AM fungal symbiosis on photosynthesis under the salt stress. Generally, improvement in photosynthetic efficiency, higher chlorophyll content and better water status has been reported in plants colonized by AM fungi under salt stress (Ruiz-Lozano et al. 2012; Beltrano et al. 2013). The main effect of AM symbiosis in imparting salinity tolerance in plants appears to be the result of enhanced photosynthetic capacity through elevated gaseous exchange, and the higher efficiency of photochemistry and non-photochemistry of PS II (Ruiz-Lozano et al. 2012; Ait-El-Mokhtar et al. 2019).

16.3.4 AM Fungi Enhance Crop Productivity

The crop or land productivity is defined by Fischer et al. (2003) as the capacity of agricultural lands to produce biomass on a sustainable long-term basis under the constraints of each agro-ecological zone. In the past century, we have seen a dramatic increase in crop productivity that has been largely due to the introduction of new crop varieties into farming systems in dryland and irrigated environments with good supplies of fertilizer and pesticides (Liu et al. 2015). However, in some areas the crop/land productivity has actually been declining in the last few decades (Gomiero 2016; Thirkell et al. 2017). High input agriculture through application of large amounts of fertilizers and pesticides with intensive monocrop cultivation on a long run is not only expensive but will also have adverse effect on the environment and soil health. Thus, there is an increasing interest currently in sustainable agriculture, which rely on limited chemical inputs and more input of biological entities. In this context, the importance of soil biota for the improvement of soil fertility and crop or land productivity through biological processes becomes a key component of a strategy toward agricultural sustainability (Giller et al. 2005; Bagyaraj and Ashwin 2016; Martínez-García et al. 2017). The majority of ecosystem processes in both natural and managed ecosystems, the soil serves as the critical and dynamic regulatory center. The soil not only houses a large proportion of the Earth's biodiversity but also, provides the physical structural substrate for most human activities. Although soils have been widely studied and classified in terms of physical and chemical characteristics, knowledge of soil biodiversity, especially AM fungi, and their function is far from complete (Brussaard et al. 2004; Bagyaraj and Ashwin 2016).

Significant increase in plant growth and yields of several plant species in agriculture, horticulture, and forestry are due to AM fungal inoculation in unsterile soils containing less or insufficient indigenous endophytes have been reported by several workers (Raghu et al. 2020a, b, 2021). The AM fungi can be inoculated to the nursery bed or root trainers to make them mycorrhizal. The seedlings thus becoming mycorrhizal could be transplanted to poly bags and then to field sites depending on the plant system. This method has been tried in several experiments and resulted in production of vigorously growing healthy seedlings of forest tree species. Further, it was observed that the inoculated seedlings performed better on out planting in the field sites including degraded forests and wastelands (Bagyaraj 2011).

Recent studies have shown that inoculation in the nursery with selected AM fungi with PGPR in nurseries or pro-trays will provide healthy and vigorously growing seedlings, which perform extremely well in the field sites. Such results have been reported recently in vegetable, medicinal and floriculture plant species (Jyothi and Bagyaraj 2018; Desai et al. 2020; Sukeerthi et al. 2020). Studies have also been carried out with forest tree species, where significant enhancement of growth in inoculated trees have been reported and have been monitored up to 6 years after field transplant (Bagyaraj and Kehri 2012; Raghu et al. 2020a, b, 2021). It is likely that inoculation with microbial consortia consisting of effective AM fungi along with PGPR will become an integral part of the nursery technology in the future. This

simple technology will not only improve the plant growth and productivity but will also lead to substantial reduction of use of fertilizers and pesticides, thus minimizing the environmental perturbations. However, there is still a wide gap in the research and development framework, and technology transfer in this field.

16.4 AM Fungi and Plant Biodiversity in an Ecosystem

In spite of importance of AM fungi is well known, its role in supporting the plant diversity is largely ignored. Recent studies revealed a positive correlation of diversity of AM fungi with frequency of occurrence of vascular plant species as well as promotion of plant naturalization in global scale (Pyšek et al. 2019; Toussaint et al. 2019). The role of AM fungal diversity toward the maintenance of plant biodiversity and the ecosystem function has been studied (van der Heijden et al. 1998a; Gianinazzi et al. 2010; Martínez-García et al. 2017; Tedersoo et al. 2020). In one of the experiments, four different native AM fungi isolated from the soils of a calcareous grassland, and their combined effect has been evaluated in 48 microcosms of the European calcareous grasslands to follow the impact of species composition and structure. It was found that among the 11 plant species, eight species (*Brachypodium pinnatum, Centaurium erythrea, Hieracium pilosella, Lotus corniculatus, Prunella grandiflora, Prunella vulgaris, Sanguisorba officinalis* and *Trifolium pratense*) were almost completely dependent on the presence of AM fungi. From the results, it was concluded that a reduction in AM fungal biodiversity from four to one leads to a decreasing biomass of several plant species. Hence, it was proposed that both plant biodiversity and ecosystem productivity will increase with increasing numbers of AM fungi, because of added beneficial effect of each single AM fungal species. Different plant species are benefited in many ways from varied AM fungi, suggesting the host preference in AM fungi. Interestingly, the blue sedge *Carex flacca* was the only plant species that did not have a symbiotic relationship with AM fungi, which showed highest biomass in non-mycorrhizal control treatment. Altering the AM fungal species in the soil had no significant effects on the biomass of the dominant perennial grass "erect brome" (*Bromus erectus*).

In another field experiment, simulating North American old-field ecosystem in 70 macrocosms, 23 AM fungal species isolated from the site were inoculated containing 1, 2, 4, 8, or 14 AM fungal species (van der Heijden et al. 1998b). Each macrocosm [($1 \times 0.75 \times 0.25$ m^3) containing 90 kg of γ-ray irradiated sand: soil (1: 1 v/v)] was showered with a seed rain consisting of 100 seeds from each of the 15 most abundant plant species of the research site. After one growing season, plants were harvested and root and shoot biomass were determined. Plant biodiversity was assessed using a Simpson's diversity index on individual species shoot-biomass data. Total shoot and root biomass, plant P, soil P, and hyphal length/g soil were also measured. The results brought out lowest plant biodiversity and productivity occurred in plants without AM fungi or with only a few AM fungal species. In contrast, plant biodiversity and productivity were highest when 8 of 14 AM fungal species were present. Increasing the AM fungal biodiversity lead to increased hyphal

foraging capacity and more efficient exploitation of soil P, improved resource use and increased productivity and plant diversity. Enhancing the plant biodiversity results in greater ecosystem productivity (Isbell et al. 2017). Further, the results of these studies emphasize the need to protect AM fungi, and to consider these fungi in future management practices in order to maintain diverse ecosystems as they can drive the ecosystem functions such as biodiversity, productivity, and variability (van der Heijden et al. 1998a).

Recent studies have also shown that different community composition of AM fungi affects plants differently and plays a potential role in ecosystem variability and productivity (Lee et al. 2013). The AM fungi with dispersal mechanism through both biotic and abiotic routes surprisingly showed low endemism based on survey of 1014 plant roots, sampled worldwide (Davison et al. 2015). In addition, functional groups of plants are known to be in association with distinct AM fungal communities (Davison et al. 2020). Such plant-AM fungal relationships and specificities are responsible for diversity of plant species and, in turn, the functional diversity of ecosystem.

16.5 AM Fungi and Ecosystem Services

Ecosystem services are "the benefits people obtain from ecosystems" as defined by the Millennium Ecosystem Assessment (MEA 2003). Human society derives benefits from a multitude of resources and processes from the natural and managed ecosystems services to which soil organisms make a crucial contribution. These ecosystem services include the products like food, and processes like nutrient transfer. Growing human population has led to increased demand for natural resources of the ecosystems, and greater global consumption of natural resources is responsible for decline in ecosystem services. Most of the people have been under the impression that the ecosystem services are free of cost, invulnerable and infinitely available, thus lack a formal market and traditionally ignored from society's balance sheet. Since 1997, collaborative efforts of ecologists and economists started estimating the annual value of the services that ecosystems provide (Fisher and Turner 2008). In this context, the ecosystem services rendered by soil biota in maintaining soil quality, plant health, and soil resilience are extremely important (Smith and Read 2008; Martínez-García et al. 2017). In particular, soil microorganisms forming mutually beneficial relationships with plant root systems have become a target of increasing interest in agricultural research and development because, they offer a biological alternative to promote plant growth and reduce the expenditure in sustainable agriculture (Hart and Trevors 2005; Bagyaraj 2011; Parkash et al. 2019).

The ubiquity of AM fungi occurring partly within the root and partly in soil makes them a key functional group of soil biota, which by their nutritional and non-nutritional activities profoundly influence the ecosystem processes that contribute to the ecosystem services. Some of the key roles that AM symbiosis can play as

Table 16.1 Functions of AM fungi responsible for ecosystem services

Function of AM fungi	Resulting ecosystem services
Increased rooting development of a complex and ramifying mycelial network in soil	Increased plant/soil adherence and soil stability (binding activity improves soil structure)
Increasing mineral nutrient and water uptake by plants	Promote plant growth and reducing fertilizer requirement
Buffering effect against abiotic stresses	Increased plant resistance to drought, salinity, heavy metals and depletion of mineral nutrients
Secretion of polysaccharides and glomalin into the soil	Increased soil conditioning, soil stability, water retention and resistance to soil erosion
Modification of plant metabolism and physiology	Bioregulation of plant development and increase in plant quality for human health
Protection against root pathogens	Increased plant tolerance to soil-borne plant pathogens
Diversity of AM fungi	Measure of soil health and productivity

an ecosystem service provider to guarantee plant productivity and quality products in emerging systems of sustainable agriculture is given in Table 16.1.

Earlier studies with AM fungi and plants have shown that simultaneous colonization and network formation by a diverse set of fungi can synergistically promote coexistence and diversity of plant species, compared to a system with a low diversity of fungi (van der Heijden et al. 1998a). Field studies in various natural ecosystems also suggest that mycorrhizal fungal and plant diversity are positively related (Tedersoo et al. 2016, 2020). The appropriate management of ecosystem services rendered by AM fungi will have impact on natural resource conservation and utilization with an obvious net gain for human society (Gianinazzi et al. 2010; Powell and Rillig 2018). Thus, the mycorrhizal symbiosis is an essential component of most of the plants and the challenge for agriculture today lies in the possibility to availing advantage of the numerous ecosystem services especially biofertilization, bioprotection, bioregulation, bioremediation, and soil stabilization (Alguacil et al. 2012; Lee et al. 2013).

16.6 Conclusions

Soil is a storehouse of huge variety of organisms. These organisms make significant contributions toward not only production but also toward regulatory functions of ecosystems (e.g., carbon sequestration, regulation of greenhouse gases, soil conservation, and so on). Species as well as genetic diversity of soil organisms are more important from the point of inventory and their attributes in evaluation of ecosystem functions. Some organisms mainly AM fungi, rhizobia, PGPR, soil-borne pests and pathogens, either directly benefit or harm the plant growth. It is very well established that inoculation with efficient AM fungi improves the growth and yield of crop plants and forest trees, and reduces use of phosphatic fertilizer. The AM fungal inoculation significantly enhancing the growth of crop plants and forest tree

seedlings in the nursery is well documented. Such enhancement of productivity happens particularly in soils deficient in nutrients (mainly phosphorus). The improved plant growth is attributed to better uptake of nutrients that are less mobile in soil solution (e.g., Cu, Zn, and P), better water uptake, and control of root pathogens and production of plant growth regulators. In this context, the ecosystem services rendered by AM fungi in maintaining soil quality, plant health and soil resilience are extremely important. A key limitation to the full recognition of AM fungal contributions to the soil processes as well as ecosystem services have been the difficulty of demonstrating these linkages under large-scale field conditions and natural ecosystems. It appears that the diversity of AM fungi is also a major factor contributing to the maintenance of plant diversity and geographic distribution. More investigations on the aboveground plant diversity and belowground AM fungal diversity are needed in order to precisely understand specific contributions of AM fungi to ecosystem functions and land productivity. The appropriate management of ecosystem services rendered by AM fungi will thus have a profound impact on plant diversity, crop productivity, and ecosystem stability or sustainability. The economic implications of such biotechnological management to crop productivity and other ecosystem services should be critically evaluated at different spatial as well as temporal scales for the benefit of human society in the context of climate change and global warming.

16.7 Future Studies

Our knowledge on the impact of different production strategies on both the diversity of AM fungal communities and its relationship with crop or land productivity is limited. Future study has to focus on the development of production strategies, which mimic the natural processes through: (a) use of organic fertilizers promoting AM fungal colonization and effectiveness; (b) promotion of mixed cropping to increase the AM fungal potential and diversity; (c) diversification of crop rotations with limited use of non-mycorrhizal crops in order to increase the AM fungal populations and diversity; (d) inoculation with mixed AM fungal consortium to overcome the detrimental effects of management practices on AM fungal populations. The large-scale exploitation of AM fungi into plant production systems has so far been hampered by: (a) use of selected crop varieties those are recalcitrant to mycorrhizal fungi; (b) decreased implementation of crop rotation systems; and (c) excessive chemical inputs.

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Mycoremediation: A Natural Solution for Unnatural Problems

17

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Abstract

The rapid and unchecked human population growth has contributed to accelerated urbanization and industrialization causing severe environmental consequences. Human activities such as agriculture, industrial, forest fires, sewage and waste disposal, ocean oil spills, surface petroleum pollution, crude oil transport incidents, etc., aid in the incremental production of persistent-toxic contaminants, heavy metals, polycyclic aromatic hydrocarbons, pesticides, fungicides, antibiotics in various ecosystem niches. Mycoremediation is an ecofriendly and relatively cheaper way to manage all these pollutants and restore the ecosystem. Biological approaches based on industrial and environmental biotechnology focus on the creation of "clean technologies" which emphasize in some useful way on maximum output, reduced waste generation, waste treatment and conversion. Further, these clean technologies focus on the use of biological methods for the remediation of waste. One such biological method is mycoremediation, which is based on the use of fungi and mushrooms to remove waste from the environment. It is an in situ remediation strategy that utilizes the ability of the fungus to recycle. Fungal associations in nature are known to break

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_17

down complex substances into simpler ones by producing a wide range of extracellular enzymes that are well exploited for toxic waste degradation and remediation of polluted sites. This chapter deals with different sources of pollutants and ecological niches where mycoremediation can be exploited. Also, different fungi used for mycorestoration and their mechanism will be discussed. An attempt has also been made to highlight past experiences in which mycoremediation has proved to be an effective and beneficial strategy for restoring the ecosystem.

Keywords

Mycoremediation · Mycorestoration · Waste treatment · Extracellular enzymes · Persistent contaminants

17.1 Introduction

The worldwide rapid increase in human population has led to an expansion in agriculture, urbanization, and industrialization. Consequently, serious environmental hazards are apparent. The poor management of effluents and toxic chemicals coming out from the households, industries, agriculture, pharmaceuticals has made pollution a matter of grave concern (Adenipekun and Lawal 2012). Some of the synthetic organic compounds present in the effluents, like xenobiotics, that do not occur naturally in the biosphere and are resistant to degradation by indigenous flora and fauna, are deteriorating the already crippling ecosystem (Atashgahi et al. 2018).

Increased agricultural production with the rising global demands accounts for increased and erroneous application of agrochemicals in the soil system. This regular dumping of chemicals has made agriculture the main source of water pollution, which has only been increasing over the years. This makes the future of agriculture even worse with rapid land degradation, salinization of soil, reduction of soil fertility, genetic diversity, and loss of organic matter worldwide (Arias et al. 2008). All these toxic chemicals, pollutants, and residues have shown hazardous effects not only to the environment but to human health as well. These chemicals include polycyclic aromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, 1,1,1-trichloro–2,2-bis (4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene, and trinitrotoluene. These are recalcitrant and highly resistant to degradation. They result from burning of fossil fuels, coal mining, burning of wood, and various agriculture and industrial applications (Adenipekun and Lawal 2012). The large amount of agro-industrial waste and its disposal by burning has also posed serious environmental concerns (Kumla et al. 2020).

The task of managing and cleaning up these pollutants from the ecosystem is a matter of immediate concern. Over the years, innumerable ways have been recommended for the same, but bioremediation stands apart in being the most rapid, cost-effective, and ecologically sound technique. Bioremediation is a waste management approach in which organisms are used to eliminate or reduce

contaminants from polluted sites. It is a process where organic wastes are biologically degraded under controlled conditions to the levels below their toxic concentrations. By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring microorganisms such as bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment (Kumar 2017).

Depending upon the kind of living organisms used, the bioremediation may be (a) microbial remediation (engaging microorganisms like bacteria and fungi), (b) phytoremediation (engaging the plants), and (c) mycoremediation (engaging the mushrooms) to heal contaminated and damaged lands and waters (Darwish 2013). In this chapter, we shall discuss in detail about mycoremediation, its working, the mode of action, remediation of major pollutants along with its advantages and constraints.

17.2 Mycoremediation

The term mycoremediation, coined by Paul Stamets, can be broken down as myco (fungus) and remediation (to clean, resolve, or correct). It entails the use of fungi, mainly mushrooms, to produce a simple but effective biomass capable of degrading environmental and industrial contaminants (Dutta and Hyder 2019). According to Paul Stamets "*Fungi recycle and rebuild healthy soil in the area following any contamination incident (oil, spills, chemical leak, radiation agent)*" (Stamets 2005).

Fungi, being extremely unique and omnipresent, are able to colonize all natural environments (soil, air, water), where they play a crucial role in maintaining the ecosystem's equilibrium (Gupta and Shrivastava 2014). Saprophytic fungi is known to play an important role in decomposition because of their ability to degrade the lignocellulose matrix in litter releasing extracellular enzymes like oxidases, peroxidases, and laccases (Kubartová et al. 2009; Cragg et al. 2015). They are almost solely responsible for the breakdown of all the woody material in the world. As a matter of fact, the enzymes that fungi have developed over millions of years, to break the chemical bonds in cellulose and lignin, have also been shown to degrade many toxic and highly persistent chemicals. Owing to these special abilities, fungi can actually heal the land and get rid of the toxic pollutants. Three qualities of fungi that are of special interest to be exploited for the purpose of remediation are: their ability as chemical destroyers, heavy metal chelators, and ultra-fine water filters. In addition to this, they also possess other remediating properties such as pH correction and soil building and some species are also known to improve plant health (Darwish 2013).

The fungi have ability to transform a variety of hazardous chemicals (Alexander 1994). In nature, they serve as the most powerful decomposers by secreting strong degrading enzymes. This potential is attributed to their aggressive growth, great biomass production, and extensive hyphal system (Ashoka et al. 2002; Elekes and Busuioc 2010). They not only decompose complex polymers such as cellulose,

hemicelluloses, lignin, etc., present in the ecosystem but also have the ability to store, release, and accumulate various elements and ions (D'Annibale et al. 2006).

Mycoremediation in nature can help in addressing two main forms of pollution: microbial and chemical contaminants. In microbial mycoremediation, the target of the remediating fungi is to remove a pathogenic microorganism from a system by disrupting its multiplication, by preventing cell wall synthesis and division, and by eroding cell membrane. It can also act indirectly by enforcing species exclusion (to increase in number and form very large colonies that prevent the entry of pathogenic organisms), by altering the system pH (making it unfavorable for the pathogenic organism), or by altering the availability of nutrient resources needed for the growth of pathogenic organisms. Microbial mycoremediation can be used in cases of fecal coliforms in water, soil, manures and other livestock waste, and failing septic systems (Cotter 2014). In chemical mycoremediation, the target of remediating fungi is the breakdown or removal of the toxic pollutant present in a system. In this case, the fungi secrete a variety of enzymes to cleave the chemical molecule into simpler units, making it more readily available for the degradation by other indigenous organisms. Chemical remediation can be used against a variety of agricultural and industrial pollutants such as: herbicides, pesticides, dyes, polycyclic aromatic hydrocarbons, pentachlorophenol, etc. (Cotter 2014).

17.3 How Does Mycoremediation Work?

Fungi exhibit unique bioremediation properties due to their excellent decomposing ability and wider adaptability to all types of environments. Various fungal species can break down almost all kinds of naturally occurring organic compounds by the production of a plethora of enzymes that allows them to use these compounds as a carbon source. This includes compounds such as pectin, cellulose, and hemicellulose and even more complex and resistant polymers like keratin, chitin, and lignin (Gupta and Shrivastava 2014). During mycoremediation, this fungal capability to decompose complex polymers in nature is exploited in degrading a wide variety of toxic waste/pollutants from the contaminated sites (Kulshreshtha et al. 2013; Purnomo et al. 2013).

In the last few decades, many mycologists have tried the use of various fungal species in the degradation of organic compounds. The discovery of the white rot fungi (*Phanerochaete chrysosporium*) in bioremediation has brought greater success and thus initiated the research throughout the world on mycoremediation (Fig. 17.1), establishing the fact that fungi can be successfully used in bioremediation (Singh 2006).

Bioremediation of waste using fungi occurs through the process of biodegradation, biosorption, and bioconversion (Akinyele et al. 2012; Kulshreshtha et al. 2013; Kumhomkul and Panich-pat 2013; Prasad and Sachin 2013). Over the years, there have been many studies on the role of enzymes in the degradation process, degradation products formed, and the conditions that favor the process. Also, many scientists have tried to understand the mechanism of how biosorption and

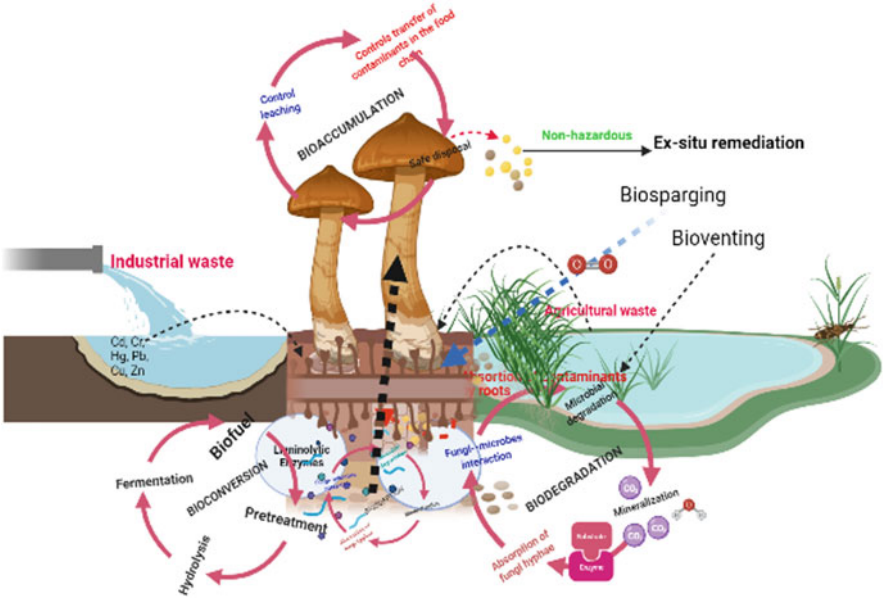

Fig. 17.1 Processes in mycoremediation

bioconversion assists in the process of mycoremediation (Novotny et al. 2004; Akinyele et al. 2011; Zhu et al. 2013).

17.3.1 Biodegradation

Biodegradation can be described as the ultimate degradation and recycling of a complex molecule into its mineral constituents. It results in complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3, and other inorganic compounds by the action of living organisms primarily fungi (Kulshreshtha et al. 2014). Different fungi degrade a variety of waste and toxic products, including field wastes (weeds, straw, paddy straw, jute fibers), animal wastes (animal dung, dead bodies), agro-industrial wastes (sugarcane molasses, peels), petroleum hydrocarbons, pharmaceutical wastes, dyes and detergents, and woolen mill waste, making them non-hazardous for the environment by either reducing or recycling (Barh et al. 2019).

These remediating fungi along with their fruiting bodies produce extracellular peroxidases, ligninases (lignin peroxidase, manganese dependent peroxidase and laccase), cellulases, pectinases, xylanases, and oxidases (Chandra 2019), which in addition to biomolecules degrade polymeric, recalcitrant pollutants such as nitrotoluenes (Bilal and Iqbal 2020), polycyclic aromatic hydrocarbons (PAHs) (Shahsavari et al. 2019; Lee et al. 2020), organic and synthetic dyes (Arunprasath et al. 2019; Salem et al. 2019), and pentachlorophenol (Xiao and Kondo 2020). In

the last decade, mushrooms have also been shown to degrade polymers such as plastics (da Luz et al. 2013). Because of the involvement of various biochemical systems, interactions of ligninolytic enzymes with the cytochrome P450 monooxygenase system, hydroxyl radicals, and the quantity of H_2O_2 produced by the mushroom, the biodegradation mechanism is quite complex (Kulshreshtha et al. 2014).

17.3.1.1 Mode of Action of Fungi in Degradation

The ability of the fungi to degrade the complex chemical bonds of synthetic compounds is largely due to the secretion of extracellular lignocellulolytic enzymes. These biocatalysts are responsible for degradation of lignin and cellulosic materials. The major lignocellulolytic enzyme producers are white rot fungi, brown rot fungi, and soft rot fungi (Manavalan et al. 2015).

Ligninolytic Enzymes

Ligninolytic enzymes catalyze the breakdown of lignin compounds. Peroxidases, manganese peroxidases, and laccases are major lignin degrading enzymes.

Peroxidases are secreted by microsomal or cytosolic systems and are found in all kingdoms of life. Fungal lignin peroxidases are globular glycoproteins that are primarily helical glycoproteins of 30–50 kDa and with an isoelectric point of 3.2–4.0 (Hirai et al. 2005; Asgher et al. 2012). These were reported for the first time from *Phanerochaete chrysosporium*, a white rot fungus. These enzymes, in addition to lignin, can degrade dyes and a variety of recalcitrant and persistent pollutants (Shrivastava et al. 2005). The biological activity of these enzymes isolated from different sources exhibits different optima for pH (2–5) and temperature (35–55 °C) (Asgher et al. 2008). Most of the lignin peroxidases contain heme proteins and utilize hydrogen peroxide (H_2O_2) or organic hydroperoxides (R-OOH) as their substrates (Bansal and Kanwar 2013). These enzymes catalyze the oxidation reactions, which result in the formation of free radicals (e.g., phenoxyl and aryl cation radicals), reactive cations (e.g., Mn^{3+}), or anions (e.g., OCl^-) which further assists in the breakdown of lignin and humic substances and oxidation of toxic compounds (Hofrichter and Ullrich 2011).

Manganese Peroxidases are secreted as multiple isoforms. These are extracellular glycoproteins (molecular weight, 32 to 62.5 kDa) that contain an iron protoporphyrin as the prosthetic group, and show maximum biological activity at pH 4 to 7 and temperature 40 °C to 60 °C (Lobos et al. 1994; Asgher et al. 2008). These were first discovered in *Phanerochaete chrysosporium* (Glen and Gold 1985; Paszczynski et al. 1985). The catalytic mechanism of manganese peroxidases differs from lignin peroxidases in utilizing Mn^{2+} as the electron donor. These enzymes cause oxidation of various phenolic substrates such as simple phenols, amines, dyes, and phenolic lignin model compounds (Hofrichter et al. 2010).

Laccases are extracellular N-glycosylated multi-copper blue oxidases with their molecular weight ranging from 38 to 150 kDa. These enzymes were discovered for the first time from *Toxicodendron vernicifluum* (*Rhus verniciflua*), a Japanese

lacquer tree (Yoshida 1883). Laccases are produced by a wide variety of wood-degrading fungi, such as *Trametes versicolor*, *Ganoderma lucidum*, *Phanerochaete chrysosporium*, and *P. eryngii* (Baldrian 2006; Chen et al. 2012; Manavalan et al. 2013). The optimum pH and temperature of laccases range from 2 to 10 and 30 to 70 °C, respectively (Murugesan et al. 2006; Wang and Ng 2006; Sun et al. 2012). They have been used increasingly for use in dye decolorization applications (Sanlier et al. 2013).

Cellulolytic Enzymes

The cellulose degrading enzymes from various white rot fungi are divided into three major groups, namely Endoglucanases, Cellobiohydrolases, and Beta-glucosidases (Elisashvili et al. 2009; Manavalan et al. 2015).

Endoglucanases catalyze the random cleavage of internal bonds in the cellulose chain and release cellobiose or cello-oligosaccharide units. They can be isolated from a variety of white rot fungi, namely *Ganoderma lucidum*, *G. applanatum*, *G. boninense*, *Ganoderma neo-japonicum*, *G. australe*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *P. gibbosa*, *Trametes hirsuta*, *T. ochracea*, *T. pubescens*, *T. versicolor,* and *Fomes fomentarius* (Elissetche et al. 2007; Elisashvili et al. 2009; Yeoh et al. 2012). The maximum biological activity of these enzymes is observed at pH 4.0–6.0 (Kaur et al. 2007; Jagtap et al. 2014).

Cellobiohydrolases are monomeric proteins with little or no glycosylation with their molecular weight ranging from 39 to 65 kDa. They cleave the chains of cellulose at their terminal ends to release cellobiose or cello-oligosaccharide units (Dashtban et al. 2009). These can be isolated from a variety of white rot basidiomycetes such as *Ganoderma lucidum*, *G. applanatum*, *G. boninense*, *G. neo-japonicum*, *G. australe*, *I. lacteus*, *Dichomitus squalens*, *Schizophyllum commune*, *Phanerochaete chrysosporium*, *Auricularia fuscosuccinea*, *Pleurotus giganteus*, *P. gibbosa*, *P. eryngii*, *P. ostreatus*, and *P. sajor-caju* (Rouau and Odier 1986; Adav et al. 2012; Manavalan et al. 2012; Tsujiyama and Ueno 2013). The optimum pH and temperature for the maximum biological activity of the enzyme are 4.0–5.0 and 37–60 °C, respectively (Rouau and Odier 1986; Levin and Forchiassin 1997; Hamada et al. 1999).

Beta-glucosidases are monomeric, dimeric, or even trimeric proteins that act on cellobiose or gluco-oligosaccharides and release glucose molecules (Dashtban et al. 2009). These enzymes can be isolated from many basidiomycetes such as *Ganoderma neo-japonicum*, *P. chrysosporium*, *Ceriporiopsis subvermispora*, *S. commune*, *A. fuscosuccinea*, *P. ostreatus*, *Trametes gibbosa*, and *T. versicolor* (Bhattacharjee et al. 1992; Adav et al. 2012; Jagtap et al. 2014). The molecular weight of these enzymes ranges between 35 and 64 kDa with optimum pH and temperature for their maximum activity at 3.5–5.5 and 45–75 °C (Levin and Forchiassin 1997; Baldrian 2006).

17.3.2 Biosorption

The second most important process of removing heavy metals and pollutants from the environment through fungi is biosorption. It is an alternative process which, along with the remediation of industrial effluents, can also recover the metals present in the effluents. The process is based on the adsorption of metallic ions or pollutants from the effluent by living or dried biomass, which often exhibits a marked tolerance toward metals and other adverse conditions (Gavrilescu 2004). Biosorption can be defined as the metabolism independent binding of metal ions to negatively charge free groups present in the microbial cell wall (Dutta and Hyder 2019). The biosorption of heavy metals from the aqueous solutions is primarily done by soil fungi. It occurs by the process of surface binding, including ion-exchange reactions, precipitation, and covalent binding with the functional groups present on the cell surface of fungi. The major functional groups that are known to bind metals include carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups (Kapoor and Viraraghavan, 1995).

The uptake of pollutants and heavy metals by mushrooms involves a combination of two processes: (i) bioaccumulation, i.e., active metabolism-dependent processes, which involves the transport of molecule into the cell and further partitioning into intracellular components and (ii) biosorption, i.e., the covalent binding of pollutants to the biomass without requiring metabolic energy. Functional groups that are involved in the metal binding are polar groups of proteins, amino acids, lipids, and structural polysaccharides such as, chitin, chitosan, glucans (Kulshreshtha et al. 2014). Biosorbents are the products that are prepared from mushroom mycelium and spent mushroom compost to carry out the task of cleaning the environment through biosorption.

17.3.3 Bioconversion

Bioconversion is the conversion of effluents coming out from industries and agro-industries into useful forms with the help of living organisms. It is a biological process, in which microorganisms, like bacteria, fungi and detritivores, or their enzymes turn organic waste and soil contaminants into usable commodities or energy sources. Paper, auto-fluff, tires, fabric, construction materials, municipal solid waste (MSW), sludge, sewage, and other products derived from plant or animal waste are among the raw materials for bioconversion. Ethanol, wine, and, most crucially, mushrooms are all bioconversion products (Barh et al. 2019). The process of bioconversion involves three steps: Pretreatment, Hydrolysis, and Fermentation.

(a) Pretreatment: This step makes the cellulose accessible for the action of cellulolytic enzymes. It enhances the hydrolysis processes and increases the product yield. Pretreatment to industrial waste can be given by following chemical method using acid or alkali or by physiochemical method using steam explosion or by biological method using living organisms. Pretreatment of waste or

effluent is advantageous in terms of low energy requirement, minimal waste production, and no environmental side effects (Shi et al. 2008).

(b) Hydrolysis: In this step, cellulose and hemicellulose are broken down into monomeric soluble sugars (hexoses and pentoses).

(c) Fermentation: In the last and final step, the hydrolytic products are brewed into beneficial products such as ethanol. *Pleurotus ostreatus*, *Flammulina velutipes*, and *Agaricus blazei* are employed in wine production and have a lot of potential. These mushrooms are also thought to have protective/curative effects against cancer and thrombosis (Okamura et al. 2001).

Agricultural wastes, forestry wastes, woody material, grass, municipal solid wastes, and other lignocellulosic residues have great potential for bioconversion and can be effectively used for the cultivation of mushrooms which can be further used as a commercial product. This way bioconversion serves two important functions, on the one hand, it provides a healthy and nutritious food, i.e., mushrooms, and on the other hand, it utilizes the industrial effluents as a substrate for cultivating mushrooms, hence help in removing the pollutants (Barh et al. 2019). The choice of the substrate for the cultivation of mushroom is usually determined by the regional availability of the material. Mushroom cultivation has been successfully practiced on various industrial wastes such as effluents from pulp and paper mill (Singhal et al. 2005; Dulay et al. 2012) and solid sludge and effluents from handmade paper and cardboard industries (Kulshreshtha et al. 2010).

17.4 Types of Mycoremediation

Mycoremediation depending upon where it is done can be of two types: in situ and ex situ.

17.4.1 In Situ Mycoremediation

It is done at the contaminated site and does not require any soil excavation. It is an efficient and cost-effective technique as there are no excavation charges (Kumar 2017). In this technique, physicochemical properties of the contaminated site such as moisture content, status of electron acceptor, nutrient availability, pH, and temperature play a very important role for successful remediation (Philp and Atlas 2005). Another important factor that influences the success of in situ mycoremediation project is soil porosity. The following strategies are followed for in situ mycoremediation (Fig. 17.2):

17.4.1.1 Biosparging

It involves the injection of air into the soil subsurface below the water table to increase groundwater oxygen concentration and stimulate the rate of microbial activities to upsurge the biological degradation of contaminants by naturally

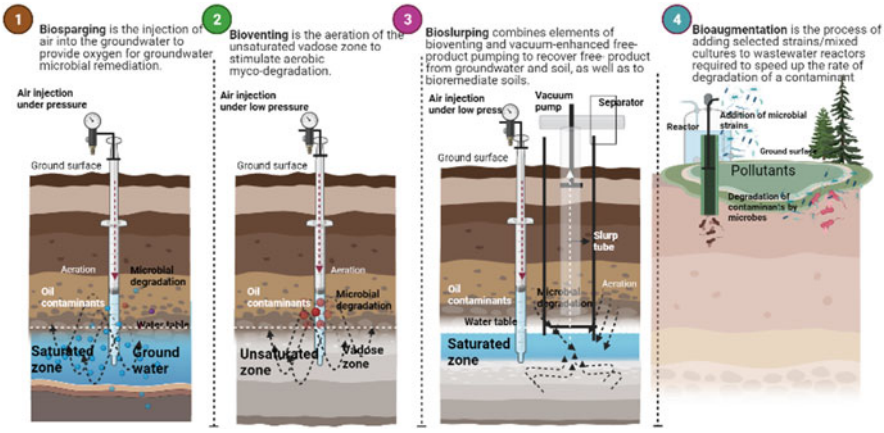

Fig. 17.2 In situ mycoremediation strategies

occurring microorganisms (Kumar 2017). The injected air in the saturated zone results in the upward movement of volatile organic compounds to the unsaturated zone and boosts the microbial degradation. It improves soil–groundwater interface by increasing mixing in the saturated zone. Biosparging's effectiveness is determined by two primary aspects. First is soil permeability, which determines pollutant bioavailability to microorganisms and second is pollutant biodegradability (Philp and Atlas 2005). Installation of small-diameter air injection sites is simple and inexpensive, allowing for a lot of flexibility in the system's design and construction (Das and Dash 2014).

17.4.1.2 Bioventing

Bioventing is a potential new technology that enhances the spontaneous in situ biodegradation of any aerobically degradable substance by supplying oxygen to the soil microorganisms already there (Das and Dash 2014). It delivers controlled stimulation of airflow to indigenous microbes and provides just enough oxygen to sustain their activities. Nutrients and moisture are also added to enhance microbial activity and subsequently, the remediation process (Philp and Atlas 2005). This technique has grown in popularity, particularly for rehabilitating locations that have been contaminated by light spilled petroleum compounds (Höhener and Ponsin 2014).

17.4.1.3 Bioslurping

This technique combines vacuum-assisted pumping, soil vapor extraction, and bioventing to remediate hydrocarbon-contaminated sites by indirect oxygenation and pollutant biodegradation stimulation (Gidarakos and Aivalioti 2007). A "slurp" reaches into the free-product layer and sucks liquids (free products and soil gas) from there. The pumping process causes light non-aqueous phase liquids (LNAPLs) to rise to the surface, where they are isolated from water and air. It can increase free-

product recovery efficiency while avoiding the extraction of significant amounts of groundwater. After removing all free products, the system may be readily converted to a traditional bioventing system to finish the remediation process (Kim et al. 2014). The adoption of this approach raises concerns that creating vacuum on a deep, permeable site with a variable water table could result in saturated soil lenses that are difficult to aerate (Philp and Atlas 2005).

17.4.1.4 Bioaugmentation

It is the introduction of effective remediating fungal strains or a genetically designed strain for the treatment of contaminated soil and water. It is most typically utilized to restart activated sludge bioreactors in municipal waste water treatment plants (Kumar 2017).

17.4.2 Ex Situ Mycoremediation

It involves the excavation of contaminated soil from the polluted site and its subsequent transportation to another site for treatment. In contrast to in situ mycoremediation, ex situ mycoremediation is expensive and may also lead to environmental pollution during the transport of pollutants from one site to another (Kumar 2017). These techniques are considered based on their cost of treatment, depth of pollution, type of pollutant, degree of pollution, and geographical location of the polluted site (Philp and Atlas 2005). The following strategies are undertaken for ex situ mycoremediation.

17.4.3 Land Farming

It is one of the simplest bioremediation techniques in which the contaminated soils are usually excavated, spread over a prepared bed, and periodically tilled until pollutants are degraded. The idea behind this is to stimulate indigenous biodegradative microorganisms and promote their activity (Kumar 2017). Depending upon the site of soil treatment, this technique can also be regarded as in situ bioremediation technique in some cases (Nikolopoulou et al. 2013). It is an easy technique to design and implement, and can be used to treat large volumes of contaminated soil with minimal energy requirement and environmental impact (Maila and Cloete 2004). Some of the limiting factors of this technique are: the requirement of large operating space, reduction in microbial activities due to unfavorable environmental conditions, additional excavation cost, and reduced efficacy in inorganic pollutant removal (Khan et al. 2004; Maila and Cloete 2004).

17.4.4 Biopiling

It involves the aboveground piling of excavated soils from the contaminated sites which is then followed by nutrient amendment and aeration to promote the microbial remediation. Aeration, irrigation, nutrient and leachate collecting systems, and a treatment bed are the main components of this technology. This technique is being increasingly preferred due to its cost-effectiveness (Whelan et al. 2015). Although this method requires lesser space as compared to other ex situ bioremediation approaches, it has some glaring limitations such as, high maintenance and operational cost and high dependency on presence of electricity, especially in remote areas, which is essential for uniform distribution of air in contaminated piled soil (Sanscartier et al. 2009).

17.4.5 Windrows

This technique involves the periodic turning of piled polluted soil to promote bioremediation by increasing degradation activities of indigenous and/or transient hydrocarbonoclastic bacteria present in polluted soil. The periodic turning of polluted soil, together with addition of water results in increased aeration, uniform distribution of pollutants, nutrients and microbial activities, thus quicken the rate of bioremediation (Barr et al. 2002).

17.4.6 Bioreactors

Bioreactor is a vessel in which raw materials are converted into specific products following a series of biological reactions. The different operating modes of bioreactor are: batch, fed-batch, sequencing batch, continuous, and multistage. The polluted soils are fed into a bioreactor in the form of dry matter or slurry. This technique has excellent control over the bioprocess parameters such as temperature, pH, agitation and aeration rates, and substrate and inoculum concentrations. The ability to control and manipulate process parameters in a bioreactor means that the biological reactions taking place inside the reactor can be effectively enhanced to reduce the time required for the bioremediation process (Mohan et al. 2004).

17.5 Mycoremediation of Major Pollutants by Fungi

Fungal potential to alter polluted soils and repair devastated landscapes has long been known. The consequences of persistent hazardous pollutants on public health and the environment, including polycyclic aromatic hydrocarbons, pesticides, herbicides, insecticides, heavy metals, dyes, and pharmaceuticals, as well as the involvement of various fungi in their breakdown, are explored in this section.

17.5.1 Degradation of Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are synthetic compounds obtained through chlorination of biphenyls. They are composed of a biphenyl molecule (two benzene rings linked by a C–C bond) that carries 1–10 chlorine atoms. There are various industrial applications of PCBs such as dielectric fluids, solvent extenders, hydraulic fluids, flame-retardants, heat transfer fluids, and organic diluents. Although the current use of PCBs is widespread and expanding regularly, these compounds are contributing huge damage to the environment by percolating into the soil and sedimentation due to inadequate waste disposal (Yadav et al. 1995; Pointing 2001). Today, PCBs are considered as one of the world's most hazardous contaminants, and hence, are of topmost public concern. The carcinogenic, teratogenic, and endocrine-disrupting aspects of these xenobiotics have been well documented (Choi et al. 2004; Casida 2017). The most problematic property of PCBs is their tendency of bioaccumulation in lipid tissues and adipose tissue of animals and humans and organic components of the soil (Crinnion 2011).

Some fungi, including wood-degrading basidiomycetes, are well known for their role in PCBs removal. The fungal hyphae can penetrate into the polluted matrix with ease. Additionally, their extracellular oxidative enzymes can scavenge even the scarcely bioavailable contaminants using nonspecific radical-based reactions. Numerous white rot fungi were tested for their ability to decompose PCBs (Covino et al. 2016). Among the ligninolytic white rot fungi that mineralizes xenobiotics, *Phanerochaete chrysosporium* is most extensively studied and it can degrade polychlorinated biphenyls (PCBs), dioxins, and other chloro organics (Bumpus et al. 1985; Pointing 2001; Kamei et al. 2006). Several other white rot fungi including *Lentinus edodes*, *Trametes versicolor*, *Bjerkandera adusta*, *P. magnoliae*, *Irpex lacteus*, *Pycnoporus cinnabarinus*, *Phlebia brevispora*, and *Pleurotus ostreatus* can successfully perform PCB removal (Kamei et al. 2006; Cvancarova et al. 2012). However, only a few white rot species have been tested on real PCB-contaminated soils. Thus far, *P. ostreatus* is apparently the most efficient known PCB-degrading organism (Kubatova et al. 2001; Chun et al. 2019).

17.5.2 Degradation of Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are among the major pollutants that are produced due to the incomplete combustion of organic materials like wood, coal, and petroleum. These toxic compounds can enter the water, air, and soil by various human or natural activities (Pozdnyakova 2012; Akhtar and Mannan 2020). A report by Clemente et al. (2001) states that contamination of soil, air, freshwater (groundwater and surface water) with hydrocarbons, especially PAHs has drawn public concerns because of their toxic, carcinogenic, and mutagenic properties. Hence, the removal of these pervasive, persistent, toxic chemicals from contaminated water and soil requires immediate attention (D'Annibale et al. 2005).

Use of Ligninolytic enzymes such as laccase, lignin peroxidase, and manganese peroxidase, released by several fungi is a promising prospect for the degradation and removal of PAH from contaminated sites. But these enzymes are profligate and can also act on other substrates, which are structurally similar to lignin. The biphasic treatment of PAH can overcome this problem of lignin degradation (Bhattacharya et al. 2013). In biphasic treatment, during the first phase *Phanerochaete chrysosporium*, a white rot fungus was cultured under nutrient rich conditions where two genes (pc2 and pah4) coding for cytochrome P-450 monooxygenases were upregulated. In phase two, the cells were exposed to nutrient deficient conditions. This led to the production of extracellular ligninolytic enzymes. This concerted effect of ligninolytic enzymes and P-450 monooxygenases was reported to be more efficient in degrading the PAH. *Agaricus campestris* showed significant reduction in the total petroleum hydrocarbons from 2744.72 mg/l in control to 503.08 mg/l in the contaminated minimal salt solution (Adongbede and Sanni 2014). In an investigation on use of *Pleurotus pulmonarius* for mycoremediation of petroleum hydrocarbon polluted soil was conducted over a period of 62 days. Hydrocarbon (diesel, petrol, spent petrol engine oil, and spent diesel engine oil in ratio, 1:1:1:1, respectively) polluted soil in 2.5%, 5%, 10%, and 20% concentrations were inoculated and incubated with pure culture of *P. pulmonarius*. The reduction was more significant ($p < 0.05$) in soil inoculated with *P. pulmonarius*, suggesting that it can be utilized for cleaning soils polluted with moderate levels of petroleum product mixture. Fungal species belonging to genera of *Mortierella*, *Trichoderma*, *Fusarium*, *Gibberella,* and *Penicillium* were identified for their ability to grow on specific hydrocarbon substrates (Horel and Schiewer 2020).

17.5.3 Degradation of Heavy Metals

The metallic elements, which have a specific mass of more than 5 gcm^{-3} and are able to form sulfides, are termed as heavy metals. Among these elements, Zn, Cu, Mn, Ni, and Co are essential nutrients and are toxic at high concentration, whereas elements such as Cd, Pb, As, and Hg are nonessential without any known biological function and are toxic at low concentration (Dutta and Hyder 2019).

The common metals found in the polluted sites are arsenic, chromium, mercury, copper, cadmium, lead, silver, and nickel. These heavy metals are highly persistent in nature and are extremely difficult to remove. Their deposition in the environment may cause serious threats to biodiversity and human health. Majority of these heavy metals are known to be mutagenic and carcinogenic (Khan et al. 2019). Their deposition in the environment can be due to natural means, such as volcanic eruption, soil erosion, and weathering of earth's crust, or by anthropogenic means such as effluents from industries like paint, textile, metal parts, and fertilizer, etc. Other sources like mining, electronic wastes, leaded petrol, fungicides, insecticides, preservatives, and combustion of fossil may also result in their deposition (Morais et al. 2012). In a study made by Singh et al. (2015), various fungal species were isolated from an agricultural land with arsenic contamination. Upon analysis, the

isolated fungi (*Aspergillus*, *Rhizomucor*, *Fusarium*, and *Emericella* species) were found to not only grow and survive in high concentrations of arsenic but were also shown to improve the growth and yield of plants when they were watered with sterile water containing arsenic. These fungi were able to improve the soil enzyme activity and its physiochemical properties, hence assisting in natural remediation.

Another study made by Abu-Elsaoud et al. (2017) suggested the role of indigenous fungi in improving the plant growth and yield in a highly contaminated agricultural site. The heavy metal pollutant was zinc and the mycorrhizal fungus, *Funneliformis geosporum*, was able to reduce the zinc accumulation in wheat plants and thereby reduce the effect of the pollutant. *Pleurotus ostreatus*, along with surfactants, was also able to effectively remove manganese from the contaminated water through bioaccumulation. Surfactants helped in the process by increasing the surface area and metal binding sites on the fungal hyphae (Wu et al. 2016). *P. ostreatus*, the same fungus can also remove other heavy metals such as lead, zinc, chromium, cobalt, copper, and nickel which are present in the effluents coming out of coal washery. Other fungi such as *Trichoderma ghanense, Fomitopsis meliae*, *Rhizopus microspores*, and *Absidia cylindrospora* have also shown the ability to remove heavy metal accumulation of arsenic, lead, copper, cadmium, and iron (Albert et al. 2018; Oladipo et al. 2018). A study conducted in Pakistan reported the ability of *Aspergillus* species to remove lead and mercury contamination from the industrial soil (Khan et al. 2019).

17.5.4 Degradation of Agricultural Wastes

Agrochemicals are responsible for causing various environmental and health hazards due to their non-degrading and highly persistent nature. The chemicals that are present in the pesticides and herbicides are known to be carcinogenic, endocrine disruptors, neurotoxic and have lethal effects on the reproductive system and various important organs like liver and kidney (Purnomo et al. 2011; Maqbool et al. 2016).

There have been many studies conducted on the remediation of agricultural chemicals using fungi, which have focused mostly on the study and use of indigenous fungi growing in the contaminated sites. Similarly, fungal species such as *Aspergillus tamarii* and *Botryosphaeria laricina*, which were isolated from an agricultural field that had previously been exposed to endosulfan, were not only endosulfan tolerant but also had the ability to degrade the toxicant and its metabolites such as, endosulfan sulfate, alpha endosulfan, and beta endosulfan, by using them as their substrate for carbon and energy (Silambarasan and Abraham 2013).

Complete degradation of herbicide atrazine was achieved using the enzyme extracts from *Trametes maxima* and co-culture of *T. maxima* and *Paecilomyces carneus* (Chan et al. 2016). *Pleurotus ostreatus*, a white rot fungi, can degrade Aldrin and its metabolite dieldrin through epoxidation and hydroxylation reactions (Purnomo et al. 2017).

Another very potent herbicide, glyphosate, was shown to be effectively degraded by fungal species, *Penicillium spiculisporus*, *Aspergillus flavus*, and *Penicillium*

verruculosum, isolated from herbicide contaminated farms (Eman et al. 2013). The ability of two fungal species, *Trichoderma longibrachiatum* and *Aspergillus oryzae*, to degrade the insecticide imidacloprid was tested by Gangola and coworkers (2015). They reported that maximum degradation of imidacloprid (92%) was observed when both the fungal strains were used as a consortium. Another study with a view to isolate and evaluate the fungal species was conducted based on their remediation potential against cypermethrin and two species of *Fusarium* had shown significant degradation potential of about 66 and 70 percent (Kaur et al. 2015). *Pleurotus pulmonarius* have shown the potential to degrade the pesticide dichlorvos (2,2-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP) in the contaminated soil as the rate of its degradation was higher in soils mixed with growing spawns of *P. pulmonarius* (Njoku et al. 2018). In another study, *Aspergillus glaucus* was found to be able to degrade fipronil and its metabolite fipronil sulfone (Gajendiran and Abraham 2017).

17.5.5 Degradation of Dyes

Synthetic dyes are extensively used in the textile, paper, cosmetic, food and pharmaceutical industries, owing to their high stability, wide varieties of color, and relatively cost-effective in their synthesis when compared to natural dyes. These are also involved in printing industries, color photography, and as additives in petroleum products. The degradation and removal of dyes from the environment is crucial due to their highly toxic, recalcitrant, persistent, and carcinogenic nature (Dutta and Hyder 2019). It is because of their chemical stability, resistance to fading, and the ability to remain unaffected by light and microbial degradation. For the industries using them, it is a lot easier and cheaper to directly dump the effluents in the environment rather than purifying them, which then serves as one of the prime pollutants in nature (Akhtar and Mannan 2020).

Aspergillus flavus was found to accumulate and degrade the Congo red dye. The fungus was isolated from the soils surrounding a paper processing industry (Bhattacharya and Das 2011). Similarly, laccases were also capable of degrading dyes. The mushroom *Lenzites elegans* produces laccases which were able to degrade synthetic dyes such as Congo red, malachite green, and brilliant green (Pandey et al. 2018). *Trametes hirsuta* was also able to degrade dyes such as anthraquinone dyes, indigoid dyes, triarylmethane dyes, and azo-dyes (Abadulla et al. 2000). Another strain, *Trametes versicolor* can also degrade various azo anthraquinone dyes (Yang et al. 2017).

17.6 Advantages of Mycoremediation

The process of mycoremediation aggravates the fungal growth to rapidly increase their population by making the environmental conditions favorable for them to degrade and detoxify maximum amount of pollutants. The fungi, being nature's

most potent decomposer, produce an array of nonspecific enzymes, which are able to act on various environment pollutants. The advantages of mycoremediation over other remediating technologies are:

(a) It involves the use of natural organisms, such as mushrooms. Hence, no introduction of synthetic or harmful chemicals in the environment.
(b) It is a natural and cost-effective method of cleaning up the waste as it does not require building up of new structures or the use of expensive machines.
(c) The process is ecofriendly and is applicable on a variety of substrates.
(d) It is much safer than most of the other alternatives of bioremediation.
(e) Complete degradation of the target product can be achieved. The pollutants are completely broken down to harmless products such as water, carbon dioxide, and cell biomass.
(f) The end products of mycoremediation are natural products such as mushrooms and ethanol, not some synthetic chemical.
(g) The technology is simple to understand and practice. It does not require trained personnel.
(h) The technology shows visible improvement with immediate results. It is much quicker than other techniques.
(i) It is equally effective for various recalcitrant, persistent, and toxic pollutants like polyaromatic hydrocarbons, antibiotics, herbicides, insecticides, antifungal drugs, algal bloom, cyanotoxins, detergents, heavy metals, and plastic.
(j) The technique requires low maintenance with useful and edible end products.

17.7 Constraints of Mycoremediation

The use of higher fungi like mushrooms to clean up the polluted sites has been shown to yield promising results, but a great deal still remains to be found out about the basic knowledge of how the fungus removes pollutants. There are various constraints in the effective and widespread usage of mycoremediation. Boopathy (2000) has discussed some of the constraints in the bioremediation technologies.

One of the major limitations of this technique is its dependency on the natural environmental conditions. If unfavorable abiotic conditions prevail for a longer time, the process of mycoremediation can be very slow and might result in partial or incomplete removal of pollutants from the contaminated site, which can further aggravate the situation (Barh et al. 2019). Also, it is difficult to hypothesize how effectively the given fungal species will be able to remediate in the field conditions based on the laboratory results (Sharma 2012; Gnanasalomi et al. 2013). Moreover, it is necessary to study the adaptability and effectiveness of the chosen microbial strain against the prevailing situations and pollutants present in the contaminated site, respectively. As mycoremediation is highly influenced by the physicochemical properties of the contaminated site under in situ management, it makes the mycoremediation process more painstaking and points to the crucial need of

thorough planning and predesigning along with regular monitoring. Only then, it can be effectively practiced under field conditions (Barh et al. 2019).

Another limitation of this technology is that mycoremediation is a slower process than other techniques such as incineration and might not be equally effective for more toxic and persistent pollutants. Another problem that can sometimes occur is that instead of being harmless and nontoxic, degraded products can be even more toxic and persistent than the parent compound.

Another major constraint in practicing mycoremediation is the lack of awareness on mycoremediation methods among the communities and the unavailability of inoculum of effective fungal strains (Kumar 2017).

17.8 Future Prospects and Challenges

Over the years, studies have shown the successful remediation of waste and toxic pollutants with either the addition of required fungal strains to the soil or the enhancement of indigenous microbial population. Whether the fungal strains are native or introduced, the knowledge and understanding of their degrading ability and detoxification mechanism are critical for the correct understanding of mycoremediation. To make this technology applicable at larger scale it still requires much more work and effort to be done. Since the technology has been proven successful, it requires appropriate funding so that certain products can be developed and made available for licensing and commercialization.

Researchers also feel that this technology can be more successful than other technologies, in being a lot faster and cost-effective, but still the complete potential of fungal strains in cleaning up the environment remains underexploited. This offers a huge potential in the future for the complete exploitation of nature's most potent decomposer (Thakur 2014).

The underlying mechanism of fungi in breaking the harmful pollutants needs further attention and efforts from the scientific community. Modern techniques such as functional and whole proteomic studies can be utilized to understand this mechanism, which might reveal different genes and proteins that play a crucial role in the mycoremediation process. Through this information, there is a scope to model genetically improved fungi for more efficient and rapid degrading ability.

The research should also focus on the mass production of effective fungal strains for the purpose of remediation at large scale. The indigenous or native fungi growing in the polluted sites needs to be further exploited as they are already adapted to service in harsh environments with higher concentration of pollutants (Akhtar and Mannan 2020). Further, there is a need to continuously isolate and characterize more effective fungal strains having higher potential to degrade and detoxify various organic and inorganic pollutants (Barh et al. 2019).

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Fungal Mediated Effective Exploitation of Uncongenial Wastes from Environment

18

Sana Sheik and Sareen Sheik

Abstract

In recent years the increased production of obsolete electronic wastes, hazardous dyes, thermoset polymers has greatly increased the amount of waste materials rapidly filling the landfills of the globe. Physical incineration and chemical processes using strong acids are hazardous and quite expensive for treatment. Biological approaches, however, are valuable alternatives and mycoremediation is one such potential source for restoring ecosystems. The ubiquitous fungi and its secreted enzymes readily facilitate biodeterioration of wastes. This chapter presents the ecological and biotechnological knowledge, primarily of different fungi colonizing wastes and their assessment from toxic compounds to simpler non-toxic biodegradable compounds. The toxic metabolites are used by the heterotrophic fungi as a carbon source, whereas certain extracellular enzymes released by the fungi assist in degradation, subsequently restoring the ecosystem.

18.1 Introduction

Bioremediation is the reclamation of polluted sites by decontamination of soil and water using microorganisms as bioremediators (Rhodes 2013). Likewise, mycoremediation is the usage of fungi upon contaminated sources for its decontamination. Mycoremediation is a cost-effective and less hazardous means of engaging fungi to remove toxic pollutants for instance, plastics, paints, hazardous chemicals, thus yielding a cleaner environment. Fungi are ubiquitous in nature hailing from a

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_18

wide range of heterogeneous environments with an ability to colonize both abiotic and biotic surfaces (Joutey et al. 2013). Fungi ranging from micro fungi to macro fungi, pathogenic to saprophytic, epiphytic to endophytic are equipped with the potential ability to degrade uncongenial wastes from surroundings. Besides, their heterotrophic nature enables them to consume the waste, thereby releasing extracellular enzymes (Joutey et al. 2013). Moreover, fungal biomass has the ability to absorb pollutants, thus resulting in a cleaner environment (Joutey et al. 2013).

Therefore, mycoremediation is a waste management technique to neutralize the contaminants, for instance, bisphenol, with mere assistance of detoxification by fungi. Fungi can be used in the treatment of contaminated soil surface, water streams due to organic/metal contaminants, removal of organic pollutants and volatile organic compounds from air by utilizing the isolated extracellular enzymes such as laccase, manganese peroxidase, lignin peroxidase instead of whole fungi (Chandrakant and Shwetha 2011). Basically, main toxic pollutants are due to the anthropogenic inputs, the pesticides used in agriculture, the industrial wastes, polycyclic aromatic hydrocarbons (PAHs) those arising from coal, tar, oil, and similar substances; polychlorinated biphenyls (PCBs) are present in plastics as softeners, paints, pesticides, electronic circuit boards; dioxins present in fly ashes; and so on (Kumar 2017). Dioxins and PCBs are recalcitrant substances for biodegradation. The role of fungi in degradation of recalcitrant compounds would be remarkably beneficial to the environment. The ecological and biochemical capacity of fungi to degrade environmental chemicals and decrease the risk associated with metals and metalloids through chemical modification or its bioavailability makes them a potent bioremediation agent (Yadav et al. 2019).

Mycoremediation involves the in situ biosparging remediation technique that facilitates biological degradation by adequate supply of air in the ground table, bioventing technique involves low air flow rate and sufficient oxygen to sustain microbial activity, whereas bioaugmentation, which involves group of natural microbial strains, treats contaminated soil or water especially municipal wastewater. Mycoremediation further involves ex situ remediation of contaminants using techniques such as land farming, composting, biopiling, bioreactors, etc. (Azubuike et al. 2016).

18.2 Fungal Species Involved in Bioremediation

18.2.1 Wood Degrading Fungi

White rot fungi, for example, *Phanerochaete chrysosporium*, have effectiveness in degrading a wide range of organic molecules due to its release of extracellular lignin modifying enzymes, with a low substrate-specificity. Most of them are species of the Class *Basidiomycetes* and also, the family *Xylariaceae* of *Ascomycotina*. *Phanerochaete chrysosporium* has potential to degrade lignin. Brown rot fungus such as *Serpula lacrymans* degrades cellulose and soft rot fungus *Chaetomium*

degrades both cellulose and lignin but with lesser effectiveness lesser than brown rot fungi (Kumar 2017).

18.2.2 Biosorption of Heavy Metal by Fungi

Soil fungi play a vital role in biosorption of heavy metals from aqueous solutions. Biosorption of metal ions primarily occurs by surface binding, including ion exchange reactions and complexation with the functional groups present on the cell surface (Abbas et al. 2014).

There are several fungi such as *Aspergillus niger*, *Aureobasidium pullulans*, *Ganoderma lucidum*, *Penicillium* sp. (Loukidou et al. 2003; Say et al. 2003), which efficiently recover heavy metals from the contaminated soil environment.

Ramasamy et al. (2011) observed that *A. fumigatus* is suitable for the removal of Pb (II) ions from the electronic waste aqueous solution (containing Pb 100 mg/L) through batch sorption with adsorption capacity of 85.41%. Also, *Mucor* spp., *A. carbonarius*, *A. niger*, *Rhizopus* spp., *Saccharomyces cerevisiae*, *Botrytis cinerea*, *Neurospora crassa*, *Phanerochaete chrysosporium*, and *Lentinus* soil fungi are found to be useful in heavy metal biosorption.

Mycorrhizal fungal degradation also termed as mycorrhizoremediation technology is employed for reclamation of soils and sediments that have been polluted by industries through phytoremediation. The proteins in the cell walls of AM fungi appear to have the ability to absorb potentially toxic elements by sequestering them (Kumar 2017). Mycorrhizal fungi growing as symbionts with plant roots have the ability to degrade pollutants in soil. Ectomycorrhiza, (ECM), arbuscular mycorrhiza (AM), and ericoid mycorrhiza (ERM) can increase the tolerance to heavy metals.

Mushrooms can uptake heavy metals from the soil by means of substrate mycelia (Raj et al. 2011). Mushroom varieties such as *Armillaria mellea*, *Polyporus squamosus*, and *Polyporus sulphureus* obtained from East Black Sea Region are found to accumulate heavy metals like Hg, Pb, Cd, and Cu. Among them *Armillaria mellea* is shown to accumulate higher concentrations of Hg^{2+} as the concentration of mercury increases in the soil (Demirbaş 2002).

There are several mushroom species identified till date to remove the heavy metals from the contaminated resources. The important species are *Galerina vittiformis*, *Hypholoma capnoides*, *Marasmius oreades*, *Agaricus bisporus*, *Lentinus squarrosulus*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pleurotus tuber-regium*, *P. ostreatus*, *P. pulmonarius*, and *Trametes versicolor* (Adenipekun and Lawal 2012).

18.2.3 Hydrocarbon Degrading Fungi

Conversely, requirement of fungal degradation is needed for pollutant classes, i.e., dioxins, 2,4,6-trinitrotoluene, synthetic drugs, or endocrine-disrupting chemicals found in medium as these are inefficiently degraded by bacteria (Harms et al.

2011; Mnif et al. 2011; Macellaro et al. 2014). Unlike bacteria, the fungal phytoremediation does not require an absolute water phase, as fungus can grow in the air–water interface. However, the water phase acts as a carrier for nutrient transport for hydrophobic organic contaminants. Mushroom releasing enzymes play an important ecological role using biodegradation, biosorption, and bioconversion methods decontaminating environmental pollutants. Moustafa (2016) reported fungus *Lichtheimia ramosa* as oil degrader. *Agaricus campestris* significantly reduced the total petroleum hydrocarbons (Adongbede and Sanni 2014). *Stropharia gosoannulata* was found to be the most efficient strain of basidiomycete for the removal of a variety of polycyclic aromatic hydrocarbons (Steffen et al. 2002). White rot basidiomycete *Phanerochaete chrysosporium* is well known to mineralize 2,4-dinitrotoluene. Fungi that degrade chlorinated aromatic compounds are *Phanerochaete chrysosporium*, *T. versicolor*, *Irpex lacteus*, and *Ganoderma lucidum* (Marco-Urrea et al. 2008a), *P. ostreatus* (Kubatova et al. 2001), *Dichomitus squalens* (Reddy et al. 1997), *Pleurotus pulmonarius* (Masaphy et al. 1996), *Phlebia lindtneri* (Kamei and Ryuichiro 2005), *Trametes versicolor* (Marco-Urrea et al. 2008b), *Gloeophyllum trabeum*, *Fomitopsis pinicola*, and *Daedalea dickinsii* (Purnomo et al. 2008). Malathion (an insecticide and neurotoxin) breakdown was successfully done using *Trichoderma viride* and *Pseudomonas* (Matsumura and Boush 1966).

The biodegradation of crude oil hydrocarbons by endophytic fungi *Verticillium* sp. and *Xylaria* sp. has proved them to be potential biodegraders of hydrocarbons that could be used in bioremediation processes (Marín et al. 2018). Fungal genera, namely *Aspergillus*, *Penicillium*, *Talaromyces*, *Amorphoteca*, *Neosartorya*, *Cephalosporium* have been isolated from petroleum sites and were noted to play an important role in bioremediation of oil spills (Koul and Fulekar 2013).

18.2.4 Polymer Degrading Fungi

The prerequisite condition for biodegradation is that the microorganism should be able to use the polymer as its sole source of carbon. The polymer degradation in general requires the adhesion and surface erosion of the films and fungal colonization on the plastic surface (Fig. 18.1). *Lasiodiplodia theobromae*, a fungal endophyte efficiently degraded LDPE film (Sheik et al. 2015). The fungi, *Penicillium oxalicum*, *Penicillium chrysogenum*, *Myceliophthora* sp., *Phanerochaete chrysosporium*, and *Trametes versicolor*, exhibit the ability to degrade polyethylene (Iiyoshi et al. 1998; Khalil et al. 2013; Ojha et al. 2017).

18.2.5 Dyes Degrading Fungi

Aspergillus spp. degraded Coomassie Brilliant Blue (CBB), dye used in the textile industry (Aditee et al. 2014). Muthezhilan et al. (2008) isolated and screened fungal isolates for their decolorization against methylene blue, gentian violet, crystal violet,

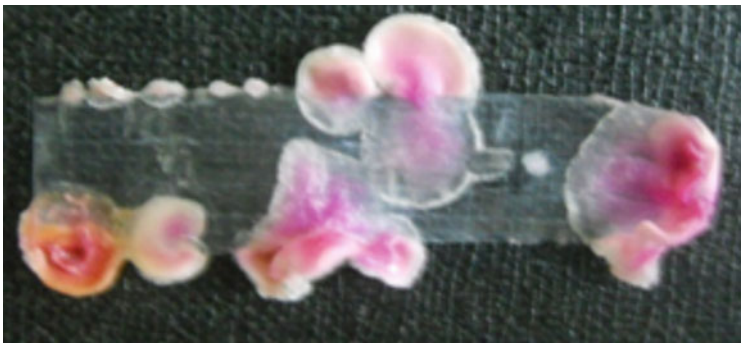

Fig. 18.1 Adherence of fungi to plastic film

cotton blue, Sudan black, malachite green, methyl red, and carbol fuchsin of which *A. ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum*, and *Fusarium* decolorized maximum number of dyes. Vinciguerra et al. (1995) have reported the decoloring reactive dye ability of *Lentinus edodes*.

18.3 Enzymes Responsible for Biodeterioration of Wastes

Massive number of microbes such as *Aspergillus*, *Pleurotus*, *Coriolus*, etc. play an important role in mycoremediation by releasing powerful microbial enzymes. Various classes of enzymes are responsible for bioremediation of pollutants, such as oxygenases peroxidases, hydrolases, lipases, phosphotriesterases, etc. Conversion of toxic chemicals to non-toxic biodegradable compounds is portrayed by the presence of microbial enzymes. Mushrooms can produce extracellular peroxidases, ligninase (lignin peroxidase, manganese-dependent peroxidase, and laccase), cellulases, pectinases, xylanases, and oxidases (Nyanhongo et al. 2007).

18.3.1 Lignin-Degrading Enzymes

White rot fungi, for instance, *Pleurotus*, produce lignin-degrading enzymes that catalyze the oxidation of xenobiotics in addition to the degradation of lignin. They consist of peroxidases, laccases, and other enzymes involved in the formation of free radicals, ROS, and H_2O_2 that cleave the carbon–carbon and carbon–oxygen bonds of the lignin/xenobiotic by means of a free radical mechanism (Reddy and Mathew 2001).

Fungi produce extracellular oxidative enzymes which completely mineralize lignin and carbohydrate components of wood to carbon dioxide and water. However, lignin is composed of many different aromatic rings in long varied chains, the fungal enzymes for mineralization are non-specific and frequently can also mineralize polycyclic aromatic hydrocarbons (Mai et al. 2004).

18.3.2 Laccase Enzymes

Laccase has copper in their active sites and are produced by a variety of fungi such as species of *Cryptococcus*, *Penicillium*, *Agaricus*, and *Pleurotus* (Mikolasch and Schauer 2009). Also, fungal endophytes such as *Monotospora* sp., in *Cynodon dactylon*, *Phomopsis longicolla* of *Bixa orellana*, *Discosia* sp. from *Calophyllum inophyllum* followed by *Chaetomium* sp. from *Alpinia calcarata* produce laccase enzymes in culture conditions (Wang et al. 2006; Sunitha and Devi 2013). Laccase enzymes degrade polyethylene due to conserved copper binding sites which couple the oxidation of a substrate with the cleavage of dioxygen bonds, leading to the capability to degrade plastics particularly polyethylene (reference). Some of the endophytic fungi that produce laccase enzymes were *Cunninghamella echinulata*, *Pestalotiopsis* sp., *Hypoxylon anthochroum*, *Paecilomyces lilacinus*, *Aspergillus* sp., *Curvularia clavata*, *Curvularia pallescens*, *Fusarium fusaroides*, *Phanerochaete* sp., from *Humboldtia brunonis* and *Lasiodiplodia theobromae* from *Psychotria flavida* (Sheik et al. 2015).

18.3.3 Cellulolytic Enzymes

Fungal cellulolytic enzymes and the enzyme producers are listed in Table 18.1.

Table 18.1 Fungal Enzymes

Enzymes	Fungi
Cellulases	*Trichoderma reesei*
Pectinases, hemicellulases	*Rhizopus microsporus*
Chitinases	*Fusarium*, *Nigrospora*, *Lasiodiplodia* sp.
Amylases	*Aspergillus niger*, *Penicillium sp.*, *Chrysosporium* sp.
Proteases	*Penicillium*

18.3.4 Glyphosate Degradation Fungi

Glyphosate disappeared rapidly in liquid Czapek-Dox medium containing 1% sucrose by *Aspergillus flavus* WDCZ2 (99.6%), *Penicillium spiculisporus* ASP5 (95.7%), and *P. verruculosum* WGP1 (90.8%) ((Eman et al. 2013). However, glyphosate almost disappeared in *P. spinulosum* ASP3 (98.8%), *P. spiculisporus* ASP5 (98.1%), *A. tamarii* PDCZ1 (96.7%), and *A. flavus* WDCZ2 (90.6%) (Eman et al. 2013).

18.4 Conversion of Toxic Chemicals to Non-toxic Biodegradable Compounds

Fungi have the potential to transform recalcitrant, toxic pollutants such as oils, diesels, petroleum hydrocarbons, etc., to simpler non-toxic compounds. Bioremediation includes the utilization of microorganisms and their toxic compounds for the degradation and change of toxins into another structure, which is less dangerous for living organisms. Microbial biotransformation is a process by which organic compounds are transformed from one form to another to reduce the persistence and toxicity of the chemical compounds. In recent years, interests in the biotransformation of various pollutants using microorganisms have gathered much momentum in order to clean up the polluted environment (Pajouhesh and George 2005). The ubiquitous fungi have the ability to survive in all ecosystems and are capable of regulating the nutrient as well as energy flow through their mycelial networks, and hence, are considered as natural and true ecosystem engineers (Lawton and Jones 1995).

The term "biodegradation" is used to describe the ultimate degradation and recycling of complex molecule to its mineral constituents (Kumar 2017). The uptake of pollutants/xenobiotics by fungi mainly involves bioaccumulation and biosorption. According to Mar'in et al. (1997), the polar groups of proteins, amino acids, lipids, and structural polysaccharides (chitin, chitosan, glucans) may be involved in the process of biosorption.

Interaction of fungi with metals includes mobilization and immobilization in the mycosphere, sorption to cell walls, and uptake into fungal cells. Thereafter, chemical transformation, translocation, and metabolization along with reactions of pollutants on fungal enzymes such as extracellular oxidoreductases/cell-bound enzymes allow fungi to act on various metal pollutants (Harms et al. 2011; Kumar 2017). Hence, the role of filamentous fungi becomes important where translocation of essential factors necessitates for the transformation or detoxification of environmental chemicals.

18.4.1 Biotransformation by Fungi

Cunninghamella elegans has shown the ability to biotransform potential pollutants bearing the pentafluorosulfanyl (SF5-) functional group (Kavanagh et al. 2014). The

breakdown of the aromatic dipentyl phthalate to butanediol by *Fusarium culmorum* and complete degradation of di(2-ethyl hexyl) phthalate by the white rot fungus *Pleurotus ostreatus* have been reported (Ahuactzin-Perez et al. 2016, 2018). Higher biotransformation rate of bisphenyl A observed with *Phoma* sp. may be attributed to laccase enzymes (Carstens et al. 2020). Laccase enzymes potentially decolorized azo dyes by oxidizing their bonds and transforming them into less harmful substances in the environment (Legerska et al. 2016). Laccase produced by *R. practicola* has the ability to degrade and biotransform phenolic compounds (Strong and Claus 2011). The degradation pathway of glyphosate produces the major metabolite aminomethylphosphonic acid (AMPA) and ultimately leads to the production of water, carbon dioxide, and phosphate (Forlani et al. 1999).

Under ligninolytic conditions, the white rot basidiomycete *Phanerochaete chrysosporium* mineralized 2,4-dinitrotoluene (I). The pathway for the degradation was elucidated by the characterization of fungal metabolites and oxidation products generated by lignin peroxidase (LiP), manganese peroxidase (MnP), and crude intracellular cell extracts (Valli et al. 1992). Stahl and Aust (1993a, b) provided evidence that TNT is reduced by a plasma membrane redox system in *P. chrysosporium* that requires live and intact mycelia. Any conditions that disrupt the integrity of the plasma membrane destroy the reductase activity. The presence of compounds known to inhibit the membrane redox systems also inhibits TNT reductase (Stahl and Aust 1993a, b).

18.5 Conclusion

Different consortia of fungi can bring about reclamation of soil environment. Several factors influencing the mycoremediation include species of fungi and their enzymes, their association strength and interaction with pollutants, physical and chemical properties of contaminants for further biotransformation, and biophysical aspects such as temperature, pH, salinity, and metal characteristics for biosorption and degradation. An increasing trend toward energy- and cost-efficient mycoremediation contemporary research for the reclamation of contaminated natural resources, i.e., land, water, and air is the need of the hour.

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Part III

Disease and Control Management

Impact of Fungi on Agriculture Production, Productivity, and Sustainability

19

Lan Jing and Yan Lu

Abstract

Fungi are a group of eukaryotic organisms and sources of food, organic acids, alcohol, antibiotics, growth-promoting substances, enzymes, and amino acids. They include microorganisms like molds, yeasts, and mushrooms. The primary functions of filamentous fungi in the soil are to degrade organic matter and help in soil aggregation. Certain *Alternaria*, *Aspergillus*, *Cladosporium*, *Dematium*, *Gliocladium*, *Humicola*, and *Metarhizium* manufacture organic compounds in the soil and therefore could also be necessary for the maintenance of soil organic matter. However, some fungi are the most important plant pathogens, causing major diseases like rusts, smuts, Dutch elm disease, chestnut blight, etc. Plant growth regulators and chemical fertilizers are applied to increase crop production. The application of these chemicals has negative impacts on human health and the environment. Fungi as biological agents to control plant pathogens, insects, and nematode pests is well documented in the management of nutrient cycling through better use of saprotrophic and symbiotic processes.

Keywords

Fungi · Plant pathogens · Agriculture · Sustainability

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_19

19.1 Challenges and Threats of Plant Pathogenic Fungi on Agricultural Productivity and Economy

The fungal kingdom includes at least 6 million eukaryotic species and is remarkable for its profound impact on global health, biodiversity, ecology, agriculture, manufacturing, and biomedical research. Approximately 8000 species of fungi and Oomycetes are associated with plant disease.

Losses due to (a) *Magnaporthe oryzae* (rice blast), (b) *Puccinia graminis* (wheat rust), (c) *Ustilago maydis* (maize smut), (d) *Phytophthora infestans* (potato late blight), and (e) *Phakopsora pachyrhizi* (soybean rust) vary regionally but pose a current and growing threat to food security (Pennisi 2010).

M. oryzae is the most destructive pathogen of rice worldwide, causing a 10–35% harvest loss. Destruction can be swift but variable, with up to 100% loss in some paddies. Some analysts estimate that each year blast destroys harvests that could feed 60 million people, at the cost of some $66 billion (Pennisi 2010).

In 1998, *P. graminis* Ug99 appeared in Uganda (Stokstad 2007). By 2004, its spread prompted Green Revolution pioneer Norman Borlaug to launch a global research initiative to address the threat. Ug99 has since shown up in Yemen and Iran and threatens wheat crops throughout the Middle East and West Asia.

U. maydis occurs worldwide and can cause losses from a trace to 10% (Shurtleff 1980). Losses occur when galls replace the kernels of the ear. Losses due to *U. maydis* on sweet corn can be much more significant due to the high susceptibility of flint corn, an ancestor of sweet corn (Bowjanowski 1969). In Mexico, the immature galls of infected ears are eaten as a delicacy known as *huitlacoche*. It replaces the kernels of corn with mushroom-like tumors or *galls* consisting of bluish black spores, fungal threads, and enlarged corn cells. In addition to its use in cuisine, it can also use corn smut to make hay for cattle and sheep.

International Potato Center in Peru reports that yield losses in developing countries due to potato late blight (*P. infestans*) are about $2.75 billion annually. Fungicide applications can be 10% of overall production costs. Soybean rust also referred to as Asian soybean rust (*P. pachyrhizi*) is an aggressive pathogen. Yield losses can be severe with this disease, and 10–80% losses have been reported.

More recently, however, there have been new disease incursions, such as myrtle rust (*Austropuccinia psidii*) and ash dieback (*Hymenoscyphus fraxineus*) (Rafiqi et al. 2018). Both these diseases are spreading worldwide, challenging not only their hosts per se but with myrtle rust having the potential to spread to other *Myrtaceae* species.

Global food insecurity is caused by pre- or postharvest loss of calorie crops and loss of cash crops, where economies of countries depend upon export revenues (Fones et al. 2020). The top five global calorie crops are wheat, rice, maize, oil palm, and soybean, as derived from measuring crop yield with the metric of calories *per capita* per day (FAOSTAT 2016). Of these, wheat and soybean harvests are currently under threat from newly emerged fungal and oomycete pathogens. They are, respectively, the wheat blast fungus, *Magnaporthe oryzae*; the soybean rust fungus, *P. pachyrhizi* (Langenbach et al. 2016; Whitham et al. 2016); and the soybean

oomycete pathogen *Phytophthora sojae* (Whitham et al. 2016). The wheat blast fungus *Magnaporthe oryzae* (also known as *Pyricularia graministritici*) was first described in 1985 in Brazil. It subsequently spread across central and southern Brazil (1988) and has since been reported in Bolivia, Paraguay, and Argentina (1996–2007) (Ceresini et al. 2019). In 2016, the fungus arrived in southwestern Bangladesh (Islam et al. 2016) and has since devastated the wheat harvest in eight districts, with total loss of crop and accounting for the loss of circa 3.5% of the total Bangladeshi wheat harvest (abstracted from 2016 USDA Foreign Agricultural drive BG6005; https://www.fas.usda.gov/data/Bangladesh-grain-and-feed-annual).

Bananas are considered a major cash crop. The livelihoods of tens of thousands of those dwelling in Africa, Central and South America, and Asia depend and face the challenge of Panama disease of bananas caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (TR4). Bananas (*Musa* spp.) are the most consumed, the cheapest, and the most traded global fruit (Ploetz 2015), the fifth most traded agricultural product. Approximately 70% of production is of the Cavendish cultivar, and the fruit is widely consumed and regarded as a staple diet (Ploetz 2015).

TR4 Infection of Cavendish and many other banana varieties (Ploetz 2015) causes severe vascular wilt disease. TR4 was first described in Malaysia, Indonesia, and China (the 1990s) and then Australia (1997), Jordan and Mozambique (2013), Europe (2018), and Turkey (2019) (Özarslandan and Akgül 2020), and its first incursion into Latin America, notably Colombia, was recorded in 2019 (Garcia-Bastidas et al. 2019) and renamed as *Fusarium odoratissimum* will likely have devastating consequences to the banana industry and exports worldwide. The recent report of the spread of TR4 into the Indian subcontinent is of major concern since India is the largest producer of bananas worldwide (Thangavelu et al. 2019).

In crop plants, fungi cause more economic damage than any other group of microorganisms, with annual losses estimated at more than 200 billion $US (Birren et al. 2002). Reduction of yield and contamination of food and fodder by highly toxic secondary metabolites called mycotoxins represent significant problems in agriculture.

The *Aspergillus* genus is saprophytes found worldwide in soil, forage, food, dust, organic debris, and decomposing matter (Gugnani 2003). Although they are considered weak plant pathogens (Gugnani 2003), there are two species, *Aspergillus flavus* and *Aspergillus parasiticus*, which produce potent toxins (aflatoxins) on certain crops. Especially susceptible to these species are oilseeds and nuts such as peanuts, corn, and cottonseed. *A. flavus* is the dominant of the two on corn and cottonseed (Hill et al. 1985; Klich 1986).

In contrast, *A. parasiticus* is more prevalent on peanuts than on other crops (Diener et al. 1987) and, generally, constitutes approximately 10–30% of the aflatoxin producing fungi on peanuts (Hill et al. 1985). Corn hybrids with high oil content are at greater risk of aflatoxin contamination than regular hybrids during some growing seasons (Severns et al. 2003).

Fusarium species are commonly found on cereal grains where the growth of these fungi, in addition to contamination by their toxins, can render these grains unsafe for consumption (Miller 1994; Krysinska-Traczyk et al. 2001; Saberi-Riseh et al. 2004).

Fusarium mycotoxins are considered one of the five major mycotoxin groups affecting human health (Pitt 2000).

F. oxysporum and *F. verticillioides* (formerly known as *F. moniliforme*) produce fumonisins that are cytotoxic to several mammalian cell lines (Abbas et al. 1998; Sewram et al. 2005). Fumonisins are found in corn and corn-based foods worldwide (Bullerman 1996). These mycotoxins play a role in diseases affecting human neural tube development in populations consuming fumonisin-contaminated maize (Carratù et al. 2003; Marasas et al. 2004). They are reported to induce equine leukoencephalomalacia and pulmonary edema in swine (Gelderblom et al. 1988, 1996; Marasas et al. 1988; Harrison et al. 1990). Fumonisins are also implicated in esophageal cancer after being found in homegrown maize in an area of the Transkei region of South Africa, which has a high incidence of this disease (Syndenham et al. 1991).

Certain *Fusarium* species produce the types A and B trichothecenes (Edwards 2004). The type A trichothecenes include T-2, HT-2, T-2 triol, T-2 tetraol, neosolaniol, di- and 15-monoacetoxyscirpenol (DAS, MAS), and scirpentriol. Type B trichothecenes include nivalenol, deoxynivalenol (DON), 3- and 15-acetylDON, fusarenon-X, zearalenone and its two derivatives, and -zearalenone (Schollenberger et al. 2004). These toxins affect the immune system of laboratory animals, resulting in increased susceptibility to various microbial diseases (Pestka and Smolinski 2005). *Fusarium graminearum* produces DON (also known as vomitoxin), which causes vomiting and feed refusal in swine (Diekman and Green 1992; Pitt 2000). In humans, the symptoms of DON toxicity include anorexia, nausea, vomiting, headache, abdominal pain, diarrhea, chills, giddiness, and convulsions (Yoshizawa 1983). DON is also a potent inducer of human lymphocyte cytokine production (Meky et al. 2001).

Zearalenone is an estrogenic toxin produced by *F. graminearum* and related species (Pitt 2000). On wheat, *F. graminearum* produces zearalenone which causes genital problems in domestic farm animals, especially pigs (Pitt 2000). This toxin seriously affects livestock reproduction due to its estrogenic activity (Diekman and Green 1992) and is implicated in precocious pubertal changes in children (Kuiper-Goodman et al. 1987).

19.2 Role and Application of Fungi in Soil Fertility Improvement and Farm Productivity

Soils are the most diverse and complex habitat on this planet, formed by biological, chemical, and physical processes, all persisting in parallel (Young and Crawford 2004). Some of the best agricultural soils in many countries are under tremendous pressure as populations grow, cities expand, and interest in the use of biofuels increases. In the last decade, people have realized the complex characteristics of soil and studied the soil fungal community and its influence process on agricultural production.

Fungi widely existing in soil, air, water, and other environmental conditions are one of the most important biological groups on the earth. They are closely related to human production and life. Although fungi may be numerically less abundant than bacteria, fungi can account for as much as 78–90% by weight of the soil microbial biomass (Kjoller and Struwe 1982; Lynch 1983). These fungi may be free-living saprotrophs, pathogens, or in mutualistic associations with plant roots. Because of the soil fungus silently worked, the earth will have the spring and autumn fruitful endless. So, what exactly do fungi do in the soil?

19.2.1 Form the Real Soil

Soil is not a simple combination of soil particles and fertilizer. As an active component of soil, soil fungi contribute to the formation of large aggregates of soil particles through the exchange of oxygen and carbon dioxide and the secretion of organic acids in their life process, finally forming the real soil and improving soil water storage capacity. The biomass and life activities of soil fungi are closely related to the formation and development of soil.

19.2.2 Decomposing Organic Matter

Second, the most significant effect of soil fungi is decomposing organic matter. In nature, most fungi exist in the saprophytic state. They can live on the remains of dead plants and animals, converting biomolecules into smaller nutrients that other organisms can use. Fungal biomass represents a significant pool of available nutrients in soils. Especially in agricultural and forest ecosystems, their activities can release nutrients back into the biosphere for reuse by other organisms. This contributes to biosphere carbon and oxygen cycling and affects soil organic matter dynamics (Kjoller and Struwe 1982; Langley and Hungate 2003; Zhu and Miller 2003; Six et al. 2006). No organism on Earth can degrade cellulose and lignin as much as fungi. Saprophytic fungi also have some unique ecological habits such as they can grow in nitrogen-deficient environments and are particularly good decomposers of nutrient-poor plant polymers (Paustian and Schnürer 1987). Because fungi are generally acid-tolerant, they can grow normally in soils with a pH of 5.0 or lower and thus play an even more critical role in acidic soils. At the same time, the development of bacteria and actinomycetes is limited. In acidic soils, normally growing fungi take over the role of bacteria and actinomycetes in the decomposition of organic matter. Fungi can produce some enzymes, such as protease, cellulase, phosphatase, etc., to decompose complex organic matter that is difficult to degrade. In particular, they can absorb phosphorus from the soil for the plant body to absorb. In soil, half or even 95–99% of phosphorus is insoluble and cannot be directly absorbed and utilized by the plant body. Fungi use their unique enzyme system to secrete some extracellular enzymes and organic acids to convert

insoluble organophosphorus into soluble inorganic phosphorus for host plants to absorb (Gong et al. 1997).

19.2.3 The Effect of Mycorrhizal Fungus

In addition, fungi can connect plants and deliver nutrients through a network of hyphae. The hyphae of a fungus, like a mycorrhizal fungus, live partly inside a plant's root system and partly extend into the soil. Fungal hyphae may also translocate mineral nitrogen to nitrogen-poor substrata where the absolute amount of nitrogen in a decomposing substrate increases during the early stages of decomposition (Aber and Melillo 1982; Holland and Coleman 1987; Frey et al. 2000). Also, the lateral and upward movement of ^{15}N-label inorganic nitrogen from mineral soil to decomposing litter has been demonstrated (Frey et al. 2000).

In grassland and agroecosystems, the mycorrhizal fungus biomass can represent 20–30% (Miller et al. 1995; Olsson et al. 1999; Leake et al. 2004). The importance of mycorrhizal fungi as symbionts provides a direct physical link between primary producers and decomposers. Mycorrhizal fungal influences on host functions can affect the nutrient accumulation and alter nutrient ratios in plant tissues. In addition, mycorrhizae can affect plant nutrient uptake by affecting a host's growth rate and by influencing mineral ion uptake (Smith and Read 1997). Plant roots and mycorrhizal fungi can secrete some substances and enzymes to promote soil organic matter decomposition and provide carbohydrates and other carbohydrates for mycorrhizal fungi. These processes significantly promote soil C and N cycles, increase soil fertility, and promote soil carbon capture function (Richard et al. 2012).

The common kinds of mycorrhizae include ectomycorrhizas, VA mycorrhiza, ectendomycorrhizas, Orchid mycorrhiza, Arbutoid mycorrhiza, Monotropoid mycorrhiza, Ericoid mycorrhiza, and so on. Each species has unique properties fitting it in some way for particular conditions (Harley 1989). Arbuscular mycorrhizal symbioses have been shown to benefit the growth of many field crops in large part due to the extensive hyphal network development in soil, more efficient exploitation of nutrients, and enhanced plant uptake (Smith and Read 1997). Application of these arbuscular mycorrhizal (AM) fungi is generally useful for overcoming heavy metal problems, alleviating soil stress, and ultimately increasing agricultural production (Siddiqui et al. 2008). AM fungi occur in the soil of most ecosystems, including polluted soils. By acquiring phosphate, micronutrients, and water and delivering a proportion to their hosts, they enhance the host's nutritional status (Siddiqui et al. 2008).

Rhizosphere fungi communities have been shown to directly affect soil fertility by carrying out essential processes that contribute to nutrient cycling and enhancing soil structure and plant growth and health (Mader et al. 2002; Wu et al. 2005; Miransari et al. 2007; St-Arnaud and Vujanovic 2007).

19.3 Role of Fungal Biocontrol Agents for Sustainable Agriculture

Nature published an editorial, "Sustainable Agriculture," the key message is achieving food security is possible if we better understand the complexity of the agricultural system and re-design practices accordingly (Sustainable Agriculture 2018). Sustainable agricultural systems employ natural processes to achieve acceptable productivity and food quality levels while minimizing adverse environmental impacts (Harrier and Watson 2004). Sustainable agriculture must, by definition, be ecologically sound, economically viable, and socially responsible. Sustainable food systems aim to provide sufficient and nutritious food while maximizing climate resilience and minimizing resource demands and negative environmental impacts. The overarching strategy is to improve agricultural production systems, enhance agricultural sustainability and efficiency while protecting the environment.

Food demand has been rising rapidly and has taken a huge environmental toll: degradation of agricultural land, pollution of rivers and aquifers from agrochemicals, increased freshwater consumption, greenhouse gas emissions from farming and land-use changes, loss of farm biodiversity, and other negative consequences. All of these environmental impacts severely erode our ability to continue to feed our growing population. However, feeding an increasing global population requires continued improvements in agricultural efficiency and productivity. Estimates indicate that output will need to double by 2050 to accommodate population growth and shift consumption preferences for more meat and dairy products (Robertson and Swinton 2005).

In the face of such trends, ecological restoration will become an apparent, effective, and essential strategy for preserving biodiversity in various parts of the world. Several research areas with fungi promise to improve agricultural production systems (Miller and Lodge 2007).

Increasingly, the microbial interactions of plants in agricultural production are being examined for their potential to increase productivity as an alternative to the use of agrochemical amendments that often have adverse environmental effects (Bakker et al. 2012). Colonizing microbes provide many services to their host plants, including nutrient transfer (Cardoso and Kuyper 2006; Muñoz et al. 2016), growth promoters' production, and developmental changes (Lehman et al. 2012).

Biocontrol fungi have been employed to suppress insects, nematodes, and other fungi. These fungi are primarily drawn from two major clades of fungi: *Entomophthorales* and *Ascomycota*. By far, the richest source of biocontrol fungi has been the order *Hypocreales* in the *Ascomycota*. Strains of several species are produced commercially and more receive active research for commercialization (e.g., *Trichoderma*: T-22, BioWorks Victor, NY 14564 and Rootshield1, Arbico Organics, Oro Valley, AZ 85737-9531; *Metarhizium* Met52, Novozymes Biologicals). Numerous species of *Hypocreales* are insect pathogens, with a concentration in the families *Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae* (Sung et al. 2007). Fungi infecting nematodes are found in most families of *Hypocreales*. Several species have been developed for commercially available products, including *Hirsutella*

minnesotensis, *Pochonia chlamydosporia*, and *Purpureocillium lilacinum* (Li et al. 2015). Fungal pathogenesis is a less common but widespread ecological trait in *Hypocreales* gained after shifts from other hosts on multiple occasions (Sung et al. 2008). The genus *Tolypocladium* contains species predominantly infecting false truffles of the genus *Elaphomyces*, although several species are also insect pathogens (Bushley et al. 2013; Quandt et al. 2014, 2016). In the early 1930s, the biocontrol capability of *Trichoderma* was introduced (Weindling 1932). Thus far, several species of *Trichoderma* are the only species developed as biocontrol agents against fungi responsible for crop failure (Roberts et al. 2010). *Trichoderma* species infect many plant pathogenic fungi and oomycetes. And commercial products of *Trichoderma* are available as biopesticides or soil improvements or as enhancers for plant growth (Chet 1987; Zhou and Everts 2007; Vinale et al. 2008a, b; Contreras-Cornejo et al. 2016). Dennis and Webster (1971) reported that *T. harzianum* could control *Rhizoctonia solani* and *Pythium ultimum*. It is also found that strains of *T. virens* were recognized as an efficient biocontrol agent (Howell 1998). Sallam et al. (2019) found that *T. atroviride* and *T. longibrachiatum* could antagonize *Fusarium oxysporum* f. sp. *lycopersici*. Swain et al. (2018) reported that *T. erinaceum* could control three soil-borne plant pathogens, i.e., *R. solani*, *Sclerotium rolfsii*, and *Sclerotium oryzae*, effectively under a controlled condition and *R. solani* and *Helminthosporium oryzae* under field condition.

When not infecting other hosts, some hypocrealean fungi are able to interact intimately with plants in several ways. Rhizosphere competence has been observed in many species, including the nematode pathogen *P. chlamydosporia* (Macia-Vicente et al. 2009; Larriba et al. 2015) and *Ophiocordyceps sinensis* (Zhong et al. 2014). *Tolypocladium* species have been found as endophytes in the bark of rubber trees (Gazis et al. 2014), and *B. bassiana* has been induced to grow as a foliar endophyte (Vega et al. 2008). Species of both *Metarhizium* and *Beauveria* are commonly isolated from bulk soil and rhizosphere habitats; however, it appears that *Metarhizium* is uniquely adapted to the rhizosphere environment (Hu and St. Leger 2002; Fisher et al. 2011). Strains of *M. anisopliae* have been shown to penetrate root tissues (Sasan and Bidochka 2012) and exchange nitrogen with plants (Behie et al. 2012).

Studies have found that about 90% of vascular plants have symbiotic fungi in their roots. The existence of symbiotic fungi brings great benefits to plant colonization and growth. For example, mycorrhizal fungi can expand the surface area of plant roots and increase the area of nutrient absorption. Antibiotic substances can be secreted to improve plant disease resistance. It can form mycelial sheaths to protect plant roots from the toxic effects of heavy metal ions. Arbuscular mycorrhizal symbiosis also increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (Subramanian and Charest 1999; Aliasgarzad et al. 2006; St-Arnaud and Vujanovic 2007). The establishment of AM fungi in the plant root has been shown to reduce the damage caused by soil-borne plant pathogens with the enhancement of resistance in mycorrhizal plants. AM fungi generally reduce the severity of plant diseases to various

crops suggesting that they may be used as a potential tool in disease management. AM fungi modify the quality and abundance of rhizosphere microflora and alter overall rhizosphere microbial activity. These fungi induce changes in the host root exudation pattern following host colonization which alters the microbial equilibrium in the mycorrhizosphere. The AM fungi are included in the phylum *Zygomycota*, order *Glomales* (Redecker et al. 2000). AM fungi are a major component of the rhizosphere of plants and may affect the incidence and severity of root diseases (Linderman 1992).

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Efficacy of Seed Treatments with *Bradyrhizobium japonicum* to Reduce Occurrence of Soybean Sudden Death Syndrome in Early-planted Soybeans

20

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Abstract

Previous studies showed that cool soil temperatures are favorable factors for *Fusarium virguliforme* (FV) infection, the causal agent of soybean sudden death syndrome (SDS). *Bradyrhizobium japonicum* (BJ), a nitrogen-fixing α-Proteobacterium symbiont forms nodules on soybean, is widely present in the US soils. The minimum temperature for BJ to infect soybean has been reported to be 17.5 °C, and for *F. virguliforme*, 15 °C. Soybeans planted early into colder soil had a greater incidence of SDS. In this study, a BJ strain was applied to seeds before and after germination and was evaluated for SDS suppression in controlled conditions. Results showed that seed germination had a significant influence on SDS incidence under low temperatures. BJ treated seeds planted in FV-infested soil in clay pots or foam cup experiments significantly ($P < 0.05$) reduced SDS incidence, both germinated and non-germinated seed at 15 °C compared to 25 °C at the V3 growth stage. BJ inoculated on vermiculite substrate as soil inoculant treatments showed the most effective treatment. Non-germinated treated seed had

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_20

significantly higher nodule counts than the germinated ones at 20 °C but detected no significant effects for plant biomass. This study suggested that either seed treatments or soil inoculants could provide better nodulation and health benefits in early-planted soybean. Also, BJ may act as a biocontrol agent against SDS.

Keywords

Soybean · *Fusarium virguliforme* · *Bradyrhizobium japonicum* · Seed treatments · Early planting

20.1 Introduction

Soybean sudden death syndrome (SDS) is among the most important soil-borne fungal diseases in US soybean production. SDS was ranked as one of the most damaging diseases on soybean from 2006 to 2009 (Koenning and Wrather 2010). In the USA, SDS was first reported in Arkansas in 1971 (Roy 1997). After that, Hirrel (1987) named the disease SDS because of the sudden development of foliar symptoms. Recently, Navi and Yang (2016b) compiled reports of SDS occurrence within the USA and other countries.

Numerous factors have been hypothesized that contribute to SDS prevalence and soybean yield decline, including soybean cyst nematode (McLean and Lawrence 1993; Xing and Westphal 2006; Marburger et al. 2014). Wet and cool soil is believed to trigger SDS development (Scherm and Yang 1996; Scherm et al. 1998). The most excellent foliar symptoms of SDS were observed in soil temperature ranging from 22 °C to 24 °C, and root severity and the temperature had a negative correlation in which the highest severity was observed at 15 °C and the lowest at 30 °C. In addition, increased SDS foliar symptoms were observed with increased soil moisture (Scherm and Yang 1996).

In Iowa, SDS was first reported in 1993 (Yang and Rixvi 1994). After an SDS outbreak in 2010, *F. virguliforme* (FV) was reported extensively distributed in most Iowa soils (Leandro et al. 2013). Infection of FV is generally favored by cold, wet conditions, which are very common in early-planted soybean in Iowa and other Midwest regions. Leandro et al. (2013) showed that yield losses by SDS in epidemic years in Iowa were dependent on cool soil temperature and high precipitation in the early part of the growing season. Typical soil temperatures in Iowa in the early part of the season range from 10 °C to 15 °C (Leandro et al. 2013). Delayed planting has been one of the management tools to reduce SDS severity (Hershman et al. 1990; Rupe and Gbur Jr 1995; Wrather et al. 1995). However, this practice may limit yield potential (Roy et al. 1997). A recent study (Marburger et al. 2016) on planting dates in Wisconsin, USA, suggested that planting in early May provided maximum yield potential despite the higher development of SDS symptoms.

Soybean requires 25 °C to 30 °C temperatures in the root zone for optimal symbiotic activity. According to Zhang et al. (1996), a delay in nodulation in the cold occurs because of inhibited nod gene expression under suboptimal root zone temperature in soybean. Cool soil temperatures are considered one of the most important factors limiting soybean growth and biological nitrogen fixation. Lynch

and Smith (1993) indicated that the onset of nitrogen fixation was delayed 2.5 days linearly for each decreased degree from 25 °C to 17 °C and was delayed 7.5 days when soil temperature was below 17 °C. The sensitivity of atmospheric nitrogen fixation to low temperatures in soybean is a significant limitation when adopting this crop in a cool spring season (Zhang et al. 1995). Pre-incubation of rhizobacterial cells with plant-to-bacteria signal compounds as inducers of enhanced plant growth, photosynthetic rates, and nitrogen fixation could alleviate lentils' low root zone temperature stress (Lee 2009). A 2-year study conducted by Zhang et al. (2003) indicated a low-temperature tolerant *Bradyrhizobium japonicum* inoculant improved nodulation and nitrogen fixation in soybean under cooler growing temperatures.

Legume-*Rhizobium* mutualism has been widely documented, especially for nitrogen fixation and plant health promotion. In soybean, *rhizobia* play an essential role as biocontrol agents against root rot diseases caused by *Macrophomina phaseolina*, *Fusarium solani*, and *Fusarium* spp. (Chakraborty and Purkayastha 1984; Buonassisi et al. 1986; Omar and Abd-Alla 1998; Al-Ani et al. 2012). Similarly, seed treatments with *Rhizobium* sp. significantly suppressed *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Arfaoui et al. 2005; Singh et al. 2010), root rot caused by *Rhizoctonia solani* (Hemissi et al. 2011) in chickpea, and *Pythium* damping-off of field pea and lentil (Huang and Erickson 2007). *B. japonicum* inoculum used either as soil inoculant or seed treatments may offer SDS management in early-planted soybeans. Therefore, the objectives of this study were (1) to evaluate the effects of *B. japonicum* inoculum and soil temperature on SDS occurrence and (2) to quantify the impacts of *B. japonicum* inoculation and soil temperature on nodule and plant biomass under SDS pressure.

20.2 Materials and Methods

20.2.1 Soybean Cultivar and Isolates Collections

Soybean cultivar P22T61RR (Pioneer Hi-Bred International, Inc., Johnston, IA), which is moderately resistant to SDS, was used in all experiments carried out in the greenhouse from 2014 to 2016.

A *B. japonicum* (ISU-BJ) strain isolated from healthy plants in a field experiment at Iowa State University in 2013 was used in experiments conducted in 2014, 2015, and 2016. Nodules were collected, and rhizobia were isolated following methods described by Somasegaran and Hoben (2012). The ISU-BJ strain was cultured on yeast mannitol agar (YMA) and was sub-cultured on yeast mannitol broth (Vincent 1970). Flasks (250 mL) were incubated on a shaker at 125 rpm at 25 °C for 7 days. Later, the culture was diluted with sterile yeast mannitol broth to an OD620 of 0.08, which was equivalent to about 108 cells mL^{-1}; used this concentration of BJ in all the experiments.

F. virguliforme isolate Fsg-ISU1 (Mbofung et al. 2012) originated from a field at Boone, Iowa, was sub-cultured on potato dextrose agar (PDA) amended with 100 mg/L streptomycin. *F. virguliforme* (FV) inoculum was fermented on white sorghum grain following the methods of Hartman et al. (1997) and Navi and Yang (2016a).

20.2.2 Efficacy Tests of ISU-BJ Strain Against Soybean Sudden Death Syndrome, 2014 and 2015

An experiment with eight treatments was conducted in the Plant Pathology Greenhouse, Iowa State University. Two separate runs were carried out. The first run was from March 2014 to May 2014, while the second from April 2015 to June 2015. Commercially untreated seeds of P22T61RR were surface-sterilized following the method described by Somasegaran and Hoben (2012). They were placed in 10 cm diameter glass Pyrex® Petri dishes lined with one layer of moist sterilized germination sheets at 30 seeds/plate. The plates were incubated in the dark at 25 °C for 5 days. Subsequently, uniformly germinated seeds were selected and were inoculated by submersion in BJ suspension (108 cfu/mL) at 25 °C for an hour. Similarly, surface-sterilized seeds of P22T61RR were taken in a Ziploc bag, transferred 10 mL of a 108 cfu/mL suspension to the bag, mixed thoroughly, and incubated at 25 °C for an hour.

20.2.2.1 FV Seed Treatments

The method described above for BJ seed treatments was followed for FV seed treatments but with FV-spore suspension at 106 spores mL^{-1}. Control treatments included surface-sterilized seeds not treated with BJ but planted in FV-infested soil; seeds not infested with FV but planted in BJ-infested soil. The controls were to assess the baseline effects of BJ treatments on the growth of soybean seedlings, nodule development, FV infection, and SDS foliar symptom development and to create conditions where both the microorganisms are initially present in the soil. The details of seed treatments combinations are provided in Table 20.1.

20.2.2.2 Soil Treatments with BJ

Preparation of moist vermiculite inoculant of BJ was described by Paau (1998). The vermiculite-based inoculant was then incorporated into a steam-pasteurized potting mixture (2 parts alfisol and 1 part sand) at a rate of 2% vermiculite (volume/volume). Soil treatment with FV: steam-pasteurized soil was mixed with FV-infested sorghum grain at 2% (volume/volume).

In summary, based on germinated or non-germinated seed, eight treatments were divided into two groups: germinated seed inoculation and non-germinated seed inoculation. Based on soil infestation either with BJ and/or with FV, planted the treated seed either with FV or with ISU-BJ and untreated seed were in those infested soils (Table 20.1). We evaluated these treatments for SDS incidence, severity, and nodulation.

20.2.2.3 Planting

Seeds of individual treatments were planted at seven seeds per 10-cm diameter clay pot (filled with the potting mixture) at an approximate depth of 3 cm. Planted each treatment in 10 clay pots, and each pot was considered a replication. Treatments were randomly arranged and were incubated for 10 days in three growth chambers. Placed three sets of treatments in three growth chambers, which were set at 15 °C,

Table 20.1 Treatment descriptions of two experiments on effects of treatments with ISU-*Bradyrhizobium japonicum* strain on soybean sudden death syndrome during 2014, 2015, and 2016

Year	Seed inoculation	Soil infestation	Description
2014 (run 1) 2015 (run 2)	ISU-BJ[a]	FV[b]	Germinated and ISU BJ-inoculated seeds planted in FV-infested soil
	FV	ISU-BJ	Germinated and FV-inoculated seeds planted in ISU BJ-infested soil
	None	FV	Germinated seeds planted in FV-infested soil
	None	ISU-BJ	Germinated seeds planted in ISU BJ-infested soil
	ISU-BJ	FV	Non-germinated and ISU BJ-inoculated seeds planted in FV-infested soil
	FV	ISU-BJ	Non-germinated and FV-inoculated seeds planted in ISU BJ-infested soil
	None	FV	Non-germinated seeds planted in FV-infested soil
	None	ISU-BJ	Non-germinated seeds planted in ISU BJ-infested soil
2016 (2 runs)	ISU-BJ	FV	Germinated and ISU BJ-inoculated seeds planted in FV-infested soil
	ISU-BJ +bioAPT[c]	FV	Germinated and ISU BJ + bioAPT-inoculated seeds planted in FV-infested soil
	FV	ISU-BJ	Germinated and FV-treated seeds planted in ISU BJ-infested soil
	None	FV	Germinated seeds planted in FV-infested soil
	None	ISU-BJ	Germinated seeds planted in ISU BJ-infested soil
	None	none	Germinated seeds planted in non-infested soil
	ISU-BJ	FV	Non-germinated and ISU BJ-inoculated seeds planted in FV-infested soil
	ISU-BJ +bioAPT	FV	Non-germinated and ISU BJ + bioAPT-inoculated seeds planted in FV-infested soil
	FV	BJ	Non-germinated FV-treated seeds planted in ISU BJ-infested soil
	None	FV	Non-germinated seeds planted in FV-infested soil
	None	ISU-BJ	Non-germinated seeds planted in ISU BJ-infested soil
	None	None	Non-germinated seeds planted in non-infested soil

[a] *Bradyrhizobium japonicum* isolate obtained from Iowa State University
[b] *Fusarium virguliforme*
[c] Microbial carrier (American Peat Technology®) used treated together with *Bradyrhizobium japonicum*

20 °C, and 25 °C with 90% relative humidity and 16 h light/8 h dark cycle. Watered pots placed in growth chambers once daily. After 10 days of incubation in growth chambers, pots were shifted to the greenhouse benches at 26 ± 2 °C in 16 h light/8 h dark photoperiod using a 400 W E-Ballast, Metal Halide type M59 bulb (Hydrofarm Inc. 2249 S. McDowell Ext., Petaluma, CA). Plants in each pot were thinned to five plants, arranged in randomized complete block design, and incubated for 40 days. Watered plants twice daily to maintain saturated soil moisture.

20.2.2.4 Evaluation

Plants were rated for SDS infection when the first foliar symptom was observed and continued every 10 days until the V3 growth stage (30 days after planting). When plants were at the V4 growth stage, 15 plants from three pots were randomly sampled from each treatment to determine total nodule numbers and the number of nodules on primary roots.

20.2.3 Seed Treatments with ISU-BJ Strain in 2016

In 2016, two runs of greenhouse experiments with 12 treatments were conducted from March to July for additional evaluation of ISU-BJ seed treatments followed by treatments with bioAPT® microbial carrier (American Peat Technology, LLC, 36203 350th Avenue Aitkin, MN) at 10 g/kg seed (Table 20.1). In treatment 1, uniformly germinated seeds were submerged-inoculated in ISU-BJ strain suspension (108 cfu/mL) and planted in 2% FV-infested potting mixture. In treatment 2, the germinated seed was treated with ISU-BJ by submerge-inoculation followed by sterile bioAPT microbial carrier added at 10 g/1 kg seed and planted in 2% FV-infested potting mixture. Planted germinated seeds inoculated with an FV-spore suspension at 106 spores/mL in ISU-BJ infested soil (treatment 3). Treatments 4 and 5 were two positive controls. Treatment 4 included water-treated germinated seeds planted in 2% FV-infested potting mixture and treatment 5 with germinated seeds planted in BJ-infested soil. Treatment 6 with a water-treated germinated seed planted in the potting mix without infestation served as a negative control. The treatment group (treatment 6–12) was similar to the first set but with non-germinated seeds (Table 20.1). Each treatment was planted in 20 cups, as described below.

20.2.3.1 Planting

Seeds of individual treatment were planted at seven seeds per 9-cm diameter foam cup (filled with the potting mixture) using the planter developed by Navi and Yang (2016b). The incubation and evaluations were similar to experiments in 2014 and 2015, except at growth stage V4, 25 plants from 5 cups per treatment were randomly collected, washed, and air-dried to determine the total nodules and fresh weights for roots and shoots. Dry weights of roots and shoots were determined after air drying on greenhouse benches for three weeks. Disease assessment was made until growth stage R1.

20.2.3.2 Evaluation

Foliar disease incidence (DI) was determined as percentage of symptomatic plants in a pot or a cup. Disease severity (DS) was recorded on a 1–9 scale, where 1 = 0–10% chlorotic (C), 1–5% necrotic (N); 2 = 10–20% C, 6–10% N; 3 = 20–40% C, 10–20% N; 4 = 40–60% C, 20–40% N; 5 = greater than 60% C, greater than

40% N; 6 = up to 33% premature defoliation; 7 = up to 66% premature defoliation; 8 = greater than 66% premature defoliation; and 9 = premature death (Njiti et al. 1996, 1998).

20.2.4 Statistical Analysis

The mixed model procedure (PROC MIXED) of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to perform Analysis of Variance (ANOVA). The ANOVA contained fixed effects for the experimental run, seed condition and treatment level, and two and three-way interactions. Data were analyzed separately by experimental temperatures (by 15, 20, or 25 °C). Data of disease incidence and disease severity were analyzed for either treatment with FV-inoculated seeds or FV-infested soil. ANOVA analyses were performed in Type 3 Tests of Fixed Effects. In LSMEANS procedure, Fisher's least significant difference (LSD) test was applied to detect differences in means for foliar disease incidence and disease severity, nodule counts, fresh and dry weights with $\alpha = 0.05$ (Steel and Torrie 1980). Data for nodule counts and plant biomass in fresh or dry weights (in the 2016 experiment) were available for all treatments. Orthogonal contrasts were conducted to compare the effects of BJ seed treatments versus non-treated seeds planted in FV-infested soil (control). These contrasts were for the mean average for the two runs in 2016.

20.3 Results

20.3.1 Efficacy of ISU-BJ Strain Treatments on Sudden Death Syndrome in 2014 and 2015

Disease incidence and severity: Experiments conducted in 2014 and 2015 had two independent runs (run 1 in 2014 and run 2 in 2015). There was no significant difference in SDS incidence in treatments at 15 °C ($P = 0.2793$) or 20 °C ($P = 0.7695$) between the 2 years. Although a significant difference was observed between the 2 years for disease incidence at 25 °C, a year is not associated with interactions except in year × seed. Similarly, there was no significant difference in disease severity at 20 °C ($P = 0.5791$) or 25 °C ($P = 0.3921$) between the 2 years, but there was at 15 °C ($P = 0.0494$). However, interactions between year and other effects (treatment, seed) had no significant impact on incidence and severity. Because the disease incidence (DI) and disease severity (DS) at each temperature generally did not differ between the 2 years, data from the 2 years were analyzed together as a blocked design with data from each year assigned as a block.

Differences among main effects on DI and DS were observed at each temperature. The only significant difference between non-germinated seeds and germinated seed treatments on DI and DS was for DI at 15 °C ($P = 0.0063$) and 25 °C ($P = 0.0059$). More specifically, in the presence of FV-infested soil, germinated seed treated with BJ reduced SDS incidence by 19.3% at 15 °C and 15.9% at 25 °C compared with

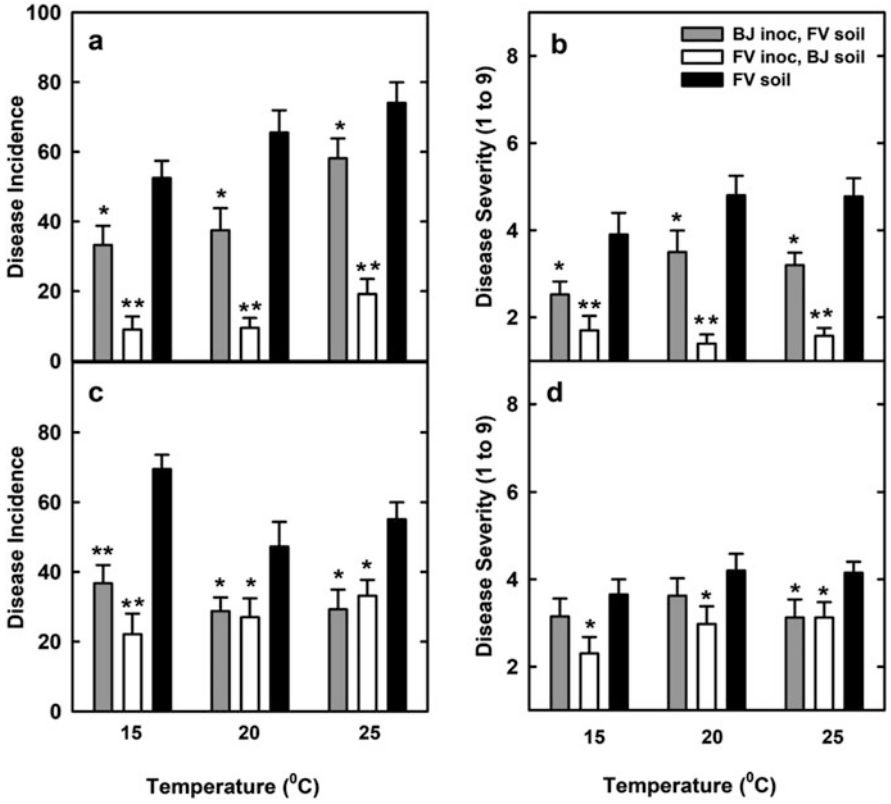


Fig. 20.1 Effects of *Bradyrhizobium japonicum* seed treatments under different temperatures on soybean sudden death syndrome were measured by incidence (%) and severity (scale 1–9) at V3 growth stage in greenhouse conditions. Germinated seeds (**a**, **b**) or non-germinated seed (**c**, **d**) were treated either with ISU-BJ strain of FV in experiment with 2 runs (run 1 in 2014 and run 2 in 2015). Incidence (%) was calculated based on the percentage of plants in pot that showed visible foliar symptoms. Incidence was assessed for each pot (5 plants/pot) and 10 pots/treatment. Severity was scored on scale from 1 to 9 based on the percentage of leaf damage. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of the mean values. Treatments are including BJ inoc-FV soil (seeds inoculated with BJ and planted in FV-infested soil); FV inoc-BJ soil (seeds inoculated with FV and planted in BJ-infested soil); and FV soil (non-treated seeds planted in FV-infested soil) as control treatment. An asterisk (*) above a bar indicates treatment was significantly different from control treatment ($P \leq 0.05$). Two asterisks (**) indicate significantly different at $P < 0.0001$

untreated seeds (52.5% at 15 °C and 74% at 25 °C) (Fig. 20.1a). Similarly, in non-germinated treated seeds with BJ, there was a 32.8% reduction in SDS incidence at 15 °C and a 25.9% reduction at 25 °C. The variables of treatment ($P < 0.0001$), as well as the interaction of seed (i.e., seed state at inoculation) and treatment, had significant effects on DI and DS. Except for interaction between seed × treatment level on DI ($P = 0.3721$) and DS ($P = 0.4167$) at 15 °C. Seed treated with BJ

germinated in FV-infested soil reduced 1.38; 1.3- and 1.58-point DS at 15 °C, 20 °C, and 25 °C compared with untreated seeds, respectively. Seed treated with BJ on non-germinated reduced 0.5; 0.6- and 1.0-point DS at 15 °C, 20 °C, and 25 °C compared with untreated controls, respectively.

Overall, a significant reduction in DI and DS was observed in germinated seeds inoculated with FV and planted in BJ-infested soil ($P < 0.0001$). There were significant reductions ($P < 0.01$) in SDS development in all three temperatures associated with BJ inoculation compared with non-treated seeds planted in FV-infested soil (Fig. 20.1). However, treatment with BJ did not affect DS on non-germinated seeds at 15 °C ($P = 0.3505$) and 25 °C ($P = 0.3116$). Non-treated seeds planted in BJ-infested soil did not show any SDS foliar symptoms (data not shown).

20.3.1.1 Nodules Count

Main effects of seed germination state, treatment, and interaction between seed × treatment were not detected on total nodule number at 15 °C and 20 °C, but were at 25 °C (treatment ($P = 0.0190$) and seed state ($P = 0.0333$) (Fig. 20.2a–c)). Unlike total nodule number, several nodules on primary roots at all three temperatures

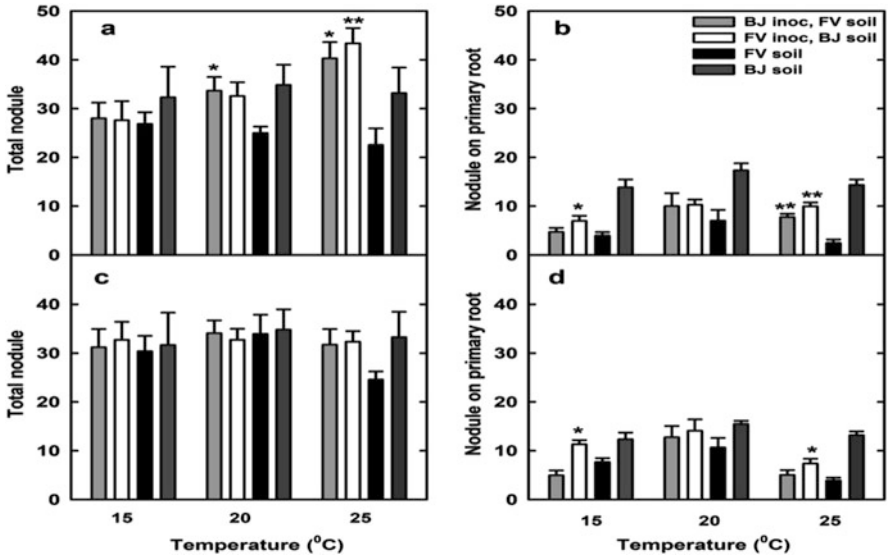


Fig. 20.2 Effects of *Bradyrhizobium japonicum* seed treatments under different temperatures on total nodule and nodule count on primary root per plant at V4 stage in greenhouse conditions. Treatments were applied on germinated (**a**, **b**) or non-germinated (**c**, **d**) seeds in experiment with 2 runs (2014, 2015). Fifteen plants in 3 pots per treatment were randomly collected, washed, and air-dried to count nodule on primary root and total nodule. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of the mean values. An asterisk (*) above a bar indicates mean value was significantly different from mean value of water-treated seed planted in FV-infested soil ($P \leq 0.05$). Two asterisks (**) indicate significantly different at $P < 0.0001$

showed differences ($P = 0.05$) by treatment level and seed status. In non-germinated BJ-inoculated seed planted in FV-infested soil, the number of nodules on primary roots produced were not different from non-treated seeds planted in FV-infested soil at 15 °C and 20 °C but significantly higher at 25 °C ($P < 0.0001$) (Fig. 20.2b). In contrast, on germinated seeds, this number was not significant (Fig. 20.2d).

Generally, the presence of BJ increased nodules on pre-germinated seeds planted in FV-infested soil more than that of non-germinated seeds. Recorded the mean number of total nodules (in three randomly sampled pots) lowest from water-treated germinated seeds planted in FV-infested soil at 25 °C. In contrast, recorded the highest number of FV-treated seeds planted in BJ soil at 25 °C (Fig. 20.2). Found difference between 2 years on nodule counts on the primary root (Supplemental Figs. 20.S1 and 20.S2).

20.3.2 Efficacy of ISU-BJ Strain Seed Treatments on Soybean Sudden Death Syndrome in 2016

Disease incidence and severity: There was no significant effect between two runs on DI at V1, V3, and R1 growth stages when data were analyzed separately by the temperature at 15 °C and 20 °C, but a significant difference was observed on DI at 25 °C. However, it did not detect two-way interactions between run × seed and three-way interactions of run × seed × treatment. There were no significant effects of seed germination on DI except for DI at V1 and V3 at 25 °C ($P = 0.0457$ and $P = 0.0144$, respectively). Detected treatment and interactions between treatment and seed germination state at $P < 0.001$. Generally, at growth stage V3, BJ treatment of germinated seeds reduced DI significantly at the lowest temperatures ($P < 0.001$). Non-germinated seeds inoculated with FV and planted in BJ-infested soil had the lowest DI in all three temperatures (Fig. 20.3). On average, at V3 growth stage, seed treatment with BJ reduced DI significantly by 32% at 15 °C and only dropped by 5.3% at 25 °C compared with the untreated seed planted in FV soil (81.2% at 15 °C and 67.5% at 25 °C) (Fig. 20.3b). Similarly, in non-germinated treated seeds with BJ, there was a 16.4% reduction in SDS incidence at 15 °C and a 22.1% reduction at 25 °C (Fig. 20.3e). Non-germinated seed treated with BJ had significantly lower DI at all stages in all three temperatures (Figs. 20.3d–f) except at the V1 stage at 25 °C (Fig. 20.3e). BJ treated without a microbial carrier (bioAPT) on germinated seed was not statistically different from treatment with the microbial carrier at 15 °C and 25 °C. However, non-germinated seeds treated with BJ + bioAPT reduced DI significantly at all temperatures compared with BJ-inoculated seeds without the carrier.

Overall, foliar severity at different growth stages reduced significantly with BJ inoculated on germinated or non-germinated seeds compared with a water-treated seed planted in FV soil except at V1 at 20 °C and 25 °C (Fig. 20.4). Germinated seeds treated with BJ had a significant effect on DS reduction only at 15 °C ($P \leq$

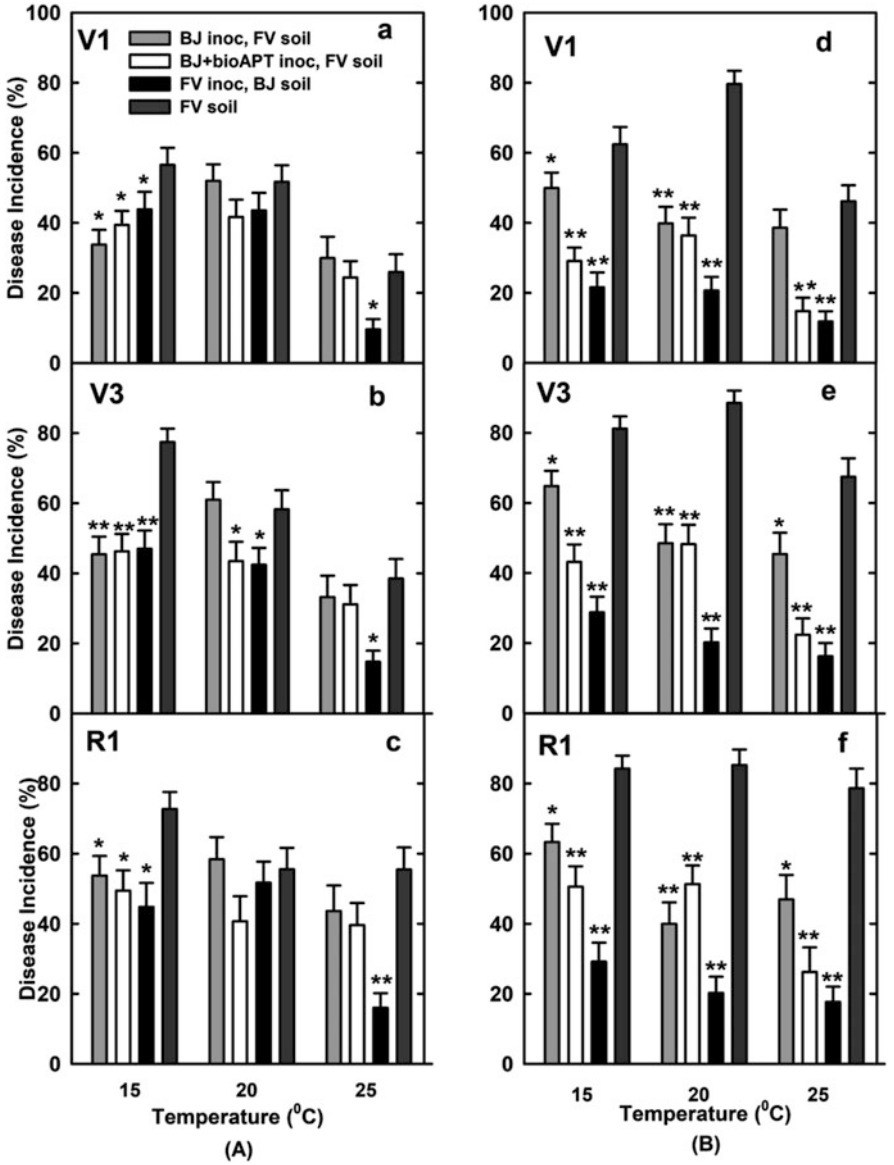


Fig. 20.3 Efficacy tests of seed treatments with ISU strain of *Bradyrhizobium japonicum* on soybean sudden death syndrome incidence under different temperatures in experiment in 2016 (combined data from two independent runs). Germinated (**a**) or non-germinated (**b**) seeds were treated with BJ or BJ plus bioAPT or FV. Disease incidence (%) was taken at V1, V3, and R1 growth stages. Incidence (%) was calculated based on the percentage of plants in cup showed visible foliar symptoms. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of mean values. An asterisk (*) indicates mean of treatment differed from mean of water-treated seed planted in FV-infested soil (control treatment) at $P \leq 0.05$, while two asterisks (**) indicate differences at $P < 0.0001$

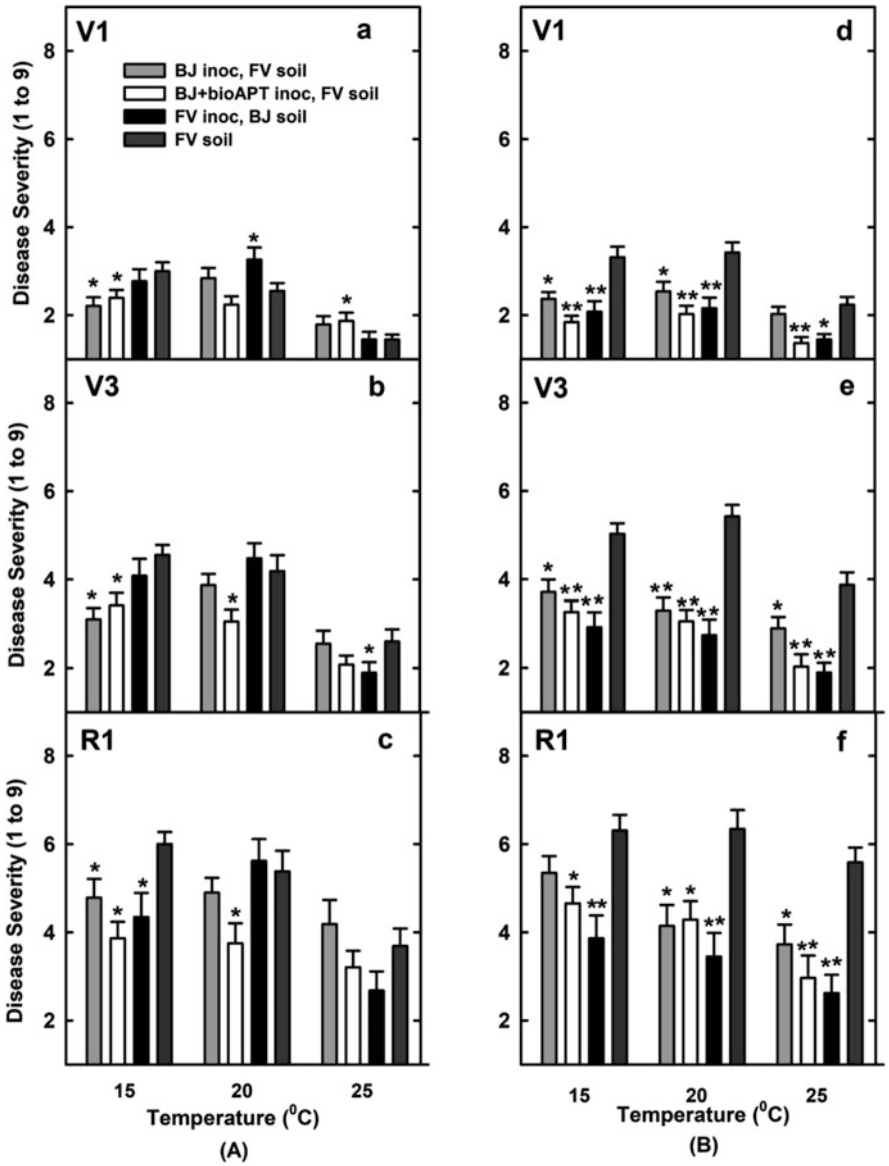


Fig. 20.4 Efficacy tests of seed treatments with ISU strain of *Bradyrhizobium japonicum* on soybean sudden death syndrome foliar severity under different temperatures in experiment in 2016 (combined data from two independent runs). Germinated (**a**–**c**) or non-germinated (**d**–**f**) seeds were treated with BJ or BJ plus bioAPT or FV. Severity was taken at V1, V3, and R1 growth stages. Incidence was assessed on scale from 1 to 9 based on percentage of leaf damage in each cup. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of mean values. Asterisks indicate: ** significance at $P < 0.0001$; * significance at $P \leq 0.05$

0.05) but not at 20 °C and 25 °C (Fig. 20.4a–c). Whereas in non-germinated seeds treated with BJ, DS decreased significantly compared with the non-treated seed at all three temperatures (Fig. 20.4d–f) (0.5, 0.58, and 1.03-point decrement on DS at 15 °C, 20 °C, and 25 °C, respectively). When compared between BJ only treat seeds and treatment with BJ treated together with the carrier, there were significant differences on both seed germination states ($P \leq 0.05$). DS was lower on non-germinated seed treated with BJ + bioAPT than BJ alone (Fig. 20.4d–f). Detected differences for DS between the two runs. Results for each run were presented in Supplemental Figs. 20.S3 and 20.S4.

20.3.2.1 Nodules Count and Plant Biomass

There were no significant effects of seed status on the total nodule number variable. However, detected significant effects of seed status on the biomass (fresh shoot, fresh root, dry shoot, and dry root weights) only in seeds planted at 25 °C ($P \leq 0.05$). Those weights were larger on germinated seeds than non-germinated seeds. Treatment had significant effects on nodule counts and plant biomass ($P \leq 0.05$) except dry root weights at 20 °C and 25 °C. There were no significant interactions between treatment level and seed status on nodule number and plant biomass (Fig. 20.4). There were significantly higher nodules formed on plants from non-germinated BJ treated seed at 20 °C than the plant from non-treated sources at the same planting condition ($P = 0.0231$) (Fig. 20.4e).

20.4 Discussion

Experiments from 2014 to 2016 were to test seed treatments with BJ under different soil temperatures on soybean sudden death syndrome occurrence on the development of nodules and plant biomass. We believe this is the first report of the effects of BJ treatments on SDS occurrence under different temperature regimes. Results of this study confirm for the first time in greenhouse conditions that BJ inoculation affected SDS foliar symptoms expressions as measured based on DI or DS. This study also seeks to answer how farmers can balance yield with disease control when early planting is optimal for both (Fig. 20.5).

ISU-BJ strain used in this study was isolated from nodules collected from Iowa in 2013. In a previous study in 2013, plants with lower SDS severities showed greater nodules per plant than plants with higher SDS severities in greenhouse conditions. We collected nodules from plants that had survived with light SDS symptoms at growth stage R1. Subsequently, an isolate of BJ was identified based on the 16S rRNA sequence, and results showed that the strain has 99% identity identical with USDA 6. In this study, we used this strain to test our hypothesis. It was challenging to interpret the differences among replicates; however, our data showed that seeds treated with BJ or infested in soil seedling stages suppressed SDS (Fig. 20.6).

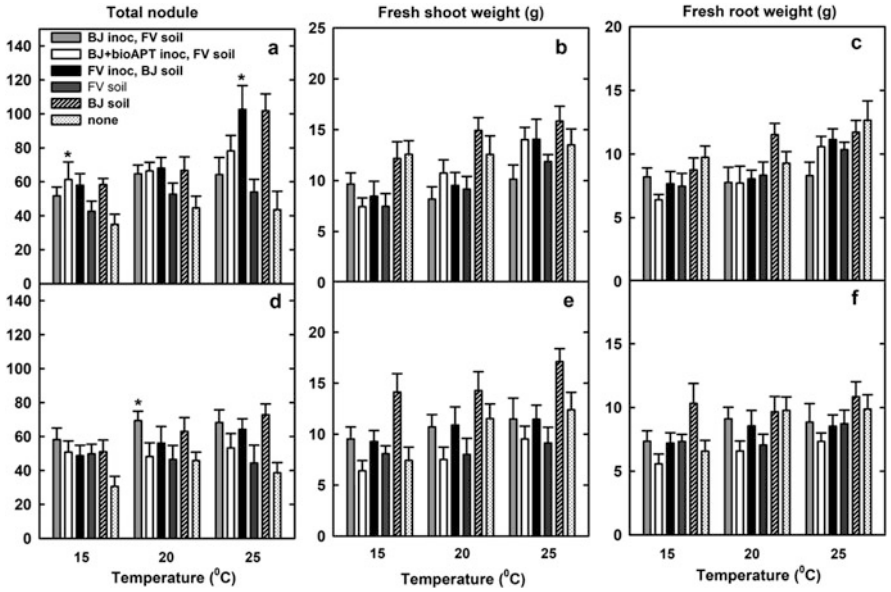


Fig. 20.5 Effects of seed treatments with ISU-BJ strain on total nodule count, fresh shoot, and fresh root weights (gram) at three different temperatures in experiment March 2016. Treatments were applied on germinated (**a**–**c**) or non-germinated (**d**–**f**) seeds. Samples were taken at growth stage V4. Means were calculated based on Fisher's protected least significance difference. Asterisk (*) indicates significant differences ($P \leq 0.05$) between BJ treatment with non-treated seeds planted in FV soil

In this study, treatments were either designed to create conditions favorable for initial colonization of soybean roots by BJ or by FV via addition to seeds and subsequent exposure to soils infested with FV or BJ. Soybean seeds were either pre-germinated or not to evaluate possible scenarios for initial root colonization by BJ or FV. Pre-germinated seeds were exposed to a higher initial BJ or FV inoculum (by increased seed surface area and the presence on radicles). In contrast, non-germinated seeds were exposed to lower initial concentrations of BJ or FV inoculum. It can explain that pre-germinated seeds were overwhelmed by higher exposure to FV inoculum and exudates from young roots. BJ treatment on germinated seed performed their efficiency differently and unpredictably.

At each temperature, treatments with BJ inoculum reduced SDS occurrence compared with untreated controls. Treatments with FV-inoculated seed planted in BJ-infested soil showed the best results in controlling SDS at all three temperatures. This can result from a higher initial BJ inoculum level added in the soil than the level used to treat directly on the seed. Mainly, clay pots used in experiments of 2014 and 2015 had better BJ efficiency of BJ-infested soil on SDS than foam cups used in 2016. Additionally, BJ treatments showed a reduction of SDS at 25 °C compared to the other two temperatures when planted in foam cups (Fig. 20.3). This can be explained by the different conditions when plants grow in greenhouse conditions, for

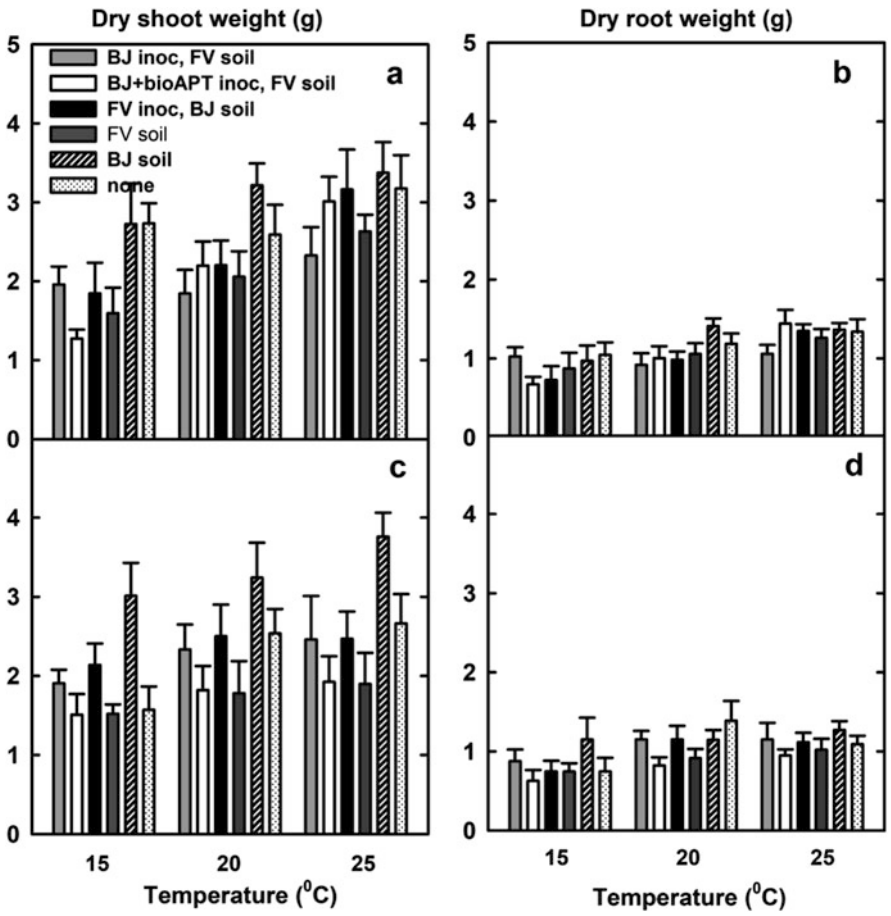


Fig. 20.6 Effects of seed treatments with ISU-BJ strain on dry shoot and dry root weights (gram) at three different temperatures in experiment March 2016. Treatments were applied on germinated (**a**, **b**) or non-germinated (**c**, **d**) seeds. Samples were taken at growth stage V4. Means were calculated based on Fisher's protected least significance difference

example, light, temperature, or type of pot. This would lead to a change in the performance of BJ strains which in turn results in nodulation and plant biomass differences between runs as the difference of each biological replication. Although different growth chambers selected to set up temperature for planting and shifting pots/cups from growth chambers to greenhouse bench may have changed the performance of BJ treatments in experimental replications. The reduction of SDS occurrence in treatment with BJ in experiments confirmed that BJ could act against SDS pathogen.

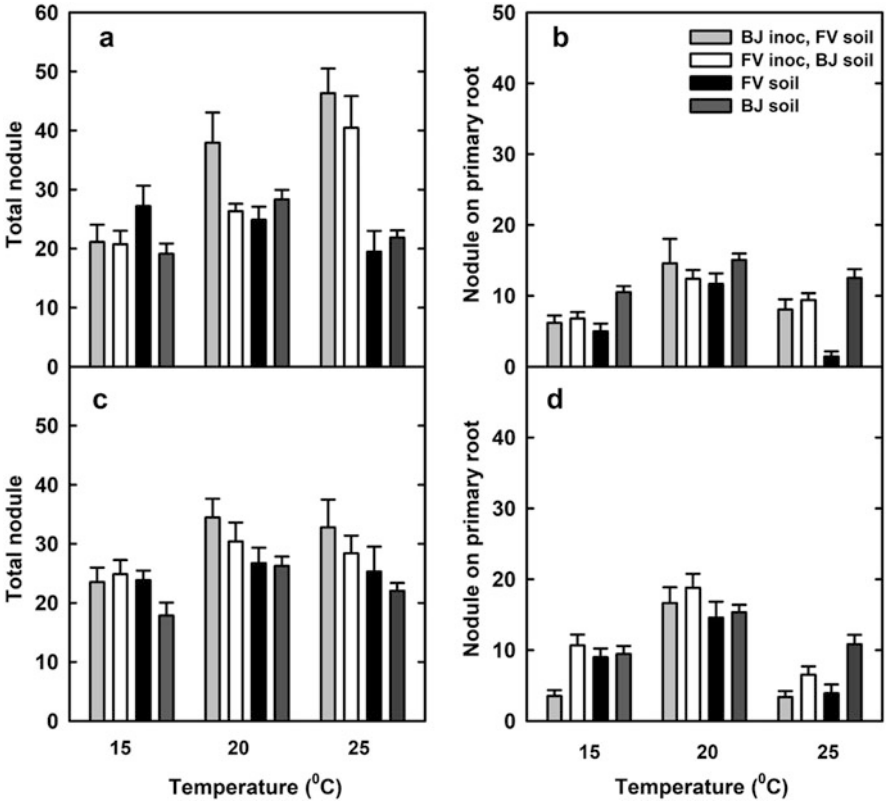
Planting soybean in cool, wet soils in an early-season increases the risk of SDS (Scherm and Yang 1996; Roy et al. 1997; Leandro et al. 2013), suggesting that young roots in such conditions are most susceptible to FV infection. Our findings

showed SDS incidence and severity were reduced significantly when BJ was treated on seeds or adding in the soil at lower soil temperature, which favors FV infection. The findings suggested that BJ infection can happen before FV or at the same time with FV infection. In addition, our observations indicated that better nodulations in BJ treatments could be the main effects on promoting plant health, nitrogen fixation, and increase metabolisms, including secondary metabolites that can trigger plant defense to SDS infection. This was drawn based on the higher number of nodules in seed treated with BJ or BJ amended in soil, compared with the untreated controls planted in FV-infested soils. Also, the number of nodules produced on primary roots assessed in 2014 and 2015 experiments was significantly different on BJ seed treatments. Other mechanisms may be involved in the suppression of fungal pathogens by potential *Bradyrhizobium* strains. These mechanisms may include the production of toxic metabolites such as rhizobitoxine (Chakraborty and Purkayastha 1984), siderophore (Nambiar and Sivaramakrishnan 1987; Guerinot et al. 1990), hydrogen cyanide (Antoun et al. 1998), and plant growth promotion (Siddiqui and Mahmood 1995). BJ has been used as plant growth-promoting rhizobacteria (PGPR) on many row crops and vegetables (Carletti et al. 1994; Antoun et al. 1998; Biswas et al. 2000). These studies indicated that growth physiology and root morphology were the main causes of higher yields and increased dry matters than biological nitrogen fixation. A 2-year study conducted by Zhang et al. (2003) also indicated that using low-temperature tolerant *B. japonicum* inoculant could improve nodulation and nitrogen fixation of soybean plants in a cool growing season. It has been suggested that induced systemic resistance (ISR) that results from a long-distance signaling mechanism causes the colonization density of the symbionts.

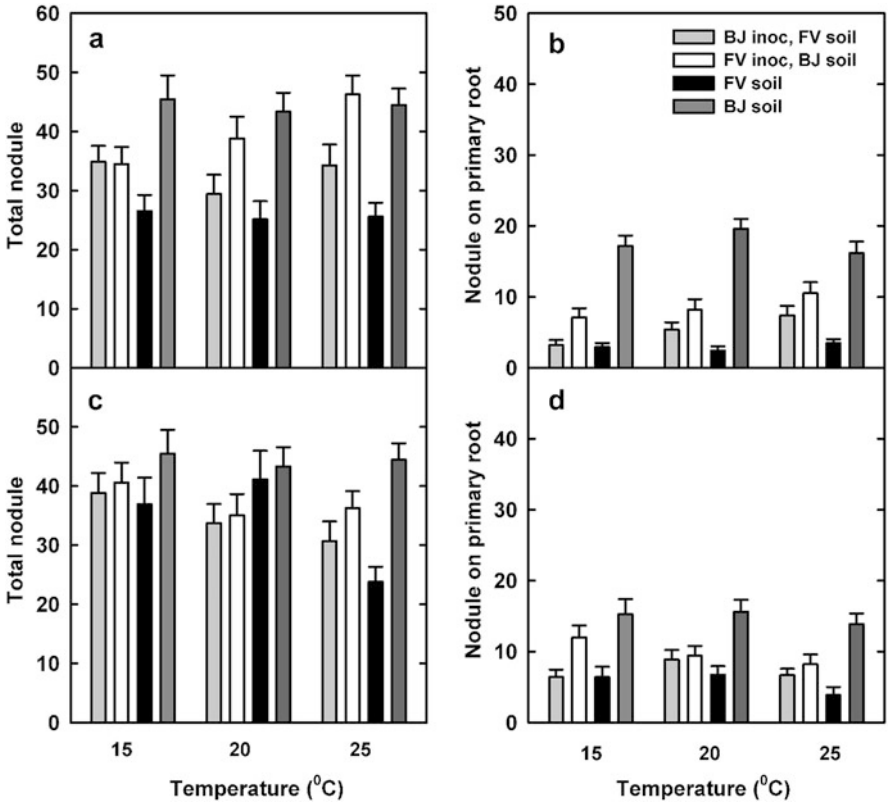
In summary, our results showed that BJ incorporation as a seed treatment or soil inoculant could suppress SDS on early-planted soybean. Our results confirmed the findings by Gongora-Canul and Leandro (2011) that foliar severity is more severe when FV is inoculated on early plant age. The present study results have indicated that it is vital to introduce BJ initial populations by seed treatments or soil inoculant in a short growing season in Iowa to reduce SDS occurrence. A limitation of this study is that the effects of BJ seed treatment against SDS were evaluated on foliar disease incidence and severity. Future work should investigate root rot severity which can be measured under controlled conditions.

Acknowledgments The authors thank Iowa Soybean Association, Iowa (USA), and Vietnam International Education Development (Vietnam) for funding this research. We thank Huaiqing Wu, Department of Statistics, and Sharon Eggenberger, Department of Plant Pathology and Microbiology, for their technical assistance in data analysis.

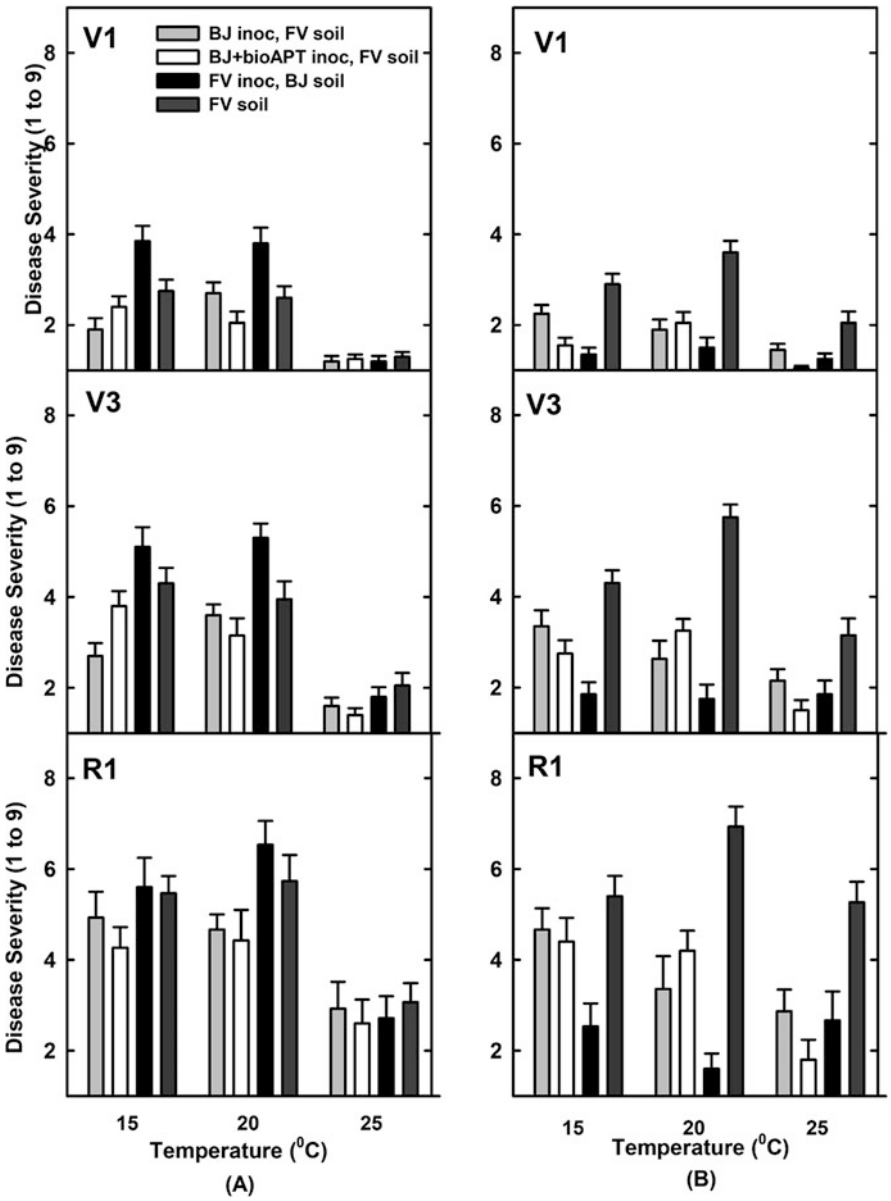
Appendix



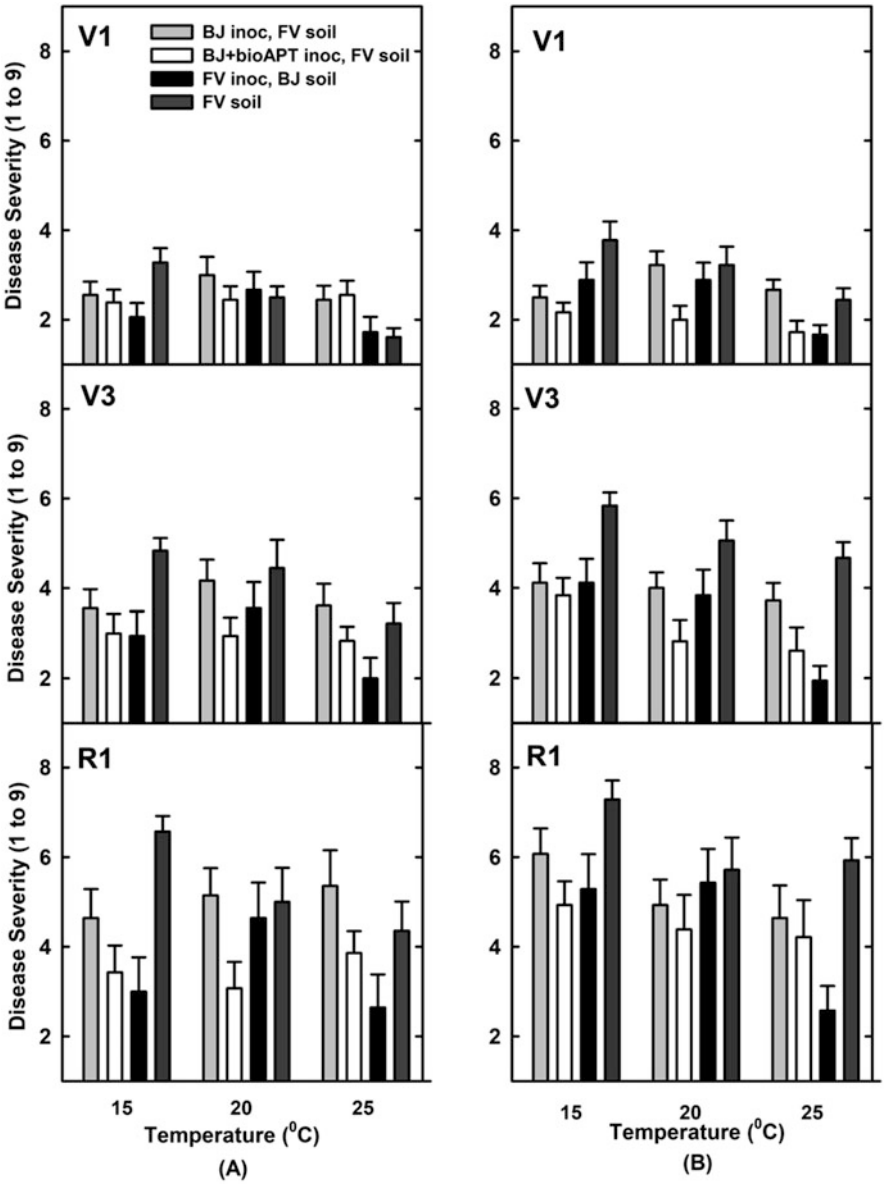
Supplemental Fig. 20.S1 Effects of *Bradyrhizobium japonicum* seed treatments under different temperatures on total nodule and nodule count on primary root at V4 stage in greenhouse conditions. Seeds were treated after germination (**a**, **b**) or before germination (**c**, **d**) in experiment in 2014 (run 1). Fifteen plants in 3 pots per treatment were randomly collected, washed and air-dried to count nodule on primary root. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of the mean values



Supplemental Fig. 20.S2 Effects of *Bradyrhizobium japonicum* seed treatments under different temperatures on total nodule and nodule count on primary root at V4 stage in greenhouse conditions. Seeds were treated after germination (**a**, **b**) or before germination (**c**, **d**) in experiment 2015 (run 2). Fifteen plants in 3 pots per treatment were randomly collected, washed and air-dried to count nodule on primary root. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of the mean values



Supplemental Fig. 20.S3 Effect of seed treatments with ISU-BJ strain on disease severity under different temperature in experiment in March 2016 (run 1). Germinated (**a**) or non-germinated (**b**) seeds were treated either with BJ, BJ plus bioAPT or FV. Severity was scored on scale from 1 to 9 based on the percentage of leaf damage. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of mean values



Supplemental Fig. 20.S4 Effect of seed treatments with ISU-BJ strain on disease severity under different temperature in experiment in March 2016 (run 2). Germinated (**a**) or non-germinated (**b**) seeds were treated either with BJ, BJ plus bioAPT or FV. Severity was scored on scale from 1 to 9 based on the percentage of leaf damage. Means were calculated based on Fisher's protected least significance difference. Error bars are standard errors of mean values

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Biocontrol Potential of *Bradyrhizobium japonicum* Against Soybean Sudden Death Syndrome in Irrigated and Non-irrigated Fields

21

Tra Huynh, Shrishail S. Navi, and X. B. Yang

Abstract

Studies were conducted from 2015 to 2017 to investigate the efficacy of *Bradyrhizobium japonicum* (BJ) seed treatments on soybean sudden death syndrome (SDS) caused by *Fusarium virguliforme* grain yields. Commercially untreated soybean seeds were treated separately with BJ strains such as ISU-BJ and USDA 110 in 2015 and USDA 30, USDA 31, and USDA 110, either alone or combined with microbial carrier bioAPT or commercial plant protectant Heads Up in 2016 and 2017. Trials were set up in randomized complete block designs in four replications with 3 m wide × 5.3 m long plots at Hinds Research Farm, Iowa State University, Ames, Iowa. Plots were evaluated for stand counts, SDS incidence (%), severity (%), and foliar disease index (FDX), and grain yields. At the R6 growth stage, BJ-treated plots showed lower FDX than untreated plots and an average yield increase of 4.4% in irrigated fields and 0.8% in non-irrigated fields compared with untreated plots. Also, higher nodule counts and shoot and root weights per plant at V5 were observed in BJ-treated plots than in untreated

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_21

plots in field conditions. The findings indicated that BJ seed treatments could be one of the management approaches to control SDS.

Keywords

Fusarium virguliforme · *Bradyrhizobium japonicum* · Soybean · Seed treatments · Irrigation

21.1 Introduction

Soybean [*Glycine max* (L.) Merr.] is an important oilseed crop in the USA. Soybean sudden death syndrome (SDS) is one of the most significant soil-borne diseases in the USA and several other countries in the world. In North America, SDS is caused by *F. virguliforme* (Aoki et al. 2003), in South America, *F. virguliforme*, *F. tucumaniae*, *F. brasiliense*, *F. cuneirostrum* (Aoki et al. 2005), and *F. crassistipitatum* (Aoki et al. 2012), and in South Africa, *F. virguliforme* (Tewoldemedhin et al. 2014) and *F. brasiliense* (Tewoldemedhi et al. 2017). Navi and Yang (2016c) have compiled different species of *Fusarium* causing SDS in different parts of the world.

Infection of *F. virguliforme* (FV) is favored by cool, wet soil conditions (Scherm and Yang 1996), prevalent in early planted soybeans. A phytotoxin is produced after fungal colonization and translocated in the plant through xylem vessels (Navi and Yang 2008). Soybean infected with SDS show symptoms during the seedling stage and with the most dramatic symptoms in reproductive growth stages (Navi and Yang 2016c). Chlamydospores, the survival structure of this fungus, can withstand wide soil temperature fluctuations and resist desiccation (Roy et al. 1989). Macro-conidia, the primary type of asexual spores of this fungal genus, can be rain-splashed, scattered by water runoff, or dispersed by air during harvest, potentially resulting in the external infestation of seeds that can contribute to dissemination (Roy 1997). The SDS has been reported in 21 soybean-producing states in the USA and the corresponding economic losses from $15.7 million in 1988 to $669.2 million in 2010 (Navi and Yang 2016c). In a review, several management options of SDS have been compiled, including screening techniques for resistance screening (Navi and Yang 2016c).

There are a few reports of using *Bradyrhizobium* spp. in the management of row crop diseases (Tu 1978; Chakraborty and Purkayastha 1984; Buonassisi et al. 1986) considering it as a plant growth-promoting rhizobacteria (Jordan 1982; Kuykendall et al. 1992; Carletti et al. 1994; Antoun et al. 1998; Biswas et al. 2000). Studies conducted in the controlled environments on the effects of seed treatments with *Bradyrhizobium* strains showed suppression of SDS incidence and severity (Huynh et al. 2016, 2021). Therefore, in this article, results of field tests of BJ as a potential biocontrol agent to suppress SDS in irrigated and non-irrigated field conditions are provided. Also, field studies would complement the central hypothesis tested in the greenhouse (Huynh et al. 2016, 2021) that early infection of BJ can enhance plant health and suppress early-stage SDS infection.

21.2 Materials and Methods

21.2.1 Soybean Cultivars, Isolates of Bradyrhizobium and Fusarium

Commercially untreated roundup ready soybean varieties P22T61RR and P22T73RR with moderately resistant SDS ratings were procured from DuPont Pioneer, Johnston, IA 50131-0184. *Bradyrhizobium japonicum* (BJ) strain from Iowa was obtained from Dr. Xun Li and tested BJ strain USDA 110 obtained from Dr. Gwyn Beattie, Iowa State University, in 2015 field tests. In 2016 and 2017, in addition to the above strains, USDA 30 and USDA 31 obtained from USDA-ARS National Rhizobium Germplasm Resource Collection, Beltsville, Maryland, USA, were tested (Table 21.1). All BJ strains were maintained on Yeast Mannitol Agar (YMA) and were sub-cultured on yeast mannitol broth (YMB) as described by Vincent (1970). BJ strains were grown in flasks (250 mL) on VWR DS-500E Orbital Shaker at 125 rpm at 25 °C for 7 days. Later, the culture was diluted with sterile yeast mannitol broth to an OD620 of 0.08, equivalent to about 108 cells mL^{-1} (Steel and Torrie 1980). *Fusarium virguliforme* (FV) isolate Fsg-ISU1 (Mbofung et al. 2012) originated from an Iowa field that was sub-cultured on Potato Dextrose Agar (PDA) Petri dishes supplemented with 100 mg/L streptomycin sulfate to suppress bacterial growth. *The* FV inoculum was fermented on white sorghum grains following the

Table 21.1 Experiment descriptions, field number, planting and harvesting, and SDS scoring dates at ISU Hinds Research Farm, Ames, IA

Year/ field	Date planted	Date harvested	Irrigation[a]	Soil temp[b] (°C)	Seed treatments
2015					
					1. ISU-BJ[c]
Field 2	4 May	23 September	No	17.2	2. USDA 110[c]
Field 3	4 May	26 September	Yes	17.2	3. Positive control[d]
2016					
					1. USDA 30[c]
Field 1	16 May	29 September	No	12.7	2. USDA 31[c]
Field 5	14 May	29 September	Yes	11.5	3. Heads Up[c]
					4. USDA 30 + Heads Up[c]
					5. USDA 31+ Heads Up[c]
2017					
Field 2	9 May	19 October	No	15.8	6. USDA 110[c]
Field 3	9 May	19 October	Yes	15.6	7. Negative control[e]
					8. Positive control[d]

[a]Overhead impact sprinklers ran from growth stage R1–R6 to provide 2.5 cm^3/week
[b]Soil temperature (10 cm depth) recorded at planting
[c]Treated seeds planted with FV inoculated sorghum grain
[d]Untreated seeds planted together with FV inoculated sorghum grains
[e]Untreated seeds planted in non-FV sorghum grain

method of Navi and Yang (2016a). The dried inoculum was transferred to brown paper bags and stored at 4 °C for field studies.

21.2.2 Effects of *Bradyrhizobium japonicum* Seed Treatments on SDS in 2015

In two studies, each with three treatments was set up in a randomized complete block design (RCBD) with four replications at the Hinds Research Farm, Ames, Iowa (42°N latitude, 93.6° W longitude). Individual plot sizes were 3-m wide × 5.3 m long with 76 cm row spacing and an alleyway of 0.8 m between plots. Seeds of P22T61RR were treated separately with ISU-BJ and USDA 110 strains at 10 mL/kg seed (108 CFU mL^{-1} concentration); YMB. The treated and untreated control seed (at 700 per plot or 10 seed per linear plot) and FV inoculum (at 4cc per linear foot) per plot were planted together using ALMACO 4-Row SeedPro Precision Vacuum planter. In one trial, plots were irrigated on non-rainy days using overhead impact sprinklers from R1 to R6 to provide a total of 2.5 cm/week or 6-h/week, while other trials were not irrigated.

21.2.3 Effect of *Bradyrhizobium japonicum* Seed Treatments on SDS in 2016 and 2017

Similar to studies in 2015, two seed treatments trials, each with eight treatments, were set up in an RCBD at Hinds Research Farm, Ames, IA. Plot dimensions, seed rates, the concentration of BJ strains, FV inoculum, planter, and irrigation were similar to that of 2015 tests, except the variety P22T73RR (Table 21.1). Seed of P22T73RR was treated separately as follows: (1) USDA 30 at 10 mL/kg seed (108 CFU mL^{-1}) mixed simultaneously with bioAPT at 10 g/kg, (2) USDA 31 at 10 ml/kg (108 CFU mL^{-1}) together with bioAPT at 10 g/kg, (3) Heads Up® (Heads Up Plant Protectant Inc., 3002 Millar Avenue, Saskatoon, SK, Canada) alone 5 ml/kg (application rate at 1 g mixed in 1 L water for 163.3 kg seed), (4) USDA 30 at 5 ml/kg (108 CFU mL^{-1}) + Heads Up, (5) USDA 31 at 5 ml/kg (108 CFU mL^{-1}) + Heads Up, (6) USDA 110 at 10 ml/kg (108 CFU mL^{-1}), (7) YMB-treated seeds planted together with autoclaved sorghum grains served as negative control, and (8) YMB-treated seeds planted together with FV inoculum served as a positive control (Table 21.1). In one trial, plots were irrigated on non-rainy days using overhead impact sprinklers from R1 to R6 to provide a total of 2.5 cm/week or 6-h/week, while the other trial was not irrigated.

21.2.4 Data Collection

In each trial, soil temperature (°C), soil moisture at planting, and monthly precipitation were obtained from the weather station through a public service website (https://

mesonet.agron.iastate.edu; https://w2.weather.gov/climate/). Stand counts were recorded 25 days after planting and subsequently at every 10 days until the V3 growth stage. To measure nodule numbers, shoot, and root weights, in 2016 and 2017, five plants were uprooted with a shovel from the center of two rows at the V5 growth stage (Fehr et al. 1971). Rinsed the uprooted plants in low-pressure running tap water and recorded nodule numbers on a plant basis. Subsequently, plants were cut into shoot and root, and fresh and dry weights (g) were recorded.

SDS disease incidence and severity were recorded in each plot both in the V3 growth stage and in reproductive growth stages from R1 to R7 (Njiti et al. 1996). The disease incidence percent (DI) was calculated as the percentage of the SDS symptomatic plants out of the total plants in a plot. The SDS disease index (DX) was calculated as DI × DS/9 (Njiti et al. 1998). At maturity, plots were harvested using an ALMACO Research Plot Combine. Yields were adjusted to 13% grain moisture and measured in bushel per acre. Subsequently, converted yield in bushel/acre to kg/ha.

21.2.5 Statistical Analysis

Results were analyzed by analysis of variance using the Statistical Analysis System (version 9.4, SAS Institute Inc., Cary, NC). Analyzed data for each trial in non-irrigated or irrigated fields separately. Data analysis was performed using the PROC ANOVA procedure. If the model was significant, the least significant difference (LSD) test was applied to compare the means at the 0.05 level of significance (Steel and Torrie 1980). When differences occurred at levels of significance between 0.05 and 0.1, they are noted in the text.

21.3 Results

21.3.1 Effects of *B. japonicum* Seed Treatments on SDS, 2015

The stand count in the BJ-treated plot differed from the untreated plots in the non-irrigated but not in irrigated trial. The DI in V3–V4 growth stages and at R3 was significantly lower in BJ-treated plots than untreated plots in non-irrigated and irrigated trials (Table 21.2). In the R4–R6 growth stages, SDS-DI and SDS-DX were not statistically different among the treatments in both fields. However, at R6, SDS-DI in BJ-treated plots was 24% lower than untreated plots (Table 21.2), and at R6, FDX ratings in BJ-treated plots were lower than untreated plots both in non-irrigated and in irrigated fields (Fig. 21.1). Generally, DX values were higher in plots planted with FV (positive control) than BJ-treated plots in both field trials (Fig. 21.1b). There were no significant differences in grain yields among the treatments (Table 21.2). Grain yields in the non-irrigated trial were lower than in the irrigated trial; this was likely due to severe flooding in the non-irrigated plots toward maturity in 2015.

Table 21.2 Effects of ISU-BJ seed treatments on stand count[a] and sudden death syndrome (SDS) disease incidence in field trials in 2015. Values are means ± standard error of four replications. Means with the same letter within each parameter are not significantly different at $P < 0.05$ according to Fisher's protected least significant difference test

Non-irrigated	Stand count[a]	SDS disease incidence (%)[b]				Yield (kg/ha)
		V3–V4	R3	R4–R4.5	R5–R6	
ISU-BJ	632.6 ± 10.8a	2.45 ± 2.29b	0.00 ± 0.0b	11.43 ± 3.88a	11.75 ± 3.73a	1840 ± 87a
USDA 110	642.0 ± 9.0a	0.00 ± 0.0b	0.00 ± 0.0b	10.31 ± 3.54a	11.76 ± 4.31a	1897 ± 72a
Positive control[c]	648.6 ± 12.6a	8.41 ± 1.15a	2.86 ± 0.6a	29.10 ± 7.86a	36.26 ± 8.67a	1893 ± 53a
Irrigated						
ISU-BJ	610 ± 20.6b	1.36 ± 1.15b	0.33 ± 0.22ab	24.2 ± 7.3a	27.7 ± 6.9a	3218 ± 105a
USDA 110	689 ± 17.7a	0.53 ± 0.18b	0.00 ± 0.0b	35.9 ± 2.4a	36.16 ± 2.2a	3254 ± 112a
Positive control[c]	577.6 ± 18.8b	7.39 ± 1.15a	0.86 ± 0.13a	39.64 ± 1.3a	32.21 ± 1.46a	3185 ± 110a

[a]Stand counts recorded at V3 growth stage. Stand count mean value presented for averaged plot size of four rows with 5.3 m length with 75 cm row spacing

[b]SDS incidence was rated as the percentage of the plants in a plot that show visible foliar symptoms of SDS

[c]Untreated seeds planted together with FV inoculated sorghum grains

21.3.2 Effects of *B. japonicum* Seed Treatments on SDS, 2016 and 2017

No significant differences in stand counts were observed in seeds treated with BJ strains (either alone or in combination with Heads Up) compared with untreated controls either in irrigated or in non-irrigated field trials (Table 21.3). There was a significant effect of seed treatments on DX at different stages in the 2016 field test (Fig. 21.2). DX was higher in the irrigated field (Fig. 21.2b). In the irrigated field, DX at growth stage R4 in BJ-treated plots either alone or with Heads Up plots were from 1% to 3% lower than untreated plots planted with positive control. However, from R6 to R7, BJ-treated plots had 10–15% lower DX than positive control plots. In irrigated conditions, seed treatments with USDA 30 and 31 showed a lower reduction of SDS than USDA 110 and Heads Up. Whereas, in a non-irrigated trial, DX was low (from 0 to 2 points) in which USDA 31 plots showed the lowest DI followed by USDA 30 and their combination with Heads Up. In 2017, we did not observe any SDS development.

In 2016, there were significant differences in nodule counts among treatments in a non-irrigated field ($P = 0.03$). The highest nodule count was recorded in positive control plots followed by USDA 31 and USDA 31 + Heads Up and lowest counts in USDA 110 plots in the irrigated field (Table 21.4). In the irrigated field, detected no significant effects on nodule counts among treatments ($P = 0.26$). The lowest nodule

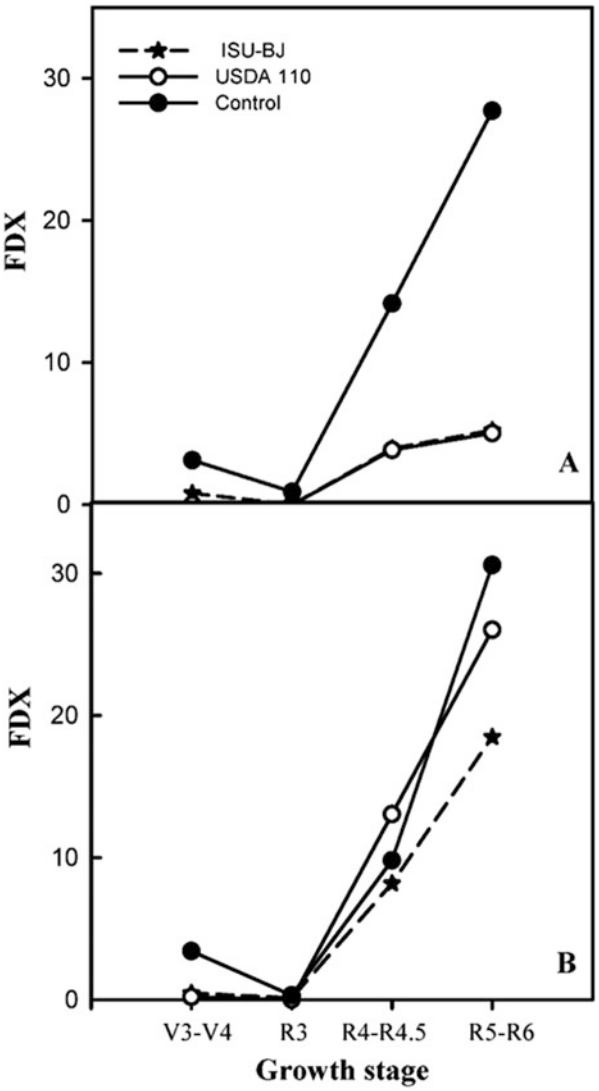

Fig. 21.1 Effects of seed treatments with two *Bradyrhizobium japonicum* strains on foliar soybean sudden death syndrome disease index in (**a**) natural and (**b**) irrigated fields in 2015. Foliar disease index (FDX) was calculated using the formula FDX = DI × DS/9, where DI as percentage of diseased plants in plot, DS as visual estimation of leaf damage level in a plot scaled from 1 to 9

count was accounted for Heads Up seed treatments followed by USDA 110 (Table 21.4). Overall, there were no consistent differences in nodule number among treatments across years or plots with distinct irrigation statuses. Fresh and dry weights (g) of roots and shoots also were recorded in 2016 and 2017. Generally, there was no significant effect of treatments on plant biomass ($P > 0.05$) either in irrigated or in non-irrigated fields (Tables 21.5 and 21.6; Supplemental Tables 21.S1 and 21.S2).

In non-irrigated fields, yield responses showed differences across years. In 2016, Heads Up seed treatments produced the highest yield (4494 kg/ha) followed by

Table 21.3 Effects of seed treatments on stand counts measured at V3 growth stages for field experiments in 2016 and 2017 at ISU Hinds Farm, Ames, IA

	2016		2017	
Treatments	Non-irrigated	Irrigated	Non-irrigated	Irrigated
USDA 30	639.75 ± 12.3a	613 ± 23.9a	639.25 ± 61.5a	548 ± 19.7a
USDA 31	647.5 ± 24.7a	632.5 ± 30.4a	591.75 ± 46.7ab	485.25 ± 27.3b
Heads Up[a] (HU)	689.75 ± 25.5a	630.5 ± 27.1a	657 ± 54.2a	555.75 ± 16.9a
USDA 30+HU	658.25 ± 27.7a	673.5 ± 14.6a	582.25 ± 39.6ab	551.5 ± 19.1a
USDA 31+HU	661.5 ± 27.2a	643 ± 34.3a	521.5 ± 87b	506 ± 23.6ab
USDA 110	620.75 ± 39.9a	659 ± 22.8a	579 ± 47ab	510.25 ± 26.2ab
Negative control	637.5 ± 25.7a	633.5 ± 47.8a	584 ± 57.8ab	533.25 ± 12.1ab
Positive control	601.5 ± 32.9a	623.5 ± 28.1a	603.5 ± 35.5ab	524.5 ± 13.7ab

Note: Values are means and standard errors of 4 replicates (plots). Means within each column followed by same letter are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference

[a]Commercial product originated from plant extracts treated seeds at application rate of 600 mL/100 kg seed

USDA 31 (4461 kg/ha) and negative control (4401 kg/ha) (Table 21.7), while USDA 110 had the lowest yield (4230 kg/ha). Treatments with BJ had a yield reduction of 1.5% compared with controls. Conversely, in irrigated fields, average yields in plots with USDA-BJ seed treatments were greater by 4.3% compared with controls. Seed treatments with USDA 30 and 31 significantly affected increased grain yields compared with the positive control ($P = 0.01$). However, negative control treatments showed the lowest yields (3813 kg/ha) (Table 21.7). In 2017, although there was no significant effect of treatments on yields at $P = 0.05$ among treatments, an average yield increase of 3% and 7.3 % was recorded in USDA-BJ treatments compared with control treatments either in non-irrigated and irrigated fields, respectively. Seed treatments with BJ combined with Heads Up did not increase yields compared with BJ alone. In 2016 and 2017, BJ-treated seeds increased yields by 1.5% and 4.95% compared with BJ + Heads Up treatments in non-irrigated and irrigated fields, respectively.

21.3.3 Weather Data

Precipitations during growing seasons from May 1 to October 1 were different in 2015, 2016, and 2017. In 2015, it received higher rainfall than in 2016 and 2017 (43% higher than 21.42 mm average over 30 years). While in 2016, 27% more, and in 2017, 24% less than the 30-year average. Soil temperatures at planting dates in three years ranged from 11.50 °C to 17.50 °C (Fig. 21.3).

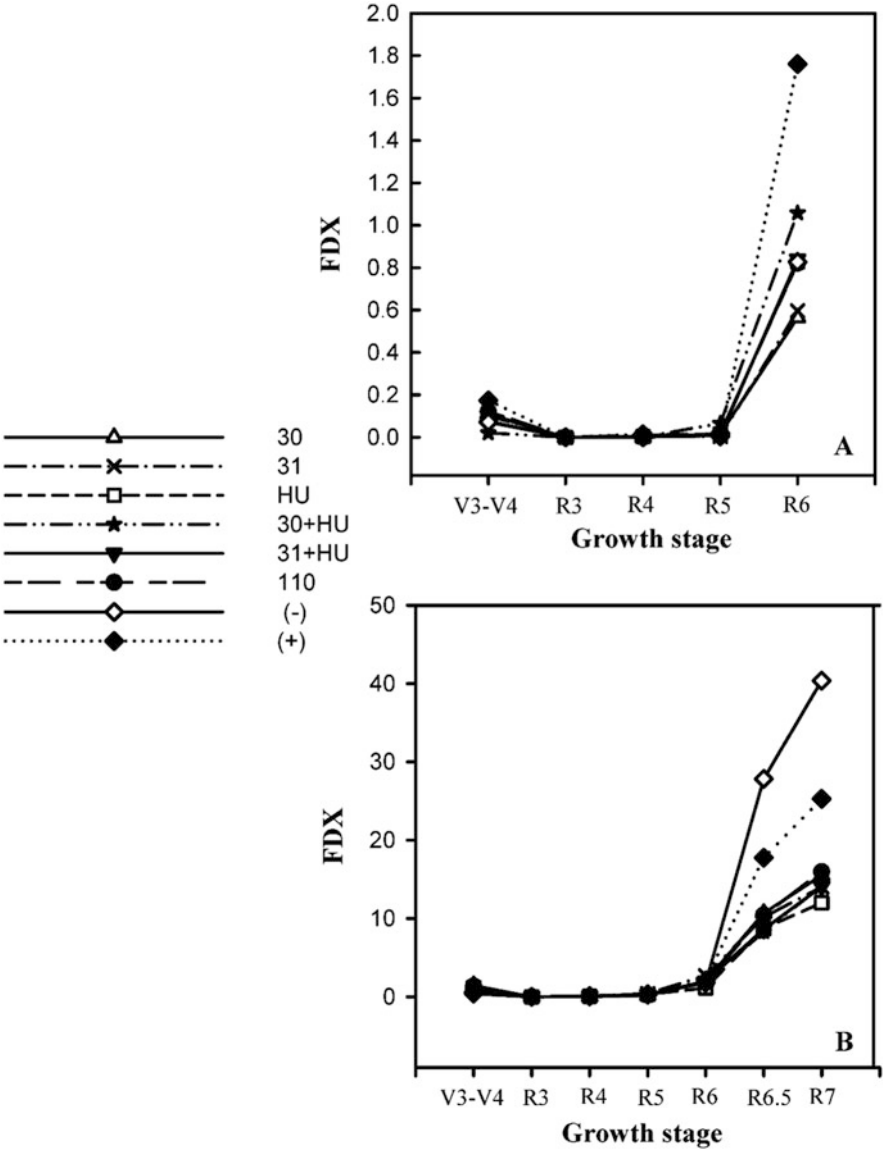

Fig. 21.2 Effects of seed treatments with two *Bradyrhizobium japonicum* strains on foliar disease index in natural (**a**) and irrigated (**b**) fields in 2016. Soybean growth stages were recorded differently in each field. Foliar disease index (FDX) was calculated using the formula FDX = DI × DS/9. (–): is negative control treatment, (+): positive control treatment

Table 21.4 Effect of USDA strains and heads up seed treatments on total nodule count per plant under field condition at Hinds Farm, Ames, IA in 2016 (A) and 2017 (B)

	2016		2017	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated
USDA 30	32.7 ± 4.01a	27.6 ± 2.97ab	19.7 ± 2.98b	27.5 ± 3.63a
USDA 31	28.5 ± 1.68abc	27.4 ± 2.43ab	18.5 ± 1.94b	22.8 ± 2.13a
Heads Up (HU)	29.6 ± 3.72ab	20.7 ± 1.67b	21.7 ± 3.21ab	26.4 ± 2.38a
USDA 30+HU	31.2 ± 2.51a	27.7 ± 4.27ab	23.3 ± 2.63ab	24.4 ± 2.25a
USDA 31+HU	25.4 ± 3.88abc	29.7 ± 2.19ab	27.9 ± 3.11a	24.0 ± 2.34a
USDA 110	21.3 ± 2.31bc	22.8 ± 2.5ab	22.4 ± 2.42ab	24.9 ± 1.87a
Negative control	20.4 ± 2.35c	29.9 ± 4.61a	21.1 ± 2.10ab	26.7 ± 2.17a
Positive control	21.5 ± 2.37bc	31.6 ± 3.9a	23.8 ± 2.05ab	24.5 ± 2.35a
P value	0.03	0.26	0.3	0.91

Note: Values are means and standard errors of 10 replicates (plants). Means within each column followed by same letter are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference

Table 21.5 Effect of USDA strains and Heads Up seed treatments on fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), and dry root weight (DRW) in non-irrigated field at ISU Hinds Farm, Ames, IA in 2016

		Growth parameters		
Treatment	FSW (g)	DSW (g)	FRW (g)	DRW (g)
USDA 30	7.88a	1.77a	1.81a	0.36a
USDA 31	9.14a	2.28a	1.83a	0.37a
Heads Up (HU)	9.40a	2.31a	2.09a	0.46a
USDA 30+HU	7.59a	1.67a	1.72a	0.41a
USDA 31+HU	7.75a	1.87a	1.25a	0.29a
USDA 110	8.62a	1.91a	1.628a	0.34a
Negative control	8.89a	1.92a	1.71a	0.28a
Positive control	9.18a	1.98a	1.783a	0.33a

Note: Values are means of 10 plants for each treatment. Means within each column followed by same letter are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference

21.4 Discussion

Field studies confirmed the greenhouse studies (Huynh et al. 2016, 2021) that seed treatments with BJ were effective in reducing SDS. It further demonstrated the effectiveness of BJ seed treatments against SDS occurrence under field conditions. The study showed that seed treatments with BJ did not affect stand count compared with untreated negative control. Therefore, seed treatments with effective BJ strains may be good practice for field use. Further studies on using BJ treatment as other biocontrol agents are suggested because of the potential for the nitrogen-fixing

Table 21.6 Effects of seed treatments with USDA-BJ strains and Heads Up, on fresh and dry weights (g) of shoot and root in irrigated field in 2016 field tests. Means were calculated based on Fisher's protected least significance difference

		Growth parameters		
Treatment	FSW (g)	DSW (g)	FRW (g)	DRW (g)
USDA 30	10.85a	2.42a	1.86a	0.43a
USDA 31	9.24a	1.57a	1.89a	0.35a
Heads Up (HU)	7.93b	1.56a	1.57a	0.24a
USDA 30+HU	8.62a	1.97a	1.49a	0.28a
USDA 31+HU	9.66a	2.29a	2.14a	0.35a
USDA 110	8.07b	2.17a	1.41a	0.24a
Negative control	9.13a	2.12a	1.67a	0.39a
Positive control	8.15ab	2.1a	1.91a	0.41a

Note: Data presented the means of a sample size of 10 plants per plot. Means with the same letter (s) within each parameter are not significantly different according to Student's *t* test at $P = 0.05$

Table 21.7 Effect of USDA strains and Heads Up seed treatments on soybean yield (kg/ha) under field condition at ISU Hinds Farm, Ames, Iowa in 2016 and 2017

	2016		2017	
Treatment	Non-irrigated	Irrigated	Non-irrigated	Irrigated
USDA 30	4251 ± 31a	4081 ± 181a	3390 ± 134ab	4938 ± 168a
USDA 31	4461 ± 157a	4079 ± 133a	3378 ± 106ab	4976 ± 165a
Heads Up (HU)	4494 ± 162a	4038 ± 111a	4543 ± 350a	4870 ± 243a
USDA 30+HU	4339 ± 37a	3837 ± 182a	3359 ± 318ab	4751 ± 172ab
USDA 31+HU	4264 ± 245a	3957 ± 181a	3371 ± 78ab	4610 ± 132ab
USDA 110	4230 ± 189a	3984 ± 46.82a	3642 ± 195a	5029 ± 196a
Negative control	4401 ± 188a	3813 ± 147a	3435 ± 61ab	4920 ± 121a
Positive control	4362 ± 138a	3994 ± 107a	3294 ± 102ab	4302 ± 180b
P value	0.87	0.4	0.4	0.1

Values are means and standard errors of 4 replicates (plots). Means within each column followed by same letter are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference

bacteria to control SDS, improve soil fertility, increase crop productivity, and reduce the negative environmental impact of chemical use.

Effects of BJ seed treatments on suppressing SDS under field conditions varied over the years of the study. In 2015, ISU-BJ and USDA 110 had significantly reduced SDS in both fields (irrigated or non-irrigated). This can be explained by the tremendous cumulative rainfall in late July and August during SDS onset at flowering stages on soybean. The performance of BJ in both fields was the same in reducing SDS occurrence. Our data showed the performance of USDA 110 in greenhouse experiments exhibited lower modulation and SDS suppression (Huynh et al. 2021) compared with other USDA-BJ strains; however, its effects under field condition on nodulation and yield were the same with other USDA strains even achieved higher yields in 2017 season compared with control. This result supports

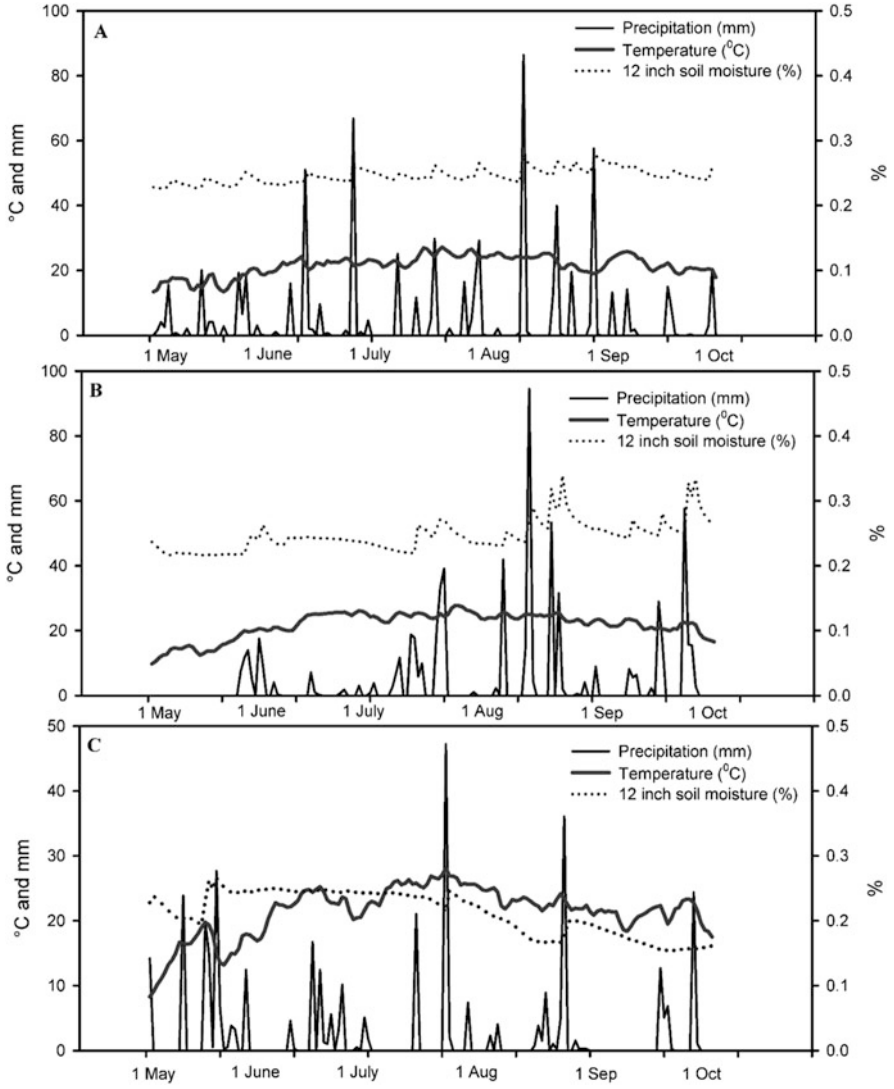

Fig. 21.3 Weather data at Ames (station AEEI4) during growing seasons 2015 (**a**), 2016 (**b**), and 2017 (**c**)

the finding of Zhang et al. (2003) that USDA 110 was the isolate that performs better at a lower temperature in field conditions.

The change in precipitation that is lower in recent years reflects a usual trend due to global climate changes. These changes, in turn, make the indigenous BJ population no longer adapt to their soybean host, which is also being changed in their genetic background. Conversely, a study by Yang and Feng (2001) predicted that the highest soybean fungal disease diversity was in the regions with the most variable climatic parameters. Overall, there might be an explanation for yield responses in our experiments together with unpredictable changes in SDS occurrence.

The purpose of this study was to test the applicability of our findings in greenhouse studies to field conditions. The results showed that seed treatments with a microbial carrier should be an option as a management tool. Our findings also indicated that BJ seed treatments acted better than a commercial product Heads Up in suppressing SDS. Our study is the first in comparing effects of a commercial product used as biological seed treatment with BJ strains on SDS development, although Heads Up treatment studies of 11 years compiled by Navi and Yang (2016b). In 2016, Heads Up seed treatments were used as Systemic Acquired Resistance (SAR) inducer mainly to compare its effect on effect in combination with BJ strains. Overall, SDS occurrence difference among those treatments was not observed; however, there were differences in dry biomass and nodule counts in which treatments either with BJ alone or with Heads Up had higher weights than treatments with Heads Up alone. This indicates seed treatments with BJ producing better modulation and higher biomass.

In 2017, treatments of 2016 repeated. We did not observe SDS during 2017. However, yield differences were observed in irrigated fields. The highest yield in USDA 110 treatments should explain the adapted strain in the US soil. The present study results have indicated that the appropriate use of BJ inocula as seed treatments to introduce the BJ population, which can adapt in a short growing season in Iowa, can be a good practice to reduce SDS. Further studies are recommended to explore the use of BJ strains on other diseases caused by *Fusarium* spp. or other soil-borne pathogens in legume and non-legume crops.

Acknowledgments This study was funded by Iowa Soybean Association and Vietnam International Education Development (VIED). Also, thanks to Sharon Eggenberger, Department of Plant Pathology and Microbiology, for technical assistance in data analysis.

Appendix

Supplemental Table 21.S1 Effects of seed treatments with USDA-BJ strains and Heads Up, on fresh and dry weights (g) of shoot and root in non-irrigated field in 2016 field tests. Means were calculated based on Fisher's protected least significance difference. Data presented the means of a sample size of 10 plants per plot. Means with the same letter(s) within each parameter are not significantly different according to Student's *t* test at $P = 0.05$

	Growth parameters			
Treatment	FSW (g)	DSW (g)	FRW (g)	DRW (g)
USDA 30	6.8b	3.27b	1.45b	0.72a
USDA 31	7.0b	3.52b	1.63b	0.83a
Heads up (HU)	7.7b	3.68b	1.68b	0.77a
USDA 30+HU	10.03ab	4.13ab	1.76ab	0.78a
USDA 31+HU	9.6ab	4.61ab	1.896ab	0.83a
USDA 110	10.3ab	3.25b	2.167a	0.98a
Negative control	10.1ab	3.96ab	1.778ab	0.75a
Positive control	12.17a	5.22a	2.22a	0.84a

Supplemental Table 21.S2 Effects of seed treatments with USDA-BJ strains and heads up, on fresh and dry weights (g) of shoot and root in irrigated field in 2016 field tests. Means were calculated based on Fisher's protected least significance difference. Data presented the means of a sample size of 10 plants per plot. Means with the same letter(s) within each parameter are not significantly different according to Student's *t* test at $P = 0.05$

	Growth parameters			
Treatment	FSW (g)	DSW (g)	FRW (g)	DRW (g)
USDA 30	10.9a	2.38a	1.87a	0.73a
USDA 31	11.64a	2.79a	1.73a	0.70a
Heads up (HU)	12.08a	3.07a	1.99a	0.7a
USDA 30+HU	12.82a	3.47a	2.07a	0.83a
USDA 31+HU	9.9a	3.12a	1.93a	0.8a
USDA 110	10.35a	2.89a	1.72a	0.79a
Negative control	10.98a	3.06a	1.81a	0.74a
Positive control	12.01a	3.03a	2.34a	0.82a

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Fungal Biocontrol Agents: An Eco-friendly Option for the Management of Plant Diseases to Attain Sustainable Agriculture in India

22

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Abstract

Intensive use of agrochemicals has adverse effects on humans and environmental conditions. Increased consumer demands for safe food have triggered research on the development of safe and eco-friendly biopesticides. Fungi-based biocontrol agents help minimize disease(s) pressure, safer food and feeds, with minimum undesirable impact on human health and environmental conditions. The use of antagonistic endophytes and *Trichoderma* as biocontrol agents is recently drawing particular attention to managing some of the major plant diseases. *Trichoderma* spp. has been widely used against many plant pathogens. It produces different secondary metabolites and enzymes such as chitinase, proteases, and β-1,3-glucanase and helps induce plant growth defense, systemic resistance and competes against plant pathogens. Fungi, including species of *Trichoderma*, *Gliocladium*, *Aspergillus*, *Fusarium*, and *Paecilomyces* species, antagonize plant pathogens, mycoparasitic pathogens, and trigger systemic acquired resistance. Research related to genetic manipulation to enhance virulence-based biocontrol agents is increasing, but it is not as widely explored as in bacteria-based biocontrol agents. The combination of fungi-based biocontrol agents and biofertilizers contributes to sustainable agriculture, not necessarily with some challenges.

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_22

Keywords

Fungal biocontrol agents · Mechanisms · Methods of application · Formulations · Management of plant diseases

22.1 Introduction

The recent challenge in the agricultural sector is to increase yield and decrease plant disease to a minimum level. Traditional methods such as fungicides, nematicides, herbicides, and fertilizer are among general methods in plant disease management. Although these mechanisms can control plant disease and suppress plant pathogens, they are not eco-friendly. Diseases caused by plant pathogens adversely affect global crop productivity and account for 20–40% yield losses annually in various agricultural and horticultural crops. In India, 57,000 metric tons of synthetic pesticides were used during 2016–2017 to control plant pathogens and insect pests, whereas biopesticide consumption was only 6340 metric tons. The development of resistance due to the continuous use of pesticides in modern farming and the increased availability of pesticide residues in vegetables, cereals, and grains has generated many problems. Moreover, the unregulated and indiscriminate use of chemical pesticides causes pollution of soil, water, and air and decreases the soil microflora and fauna. Beneficial rhizosphere microorganisms could be exploited to provide sustainable solutions in reducing the application of pesticides for agricultural crop production (Sehrawat and Sindhu 2019). Growing public concerns on the overuse of pesticides in agriculture and their effects on the environment have led to research on safe and eco-friendly options for managing pests and pathogens. Different approaches have been used for the mitigation of plant diseases. Beyond good agronomic and horticultural practices, producers often rely heavily on chemical fertilizers and pesticides. However, the environmental pollution caused by undesirable use and unintended misuse of agrochemicals and pesticides has culminated in considerable changes in people's approaches toward pesticides in agriculture (Jyoti and Singh 2016). Today, there are strict regulations on chemical pesticides, and there is a radical move to remove the most hazardous chemicals from the market.

The terms "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology, most notably in entomology and plant pathology. The term biocontrol can be defined as a reduction of inoculum density or disease-producing activity of a pathogen or a parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment of host or antagonist by mass introduction of one or more antagonists (Baker and Cook 1974). Biological control is a highly supportive approach for disease management, and it is precious to make an eco-friendly environment. Biological control plays a vital role in managing plant disease without disturbing flora and fauna and increasing soil fertility. There is an increasing trend for the exploitation of fungi to control plant diseases, and the most exploited are *Trichoderma*, *Gliocladium*, and *Paecilomyces*. Fungal biological control is an

exciting and rapidly developing research area with implications in plant productivity. Hence, this chapter aims to review the method of activity of fungal biocontrol agents with particular reference to most potential bioagent *Trichoderma* and *Paecilomyces* for the control of plant diseases.

22.2 Fungal Based Biocontrol Agents, Formulations, Mechanisms with Some Success Stories

22.2.1 *Trichoderma* spp.

Trichoderma spp. are free-living fungi that are common in soil and root ecosystems. *Trichoderma* has long been recognized as biocontrol agents for controlling plant diseases and enhancing root growth and development, crop productivity, resistance to abiotic stresses, uptake, and use of nutrients. Many successful products based on different species of *Trichoderma* have been commercialized in India (Kumar et al. 2014).

The first description of the genus *Trichoderma* was made by Persoon (1794). Tulasne and Tulasne (1860) suggested the sexual state of a *Hypocrea* species in 1865. Cook and Baker (1983) described genus *Trichoderma* as a common soil inhabitant, and the conidiophores are terminating in phialides. This fungus acts as a potential biocontrol agent because it stimulates plant resistance, plant growth, and development, resulting in increased crop production and antagonistic activity, viz. mycoparasitism, antibiotics, competition for nutrients, and also induces systemic resistance in plants (Harman et al. 2004).

The different species of the genus *Trichoderma/Hypocrea* were challenging to be distinguished morphologically. It was even proposed to reduce taxonomy to only a single species, *T. viride* (Schuster and Schmoll 2010). Nowadays, new species can be easily identified due to the development of TrichOKEY, where an oligonucleotide barcode and TrichoBLAST became a search tool (Druzhinina and Kubicek 2005; Kopchinskiy et al. 2005). Recently, according to Kamala et al. (2015) *Trichoderma* species belongs to the division Ascomycota, subdivision Pezizomycotina, class Sordariomycetes, subclass Hypocreomycetidae, order Hypocreales, and family Hypocreaceae. Chaverri et al. (2015) have reidentified the ubiquitous *Trichoderma harzianum* into 14 new species with various characteristics.

22.2.1.1 Mechanism of *Trichoderma* spp. Against Plant Pathogens

Trichoderma spp. are ubiquitous soil fungi. By their ability to decompose organic matter, they are free-living in the soil as Saprophytes. However, these species can also live on other fungi and colonize plant roots in the rhizosphere. *Trichoderma* spp. produce a range of hydrolytic enzymes that make them useful in industry (Mach and Zeilinger 2003). These fungi can parasitize some of the major plant pathogenic fungi that make them useful as biofungicides (Hjeljord and Tronsmo 1988; Mukhopadhyay et al. 1992; Mukhopadhyay and Mukherjee 1996; Chet et al. 1998; Harman and Bjorkmann 1998). *Trichoderma* spp. produce various kinds of

secondary metabolites in abundance, including antibacterial and antifungal antibiotics (Zeilinger et al. 2005). Some of the species/strains of *Trichoderma* spp. are plant growth promoters and inducers of SAR in plants (Harman et al. 2004). Mechanisms of *Trichoderma* spp. based biocontrol agents are mycoparasitism, antagonism, antibiosis, and competition for nutrients or space, among others which may operate independently or together to suppress plant pathogens (Harman et al. 2004).

22.2.1.2 Mycoparasitism

Haran et al. (1996) proposed the mycoparasitic activity (hyperparasitism) of *Trichoderma* spp. as one of the major mechanisms. Mycoparasitism implies the direct strike of one fungal species on another and is among the most critical antagonistic mechanisms expressed by *Trichoderma* spp. Firstly, the identification between *Trichoderma* and the target fungus is mediated by the binding of carbohydrates present in the cell wall of *Trichoderma* to the lectins of the other one. This is followed by hyphal twirling and appressoria development, which encompasses many osmotic compounds like glycerol. After successful penetration, *Trichoderma* attacks the host's cellular machinery by generating numerous fungitoxic cell wall degrading enzymes (CWDEs), such as glucanases, chitinases, and proteases (Harman et al. 2004). The cumulative action of these compounds causes the dissolution of the host cell walls, which ultimately results in parasitism of the target fungus. It has been observed that gaps can be generated at the location of appressoria formation, which facilitates the direct access of *Trichoderma* hyphae into the lumen of the target fungus, which then proceeds to kill the pathogenic fungus (Kumar 2013). Furthermore, biocontrol agents degrade the cell wall of the target fungus and inactivate its enzymes (e.g., pectinases, etc.), which are essential for pathogenic fungus to colonize and penetrate the plant tissues (Harman et al. 2004). As we know, fungal cell walls are mainly composed of chitin and 1,3-glucan. Chitinases and 1,3-glucanases lytic enzymes synthesized by *Trichoderma* spp. are supposed to be responsible for their mycoparasitic actions leading to the degradation of phytopathogenic fungal cell walls (De La Cruz et al. 1992; Geremia et al. 1993). In addition, other CWDEs, including those hydrolyzing minor polymers (like proteins, 1,6-glucans, 1,3-glucans, etc.), further ensure the complete and adequate disintegration of fungal mycelial or conidial walls by *Trichoderma* spp. (Geremia et al. 1993). A chitin-induced subtilisin-type serine proteinase was previously depicted in a *Trichoderma harzianum* mycoparasitic strain (De La Cruz et al. 1992).

The first *Trichoderma* secretome analyzed under confrontation with *R. solani* was that of *T. harzianum* EST 323. Two-dimensional gels (2-DE) and liquid chromatography mass spectrometry (LC-MS/MS) analysis identified seven CWDEs, including a chitinase, a cellulase, xylanase, a β-1,3-glucanase, a β-1,6-glucanase, a mannanase, and a protease. Enzyme activity staining showed that the xylanase, β-1,3-glucanase, β-1,6-glucanase activities only occurred in media containing *R. solani*. Eight out of 43 spots on the 2-DE were identified by LC-MS/MS. These proteins included two proteases, two β-glucosidases, two glycoside hydrolases, one endochitinase, and one amino acid oxidase (Tseng et al. 2008). Proteomic analysis to determine the changes

on the secretome of *T. harzianum* in response to glucose, a mixture of glucose and deactivated *Botrytis cinerea* mycelia, deactivated *B. cinerea* mycelia, or deactivated *T. harzianum* mycelia was performed. Ninety-one out of 100 excised proteins were analyzed, and some of them were sequence identified. Two endochitinases and one l-amino acid oxidase (LAAO) were exclusively induced in media containing deactivated *B. cinerea* mycelia as the sole carbon source. Media containing deactivated *B. cinerea* mycelia showed high CWDE activities, including chitinase, cellulase, xylanase, β-1,3-glucanase, β-1,6- glucanase, and protease, than in other media. These results confirm the critical role of CWDE in the antagonism by *Trichoderma* and indicate that cell walls are the primary target of *Trichoderma* during mycoparasitism (Tseng et al. 2008; Yang et al. 2009).

22.2.1.3 Antibiosis

Antibiosis is one of the critical attributes in deciding the saprophytic ability of the fungus. A range of antibiotics produced by species of *Trichoderma* and *Gliocladium*, which has been suggested as a mode of action of both fungi against plant pathogens, was reported by Weindling (1934). Manibhusanrao et al. (1989) reported that antibiotics like trichodermin, suzukacillin, and alamethicin produced by *T. harzianum* influence morphological or physiological sequences leading to its successful penetration. *Trichoderma* spp. and *Gliocladium* spp. inhibited the growth of a broad range of soilborne fungi, viz. genera of *Fusarium*, *Macrophomina*, *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, *and Verticillium* (Zaher et al. 2013; Ragab et al. 2015; Chen et al. 2016). Do Nascimento Silva et al. (1998) demonstrated the in vitro antagonistic potential of 3 *Trichoderma* spp. against *Colletotrichum gloeosporioides* on passion fruit. Bhagat and Pan (2010) screened 12 isolates of *Trichoderma* spp. in vitro against *R. solani* Kuhn. causing root and collar rot of French bean (*Phaseolus vulgaris* L.) by dual culture tests and production of volatile and non-volatile antibiotics, and it was found that all the isolates significantly inhibited the mycelial growth of *R. solani.*

It is well known that *Trichoderma* spp. produce a plethora of nonpolar compounds of low molecular weight cataloged as secondary metabolites, including pyrones, terpenoids, steroids, and polyketides. *Trichoderma* spp. also produces siderophores and many peptaibiotics known as peptaibols, which contain high-frequency nonstandard amino acids (Degenkolb et al. 2006), which play an important role in signaling process development.

22.2.1.4 Competition

Bioagents compete for nutrients and space with pathogens, and thus, it is the injurious effect of one microorganism on another due to the utilization or removal of some resources from the environment. Competition between iron-containing siderophore of *Trichoderma* and wood decay Basidiomycete's fungi was investigated (Srinivasan et al. 1995).

Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens (Chet et al. 1997). For instance, in most filamentous fungi, iron

uptake is essential for viability. Under iron starvation, most fungi excrete low molecular weight ferric iron specific chelators termed siderophores to mobilize environmental iron (Eisendle et al. 2004). Subsequently, iron from the ferrisiderophore complexes is recovered via specific uptake mechanisms. In *Aspergillus fumigatus* and *Aspergillus nidulans*, the carbon source negatively regulates siderophore biosynthesis (Eisendle et al. 2004). In *Ustilago maydis*, gene products related to iron uptake affect plant disease development (Mcintyre et al. 2004). Some *Trichoderma* BCAs produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Chet and Inbar 1994).

For this reason, soil composition influences the biocontrol effectiveness of *Pythium* by *Trichoderma* according to iron availability. In addition, *T. harzianum* T35 controls *Fusarium oxysporum* by competing for rhizosphere colonization and nutrients, with biocontrol becoming more effective as the nutrient concentration decreases (Tjamos et al. 1992). Competition has proved to be particularly important for the biocontrol of phytopathogens such as *Botrytis cinerea*, the primary pathogenic agent during the pre-and post-harvest in many countries (Latorre et al. 2001). The extraordinary genetic variability of this fungus makes it possible for new strains to become resistant to essentially any novel chemical fungicide it is exposed to (Latorre et al. 2001). The advantage of using *Trichoderma* to control *B. cinerea* is the coordination of several mechanisms simultaneously, thus making it practically impossible for resistant strains to appear. Among these mechanisms, the most important is nutrient competition since *B. cinerea* is particularly sensitive to the lack of nutrients. *Trichoderma* has a superior capacity to mobilize and take up soil nutrients compared to other organisms. The efficient use of available nutrients is based on the ability of *Trichoderma* to obtain ATP from the metabolism of different sugars such as those derived from polymers widespread in fungal environments: cellulose, glucan, and chitin, among others, all of them rendering glucose (Chet et al. 1997). The key components of glucose metabolism include assimilation enzymes and permeases and proteins involved in membrane and cell wall modifications. While the role of the glucose transport system remains to be discovered, its efficiency may be crucial in competition (Delgado-Jarana et al. 2003) as supported by the isolation of a high-affinity glucose transporter Gtt1, in *Trichoderma harzianum* CECT 2413. This strain is present in environments very poor in nutrients, and it relies on extracellular hydrolases for survival. Gtt1 is only expressed at very low glucose concentrations, i.e., when sugar transport is expected to be limiting in nutrient competition transformant derivative that carried an additional copy of the transporter gene. Only two other genes encoding glucose transporters have been described in filamentous fungi (Franken et al. 2002), one of them in *Uromyces fabae*. This basidiomycete has an ATPase and a proton-coupled glucose transport system that is expressed during the infection of *Vicia faba*. This suggests an additional, antagonistic role for Gtt1, allowing the fungus to obtain energy from hydrolyzed polymers and rapidly transport sugar into the cells. Consequently, transformants able to transport glucose more rapidly than the wild-type (Delgado-Jarana et al. 2003) should be more efficient BCAs. This would serve as an advantageous mechanism of nutrient competition during mycoparasitism interactions.

22.2.1.5 Molecular Mechanisms of Biocontrol by *Trichoderma* spp.

Trichoderma spp. produce several types of chitinases, both endo, and exo that exhibit mucolytic activities. Many new chitinases are being discovered, and a genome-wide search would reveal more. The hydrolytic enzymes are generally induced by specific substrates (like chitin, glucan, fungal cell wall) and repressed by glucose. Lorito et al. (1996) observed that mycoparasitic interaction relieves the Cre1 catabolite repressor protein's binding to promoter sequences of the ech42 gene in *T. harzianum*. Expression of ech42 gene of *T. atroviride* under carbon starvation is antagonized via a BrlA-like *cis*-acting element (Brunner et al. 2003). The regulation of expression of two major chitinase genes (ech42 and nag1, encoding CHIT73) of *T. atroviride* is triggered by different regulatory signals (Mach et al. 1999). The direct evidence on the role of hydrolytic enzymes in biocontrol came from the gene knockout studies, where a gene is selectively deleted through homologous recombination or inactivated by antisense/RNAi. Disruption of ech42 in *T. harzianum* resulted in almost no endochitinase42 activity, whereas strains carrying multicopy of this gene exhibited up to a 42-fold increase in enzyme activity (Carsolio et al. 1999). Woo et al. (1999) disrupted ech42 in *T. harzianum* P1 (*T. atroviride*) and showed reduced biocontrol activity against *B. cinerea* on bean leaves. However, interestingly, the biocontrol activities of the disruptants were enhanced against *R. solani* and remained unaltered against *P. ultimum*. The observation that *Trichoderma* spp. colonize plant roots and induce systemic resistance against a wide range of fungal, bacterial, and viral pathogens can be considered a breakthrough in biocontrol research (Harman et al. 2004). Inoculation of roots of cucumber seedlings with conidia of *T. harzianum* T-203 (*T. asperellum*) in an aseptic hydroponic system resulted in induction of defense responses (Yedidia et al. 2003). Electron microscopy of ultrathin sections from *Trichoderma* treated roots revealed penetration of the mycoparasite into the roots, restricted mainly to the epidermis and outer cortex. *Trichoderma* colonization strengthened the epidermal and cortical cell walls and deposition of newly formed barriers, these typical host reactions being found even beyond the sites of potential fungal penetration. The inoculation of *Trichoderma* initiated increased peroxidase and chitinase activities, both in roots and leaves. Later on, the authors showed that inoculation of cucumber roots with *Trichoderma* induced an array of PR proteins (Yedidia et al. 2000). Inoculation of cucumber roots with *T. asperellum* reduced the inoculum load of *Pseudomonas syringe pv lachrymans* up to 80% when inoculated on leaves (Yedidia et al. 2003), thus providing direct evidence on induced defense-mediated protection of crop plants in response to *Trichoderma* inoculation. The protection afforded by the biocontrol agent was associated with the accumulation of mRNA of two defense-related genes: the phenylpropanoid pathway gene encoding phenylalanine ammonia-lyase (PAL) and the lipooxygenase pathway gene encoding hydroxyperoxidase lyase (HPL). Recently using the gene knockout approach, a hydrophobin TasHyd1 has been demonstrated in root colonization by *T. asperellum* (Viterbo and Chet 2006). In a significant finding, Shoresh et al. (2006) identified a MAPK (TIPK—*Trichoderma* induced MAPK) in cucumber, antisense-mediated silencing of this gene made plants susceptible even after inoculation of roots with *T. asperellum*. It

was thus proved that *Trichoderma* exerts its positive effects on plants through the activation of a *MAPK* gene involved in signaling the pathway of defense response A definite role of phytoalexin induction in biocontrol has recently been demonstrated by Howell and Puckhaber (2005), who showed that the "P" strains of *T. virens* failed to stimulate phytoalexin synthesis in cotton and were ineffective as biocontrol, while the "Q" strains that stimulated phytoalexin biosynthesis were effective. This difference was attributed to the ability of "Q" strains to produce the 18-kDa elicitor protein. Recently, three groups independently identified a homolog of SnodProt proteins, variously named as SnodProt1 (GV Sible and PK Mukherjee, unpublished; GenBank Acc. no. DQ494198), (Djonovic et al. 2006) from *T. virens*, and Epl1 (Seidl et al. 2006) from *T. atroviride*. Olson and Benson (2007) have studied three root-colonizing fungi, binucleate *Rhizoctonia* (BNR) isolates BNR621 and P9023 and *T. hamatum* isolate 382 (T382), for suppression of Botrytis blight in geraniums by induction of host systemic resistance. Resistance to Botrytis blight was observed in geraniums transplanted into potting mix amended with formulations of P9023 and T382 2 weeks before inoculation with *B. cinerea* when grown under environments either highly or less conducive to disease development. Restriction of lesion development may play a role in the suppression of Botrytis blight in geraniums. This may be the first to demonstrate induced systemic resistance by BNR fungi to a foliar pathogen and support additional research into the use of T382 in an integrated management program for *B. cinerea* (Olson and Benson 2007). Research on the specific effects of induced systemic resistance should be continued with additional pathogens since there is some indication of pathogen specificity in the suppression method.

Mukherjee et al. (2004) studied the role of the G-proteins TgaA and TgaB in *T. virens*. Deleting these genes individually did not affect the hyphal coiling of *R. solani*, but TgaA was involved in the parasitism of sclerotia of *S. rolfsii*. Deletion of the MAPK TmkA in *T. virens* resulted in attenuation of sclerotial parasitism of *S. rolfsii* and *R. solani*. In contrast, the hyphal parasitism was unaltered. Reithner et al. (2007) had examined the function of the tmk1 gene encoding a MAPK during fungal growth, mycoparasitic interaction, and biocontrol was examined in *T. atroviride*. Dtmk1 mutants exhibited altered radial growth and conidiation and displayed de-regulated infection structure formation without a host-derived signal. In confrontation assays, tmk1 deletion caused reduced mycoparasitic activity, although attachment to *R. solani* and *B. cinerea* hyphae was comparable to the parental strain. Under chitinase-inducing conditions, nag1 and ech42 transcript levels and extracellular chitinase activities were elevated in a Dtmk1 mutant. In contrast, upon direct confrontation with *R. solani* or *B. cinerea*, a host-specific regulation of ech42 transcription was found, and nag1 gene transcription was no more inducible over an elevated basal level. These findings strongly suggest the presence of further, still unknown, mycoparasitism related factors that are missing in our Dtmk1 mutants and therefore affected by a signaling pathway involving Tmk1.

22.2.1.6 *Trichoderma*: Formulations and Application Methods

The success of biological control of plant pathogens using *Trichoderma* spp. does not rely solely on effective antagonists but also the methods of applications such as seed treatments (solid/liquid coating), in-furrow soil application, and or foliar applications. The following are examples of effective modes of delivery and application of *Trichoderma.*

22.2.1.7 Success Stories of Seed Treatments from the Indian Agricultural Perspective

Seed treatments or seed coating is one of the most effective methods of application of *Trichoderma* spp. in the agricultural system (Mathre et al. 1999). *Trichoderma* spp. is delivered in the infection court (surface of seed coat) as a protectant at planting. This method should limit the growth of competitive microflora and provide conducive growth for the biocontrol agent (Cumagun 2014). Seed treatments using seed dressing formulations, Pusa 5SD has been proven more effective than soil application formulations, Pusa Biogranule 6 (PBG 6) and Pusa Biopellet 16G (PBP 16G) in managing wet root rot of mung bean caused by *R. solani* (Dubey et al. 2011).

Seed coating with *Trichoderma* spp. is one of the easy and effective methods of delivering the antagonist to manage seed/soilborne diseases. Seed is coated with dry powder/dust of *Trichoderma* just before sowing. For commercial purposes, dry powder of antagonist is used at 3–10 g/kg seed based on seed size (Mukhopadhyay et al. 1992). Propagules of biocontrol agents germinate on the seed surface and colonize the roots of germinated seedlings and rhizosphere (Tiwari 1996; Kumar et al. 2009). *T. harzianum*, *T. virens*, and *T. viride* are effective seed protectants against *Pythium* spp. and *R. solani* (Mukherjee and Mukhopadhyay 1995). The rice seed treated with two antagonistic fungi, *viz*. *T. viride*, and *T. harzianum*, *were found effective in controlling the* sheath blight of rice and increasing the crop's yield (Das and Hazarika 2000). In another study, *T. viride* was an efficient agent to control the *R. solani* toxin activity against the same disease (Sriram et al. 2000). Singh and Maheshwari (2001) reported that seed treatment with bioagents like *T. viride*, *T. harzianum*, and *G. virens* was found helpful in combating the loose smut of wheat. *Trichoderma*, being a growth-promoting agent, also helps increase crop yield, which has been demonstrated by the application of *T. harzianum* (Th3) in irrigated and dry areas of Kota and Jaipur districts Rajasthan, which is also ecologically competent (Sharma et al. 2012). Seed treatment with *T. harzianum*, *A. sativum*, and *A. indica* on par with the foliar spray of mancozeb showed results against *Alternaria* blight disease of mustard caused by *A. brassicae* and *A. brassicicola* and increasing the yield (Jagana et al. 2013). Seed treatment with *Trichoderma* species inhibited the growth of oilseed-borne fungi like *Aspergillus flavus*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *Rhizopus nigricans*, *Penicillium notatum*, and *Penicillium chrysogenum*, which affects oilseed crops like soybean, sesame, and sunflower (Jat and Agalave 2013). More examples are listed below in Table 22.1.

Table 22.1 Seed treatment methods recommended for different crops by TNAU Agritech Portal, 2014 (Sharma et al. 2015)

Crop	Disease	Seed treatment
Sugarcane	Root rot, wilt	*Trichoderma* spp. 4–6 g/kg of seeds
Rice	Sheath blight	*Trichoderma* 5–10 g/kg of seeds
Chilly	Anthracnose and damping off	*T. viride* 4 g/kg
Pigeon pea	Wilt and Root rot	*Trichoderma* spp. at 4 g/kg of seeds
Tomato	Damping off, wilt, and early blight	*T. viride* at 2 g/100 g seeds
Coriander	Wilt	*T. viride* at 4 g/kg of seeds
Leguminous vegetables	Soilborne infection of fungal disease	*T. viride* at 2 g/100 g seeds
Sunflower	Seed rot	*T. viride* at 6 g/kg of seeds
Cruciferous vegetables	Soil/seed-borne diseases (damping off)	Seed treatment with *T. viridae* at 2 g/100 g of seeds

22.2.1.8 Seed Biopriming

Seed priming is when the seeds are hydrated to allow the metabolic process of germination to take place but not sprouting. Priming enhances the biocontrol of *Trichoderma* by regulating water levels in the seed. It allows effective colonization of seed surface before planting, given the right pH and matrix material (Harman and Bjorkmann 1998). Seed priming of three rhizosphere isolates of *T. harzianum* enhanced growth and induced resistance in sunflower against downy mildew caused by *Plasmopara halstedii* (Nagaraju et al. 2012).

Treating seeds with biocontrol agents and then incubating under warm and moist conditions until before the emergence of radical is referred to as biopriming. This technique has potential advantages over the simple coating of seeds as it results in rapid and uniform seedling emergence. *Trichoderma* conidia germinate on the seed surface and form a layer around bioprimed seeds. Such seeds tolerate adverse various soil conditions better. Biopriming could also reduce the amount of biocontrol agents that are applied to the seed. Seed biopriming is successfully used in tomato, brinjal, soybean, and chickpea in the Tarai region of Uttaranchal (Mishra et al. 2001). Three rhizosphere competent microbial strains, *viz.*, *P. fluorescens* OKC, *Trichoderma asperellum* T42, and *Rhizobium* sp. RH4, individually and in combination in bioprimed seeds of chickpea and rajma in pots and fields, showed higher germination percentage and better plant growth in both the crops than non-bioprimed control plants. It was also observed that the combined application of the microbes enhanced seed germination and plant growth better than their application. Among the combinations, all combinations comprising *Trichoderma* showed better results than the others. The triple microbial combination demonstrated the best results in seed germination and seedling growth in both chickpea and rajma (Yadav et al. 2013).

22.2.1.9 Liquid Coating

Liquid coating is a seed coating system that involves the application of *Trichoderma* to the seed with an aqueous adhesive or binder (pelgel or polyox-N-10) and a particulate material (Agro-lig or muck soil) to optimize pH level, including a bulking agent (Taylor et al. 1991). The Agro-lig is reported to have physical and chemical characteristics which favor the growth of the fungus.

22.2.1.10 Soil Application

Soil is the home for both beneficial and pathogenic microbes. Delivering *Trichoderma* spp. to the soil will increase the population dynamics of augmented fungal antagonists and thereby suppress the establishment of pathogenic microbes onto the infection court. There are several reports on applying biocontrol agents to the soil either before or at the time of planting for control of a wide range of soilborne fungal pathogens (Baby and Manibhushanrao 1996; Kumar et al. 2009; Kumar 2010). Soil application of *T. viride* either alone or in combination with other treatments significantly reduced red rot caused by *C. falcatum* (Reddy et al. 2009). Srivastava et al. (2010) suggested that the soil application of *T. viride* was best in controlling seedling blight, color rot, stem rot, and root rot disease of Jute. Soil application of organic preparation of *Trichoderma* was effective in managing seed-borne pathogenic fungi *F. oxysporum*, *F. moniliforme*, *F. solani*, *B. theobromae*, *A. alternata*, and *R. solani* and in the seedling establishment of *Dalbergia sissoo* Roxb (Mustafa et al. 2009). *Trichoderma* can colonize farmyard manure (FYM), and therefore the application of colonized FYM to the soil is more appropriate and beneficial. This is the most effective method of application of *Trichoderma*, particularly for the management of soilborne diseases. Soil application of *T. asperellum* strain GDFS1009 granules produces beneficial effects on maize growth and resistance to stalk rot caused by *F. graminearum* (He et al. 2019).

22.2.1.11 Root Treatment

Seedling roots can be treated with spore or cell suspension of antagonists either by drenching the *Trichoderma* in nursery beds or by dipping roots in *Trichoderma* suspension before transplanting. Root dipping in antagonist's suspension not only reduces disease severity but also enhances seedling growth in rice, tomato, brinjal, chili, and capsicum (Singh and Zaidi 2002). There are also reports on the reduction of sheath blight disease of rice by root dip of seedlings before transplantation (Vasudevan et al. 2002).

22.2.1.12 Foliar Spraying/Wound Dressing

The efficacy of biocontrol agents for foliar diseases is greatly affected by the fluctuation of microclimate. Phyllosphere is subjected to diurnal and nocturnal, cyclic and non-cyclic variation in temperature, relative humidity, dew, rain, wind, and radiation. Hence, the water potential of phylloplane microbes will constantly be varying. It will also vary between leaves or the periphery of the canopy and on sheltered leaves. Higher relative humidity could be observed in the shaded, dense region of the plant than that of peripheral leaves. The dew formation is greater in the

center and periphery. The concentration of nutrients like amino acids, organic acids, and sugars exuded through stomata, lenticels, hydathodes, and wounds varies highly. It affects the efficacy and survival of antagonists in phylloplane (Andrews 1992). The liquid suspension of *Trichoderma* has been successfully applied to the aerial plant parts for the biocontrol of *Alternaria* leaf spot of *Vicia faba* (Kumar et al. 2002). Khan and Sinha (2005, 2007) emphasized the usefulness of *T. harzianum* and *T. virens* in foliar sprays and talc-based formulations to reduce disease incidence of sheath blight of rice. Sharma et al. (2012) carried out field trials in Rajasthan on the groundnut root rot disease caused by multiple pathogen complex mainly *A. niger*, *A. flavus*, *S. rolfsii*, *Thielaviopsis basicola*, *R. solani*, and *P. aphanidermatum* by the application of *T. harzianum* in the form of powder and liquid bioformulation found effective in controlling disease in the field. Singh et al. (2000) managed citrus scab caused by *Elsinoe fawcettii*. They found that *T. harzianum* and *E. purpurascens* reduced the disease incidence in the field on spraying by 17.8 and 10%, respectively. Though the foliar application of *Trichoderma* reduces the severity of diseases under field conditions, it is not technically feasible due to increased dosage and economy realized from the crop. Hence, dosage and frequency of application have to be standardized based on the crop value, which could be a reliable and practical approach.

22.2.1.13 Types of Formulations

Significant research on biocontrol is centered on the use of spores of *Trichoderma* directly to seed. Technologies become viable only when the research findings are transferred from the lab to the field. Though *Trichoderma* has excellent potential in managing diseases, it could not be used as spore suspension under field conditions. Thus, the culture of *Trichoderma* should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization, and field use.

For the successful development of a formulation of biocontrol agents, it is important not only to provide a substrate that will promote the synthesis of the desired enzymes, which help in its biocontrol mechanisms but also to provide sufficient substrate so as not to limit the synthesis of the enzymes at the time they are required. These include the utilization of a large number of agro-wastes as a substrate for the mass production of *Trichoderma*, the use of a wide variety of solid substrates with less expenditure, and higher reproducibility (Kulkarni and Shalini 2007). Two types of formulation used widely are either liquid or solid formulations (Table 22.2).

A deep tank fermentation system is employed in a liquid formulation, making it a preferred approach for biomass production in Europe and North America (Churchill 1982). Inexpensive growth media such as molasses and brewer's yeast are used for production in liquid formulation (Papavizas et al. 1984). The advantage of this formulation is the optimization of biomass production and quality which allows control of nutrients, pH, temperature, and other environmental factors, thus reducing contamination (Whipps 1997).

Table 22.2 Substrates used for production of *Trichoderma* spp.

Species	Substrate	References
Solid		
T. harzianum and *T. viride*	Sorghum	Reithner et al. (2007)
T. viride	Sorghum and Wheat	Bhagat et al. (2010)
T. harzianum	FYM, spent compost	Tseng et al. (2008)
T. harzianum and *T. viride*	Sawdust, rice bran	Reithner et al. (2007)
T. hamatum, *T. harzianum*, *T. viride*	Molasses and brewers yeast	Papavizas et al. (1984)
Liquid		
T. harzianum Rifai	Potato dextrose broth, V8 juice, and molasses-yeast medium	Prasad et al. (2002)
T. harzianum	Local cow urine, Jersey cow urine, butter milk, vermiwash	Parab et al. (2008)

Solid Formulation

Solid formulation or fermentation is the alternative method for inoculum production. Agricultural waste materials such as wheat and rice straw, sugarcane bagasse, ground corn cobs, sawdust, rice bran are used as food base or substrate alone or in combination for the growth of *Trichoderma*. Cumagun and Lapis (1993) used rice bran as a food base for *Trichoderma* spp. with tapioca flour as a binding agent to produce pellets. Provision of food base in the formulation should, in most cases, favor the antagonist (Papavizas et al. 1984) or a food base that can only be utilized by the antagonist in which the pathogen can be inhibited (Nelson et al. 1988). This method of formulation requires only minimal cost, especially in small-scale production. However, it is bulky as it requires considerable space for production, inoculation, and storage, including drying and milling. Solid and liquid formulations require drying to obtain stable products with prolonged shelf life (Jin et al. 1992). Spray drying is preferred among the different drying techniques for large-scale production of microorganisms containing dried powders due to its low cost (Morgan et al. 2006). Various solid formulations methods are listed in Table 22.3.

Talc-Based Formulation

In India, talc-based formulations of *T. viride* were developed at Tamil Nadu Agricultural University, Coimbatore, for seed treatment of pulse crops and rice (Jeyarajan et al. 1994). *Trichoderma* is grown in the liquid medium, mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade. The talc formulations of *Trichoderma* have a shelf life of 3–4 months. It has become quite popular in India to manage several soilborne diseases of various crops through seed treatment at 4–5 g/kg seed. Several private industries produce large quantities of talc formulations in

Table 22.3 Various formulations of *Trichoderma* spp. (Source: Pandya 2012)

Formulation	Ingredient
Talc based	Trichoderma culture biomass along with medium: 1 l, talc (300 mesh, white color): 2 kg and CMC: 10 g
Vermiculite-wheat bran based	Vermiculite: 100 g, wheat bran: 33 g, wet fermenter biomass: 20 g and 0.05N HCL: 175 ml
Wheat bran based	Wheat flour: 100 g, fermenter biomass: 52 ml and sterile water: sufficient enough to form a dough
Wheat flour-kaolin	Wheat flour: 80 g, Kaolin: 20 g and fermenter biomass: 52 ml
Alginate prills	Sodium alginate: 25 g and wheat flour: 50 g and fermenter biomass: 200 ml

India for supply to the farmers. The annual requirement of *Trichoderma* has been estimated as 5000 tons to cover 50% area in India (Jeyarajan 2006).

Vermiculite-Wheat Bran-Based Formulation

Trichoderma is multiplied in a molasses-yeast medium for 10 days. 100 g vermiculite and 33 g wheat bran are sterilized in an oven at 70 °C for 3 days. Then, 20 g of fermented biomass, 0.05 N medium, and concentrated or entire biomass with HCl are added, mixed well, and dried in the shade (Lewis 1991).

Pesta Granules-Based Formulation

Fermenter biomass (52 ml) is added to wheat flour (100 g) and mixed by gloved hands to form a cohesive dough. The dough is kneaded, pressed, and folded by hand several times. Then one mm thick sheets (pesta) are prepared and air-dried till it breaks crisply. After drying, the dough sheet was ground and passed through a mesh and granules were collected (Connick et al. 1991).

Alginate Prills Based Formulation

Sodium alginate is dissolved in one portion, and distilled water (25 g/750 ml) and food base are suspended in another portion (50 g/250 ml). These preparations are autoclaved and, when cool, is blended with biomass. The mixture is added dropwise into $CaCl_2$ solution to form spherical beads, which are air-dried and stored at 5°C (Fravel et al. 1999).

Press Mud-Based Formulation

Press mud is available as a byproduct of the sugar factory, and this can be used as a substrate for mass multiplication of *Trichoderma*. The method involved uniformly mixing of 9 days old culture of *T. viride* prepared in potato dextrose broth into 120 kg press mud. Water was sprinkled intermittently to keep it moist. Gunny bags covered this to permit air movement and trap moisture under shade. Within 25 days, nucleus culture for further multiplication becomes ready. The same was added to 8 tons of press mud, mixed thoroughly, and incubated for eight days under shade conditions before being applied in the field. By this, we added 8000 times more inoculums in the soil than the recommended doses of biopesticides, which rapidly

get established, showing rapid and visible effect. Similarly, other substances could also be effectively used for the multiplication of different bioagents at the mass level (Sabalpara 2014).

Coffee Husk-Based Formulation

In Karnataka, India, Sawant and Sawant (1996) developed a *Trichoderma* formulation based on the coffee husk, waste from the coffee curing industry. This product effectively managed *Phytophthora* foot rot of black pepper and is widely used in Karnataka and Kerala.

Banana Waste-Based Formulations

The mass multiplication protocol of *Trichoderma* sp. in banana waste was proposed by Balasubramanian et al. (2008). For the same banana waste, urea, rock phosphate, culture of *Bacillus polymyxa*, *P. sajor-caju*, and *T. viride* are used. A pit of other banana waste, *viz*. sheath pseudostem and core, is chopped in the length of 5–8 cm. A pit is prepared, and different ingredients are placed in five different layers. Each layer contains one tone banana waste, 5 kg urea, 125 kg rock phosphate, and one-liter broth culture of *B. polymixa*, *P. sajor-caju*, and *T. viride*. Five different layers are prepared similarly and mixed thoroughly in Banana. Banana waste is decomposed within 45 days, and enriched culture is mass available for field application.

Liquid Formulation

Oil-Based Formulations

They were prepared by mixing the conidia harvested from the solid-state/liquid state fermentation with vegetable/mineral oils in a stable emulsion formulation. In such formulations, microbial agents are suspended in a water-immiscible solvent such as a petroleum fraction (diesel, mineral oils) and vegetable oils (groundnut, etc.) with the aid of a surface-active agent. This can be dispersed in water to form a stable emulsion. Emulsifiable concentrates require a high concentration of an oil-soluble emulsifying agent to rapidly form a homogenous emulsion on dilution in water. The oils used should not have toxicity to the fungal spores, plants, humans, and animals. Such formulations of *Trichoderma* are now being used as foliar sprays. Oil-based formulations are suitable for foliar sprays under dry weather and have a prolonged shelf life. The spores can survive longer on the plant surface, even during the dry weather, as the spores are covered by oil that protects them 5°C from drying. Batta (2005) developed an emulsion formulation of *T. harzianum* to control post-harvest decay of apple caused by *Botrytis cinerea*. Invert-emulsion formulation of *T. harzianum* with a shelf life of 8 months has been developed using indigenous constituents at the erstwhile Project Directorate of Biological Control (PDBC) in India. This formulation has been found to be effective against soilborne diseases of groundnut.

Adjuvants, Spreaders, and Stickers

Performance of *Trichoderma* in the formulations can be increased by incorporating water-soluble adjuvants, oils, stickers, and emulsions. It increases the efficacy of biocontrol agents by supplying nutrients and protecting the microbes from desiccation and death (Connick et al. 1991; Bateman et al. 1993; Barnes and Moore 1997; Green et al. 1998; Ibrahim et al. 1999). The incorporation of carboxymethyl cellulose (CMC) in formulations serves as stickers in the uniform seed coating of microbes. Though adjuvants and stickers increase the efficacy of bio-products, it has its demerits. Adjuvants/stickers in the formulations will be diluted when exposed to rain or heavy dew. It would alter the efficacy of formulations by reducing the establishment or colonization of *Trichoderma* onto the infection court. Sometimes spray application of emulsions or oil-based formulations may be toxic to plants.

Consequently, comprehensive knowledge on the usage of adjuvants, stickers is crucial for increasing the efficacy of formulations. Some commercial products of *Trichoderma* spp. available in India are listed in Table 22.1.

22.2.1.14 Characteristics of *Trichoderma* for Formulation Development

To develop a successful *Trichoderma* formulation, *Trichoderma* spp. should possess (Jeyarajan and Nakkeeran 2000)

1. High rhizosphere competence
2. Highly competitive saprophytic ability
3. Enhanced plant growth
4. Ease for mass multiplication
5. Broad-spectrum of action
6. Excellent and reliable control
7. Safe to environment
8. Compatible with other bioagents
9. Should tolerate desiccation, heat, oxidizing agents, and UV radiations.

22.2.2 *Paecilomyces* spp.: Formulations, Mechanism with Some Success Stories

The fungus *Paecilomyces lilacinus* (Thom) Samson, a nematode egg parasite, is currently used as a biological control agent against various plant–parasitic nematodes. The genus *Paecilomyces* was first described (Bainier 1907) as closely related to Penicillium and comprising only one species, *P. variotii* Bainier. The genus Paecilomyces has many species, both pathogenic and saprophytic, and can be found in a wide range of habitats, including soil (Samson 1974).

22.2.2.1 Mechanisms

Paecilomyces, microbial mechanisms involved in disease suppression have been direct, such as parasitism, competition or antibiosis, and indirect, which involve

plant protection through induced systemic resistance (ISR) mechanisms (Di Francesco et al. 2016; Lugtenberg et al. 2017; Obrien 2017; Latz et al. 2018).

22.2.2.2 Parasitism

Chitinase production by *P. javanicus* leads to mycelia inhibition of *Aspergillus nidulans*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, and *Sclerotium rolfsii* (Chen et al. 2007). Various studies refer to the nematicidal activity of *Paecilomyces.* Species of this genus, namely *P. lilacinus*, can penetrate both the eggshells and structural components of juvenile and adult stages of different species of nematodes through spore germination and subsequent hyphal branching and appressoria formation (Khan et al. 2006; Dong et al. 2007). Regarding the production of lytic enzymes causing a nematicidal effect, the synthesis of amylases, lipases, proteases, and chitinases associated with this species has been described (Morton et al. 2004; Park et al. 2004; Khan et al. 2006; Gortari et al. 2008; Gine and Sorribas 2017). Overexpression of genes regulating the synthesis of these enzymes increases *P. lilacinus* virulence and parasitic ability against *Meloidogyne incognita*, *Panagrellus redivivus*, and *Caenorhabditis elegans* (Wang et al. 2010; Yang et al. 2011).

22.2.2.3 Competition

In vitro synthesis of hydroxamate and carboxylate siderophores such as ferrirubin trihydroxamate has been described mainly in *P. lilacinus* and *P. variotii* (Vala et al. 2000; Renshaw et al. 2002; Moreno-Gavíra et al. 2020). While this mechanism directly impacts control, competition is often accompanied by other mechanisms (Latz et al. 2018). The rapid growth of *Paecilomyces* species prevents the development of specific pathogens (Adebola and Amadi 2010; Arora et al. 2017). For instance, spraying sunflower seeds with *P. variotii* spores prevents penetration and infection by the pathogen *Macrophomina phaseolina* (Anis et al. 2010). However, this competition can sometimes harm the rest of the beneficial microbiota (Yu et al. 2015).

22.2.2.4 Antibiosis

The production of secondary metabolites with antimicrobial effect by *Paecilomyces* species has been widely described. Among them, we can highlight the synthesis of alkaloids, phenolic compounds, volatile organic compounds, steroids, flavonoids, peptides, polyketides, quinones, and terpenoids (Mousa and Raizada 2013; Lugtenberg et al. 2016). Li et al. (2020) recently described a total of 148 active metabolites produced by different *Paecilomyces* species that can be used for drug or agrochemical development. In the following sections, we will show the importance of these metabolites in the biological control of pests and diseases.

22.2.2.5 Management of Plant Diseases by *Paecilomyces*

The effectiveness of *Paecilomyces* against different species of plant pathogenic bacteria is reported. *Paecilomyces variotii* isolated from municipal solid waste compost showed a reduction in 27% of diseases caused by *X. campestris* in melon

and a decrease in the pathogen population (Suarez-Estrella et al. 2013). Sornakili et al. (2020) reported the inhibition of *Erwinia carotovora*, *Xanthomonas*, *oryzae* PV. *oryzae*, and *Ralstonia solanacearum* with in vitro inhibition between 13 and 45% using *P. tenuis*, an endophyte isolated from rice leaves.

Paecilomyces species have shown their antagonistic effect against plant pathogenic fungi causing root and aerial plant diseases through various mechanisms. Plasmolysis in spore germ tubes or hyphal melanization in *Pyrenophora tritici-repentis* (Larran et al. 2016), hyphal lysis in *Moniliophthora roreri* was caused by *Paecilomyces* sp. (Suarez-Estrella et al. 2013), mycoparasitism of *F. oxysporum* caused by *P. variotii* and *P. lilacinus* (Jacobs et al. 2003), or antibiosis against *R. solani* (Horn et al. 1992) were reported.

Yang et al. (2015) observed inhibited *S. sclerotiorum* mycelial growth and sclerotia germination and reduced disease severity after using *P. lilacinus* on a rapeseed crop. In tomatoes, spraying *P. variotii* spores on the leaves significantly reduces damage caused by *Alternaria solani* (Varma et al. 2008). Conversely, the increase in polyphenols and antioxidant activity due to *P. lilacinus* on okra roots improves plant development and control of various phytopathogenic fungi causing root rot (Shafique et al. 2015).

As a hematophagous fungus, *Paecilomyces* has been widely studied and can be found in various biological formulations for agricultural use (Dong et al. 2007). There are many examples where *Paecilomyces* spp. act as nematicidal agents, especially against *Meloidogyne* spp. and against other genera such as *Globodera* (Lima-Rivera et al. 2016), *Rotylenchulus*, *Heterodera*, *Xiphinema*, or *Pratylenchus* (Favre-Bonvin et al. 1991). One example is the use of *P. lilacinus* and *P. fumosoroseus* against *M. incognita* or *M. javanica*, which drastically reduces their populations (Favre-Bonvin et al. 1991; Walters and Barker 1994; Siddiqui and Akhtar 2009; Nesha and Siddiqui 2017) in in vitro (Khan et al. 2004; Perveen and Shahzad 2013) and field tests (Brand et al. 2004; Saha et al. 2016). The spores of these species must germinate on the host to penetrate and colonize its surface to modify its physiology (Favre-Bonvin et al. 1991). *Paecilomyces* act according to the fungal and nematode species it parasitizes.

Paecilomyces spp. can act at different nematode developmental stages by infecting eggs, young or adult nematodes. Nematode eggshell is the main barrier against parasite agents and resistance to chemical nematicides and biological compounds. *Paecilomyces* species can secrete enzymes to degrade this barrier and deploying mechanisms involved in nematode parasitism (Roumpos 2005; Sexton and Howlett 2006). Thus, observations have shown that *Meloidogyne incognita* eggs at early stages of development are more vulnerable than eggs containing fully developed juveniles, although the latter is also affected (Jatala et al. 1980; Dunn et al. 1982; Eapen et al. 2005). Williams et al. (1999) confirm that eggs are parasitized by *P. lilacinus* at all stages, including unhatched juveniles. Egg infection occurs when hyphae lie flat on the egg surface, and appressoria are formed. Then, the fungus spreads, and conidiophores are formed. Studies carried out by Khan et al. (2006) concluded that said juveniles show various degrees of deformities and developmental abnormalities, such as reduced mobility inside the eggs. Different

studies show the significant role of proteases and chitinases in the penetration of the fungus through eggshells. Thus, *M. arenaria* eggshells showed vitelline membrane disaggregation and chitin and lipid layer destruction after using *P. lilacinus* (Morgan-Jones et al. 1984).

Evidence shows that various hydrolytic proteins, such as proteases (mainly serine proteases), collagenases, and chitinases, are involved in nematode cuticle penetration and subsequent cell degradation (Huang et al. 2004; Morton et al. 2004; Ahman et al. 2002; Yang et al. 2011; Pau et al. 2012). Likewise, different secondary metabolites produced by *Paecilomyces* also play a significant role in nematode control (Yan et al. 2011). Nematode control effectiveness using *Paecilomyces* depends on the crop itself, affecting fungal activity in many cases (Al-Hazmi et al. 2017). Thus, using an antagonist in combination with organic substances increases parasitism by *Paecilomyces* in both eggs and larvae of nematodes (Siddiqui and Futai 2009).

22.3 Conclusions

The growth of agricultural production has led to several new challenges, making further growth possible only if these challenges are met appropriately and timely due to excessive use of chemical fertilizers and pesticides becoming a matter of concern. So, biological control can be an alternate system, which may play an essential role in achieving the goal of agriculture agents. Different methods have been tried to test the efficiency of these biocontrol agents, which involves mutation and protoplasm fusion via chemical agents. There is a need for producing these biological agents with better environmental factors for the growth of biocontrol agents. The main challenge is the cost associated with the formulation and safety assessment during the production of commercial biocontrol agents. The biocontrol approach emerged as the promising alternative approach, which provides and ensures a sustainable management system.

The success of biopesticides to suppress pests and diseases depends on the availability of microbes as a product or formulation, which facilitate the technology to transfer from lab to land. The constraints to biopesticides development and utilization mirror some of those factors that limit the development worldwide. Some constraints include lack of the right screening protocol for the selection of promising candidates of *Trichoderma*, inconsistent performance, and poor shelf life, awareness, training, and education shortfalls.

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Challenges and Threats Posed by Plant Pathogenic Fungi on Agricultural Productivity and Economy

23

Garima Anand and Kunhiraman C. Rajeshkumar

Abstract

Fungi represent the largest group of plant pathogens that causes up to 14% crop yield losses annually, rendering it to be one of the prime challenges in achieving global food security and agricultural sustainability. Fungal diseases significantly affect crop yield and productivity, thereby dwindling the global economy at large. The Irish famine, Coffee rust in Ceylon, Great Bengal famine, and Southern corn leaf blight in the USA are examples of fungal disease outbreaks that have been major economic downfall causing millions to die of starvation. To indemnify the devastations of plant pathogens, agricultural biotechnology has emerged as a potential tool to boost crop productivity and thereby the world economy. It has gained colossal acceptance with an expansive growth in recuperating agriculture efficiency with emphasis on technological innovations, development of disease-resistant varieties, employment of effective disease management strategies, and implementation of plant protection schemes. One of the primary challenges faced by agriculturists is to feed the booming population without jeopardizing the arenas of food security, sustainable use of natural resources, and ecosystem resilience. Intensification of agriculture perpetuates the existence of monocultures in fields that pose a serious threat to increased disease epidemics. Facilitation of a dynamic agro-ecosystem that includes integrated crop management systems is significant in improving pathogen resistance. Precise identification of a pathogen, appraisal of its impact in field and productivity, host interaction, epidemiological studies, and understanding the conducive conditions for its proliferation and

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_23

dissemination are the other important challenges faced by agriculturists. Although better crop productivity has been achieved with the use of pesticides and fungicides, their recurrent use has exerted ecological and environmental pressures on agricultural ecosystems and human health. Similarly, understanding the role of climate change on the impact of fungal pathogens and the development of suitable management programs are the need of the hour that yet need to be largely employed worldwide. While the economic impact is not only restricted to the crop or yield loss, the abated quality of crop products also has a significant impact on the revenue. This demands for the creation of a global network of stakeholders that can enforce crop protection schemes and fortify the crop demand. This chapter aims to understand the intricate challenges and threats posed by fungal pathogens on agricultural productivity and the reliable solutions to strengthen and improve global agricultural production and sustainability.

Keywords

Fungal pathogens · Agricultural productivity · Economic loss · Ecosystem sustainability · Virulence factors · Climate change

23.1 Introduction

Fungal diseases pose a serious threat to agriculture with major vulnerability to the crop sector followed by allied threats to the environment. Fungi cause catastrophic diseases in plants as they sporulate prolifically, producing numerous spores that provide primary inoculum to infect plants (Agrios 2005). The latent period, i.e., spell between the primary and secondary inoculum with an upsurge of generation of spores is typically a few days, thereby increasing the pathogen load (Suffert and Thompson 2018). Moreover, the spores are commonly transmitted across fields by wind and water (rain-splash) leading to an easy mode of dissemination. Fungi cause infection and produce compounds and enzymes that hamper the plant machinery and are toxic for the plant (Bergamin et al. 1997). Furthermore, the nutrient balance gets disrupted as the pathogen feeds from the plethora of nutrients stored in the economically valuable part of the plant (Agrios 2005). Any pathogen attack also leads to a deregulation of the plant hormone balance and causes the induction or inhibition of plant growth regulators, thereby limiting the crop yields (Strange 2003; Strange and Scott 2005). The quality of the economically valuable part is also widely affected due to the variable regulation of plant growth regulators.

In order to subside the effects of fungal pathogens on crop productivity, an in-depth understanding of the threats associated with optimum agricultural productivity supporting ecological resilience is required. This chapter lays down the serious threats and the present challenges agriculturists face to eradicate these risks from the system. The potential solutions to strengthen and improve global agricultural production and sustainability are also highlighted.

23.2 Fungal Diseases: A Menace for Agriculture

Pathogenic fungi, which are a major threat to plants and animals lead to about 65% of the total pathogen driven loss of the crops (Fisher et al. 2012). This substantiates for the pollinator loss (Casadevall 2017) causing major losses in the overall agricultural activities (Savary et al. 2012). Fungal diseases are known to devastate one-third of the food crops annually (Fisher et al. 2012). The overall economic losses are enormous, with a massive impact on global poverty. Statistics (2009–2010 world harvest) (www.fao.org or FAOSTAT) analyses the fungal induced losses in five economically important crops, i.e., rice, wheat, maize, potato, and soybean, and states that the losses incurred by fungal diseases were huge enough to feed 8.5% of the seven billion population in the year 2011 (Fisher et al. 2012). Such reports dread the even bigger losses and economic challenges we face with an upsurge of fungal load and the ever-increasing population to feed (Palmgren et al. 2015).

Table 23.1 lists the top ten fungal pathogens responsible for affecting colossal losses in crop plants across the world. The Irish famine, Coffee rust in Ceylon, Great Bengal famine, and Southern corn leaf blight in the USA are examples of fungal disease outbreaks that have been major economic downfall causing millions to die of starvation (Bourke 1964; Padmanabhan 1973; Strange and Scott 2005). The menace lies more in areas where specific crops exclusive to a particular region are cultivated. The tropics are well known for numerous high-valued crops, including coffee, cacao, spices, and nuts that are presently at high risk for fungal infections (Drenth and Guest 2016).

Table 23.1 The top ten most notorious fungi ranked by the extent of economic losses that they incur along with their hosts. (Adapted from Dean et al. 2012)

Rank	Fungus	Disease caused	Host plant/s
1	*Pyricularia oryzae*	Rice blast	Rice and wheat
2	*Botrytis cinerea*	Gray mold	More than 200 host plants
3	*Puccinia* spp.	Rust	Wheat
4	*Fusarium graminearum*	Head blight	All cereals
5	*Fusarium oxysporum*	Vascular wilt	Multiple hosts
6	*Blumeria graminis*	Powdery mildew	Grasses
7	*Zymoseptoria tritici*	Septoria blotch	Wheat
8	*Colletotrichum* spp.	Spots and blights	All crops
9	*Ustilago maydis*	Corn smut	Corn
10	*Melampsora lini*	Flax rust	Flax

23.3 Threats, Challenges, and Remedial Measures

The major challenges posed by plant pathogenic fungi on agricultural productivity and economy in all the three facets of the disease triangle have been shown in Fig. 23.1. Agriculturists must overcome these challenges for agricultural sustainability and subsistence.

23.3.1 Inadequacies in Fungal Identification

One of the major challenges in maintaining the global food security is the current inadequacy in fungal identification (Crous et al. 2016). Fungal plant pathogens are mostly linked to their morphology and symptomology for identification. The fundamental descriptions based on variabilities, mating types, and host specificity in turn get ignored. Most of the current descriptions of fungal species even lack primary genetic data to throw light on some of the issues. This information is critical for precise fungal pathogens identification and understanding their potential impact on crop productivity and yield. Moreover, many fungi remain unknown and unspecified until they cause a considerable yield loss in crop plants. The other concerns for fungal taxonomists include the reliable identification of pathogen to the disease level, i.e., the identification of formae speciales, pathovars, biovars, and races, which is the key for the development of proper disease management strategies (Fones et al. 2020).

With an increase in international trade in plant and plant products, allied with the emergence of pathogens to new areas, accurate identification of fungal pathogens is indeed essential (Desprez-Loustau et al. 2007; Hantula et al. 2014; Wingfield et al.

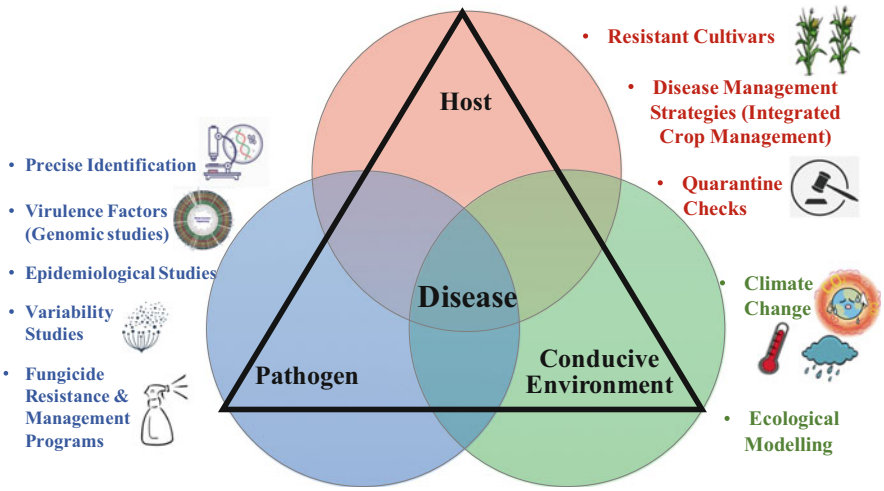

Fig. 23.1 Disease triangle depicting the interactions among pathogen, host, and environment that leads to disease in plants (Scholthof 2007)

2015). The importing countries are exposed to the accidental introduction of new emerging pathogens. A major challenge lies in ensuring a check on quarantine protocols that include specific trade restrictions from areas of high disease incidence, management of goods, and examination of infectious material to reduce the risk associated with the spread of inoculum. Most of the fungal diseases are specified to symptoms that are associated with a specific stage of the life cycle of the pathogen (Wingfield et al. 2012). Many pathogens often remain undetected as latent infections in apparently healthy-looking plants and seeds that may be overlooked in early inspections (Palmer and Skinner 2002; Slippers and Wingfield 2007). A comprehensive analysis of pathogens that remain ignored may represent species complexes that may pose a serious threat in the future (Crous and Groenewald 2005). Pathogen detection and identification are therefore imperative to effectively cope up with the current threats posed by fungi found in commercially transacted plants and plant products. A single fungal pathogen species may be represented as a complex or a pool of various sexes or mating types that may be regulated via various virulence factors and pathogenicity genes. It may cause variability in its response to the host or to the environment. Moreover, many fungal pathogens may also exemplify cryptic species that may cause serious problems both in understanding the nature of the pathogen and substantiating proper quarantine management programs for them (Crous and Groenewald 2005; Perez et al. 2012; Sakalidis et al. 2013).

23.3.2 Current Situation of Disease Management Strategies

The increasing world human population demands higher crop yields and better quality of crop products. However, limited arable lands with draining natural resources question the potential of agronomic practices (Ray et al. 2013). The current shift in agriculture pursuits such as making it large scale and intensive with specialized cultivation has disturbed the host–pathogen co-evolution process. Consequently, pathogens continue to evolve at a much higher rate, but we tend to maintain the plants with traits of interest that subjugate the evolutionary responses in host defense against the pathogen (McDonald and Stukenbrock 2016). Moreover, practices of monoculture with high input of fertilizers and pesticides facilitate the rapid evolution of fungal pathogens that may even lead to epidemics.

One of the major challenges includes the planning and execution of proper disease management programs specific to individual crops. The lack of epidemiology models for plant pathogens is a major limitation that requires the immediate attention of agriculturists. Often, the models based on regulating the output of crops concentrate on the yield of the plant (Madden and Nutter 1995). They do consider a simple calculation based on the logistic growth of epidemics but are unable to predict the actual scenario in terms of pathogen outbreaks (Bebber et al. 2019). This is due to the absence of understanding of the interaction between each crop–pathogen duo. The population size of the pathogen can also vary considerably in a single season. Furthermore, the spatial, temporal proliferation, vector preferences for dispersal of spores and spread of a fungal disease should also be considered (Cunniffe et al.

2015). Improper disease management strategies also include the costs of various associated externalities. Understanding the externalities is also essential to gain insights into the economic analysis of any crop disease. These externalities emerge with a substantial negation of the cost of plant disease management approaches in calculating the overall economic benefit obtained for a crop. The negative externalities include the effects of environmental pollution, overuse of fertilizers and pesticides, toxin production, ecological damage, depletion of natural resources that come as hidden costs overlaying the economic benefit from a crop. This also engages with various stakeholders including conservationists, urban developers, nursery developers, agriculturists, and farmers to gain an overall insight of the problem. This may help in the institution of pragmatic policies to combat disease damage and grasping the concepts of novel pathogen emergence.

23.3.3 Climate Change

The role of climate change on the impact of fungal pathogens is one of the major concerns alarming the agronomists in the current scenario. Agricultural productivity is highly sensitive to climate change (Bebber et al. 2013). A shift in climate parameters such as average temperature and rainfall plays a massive role in determining crop productivity. The interannual climate variability and the temperature shocks at specific phenological stages during the growth have serious implications on the crop yield and productivity. As high temperature and humidity support an even higher fungal infection subsequent disease, climate change is considered a huge threat on agricultural productivity. Global warming and the allied climate changes have augmented the incidence of several fungal diseases (Manning and Tiedemann 1995; Garcia-Solache and Casadevall 2010). The serious concerns for climate change and the linked increase in fungal diseases have even alarmed agriculturists for a pandemic of fungal origin in times to come (Casadevall 2017).

Development of new crop production strategies is therefore the need of the hour. The challenge lies in modeling the complex relationships between crop productivity and predicted climate scenarios (Schlenker and Roberts 2009; Bebber et al. 2016). The challenge for scientists here lies in harboring plant genetic resources to tailor crop plants for adverse conditions via transgenic approaches. The employment of these approaches has been established to enhance crops for traits of economic significance. However, the focus now also needs to shift to redesign crops to ameliorate from biotic and abiotic stresses. Biotic stress, more importantly, fungal and bacterial pathogens have an enhanced effect with increased temperatures and humidity (Agrios 1997, 2005). The altered climate, therefore, poses a higher threat by these pathogens to crop plants. An amalgamation of biotechnological tools, including genetic engineering and transgenics along with conventional breeding permits the agriculturists to transform crops with desired traits.

23.3.4 Virulence Characteristics of Pathogens

Fungal pathogens are responsible for causing disease by subverting or eluding the host defense machinery through an array of virulence factors that ultimately causes pathogenesis (Cross 2008). Secretion of cell wall degrading enzymes (Huang and Allen 1997; Herron et al. 2000; Yakoby et al. 2001; Rouanet et al. 2004) and phytotoxins (Kimura et al. 2001; Wolpert et al. 2002; Möbius and Hertweck 2009) act as virulence factors leading to disease visible as symptoms in plants. Studies elucidating the role of an array of genes involved in defense responses at penetration, infection, and post-infection and the allied pathways have been carried out (Pontes et al. 2020). However, in addition to interspecies variability the pathogens exhibit huge variability at the intraspecies level. This variability within species often gets ignored while planning control strategies causing such plans to fail at large. Variability and diversity analyses of fungal pathogens are therefore marked as an important arena for further studies. Genomic and gene deletion studies have brought into light the various mechanisms through which a pathogen infects and further revealed how the disease progresses through a plant (Pandey et al. 2016). The mechanisms when studied in detail signify the importance of every single enzyme and phytotoxin (Strange 2007) for disease progression. Although a large number of fungal pathogens have been identified, the reports on virulence factors still stay abstruse (Reino et al. 2004; Casadevall and Pirofski 2009). Research on virulence factors using molecular biology and biochemical tools involves the comparison of wild and mutant type pathogen for characterizing their functional roles in determining pathogenicity. Regulating the expression of these factors may have direct implications in disease control strategies. These strategies are the current aim and a foremost challenge for researchers and agronomists for the development of new disease control methods for subsistence food production. Although the mechanisms are complex and require an extensive understanding of host–pathogen interactions, the outcome targets safer and specific strategies for crop disease control.

Therefore, it is imperative to gain a better understanding of the mechanisms of pathogen–plant interactions to devise new and superior biocontrol strategies. This in fact is the future for disease management programs.

23.3.5 Pathogen Evolution and Fungicide Resistance

The agrarian ecosystems are stable populations of genetically similar plants grown at a high density spanning large areas. Moreover, the composition of species may remain unchanged not across regions but also through the years. This makes the environment extremely conducive for the emergence and dissemination of fungal pathogens. With a homogeneous crop population as host, the fungi uphold a huge population load in fields that are easy to disperse. The fungal genetic and population genomic studies reveal the underlying mechanisms responsible for the pathogen evolution (Raffaele and Kamoun 2012; Möller and Stukenbrock 2017). These may include processes such as speciation, adaptive evolution, and dispersion across

different agricultural ecosystems. It is also useful to know the evolution of a fungal species in fields sprayed with fungicides or across fields with crops containing pathogen-resistant genes. The time of dissemination across fields, states, countries, and continents reveals the significance of human intervention that can be lethal for agro-ecosystems. Anthropogenic activities leading to the pathogen spread are responsible for the emergence and introduction to naive fields and crops (Fones et al. 2020). The introduced fungus may trade genetic material with local non-virulent strains through horizontal gene transfer processes (Raffaele and Kamoun 2012; Croll and McDonald 2017). This may lead to the establishment of new aggressive fungal strains in healthy fields. Few examples that confirm this phenomenon include the expansion of wheat blast to wheat growing countries of Asia leading to crop losses of up to 50% (Brasier and Kirk 2010; Mottaleb et al. 2018), chestnut blight, Dutch elm disease, ash dieback that have caused more than 90% damage to the respective crop plants (Raffaele and Kamoun 2012).

To date, fungicides are the chief components of disease control management programs. With the fungicide chemical market boosting, it alarms to focus on the ecosystem health of the crop fields (Oliver and Hewitt 2014). Agriculture today cannot thrive without the arsenal of fungicides. However, this approach is expensive and short-lived as the pathogens gain resistance or become tolerant against them (Bosch et al. 2018; Elderfield et al. 2018). With monocultures being the prominent form of farming, the pathogens proliferate enormously infecting the crop plants through various growing seasons (Fisher et al. 2012). New strains of the pathogen evolve that are resistant to the generic fungicides. The fields become feeding and breeding grounds for pathogen surge and not only sustain large populations but contain strains with high genetic variability. Favoring the evolution of the fungal pathogens, a complete wipe down of control strategies and disease management programs occurs.

The most critical challenge calls for the re-designing of the agricultural management programs that support the dynamic diversity of the pathogen considering the rate of evolution and the corresponding resilience in fungal pathogens.

23.4 Conclusions

Fungal phytopathogens have created havoc in the agricultural sector through centuries. Our current agronomy practices emphasize excessive monoculture practices that bring allied threats for the emergence of new fungal pathogenic strains and fungicide resistance. Globalized markets with no quality and quarantine inspections and unrestrained anthropogenic activities also introduce fungal loads from one region to another (McDonald and Stukenbrock 2016). The trade of crop products through the international food market has major repercussions on food security as it dives into the massive blowout of fungal pathogens. Climate change also fosters the advent of pathogens in otherwise marked healthy fields and uncontaminated areas. The adversity is aggravated with insufficiencies of systematic and precise identification of pathogenic fungi (Stukenbrock and McDonald 2008). Our

approach lacks fundamental revisions on taxonomic information along with the understanding and identification of cryptic fungi. Encouraging studies on fungal taxonomic identification is the need of the hour. It is therefore remedial to bring a series of amendments to the current management programs for disease control. The Green revolution boosted global agricultural productivity through the prolog of fertilizers and fungicides/pesticides along with dwarf and disease-resistant varieties (Evenson and Gollin 2003). The testimony of time teaches us how reckless acts of overuse have contributed to the loss of biodiversity, depleted resource inputs, degraded ecosystems, and worsened pathogen outbreaks across the globe. Cognizing the atrociousness of the risks associated with the current scenario, identifying the threats to agricultural productivity and economy, it is upsetting to see the lack of global urgency to tackle them. A thorough ratification of management programs is therefore a prerequisite for fundamental control. It is also necessary that the studies on diverse hosts, fungal pathogens and their interactions be considered as a priority for researchers. Investigation on environment–host–pathogen interactions and the effect of climate change on these interactions should also be fueled with enough funding to motivate this arena of studies.

In conclusion, the present challenges withhold the redressal of planned strategies to restrain fungal pathogenesis. A stringent global biosecurity protocol for international trade and the development of suitable management programs are immediate concerns for sustaining agricultural productivity and thereby the economy at large.

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Challenges Faced by Farmers in Crops Production Due to Fungal Pathogens and Their Effect on Indian Economy

24

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Abstract

Indian agriculture is considered a global powerhouse. It is the second largest producer of rice, wheat, sugarcane, fruits, vegetables, cotton, and tea. In India, the agri sector employs around 60% of the population and contributes about 17% to the total GDP. One of the major constraints Indian agriculture facing is its low yield, which is 30-5% lower than those of developing countries. The challenges stagnating agricultural productivity in India include outbreaks of pests and diseases, poor soil fertility, unavailability of sufficient water, and climate change. Among all the factors, plant pathogens especially fungal pathogens are of key concern, and they are a major yield-limiting factor in agriculture. According to Punjab Agricultural University in 2007, 26% of yields got lost due to plant diseases. Pest and diseases cause over INR 290 billion per annum losses of crops in India. Out of 30,000 plant diseases recorded from different countries, around 5000 occur in India. The fungal infections related decline in crop yield in India is believed to be 5 million tons per year, approximately. In 2012, fungal diseases ruined at least 125 million tons of the crops like wheat, rice, soybeans, maize, and potatoes. The global damage to rice, wheat, and maize by the fungi accounts for $60 billion each year. This shows serious economic implications, resulting in production losses, market declines, and increased unemployment in

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_24

the food and agriculture sector. Thus, there is an urgent need to emphasize the problem of plant diseases so that preventive measures can be taken up. Plant pathology has a special role to meet new challenges for sustainability and advancements of Indian agriculture.

Keywords

Agriculture · Plant pathology · Fungi · Economy · Challenges

24.1 Introduction

Microorganisms such as molds, yeasts, and several others are used since historic times. They survive in the tissues of dead or alive plants. Fungi differ greatly from the other organisms of the biological system; they constitute the principal decomposers of organic matter. Fungi are huge components of organic waste and target cellulose, lignin, gums, and some other complex organic substances. Fungi are an ensemble of eukaryotic cells, source of food supply, alcohol, antibiotics, enzymes, and amino acids. They also play an important role in various physiological processes such as mineral and water uptake, chemical transition, stomatal movement, and biosynthesis of compounds such as biostimulants, auxins, lignan, and ethylene to improve plant flexibility in detecting and coping with ecological stress such as drought, salinity, extreme temperature, and significant metals. Fungi can also be involved in a large variety of soil responses from acid to alkaline. In various physiologies, fungi perform a fundamental function together (Yuvaraj and Ramasamy 2020). Fungi have also evolved a variety of techniques for colonizing plants, and such interactions produce a wide range of effects, from good interactions to mortality in host countries. In terms of plant diseases, fungi are likely the most complex category of ecologically and economically significant threats. Phyla Ascomycota and Basidiomycota typically include fungal plants pathogens. Plant fungal pathogens of Ascomycota are grouped into different classes such as Dothideomycetes (e.g., *Cladosporium* spp.), Sordariomycetes (e.g., *Magnaporthe* spp.), and Leotiomycetes (e.g., *Botrytis* spp.). Similarly, rusts and smuts are the two principal groups of plant pathogens in Basidiomycota (Doehlemann et al. 2016).

24.2 Crop Losses in India

The crop loss, which is the difference between the healthy crop yield and the sick crop yield, is mostly stated in financial terms. An evaluation of crop loss is the main objective of the disease evaluation. However, it is a tough task due to the non-availability of an easy methodology to quantify the amount of loss of income due to diseases. The difference in yield of crop, quality, and loss of market value should be assessed in determining the yield loss of any disease. The estimation of losses in diseases such as smuts, root rots, ergot, etc., which cause nearly 100%

damage to crops, is easy to make. However, it is difficult to determine losses for diseases, which partially impair production in many respects (Sharma and Karthikeyan 2017).

Comparisons between crops cultivated in various crop seasons or locations are not valid in calculating the loss of output owing to diseases, because other factors are not the same. For accurate comparisons, the disease-free plots should be compared with those having different diseases in nearby. To evaluate the yield loss on a regional level, it is possible to use formulas based on fungicide trials to use the disease incidence data collected from the study. These data are generally used in the model of the critical point and the regression equations. Various models based on loss estimates of many diseases appear to be more reliable. The data are utilized to build a multi-dimensional model that measures the start date, the form of the pattern of disease, the host variety, and the loss of yield as the dependent variables.

Wheat rusts annually cause an economic loss of INR 4 billion. In outbreak years, there are greater losses of INR 50 billion. The total annual loss of wheat from loose smut (which is roughly INR 5 billion) is expected to be 3%. Some other fungal diseases like red rot in sugarcane, rice blast and blight, Karnal bunt in wheat, apple scab, and mango malformation are the causes of massive losses.

Mycotoxins like aflatoxins, fumonisin, etc. occurring in the infected fruits and grains may cause insanity, paralysis, stomach disease, and hepatic cancer in animals and humans when consumed. The money expended on plant disease control is also a waste as this money can be avoided in the absence of diseases. Besides the yield loss due to plant diseases, there are several other consequences for the transport and agri-based industry. For instance, the farm products containing pathogens and pesticide residues are always at risk of quarantine causing additional losses (Sharma and Karthikeyan 2017).

24.3 Major Crops Affected by Fungi

The study conducted at the Indian Institute of Technology, Chennai claims that fungi cause more harm to main crops—such as rice, potato, tomato, and ginger—than bacteria and viruses (Yadav et al. 2020). Plant diseases triggered by fungi restrict plant growth, generate marks, influence blossoms and fruits, and eventually kill the infected plants. Fungi are the cause of around 8000 types of plant diseases, of which prominent have been mentioned in Table 24.1. Between 1998 and 2018, scientists led by Sachin S. Gunthe collected data from different sources and reported 4000 records of plant disease associated with fungi in India. In the previous 20 years, it has been noticed that 69 fungal infections have negatively affected 39 plants (Yadav et al. 2020).

The *Puccinia striiformis* affected wheat crops 12 times throughout this period. Additionally, nine kinds of fungi including *Pyricularia oryzae* were discovered to harm paddy. The *Phytophthora infestans* devastated plants such as potatoes, tomatoes, and ginger at 14 sites in India. The incidence of fungal diseases is connected to the mixture from surface air with top layer air and so increases the

Table 24.1 Major crop/plant affected by fungal diseases

S. No.	Crop/plant	Disease
1.	Sugarcane	Red rot
2.	Soybean	Rust
3.	Cotton	Wilt
4.	Rice	Blast
5.	Wheat	Rust, powdery mildew
6.	Potato	Late blight
7.	Maize	Downy mildew

Table 24.2 Major fungal diseases of wheat

S. No.	Disease	Pathogen
1	Stem rust (black rust or cereal rusts)	*Puccinia graminis* f. sp. *tritici*
2	Leaf rust or brown rust	*Puccinia triticina* (Syn. *Puccinia recondita* f. sp. *tritici*)
3	Stripe or yellow rust	*Puccinia striiformis* f. sp. *tritici*
4	Hill bunt or stinking smut or common bunt	*Tilletia caries* and *Tilletia foetida*
5	Karnal bunt or partial bunt	*Tilletia indica* (Syn. *Neovossia indica*)
6	Loose smut	*Ustilago nuda* var. *tritici.* (Syn. *U. segetum* var. *tritici)*
7	Flag smut	*Urocystis agropyri* (Syn. *U. tritici*)
8	Powdery mildew	*Blumeria graminis*

concentration of fungal bioaerosols. The concentration of bioaerosol peaks in January and begins to fall in February (Yadav et al. 2020).

24.3.1 Wheat

Wheat (*Triticum aestivum* L.) is the second largest cereal crop in India, just next to rice. It is one of the main food grains of Indian diet. It is rich in carbohydrates, proteins, and vitamins and offers equitable nutrition. Globally, India is the fourth-largest producer of wheat after Russia, the USA, and China and represents 8.7% of total wheat global output. It is grown in many states of India generally from September to December. Weeds, diseases, and pests significantly affect the quality as well as production of wheat. There are varieties of fungal diseases causing output losses in wheat that are very difficult to identify by the farmers (Table 24.2) (https://icar.org.in/node/8098).

24.3.2 Rice

Rice is one of the major food safety plants. It feeds about 2.7 billion people worldwide. A nasty disease caused by *Magnaporthe oryzae* and called rice blast,

Table 24.3 Major fungal diseases of rice

S. No.	Disease	Pathogen
1.	Blast	*Pyricularia grisea* (*P. oryzae*)
2.	Brown spot	*Helminthosporium oryzae*
3.	Sheath rot	*Sarocladium oryzae*
4.	Sheath blight	*Rhizoctonia solani*
5.	False smut	*Ustilaginoidea virens* (telomorph *Villosiclava virens*)
6.	Grain discoloration	*Ustilaginoidea virens*

Table 24.4 Major fungal diseases of soybean

S. No.	Disease	Pathogen
1.	Stem rot	*Rhizoctonia solani*
2.	Rust	*Phakopsora pachyrhizi*
3.	Stem rot	*Sclerotinia sclerotiorum*
4.	Leaf blight	*Septoria glycines*

despite the finest management methods, has afflicted the crop. This fungal pathogen damages practically every part of the plant but is most harmful during planting phase. In India, rice blast is a common disease, which is more widespread in locations having high humidity and low temperature at night (https://icar.org.in/node/4461). Other important fungal diseases of rice are given in Table 24.3. In the near future, there is little opportunity to increase the area under paddy cultivation. Thus, the extra demand of rice must be met with an increase in the crop productivity (Kumar and Ladha 2011). The maximum yields can only be attained per unit of the land area if there is a provision for the protection of the crop from its enemies coupled with high-yielding crop varieties (Srivastava et al. 2010).

24.3.3 Soybean

Soybean (*Glycine max*) is a Chinese crop that was brought to India millennia ago via Himalayan routes. Therefore, on a modest scale soybean has historically been grown in Himachal Pradesh, Uttar Pradesh, Uttarakhand, Eastern Bengal, the Khasi Hills, Manipur, the Naga Hills, and areas of central India including Madhya Pradesh. At present, it is an important oilseed crop of India. The area under soybean cultivation is increasing. For instance, soybean cover in 2003 was 6 million hectares, which increased in 2011–2012 up to 9.33 million hectares. Consequently, India has become the world's fifth-largest soybean producer (Dutta et al. 2013).

Madhya Pradesh and Maharashtra are the two topmost soybean-producing states followed by Rajasthan in India and account for 86% of the country's total acreage and production (Dutta et al. 2013). The majority of soybean crop in India is planted in the month of July but in the central and southern states, it is planted in the spring.

Among major fungal diseases of soybean (Table 24.4), the Asian soybean rust disease caused by *Phakopsora pachyrhizi* was discovered for the first time in India, in 1951 (Sharma and Mehta 1996). In the Northeastern Hill region, frogeye leaf spot

(*Cercospora sojina*), rust (*Phakopsora pachyrhizi*), powdery mildew (*Microsphaera diffusa*), and purple seed stain (*Cercospora kikuchii*) have all been observed in moderate to severe forms (Prasad et al. 2003).

24.3.4 Potato

Globally, after wheat, rice, and maize, potato is the most important food crop. Farmers in developing nations will have to treble their productivity to feed the expanding population during the next three decades, when the world population is estimated to expand by about 100 million per year, putting further strain on land, water, and other resources (Zandstra 2000). For such situation, the potato has great potential as a source of food for millions of people, particularly in developing nations including India. In the majority of emerging nations including India, potato production and consumption are growing. In fact, developing-countries potato production has surpassed that of developed-countries potato production. Potatoes are farmed in India under several weather conditions, from tropical, subtropical to humid highlands (Sharma 2013).

Diseases may strike potatoes at any stage of development, including storage. They may have an impact on the foliage, tubers, or both. Pathogens that thrive in a warm environment can damage the crop. The consequences of the historical potato famines in Europe, especially in Ireland, caused by late blight, have been widely chronicled (Woodham-Smith 1962). Tuber diseases such as common scab, black scurf, dry rots, and soft rot may not completely destroy the crop, but they can significantly affect its quality and marketability. The disease situation may vary from time to time as resistant cultivars and improved cultural methods are introduced, necessitating frequent inspection (Khurana 1998). Any environmental changes, such as global warming, may potentially influence diseases (Khurana 1998). A number of reviews related to fungal and bacterial diseases are accessible in Indian context (Khurana 1998). Around 160 diseases can harm the potato crop, of which fungi, bacteria, and viruses cause 50, 10, and 40, respectively, and the rest by non-parasitic or unknown causes. Some of the major fungal diseases of potatoes, which are commonly found in India, are listed in Table 24.5.

24.3.5 Maize

In terms of area, India is the world's fifth-largest country to cultivate maize, yet it falls to the tenth position based on production. In the Asia region, 37% of the acreage and 34% of output are next to China (Anonymous 1973). In India, maize is attacked not just by downy mildews, but also by leaf and sheath, stalk reds, rust and smuts and ear-reds, all of which sum to around 35 in number. Payak and Renfro (1973) calculated the national yearly decrease in grain yield, ascribable to diseases, at 3,706,450 quintals. Some major fungal diseases of maize, which are common in Indian states, are listed in Table 24.6.

Table 24.5 Major fungal diseases of potato

S. No.	Disease	Pathogen
1.	Late blight	*Phytophthora infestans*
2.	Early blight	*Alternaria solani*
3.	Black scurf	*Rhizoctonia solani*
4.	Charcoal rot	*Macrophomina phaseolina*
5.	Silver scurf	*Helminthosporium solani*
6.	Fusarium wilt and dry rot	*Fusarium* spp.
7.	Verticillium wilt	*Verticillium albo atrum*
8.	Sclerotium wilt	*Sclerotium rolfsii*
9.	Sclerotinia wilt	*Sclerotinia sclerotiorum*

Table 24.6 Major fungal disease of maize

S. No.	Disease	Pathogen
1.	Banded leaf and sheath blight of maize	*Rhizoctonia, solani* f. sp. *sasakii*
2.	Northern maize leaf blight or Turcicum leaf blight of maize	Anamorph (asexual phase): *Exserohilum turcicum* (syn. *Helminthosporium turcicum*)
		Teleomorph (sexual phase): *Setosphaeria turcica*
3.	Brown spot of maize	*Physoderma maydis*
4.	Head Smut of maize	*Sporisorium reilianum* (Syn. *Sphacelotheca reiliana*; *Ustilago reiliana*)
5.	Common smut of corn (Syn. boil smut, blister smut)	*Ustilago maydis* (Syn. *Ustilago zeae*)
6.	Downy mildew of maize	*Peronosclerospora philippinensis*, *P. sorghi*, *P. sacchari*, *P. maydis*, *Sclerospora graminicola*, *S. rayssiae*
7.	Brown stripe downy mildew of maize	*Sclerophthora rayssiae* var. *zeae*
8.	Philippine downy mildew of maize	*Peronosclerospora philippinensis*

24.4 Impact of Diseases on Indian Economy

Fungi symbolize a huge danger to food safety given that they cause diseases in crops, which make up the largest proportion of worldwide food consumption. It has been estimated that fungal infections are one of the damaging diseases in paddy cultivation. For example, *Magnaporthe oryzae* causing rice blast has been ranked on the top in the most academically and commercially relevant "top ten" list of fungal infections for its catastrophic ability to disrupt world food supplies (Ou 1980; Dean et al. 2012). Other important fungal plant diseases are soybean rust (*Phakopsora pachyrhizi*), wheat stem rust (*Puccinia graminis*), maize corn smut (*Ustilago maydis*), and potato late bite (*Phytophthora infestans*).

In the agriculture history, there are many examples of fungal diseases that have hampered food safety, substantially. The oomycete *P. infestans*, for instance, caused the famine of Irish potatoes in the 1840s, which resulted in one million deaths from hunger and one million displaced, and led to a 20% fall in Ireland's population (Fry and Goodwin 1997). This catastrophe was not only a humanitarian disaster but also altered the political and economic history of Europe. In India, the 1943 Bengal famine caused by *Cochliobolus miyabeanus* led to the deaths of 2–3 million people because of population dependency on rice as a key source of sustenance (Padmanabhan 1973). Similarly, the outbreak of southern corn leaf blight (*Cochliobolus heterostrophus*) led to major crop failure in the USA (Ullstrup 1972).

In India, on average 20–30% of the food produced by the farmers is lost annually because of pests and diseases. The fungal diseases cost $60 billion per annum to rice, wheat, and maize alone. Annually, fungal diseases annihilate 125 million tons of rice, wheat, maize, potato, and soybean, which is sufficient to feed more than 600 million people (Fisher et al. 2012). Consequently, management of pests and diseases via primary plant protection is crucial for food safety of the rising human population of the country. In the 1960s and 1970s, the "Green Revolution" raised crops productivity significantly and made India independent on food supplies. Apart from high-yielding crops, chemical fertilizers and irrigation pesticides have played an important role in the success of the Green Revolution. The availability and application of safe and efficient pesticides are important for the continuous improvement in farm productivity and production. Among three varieties of wheat rust diseases, leaf rust (*Puccinia triticina*) is more common, world over and thus leads to larger production losses than any other wheat rust. Leaf rust may cause grain production losses above 50% in the event of severe outbreaks, if no fungicides are administered. During the 1970s and 1980s, Indian wheat production encountered major rust related issues, which were eventually controlled efficiently (https://icar.org.in/node/4531). Worldwide, over 678.7 million tons of rice is produced on 161 million hectares, yearly (FAO-Food and Agriculture Organization 2009). The contribution of Asia in total rice production is around 90% (143 million hectares of production area, 612 million tons) (FAO-Food and Agriculture Organization 2009). Rice provides more than 3 billion Asians between 30 and 75% of their total calories (Braun and Bos 2004; Khush 2004). To fulfill world rice demand, rice production must be increased almost 114 million tons, which is the equivalent of a total 26% increase, by 2035. Paddy infections are one of the major important biotic variables affecting rice production. The world's average annual losses from rice diseases are estimated at 10–15% that reach up to 75% in epidemic conditions. Rice diseases were almost inconsequential in the tropical Asia when old cultivars, which were disease resistant but low yielding, were routinely cultivated on low-fertility soils (Areygunawardena 1968). However, with growing need for worldwide rice supplies and green revolution high-yielding varieties are being now preferred. Further, high fertilization, irrigation, and intense cultural behavior have led to a substantial increase in the incidence and severity of rice infections in several nations or countries (Teng 1990). Rice blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia solani*), bacterial blight (*Xanthomonas oryzae*), and tungro virus disease are the principal rice

diseases caused by various pathogens that cause severe economic losses, mainly in Southeast and South Asia (Ling 1980). The main fungal diseases observed during pre-independent India and before the introduction of high-yielding varieties were the rice blast and brown spot. However, after introduction of high-yielding varieties bacterial blight, tungro, and sheath blight became serious diseases. Recently there has been a significant loss of output due to fungal diseases such as sheath red, false smut, stem rot, and grain discoloration that were once less important and appearing intermittently. Of the total loss of yield owing to rice diseases, 35% is attributable to blast, 25% to sheath-bull, 20% to bacterial blight, 10% to tungro, and 10% to other diseases. Fungicides of INR 380 crores were used on rice, of which INR 280 crores were spent on blast and sheath blight, and the proportion of fungicides employed against brown spot, blackened grain, stamping red, and false smut was INR 100 crores, in India for the year 2010–2011 (Prasanna et al. 2011).

24.5 Conclusions and Future Prospective

The task of agriculture, which has received attention, is to satisfy the food security demands of the country. Besides this, other goals are to enhance food quality, as well as to safeguard the ecological and natural environment. High crop productivity is a necessary component of a thriving agriculture business. There are several reasons that lead to decline in the crop yield. Application of improved pre- and post-harvest technology is essential to reduce the losses in crop productivity and to increase the income of farmers. For effective management of plant diseases, their impact on economy and sociology and ecology of management approaches through a full understanding of plant epidemic mechanisms and healthy agro-ecosystems functioning are required to make plant disease management sustainable.

The general impact of fungal diseases on human health goes beyond human infections as fungi damage one-third of food crops yearly that results in economic losses and worldwide poverty (Fisher et al. 2012). Fungal diseases induced losses in five major crops, namely rice, wheat, maize, potatoes, and soybean are evident from world harvest statistics for the year 2009–2010 (www.fao.org/FAOSTAT1). Almost 61% of the world's human population does not have sufficient food. The current crop production would be sufficient to feed 8.5% of seven billion people by simply mitigating the ongoing crop losses (Fisher et al. 2012). In addition to major food crops, there are other numerous high-value tropical plants such as banana, coffee, cacao, and mango and several nuts and spices in which fungi cause infection (Drenth and Guest 2016). All these have worldwide economic ramifications because of our dependency on crops grown in tropical regions, which further exacerbate due to lack of biodefense and preparation.

Fighting against fungal diseases will remain the major problem of plant pathology. Fungi are a varied group of organisms that have a significant agricultural impact, as they have a wide range of lifestyle and significant genetic flexibility, allowing pathogens to invade new hosts fast, to acquire pesticide resistance, or to break apart the resistance of R-gene mediated seedlings. The battle against fungal

infections will thus continue to be a key problem in future plant pathology. We need to improve our knowledge of the processes for fungal virulence, the way pathogenic characteristics evolve, the molecular foundation for host adaptability, how to make crop resistance more sustainable, and to create novel approaches to manage pathogenic plant fungi. Apart from these qualities, pathogenic plant fungi are also intriguing creatures, which have a vast array of colonization methods. Studying the molecular mechanisms behind these intricate interactions is a good approach for understanding host–parasite relationship. Effector proteins are useful molecular instruments to research plant pathways because they are precisely suited to specific cellular activities in the host. In addition to effector proteins, the activities of micro RNAs in plant–fungus interactions are little recognized. Similarly, the role of secondary metabolites in biotrophic interactions also requires extended focus. As of now, the interactions between plants and fungi have mostly been examined individually, but this is quite unnatural since plants are constantly colonized by a complex microbiome, in which different microorganisms affect one another and affect the plants in several ways. The results of pathogenic infections are becoming increasingly obvious with multitrophic interactions. For this reason, the impact of microbial communities on pathogen growth and plant healing should be considered in the future research on plant diseases. Considering ecological aspects of plant diseases, future studies need to focus on: (a) epidemic and evolutionary plant disease patterns in changing environment, (b) philosophy on agricultural production, and (c) the role of ecological aspects in agricultural productivity and crop health.

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Understanding the Various Strategies for the Management of Fungal Pathogens in Crop Plants in the Current Scenario

25

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Abstract

Agricultural sector is being continuously under threat since a long time due to losses associated with phytopathogens. In order to surmount this problem, majority of farmers highly depend on the chemical fungicides to protect the crop plants and balance productivity. However, frequent use of such fungicides leads to contamination of soil, water, and air along with the rise of resistant fungal strains. These new fungal strains are becoming a matter of concern as they are constantly modifying themselves at genetic level and therefore difficult to control with conventional methods. In lieu of this, molecular tools and techniques have proven to be valuable in understanding plant–pathogen interactions and help to identify various host as well as fungal genes that play a crucial role in this interaction. Genes that are associated with the production of antimicrobial peptides, phytoalexins, hydrolytic enzymes, and defense-signaling molecules are the prime target for the management of phytopathogens. In addition, emerging areas such as CRISPR-CAS and RNAi have been successfully employed for controlling fungal pathogens. Nanobiotechnology has also become a novel alternative approach for plant disease management. Nano forms of various elements (carbon, silica, silver, etc.) are being used for their efficiency to control plant diseases. In contrast, biofungicides that comprises consortium of living organisms provide an eco-friendly approach for sustainable agriculture over

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_25

chemical fungicides. With this background, an attempt has been made in this chapter to highlight the various strategies that have been adopted in recent years for the management of fungal pathogens in crop plants. The chapter will also discuss the application of biofungicide and its advantages over conventional methods.

Keywords

Biofungicides · CRISPR/CAS · Innate immunity · Nanofungicides · RNA interference

25.1 Introduction

Fungal diseases are responsible for causing significant yield losses in a number of crop plants that has led to alarming situation all over the world due to concerns over global food security. Simultaneously, global food demand will also be soaring to extreme levels by 2050 owing to rise in population index (He et al. 2016). In order to feed the ever increasing population, new strategies are being continuously evaluated for minimizing the crop losses due to various fungal phytopathogens and at the same time augmenting growth and productivity of different crops. Plant breeding techniques and genetic improvements have shown significant prospects in management of losses incurred due to fungal pathogens. A number of transgenic plants have been raised with *R* gene as well as other genes involved in plant innate immunity (Nelson et al. 2018).

Plant immunity is an amalgamation of various cellular and subcellular components involving receptors proteins, transcription factors, elicitors, and antimicrobial genes (Andersen et al. 2018). Most of the present studies are utilizing advanced genomic and metabolomics techniques to identify regions of genome responsible for susceptibility or resistance in plants and virulence in pathogens and deduce this knowledge for creating resistant plants and avirulent pathogens (Piquerez et al. 2014; Aylward et al. 2017). Studies are also focused on to investigate complex interactions occurring between plant and pathogens during pathogenesis and examine the role of various genes and proteins owing to secretion of toxins, metabolites, etc. for disease manifestation by pathogens (Miller et al. 2017; Chen et al. 2019a).

Clustered regularly interspaced short palindromic repeat (CRISPR/CAS9) and RNA interference technologies have emerged as key molecular techniques that have shown significant prospects in plant disease control (Borrelli et al. 2018). Both of these techniques have been used substantially to control growth of different fungal pathogens and prevent losses occurring due to fungal pathogens in plants (Goulin et al. 2019; Ganpatrao 2020). CRISPR/CAS9 utilizes Cas9 endonuclease to target specific loci on host genome and create knockout mutants, e.g., knockout mutants of susceptible gene loci (SLO) in different plant systems showed decline in disease expression in response to pathogen attack (Naghmeh et al. 2016; Yin and Qiu 2019).

At the same time, CRISPR/CAS9 has been utilized for genome editing of fungal pathogens such as *Ustilago maydis*, *Botrytis cinerea* and through these specific regions are disrupted that may be responsible for its virulence. Enormous efforts have been made to investigate the role of various genes responsible for toxins; metabolites, etc. that is known to play a vital role in plant–pathogen interactions (Pontes et al. 2020). CRISPR/CAS9 possesses wide applicability in plant biology due to its approach in targeting and modifying host genome for a large number of agronomic traits and producing new genetically better varieties (Chen et al. 2019a, b). Affirmative response of CRISPR/CAS9 genome editing has been well observed in oilseed crops (Subedi et al. 2020), fruit crops (Wang et al. 2019), and many others (Khatodia et al. 2016).

RNA interference (RNAi) exhibited remarkable progress in the field of plant pathology in lieu of its significant role in crop protection. RNAi encompasses use small interfering RNA (SiRNAs), double strand RNA (dsRNA) for targeting specific gene sequences and generating knockout mutants that show loss of function (Ganpatrao 2020). RNAi also brings about host-induced gene silencing (HISC), spray-induced gene silencing (SIGS), and fungal induced gene silencing (FIGS), all of which utilizes dsRNA, sources of which may vary but the target genes are highly sequence specific. RNAi has been shown to inhibit growth of different fungal pathogens affecting various economically important crop plants.

Nanotechnology has shown a new way to plant biologists and its amalgamation with biotechnology delivers new approaches to enhance plant growth, development, and protection from pests and pathogens (Worrall et al. 2018; Shang et al. 2019). An important idea that has shown favorable response in plant pathology is the role of nanobiotechnology for devising methods for fungal disease management and constructive efforts have been made in this field for development of nanofungicides. Elmer and White (2018) reviewed the antifungal effects of various metallic, metalloids, and non-metallic nanoparticles and their application in plant disease management and disease diagnostics. Additionally, there are reports of various biogenic nanoparticles in regulating growth of different fungal pathogens (Ali et al. 2020). Among the different nanoparticles, silver (Ag) nanoparticles have been proven to be highly effective followed by copper (Cu), zinc (Zn), gold (Au), silica (Si), etc. These nanoparticles may be applied directly to plant as foliar nanosuspension or may be used in the form of some commercial formulation in the form of nanofungicides (Mittal et al. 2020). Apart from the antifungal effects of nanoparticles, these nanoparticles also find their application as nanofertilizers due to small size, high reactivity, and better absorption to target cells and form the basis of precision agriculture (Duhan et al. 2017).

Conventional agriculture employs extensive use of synthetic fertilizers for augmenting crop productivity and chemical fungicides for the management of plant pathogens. Both of them are extremely harmful for the environment due to their toxic effects on ecosystem and at the same time on the crop quality. To overcome such toxic effects and improve crop quality, application of biofungicide to regulate growth of different fungal pathogens is the best suited method. Biofungicides can be defined as use of biologically living organism/consortia for the management of fungal pathogens. These biofungicides can be synthesized from

any living source like bacteria, fungi, lichens, algal and plant extracts, etc. and may be used in the form of liquid suspension or in the form of solid consortia that can be applied in the fields. Similar to these biofungicides, a number of biocontrol agents have also been utilized for controlling growth of pathogens in the fields that is an eco-friendly approach (Ram et al. 2018). Recently, Raymaekers et al. (2020) comprehensively reviewed the potential biocontrol agents that are being used in management of plant pathogens.

An attempt has been made through this chapter to provide an in-depth knowledge of various strategies that can be adopted to enhance disease resistance in plants towards fungal pathogens. The chapter will highlight upon the constituents of plant innate immunity complex and other related tools that can boost immunity in plants. The chapter will discuss about modern day tools such as CRISPR/CAS9 and RNAi and their application for controlling the growth of fungal pathogens in plants. The chapter will also provide recent insights into the emerging concept of biofungicides and nanofungicides for the pathogen control in the coming decade.

25.2 Strategies for the Management of Fungal Pathogens in Crop Plants

25.2.1 Strengthening Plant Innate Immunity

Plant innate immunity is maintained by two types of system one of which is mediated by different cell surface receptors that help to sense microbial infection and activating downstream cascade of events (Bentham et al. 2020) (Fig. 25.1). The second type of innate plant immune response is facilitated by *R* genes (Bentham et al. 2020). Cell surface receptors can be categorized into receptor-like kinases (RLKs) and receptor-like proteins (RLPs), trans-membrane in nature commonly referred to as pattern recognition receptors (PRRs) and recognize molecular structural signals of pathogens called as pathogen associated molecular patterns (PAMPs) (Dodds and Rathjen 2010). Wu et al. (2018) reviewed the different categories of receptors present both on the cell surface and in the interior of cell that play a crucial role in plant innate immunity towards pathogen. Recognition mediated by PRRs will bring about activation of immune response in host which is defined as PAMP triggered immunity (PTI) (Dodds and Rathjen 2010) and is considered as one of the dynamic form of resistance possessed by the plant system towards the non-selective pathogen. These PAMPs may be bacterial flagellin or components of fungal cell wall such as chitin and this PTI could be one of the prime targets for inducing resistance in a variety of crop plants. Saijo et al. (2018) showed different PRRs in plants systems that recognize various fungal PAMPs including oomycete, thereby activating PTI. Studies have shown that with advancement in genomic and molecular tools these PRRs could be engineered in different plants and may provide protecting against pathogens (Salomon and Sessa 2012). A recent study demonstrated that resistance can be augmented in *Nicotiana* plants against *Agrobacterium tumefaciens* by expressing a receptor, i.e., FLS2 (FLAGELLIN SENSING 2) from wild grape

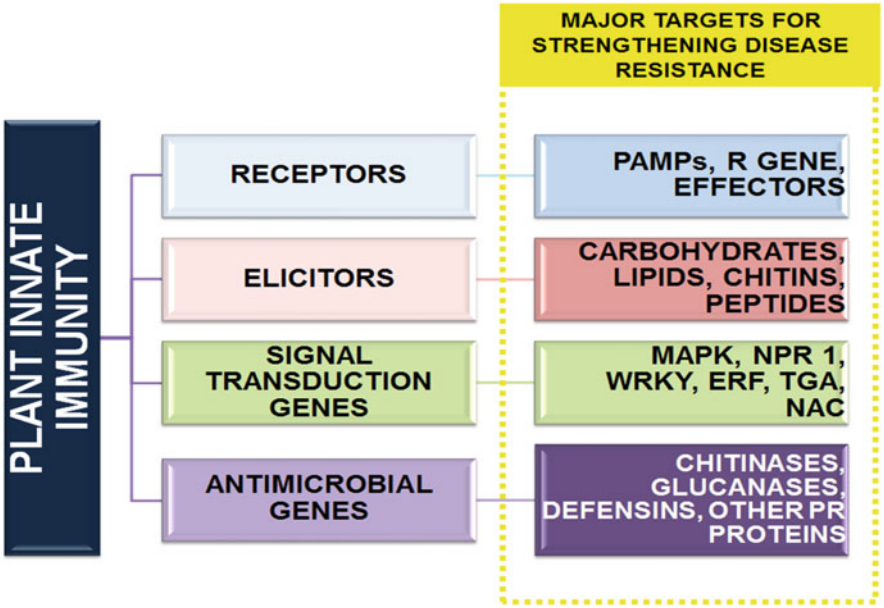

Fig. 25.1 Elements of plant innate immunity to strengthen plant defense against fungal pathogens. Plant immunity is achieved by the presence of various receptor proteins that are located on cell surface or intracellular and contribute to triggering different types of immune response. Elicitors also play a key role in augmenting immune responses that may help in initiating cascade of events with respect to different pathogens. Simultaneously, a number of transcription factors affect various metabolic pathways and interact with defense genes in order to bring out their transcription and further prevent the plants. Plants possess number of antimicrobial genes that restrict the infection of fungal pathogens such as chitinases, glucanases, and many others (*PAMPs* pathogen associated molecular patterns, *R gene* resistance genes, *MAPK* mitogen activated protein kinases; *NPR 1* non expressor of pathogenesis-related protein 1, *PR* proteins-pathogenesis-related proteins)

(Fürst et al. 2020). Previous study carried out in rice showed that overexpression of SERK 1 gene (SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1) led to increased resistance towards infection of *Magnaporthe grisea*. The study also confirmed the role of *SERK* 1 in defense by investigating its expression levels in response to exogenous treatment of salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) and it was noted that all defense signaling molecules upregulated the expression of *SERK* 1 (Hu et al. 2005).

In contrast to these surface receptors, plant immune response is also modulated by intracellular receptors commonly referred to as resistance proteins (R proteins). The receptors recognize effector molecules that are secreted by pathogen inside the cell and induce immune responses that are generally referred to as effector triggered immunity (ETI) (Jones and Dangl 2006). The R proteins are characterized by having two conserved domain that are nucleotide binding (NB) and leucine rich repeat (LRR) OR NB-LRR. These R genes hold great importance in plant disease management strategy as through genetic modifications these R genes can be introduced in

Table 25.1 Utilization of CRISPR/CAS9 in targeted mutagenesis of different genes for the control of fungal pathogens in different crop plants

Name of pathogen	Host plant	Gene targeted	References
Blumeria graminis f. sp. *tritici* (Bgt) (powdery mildew)	*Triticum aestivum* (bread wheat)	*MLO-A1* *MLO-B1* *MLO-D1*	Wang et al. (2014)
Phytophthora capsici	*Solanum lycopersicum* (tomato)	*DMR6*	de Toledo Thomazella et al. (2016)
Magnaporthe oryzae (rice blast)	*Oryza sativa* (rice)	*OsERF922*	Wang et al. (2016)
Oidium neolycopersici (powdery mildew)	*Solanum lycopersicum* (tomato)	*SlMlo1* to *SlMlo16*	Nekrasov et al. (2017)
Blumeria graminis f. sp. *tritici* (Bgt) (powdery mildew)	*Triticum aestivum* (bread wheat)	*TaEDR1*	Zhang et al. (2017)
Phytophthora tropicalis	*Theobroma cacao* (cacao)	*TcNPR3*	Fister et al. (2018)
Botrytis cinerea	*Vitis vinifera* (grapevine)	*VvWRKY52*	Wang et al. (2018)
Botrytis cinerea	*Solanum lycopersicum* (tomato)	*SlMAPK3*	Zhang et al. (2018)
Erysiphe necator En. NAFU1 isolate (powdery mildew)	*Vitis vinifera* (grapevine)	*VvMLO3* *VvMLO4*	Wan et al. (2020)
Oidium neolycopersici (On) (powdery mildew)	*Solanum lycopersicum* (tomato)	*PMR4*	Martínez et al. (2020)

different host and provide resistance against fungal pathogens as evident from recent review of Salomon and Sessa (2012).

A number of studies have shown that various genes involved in signal transduction pathways, elicitors, and hormones can also be targeted to boost plant innate immune responses (Salomon and Sessa 2012; Seo and Choi 2015; Andersen et al. 2018) (Fig. 25.1). Among the signal transduction events during plant–pathogen interactions, mitogen activated protein kinases play an important role in mediating defense response against different categories of fungal pathogens (Meng and Zhang 2013). Diverse MAPKs are triggered in response to PAMPs or PTI and activate defense response by initiating transcriptional reprogramming of downstream transcription factors associated with defense genes. A number of transcription factors like WRKY (Phukan et al. 2016), NAC (Yuan et al. 2019), bHLH and bZIP (Seo and Choi 2015), ERF (Gutterson and Reuber 2004) can also be directed through genetic engineering techniques into the plants for protection against a variety of fungal pathogens (Fig. 25.1).

Table 25.2 Utilization of RNAi technique for host-induced gene silencing for the control of fungal pathogens in different crop plants

Name of pathogen	Host plant	Gene(s) targeted in pathogen	References
Sclerotinia sclerotiorum	*Nicotiana tabacum* (tobacco)	*chs* (chitin synthase)	Andrade et al. (2016)
Fusarium oxysporum f. sp. *lycopersici*	*Solanum lycopersicum* (tomato)	*FOW2* (Zn (II) 2Cys6-type transcription regulator), *chsV* (chitin synthase V)	Bharti et al. (2017)
Rhizoctonia solani	*Oryza sativa* (rice)	*PMK1* (pathogenicity MAP kinase 1)	Tiwari et al. (2017)
Puccinia striiformis f. sp. *Tritici*	*Triticum aestivum* (wheat)	*FUZ7* (MAP kinase gene)	Zhu et al. (2017)
Puccinia triticina	*Triticum aestivum* (wheat)	*PtMAPK1* (MAP kinase), *PtCYC1* (cyclophilin)	Panwar et al. (2018)
Puccinia striiformis f. sp. *Tritici*	*Triticum aestivum* (wheat)	*PsCPK1* (protein kinase A catalytic subunit gene)	Qi et al. (2018)
Verticillium dahliae	*Gossypium hirsutum* (cotton)	*RGS1* (regulator of G protein signaling)	Xu et al. (2018)
Magnaporthe oryzae	*Oryza sativa* (rice)	*AP1* (bZIP transcription factor)	Guo et al. (2019)
Botrytis cinerea	*Arabidopsis thaliana* *Solanum tuberosum* (potato) *Solanum lycopersicum* (tomato)	*TOR* (target of rapamycin)	Xiong et al. (2019)
Fusarium oxysporum f. sp. *lycopersici*	*Solanum lycopersicum* (tomato)	*ODC* (ornithine decarboxylase)	Singh et al. (2020)

Pathogenesis-related proteins (PR proteins) are low molecular weight antimicrobial proteins that are produced in response to pathogen attack (necrotrophs and biotrophs) (Ali et al. 2018). Several classes of PR proteins have been reported in plant system like PR-1, PR-2, PR-3, PR-4, PR-5, PR-8, PR-10 and functional attributes of many of the PR-proteins have been quite well discovered (Ali et al. 2018). These PR proteins have great prospects in future plant disease management and transgenic plants expressing PR gene can be raised that will improve their resistance against fungal pathogens. At the same time, PR-proteins show differential regulation with respect to hormones such as salicylic acid (SA) responsible for systemic acquired resistance (SAR) (Sudisha et al. 2012) and jasmonic acid

Table 25.3 Evaluation of different nanoparticles for antifungal activity under *in vitro* conditions

Types of nanoparticles	Name of fungus	Effective concentration	References
Silver nanoparticle (AgNPs)			
AgNP	*Candida albicans*	50, 100 µg/ml	Paul and Yadav (2015)
AgNP + polyene amphotericin B (AmB)	*C. albicans* *Aspergillus niger*	0.0014 mol/L, 0.0019 mol/L	Tutaj et al. (2016)
AgNp	*Rhizoctonia solani*	0.0019 mol/L	Koduru et al. (2018)
Chitosan coated AgNP	*A. terreus* *A. flavus*	5 mM	Kailasa et al. (2019)
AgNPs	*Fusarium* sp. *Alternaria* sp.	100 µg/ml	Win et al. (2020)
AgNPs	*F. chlamydosporum* *A. flavus*	150, 200 ppm	Yassin et al. (2021)
Zinc oxide nanoparticle (ZnONPs)			
ZnONPs	*A. flavus*	80,100 µg/ml	Navale et al. (2015)
ZnONPs	*A. niger*	75, 100 µg/ml	Shah et al. (2015)
ZnONPs	*Alternaria alternata*, *Penicillium expansum*	16 µg/ml	Jamdagni et al. (2018)
ZnONPs	*Erythricium salmonicolor*	6 mmol/L	Arciniegas-Grijalba et al. (2017)
Cashew gum-capped ZnONPs	*Candida parapsilosis*	75 µg/ml	Souza et al. (2020)
Gold nanoparticles (AuNPs)			
AuNPs	*A. flavus*, *A. niger*	50 µg/ml	Piruthiviraj et al. (2016)
AuNPs	*C. albicans*	1.25 mg/ml	Aljabali et al. (2018)
AuNP	*C.* albicans	40 mg/L	López-Lorente et al. (2019)
Silica nanoparticles (SiNPs)			
Silica coated ferric oxide nanoparticles	*Candida albicans*	15 mg/ml	Asab et al. (2020)
Cobalt nanoparticles (CoNPs) Nickel nanoparticles (NiNPs)	*Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Dematophora necatrix*	500 ppm	Sharma et al. (2017)
Magnesium oxide nanoparticles (MgONPs)	*Thielaviopsis basicola* *Phytophthora nicotianae*	500 µg/ml	Chen et al. (2020)

Table 25.4 Evaluation of different nanoparticle for management of fungal pathogens in different crop plants

Types of nanoparticles	Host plant (fruit/seed)	Name of pathogen	Effective conc.	References
Silica nanoparticles (SiNPs)				
SiNP	*Lycopersicon esculentum*	*Alternaria solani*	300, 400 µg/ml	Derbalah et al. (2018)
Chitosan coated SiNP	*Citrullus Lanatus* (seeds)	*Fusarium oxysporum*	500 mg/L	Buchman et al. (2019)
Mesoporous SiNP + essential oils of lemon grass/clove oil	*Triticum aestivum*	*Gaeumannomyces graminis* var *tritici* (Ggt)	100 µg/ml	Sattary et al. (2020)
SiNP coated with Cu-ultrathin film	*Piper nigrum*	*Phytopthora capsici*	125 ppm, 150 ppm	Hai et al. (2021)
Zinc oxide nanoparticles (ZnONPs)				
ZnONP	*Coffea Arabica*	*Erythricium salmonicolor*	6 mmol/L	Arciniegas-Grijalba et al. (2017)

(JA) and ethylene (Et) responsible for induced systemic resistance (ISR) (Romera et al. 2019). Therefore, it can be established that by exploiting levels of these phytohormones innate immunity of plants can be enhanced via increased expression of respective PR-proteins.

25.2.2 CRISPR/CAS9 Technology

CRISPR/CAS system was first discovered in bacteria and found to be associated with their adaptive immunity as it protects bacteria from any foreign DNA like that of bacteriophage, plasmid, etc. (Hille and Charpentier 2016). CRISPR/CAS system consists of an array of repeats (palindromic tandem repeats) located at CRISPR loci in genome and also Cas proteins whose genes are also positioned near CRISPR loci (Jiang and Doudna 2017). Several CRISPR/CAS systems have identified on the basis of Cas proteins such as class I which includes type I/III/IV, whereas class 2 comprises of type II/V/VI and discovery of this type II has been a major landmark for genome editing in plant system (Barman et al. 2020). The type II CRISPR/CAS is the most widely used system for genome editing in plants that employs Cas9 protein which is endonuclease initially isolated from bacteria *Streptomyces pyogenes* and used to produce a double strand DNA break (DSB). There are two signature domains of this Cas9 popular marked as RuvC and HNH responsible for its effective functionality, i.e., cleavage of complementary DNA. Another central portion of this CRISPR/CAS9 system is single guided RNA (sgRNA) composed of 20 nt user defined sequences which assist in targeting specific region of genome. In addition, sgRNA forms a complex with Cas9 (Cas9/sgRNA) and guide the

Table 25.5 Utilization of different sources of biofungicide for the management of different phytopathogenic fungi in various crop plants

Source of biofungicide	Pathogenic fungi	Disease	Host	References
Microbes				
Aureobasidium pullulans	*Monilinia laxa, M. fructicola, M. fructigena*	Brown rot	*Prunus* sp.	Mari et al. (2012a)
Paenibacillus polymyxa SCHC33	*Botrytis cinerea*	Gray rot	Grapevine	Santiago et al. (2016)
Trichoderma spp.	*Fusarium oxysporum f.* sp. *Melonis*	Melon wilt	*Cucumis melo*	Gava and Pinto (2016)
Bacillus subtilis V26	*Botrytis cinerea*	Tomato fruit rot	*Solanum lycopersicum*	Kilani-Feki et al. (2016)
Trichoderma harzianum Ths97	*Fusarium solani*	Root rot	*Olea europaea*	Amira et al. (2017)
Pseudomonas fluorescens, 1–112, 2–28, and 4–6	*Botrytis cinerea*	Gray mold decay	*Malus domestica*	Wallace et al. (2018)
Entoleuca sp.	*Rosellinia necatrix*	White root rot	*Persea americana*	Arjona-Girona and López-Herrera (2018)
Bacillus amyloliquefaciens strain QST 713	*Blumeria graminis f.* sp. *tritici, f.* sp. *hordei, f.* sp. *avenae*	Powdery mildew	*Cereals*	Matzen et al. (2019)
Schwanniomyces vanrijiae Galactomyces geotrichum Pichia kudriavzevii	*Monilinia fructigena*	Brown rot	*Malus pumila*	Madbouly et al. (2020)
Bacillus subtilis V26	*Fusarium* spp.	*Fusarium* wilt and tuber dry rot	*Solanum tuberosum*	Khedher et al. (2021)
Paenibacillus sp. PNM200	*Fusarium oxysporum f.* sp. *lycopersici*	Tomato vascular wilt	*Solanum lycopersicum*	Vinchira-Villarraga et al. (2021)
Aureobasidium pullulans	*Monilinia laxa*	Brown rot	*Prunus persica*	Di Francesco and Baraldi (2021)
Plant extracts				
Azadirachta indica	*Alternaria solani*	Early blight	*Solanum tuberosum*	Jabeen et al. (2013)
Terminalia nigrovenulosa	*Fusarium solani*	Root rot	*Cucumis sativus*	Nguyen et al. (2013)

(continued)

Table 25.5 (continued)

Source of biofungicide	Pathogenic fungi	Disease	Host	References
Lawsonia inermis Psidium guajava	*Fusarium oxysporum*	*Fusarium* wilt	*Lycopersicon esculentum*	Neela et al. (2014)
Syzygium aromaticum Cinnamomum zeylanicum	*Fusarium oxysporum f.* sp. *lycopersici*	*Fusarium* wilt	*Lycopersicon esculentum*	Yeole et al. (2016)

movement of this complex using Watson Crick pairing towards the predetermined protospacer adjacent motif (PAM).

Genome sequences for large number of crops are now available and based upon those specific sites on the genome can be targeted for increasing crop yield, nutrient content, disease resistance, etc. CRISPR/CAS9 has been exploited in different ways to employ disease resistance and augment immunity in plants (Maurya et al. 2019). Concomitantly, CRISPR/CAS9 successfully imparted disease resistance in various crop plants against number of fungal phytopathogens (Mukhtar et al. 2019; Tyagi et al. 2021).

The prime mechanism through which CRISPR/CAS9 has been used in controlling fungal pathogens is by targeting host susceptibility (*S*) genes and creating knockout mutants (Tyagi et al. 2021). One such example is MLO (mildew resistance locus O) that belongs to host *S* gene responsible for plant defense. A study by Wang et al. (2014) showed CRISPR/CAS9 mediated targeted mutagenesis of three homoallelic genes of *MLO* family, i.e., *TaMLO-A1*, *TaMLO-B1*, and *TaMLO-D1* in bread wheat and it was observed that the mutants of *tamlo-a1* gene were found to be highly resistant against infection of powdery mildew pathogen (*Blumeria graminis*). CRISPR/CAS9 facilitated resistance of grapevine plants to powdery mildew pathogen (Wan et al. 2020). The work showed successful targeted mutagenesis of grapevine *MLO* family genes *VvMLO3* and *VvMLO4* and decreased expression of *VvMLO3* which led to decline in infection of powdery mildew pathogen in grapevine. This *VvMLO3* is required for infection of powdery mildew pathogen and mutation in this caused loss of infection by virulent pathogen (Wan et al. 2020). Successful application of targeted mutagenesis of MLO gene family has also been observed in tomato leading to enhanced resistance against infection of powdery mildew pathogen (*Oidium neolycopersici*) (Nekrasov et al. 2017). CRISPR/CAS9 was used to generate loss of function mutants *SlMlo1* gene that is a main factor of generating resistance against pathogen (Nekrasov et al. 2017).

In a different kind of study, CRISPR/CAS9 was utilized to control pathogenicity of powdery mildew pathogen in tomato (Martínez et al. 2020) by carrying targeted mutation in tomato *PMR* gene. Tomato *pmr* mutants thus obtained demonstrated reduced susceptibility towards powdery pathogen. Another study depicted that mutagenesis of *DMR6* (downy mildew resistance 6) ortholog in tomato using CRISP/CAS9 confer resistance against *Pseudomonas syringae* pv. tomato, *Phytophthora capsici* and *Xanthomonas* spp. Tomato plants with *dmr6* mutated

gene showed increased levels of salicylic acid that might have induced resistance against fungal phytopathogens (de Toledo Thomazella et al. 2016). Mutagenesis of *TcNPR3* (non-expressor of pathogenesis-related 3) gene with CRISPR/CAS9 assisted in improving resistance of cacao plants from infection of *Phytophthora tropicalis* (Fister et al. 2018). This *TcNPR3* has been found to be negative regulator of defense response in cacao plants; therefore, mutant plants showed increased defense response against the pathogen infection (Fister et al. 2018).

Magnaporthe grisea is an important pathogen of rice plant that causes blast disease and is responsible for extensive losses in yield and productivity all over the world. CRISPR/CAS9 has been known to impart resistance to rice plants against *M. grisea* by inducing mutation in *OsERF922* gene (Wang et al. 2016); this *OsERF922* belongs to an ethylene response transcription factors (ERF) gene that is a shown to impart resistance against various abiotic and biotic stresses in plants. Similarly, a study showed that wheat plants with mutant *taedr1* gene showed significant disease resistance against the powdery mildew pathogen, i.e., *Erysiphe cichoracearum*. This *TaEDR1* belongs to group of enhanced disease resistance genes *(EDR)* genes that are negatively correlated with defense of plants and site directed targeted mutagenesis through CRISP/CAS9 of *TaEDR1* gene improved resistance of wheat plants against powdery mildew pathogen in this study (Zhang et al. 2017). Another important class of transcription factors that play an imperative role in plant–pathogen interactions are WRKY and manipulation in expression of WRKY gene in plants alters their resistance patterns to different pathogens. Wang et al. (2018) demonstrated knockout of *VvWRKY52* transcription factor gene using CRISPR/CAS9 in *Vitis vinifera* and first generation mutant *(vvwrky52)* plants represented significant resistance against *Botrytis cinerea.*

In large number of filamentous fungi, CRISPR/CAS9 has been used to bring about gene addition and deletion at specific sites in their genome popularly referred to as fungal genome editing. Interestingly, CRISPR-Cas genome editing using single guided RNA (sgRNA) ribonucleoprotein complex has been well performed in different fungal pathogens like *Botrytis cinerea* (Leisen et al. 2020) and *M. grisea* (Foster et al. 2018). There are other reports also where CRISPR/CAS9 has been utilized such as manipulation of genome in *Ustilago maydis* (Schuster et al. 2016), gene disruption of osmosensor gene in *Leptosphaeria maculans* (Idnurm et al. 2017), endogenous gene tagging in *Fusarium oxysporum* (Wang and Coleman 2019). Fungal genome editing through CRISPR/CAS9 has now became appreciated in plant disease management. Muñoz et al. (2019) has substantially reviewed the application of this strategy for controlling growth of various fungal pathogens. Li et al. (2018) demonstrated generation of pathogenicity mutant of *Sclerotinia sclerotiorum* by utilizing this approach. A list of studies summarizing the application of CRISPR/CAS9 for utilization for the management of fungal pathogens has been shown in Table 25.1.

25.2.3 RNA Interference

RNA interference (RNAi) has been described as a naturally occurring phenomenon associated with the repression of gene functions (Dykxhoorn and Lieberman 2005). RNAi has been developed as a powerful biotechnological tool in the field of agricultural sciences to expedite the silencing of specific genes for inducing stress tolerance, resistance to several pathogens, etc. for crop improvement (Manoharan 2004; Guo et al. 2016). In eukaryotes, RNA silencing is initiated by the synthesis of double-stranded RNA (dsRNA) and hairpin loop RNA (hpRNA) also referred to as small interfering RNAs (SiRNAs, Carthew and Sontheimer 2009). These SiRNAs are short fragments of RNA usually 20–24 nucleotides long which are synthesized from the long dsRNA by the activity of a ribonuclease enzyme named Dicer. These short stretches of dsRNA with two nucleotide overhangs at the 3′ end bind to the Argonaute proteins (AGO) post-degradation of the sense strand and thereby constitutes the catalytic component of the RNA-induced silencing complex (RISC, Lam et al. 2015). The RISC complex in conjugation with the antisense strand of the small RNAs takes part in the sequence specific degradation of mRNA, thereby inflicting post-transcriptional gene silencing in plants (Kamthan et al. 2015). Apart from that, other interfering RNAs known as micro-RNAs (miRNAs), 18–25 nucleotides long are also synthesized by Dicer, but from the single stranded RNAs which are later folded to form double-stranded regions (Wahid et al. 1803). The miRNAs also bind to the AGO proteins following the degradation of one of the strands of miRNA and then results into the cleavage of multiple target mRNAs by binding to the partially complementary mRNA strands (Wahid et al. 1803; Lam et al. 2015).

Plant–pathogen interactions are defined by the interaction between the virulence genes of the pathogen and resistance of the host plant. Cai et al. (2018) have observed that the migration of small RNAs (sRNAs) from the pathogen to host plant and vice versa plays a critical role in determining the resistance or susceptibility of host. In this connection, they have observed in *Arabidopsis* plants challenged with the fungal pathogen—*Botrytis cinerea*, sRNAs are secreted in exosomal vesicles at the infection site that were responsible for the silencing of the virulence genes in the pathogen. Also, Zhao et al. (2016) studied the expression level of *Argonaute* (*AGO*) genes in *Brassica napus* challenged with *Sclerotinia sclerotiorum*. They found that the host–pathogen interaction was modulated by the expression levels of *ARGONAUTE* genes, *viz. AGO2*, *AGO3*, *AGO5*, *AGO*7 in the host which determined the level of infection caused by the pathogen. This type of host-induced gene silencing can be mimicked for engineering host plants for restricting the invasion of pathogen, which has been popularly designated as RNA interference (RNAi technology) (Ghag 2017).

RNAi technology has been employed widely for the development of transgenic plants overexpressing small stretches of dsRNA that generates small interfering RNAs (siRNA), thereby interfering with the functionality of virulence related genes of the pathogens (Majumdar et al. 2017). In this connection, Bharti et al. (2017) have described the transgenesis of a hairpin RNA cassette in tomato plants

corresponding to the *FOW2* (zinc 2Cys6 type transcription regulator) and *chsV* (class V chitin synthase) genes of *Fusarium oxysporum* that were important for the pathogenicity of the fungal strain. Further, they exhibited that the transgenic plants expressing the hairpin RNA could induce host-induced gene silencing of the pathogenic genes, thereby improving the resistance to vascular wilt. Singh et al. (2020) also reported the use of host-induced gene silencing of an *ODC* gene (*ornithine decarboxylase*) of *Fusarium oxysporum* f. sp. *lycopersici* that was required for the normal growth and development of the fungal pathogen. In this connection, they cloned the *ODC* gene fragment in a hairpin RNA construct for the development of transgenic tomato plants, the expression of which enhanced the resistance of tomato plants against Fusarium wilt. Similarly, transgenic wheat lines expressing dsRNA can target the *PsFUZ7* (a MAP kinase gene) transcripts of the *Puccinia striiformis* f. sp. *tritici* (Zhu et al. 2017). *PsFUZ7* gene played an important part in the virulence of *Puccinia* by promoting the hyphal development and infection; therefore, the silencing of this gene enhanced tolerance level of wheat plant. Similarly, Panwar et al. (2018) targeted the *MAPK1* (MAP kinase) or *CYC* (cyclophilin) gene of *Puccinia triticina* by using RNAi technology to express the hairpin RNA constructs in transgenic wheat lines. Tiwari et al. (2017) have reported the control of *Rhizoctonia solani*—the causal agent of sheath blight disease of rice by RNAi technique. According to them the transgenic rice lines expressing the siRNAs corresponding to the *PMK1* (*Pathogenesis MAP Kinase 1*) homologues RPMK1-1 and RPMK1-2 of the pathogen (crucial for appressorium formation and host penetration) displayed decrease in levels of fungal infections. Niu et al. (2017) working in the same line demonstrated the inhibition of reproductive genes like *dvvgr* and *dvbol* to reduce the fecundity of corn rootworm beetles. They have shown that the transgenic maize lines expressing the dsRNA corresponding to the genes effectively managed the multiplication of the insect pest.

RNAi technology has also been employed to engineer the expression of microRNAs (miRNAs) in host plants infected with fungal pathogens. Zhang et al. (2016) have reported that the expression of two miRNAs, viz. miR166 and miR159 in cotton plants infected with *Verticillium dahliae* was associated with the silencing of virulence genes in the fungal pathogen. These genes *Clp-1* (*cysteine protease*) and *HiC-15* (*isotrichodermin C-15 hydroxylase*) were found to be highly conserved in their miRNA binding regions and their inhibition by the miRNAs resulted in the impairment of hyphal growth and microsclerotium formation. RNAi has also been directly used to silence the host genes that directly interacted with the virulent genes of the pathogen, thereby providing an indirect approach to enhance host tolerance. Further advancements have been made in respect of using RNAi technology to control the growth of pathogens. Several studies have reported that the direct application of dsRNA and sRNA in form of spraying (spray-induced gene silencing) is as effective as host-induced gene silencing (Wang and Jin 2017). In this context, the uptake of dsRNA by the fungi often takes place directly or via the plant cells where the dsRNA is first processed by the Dicer-like proteins and cleaved in to sRNAs (Wang and Jin 2017). This technique is highly effective as shown through the control of fungal pathogens like *Sclerotinia sclerotiorum* and *Botrytis cinerea* by

the foliar spray of dsRNAs homologous to the disease-causing genes in the pathogen (McLoughlin et al. 2018). Table 25.2 highlights some of the studies where RNAi has been utilized for crop protection against fungal pathogens.

25.2.4 Nanotechnology

Nanotechnology has played a vital role in agriculture in conjunction with biotechnology and allied fields (Patel et al. 2014; Singh et al. 2015). Management of fungal pathogens responsible for causing huge losses in crop plants using nanoparticles has fascinated the attention of scientists all over the world (Khan et al. 2019). Nanoparticles (NPs) are tiny particles whose size ranges between 1 and 100 nm (nanoscale) and are the basic units of nanotechnology. Nanoparticles possess distinct physical, chemical and biological characteristics in comparison to their respective particles at higher scale. Due to their small size, NPs possess distinct properties such as high reactivity and stability, large surface area to volume, great mechanical strength, etc. (El-Mohamedya et al. 2019). All these properties make NPs a widely used material and have enormous application in different fields like industries, pharmaceutical, agriculture, etc. (Ealia and Saravanakumar 2017).

Nanoparticles can be categorized into metallic and non-metallic depending on the source from they have been prepared. Metallic nanoparticles are synthesized from pure metals such as silver (Ag), gold (Au), copper (Cu), iron (Fe), zinc (Zn), platinum (Pt), etc. and other mixed metals iron (Fe), manganese (Mn), silica (Si), etc. These nanosized metallic particles provide protection from different phytopathogens that cause severe diseases in plants. Additionally, nanoparticles can be utilized in different forms such as in the form of foliar spray or by developing formulation specifically nanofungicides. With the introduction of nanotechnology, nanobiofungicides have gained momentum for controlling fungal pathogens of plants through the beneficial use of nanotechnology (Bhattacharyya et al. 2016; Dwivany et al. 2020; Luksiene et al. 2020). These nanobiofungicides are the best alternative approach for fungal disease management in crop plants in comparison to the conventional synthetic chemical fungicides that are hazardous and highly toxic to environment (Abd-Elsalam and Alghuthaymi 2015).

Fungicidal activity of several chemically synthesized metal nanoparticles has been depicted by previous studies both under in vitro (Table 25.3) and in vivo in different crop plants (Table 25.4) (Haq and Ijaz 2019; Win et al. 2020). Kim et al. (2012) demonstrated considerable effects of silver nanoparticles (AgNPs) in inhibiting growth of various plant pathogenic fungi, i.e., various species of *Alternaria*, *Cladosporium*, *Pythium*, *Fusarium*, etc. under in vitro conditions. Efficacy of AgNPs against fungus *Candida albicans* at a concentration of 50 and 100 μg/ml was monitored under cultured conditions by Paul and Yadav (2015). Significant antifungal activity of AgNPs against *A. flavus* and *Fusarium chlamydosporum* was also shown by recently by Yassin et al. (2021). A study demonstrated inhibitory effect of AgNPs, CuNPs (copper NPs), and combined AgNPs and CuNPs on *Alternaria alternata* and *Botrytis cinerea*. It was observed that all the three

nanoparticle treatments disrupt the fungal hyphae as well as conidial germination (Ouda 2014). A study showed that AgNPs curb the growth of pathogen isolated from infected leaf (*Alternaria alternate*) and fruits (*Alternaria citri* and *Penicillium digitatum*) of *Citrus* plants under in vitro conditions (AbdelMalek and Salaheldin 2016). Gold NPs (AuNPs) also showed significant antifungal activity at different concentrations as evident from number of studies (Piruthiviraj et al. 2016; Aljabali et al. 2018; Folorunso et al. 2019; López-Lorente et al. 2019).

Copper NPs (CuNPs) demonstrated substantial antifungal activity against *C. albicans* and *C. parapsilosis* at different concentrations (Kruk et al. 2015). Conversely, chitosan coated CuNPs showed high resistance towards *Aspergillus flavus* (Morsi et al. 2017). At a concentration of 20 mM, CuNPs greatly inhibit the growth of *Aspergillus brasiliensis* and *C. albicans* (Hassan et al. 2018). Antifungal effect of CuNPs against some of pathogenic fungi has been well observed through work of Pariona et al. (2019). It was witnessed that fungi like *Fusarium* sp., *Phoma destructiva*, *Curvularia lunata*, *Alternaria alternate*, *Fusarium oxysporum*, *Penicillium italicum*, *Penicillium digitatum*, and *Rhizoctonia solani* showed disruption in their hyphal membrane and generation of reactive oxygen species in their mycelium. A study revealed favorable response of Cu-chitosan NPs against *Alternaria solani* and *Fusarium oxysporum* both under in vitro and in vivo conditions responsible for causing disease in tomato (Saharan et al. 2015).

Zinc oxide NPs (ZnONPs) are also known for its antimicrobial activity against fungus *Aspergillus niger* at 75 and 100 μg/ml (Shah et al. 2015). A study showed efficacy of ZnONPs against *Aspergillus flavus* at a concentration of 80 and 100 μg/ml (Navale et al. 2015). Antifungal activity of ZnO has been observed in study of Jamdagni et al. (2018) where it inhibited the growth of *Alternaria alternata* and *Penicillium expansum* at 16 μg/ml. Interestingly, cashew gum-capped ZnONPs inhibits the growth of *Candida parapsilosis* at 75 μg/ml (Souza et al. 2020).

A study demonstrated significant role of iron oxide nanoparticle (FeONPs) and magnesium oxide nanoparticles (MgONPs) in restricting growth of number of rot causing fungi (*Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum*, and *Rhizoctonia solani*) (Koka et al. 2019).

Silica NPs have been momentously used in controlling number of phytopathogens due to their high antimicrobial activity against various fungi. A study showed that infection of *Alternaria solani* that causes early blight disease in *Lycopersicon esculentum* (Tomato) can be resisted by application of SiNPs (Derbalah et al. 2018). In a different study, positive effects of chitosan coated SiNPs were noted to control the impact of *Fusarium oxysporum* in *Citrullus lanatus* (Buchman et al. 2019). Furthermore, the ability of SiNPs coated with Cu ultrathin film and suspended in carboxymethyl cellulose to resist *Phytophthora capsici* infection in *Piper nigrum* has also been observed (Hai et al. 2021). Infection of *Gaeumannomyces Graminis* var *tritici* (Ggt) that causes take all disease in *Triticum aestivum* (wheat) can be subdued by SiNPs loaded with essential oils such as clove oil at 100 μg/ml (Sattary et al. 2020).

There are reports of antifungal activity of some nanoparticles synthesized from transition metals like cobalt (Co) and nickel (Ni) against crucial phytopathogens (*Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Dematophora necatrix* (Sharma et al. 2017). Additionally, nanoparticles synthesized from inorganic metal oxides like magnesium oxide (MgO) also retain antifungal activity as evident from the work of Chen et al. (2020). The work showed reduced spore germination of *Phytophthora nicotianae* and *Thielaviopsis basicola* under in vitro conditions in response of MgO nanoparticles. Simultaneously, the results were further confirmed under greenhouse experiment where reduced disease severity of tobacco black shank and black root rot disease was seen in plants treated with suspension of MgO nanoparticle.

Biogenic nanoparticles have shown positive results in restricting growth of various plant pathogenic fungi. Win et al. (2020) showed significant potential of silver nanoparticles synthesized from fungus *Alternaria* in limiting *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium tricinctum*, *and Alternaria* sp. under culture conditions. Silver nanoparticles synthesized from *Streptomyces clavuligerus* substantially regulated growth of *Fusarium oxysporum* both under in vitro and in vivo (El-Waseif et al. 2019). Similar reports were seen through studies of Mishra et al. (2014), where the biosynthesized AgNPs from *Serratia* sp. BHU-S4 exhibited antifungal effects against *Bipolaris sorokiniana* that is responsible for spot blotch disease in wheat. The study revealed that the biosynthesized AgNPs affect conidial germination under in vitro conditions that the antifungal effects were further confirmed under in vivo conditions by detached leaf assay and under greenhouse by artificial inoculation of pathogen along with AgNPs suspension (Mishra et al. 2014). Apart from this, biogenic nanoparticles synthesized from various fungi have been also observed to play a substantial role in plant disease management. Biogenic CuNPs also showed antifungal activity against *F. oxysporum*, *Drechslera sorghicola*, *Rhizopus stolonifer*, and *Alternaria carthami* at 0.01, 0.03, 0.06 mg/ml (Sattary et al. 2020). CuNPs biosynthesized using *Syzygium aromaticum* restrict growth of *Aspergillus niger*, *A. flavus*, and *Penicillium* sp. (Rajesh et al. 2018). Alghuthaymi et al. (2015) comprehensively reviewed the synthesis of myconanoparticles from various fungi such as *Fusarium*, *Aspergillus*, *Verticillium*, *Penicillium*, *Trichoderma*, etc. and discussed their antifungal effects.

In recent times, nanocomposites have been shown to possess relatively enhanced antifungal activity than their pure nanomaterial counterparts (Alghuthaymi et al. 2021). Nanocomposites may be defined as the hybrid materials that may have multiphases and one of the phases is at nanoscale (Sen 2020). At the same time conjugated nanoparticles also act as an excellent source for protection against phytopathogens. In one of the study, fabricated AGNPs such as chitosan coated AgNPs also showed high antifungal effect against *Aspergillus terreus* and *A. flavus* at 5 mM concentration under in vitro conditions (Kailasa et al. 2019). Another study reported AgNPs encapsulated with polyene amphotericin B (AmB) actively resist infection by *C. albicans* and *A. niger* at a concentration of 0.0014 mol/L (Tutaj et al. 2016).

25.2.5 Biofungicides

Biofungicides are the products of biological organisms that possess fungitoxic or fungistatic activity towards phytopathogenic fungi (Tomaso-Peterson 2006). Biofungicides are usually produced as formulations and suspensions and are applied as seed treatment, soil treatment, or foliar sprays (Desai 2008). Due to the phytotoxic nature of fungicides (Lamichhane et al. 2018; Baibakova et al. 2019), modern research is more focused on biological approach for control of fungal pathogens (Heydari and Pessarakli 2010). The basic interaction mechanisms used by micro-organisms include hyperparasitism, predation, production of antibiotics, lytic enzymes, physical and chemical interference, competition and induction of resistance in host (Pal and Gardener 2006; Heydari and Pessarakli 2010). Conversely, plants produce various bioactive compounds that have antifungal activities towards the fungal pathogen (Zaker 2016; Nmom and Ajuru 2020).

Several investigations have employed a range of plants and micro-organisms in order to tackle plant pathogenic fungi (Zhang et al. 2010; Jagtap et al. 2013; Vergnes et al. 2014; Shehata et al. 2016; Spadaro and Droby 2016; Shuping and Eloff 2017; Choudhury et al. 2018). According to Kamaruzzaman et al. (2020) hypovirulent *Botrytis cinerea* isolate QT5-19 provided effective suppression of virulent *B. cinerea* and *Sclerotinia sclerotiorum* infection in leaves of oilseed rape through production of antifungal volatile organic compounds (VOCs). Similar result was obtained by Haidar et al. (2016) where the ability of *B. pumilus* (S32) and *Paenibacillus* sp. (S19) to secrete VOCs (1-octen-3-ol and 2,5-dimethyl pyrazine) and induce systemic resistance in grapevine that worked against fungal pathogen *Phaeomoniella chlamydospora* was reported. Chlorogenic acid, a plant phenolic secondary metabolite can induce cell lysis and spore membrane permeabilization, thereby inhibiting mycelial growth of *Sclerotinia sclerotiorum*, *Fusarium solani*, *Verticillium dahliae*, *Botrytis cinerea*, and *Cercospora sojina* (Martínez et al. 2017).

Seed priming with plant extracts provides resistance towards fungal diseases and is beneficial to plants (Mondal and Bose 2014). Priming of seeds results in altered levels of antioxidative enzyme activities like superoxide dismutase (SOD) and peroxidase (POD) (Ali et al. 2019). Application of extracts from plants like *Allium sativum*, *Allamanda cathartica*, *Azadirachta indica*, *Tagetes erect*, *Schinus terebinthifolius*, *Mirabilis jalapa*, *Thuja occidentalis*, etc., for seed priming has been reported to have biofungicidal activity towards various plant pathogenic fungi (Islam et al. 2015; Shabana et al. 2017). Inhibition of seed-borne fungus *Pyricularia grisea* causing blast disease in rice was observed by Hubert et al. (2015) after priming rice seeds with aqueous extracts of *Coffea arabica*, *Nicotiana tabacum*, *Aloe vera*, and *Chrysanthemum coccineum*. Seed biopriming with biofungicides has been proven beneficial against many fungal diseases (Reddy 2012). Bioprimed seeds of *Vigna radiata* with *Trichoderma viride*, *T. harzianum*, *T. fasciculatum*, *Pseudomonas fluorescens-I*, *P. fluorescens-II*, *P. Aeruginosa* were effective in reducing disease caused by *Alternaria alternata* (Deshmukh and Sabalpara 2019). Interestingly, *T. harzianum* biofungicide formulation primed maize seed not only showed disease resistance towards *Fusarium verticillioides*

but also increased seed germination, yield and vigor expressing both the antifungal and plant growth promoting abilities (Chandra Nayaka et al. 2010). Similar results were obtained while working with chickpea bioprimed with *T. harzianum* and *T. viride* against wilt causing *Fusarium oxysporum* f. sp. *ciceri*. (Kumar et al. 2014; Pandey et al. 2017).

Number of investigations have provided evidences for biofungicidal potential of various plant-based products towards the plant pathogenic fungi (Kumari et al. 2013; Ravikumar and Garampalli 2013; Silva et al. 2014; Mahlo et al. 2016; Akpor and Oluba 2018). Aqueous leaf extract of plants like *Azadirachta indica*, *Lycopersicon esculentum*, and *Datura* sp. was able to reduce tikka disease caused by *Cercospora arachidicola* and *Cercosporidium personatum* in *Arachis hypogaea* (Hossain and Hossain 2013). Crude extracts obtained from *Brassica oleracea* var. *italica* exhibited hyperbranching and membrane permeabilization in phytopathogenic *Colletotrichum gloeosporioides* (Pacheco Cano et al. 2018). Around 17–55% reduction in *Sclerotium rolfsii* biomass was observed after the application of methanolic extract of *Acacia nilotica* subsp. *indica* leaves (Sana et al. 2016). In vitro study using ethanolic extract obtained from triguero asparagus enriched in flavonoids showed promising results against growth and sporulation of *Fusarium oxysporum* f. sp. *asparagi*, *F. oxysporum* f. sp. *Dianthi*, and *F. oxysporum* pathogenic to asparagus, carnation, and strawberry, respectively (Rosado-Álvarez et al. 2014). Methanolic extract derived from *Vitex agnus-castus* was able to induce expression of pathogen related (*PR*) genes (*PR-1*, *PR-2*, *PR-6*) in *Lycopersicon esculentum* showing strong antifungal activity towards *Pythium ultimum* (Švecová et al. 2013). Chitinase (*Chi1 and CHI4*), β-1-3-glucanase (*Glu1*), phenyl alanine lyase (PAL), and peroxidase (POX) genes were upregulated by aqueous leaf extracts of *Jacaranda mimosifolia* that provided resistance in wheat against *Puccinia triticina* (Naz et al. 2014).

Essential oils also show effective fungicidal properties towards many phytopathogenic fungi (Cavanagh 2007; Bakkali et al. 2008; Sivakumar and Bautista-Baños 2014). Biofungicidal effects of *Thymus kotschyanus* essential oil towards plant pathogenic *Botrytis cinerea*, *Aspergillus niger*, and *Penicillium expansum* were reported by Ghasemi et al. (2020). According to Ghasemi et al. (2019), essential oil of *Cuminum cyminum* with 3-caren-10-al and cuminal as major constituents was able to inhibit the mycelial growth of three fungal pathogens, *Botrytis cinerea* (grapevine black mold), *Aspergillus niger* (grapevine gray mold), and *Penicillium expansum* (apple green molds). Incorporation of *Cinnamomum zeylanicum* essential oil with shellac and carnauba wax was found to be efficient in controlling diseases caused by *Penicillium italicum* PIRBM1 and *P. digitatum* PDRBM1 (Kouassi et al. 2012). Treatment with vapors of *Oregano vulgare* essential oil in *Vitis vinifera* cv Chasselas triggered the genes which are involved in salicylic, jasmonic acid and ethylene signaling pathways, activating PR protein accumulation and synthesis of phytoalexin and thus promoting the plant innate immunity towards *Plasmopara viticola* invasion (Rienth et al. 2019). Studies that have shown beneficial effects of biofungicides for controlling growth of phytopathohgenic fungi are depicted in Table 25.5.

Biofungicides also play an important role in the control of fungal postharvest diseases in plant products (Mari et al. 2014). Kharchoufi et al. (2018) were able to control postharvest decay causing *Penicillium digitatum* with the application of mixture of chitosan with aqueous and methanolic extract of pomegranate peel in oranges. Correspondingly, reduction in postharvest decay of *Lycopersicon esculentum* caused by *Alternaria alternata* through cell electrolyte leakage after rhamnolipids treatment was reported by Yan et al. (2015). Two strains of *Aureobasidium pullulans* (L1 and L8) produced VOCs that inhibited the growth of *Botrytis cinerea*, *Colletotrichum acutatum*, and *Penicillium expansum* causing postharvest gray mold, bitter rot, and blue mold in apple, respectively (Mari et al. 2012a, b). Mixture of Arabic gum with chitosan when applied in banana controlled postharvest anthracnose caused by *Colletotrichum musae* with delayed ripening of the fruit (Maqbool et al. 2010).

25.3 Conclusion and Future Perspectives

Plant diseases pose a significant threat to mankind due to their direct effect on global food security. Major losses in crop plants are incurred due to fungal pathogens including oomycete that have caused huge devastation in crop production throughout the world. Conventional techniques mainly depend upon usage of chemical or synthetic fungicides for the control of obnoxious fungal pathogens. However, their excessive usage has caused detrimental effects on natural environment as well as on ecosystems. Additionally, there seems to be a problem of development of resistance in pathogen towards these fungicides. Therefore, scientists should explore new methods to curb fungal pathogens and prevent losses in agricultural sector. Since the starting of twenty-first century, a lot of advancement has been observed in molecular diagnostics of plant pathogens and genome sequences of many fungal pathogens are now available easily as well as the role of various metabolites has been very well studied through proteomics. All these data should be amalgamated and utilized to regulate fungal growth in crop plants in a way that it does not cause any problem of resistance development. Genome wide analysis will help in targeting specific genes and virulence factors responsible for pathogenicity. Alongside, the research efforts should be promoted in emerging areas like development of nanofungicides and biofungicides as they are quite eco-friendly and easy to use at cheaper cost. Although initial studies have shown the beneficial effects of both nanofungicides and biofungicides under controlled conditions but to successfully commercially adopt these technologies, the evaluation should be carried out in field conditions and with different crop plants.

Acknowledgments All the authors are thankful to University of North Bengal for providing necessary facilities for writing this chapter. RS is grateful towards CSIR-UGC (File No. 09/0285 (11430)/2021-EMR-I) for granting Junior Research fellowship.

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Molecular Basis of Host–Pathogen Interaction: An Overview

26

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Abstract

The concept of plant–pathogen interaction involves individual pathways for effective defense and successful colonization. The early detection of pathogen attacks is challenging and has a limited success rate. The conventional methods include selective media and microscopic observations, plate assay, anatomical observations using staining techniques, phenotypic observations, assessment through disease scale for a particular disease, etc. The most reliable and accurate results in a short period can be obtained by polymerase chain reaction (PCR), qRT-PCR with the expression-specific primers to assess the pathogen's presence and level of expression inside the host. The complex two-way interaction between the plant and the pathogen involves disturbed plant metabolic pathways during a compatible interaction. The predictions sometimes remain incomplete and create false positives in understanding the exact members involved to confer compatible or incompatible interaction between the host and the pathogen. This complication needs in-depth understanding at the molecular level. Recent advances in the OMICS approach in understanding the mechanisms involved in plant–pathogen interactions help understand genes and expression. The proteomic and metabolomics studies help fill the pathways that provide clear insights and knowledge during the interaction.

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_26

Keywords

Systemic acquired resistance · Transcriptomics · Proteomics · OMICS approaches · Metabolomics

26.1 Introduction

Plants face a wide range of pathogens such as bacteria, oomycetes, fungi, and viruses during their lifetime. It was reported that the loss in crop production caused by pathogen infections ranged from 20% to 40%, and the direct economic loss was up to 40 billion dollars yearly (Yang et al. 2017). The life cycle of the plant involves interaction with various microorganisms in their environment. A better understanding of what makes a plant–microbe interaction detrimental or beneficial to plants would provide an essential insight into the efficient handling of microbes for agriculture production, which offers unprecedented opportunities to increase crop productivity (Swarupa et al. 2016). As sessile organisms, plants have developed coordinated responses by complex signaling networks involving phytohormones to promote their health during pathogen attack (Mauro et al. 2019). Studies are being focused on uncovering the molecular components involved in plant–microbe interaction to understand pathogen infection or symbiosis (Ashwin et al. 2017). Plant pathogens elicit an immune response through effector proteins. In turn, plant genomes encode genes that determine species-specific recognition of these effectors by a process known collectively as effector-triggered immunity (ETI) (Laflamme et al. 2020). The functional genomics strategy, including proteomics and transcriptomics, has contributed to defining gene, protein function, and expression profiles. Symbiotic and defense-related genes and proteins expressed during plant–microbe interactions have been identified and accumulated enormous datasets (Du et al. 2016). The proteomic approach has evolved in the pursuit of large-scale functional assignments of candidate proteins. Several proteins expressed during plant–microbe interactions have been identified (Mehta et al. 2008). Structural proteomics defines the primary, secondary, and tertiary structures of proteins.

In contrast, functional proteomics refers to developing and applying global (proteome-wide or system-wide) experimental approaches to assess protein function. A detailed understanding of plant–microbe interactions using a successful combination of proteomic techniques and other high throughput techniques of cell biology, biochemistry, and genomics is needed for practical application to secure and stabilize the yield of many crop plants (Lodha et al. 2013). Metabolomics plays a significant role in revealing the perturbations between two comparative analyses of healthy and diseased samples by generating the data about signaling and pathways as an output during the interaction (Castro-Moretti et al. 2020). The concept of OMICS approaches and their utilization at proper stages of experiments is essential. The selection of OMICS approaches depends on the availability of genomic data; without the genomic data, transcriptomic approaches have no role in relating the

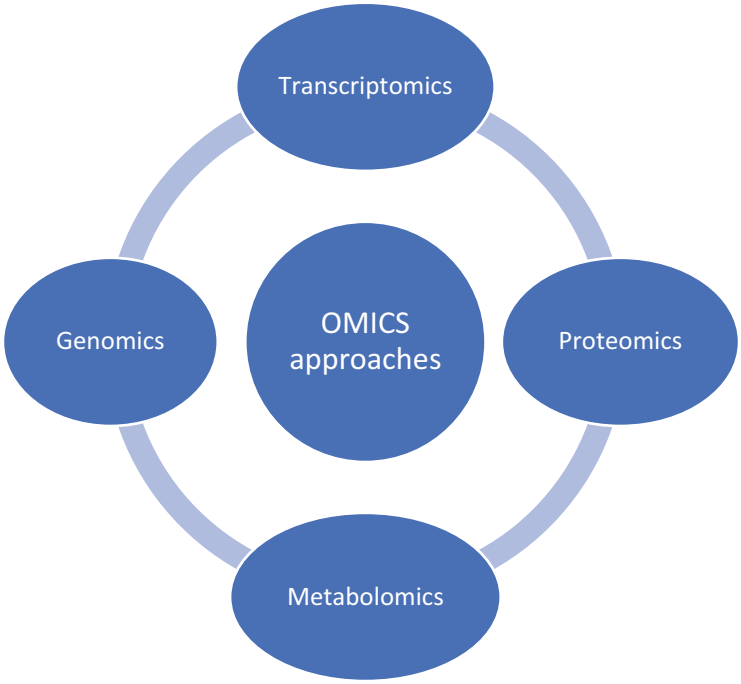

Fig. 26.1 Main pillars of OMICS approaches

molecular results, and it is impossible to go for proteomics and metabolomics. The sequence information eases the in-depth analysis as per our objective (Fig. 26.1).

26.2 What is Host–Pathogen Interaction?

The different types of constant inter-relationships can be found between both rhizosphere and phyllosphere parts of plants with continuous interaction with various microorganisms, including beneficial and pathogenic. The biotic and abiotic stresses causes plants to suffer from various issues and correspond to the lower-income for the farmers by reducing the yield. The stress includes biotic stress in which, without killing the plant, the pathogen destroys the actual metabolism of plants, thus decreasing the yield (Swarupa et al. 2016). Plants may suffer in their life cycle with different kinds of microorganisms such as viruses, bacteria, fungi, protozoa, nematodes, and face adverse effects in limiting conditions such as lack of light, nutrients and to lead normal metabolic lifestyle (Du et al. 2016).

The rhizospheric/phyllospheric pathogen community structure changes, depending upon:

- Plant genotype,
- Plant developmental stage,
- Exposure to disease-suppressive soils,
- Root exudate composition, and
- Plant hormone signaling.

Specific compounds released as root exudates mediate one-to-one, host–pathogen, or species level interactions:

- Flavonoids act as signaling compounds.
- Strigolactones stimulate mycorrhizal hyphal branching.
- Malic acid is involved in recruiting specific plant-growth-promoting (PGPR) (*Bacillus subtilis*).
- Disruption or initiation of quorum sensing (QS) in bacteria.
- Sugars and amino acids act as chemoattractants for microbes.

26.2.1 Signal Exchanges Between Host Plant and Microbes

The roles of proteins secreted by roots and their interaction with other organisms in the rhizosphere are very limited and need further exploration to conclusively determine the mechanisms at play other root exudates mediate multitrophic interactions and may disturb the interaction of pathogen in both positive and negative way. The signal exchanges between plant and pathogens such as virus, bacteria, and fungi will be studied at a molecular level by focusing on PAMP and effector-triggered immunity in most of the cases (Barnabas et al. 2016).

The defense molecules and isoflavonoids secreted by plants are due to pathogenesis secretions by pathogens outside the plant atmosphere. Perception of microbial signals results in plant protein abundance and post-translational modification (PTM) changes, which can be detected using proteomic techniques.

26.2.2 Identification of PTMs

The interaction studies are complex because of molecular-level changes such as PTM changes, which affect protein activity and protein's structure and plays a vital role in regulating various biological processes. The number of PTM changes has to be studied due to its reversible and liable nature, low abundance, and difficulties in practical approaches such as protein digestion and its ionization capacity. The PTM changes may be several, but much importance has to be given to phosphorylation. The initial PTM changes come across during the host–pathogen interaction (Ashwin et al. 2017). As an initial step during the host–pathogen interaction, phosphorylation acts as a significant signal transduction agent in which transfer of phosphoryl group is facilitated by tyrosine, serine, histidine, or threonine residue. The reversible mechanism also seen by the action of kinases involving protein phosphorylation and removal of phosphoryl group can be seen during the host–pathogen interaction.

When there is a signal that a pathogen is invading a host such as plants, the alterations in the signaling cascades can be seen and allow rapid response by plants against the pathogen attack through various effector molecules (Jayaraman et al. 2012).

26.3 Why Study Host–Pathogen Interaction?

- To understand the molecular components involved in the mechanism of pathogen infection or symbiosis
- Understanding and selecting efficient, beneficial strains with various improved traits during their interaction with a host like
  - Growth promotion
  - Imparting abiotic and biotic stress tolerance

The fascinating concept of plant–pathogen interaction is very complex. Still, it is an environmental approach and nature's rule that accommodate plant and pathogen to interact and can be the social or pathogenic relations. Still, it is negative only when it affects the yield determining factors in agriculture. The escape of the host from the pathogen is quite avoidable. Still, mechanisms to resist it play a major role and are complex to understand at the phenotypic and biochemical level, and, unfortunately, pathway-level understanding is quite obvious to say impossible. Knowing how plants control their interactions directly impacts our agricultural strategies, and thus a vital field of research.

26.4 Molecular Approaches

26.4.1 PCR-Based Specific Techniques Used for Detecting the Most Important Pathogens

The plant pathogen detection through rapid techniques with accuracy and reliability is essential in identifying particular diseases in the early stages of infection without plant's phenotypic symptoms in some cases (Palacio-Bielsa et al. 2009). The diagnosis method should be specific to take proper control measures. The immunological, biochemical, selective media approaches to identify and characterize the pathogens are less specific with an improper conclusion and time-consuming but provide fundamental background to pathogen identification (McCartney et al. 2003). Morphological characterization is lengthy and requires considerable mycological expertise, while also not efficient for detecting quiescent forms of the pathogen that can encounter in seedlings or seeds (Mullet et al. 2017). To overcome these shortcomings, the molecular-based detection of pathogen infection is challenging but most convincing technique. The conventional methods can be overheld by PCR-based techniques with rapid and specific results. PCR-based detection is nowadays a promising technique to identify even non-culturable microorganisms (Lievens et al. 2005).

The conventional methods of breeding potatoes for disease resistance is time-consuming; significant improvement in the use of DNA markers linked to genes imparting resistance to plant diseases and has helped in selection of valuable genotypes (Rogozina et al. 2019). The early detection of pathogen presence through PCR-based techniques during quarantine check helps us get rid of new pathogen entry from one region to another region. The molecular basis of plant–pathogen identification in rice sheath blight caused by *Rhizoctonia solani* using PCR techniques combined with morphological markers has been studied. The isolate RS4 showed 99% homology with *R. solani* AG1-IA based on nucleotide sequence data for ITS 5.8S-rDNA region (Suryawanshi et al. 2019). An effort to examine the contribution of rice variety Tetep toward resistance against *R. solani*; suppressive subtractive hybridization (SSH) was done and found that the photosynthetic and secondary metabolic pathways were modulated in rice in response to the *Rhizoctonia* infection (Krishnaraj et al. 2018). The biocontrol efficacy of actinobacteria against sheath blight disease was confirmed through in vitro studies. The ultimate identification of isolates potent against the disease was done through the molecular characterization and found that isolates selected belonged to *Streptomyces* spp. or *Actinopolymorpha* spp. (Shinde et al. 2016). The efficacy of transgenic plants capable of post-transcriptional gene silencing (PTGS) was assessed in available T4 seeds of four transgenic events by PCR-based screening and gene segregation analysis. The bio-efficacy test of transgenic plants from three events, viz. "A," "C," and "E" in T4 and T5 generation indicated moderate resistance against ToLCV infection (Sarita and Krishnaraj 2013).

The attack of different pathogens, such as bacteria, fungi, and viruses negatively impacts crop production. To counter such attacks, plants have developed various strategies involving the modification of gene expression, activation of several metabolic pathways, and PTM of proteins, which culminate into the accumulation of primary and secondary metabolites implicated in plant defense responses (Nguyen et al. 2019). The recent advancement in omics techniques allows the increased coverage of plants transcriptomes, proteomes, and metabolomes during pathogen attack and the modulation of the response after the infection (Yang et al. 2017). Omics techniques also allow us to learn more about the biological cycle of the pathogens in addition to the identification of novel virulence factors in pathogens and their host targets. Both approaches become essential to decipher the mechanism underlying pathogen attacks and to develop strategies for improving disease-resistant plants. The contribution of genomics, transcriptomics, proteomics, metabolomics, and metabolomics is of great importance in devising the strategy to obtain plants with increased resistance to pathogens and symbiosis (de Falco et al. 2019).

26.4.2 Omics Strategies for Understanding Host–Pathogen Interactions

Understanding gene function can be approached via several techniques:

- Genomics
- Transcriptomics (messenger, structural and regulatory RNAs)
- Proteomics (proteins and their putative PTMs and peptides)
- Metabolomics (primary and secondary metabolites)

In prokaryotes, gene finding is essentially identifying open reading frames (Mehta et al. 2008). As genomes get larger, it becomes increasingly complicated. Several sophisticated software algorithms have been designed to handle gene prediction in eukaryotic genomes. Despite considerable progress, gene prediction entirely based on DNA analysis is cumbersome and requires support from "functional genomics," i.e., transcriptomics, proteomics, and metabolomics. Indeed, genomics focuses on the static aspects of genome information. Gene prediction and annotation in a reference variety is the initial step for every crop, but this is not sufficient to get a complete insight into the biodiversity's phenotypic plasticity and agricultural potential. Functional genomics deals with dynamic aspects, reflecting environmental adaptations and allows the description of gene functions and the interactions between gene products that may provide a view of a variety/genotype (Shi et al. 2020).

26.4.2.1 Transcriptomics

The field of transcriptomics allows for the examination of whole transcriptome changes across a variety of biological conditions. Probably the easiest way to study changes on a genome-wide scale is through transcriptomics (Santos et al. 2020). The structure of RNA is homogenous and relatively simple, and therefore the analysis is the most straightforward compared to protein and metabolite analyses.

Transcriptomes study helps to identify groups of co-regulated genes, transcription factor binding sites that are enriched in those gene clusters, and predicted possible protein–protein interaction sensor complexes that potentially link the ability to detect extracellular conditions and use that information to drive changes in gene expression (Wani and Ashraf 2018). Co-regulated genes are expected to share common regulatory genomic elements, and those elements are hypothesized to be associated with mechanisms of plant microbial interactions. The combination of transcriptomic analysis with genomic sequence analysis helps to identify possible transcription factor binding sites that are enriched in the identified co-regulated gene clusters (Qi et al. 2018).

26.4.2.2 Proteomics

The proteome is defined as the entire protein complement expressed by the genome, and proteomics is the study and characterization of the complete set of proteins in a cell, tissue, or whole organism at a given time under defined conditions.

Proteomics, one of the major tools of "omics" is evolving phenomenally since the development and application of two-dimensional gel electrophoresis coupled with mass spectrometry at the end of the twentieth century. However, the adoption and application of advanced proteomics technologies in understanding plant–pathogen interactions are far less when compared to their application in other related fields of

Table 26.1 Subdivisions of proteomics

Structural	• In-depth analysis of protein structure • Compares protein structures and helps identify functions of newly discovered genes • X-ray crystallography and NMR spectroscopy
Quantitative	• Simultaneous quantitation of level differences between many proteins in different sample • Not measurement of their absolute concentration • 2D-PAGE and mass spectrometry
Functional	• Characterization of protein–protein interactions • Useful to determine the protein functions • Explains the way proteins assemble in bigger complexes • Affinity purification, mass spectrometry, and the yeast two-hybrid system

systems biology (Barnabas et al. 2016). Hence, it is important to diligently focus on the advances in various proteomic approaches and their gamut of applications in different facets of phyto-pathoproteomics. Especially, the scope and application of proteomics in understanding fundamental concepts of plant–pathogen interactions such as identification of pathogenicity determinants (effector proteins), disease resistance proteins (resistance and pathogenesis-related proteins), and their regulation by PTMs play a major role in understanding plant–microbe interactions (Handakumbura et al. 2017). The compatible interaction between *Sporisorium scitamineum* and smut susceptible genotype was studied using a proteomic approach. Many fungal virulence proteins inside the meristem sample were upregulated, making the pathogen virulent successful colonization (Arun et al. 2018). Proteomics study also has many subdivisions based on our objective (Table 26.1).

a. Structural
   - In-depth analysis of protein structure
   - Compares protein structures and helps identify functions of newly discovered genes
   - X-ray crystallography and NMR spectroscopy

b. Quantitative
   - Simultaneous quantitation of level differences between many proteins in different sample
   - Not measurement of their absolute concentration
   - 2D-PAGE and mass spectrometry

c. Functional
   - Characterization of protein–protein interactions
   - Useful to determine the protein functions
   - Explains the way proteins assemble in bigger complexes
   - Affinity purification, mass spectrometry, and the yeast two-hybrid system

26.4.2.3 Protein/Peptide Separation Techniques

Many protein and peptide separation methods have been developed to exploit differences in size, charge, and hydrophobicity. A significant amount of information has been gained from proteomic studies using classical gel-based separation, as resolved proteins can often be identified and further characterized by mass spectrometry (MS). However, MS technologies are frequently more potent for large-scale proteomics when combined with gel-free separation techniques (Handakumbura et al. 2017).

Gel-Based Separation

Two-dimensional gel electrophoresis (2-DE) involves resolving proteins by isoelectric point (p*I*) and molecular weight. 2-DE has long been utilized for identifying plant protein abundance alterations in response to microbes and remains a viable technique (Barnabas et al. 2016).

Gel-Free Separation

While gel-based separation is still frequently utilized for specific proteomic applications, gel-free techniques for separating peptides after sequence-specific digestion have become standard for large-scale shotgun proteomics (Roe and Griffin 2006). Most gel-free methods utilize two (2D LC) or more complementary dimensions of liquid chromatography, as extensive pre-fractionation of peptide mixtures significantly increases proteome coverage with MS-based peptide sequencing.

Protein Identification Using Mass Spectrometry

Mass spectrometry is the most common technique for unbiased protein identification and has been widely applied to plant–microbe proteomics. Protein/peptide ionization, ion separation, and detection are the three major steps for MS analysis. The major protein/peptide ionization techniques are matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI), while mass analyzers include time-of-flight (TOF), quadrupole, ion trap, orbitrap, and Fourier transform ion cyclotron resonance (Zhang et al. 2015).

26.4.3 Proteomics to Study Plant–Microbe Interactions Involve Some Typical Situations

- Type of interactions: beneficial or pathogenic
- Microbe involved: bacteria, fungi, virus, nematode
- Collection of samples: infection stage and part
- Members involved: two-way or three-way interactions
- Proteins involved during interactions
- PTMs mainly involved
- Cellular targets of host specifically chosen by microbes in plants (host)
- Protein and peptide separation

- Protein precipitation and collection of pure samples
- Proteomic technique involved
- Protein quantification

26.5 Plant–Pathogen Interaction

Several studies have been performed to analyze plant–pathogen interactions. Recently, functional genomic strategies, including proteomics and transcriptomics, have contributed to defining gene and protein function and expression profiles. Pathogenicity- and defense-related genes and proteins expressed during phytopathogen infections have been identified, and enormous datasets have been accumulated. However, the understanding of molecular plant–pathogen interactions is still an intriguing area of investigation. Proteomics has dramatically evolved in the pursuit of large-scale functional assignment of candidate proteins and, by using this approach, several proteins expressed during phytopathogenic interactions have been identified. We can also understand plant–virus, plant–bacterium, plant–fungus, and plant–nematode interactions reported in proteomic studies.

Compatible Plants are incapable of mounting effective anti-infectious defense responses, allowing the pathogens to complete their life cycle.

Incompatible Plants trigger a series of complex defense responses against pathogenic interactions to forestall pathogen growth.

Plants activate basal resistance, mediated by pathogen-associated molecular patterns (PAMPs) or cell wall-degrading enzymes (CWDEs), resulting in a compatible or incompatible interaction. In both interactions, several defense-related and biotic stress-responsive proteins are induced. Suppression of plant defenses by pathogen effectors leads to susceptibility in host plants. Some host plants express resistance (R) proteins, which guard against this interference and trigger a specific resistance, referred to as the hypersensitive response (HR).

Pertaining to defense mechanisms in plants, the recognition and signaling events that occur in plant cells in response to microorganism challenges need to be extremely rapid, reliable, and specific and are part of the strategy evolved by plants to survive attacks. The intracellular sensitive perception of pathogens and the recognition of pathogen-associated molecular patterns, such as lipopolysaccharides and flagellin, lead to the activation of the plant basal defense (or resistance), which is the first defense response, and trigger a generic mechanism consisting of plant cell wall thickening, papilla deposition, apoplast acidification, and signal transduction and transcription of defense genes. This generic basal defense mechanism has been observed in several incompatible plant–microorganism interactions. It is believed to corroborate the observation that most plants are resistant to invasion by most pathogens. Therefore, successful pathogens must evolve mechanisms to interfere with or suppress basal defense to colonize the host and develop the disease.

26.5.1 Basal Defense to Plant Pathogen Attack

Some plant varieties express resistance proteins that guard against this interference and trigger a specific, genetically defined hypersensitive response and subsequent programmed cell death in the basal defense. The function of the hypersensitive response is to contain the pathogen, and it is typified by various biochemical perturbations, known as generic plant responses, including changes in ion fluxes, lipid hyperperoxidation, protein phosphorylation, nitric oxide generation, and a burst of reactive oxygen species and antimicrobial compounds (Jones et al. 2004). This rapid incompatibility response effectively puts an end to pathogen invasion and prevents further disease development.

The capacity to overcome plant defense by protecting themselves from the oxidative stress-activated by the plant in response to pathogen perception is of extreme importance. Therefore, pathogens induce several genes, such as catalases and superoxide dismutase (SOD), responsible for the inactivation of H_2O_2 and O_2 (Jones et al. 2004). The importance of secretion pathways for pathogenicity has also been well established. Effector proteins expressed by the pathogen are predicted to collaborate in suppressing basal resistance through the modification of specific host proteins (Peck et al. 2001). The secretion of extracellular enzymes, such as pectin esterases, polygalacturonases, xylanases, pectate lyases and cellulases, is another essential process for colonization and pathogenicity.

26.5.2 Pathogen Specificity to Cellular Organelles Through Proteomic Analysis

Virulence factors of diverse plant pathogens act inside the plant cell by manipulating a variety of host pathways. We can list the pathogenic proteins which reach to respective cellular targets. So sequencing results from the database can give us the biological functions of those proteins. We can understand the effectiveness of pathogenesis and the type of damage by a pathogen.

26.5.3 Pathogenic Proteins and Respective Cellular Targets

Nutrient-rich plant tissues provide one of the most essential niches for the survival and proliferation of microbes. Over time, plants have evolved a complex and multilayered immune system that effectively wards off most microbial infections. Nonetheless, numerous microbes, such as bacteria, fungi, oomycetes, and viruses, have evolved to cause disease in plants (Radhakrishnan et al. 2018). Pathogens need to evade or suppress host defenses and to manipulate host cellular functions to their advantage. This is achieved through various virulence strategies, relying on sophisticated molecular mechanisms that we are only beginning to understand.

Increasing evidence indicates that the intracellular vesicle trafficking and polarized secretion pathways are essential for plant immunity against fungal and bacterial pathogens and that pathogen virulence factors may be targeting intracellular trafficking to suppress host immunity (Toruño et al. 2016). For example, the *P. syringae* effector protein HopM1 was recently shown to target AtMIN7, one of the eight guanine nucleotide exchange factor (GEF) proteins that activate ARF GTPases in *Arabidopsis*. HopM1 physically interacts with AtMIN7 and mediates its degradation through the 26S proteasome. Significantly, atmin7 mutant plants are compromised in host immunity and are more susceptible than wild-type Arabidopsis to a bacterial mutant lacking HopM1 (Li et al. 2017).

PTMs

- Phosphoprotein expression profiling: signal pathways
- Acetylated protein expression profiling: affect protein function, interactions
- Methylated protein expression profiling: epigenetic studies
- Glycosylated protein expression profiling: varying effects on molecular protein weight, pI, and protein functions

Plant–pathogen interaction and proteomics

- The first step in understanding disease resistance is currently being met by identifying the proteins expressed during plant–pathogen interactions with different organisms such as bacteria, viruses, fungi, and nematodes (Fig. 26.2).
- The next step will be to determine which proteins confer pathogenicity and disease resistance and the mechanisms by which they do so.

Importance of proteomic approach

- A significant proportion of the identified proteins related to defense and stress response represented the phenylpropanoid pathway and oxidative stress.
- It is established that sugarcane responds to *S. scitamineum* infection by modulating the genes associated with the phenylpropanoid pathway and salicylic acid (SA) mediated pathway.
- So, by understanding the proteins involved, we can develop the match between those proteins in the biological pathway and easily understand the mechanism and channel involved by the pathogen to interact with the plant.

Pitfalls of proteomic analysis

- Identification of differentially expressed proteins will be successful when the genome sequence or a large amount of sequence data are available in public databases.
- A gap in the bioinformatics pipeline for the proteomics of organisms with incomplete sequenced genomes appears to exist.

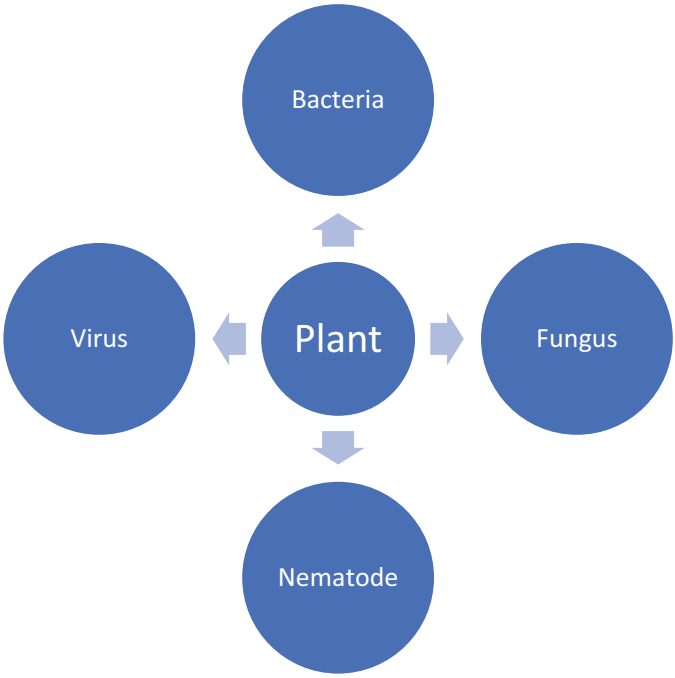

Fig. 26.2 Different pathogenic organisms affecting plants have different mechanisms of infection and interaction

- These technical limitations in proteomic studies need to be overcome to advance our knowledge of protein expression during plant–microbe interactions.
- The multiple roles of proteins are a significant barrier to progress in identifying proteins involved in processes such as plant–microbe interactions and the large number of proteins obtained with unknown functions.
- It is important to investigate further these proteins, which may present new biological functions and play important roles in the processes under investigation.

26.6 Future Perspectives on Plant–Pathogen and Approaches

- Many existing databases, including plant proteome database, plprot, PTM database, Medicago phosphoprotein database, rice proteome database, and LegProt, should be expanded and integrated in the future.
- Proteomic analyses of plant–microbe interactions have provided a better understanding of plant defense and symbiosis-induced responses. However, the lack of published work using quantitative and in vivo proteomic techniques is striking.
- While most proteomic studies provide protein identification and functional predictions, a more systematic integration of this approach will provide helpful

information that will allow for better prediction and manipulation of plant responses to symbiotic and pathogenic microbes.

Macromolecular data banks

- Sequence data banks of proteins (SwissProt); 3D-structures (PDB), references (Medline), etc.
- UniProtKB = Protein Information Resource + SwissProt + TrEMBL

Software tools

- Analysis of intrinsic properties of sequences (PROT PARAM, Glycan Mass, SignalP, etc.)
- Comparison of sequences (BLAST, PROSITE, InterPro scan, etc.)
- Identification and characterization of PMF and MS/MS data (Mascot, Phenix)
- Visualization and modeling of 3D structures (PSORT, AGADIR, MolTalk, Swiss-Model, etc.)
- Analysis and storage of proteomics data

Metabolomics

Before the advent of metabolomics, the development of genomics, transcriptomics, and proteomics contributed greatly to our understanding of plant diseases and the mechanisms that determine whether a pathogen successfully obtains nutrients and evades plant immunity. Genomics studies analyzing the genetic architecture of both plants and pathogens have been helpful to monitor how the organisms adapt to disease pressure (Möller and Stukenbrock 2017).

Metabolomics is a tool to unveil plant–pathogen interactions. The untargeted approach is qualitative and gives a global profile of many unknown metabolites in a sample. The targeted approach is quantitative and more specific, as it aims for a determined class of known compounds (Castro-Moretti et al. 2020). Among the mechanisms by which plants can control biotic or abiotic stresses, the production of secondary metabolites as defensive response is the most common feature. Metabolomics approach based on NMR spectroscopy followed by multivariate data analysis allowed for a detailed metabolite profile of plant defenses, providing essential information for breeding programs in plant crops (de Falco et al. 2019).

Using a comparative metabolomics approach, we can identify the defense-related biosynthetic pathways suppressed and induced in susceptible and resistant cultivars, respectively (Seybold et al. 2020).

Study of plant metabolomics comprises of:

- Sample preparation or extraction of bioactive molecules from the plants,
- Detection and identification of the metabolites, and
- Data processing and statistical analysis of the identified metabolites.

Modern technologies used for the study of plant metabolomics includes

- Metabolic fingerprinting and
- Metabolite profiling and targeted and non-targeted detection analysis.

26.7 Conclusion

The development of genomics, transcriptomics, and proteomics contributed greatly to our understanding of plant diseases and the mechanisms that determine whether a pathogen successfully obtains nutrients and evades plant immunity. Genomics studies analyzing the genetic architecture of both plants and pathogens have been useful to monitor how the organisms adapt to disease pressure. Transcriptomic studies have given insight as to what host genes are manipulated by pathogens in a disease setting or are reprogrammed for a successful defense response. Proteomics is the large-scale study of proteins, usually by biochemical methods. The word proteomics has traditionally been associated with displaying many proteins from a given cell line or organism on two-dimensional polyacrylamide gels. In this sense, proteomics already dates back to the late 1970s, when researchers started to build databases of proteins using the newly developed two-dimensional gel electrophoresis technique. This resulted in extensive cataloging of spots from two-dimensional gels to create databases of all expressed proteins. However, even when such gels could be run reproducibly between laboratories, determining the identity of the proteins was difficult because of a lack of sensitive and rapid analytical methods for protein characterization (such as the polymerase chain reaction and the automated sequencer for DNA analysis). In the 1990s, biological mass spectrometry emerged as a powerful analytical method that removed most of the limitations of protein analysis.

26.8 Future Prospects

As seen extensively in this review, integration between proteomics, transcriptomics, and metabolomics is key for a deep understanding of the relationship between host and pathogen. However, integrating all the datasets from the Omics is not an easy task. Linking phenotype to genotype is not as straightforward as correlating genomics data to proteomics since there is no direct link between a gene or a set of genes and a specific metabolite, as it is with DNA and mRNA (hence, protein). Nonetheless, metabolites represent the final product and drive the phenotype related to the expression of many genes (Castro-Moretti et al. 2020). Thus, future prospective plays a major role in selecting proper OMICS approaches based on our objective and availability of the database.

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Biocontrol Potential of Fungi for Pest and Pathogen Management

27

S. Shishupala

Abstract

Agriculture production is always associated with problems posed by pests and pathogens. Different groups of insect pests feed on varieties of crop plats causing severe damage. Crop plants are also attacked by various pathogens responsible for reduction in yield. Biological control has been projected as eco-friendly approach for pest and disease management. Even in integrated systems, biocontrol has been included. It is essentially based on the use of natural antagonists or making suitable conditions for the natural process to suppress diseases. The major steps involved are isolation, identification, determination of antagonistic potential of organisms, mass production, and applications. Fungi have been considered as potential antagonistic microorganisms to be used against an array of insect pests and pathogens. Fungi are also being seen as potential herbicides in weed management practices. Diverse group of fungi inhabiting soil, rhizosphere, and phylloplane are considered to be competitive biocontrol agents. Such fungi are generally fast growing, capable of killing the targets, able to produce inhibitory metabolites and enzymes. By virtue of such qualities they have become potential biocontrol agents. Entomopathogenic fungi are being currently envisaged as biocontrol agents of insect pests. *Beauveria*, *Cordyceps*, *Entomophthora, Metarhizium,* and *Verticillium* spp. are potential bioinsecticides and commercial products are made available. Likewise *Trichoderma*, *Gliocladium*, avirulent strains of pathogens are also being projected as biocontrol agents against plant pathogens. Some of these fungi are also known to enhance plant growth and induce disease resistance. The relevance of using fungal antagonists for the control of pests and pathogens has been discussed. The significance of fungal

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_27

diversity assessment and metabolomics approach for successful biocontrol is also highlighted.

Keywords

Entomopathogens · Mycoparasitism · Antibiosis · Peptaibols · Suppressive soil

27.1 Introduction

Nature has developed its own mechanisms to keep the control of every species on Earth. Ecological balance has taken over a period of time with continuous dynamic evolutionary process. However, human sustenance in this biosphere largely depended on agriculture. For this purpose selective crops are being grown in large areas available as monoculture. This provided opportunity for evolution of new pests and pathogens for adaptation into crop species being grown. Hence, plant protection measures are essential in order to prevent damage of the crop by pests and diseases. Several methods have been adopted and being developed for managing insects pests and microbial pathogens. Practical implementation of physical methods of pest management was difficult owing to large area to be monitored. The use of chemical pesticides resulted in unprecedented ecological issues as agrochemicals became serious pollutants. Renewed interest in looking for natural enemies of pests appeared to be potential in sustainable agriculture. The process of utilizing organisms against agricultural pests and pathogens was considered as biocontrol (Kolodny et al. 2020; Niu et al. 2020). The process involved identification of suitable organisms, evaluation of their biocontrol potential, and successful applications. This strategy appeared to be beneficial and later biocontrol methods provided potential alternative method in integrated pest management practices (Gangwar 2017; Tsegaye et al. 2018).

Fungi are unique group of eukaryotic microorganisms with their distinctive role in associated plants. Plant microbiome consists of microorganisms found in and on plants. Being major components of plant microbiome, fungi play a significant role in maintaining plant health. Use of fungi as bioferilizers and biopesticides is promising component in agriculture. Fungal biotechnology has opened up several possibilities of using fungi in agricultural sustenance (Patel et al. 2014; Thambugala et al. 2020; Batista and Singh 2021; Pandey et al. 2021; Pirttila et al. 2021). Recently, the role of plant microbiome for enhancement of crop yield and nutrition is subject of discussion. New insights into plant–microbe interaction mechanisms provide an opportunity to explore naturally occurring organisms for crop improvement (Kolodny et al. 2020).

Origin of biocontrol mechanisms lies in identifying suppressive soils where microbial populations keep the pathogens in-check and not allowing development of plant diseases. The organisms present in suppressive soils are the real source of biocontrol agents (Agrios 2005; Deacon 2006). Traditional methods of cultivation would provide ample opportunity for the increase in the populations of natural enemies and reducing the pathogen populations. Rhizosphere fungal communities

are extremely important in maintenance of soil health and in turn plant growth (Saba et al. 2012; Wang et al. 2020a). Even for weed control many organisms are being used (Evans 1998; Soares and Barreto 2008). It becomes imperative to enhance natural fungal populations to achieve significant biocontrol of pest and pathogens.

Use of various agrochemicals not only resulted in reduction of soil fertility but also contaminated the earth. These xenobiotics have resulted in various problems disturbing the ecological equilibrium. A conscious approach is required by using natural process/products for sustainable agriculture. Hence, biocontrol gains significance as eco-friendly approach in plant pest/disease management. Protection of food crops from pests and diseases is of highest priority owing to significant crop loss incurred. At the same time limited natural resources, intensive cultivation to meet the global human population and mitigating the requirements of sustainable agriculture are essential aspects to be addressed (Sundh and Eilenber 2020). The present chapter deals with potential of fungi as biocontrol agents to be used in crop management. Multifold mechanisms of these fungi to control insect, nematodes, and other plant pathogens have been discussed with suitable examples. Commercial products and biotechnological approach have been envisaged.

27.2 Fungal Interactions

In nature multiple interactions of fungi with other organisms occur. In soil they interact with plant roots, insects, nematodes, and microorganisms. Saprophytic, mutualistic, parasitic, and pathogenic interactions are well documented (Agrios 2005; Deacon 2006). The type of interactions occurring in rhizosphere is essential for the success of biocontrol. Even in the phylloplane and rhizoplane microbial ecosystem determines the fungal population load. Some of the fungi are extremely significant plant pathogens responsible for many diseases in plants (Agrios 2005). In soil too fungi show mutualistic or antagonistic interactions with other microorganisms. Few fungi live inside the plant tissue as endophytes without causing any deleterious effect on the host plant (Mejia et al. 2008; Larran et al. 2016; De Silva et al. 2019; Latz et al. 2021). Such interactions are dependent on both biotic and abiotic factors (Kowalska et al. 2020). Fungi are being used as biocontrol agents against insects, nematodes, and plant pathogenic fungi (Baron et al. 2019; Constantine et al. 2020; Peng et al. 2021; Upadhyay et al. 2021).

Information is available on strategies involving multi-strain biocontrol for suppressing soil-borne plant pathogens (Nega 2014; Niu et al. 2020). Adequate understanding of microbial interactions in rhizosphere is extremely important for efficacy of biocontrol. Consortium of microorganisms and rhizosphere effect need to be considered for developing biocontrol strategy. Sustainable crop management systems relay on proper understanding of fungi as biocontrol agents to ward off insect pests and nematodes. Need of complete knowledge on types of interactions occurring between insects and entomopathogenic fungi is highlighted (Baron et al. 2019). At the same time interactions of nematodes with nematophagous fungi essentially determines fate of biocontrol achieved by fungal antagonists.

27.3 Entomopathogenic Fungi

In natural environments fungi can infect insects and other invertebrate animals. Insect-pathogenic fungi may be the source for biocontrol of insects (Koiri et al. 2017; Ruiu 2018). Major entomopathogenic fungi are *Beauveria, Coelomyces, Cordyceps, Entomophthora, Hirsutella, Metarhizium,* and *Paecilomyces*. These are well-known to infect various insect groups at different stages of insect development. These fungi are necessary to keep the insect population under control and some of them are already commercially exploited as biopesticides (Deacon 2006; Carrillo et al. 2015; Ruiu 2018; Shahriari et al. 2021). Few of these are specific to group of insects and other have broad host range.

Isolation of entomopathogenic fungi is the first step toward the identification of fungal biocontrol agents. The use of various techniques for isolation of bioinsecticide candidates such as *Beauveria*, *Clonostachys*, *Lecanicillium*, *Metarhizium*, and *Purpureocillium* has been reviewed. Many of the entomopathogenic fungi are isolated from insect cadavers or from plant sources. Some of these are also endophytes. Different methods of isolation are also discussed (Sharma et al. 2020).

Entomopathogenic *Beauveria bassiana* produced blastospores when high glucose concentration is available in the medium. Genetic analysis of the fungus grown in different concentrations of glucose revealed expression of hydrophobin protein genes. Blastospore development has been implicated in application of this fungus for pest control (Mascarin et al. 2021).

Importance of secondary metabolites produced by entomopathogenic fungus *Paecilomyces* was discussed (Dai et al. 2020). An array of metabolites with various biological activities found in this fungus was responsible for biocontrol nature (Elkhateeb and Daba 2019). Enough evidences have been accumulated about potential of these fungi as biocontrol agents of insects.

27.4 Mycoparasitic Fungi

Fungi are capable of parasitizing other fungi and being referred as mycoparasites. Among these some can destroy the target fungal cells by producing various enzymes and few are capable of drawing nutrients from other fungi (Agrios 2005; Deacon 2006; Demirci et al. 2007; Bastakoti et al. 2017; Niu et al. 2020). Extensive literature is available with respect to *Trichoderma* as successful mycoparasite (Fig. 27.1). These antagonistic fungi make their hyphae penetrate into target fungal structures or coil around them and digest. The process is referred as mycoparasitism (Naydenov and Draganova 2007; Carreras-Villasen et al. 2012; Jorge 2014; Waghunde et al. 2016; Fan et al. 2020; Poveda et al. 2020).

An extensive list of fungal antagonists to control plant diseases was presented. Around 300 fungal antagonists were listed for their potential as biocontrol agents against fungal diseases of plants. These antagonistic fungi belonged to 113 genera distributed in 13 classes. Their phylogenetic relationships were also established. The

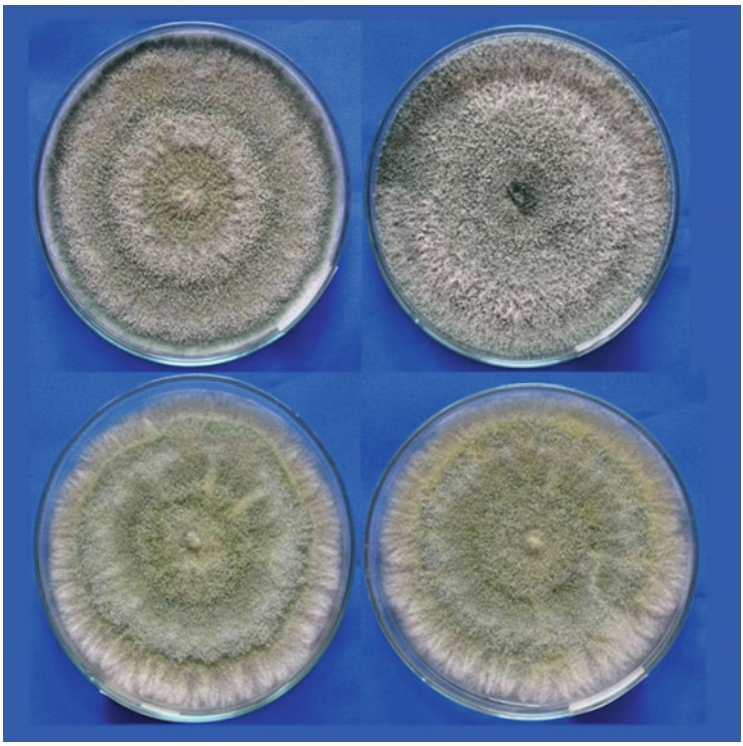

Fig. 27.1 Different *Trichoderma* isolates having potential for biocontrol

major fungal genera being used as biocontrol agents are *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, *Penicillium*, *Pichia*, *Talaromyces*, *Trichoderma,* and *Verticillium.* These fungi are reported to be effective antagonists against several plant pathogenic fungi (Thamugala et al. 2020). Biocontrol potential of *Trichoderma* was envisaged in terms of production of antifungal compounds apart from mycoparasitism. Complex interactions of *Trichoderma* with other plant pathogenic fungi also revealed the production of hydrolytic enzymes to degrade target fungi (Mukhopadhyay and Kumar 2020).

The role of phytopathogenic fungi to be used as biocontrol agents was suggested (Peng et al. 2021). Understanding the capacity of these fungi to produce pathogenicity factors, enzymes, toxins and growth regulators is essential to use them as biocontrol agents. These fungi may serve as novel beneficial resources to control plant diseases.

Fusarium oxysporum is notorious phytopathogen capable of infecting more than 100 species of plants. However, a strain of this fungus Fo47 was an endophyte with biocontrol potential. This strain had a single accessary chromosome. This analysis of chromosome-scale genome assembly was useful in understanding endophytic interactions and biocontrol ability of the fungus (Wang et al. 2020b).

Application of biocontrol strategy for the prevention of mycotoxins in food and feed stuffs was explored. Yeast and other fungi were involved in degradation or absorption of mycotoxins from the feed materials and provided a novel approach in detoxification (Nesic et al. 2021). It is possible to use biocontrol to prevent mycotoxigenic fungi and their mycotoxins (Pellan et al. 2020). Potential of biocontrol even in such aspects are being explored.

27.5 Nematophagous Fungi

Nematodes are small and thin animals living in soil and organic matter. Many of them cause plant diseases. They can live as endo- or ecto-parasites in plants. The major plant pathogenic nematodes belong to the genera *Meloidogyne*, *Heterodera,* and *Globodera* (Agrios 2005; Deacon 2006). The chemical nematicides are effective but contaminate the soil. Hence, nematophagous fungi have the potential to become biocontrol agents. In organic-rich environments many nematophagous fungi survive. Some of them trap nematodes and others parasitize eggs/cysts. The major genera of nematopagous fungi include *Arthrobotrys*, *Atricordyceps*, *Catenaria*, *Cystopage*, *Dactylella*, *Hirsutella*, *Nematophthora*, *Pleurotus,* and *Verticillium*. Some of them use adhesive hyphae, nets, knobs, constricting rings to trap the nematodes. All these fungi are mainly saprophytic and capable of using nematodes as source of nitrogen. Endoparasitic fungi can make use of nematodes as major source of nutrients. Even eggs and cysts are parasitized by such fungi. All these properties make them supreme biocontrol agents to reduce nematode diseases of plants (Fan et al. 2020; Poveda et al. 2020; Zhang et al. 2020).

Screening of 890 fungal isolates resulted in identification of *Trichoderma citrinoviride* strain capable of killing juveniles of root-knot nematode *Meloidogyne incognita*. The fungus also prevented nematode egg hatching contributing significantly for reduction of root gall formation. Apart from biocontrol efficacy, the selected strain also promoted the growth of tomato plants (Fan et al. 2020). It is interesting to understand fungi–nematode interactions. In agricultural ecosystem both fungi and nematodes interact in different ways. Diversity, ecology, and biocontrol significance of nematophagous fungi have been discussed (Zhang et al. 2020). Implications of these interactions in biocontrol of nematodes and phytopathogenic fungi are presented. Hence, it is important to explore mechanisms of biocontrol where some endophytic fungi, trigger the host defense against nematodes. Bionematicide fungus *Aphanocladium album* protected tomato plants from infestation of root-knot nematode *Meloidogyne javanica*. Significant reduction in gall formation in root was noticed. Microbial populations in rhizosphere were also increased. Successful biocontrol of nematodes was achieved (Leoni et al. 2020).

27.6 Mechanisms of Biocontrol by Fungi

Antagonistic organisms make use of several modes of action in order to prevent/kill target pathogens. Biocontrol fungi have shown an array of mechanisms by which they are able to successfully take over the pests and pathogens. A set of criteria are used to assess biocontrol ability of fungi. Mainly, fast competitive growth, establishment in rhizosphere, parasitism, production of compounds such as siderophores, antimicrobial metabolites, and enzymes are considered as direct mechanisms to control pathogens. Induction of resistance in the plants against pathogen/pests is considered to be indirect mode of action of biocontrol agents (Agrios 2005; Lyon 2007; Glick et al. 2010).

Multiple mechanisms operate in biocontrol of plant diseases by fungal antagonists. Competition of biocontrol agent to make use of available nutrients and space ahead of the pathogen is one of the key attributes. In case of seed- and soil-borne infections establishment of biocontrol agents in rhizosphere is extremely important. Rhizosphere competence is another important aspect defining success of biocontrol. Many fungal antagonists have the mycoparasitic ability to target the pathogens (Lo 1998).

The types of microbial interactions occurring on phyllosphere with reference to biocontrol are essential (Legein et al. 2020). On leaf surface complex factors influence microorganisms. Modes of action occurring between microorganisms at phyllosphere greatly influence the efficacy of biocontrol. Thus, antagonists improve the plant health.

Mechanisms of biocontrol by *Trichoderma* involved mycoparasitism, antibiosis, and competition for food and space. Different metabolic pathways will ensure all these mechanisms to be effective for successful biocontrol (Mukhopadhyay and Kumar 2020).

27.6.1 Competition and Rhizosphere Competence

To become successful biocontrol agent the organism should have competitive ability to establish in the given ecosystem. Most of the selected antagonistic fungi are capable of growing very fast to outgrow the target pathogen. For example, *Trichoderma* spp. are efficient in covering most of the available space of soil under favorable conditions. Early establishment of the biocontrol agent reduces the scope for the pathogen to establish. Reduction in pathogen inoculum obviously reduces the disease (Mathre et al. 1999; Butt and Copping 2000; Bastakoti et al. 2017).

Seed- and soil-borne infection occurs in the rhizosphere. Nature and concentration of the root exudates greatly influence the population of microorganisms in the rhizosphere. Introduced biocontrol agent should have higher level of rhizosphere competence to establish itself ahead of others to be successful (Aboutorabi 2018; Li et al. 2019; Leoni et al. 2020; Wang et al. 2020a). Even in phylloplane microbial succession determines the efficacy of biocontrol (Chen et al. 2020; Legein et al.

2020). Potential of biocontrol agent can be realized when suitable environmental conditions are provided.

27.6.2 Hyperparasitism

Fungal antagonists can directly parasitize the target pest/pathogen. Entomopathogenic fungi like *Cordyceps* can establish in the living insect and continue to grow even after the death of the insect completely mummifying the cadaver (Shishupala 2021). *Trichoderma* hyphae are known to penetrate through the target fungal conidia. They can even coil around the target hyphae resulting in digestion and ultimately death (Agrios 2005; Carreras-Villasen et al. 2012; Nega 2014). Nematophagous fungi can trap adult nematodes and digest. Some fungi can even parasitize the nematode eggs and cysts (Fan et al. 2020; Zhang et al. 2020).

Trichoderma harzianum isolate successfully inhibited mycelial growth of *Colletotrichum capsici* and *C. gloeosporioides* in dual culture plate assay. Mycoparasitism was evident by damage caused to hyphae of the anthracnose pathogen in chili. The study indicated potential of biocontrol (Sutarman et al. 2021). Hyperparasitism is one of the essential mechanisms in biocontrol.

27.6.3 Antibiosis

Production of antimicrobial secondary metabolites inhibits growth of target pathogens. Number of such metabolites has been discovered. These compounds provide advantage for biocontrol agent to suppress other competitors (Dai et al. 2020). Successful biocontrol of several pathogens have been achieved using actinomycetes group of organisms. These soil inhabitants produce an array of secondary metabolites with antimicrobial activities (Selim et al. 2021). However, such efforts of screening and selection of fungal species/strain for effective biocontrol are limited.

Peptaibols are group of antimicrobial compounds produced by species of *Trichoderma*. Extensive chemical diversity exists in this group of peptide antibiotics. They are produced by non-ribosomal synthesis involving multifunctional enzymes. Chemical diversity of these compounds can be assessed by using intact cell matrix-assisted laser desorption/ionization mass spectrometry. These peptaibols include atrovirdin, trichorozin, harzianin, trichokonin, polysporin, etc. (Shishupala, 2008, 2009). Biocontrol potential of *Trichoderma* is mainly attributed to the production of such antimicrobial peptides.

Among the several metabolites of plants, terpenes play an important role offering protection against infections. Antimicrobial, insecticidal, and weed control abilities of terpenes are significant. Chemistry, biosynthesis, and types of terpenes have been described in view of using them as biopesticides and herbicides (Ninkuu et al. 2021). It will be interesting to generate information on terpene metabolism as influenced by biocontrol agents. Analysis of metabolites produced by an organism is referred as

metabolomics and several activity-based techniques have been developed to identify the metabolites produced by biocontrol agent.

The effectiveness of biocontrol of *Trichoderma* largely depends on secreted metabolites. Biocontrol strain of *Trichoderma* was tested against rhizosphere bacteria isolated from tomato plants. The culture filtrate inhibited most non-target rhizosphere bacteria indicating antibacterial metabolites. The culture filtrate of *Trichoderma* was found to have secreted proteins, metabolites, and also volatile compounds. Interestingly, suppression of growth of *Trichoderma* was also noticed by some bacterial isolates. Effectiveness of biocontrol largely depends on metabolite-mediated interactions (Li et al. 2019).

Abdelhamid and El-Dougdoug (2020) reported the use of natural antimicrobials for control food borne pathogens. This approach aims at use of natural antimicrobials for prevention of microbial growth and inhibition of pathogen virulence factors. Non-toxigenic strains of *Aspergillus* may prove beneficial to competently remove aflatoxins from maize grains. Many other lactic acid bacteria, bacteriophages, and bacteriocins are also useful in preventing food borne pathogens.

Entomopathogenic *Paecilomyces* strain was known to produce more than 223 secondary metabolites. The metabolites characterized belong to alkaloid, peptide, polyketide, pyrone, and sterol groups. Biological activities such as antimicrobial, insecticidal, and nematicidal properties of these metabolites were useful for biocontrol (Dai et al. 2020).

27.6.4 Enzymes in Biocontrol

Many of the biocontrol agents are capable of secreting hydrolytic enzymes such as chitinases, glucanases, cellulases, etc. which are essential to digest the components of target pest/pathogen. All types of biocontrol agents including entomopathogenic, mycoparasitic, and nematophagous fungi produce different levels of these enzymes helping in biocontrol (McQuilken and Gemmell 2004; Mukhopadhyay and Kumar 2020; Poveda et al. 2020). Both constitutive and induced enzyme production have been noticed. Target pathogen cell wall is digested with these enzymes. In case of insect pest the outer covering is digested leading to death of the insects.

27.6.5 Induction of Plant Resistance

Plant defense involves both structural and biochemical basis. Timely activation of defense genes is essential to ward off the pathogens. Induction of resistance is possible with biotic and abiotic elicitors of defense. Many biocontrol agents are capable of producing suitable elicitors to activate the defense mechanisms in plants (Lyon 2007).

Some of the biocontrol agents are known to induce resistance in plants against fungal infections. This has been mainly attributed to activation of plant defense genes. Molecular evidences were made available to prove induction of resistance by

biocontrol agents in case of grapevine trunk disease caused by fungus *Phaeomoniella chlamydospora* (Yacoub et al. 2020). Transcriptome analysis of plants inoculated with biocontrol agent *Pythium oligandrum* clearly demonstrated activation of defense mechanisms through jasmonic acid/ethylene mediated reactions. Biocontrol agent was considered as inducer of plant-systemic resistance. It also affected virulence factors of the fungal pathogen at molecular level. The study clearly demonstrated suppression of the disease by induction of defense by biocontrol agent.

Influence of biocontrol agent on host defense is a significant aspect in mechanisms of action. *Trichoderma harzianum* and *T. viride* treated to soybean seeds increased the activity of defense enzyme such as β-1,3 glucanase, peroxidase, and polyphenol oxidase. Significant increase in phenolic content of leaves was also evident with these treatments. Induction of systemic acquired resistance by biocontrol agent against *Macrophomina phaseolina* was demonstrated in soybean (Intisar et al. 2021).

Entomopathogenic fungus *Metarhizium brunneum* associated with roots of cabbage plants induced changes in the leaf reflection and also plant physiology at jasmonic acid and salicylic acid signaling level. Due to manipulation of host plant, behavior of herbivorous insects changed both at green house and field conditions. This is the key mechanism for biological control (Cotes et al. 2020). The ongoing examples surely indicate the ability of biocontrol agents to induce host resistance. Indirect mode of action of these will benefit the plant to ward off the pests/pathogens. Altered plant physiology where defense genes are activated provide conditions for healthy plants to grow.

27.7 Applications and Commercial Products

Identification of suitable biocontrol agent is the preliminary step for effective implementation of biocontrol. The large scale production of either biomass or culture filtrate involves both solid-state and liquid fermentation process. Use of locally available ingredients is necessary to reduce the cost involved in production of antagonists. The biocontrol agents are being used in the field in various ways. Soil application is one of the major methods of application to prevent soil inhabiting pathogens. Furrow applications are also being done. In case of many diseases seed treatment of biocontrol with suitable adhesive is being practiced. Seedling dip methods are at nursery level. The phylloplane organisms or culture filtrates/metabolites are being used as foliar spray. Many different formulations are available in commercially developed biocontrol agents (Mathre et al. 1999; Jensen et al. 2007; Pellan et al. 2020).

Significant reduction of oospore infection in pearl millet downy mildew was achieved by soil amendment of biocontrol agents such as *Trichoderma harzianum, T. viride, Aspergillus niger,* and *Chaetomium globosum*. The culture filtrates of these organisms were found to be useful in preventing zoospore infection when applied as foliar spray on to the seedlings (Shishupala and Shetty 1989, 1990). Biocontrol

efficacy of fungal antagonists is greatly influenced by host plant, soil microbiome, and other edaphic factors. It was evident in a study on lentil genotypes and root rot pathogen *Aphanomyces euteiches*. The biocontrol agent *Trichoderma* spp. showed differential correlation based on host genotypes and root associated fungal communities. Multipartite interactions between host plant, pathogen, biocontrol agent, and other microorganisms will determine success of biocontrol (Bazghaleh et al. 2020).

Talaromyces apiculatus and *Clonostachys rosea* were found to be effective in reducing basal stem rot disease of oil palm. The mycelial growth of the pathogen *Ganoderma boninense* got reduced drastically in the presence of biocontrol agents. Individual applications of biocontrol agents or consortium helped the oil palm plants to grow better with significant reduction in disease. The study demonstrated usefulness of biocontrol even in crops like oil palm (Goh et al. 2020). Biocontrol agents have been viewed to improve plant nutrition apart from protection. Multiple functions can be achieved by using beneficial microorganisms through proper understanding of mechanisms involved in plant–microbe interaction. The alteration of plant metabolism induced by fungi seems to improve plant nutrition. Hence, it is important to ascertain potential of fungal antagonists (Kowalska et al. 2020).

Several yeast strains were identified for use of biocontrol against *Penicillium digitatum* in orange fruits. *Saccharomyces cerevisiae* and *Candida stellimalicola* were able to inhibit mycelial growth of *P. digitatum* in vitro. *Meyerozyma caribbica* also prevented the incidence of green mold in oranges. The study clearly demonstrated potential of biocontrol by yeasts in oranges (Cunha et al. 2020). Similarly, the apple fruit microbiome was studied (Bosch et al. 2021). Identification of fruit microbiome composition and diversity will help in selection of useful biocontrol agents to prevent post-harvest losses of fruits.

Post-harvest diseases are really problematic in fruit production. Both epiphytes and endophytes of fruit microbiome may determine the marketing quality of the fruits. Biological control of fruit molds is achieved by useful yeasts such as *Aureobasidium*, *Candida,* and *Pichia* spp. The yeast coating on the fruits can be effective in presenting post-harvest damages. Even genetic engineering of useful enzyme coding genes from these yeasts is found to be useful in biological control (Agrios 2005; Lindo et al. 2020; Zhang et al. 2021). Yeasts are also being seen as potential bioagents to achieve biocontrol efficiency in sustainable agriculture. Evidences have been accumulated to use selected culturable yeasts for the beneficial interactions with plants (Hernandez-Fernandez et al. 2021).

Soil microbiome is an important aspect of plant health. Efficiency of biological control of *Verticillium dahlia* in cotton largely depends on the composition of soil microbiome and host factors. Reduction in soil fungal diversity and increase in bacterial diversity was noticed when crop rotation was made with cotton-maize. Correlations were obtained with different fungal communities in soil as influenced by crop rotation (Xi et al. 2021). Thus, it is important to ascertain soil microbiome for effective use of fungal biocontrol agents. Use of microbial detoxification of mycotoxins present in animal feed was found to be useful. Many fungi involved in

such a process provide future prospects for biocontrol of mycotoxin contamination (Nesic et al. 2021).

Encapsulation of microbial biocontrol agents within biopolymers have opened up a novel formulation method. Efficient application of encapsulated microorganisms has been shown to be advantageous over traditional methods in biocontrol. Different types of biopolymers for encapsulation of microorganisms in biocontrol have been reviewed (Saberi-Riseh et al. 2021). Such technological advancements are necessary for effective use of fungal biocontrol agents.

An interesting comparison was made between susceptible and tolerant ash tree leaves for fungal microbiota in order to search for suitable biocontrol agent against fungal pathogen *Hymenoscyphus fraxineus*. Disease tolerant trees showed the presence of yeast *Papiliotrema flavescens* and filamentous fungus *Sarocladium strictum*. Both these fungi showed antagonistic property against the pathogen. Differences were observed among the different strains of antagonists with reference to inhibition of the pathogen. However, analysis of leaf colonizing fungi provided potential antagonistic fungi to be used as biocontrol agents (Becker et al. 2020).

In a field study fungal biocontrol agents were successful to reduce *Fusarium* head blight of maize and wheat. *Clonostachys rosea* and *Trichoderma atrobrunneum* found to reduce the disease caused by *Fusarium graminearum*. Various parameters showed effectiveness of biocontrol by *C. rosea* to reduce the disease incidence up to 95% under field conditions. At the same time the biocontrol agent significantly decreased mycotoxins like deoxynivalenol and zearalenone in seeds. The results are highly promising for effective implementation of biocontrol in head blight management (Gimeno et al. 2020).

In tree management aspects cutting of tree branches in undesired areas like under electric power lines or above gas pipes are required. However, mechanical cutting will allow resprouting of tree from the cut ends. Hamberg et al. (2021) reviewed the use of fungi like *Chondrostereum purpureum* as biocontrol agent to prevent sprouting of hardwood stumps. This fungus induces decay of hardwood and thus prevents sprouting. Essentially a wood rotting fungus can also be used as biocontrol agent in tree management practices.

Biocontrol is also being envisaged to protect historical monuments and prevent microbial deterioration of building stones. Fungal pathogens of forest plants were able to produce metabolites like cavoxin and epi-epoformin which showed antifungal activity. This property of fungal metabolites was exploited to prevent deterioration of archeological remains by *Alternaria alternata*, *Aspergillus niger*, and *Fusarium oxysporum.* Biological preservation of stone buildings with fungal metabolites opened up novel method of biocontrol (Masi et al. 2021).

Many formulations of biocontrol agents have been developed for commercial purpose. These biofungicides, bionematicides, and bioinsecticides are being packed and sold just like any other pesticides (Fig. 27.2). Different fungal biocontrol agents are being sold with various trade names all around the world. Some selected fungi for the control of plant pathogens, insects, and nematodes are listed (Table 27.1). Many materials like talc are being used apart from adhesives and carrier materials in these formulations. Solid mass or liquid cultures are also available depending on nature of

Fig. 27.2 Commercial fungal antagonists available in the market

the organisms and method of application. Viability of the organisms essentially determines the efficiency of biocontrol.

27.8 Conclusions and Future Perspectives

Plant microbiome has gained significance. Biocontrol as a component of integrated pest/disease management practice has made its own impact. Initial success at laboratory and greenhouse level led biocontrol to be taken up at field level. Successful field evaluation followed by commercial production was achieved in selected fungi. Fundamental understanding of antagonist's behavior in natural conditions determines the success of biocontrol. Native strains of fungal antagonists are better as they survive well under the given conditions. Potential fungal metabolites may be used as lead molecules to synthesize commercially viable compounds. Metabolite data base, biotechnological approach in combinatorial chemistry, and selection of suitable biocontrol agent are the requirements. Metabolomics and metagenomics approach are also essential. Screening and selection of efficient biocontrol species/strain is a continuous process. Technological development in order to identify metabolites need to be taken into consideration. Proper agronomic practices will also help to increase populations of antagonists suppressing pest and pathogens. Traditional methods of agriculture form the basis of biocontrol. Modern applications of biocontrol may lead to sustainable agriculture.

Acknowledgements The author is grateful to UGC and Davangere University authorities for providing necessary facilities and permission. Thanks are due to Prof. M.S. Hegde, Convener, Talent Development Centre, Indian Institute of Science for providing library facilities and Mr. B.-N. Narendrababu for photographic help.

Table 27.1 Fungal antagonists for biocontrol of pests and pathogens and their commercial products

Sl. No.	Fungal antagonist	Target species	Commercial product
1	*Ampelomyces quisqualis*	Powdery mildew fungi	AQ10 BioFungicide
2	*Anthracocystis flocculosa*	Powdery mildew fungi	Sporodex L
3	*Beauveria bassiana*	Colorado potato beetle Froghopper in sugarcane Whitefly, aphids, thrips, Corn borer	V-Cide Green Beauveria Mycotrol Botanigard Ostrinol
4	*Candida oleophila*	*Penicillium* spp. (citrus) *Botrytis cinerea* (apple)	Aspire
5	*Fusarium oxysporum*	Wilt diseases	Biofox C
6	*Hirsutella thompsonii*	Spider mites	No-Mite
7	*Isaria fumosorosea*	Whitefly	Nofly
8	*Metarhizium anisopliae*	Termites, locusts	Metarhoz Meta Bio-Metaz BioBlast Green Muscle
9	*Myrothecium verrucaria*	Nematodes	DiTera
10	*Lecanicillium lecanii*	Aphids, leafminers, mealybugs, scale insects, thrips, whiteflies	Lecatech-WP Varunastra
11	*Paecilomyces lilacinus*	Nematodes	Paecil/Bioact
12	*Paecilomyces fumosoroseus*	Insects, mites, nematodes, Thrips	Bioact WG No-Fly-WP Paecilomite
13	*Paraphaeosphaeria minitans*	*Sclerotinia sclerotiorum*	Contans WG
14	*Phlebiopsis gigantea*	*Heterobasidium annosum* (Pine root rot)	Rotstop PG IBL
15	*Pythium oligandrum*	*Pythium ultimum*	Polygandron
16	*Trichoderma harzianum*	Root rot diseases; *Pythium*, *Fusarium*, *Rhizoctonia*, *Thielaviopsis,* and *Cylindrocladium* species	RootShield Trichodex Binab T
17	*Trichoderma viride*	Soil-borne fungal diseases	Trieco
18	*Trichoderma virens*	Soil-borne pathogens; *Rhizoctonia* and *Pythium* species	Soilgard
19	*Verticillium biguttatum*	*Rhizoctonia solani*	Vetri
20	*Verticillium lecanii*	Aphids	Verticill Vertalec

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Role of Fungal Biocontrol Agents for Sustainable Agriculture

28

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Abstract

Biological control is a method of reducing the density of disease-producing infectious propagules in their active or dormant state by one or more organisms, accomplished naturally or through manipulation of the surrounding environment, host, or biocontrol agent. Generally, the microbial control agents against plant pathogens, i.e., fungi and bacteria isolated from the rhizosphere and phyllosphere region play a crucial role in controlling plant pathogens. Similarly, several microbial agents representing different species of fungi, bacteria, viruses, and protozoa help in reducing the insect pest population. Several species of higher fungi such as *Beauveria*, *Metarhizium*, *Cordyceps*, *Purpureocillium*, *Lecanicillium*, *Trichoderma, Ampelomyces,* and others are reported as potential fungal bioagents. They demonstrate diverse roles as antagonists against phytopathogens, rhizosphere colonizers, biocontrol agents against insect pests, plant growth promoters, and endophytes. Commercial uses and applications of fungal biocontrol agents have been slow mainly due to their varied efficacy under different environmental conditions and due to their host specificity. Hence, it is imperative to develop new formulations of fungal biocontrol agents with a higher degree of efficiency, stability, and survivability using biotechnological approaches. Modern techniques in biotechnology have the potential to manipulate desirable traits of these agents to improve the overall field efficacy.

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_28

Keywords

Fungal biocontrol agents · Antibiosis · Entomopathogen · *Trichoderma* · *Beauveria* · *Metarhizium* · Sustainable agriculture

28.1 Introduction

The injudicious and overuse of synthetic pesticides in agriculture and their ill effects on the environment have led researchers to use safe and eco-friendly measures to combat pest and disease attacks. The major biotic threat for agricultural crops, viz., insect pests and diseases need to be controlled in order to ensure food security. Various strategies have been used to mitigate the insect pest and diseases affecting crop plants. Despite various good agronomic and horticultural practices, producers heavily depend on chemical fertilizers and pesticides. However, the undesirable use of these synthetic chemicals has resulted in considerable adverse effects on the environment. Currently, the consumers prefer residue-free food and there is a radical move to ward off most hazardous chemicals from the agriculture. Hence, attentive efforts are being made to develop safe alternatives to replace synthetic chemicals for the management of pests and diseases. Among the few effective alternatives, biological control of insect pests and diseases is focused on as an environmentally sound approach. The development and successful adoption of the this strategy relies on the complex interaction between the plants, pests, biocontrol agents and the environment. "The biological control can be elaborated as 'reducing the density of disease-producing infectious propagules in its active or dormant state, by one or more organisms accomplished naturally or through manipulation of surrounding environment, host or biocontrol agent" (Baker and Cook 1974; Boyetchko 1999). The important factors deciding the efficacy of biocontrol strategy are target host, number, type, and source of biocontrol agent; and the intensity and time of intervention. More broadly, the term biological control encompasses the utilization of natural or fermentative products from various sources (Jyoti and Singh 2017).

Among the various microorganisms that have been reported to be potential biocontrol agents in curbing pest and disease menace, fungi are a prime group, studied in-depth and have been explored extensively. The rationale for the broader use of fungi is their diverse metabolic activities, which increase the chances of an array of isolates for biocontrol, their efficiency in suppressing the target host and their relative environmental safety. Several species of higher fungi such as *Beauveria*, *Metarhizium*, *Cordyceps*, *Purpureocillium*, *Lecanicillium*(-=*Verticillium*), *Trichoderma,* and others are reported as potential fungal bioagents, commercialized, and applied in the field (Baron et al. 2019). Nevertheless, the factors such as virulence, resistance to harsh environmental condition (UV stress, high temperature), the feasibility of mass production, sporulation efficiency on low-cost substrates, ability to cause pathogenicity under low humidity regimes, and host specificity determine the utilization of fungi at commercial scale (Pourseyed et al. 2010). This chapter discusses the role of different fungal biocontrol agents,

their mode of action against various insect pests and phytopathogens, and the possible application of these fungi for sustainable agriculture development.

28.2 Biological Control of Insect Pests and Diseases: An Overview

Kenis et al. (2017) described biological control as the introduction of an exogenous biological agent into an environment with an aim for its permanent establishment and to control pests present therein over the long term. The approaches for plant disease control include the reduction in the number of propagules and effect of the pathogen, which is accomplished by the introduction of various biological mechanisms or through the action of naturally present or introduced antagonists by molding the microenvironment to favor the growth and activity of antagonists (Baker 1987; Strirling and Stirling 1997). Generally, the microbial control agents against plant pathogens, i.e., fungi and bacteria isolated from the rhizosphere and phyllosphere region play a key role in controlling plant pathogens. These microbial agents prevent the host plant from pathogen infection and further the establishment of the pathogen in the host. The various direct and indirect mechanisms have been manifested by the biocontrol agents against plant pathogens. These include mycoparasitism, antibiosis, induced systemic resistance, growth promotion through the production of various plant hormones, extracellular secretion of hydrolytic enzymes, competition for space and nutrients between antagonist and plant pathogen, and detoxification of virulence factors (Heydari and Pessarakli 2010; Chandrashekara et al. 2012; Singh 2014; Zhang et al. 2014; Deketelaere et al. 2017). The recent studies have highlighted the importance of localized or systemic resistance induced by microbial control agents subsequent to root colonization. The bioactive molecules released by the antagonists on root epidermis and outer cortical layers cause the walling-off of pathogenic fungi or bacterial colonies (Harman 2006). Consequently, the transcriptome and proteome machinery of the plant will get altered substantially, which provides the plants additional advantages such as increased plant growth, nutrient uptake, and induction of resistance pathways in plants. Due to diverse mechanisms exhibited by microbial antagonists, they are gaining momentum as viable alternatives to synthetic pesticides with an increased level of safety and reduced environmental hazards. As a result of intensive research on microbial antagonists, commercial products, viz., *Trichoderma*-based products have been used significantly against various plant diseases (Menzler-Hokkanen 2006). Moreover, post-harvest loss of fruits and vegetables due to microbial spoilage ranges from 10 to 15% (Janisiewicz and Korsten 2002). It has been demonstrated that the effective microbial antagonist for post-harvest disease management and as a result, various microbial agents are being employed for the control of post-harvest disease worldwide and there has been ample interest in the utilization of antagonistic microorganisms against post-harvest diseases (Heydari and Pessarakli 2010; Wisniewsk et al. 2016; Nabi et al. 2017). A few examples are indicated here. Mohamed and Saad (2009) reported the strains of *Pichia anomala* as the safe

and effective antagonist against post-harvest rot of guava caused by *Lasiodiplodia theobromae*. Moreover, *Trichoderma* species have been documented as the promising bioagents against crown rot complex of banana caused by fungal pathogen *Colletotrichum musae*, *Fusarium verticilloides,* and *Lasiodiplodia theobromae* (Alvindia and Natsuaki 2008; Sangeeta et al. 2009).

The microbial agents representing different species of fungi, bacteria, viruses, and protozoa help in reducing the insect pest population. The naturally occurring entomopathogens are significant factors against insect pests infesting agricultural crops (Tanzini et al. 2001; Roy and Cottrell 2008). Normally, these entomopathogens regulate the insect pest population to the levels wherein no economic damage is recorded in crop plants and utilize insect pests as hosts during their life cycle. They are either facultative or obligate parasites attacking various insect pests, having a high potential of survivability in the environment. Among all other microbial agents against insect pests, entomopathogenic fungi (EPF) are most crucial due to various factors such as easy distribution, simple mass production protocols and techniques, access to different strains of diverse characteristics (St Leger and Wang 2010). As per the mechanisms of action of EPF are concerned, the spores are infectious propagules infecting the body of the insect host. Initially, these propagules propagate on the exterior of the insect body and later penetrate the host. As a result, the death of the infected host occurs within 4–8 days. Subsequently, the cadavers serve as the origin of new propagules, which further disseminate and thus life cycle of EPF is continued while infecting new hosts. All groups of insects may be infected with EPF and over 700 different species of fungi have been recorded as insect pathogens. Some of these fungi have a narrow host range, viz., *Aschersonia aleyrodes* infects only scale insects and whiteflies, while other fungal species have a wide host range, with individual isolates having high specificity to target pests. The general facultative parasites, such as *Aspergillus* and *Fusarium* and few obligate species, quite rarely found such as *Cordyceps* have also gained considerable attention as EPF (Sandhu et al. 2012).

28.3 Fungal Biocontrol Agents

The research and utilization of fungal biocontrol agents against insect pests and diseases have largely increased because of the key characteristic features that fungi possess such as high reproduction rate, dispersal efficiency, a short generation time, high degree of host specificity, simple culture, and maintenance in the laboratory. Besides, in the absence of a host, fungal biocontrol agents persist in the environment as saprophytes, shifting from their mode of parasitism. Since ancient times, man has attempted various alternative cultivation practices to accelerate crop production and to control pest and disease severity in crop plants (Singh and Chawla 2012; Gupta and Sharma 2014). With the findings of the efficacy of microorganisms in reducing the disease severity, various strategies have been deployed to control plant pathogens with the use of fungal biocontrol agents. Roberts (1874) introduced the term antagonism and demonstrated the antagonistic potential of *Penicillium glaucum*

against bacteria. Hartley (1921) made the first attempt of inoculating soil with potential antagonistic microorganisms. The different strains of antagonistic fungi were inoculated to the soil to control damping-off caused by *Pythium debaryanum* (Baker 1987; Gupta and Sharma 2014). Weindling (1941) documented the antagonistic potential of *Trichoderma* species against *Rhizoctonia solani* and *Sclerotium americana.* The gliotoxin was the antimycotic agent released by *Trichoderma* in controlling the plant pathogenic fungi. This was the first document to demonstrate the antimycotic antagonistic potential in plant disease management (Baker 1987; Howell 2003). The advancement in biotechnological approaches has led to an increase in the potential use of fungal biological control agents against a wide range of plant diseases. The multifarious research findings have been endorsed the new fungal biocontrol agents and their effectiveness under diverse environmental conditions. Table 28.1 depicts the taxonomic position of a few important EPF as suggested by Hibbett et al. (2007).

With respect to the potential of fungal biocontrol agents against insect pests, EPF attracts great attention. This EPF encompasses numerous phylogenetically, morphologically, and ecologically diverse fungal species. A wide range of insect hosts ranging from aquatic ecosystems to terrestrial insects is infected by these EPF at all the developmental stages: egg, larvae, pupae, nymphs, and adult. The EPF differs significantly in the mechanism of action and virulence. The degree of attachment and penetrability through the host exoskeleton regulate the extent of infection. The basic biological knowledge and socio-economic factors are the key challenges in the research and development of commercial formulations of fungal biocontrol agents. Significant achievements have been documented in different domains, but it is very crucial to amalgamate and communicate these innovations (Singh et al. 2017). There have been few important genera of EPF, effective against the field, greenhouse, storage, and household pests. For instance, EPF *Beauveria*, *Metarhizium*, *Isaria*, *Lecanicillium,* and *Hirsutella* are the key genera, which have been exploited at remarkable levels (Sharma and Sharma 2021). These are generally common habitants of the soil ecosystem, which protects them from harmful solar radiation (Meyling and Eilenberg 2007). Moreover, it has been noted that EPF interacts positively with plant roots and enhances their growth and survival longevity primarily relies on the insect for carbon and not on soil (Inglis et al. 2001). Several EPFs evolve and establish as endophytes inside the plant tissues and do not produce any conspicuous infection symptoms. The most commonly reported and naturally occurring EPF endophyte species are *Beauveria bassiana* and *Metarhizium anisopliae* (Akutse et al. 2013). These endophytic EPF sensitize the host resistance against various pathogens and insect pest attacks by releasing metabolites, which induces a systemic resistance mechanism in the plant system. The several metabolites, enzymes, and chemical signals are released upon colonization by endophytic EPF. The key secondary metabolites: benzopyranones, phenolic acids, quinones, steroids, and enzymes: β-1,3-glucanase, chitinases, amylases, laccases, and cellulases facilitate the interaction between endophyte and plant host (Zaynab et al. 2018).

Table 28.1 Outline of taxonomic position of the fungal biocontrol agents (Hibbett et al. 2007)

Basal fungi	Phylum *Glomeromycota* Phylum *Neocallimastigomycota* Phylum *Chytridiomycota* Phylum *Blastocladiomycota*		*Class Blastocladiomycetes*	*Order Blastocladiales*	*Coelomomyces*
		Subphylum Entomophthoromycotina *Subphylum Zoopagomycotina* *Subphylum Mucoromycotina*		*Order Entomophthorales*	*Conidiobolus* *Entomophthora* *Entomophaga* *Erynia*
Higher fungi (Subkingdom Dikarya)	Phylum *Ascomycota*	Subphylum *Pezizomycotina*	Class *Sordariomycetes*	Order *Hypocreales*	*Beauveria* *Paecilomyces* *Purpureocillium* *Cordyceps* *Nomurea* *Trichoderma* *Fusarium* *Verticillium*

28.4 Mechanism of Action of Fungal Biocontrol Agents

28.4.1 Mode of Action of Antagonistic Fungus Vis-À-Vis Plant Pathogens

Several interactions among the organisms are the key processes involved in the biological control of plant pathogens. The various mechanisms occur through a distended range directly related to the amount of interspecies contact and precision of the interaction. Mycoparasitism is considered the most direct type of antagonism in antagonist and phytopathogen interactions.

28.4.1.1 Mycoparasitism

The ability of any antagonistic fungi to attack the other fungal species and utilizing their nutrients are termed mycoparasitism. It involves the direct lysis of pathogen by mycoparasites (Atanasova 2013). Different events take place such as host fungus recognition followed by parasitization and finally killing them (Rabea et al. 2003). Several enzymes mediate this process, which degrades the fungal cell wall: chitinases, cellulases, etc. (Rabea et al. 2003; Horbach et al. 2011). The fungus *Ampelomyces quisqualis* parasitizes the powdery mildew pathogen, which is naturally occurring Deuteromycetes hyperparasite. During the interaction, it forms fruiting bodies (pycnidia) within the hyphae, conidiophores, and fruiting bodies of powdery mildew pathogen. Mycoparasitism eventually reduces the growth and kills the mildew pathogen. Numerous studies have shown the potential of *A. quisqualis* as a biocontrol agent against powdery mildew pathogen (Jyoti and Singh 2017). The mycoparasitic capability of *Trichoderma* species was significantly registered against various economically important plant pathogens (Harman et al. 2004). There are diverse and distinct fungi known to parasitize the living hyphae or sclerotial bodies of the plant pathogen and a single fungal pathogen can be parasitized by multiple hyperparasites. For instance, *Acremonium alternatum*, *A. quisqualis*, *Cladosporium oxysporum,* and *Gliocladium virens* have the ability to parasitize powdery mildew fungus (Kiss 2003).

28.4.1.2 Competition

The competition for space and limiting nutrients among organisms results in the biological control of various phytopathogens. Iron is one such limiting nutrient element in the rhizosphere, which determines the biocontrol efficacy of various biocontrol agents. The availability of iron depending on soil pH has been observed and it present as a ferric ion (highly oxidized state) in aerated soil (Lindsay 1979). It is an established fact that the organisms produce iron-binding ligands known as siderophores to sequester limiting nutrient iron from the microenvironment. Under the iron starvation condition, the majority of filamentous fungi secrete siderophores to uptake environmental iron (Eisendle et al. 2004). Several *Trichoderma* species are known to secrete highly efficient siderophores that sequester iron and arrest the growth of other fungi (Chet and Inbar 1994). This signifies the composition of soil in influencing the biocontrol efficacy by *Trichoderma* against *Pythium* sp. according to

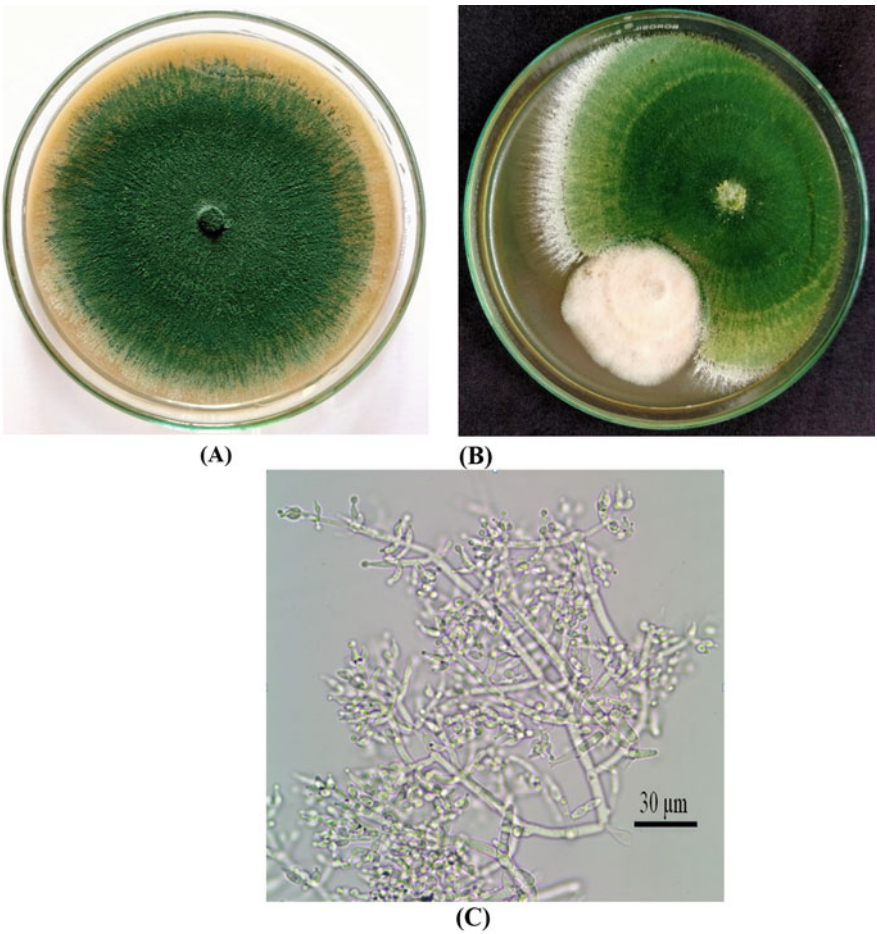

Fig. 28.1 (**a**) Fungal antagonist *Trichoderma harzianum* grown on potato dextrose agar medium (**b**) Growth inhibition of pathogen *Fusarium solani* by antagonistic *Trichoderma harzianum* (**c**) Phase contrast microscopic image of *Trichoderma harzianum* (×400 magnification)

the availability of iron. Similarly, *Trichoderma harzianum* T35 inhibits *Fusarium oxysporum* by competing for colonization site and nutrients, with biocontrol becoming more significant with a decrease in the nutrient concentration (Tjamos et al. 1992). Figure 28.1b represents the competition attribute of antagonistic fungi *T. harzianum* against phytopathogen *Fusarium solani.*

28.4.1.3 Antibiosis

Antibiosis is the process of secretion of antimicrobial moieties by the antagonistic fungi to suppress pathogenic fungi. The majority of the fungi are capable of secreting one or more diverse compounds and secondary metabolites having antimicrobial attributes. Secondary metabolites produced by several fungal biocontrol agents

constitute diverse chemical compounds. These compounds help in the biocontrol of numerous phytopathogens and involve many interactions, e.g., symbiosis, nutrient transport, differentiation, etc. (Keller et al. 2005; Mukherjee et al. 2012). Antibiotics are the important constituents of antibiosis interaction. *Trichoderma* species produce mainly three types of secondary metabolites (a) volatile antibiotics (6-pentyl-a-pyrone, derivative of iso-cyanide), (b) water-soluble compounds (heptelidic acid or koningic acid), (c) peptaibols (liner-oligopeptides of 15–22 amino acids long and rich in amino iso-butyric acid) (Mukherjee et al. 2012; Zeilinger et al. 2016). The metabolites produced by the *Trichoderma* spp. were considered as the possible elements responsible for antibiosis and they have been registered as effective biocontrol agents against a wide host range and hinder the longevity of sclerotial bodies of phytopathogens (Szekeres et al. 2005a, b). The detailed review on antibiosis and secondary metabolites produced by *Trichoderma* given by Hutchinson (1999), Hanson and Howell (2002) decipher the significance of secondary metabolites against phytopathogens *Pythium ultimum* and *Rhizoctonia solani.*

28.4.1.4 Induction of Disease Resistance

Several studies have demonstrated that various groups of chemical compounds are secreted by *Trichoderma* spp. into the zone of interaction and induce resistance mechanism in plants. Fungal proteins chiefly possess enzymatic role or other activity such as swollenin, cellulose, and xylanase are produced by *Trichoderma* species (Fuchs et al. 1989; Lotan and Fluhr 1990; Anderson et al. 1993; Martinez et al. 2001) and they appear to induce localized reaction and necrosis in plants (Bailey et al. 1991; Martinez et al. 2001; Brotman et al. 2008). Moreover, the enzyme endochitinase intensifies the defense through the initiation of plant defense-related proteins (Lorito 1998; Harman and Shoresh 2007). Furthermore, several hydrophobin-like proteins involved in inducing the synthesis of terpenoid phytoalexin were found to be released by strains of *T. virens* (Hanson and Howell 2004; Djonovic et al. 2006, 2007). Avirulence-like (*Avr*) genes are also known to synthesize a group of proteins that induce defense mechanisms in plants produced by several fungal biocontrol agents (Woo et al. 2006; Woo and Lorito 2007). Peptabiols are a different group of secondary metabolites that prime the plant defense system against pathogen attack. They are A class of short-chain peptides ($\leq$20 residues) synthesized by nonribosomal peptide synthase. The biological role of peptabiols and their antimicrobial activity has been registered in various studies (Chugh and Wallace 2001; Szekeres et al. 2005a, b; Chanikul et al. 2008). Furthermore, several small secondary metabolites of *Trichoderma* origin are known to induce expression of pathogenesis-related (PR) proteins when applied to plants as well as decrease the disease severity (Vinale et al. 2008a, b). The modulation of Ca^{2+} signal perception and H^{+} signaling are the primary steps in plant response to the interaction with metabolites (Felle et al. 2009; Vadassery et al. 2009).

Trichoderma spp. are not the only well-established fungi that enhance systemic resistance in plants, *Piriformospora indica* has very similar potential (Stein et al. 2008). Typically, the data pertaining to induced resistance have addressed disease control, but there is a good possibility that these systems may also enhance resistance

to insect pests, especially because the ethylene/jasmonate pathway is the key pathway involved in plant resistance to insects (Turlings et al. 1991; Schmelz et al. 2001). In addition, as described above, *Trichoderma* spp. have the capability to suppress the nematode infestation (Sharon et al. 2001, 2009; Harman and Shoresh 2007). Physical interaction with pathogenic and non-pathogenic microbes generates diverse defense mechanisms in the plant system. Generally, two main mechanisms are realized; systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is usually promoted by local infection, provides long-term systemic resistance to subsequent pathogen infection, correlated with the stimulation of PR genes with the involvement of the signal molecule salicylic acid (SA) (Durrant and Dong 2004). ISR is recognized because of root colonization by different non-pathogenic rhizosphere bacteria (Van-Loon et al. 1998). ISR is not SA-dependent but rather depends on constituents of the jasmonic acid (JA) signaling pathway followed by the ethylene signaling pathway.

28.4.2 Mode of Action of Entomopathogenic Fungus

Among different entomopathogens, fungi constitute the large single group and they are recognized as crucial, key disease-causing agents in insects. Several species of insects belonging to orders such as lepidoptera, coleopteran, homoptera, hymenoptera, and diptera are susceptible to mycopathogens infection.

Infection process: The mycopathogens exhibit a unique mode of action, i.e., they reach the hemocoel through the insect exoskeleton cuticle or probably through the ingestion. The ingested spores do not germinate in the gut and are eliminated from the insect body in the feces.

Conidial attachment: The attachment of conidia to the host cuticle is by a passive process and recognition of host location is a random event. The process of attachment of spore to the insect cuticle of the susceptible host represents the fundamental process in the establishment of mycosis. It was noted that the dry spores of *B. bassiana* interweaved the fascicles of hydrophobic rodlets in the outer layer. This hydrophobic rodlet appears to be specific to the conidial stage and not in the vegetative cells (Boucias et al. 1988). The nonspecific hydrophobic forces established by the rodlets play a key role in the adhesion process (Latge and Prevost 1988). Some of the chemical components like lectin, carbohydrate-binding glycoproteins have also been recognized on the conidial surface of *B. bassiana*. However, the exact mechanism of interaction between spore and cuticle remains to be ascertained (Sandhu 1995). Several factors such as availability of nutrients, water, oxygen, temperature and pH of host surface profoundly influence the germination and growth of infectious spores. Fungi with a broad host range respond to a wide array of nonspecific carbon and nitrogen source, whereas fungi with a restricted host range appear to have a specific nutritional requirement for pathogenicity (Leger et al. 1989a, b).

Establishment of infection structure: Development of appressorium and penetration peg appears to be crucial in a successful infection process (Sandhu 1995). Insect

cuticle is comprised of two layers; the outer epicuticle and the inner procuticle. The epicuticle is a thin, very complex structure, lacks chitin but comprises phenol-stabilized proteins and is covered by a waxy layer containing sterols (Hackman 1984). The procuticle accounts for a majority of the cuticle and contains chitin embedded in the protein matrix (Neville 1984). Chitin is organized in a helical fashion and appears as a lamellate structure. Conidia of EPF germinate on the host surface and differentiate into an infection structure described as appressorium. This appressorium serves as an adaptation for accruing physical and chemical energy on the exoskeleton is that access may be achieved successfully. Thus, the formation of appressorium plays a key role during mycosis and its formation may be influenced by the host surface architecture. Generally, intracellular biochemical messengers Ca^{2+} and cyclic AMP (cAMP) involvement have been documented while the cuticle surface was hard.

Cuticle penetration: During mycosis, the EPF obtain nourishment while penetrating the cuticle and insect body. The main driving force behind the entry into the host is the enzymatic degradation of cuticle and mechanical pressure as witnessed by the physical separation of lamellae while penetration of hyphae. The major extracellular enzymes produced are chitinases, lipases, esterases, and different groups of proteases. These enzymes act synergistically to facilitate the cuticle penetration and much of the attention has been concentrated on endoprotease as a crucial factor in the process. The cuticle degrading enzymes produced by *Metarhizium anisopliae* during the infection on *Calliphora vomitoria* and *Manduca sexta* have been examined through biochemical and histochemical analyses. The first enzymes produced are endoprotease termed as PR1 and PR2, aminopeptidases coinciding with the appressorium formation (Leger et al. 1989a, b). These fungi initiate their infective process when conidia are retained on the integument surface, where the germination tube starts protruding, the fungi release enzymes such as proteases, chitinases, quitobiases, lipases, and lipooxygenases. *Lecanicillium* (=*Verticillium*) *lecanii* is able to penetrate insect cuticle with its germ tube only while *B. bassiana* and *M. anisopliae* produce specific infection hyphae protruding from appressorium. After successful penetration, the fungus establishes in the hemolymph through the formation of blastospores (Fig. 28.2). However, the host specificity is determined by the physiological state of the host, structural properties of the insect integument vis-à-vis nutritional requirements of the fungus and the cellular defense machinery of the host.

Production of toxins: During mycosis the EPF secretes diverse cytotoxins which cause cellular disruption. With the consistent action of neuromuscular toxins, varied behavioral symptoms such as general or partial paralysis, sluggishness in the mycosed insects are quite apparent (Leger et al. 1987). *M. anisopliae* and *B. bassiana* produce substantial amounts of toxic moieties within their host insects. For instance, the toxins, Beauvericin, Brassinolide, Isarolide, and Beauverolides have been reported from *B. bassiana* infected insects (Hamill et al. 1969), destruxins (DTXs) and cytochalasins have been reported from *M. anisopliae* infected hosts. These toxins are known to exhibit a diverse effect on numerous insect tissues. The toxin DTX depolarizes the muscle membrane in lepidopteran pest by activating

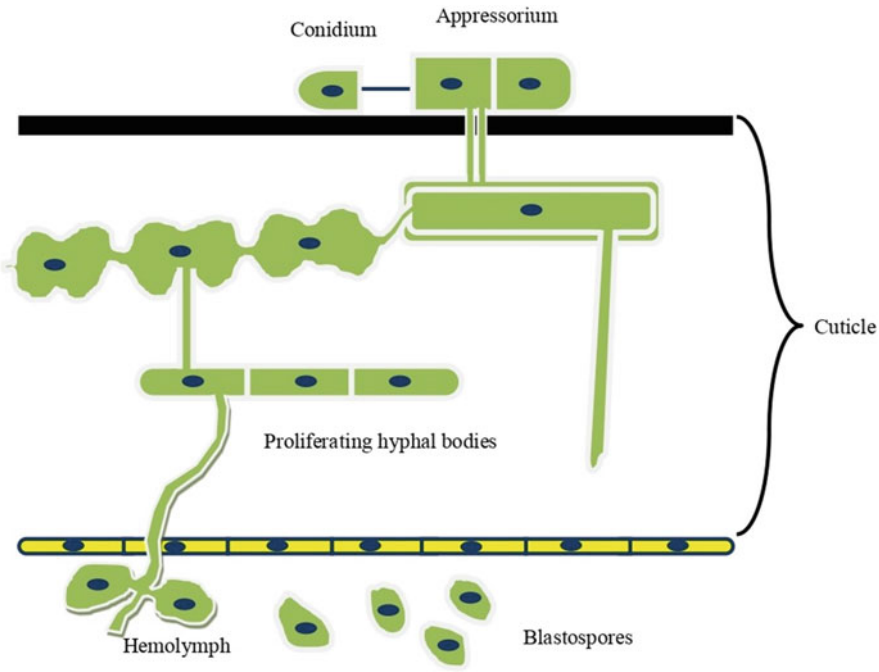

Fig. 28.2 Illustration of infection process by EPF in an insect depicting the structure of cuticle and mode of penetration and proliferation of fungal hyphae (Modified from Sandhu et al. 2012)

calcium channels and inhibits insect hemocytes (Elsworth and Grove 1977; Bradfisch and Harmer 1990). There are still numerous toxins to be described from the parasitized insects and their association in the process of mycosis needs to be explored in detail.

28.5 Fungal Mediated Biocontrol of Plant Diseases

The genus *Trichoderma* (*Hypocreales*) is one of the potential antagonists against various plant pathogens. They are primarily described by branched conidiophores producing bright green conidia (Gams and Bissett 1998). It includes several species generally found in soil. Almost 20 different species of *Trichoderma* act as biocontrol agents against various soil-borne and aerial phytopathogens have been documented. *T. harzianum, T. koningii, T. viride, T. atroviride, T. pseudokoningii, T. longibrachiatum, T. hamatum, T. polysporum,* and *T. reesei* are the most prominent species, which act as potential bioagents (Monaco et al. 1991). The genus *Trichoderma* possesses rapid growth and their primary role in nature as decomposers, decomposing mainly cellulosic materials (Jaklitsch 2009; Kubicek et al. 2009). Besides, *Trichoderma* spp. have been targets of studies of commercial exploitation due to their diverse attributes such as antibiotics production, several

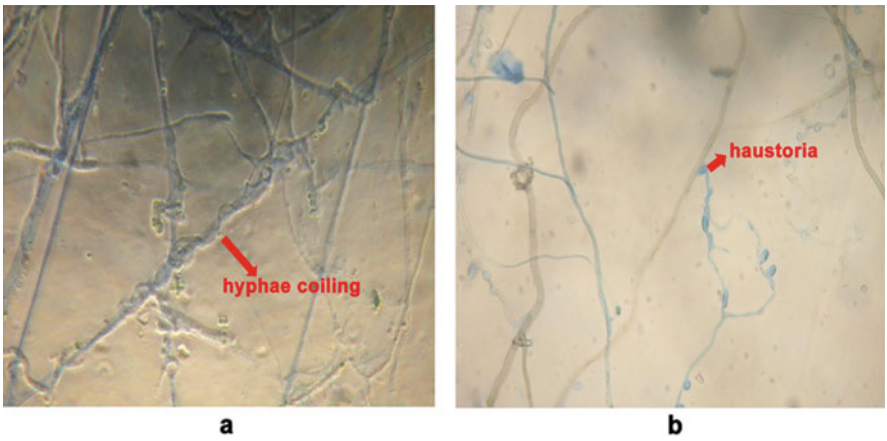

Fig. 28.3 Mycoparasitism by *Trichoderma* hyphae (**a**) coiling around the hyphae of *Rhizoctonia solani* and (**b**) producing haustoria to inhibit growth of *Macrophomina* sp. (Mukhopadhyay and Kumar 2020)

enzymes of industrial importance and their potential as biocontrol agents (Anees et al. 2010). These fungi are known to inhibit the growth of phytopathogenic fungi significantly by acting directly on the target pathogen as hyperparasite (mycoparasite), antagonist, competition, or by inducing resistance mechanism in the plants (Howell 2003). During mycoparasitism, *Trichoderma* species produce helical hyphae around the hyphae of a pathogenic fungus, forming appressoria, where several complex lytic enzymes are released and allowing penetration to the pathogen's hyphae (Fig. 28.3). Several antimicrobial compounds and secondary metabolites involved in the antagonism mechanism are produced by different species of *Trichoderma* (Vinale et al. 2008a, b), and as potential competitors for space and nutrients (Hjeljord and Tronsmo 1998). Anees et al. (2010) demonstrated the biocontrol potential of *Trichoderma gamsii* T30 against *Rhizoctonia solani* AG 2–2 in vitro and in vivo and revealed antibiosis is the best way to combat this pathogen by the selected strain of *Trichoderma* for the study.

The antagonistic potential of *Trichoderma* species against various plant pathogens has been documented in the literature. For instance, plant pathogens, viz., *Sclerotium rolsfii, Fusarium ciceris, Macrophomina phaseolina, Rhizoctonia solani,* and plant-parasitic nematodes are known to be effectively inhibited by *Trichoderma* (Spiegel and Chet 1998; Mukhopadhyay and Pan 2012). Mukherjee and Raghu (1997) noted that *Trichoderma* species and *Gliocladium virens* were highly significant in containing *S. rolsfii* on ginger rhizomes and several vegetables in storage. Li et al. (2018) showed that *Trichoderma* restricted the growth of *Fusarium oxysporum* by producing volatile compounds. Moreover, *Trichoderma* species are known to induce seed germination, seedling emergence, and plant growth promotion. Several scientists have reported disease control and enhanced plant growth and yield in vivo and under field conditions (Joshi et al. 2010;

Mukhopadhyay and Pan 2012). Seed treatment with bioagents offers protection against various seeds and seedling pathogens such as *Pythium* spp., *R. solani*, *S. rolfsii*, *M. phaseolina,* and *Fusarium* spp., (Dubey et al. 2007; Nirmalkar et al. 2017). The moisture, temperature, and inoculum levels of the pathogen (Mathre et al. 1994) besides soil pH and iron concentration (Weller 1988) might influence the efficacy of seed treatment with biocontrol agents. Pre-colonization imparts the bio-agent with a competitive advantage over seed-borne pathogens and often provides remarkable seed protection when compared to seed coating with chemicals (Harman et al. 1989).

Ampelomyces quisqualis belongs to deuteromycotina and is a naturally occurring hyperparasite on powdery mildews. It parasitizes and forms pycnidia within powdery mildew hyphae, conidiophores, and cleistothecia. This parasitism reduces growth and may eventually kill the mildew colony. The mycoparasite can directly penetrate the walls of hyphae, conidiophores, and immature cleistothecia, but may be unable to infect mature cleistothecia These mycoparasites can live up to 21 days on mildew-free host plant surfaces, where they can attack powdery mildew structures as soon as they appear (Németh et al. 2019). It has been recorded on more than 64 species in the genera *Brasilomyces, Erysiphe, Leveillula, Microsphaera, Phyllactinia, Podosphaera, Sphaerotheca*, and *Uncinula*, as well as the anamorphic genera *Oidium* and *Oidiopsis* (Falk et al. 1995). Though *Ampelomyces quisqualis* and wettable sulfur were found to be equally effective in controlling powdery mildew; *A. quisqualis* may be preferred due to its non-residual and odorless properties, *A. quisqualis* is an attractive and viable option in managing powdery mildews (Gopalakrishnan and Valluvaparidasan 2009). Nematophagous fungi are key parasites of nematodes and have been explored for plant-parasitic nematode management. *Purpureocillium* (=*Paecilomyces*) *lilacinum* is considered to be the most important in the biological control of plant-parasitic nematodes due to its capability to parasitize even nematode eggs (Atkins et al. 2005). Species of the genus *Lecanicillium* (=*Verticillium)* have also reported as potential biological control agents as described with *V.* (=*Pochonia/Metacordyceps*) *chlamydosporium* being the best illustrative for this purpose (Hajji et al. 2017). Another positive aspect of *Purpureocillium* representatives is the secretion of secondary metabolites such as phytohormones (gibberellins and auxins) that can promote plant growth and substances that prime the plant defense against the detrimental effects of biotic and abiotic stresses (Khan et al. 2012).

28.6 Fungal Mediated Biocontrol of Insect Pests

Being the component of biological control, several species of EPF are capable to infect and cause disease in insect pests and other arthropods (Vega et al. 2009; Pell et al. 2010). The majority belongs to the orders *Hypocreales* (phylum *Ascomycota*) and *Entomophthorales* (subphylum *Entomophthoromycotina*) (Hibbett et al. 2007). Generally, the EPF of entomophthorales order is considered as low risk of infecting beneficial insects such as pollinators and natural enemies of insect pests, while fungi

of hypocreales order are less selective and have a wide host range (Roy and Pell 2000). The best characterized and most explored EPF in the biological control of insect pests are *B. bassiana* and *Metarhizium anisopliae*. They are facultative pathogens and can be found in almost all ecosystems as saprophytic species that do not require an insect host to complete their life cycles (Schrank and Vainstein 2010; Ortiz-Urquiza et al. 2015).

Beauveria bassiana, a filamentous fungus, grows naturally in soils throughout the world and acts as a pathogen on various arthropods, causing white muscardine disease. It is highly adapted to particular host insects. Globally, a wide range of diverse species has been isolated from a variety of insect hosts, which are of medicinal or agricultural importance (Sandhu et al. 1993, 2001; Sandhu and Vikrant 2004; Thakur et al. 2005; Jain et al. 2008). An important feature of *Beauveria* sp. is the high host specificity infecting a variety of insect pests of agricultural and forest significance, e.g., *Helicoverpa armigera*, colorado potato beetle, codling moth, several genera of termites, whitefly, etc. Additionally, the persistence of spores in the host population and the environment provides long-term effects in suppressing the pest population, causing epizootics (Thakur and Sandhu 2010). *B. bassiana* is the anamorph (asexually reproducing form) of *Cordyceps bassiana*. The latter teleomorph (the sexually reproducing form) has been collected only in eastern Asia (Li et al. 2001). Many insect pests having sub-terranean larval stage, e.g., curculionidae weevils are highly susceptible to this white muscardine fungus (Ferron 1981; Beavers et al. 1983). Like many other species of EPF, *B. bassiana* is comprised of genetically distinct variants related to geographical location and host, which significantly influence the pathogenicity of the fungus. The spores, infectious ingredients are sprayed on affected crops as a wettable powder or an emulsified suspension and it is known to parasitize diverse arthropod hosts and therefore considered as a nonselective microbial insecticide. Various new alternative methods to disseminate infections spores of the fungus had been explored with the aim to enhance the efficiency of its transmission to target pests. Lin et al. (2017) described the possible utilization of invertebrates as vectors of EPF, enhancing the chances of contact between the host and infectious spore. Hence, this strategy ensures the effective release of fungal spores against target insect pests. Several researchers had registered this kind of application in the literature. Al Mazra'awi et al. (2006) demonstrated the apivectory, wherein *B. bassiana* spores were effectively disseminated using honeybees, *Apis mellifera*. The contaminated bees were released in canola (*Brassica napus*) fields with the aim to facilitate the contact of the fungus to the tarnished plant bug, *Lygus lineolaris*. As the bees are the main pollinators of canola, and their great movement in the field ensures wide distribution of the fungus, carrying it to the bugs on the plants. By enhancing fungus dispersal, the authors recorded increased mortality rates of up to five times higher as compared to control. Similarly, parasitoids have been regarded as vectors of EPF to the hosts during instances of foraging, as documented by Oreste et al. (2016), wherein *Encarsia formosa* acted as a passive vector to transfer the fungal infections spores from contaminated to uninfected host as a result of oviposition and host handling.

Another EPF *L. lecanii* is a widely distributed fungus, which can cause epizootics in tropical and subtropical regions, as well as in warm and humid environments (Nunez et al. 2008). Kim et al. (2008) documented the efficacy of *L. lecanii* as an effective biological control agent against *Trialeurodes vaporariorum* in South Korean greenhouses. This fungus infects nymphs and adults and colonized the underside of the leaf by means of a filamentous mycelium (Nunez et al. 2008). Furthermore, *L. lecanii* was reported to control whitefly and several aphid species in the greenhouse chrysanthemums (Hamlen 1979). *M. anisopliae* is also a potential fungus on insect pests and exploited for biocontrol of sub-terranean insect pests such as white grubs and sucking pest leafhopper (Sandhu et al. 1993) (Fig. 28.1a–d). In developed and developing countries including America, Australia, and Africa, this species is used for the control of locusts, grasshoppers, cockroaches, and termites. Using oil formulation, the green spores easily establish contact with parts of the insect, which have high relative humidity (i.e., where the cuticle is thin like the back of the neck or under the wing) where the fungus can proliferate and cause disease. The fungus is highly promising, for instance, it can kill up to 90% of locusts within 7–21 days of application, depending on the number of infectious spores. *Nomuraea rileyi* is another efficacious EPF; a dimorphic hyphomycete can cause epizootic infection in various insect pests. It has been demonstrated that many insect species belonging to lepidoptera including *Spodoptera litura, S. frugiperda* (Fig. 28.4f) and few belonging to *Coleoptera* are susceptible to *N. rileyi* infection (Ignoffo 1981). Another insect pest *Spilosoma* was found to be severely infected by *N. rileyi*, hence exploited in detail for its mycobiocontrol. The host specificity of *N. rileyi* and its green chemistry boost its use in insect pest management (Mathew et al. 1998).

28.7 Role of Fungal Biocontrol Agents in Nature and Their Commercialization

The EPF demonstrate diverse role as antagonists against phytopathogens, rhizosphere colonizers, biocontrol agents against insect pests, plant growth promoter, and endophytes. The bioactive metabolites produced by EPF such as antibiotics, volatile compounds (e.g., ammonia, hydrogen cyanide, alkyl pyrones, alcohols, esters, ketones, and lipids), and enzymes assist in containing the disease's severity of plant pathogens (Ownley and Windham 2007). It is also registered that few entomopathogens effective against various insect pests are also antagonistic to plant pathogens (Kim et al. 2008). The EPF belong to hypocreales is ubiquitous in the soil ecosystem. Generally, *Beauveria*, *Isaria,* and *Metarhizium* are isolated from soil. The soil protects EPF from harmful solar radiation and increased temperature (Inglis et al. 2001). Rhizosphere soil has abundant soil carbon and EPF interacts with plant roots for its growth and survival (St Leger and Wang 2010). The interaction with native microflora and their metabolites significantly affect the capability of EPF causing pathogenicity against insect pests. The *B. bassiana* is known to control the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Groden and Lockwood 1991). The application of conidia of

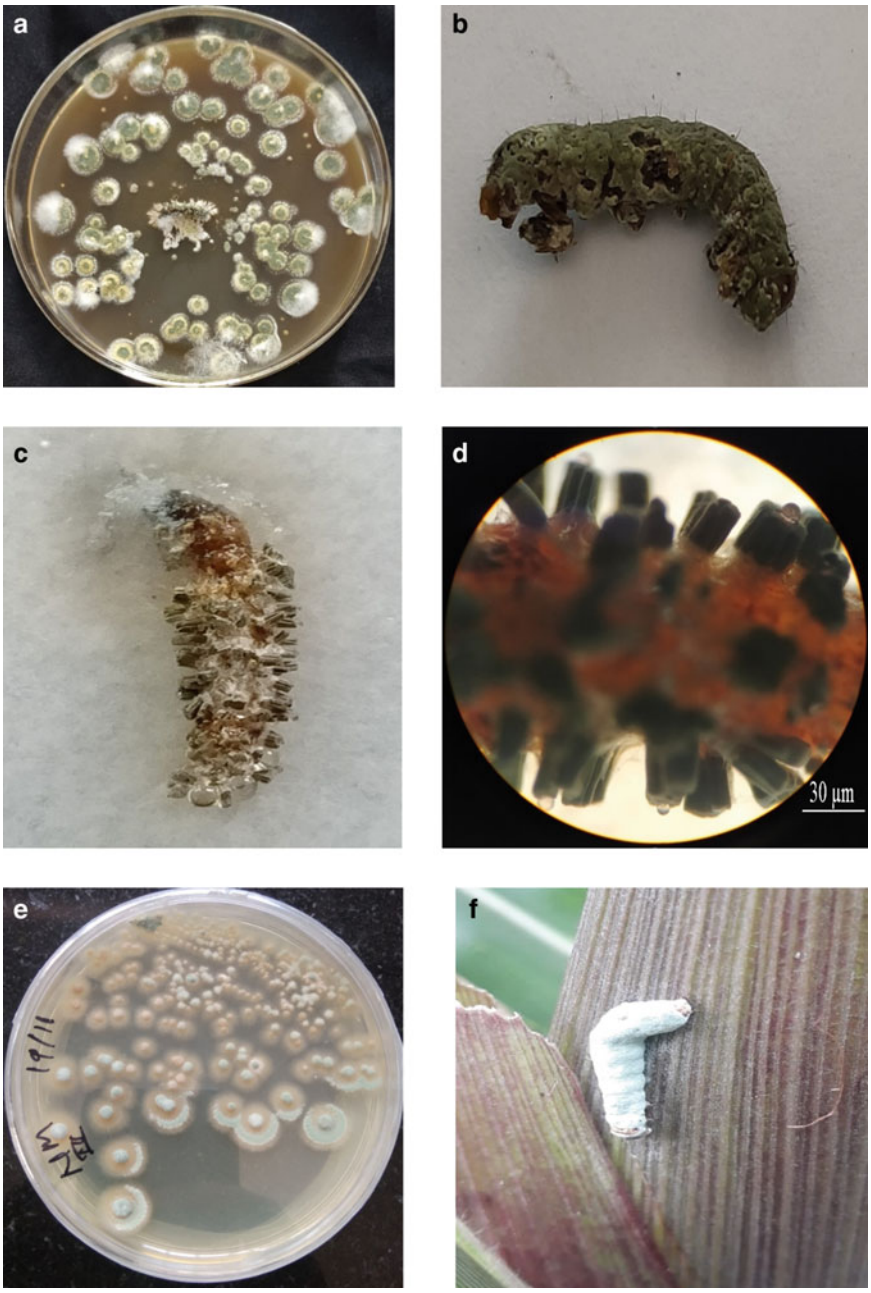

Fig. 28.4 (**a**) Entomopathogenic fungi *Metarhizium anisopliae* grown on potato dextrose agar medium, (**b**) fall armyworm, *Spodoptera frugiperda* larva colonized by EPF *Metarhizium anisopliae*, (**c**) rice moth, *Corcyra cephalonica* larva colonized by EPF *Metarhizium anisopliae*, (**d**) colonization of *Metarhizium anisopliae* on setae of *Corcyra cephalonica* (×400 magnification), (**e**) entomopathogenic fungi *Nomuraea rileyi* grown on potato dextrose agar medium, and (**f**) natural occurrence of EPF *Nomuraea rileyi* on fall armyworm, *Spodoptera frugiperda*

M. anisopliae to corn seeds before planting recorded the reduced damage from wireworms and increased plant stand and fresh weight of field corn (Kabaluk and Ericsson 2007). Furthermore, EPF also exhibits an endophytic mode of colonization in plants and acts against insect pests attack (Arnold and Lutzoni 2007). *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, and *Isaria* are some of the EPF exhibited endophytic colonization in several crop plants (Vega et al. 2008). Lately, the use of fungal pathogens is drawing special attention as mycobiocontrol agents of many insect pests as this approach is reliable, cost-effective, and environmentally safe (Wraight et al. 2001). The *B. bassiana* is efficacious against several lepidopteran, coleopteran, and hemipteran pests. *B. bassiana* has also been found to be pathogenic to the larvae of *Capnodis tenebrionis* (Linnaeus) (Coleoptera: Buprestidae) in laboratory assays as documented by El Khoury et al. (2020). Similarly, *M. anisopliae* has documented the effectiveness against weevils, cutworms, scarab beetle grubs, and termites. *L. lecanii* used against insects of Hemiptera and Thysanoptera order.

Several salient features of the EPF like enhanced virulence against the target species; no infestation in the non-target organisms; resistance toward abiotic and biotic factors of the environment are decisive in achieving adequate results in the field trials (Van Lenteren et al. 2003; Jackson et al. 2010). Several researchers have illustrated that EPF show very less impact on the non-target insects (James et al. 1995; Parker et al. 1997; Traugott et al. 2005; Nielsen et al. 2007). Application of barley kernels colonized by entomopathogenic fungus into the soil is the most commonly used method against soil-dwelling pests. This approach has been employed for the control of *Melolontha melolontha* in different crops (Keller et al. 1997; Vestergaard et al. 2002). Using fungal bands that are impregnated with entomopathogenic fungi is another successful method of biocontrol. The bands are allocated near the trunk or around the branches of the tree and they disseminate the infectious spores against the invading pests. The method was first used to control *Monochamus alternatus* which is the major carrier of wilt disease in pines caused by *Bursaphelenchus xylophilus* (Shimazu 2004). Presently, the fiber band approach gives significant outcomes in biocontrol of *Anoplophora glabripennis* and *Agrilusplani pennis* invasive species (Augustyniuk-Kram and Kram 2012).

Commercial uses and applications of fungal biocontrol agents have been slow mainly due to their varied efficacy under different environmental conditions and due to their host specificity. Hence, it is desirable to develop new formulations of fungal biocontrol agents with a higher degree of efficiency, stability, and survivability using biotechnological approaches (Heyadri and Pessarakli 2010). Commercialization of biological control agents is quite expensive as it encompasses several steps such as isolation, enrichment, identification, characterization, scaling up of mass production, development of suitable formulation, storage stability, field efficacy, focus on human and environmental safety, registration, and marketing (Punja 1997; Stirling and Stirling 1997; Janisiewicz and Korsten 2002; Montesinos 2003). A number of microbial-based products are being commercialized worldwide for the control of phytopathogens and insect pests affecting economically important crops. Generally,

Table 28.2 List of few commercialized fungal biocontrol agents against phytopathogens and their specifications (Thambugala et al. 2020)

Biocontrol agent	Product	Target pathogen(s) or crop disease	Manufacturer
Ampelomyces quisqualis	AQ10 Bio Fungicide	Powdery mildew	Ecogen Inc., USA, Israel
Ampelomyces Quisqualis	Bio Dewcon	Powdery mildew	T- Stanes & Company Ltd., India
Trichoderma virens	SoilGard	Soil-borne pathogens; *Rhizoctonia* and *Pythium* species	Certis USA
Trichoderma harzianum	RootShield	Root rot diseases; *Pythium*, *fusarium*, *Rhizoctonia*, *Thielaviopsis,* and *Cylindrocladium* species	BioWorks, Inc., USA
Trichoderma harzianum	Trichodex	Gray mold (*Botrytis cinerea*); *Rhizoctonia*, *Sclerotinia,* and *Colletotrichum* species	Makhteshim Agan Industries, Israel
Trichoderma harzianum and *T. polysporum*	Binab T	Root rot diseases, pruning wounds in ornamental, shade, and forest trees	BINAB Bio-Innovation AB, Sweden
Trichoderma viride	Trieco	Soil-borne fungal diseases	Ecosense Lab (I) Pvt. Ltd., India
Trichoderma viride	Monitor	Seed borne and soil-borne fungal diseases, Foliar pathogens *Alternaria*, *Pyricularia*, *Curvularia, Colletotrichum*	Agriland Biotech Ltd., India
Trichoderma viride	Nisarga	Collar rot, damping-off, wilt, and blight pathogens	Multiplex Agricare Pvt. Ltd., India
Paecilomyces lilacinus	Bio-nematon	Root-knot nematode, cyst nematode, burrowing nematode, lesion nematode	T-Stanes & Company Ltd., India
Paecilomyces lilacinus	Niyantran	Plant-parasitic nematode, root-knot nematode	Multiplex Agricare Pvt. Ltd., India
Trichoderma viride	Biocure-F	Root wilt, seedling wilt, loose smut disease	T- Stanes & Company Ltd., India

they are formulated as granules, wettable powders, dust, and aqueous or oil-based liquid products using different mineral and organic carriers (Ardakani et al. 2009; Nega 2014). Several microbial-based antagonists and insecticides have been patented and evaluated for commercial uses (Schena et al. 2004; Nabi et al. 2017) and these agents are regularly recommended for different crops. Some commercialized fungal biocontrol used to control plant pathogens, insect pests and their particulars are listed in Tables 28.2 and 28.3. Mycoinsecticide formulations depend on a few restricted number of fungal species such as *B. bassiana*, *B. brongniartii*, *L. lecanii*, *L. longisporum*, *M. anisopliae,* and *Paecilomyces fumosoroseus*. About 33.9% of the

Table 28.3 List of few commercialized fungal biocontrol agents against insect pests and their specifications (Butt et al. 2001)

Product	Entomopathogenic fungus	Target pests	Manufacturer
Mycotal	*Verticillium lecanii*	Whitefly and thrips	Koppert, The Netherlands
Vertalec	*Verticillium lecanii*	Aphids	Koppert, The Netherlands
Bio-Blast	*Metarhizium anisopliae*	Termites	Ecoscience, USA
Cobican	*Metarhizium anisopliae*	Sugarcane spittle bug	Probioagro, Venezuela
Conidia	*Beauveria bassiana*	Coffee berry borer	Live Systems Technology, Colombia
Ostrinil	*Beauveria bassiana*	Corn borer	Natural Plant Protection (NPP), France
CornGuard	*Beauveria bassiana*	European corn borer	Mycotech, USA
Mycotrol GH	*Beauveria bassiana*	Grasshoppers, locusts	Mycotech, USA
Mycotrol WP & BotaniGard	*Beauveria bassiana*	Whitefly, aphids, thrips	Mycotech, USA
Naturalis-L	*Beauveria bassiana*	Cotton pests including bollworms	Troy Biosciences, USA
Boverol	*Beauveria bassiana*	Colorado beetle	Czechoslovakia
Green muscle	*Metarhizium flavoviride*	Locusts, grasshoppers	CABI—BioScience, UK
PFR-97	*Paecilomyces fumosoroseus*	Whitefly	ECO-tek, USA
Pae-Sin	*Paecilomyces fumosoroseus*	Whitefly	Agrobionsa, Mexico
Vertisoft	*Verticillium lecanii*	Sucking pests aphids, jassids, thrips, mites, hoppers, mealybugs, and scale insects	Agriland Biotech Ltd., India
Metasoft	*Metarhizium anisopliae*	White grubs, termites, cutworm, mango hoppers, rice insects	
Biosoft	*Beauveria bassiana*	American bollworm, fall armyworm, cutworms, white grubs, sucking pests aphids, jassids, thrips, mites, hoppers	
Biocatch	*Verticillium lecanii*	Whitefly, jassids, aphids, thrips, mealybugs	T- Stanes & Company Ltd., India
Biopower	*Beauveria bassiana*	Borers, cutworms, root grubs, leafhoppers, aphids, mealybug	

(continued)

Table 28.3 (continued)

Product	Entomopathogenic fungus	Target pests	Manufacturer
Biomagic	*Metarhizium anisopliae*	Leaf hoppers, grasshoppers, root grubs, corn root worms, bugs, beetles, palm weevils, borers, cutworms, termites	
Baba	*Beauveria bassiana*	Shoot borer, semi-looper, thrips, and mealybug	Multiplex Agricare Pvt. Ltd., India
Metarhizium	*Metarhizium anisopliae*	Root weevils, plant hoppers, root grubs, black vine weevil, spindle bug, termites,	
Mycomite	*Paecilomyces fumosoroseus*	Red spider mites	
Varsha	*Verticillium lecanii*	Whitefly, jassids, aphids, thrips, mealybugs	
Checkmite	*Hirsutella thompsonii*	Spider mites and eryiophid mites	

mycopesticide formulations are of *B. bassiana*, followed by *M. anisopliae* (33.9%), *Isaria fumosorosea* (5.8%), and *B. brongniartii* (4.1%) (Faria and Wraight 2007). However, to enhance the share of the overall industry of mycopesticides, the killing speed, which is the significant decisive factor in their utilization needs to be enhanced (St Leger and Wang 2010). As the native isolates of these fungal biocontrol agents are deficient in terms of sufficient levels of virulence (Rangel et al. 2005), stain improvement and manipulation at the genetic level is crucial to enhance their viability and ecological fitness (Fang et al. 2005).

28.8 Future Prospects and Conclusion

The fungal biocontrol agents are host-specific and relatively safe to non-target organisms and beneficial insects. These mycobiocontrol agents have been exploited or are being assessed as an integral and essential component of IPM or IDM strategies. The modern biotechnological and genetic engineering techniques have immensely contributed toward the development of new improved strains with enhanced biocontrol efficacy. The secondary metabolites produced by various fungal antagonists will also play a key role in the future bio-control of phytopathogens. The most interesting future prospect in terms of entomogenous fungi is to devise strategies for strain improvement. The key characters need to be addressed are, e.g., enhanced killing power (reduced LD_{50}), ability to cause infection and pathogenicity at low humidity levels, stability under diverse environmental conditions, tolerance to different abiotic factors (temperature tolerance, UV tolerance), reduced LT_{50} and improved sporulation during large scale production and extension of the host. The manipulation at the genetic level targeting a single or cluster of genes, for instance, the epizootic potential of *Beauveria* spp. and

Metarhizium spp. was intensified by genetic manipulation; thereby augmenting their saprophytic potential has been documented (Wang and St Leger 2005). There is a strong urge to improve the overall efficacy of these biocontrol agents along with developing novel methods to deliver adequate inoculum at the target sites. Modern techniques in biotechnology have the potential to manipulate desirable traits of these agents to improve the overall field efficacy. Apart from agro-ecosystem, fungal biocontrol agents appear to be promising against human and animal pests such as mosquitoes, tsetse flies, fire ants, etc. exhibiting diverse targets for mycopesticides (Sharma and Sharma 2021).

Investigations pertaining to strain improvement and potential targets for entomopathogens will clear apprehensions for their improved utilization in agriculture frameworks as well as integrated pest management strategies. The detailed investigations on the metabolic and genetic potential of fungi related to the biocontrol process reveal that they demonstrate multifarious applications. *Trichoderma* species, *Metarhizium* spp. and *Beauveria* spp. can be more than biocontrol agents, associating with the roots and tissues of plants and could manifest a crucial role in sustainable agriculture by controlling the adverse effect of biotic or abiotic stresses and hence improve the quality of crops.

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Antagonistic Fungi Against Plant Pathogens for Sustainable Agriculture

29

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Abstract

The demand for the reduction of chemical pesticides and the adoption of biocompatible products, following the sustainability principles, has changed food production worldwide. Biotic and abiotic stresses cause severe losses in food, fibers, and energy, and it is essential to minimize these losses with sustainable methods. Because of that, microorganisms and biocompatible products have been developed to overcome these challenges without causing adverse environmental impacts. In addition to developing biopesticides, there is a need to integrate these products with other agricultural technologies. Therefore, integrated disease management is fundamental to the path to sustainability. For that to happen, it is necessary to understand the structure and functioning of agricultural ecosystems to carry out adequate integrated management of pests and diseases. In this sense, the understanding of plant–pathogen relationships, the effects of the environment

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V. R. Rajpal et al. (eds.), *Fungal diversity, ecology and control management*, Fungal Biology, https://doi.org/10.1007/978-981-16-8877-5_29

on these relationships, and the impacts of external interventions on the production system need to be understood. This understanding will lead to the rational use of inputs effectively and sustainably. Brazilian agricultural productivity is impacted by soil-borne plant pathogens, whose problems have been increased. The chemical control of these plant pathogens presents numerous problems, and the biological control associated with soil and crop management has been an adequate alternative. In this scenario, the development and use of biopesticides are vital factors for managing soil-borne fungi and nematodes. However, for its best performance, it is vital to consider the pathosystem, the biological target to be reached, the efficacy of the antagonist agents, compatibility in the integrated pest management; all these topics will be covered in this chapter.

Keywords

Bioprotectant · Agroecosystem · Plant disease · Biological control

29.1 Introduction

The first preoccupation about the damage caused by chemical pesticides in agriculture arose in the 1950s. However, the publication of the book "Silent Spring" in 1962 by Rachel Louise Carson led to further discussions. In the 1980s, the concern with the environmental impacts of agriculture increased, and the externalities started to be studied. In the same decade, the adverse effects of the intensive use of pesticides inside and outside the agroecosystem became evident (Campanhola and Bettiol 2003). Within the agroecosystem, the intensive use of pesticides increases their dependence due to the biological imbalances (elimination of natural enemies, antagonists), the resurgence of pests, the resistance of target organisms to active ingredients, and the emergence of new pests and diseases caused by elimination agents responsible for natural biological control (Campanhola and Bettiol 2003). Outside of agroecosystems, pesticides damage the health of the consumers and contaminate soil, water, and air.

In a large study carried out in the USA, entitled Environmental and Economic Impacts of Reducing U.S. Agricultural Pesticide Use, the impacts on the main crops were evaluated for a 50% reduction in the use of pesticides, based on the government pesticide reduction policies that were taking in the Netherlands, Sweden, and Denmark. This study revealed the possibility of reducing pesticides by strengthening public awareness policies and advances in research in biological control (Pimentel et al. 1993). In Brazil, in the 1996s, a discussion was initiated on a National Program to Rationalize Pesticides, linked to the Green Protocol Program (Soares et al. 2003). Unfortunately, the program was halted in the following decade.

Biological control is "the use of one organism to reduce the population density of another organism" (van Lenteren et al. 2020) and has been used for more than 2000 years (van Lenteren et al. 2018). Biological control is effective in controlling plant diseases, pests, and invasive plants (Barreto 2009; Bettiol and Morandi 2009;

van Lenteren et al. 2018). It is also effective in controlling pests and diseases in animals and human disease vectors (Weeks et al. 2018). According to Bale et al. (2008), the most successful, economical, and environmentally safe method for managing pests, diseases, and weeds is biological control.

The use of biological products is expanding widely around the world. According to Bueno et al. (2020), in Brazil, the area under biological control of pests and diseases with natural and microbial biocontrol agents is greater than 24.7 million ha, considering the information from 2017. If added to these numbers, the area of 5.5 million ha treated with *Bacillus thuringiensis* (that are not considered as biocontrol agents) area exceeds 30 million hectares. This expansion is related to the positive characteristics of biological control, such as those associated with men's health and productivity in crops (van Lenteren et al. 2018). The advances in the use of biological control agents are due to some factors such as the production of bioprotectants on an industrial scale; diversity of biological control agents for several targets; success cases being demonstrated; when chemical pesticides fail or are not available (van Lenteren et al. 2018). The role of consumers, who demand the reduction of pesticide residues in food, has also been significant in increasing the use of bioagents on a global scale.

Before external interventions in the production system, it is essential to know the structure and functioning of the agroecosystem (Bettiol and Ghini 2003). With this knowledge, biological control will be better managed. Four different types of biological control are recognized: natural, conservation, classical, and augmentative (Cock et al. 2010; van Lenteren et al. 2018). Natural biological control of plant disease is the type of biological control where antagonists occur naturally, reducing the incidence and severity of plant diseases without any human intervention. This type of control is active in all agroecosystems. Conservation biological control consists of human actions to protect and stimulate beneficial agents' preservation and natural increase. The induction of suppressiveness of soil-borne pathogens is an important example of biological conservation control and has been continuously expanding (Faria et al. 2020). In classic biological control, natural enemies are collected in the region of origin of the pest, pathogen, or invasive plant and then released in areas of choice to increase the number of biocontrol agents, resulting in a permanent population. Augmentative biological control is a mass application of natural enemies (parasitoids, predators, or antagonistic microorganisms) in a crop with the objective of immediate or prolonged control of pests and diseases (van Lenteren et al. 2018). Augmentative biological control is undoubtedly the best known among farmers.

In the global scenario toward the sustainability of agricultural production, associated with the loss of efficiency of chemical control of plant diseases (mainly due to the emergence of pathogens resistant to fungicides, as well as biological imbalance), agricultural production systems have been advancing in the adoption of biological control, mainly of augmentative biological control and conservation. This increase in the use of biological control agents can be seen by forecasting the annual compound growth rate (CAGR) of 14.7% until 2025 (Markets and Markets 2020). In

this scenario, the development and use of bioprotectants are essential for the management of plant diseases.

29.2 Brief History of Biological Control of Plant Diseases

Biological control of plant disease is more than an intimate interaction of the pathogen with its host influenced by the environment. Biological control is the result of an interaction among the host, pathogen, and a variety of non-pathogens that also lingers in the infection site and present potential to limit or increase the pathogen's activity or the resistance of the host (Cook and Baker 1983; Cook 1985). According to Cook and Baker (1983), "biological control is the reduction of the amount of inoculum or disease-producing activity of a pathogen, accomplished by or through one or more organisms other than a man." Antagonists for the control of plant diseases are generally fungi or bacteria isolated from the phyllosphere, rhizosphere, carposphere, and spermosphere and play an important role in the control of plant pathogens (Baker 1987; Stirling and Stirling 1997; Jyoti and Singh 2016). However, mycoviruses, microarthropods, and other organisms can be used in biological control.

The biological control of plant diseases has come to have greater practical importance and use on a large scale only in recent years. However, the first report occurred in 1874, when W. Roberts demonstrated antagonistic action of microorganisms (*Penicillium glaucum* and bacteria) in liquid cultures, responsible for introducing the word antagonism in microbiology. In 1908, M. C. Potter reported for the first time the potential of *Erwinia carotovora* and *Penicillium italicum* metabolites to inhibit plant pathogens. However, A. Fleming's discovery of penicillin in 1928 and its purification and use in medicine in 1939 stimulated studies of antagonists to control plant pathogens. Between the 1940s and 1950s, the production of antibiotics by *Penicillium, Aspergillus, Trichoderma*, and *Streptomyces* in soil was reported (Baker 1987). In Japan, Yoshii (1949), cited by Fukunaga (1965), reported for the first time the possibility of controlling rice blast caused by *Pyricularia oryzae* with antibiotics. However, the investigation with antibiotics to control rice blast intensified in 1955, and several promising antibiotics such as antiblastin, antimycin A, blastimicidine, blastimicidin A were discovered, which were not used due to toxicity to fish (Fukunaga 1965). The two most splendid successes were blasticidin S and kazugamycin, produced by *Streptomyces griseochremogenes* and *Streptomyces kazugaensis,* respectively (Fukunaga 1965; Umezawa et al. 1965; Okamoto 1972, cited by Ou 1965). These antibiotics have been commercialized to date in almost all regions of the world.

The focus of pest, disease, and weeds control during the green revolution in the early 1950s was the use of chemical pesticides, which caused serious damage to human and animal health, environment, beneficial pollinators, predators, and antagonistic microorganisms (Pimentel et al. 1992). In response, researchers concerned about these problems constituted the International Organization for Biological Control (IOBC) in 1955, which, among other activities, organized events on

biological control in several regions of the world and started a research journal-Biocontrol to disseminate information in the field of biological control.

About plant diseases, between April 7 and 13, 1963, the "Ecology of Soil-Borne Plant Pathogens: Prelude to Biological Control. Symposium on Factors Determining the Behavior of Plant Pathogens in Soil" was organized, at the University of California, Berkeley, with 310 participants from 24 countries (Baker 1987). After the symposium, the entitled Ecology of Soil-borne Plant Pathogens: Prelude to Biological Control by Baker and Snyder (1965) was published. After this event, many others were organized around the world to discuss the biological control of plant diseases. One of the great milestones in the biological control of plant diseases was the launch of the book "Biological Control of Plant Pathogens" in 1974 by Kenneth Frank Baker and Robert James Cook. This book took 5 years of work by the authors (Cook 2005) and created the theoretical basis for the biological control of plant diseases. Practically, 10 years later, Cook and Baker (1983) published the book The Nature and Practice of Biological Control of Plant Pathogens, consolidating their role in the history of biological control of plant diseases and establishing it as a science.

In Brazil, Bettiol published the first book related to biological control entitled "Biological control of plant diseases" in Portuguese in 1991. Furthermore, Bettiol et al. (2014a, b) published the book "Biological control of plant diseases in Latin America and the Caribbean." Despite its late start, Brazil is currently one of the countries using biological control of diseases and pests at a wider scale, with an area of approximately 30 million hectares (van Lenteren et al. 2018; Bueno et al. 2020).

29.3 Targets of Biological Products Based on Antagonistic Fungi and Cultivation Systems

The use of fungi as biocontrol agents is attractive due to the presence of these organisms in the most diverse environments, specificity and destruction of the host (when it acts by parasitism or predation or cell wall lysis), persistence, dispersion efficiency, ease of cultivation and maintenance in the laboratory (Thambugala et al. 2020). Also, the possibility of obtaining formulations with a long shelf life is considered adequate.

Many fungi have been commercialized as bioagents to control plant diseases in different countries, which include genera: *Ampelomyces, Arthrobotrys, Arthrobotrys, Candida, Clonostachys, Coniothyrium, Gliocadium, Metschnikowia, Myrothecium, Purpureocillium, Phlebiopsis, Pochonia, Sporotrix, Pythium,* and *Trichoderma* (Table 29.1). These agents are used to control several plant pathogenic fungi and nematodes.

The two most important biological control agents to control plant diseases in Brazil are *Bacillus* spp. and *Trichoderma* spp. (Table 29.2) (Agrofit 2021). Furthermore, a new trend was observed: forming formulations containing a mixture of biocontrol agents (Table 29.2).

Table 29.1 Fungal biocontrol agents of different plant pathogens available in the world

Biocontrol agent	Pathogen/disease	Commercial product/ country	Reference
Ampelomyces quisqualis	*Podosphaera fusca, Sphaerotheca, Eryshiphe necator, Oidium*	AQ10®/USA, Italy, UK, and other	AQ10WG (2020), Bettiol et al. (2012)
Arthrobotrys oligospora and *Arthrobotrys botryospora*	*Helicotylenchus, Meloidogyne, Pratylenchys, Radopholus*	Nemout®/USA, Costa Rica	Bettiol et al. (2012)
Aspergillus flavus atoxigenic	*Aspergillus flavus*	AF36®, Afla-Guard®, Aflasafe®/ USA, Several Country in Africa	Bettiol et al. (2012), Moral et al. (2020)
Candida sake	Postharvest disease	Candifruit®/Spain	Droby et al. (2016)
Candida oleophila	Postharvest disease	Aspire®/USA	Droby et al. (2016)
Clonostachys rosea f. *rosea* BVT- Cr-7	*Botrytis cinerea, Sclerotinia sclerotiorum, Monilinia, Pythium, Rhizoctonia*	Vectorite®/Canada	Vectorite (2020)
Coniothyrium minitans	*Sclerotinia* spp.	Contans®/Germany, USA, Canada	Patents (1996), Bettiol et al. (2012)
Gliocladium catenulatum	*Rhizoctonia solani, Pythium, Phytophthora, Fusarium, Verticillium, Alternaria, Cladosporium, Helminthosporium, Penicillium, B. cinerea, Didymella*	Prestop®/USA, Canada	Bettiol et al. (2012)
Metschnikowia fructicola	*B. cinerea* and *Rhizopus stolonifera* in postharvest	Shemer®/Israel	Droby et al. (2009)
Myrothecium verrucaria	*Meloidogyne, Pratylenchus, Trichodorus, Belonolaimus, Radopholus, Heterodera, Globodera, Tylenchulus, Xiphinema*	Ditera®/USA, Mexico, Chile, Panama, Honduras, Costa Rica	Bettiol et al. (2012)
Paecilomyces lilacinus	*Meloidogyne, Radopholus, Heterodera, Globodera, Pratylenchys, Tylenchus, Rotylenchus*	BioAct®/Germany	BioActPrime (n. d.), Bettiol et al. (2012)
P. lilacinus	*Meloidogyne incognita, M. javanica, P. brachyurus*	Nemat®/Brazil	Agrofit (2021)
Phlebiopsis gigantea	*Heterobasidium annosum*	Rotstop®/USA, Germany, Finland	Rotstop (2021), Bettiol et al. (2012)
Pochonia chlamydosporia	*Meloidogyne*	Rizotec®/Brazil	Agrofit (2021)
Pseudozyma (Sporotrix) flocculosa	*Sphaerotheca fuliginea, Sphaerotheca pannosa, Uncinula necator*	Sporodex® L/USA, Canada/Union Europe	EPA (2002), Bettiol et al. (2012), Konstantinidou-

(continued)

Table 29.1 (continued)

Biocontrol agent	Pathogen/disease	Commercial product/ country	Reference
			Doltsinis et al. (2007)
Pythium oligandrum	*Alternaria brassicae, S. sclerotiorum, B. cinerea, Tilletia carie, Peronospora parasitica, Sphaerotheca humulil*	Polyversum®/Czech Republic	Bettiol et al. (2012), Polyversum (2021)
Trichoderma asperellum	*Rhizoctonia, Pythium, Phytophthora, Fusarium, Botrytis,* and *Colletotrichum*	Bioprotection® TR/Costa Rica	Obregón-Gómez (2010), Obregón (2012)
T. asperellum	*R. solani*	Tricho-Turbo®/ Brazil	Bettiol et al. (2019a, b)
T. asperellum	*F. solani* f. sp. *phaseoli, R. solani, S. sclerotiorum*	Quality®/Brazil	Bettiol et al. (2019a, b)
Trichoderma atroviride	*Gibberella fujikuroi, Burkolderia plantarii, Burkolderia glumae, Acidovorax avenae* subsp. *avenae*	Ecohope® and Ecohope-Dry®/Japan	Ecohope (2019)
T. atroviride	*Sclerotium cepivorum, Fusarium oxysporum* f. sp. *cepae*	Tenet®/Australian, New Zeland	Tenet (2013)
T. atroviride NBT-T11 and *T. asperellum* NBT-T25	*R. solani, S. sclerotiorum, Fusarium* sp., *Pythium* sp., and *Phytophthora*	Tusal®/Spain	Grandona et al. (2004), Hermosa et al. (2001)
Trichoderma fertile	*R. solani, Fusarium, Pythium, Sclerotinia, Phytophthora*	Trichoplus 1® TM/South Africa	Trichoplus (2018)
Trichoderma harzianum	*Fusarium solani, R. solani, Sclerotinia sclerotiorum, Thielaviopsis paradoxa, Macrophomina phaseolina, Pratylenchus zeae*	Trichodermil®/Brazil	Bettiol et al. (2019a, b)
T. harzianum T22	*Rhizoctonia, Sclerotinia, Sclerotium, Fusarium, Pythium, Botrytis*	Bio-Trek 22G®, F-Stop®, Plantshield®, Rootshield®, Triaction®, Trianum®	Harman et al. (1989), Woo et al. (2014), Bettiol et al. (2019b)
T. harzianum A-34	*Phytophthora, Rhizoctonia, Pythium, Sclerotium, Fusarium*	Tricosave® A-34/ Cuba	Stefanova (2007), Stefanova et al. (2014)
T. harzianum	*R. solani, S. sclerotiorum*	Ecotrich® WP/Brazil, Paraguay, Bolivia	Bettiol et al. (2019a, b)

(continued)

Table 29.1 (continued)

Biocontrol agent	Pathogen/disease	Commercial product/ country	Reference
Trichoderma koningiopsis	*Heterodera glycines, Meloidogyne, Pratylenchus brachyurus*	Diamond®/Brazil	Bettiol et al. (2019a)
T. koningiopsis	*Pythium splendens, R. solani, S. sclerotiorum* and *F. oxysporum*	Tricotec®/Colombia	Bettiol et al. (2019b)
Trichoderma lignorum	*F. oxysporum* f. sp. *dianthi, F. oxysporum* f. sp. *lycopersici, R. solani*	Mycobac®, Trichobiol®, Trichogen®/ Colombia	Instituto Colombiano AgropecuÁrio (2018)
Trichoderma virens	*R. solani, Pythium, Fusarium*	SoilGard®/USA, Mexico	SoilGard (2019)
Trichoderma viride	*Pythium, R. solani, Fusarium, B. cinerea, S. rolfsii, Sclerotinia homeocarpa*	Bio Cure®/India, USA, Union Europe	Bio-Cure (2019)
T. viride	*R. solani, Helminthosporium oryzae, Sarocladium oryzae, Gaeumannomyces graminis*	Trifesol®/Colombia	Instituto Colombiano AgropecuÁrio (2018)
T. viride TS-3	*Phytophthora, Rhizoctonia, Pythium, Sclerotium, Fusarium*	Tricosave TS-3/Cuba	Stefanova (2007), Stefanova et al. (2014)
Trichoderma stromaticum	*Moniliophthora perniciosa* in cacao	Tricovab®/Brazil	Bettiol et al. (2019a, b), Costa et al. (2009)

Many biocontrol agents exhibit parasitism as the primary mechanism of action. It is essential to consider that many of these antagonists were isolated directly from the pathogen's structure. For example, *Coniothyrium minitans* (Trutmann et al. 1980); *Penicillium nigricans* (Utkhede and Rahe 1980, 1983); *Acremonium persicinum* and *Acremonium altematum* (Sudo 1989); *Hansfordia pulvinata* (Junqueira et al. 1989; Junqueira and Gasparotto 1991); and *Lecanicillium lecanii* (Eskes et al. 1991).

For the effectiveness of biocontrol agents, it is important to understand the structure and functioning of agroecosystems and the detailed knowledge of the pathosystems involved, as well as the best way to achieve biological targets. Associated with this, there is a need for integrated crop management because, depending on cultural management, biological control agents may have low effectiveness. Thus, in the development of a biological control agent, a fundamental step is integrating the bioagent into cropping systems.

When crops are considered in sequential crops within the same agricultural year, as is the case in Brazil of soybean production and the sequence with the sowing of corn or cotton and the following year returning to soybeans, all with intensive use of

Table 29.2 Fungi registered for biological control of plant diseases in Brazil (Agrofit 2021)

Biological control agent	Company	Commercial product	Pathogen
Clonostachys rosea	Agrivalle Brasil Ltda	Kamoi	*Botrytis cinerea*
Paecilomyces lilacinus	Agrobiológica Sustentabilidade S.A.	Nemakill, Atialy	*Meloidogyne incognita*
P. lilacinus	Ballagro Agro Tecnologia Ltda	Nettus	*M. incognita*
P. lilacinus	Ballagro Agro Tecnologia Ltda	Nemat	*M. incognita, M. javanica, P. brachyurus*
P. lilacinus	Tz Biotech Ltda	Purpureonyd FR 25	*M. incognita*
P. lilacinus CCT 7766	Agrobiológica Sustentabilidade S.A.	MNG 02/14	*M. incognita*
Pochonia chlamydosporia	Biovalens Ltda	Clamax	*M. javanica*
P. chlamydosporia	Rizoflora Biotecnologia Ltda	Rizotec	*M. javanica*
P. chlamydosporia	Vittia Fertilizantes e Biológicos S. A.	Rizo-Turbo	*M. javanica*
P. chlamydosporia	Vittia Fertilizantes e Biologicos S. A.	PC-Guard, PC-Attack	*M. javanica*
Trichoderma asperellum	Novozymes Agricultura Ltda	Trichodermax EC	*Fusarium solani* f. sp. *glycines, Rhizoctonia solani, Sclerotinia sclerotiorum*
T. asperellum	Vittia Fertilizantes e Biológicos S. A.	Tricho-Turbo	*Fusarium oxysporum, Pratylenchus brachyurus, R. solani, S. sclerotiorum*
T. asperellum	Vittia Fertilizantes e Biológicos S. A.	Tricho-Guard	*R. solani*
T. asperellum	Vittia Fertilizantes e Biológicos S. A.	Bio-Hulk	*R. solani*
T. asperellum URM-5911	Lallemand Solucoes Agrobiologicas Ltda	Organic WP	*F. solani* f. sp. *phaseoli, R. solani*

(continued)

Table 29.2 (continued)

Biological control agent	Company	Commercial product	Pathogen
T. asperellum URM-5911	Lallemand Solucoes Agrobiologicas Ltda	Quality	*F. solani* f. sp. *phaseoli, R. solani, S. sclerotiorum*
T. asperellum CBMAI 1622	Genica Inovacao Biotecnologica S.A.	Congregga	*S. sclerotiorum*
T. harzianum	Ballagro Agro Tecnologia Ltda	Tritter	*R. solani, S. sclerotiorum*
T. harzianum	Ballagro Agro Tecnologia Ltda	Ecotrich WP	*Macrophomina phaseolina, R. solani, S. sclerotiorum*
T. harzianum	Ballagro Agro Tecnologia Ltda	Predatox	*R. solani, S. sclerotiorum*
T. harzianum	Ballagro Agro Tecnologia Ltda	Rizoderma	*R. solani, S. sclerotiorum*
T. harzianum	Koppert do Brasil Ltda	Daytona	*F. oxysporum* f. sp. *phaseoli, Pratylenchus zeae, R. solani, S. sclerotiorum, Thielaviopsis paradoxa*
T. harzianum	Koppert do Brasil Ltda	Trichodermil SC 1306	*F. oxysporum* f. sp. *phaseoli*
T. harzianum	Koppert do Brasil Ltda	Trichodermil SC 1306	*P. zeae, R. solani, S. sclerotiorum, T. paradoxa, Fusarium solani* f. sp. *phaseoli*
T. harzianum	Koppert do Brasil Ltda	Trianum DS	*F. oxysporum* f. sp. *lycopersici, M. phaseolina, P. brachyurus, S. sclerotiorum*
T. harzianum	Koppert do Brasil Ltda	Trianum WG	*F. oxysporum* f. sp. *lycopersici, M. phaseolina, P. brachyurus, S. sclerotiorum*
T. harzianum	Mezfer Br Soluções Agrícolas Ltda	Natucontrol	*F. oxysporum* f. sp. *lycopersici, R. solani, S. sclerotiorum*
T. harzianum	Simbiose Ltda	Bio Zenon	*R. solani, S. sclerotiorum*
T. harzianum	Simbiose Ltda	Stimucontrol Evolution	*S. sclerotiorum*
T. harzianum	Simbiose Ltda	Stimucontrol	*R. solani, S. sclerotiorum*
T. harzianum	Simbiose Ltda	Gaia Bio	*R. solani, S. sclerotiorum*
T. harzianum	Tz Biotech Ltda	Trychonyd FR 25	*F. oxysporum* f. sp. *lycopersici*

(continued)

Table 29.2 (continued)

Biological control agent	Company	Commercial product	Pathogen
T. harzianum T-22	Koppert do Brasil Ltda	Walker	*F. oxysporum* f. sp. *lycopersici*, *S. sclerotiorum*
T. koningiopsis IBCB 56/12	Lallemand Solucoes Agrobiologicas Ltda	Lalnix Resist	*Heterodera glycines*, *M. incognita*, *P. brachyurus*
T. stromaticum	Ceplac - Comissão Executiva do Plano da Lavoura Cacaueira	Tricovab	*Moniliophthora perniciosa*
T. harzianum + *Bacillus amyloliquefaciens*	Agrivalle Ltda	Shocker	*R. solani*, *S. sclerotiorum*
B. amyloliquefaciens CCT 7901 + *T. harzianum* URM8119 + *T. asperellum* URM8120	Ballagro Agro Tecnologia Ltda	Pardella	*Colletotrichum lindemuthianum*, *R. solani*, *S. sclerotiorum*
B. amyloliquefaciens CCT 7901 + *T. harzianum* URM8119 + *T. asperellum* URM8120	Biota Innovations Indústria e Comércio de Bioprodutos Ltda	Tanus	*C. lindemuthianum*, *R. solani*, *S. sclerotiorum*

chemical pesticides, it becomes difficult to carry out an intervention based on conservationist principles. In this case, bioprotectants are used aiming at the biological target and its flooding introduction.

In this situation, when the plant protection problem is caused by nematodes (*Pratylenchus* spp., *Meloidogyne* spp.) or soil-borne plant pathogen (*Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Sclerotinia* spp. among others), biocontrol agents are applied via seed treatment or directly in the cultivation furrow at the time of sowing. These application technologies to achieve the biological target have been adopted in recent years, with a preference for use in cultivation furrows, having seen the compatibility problems of the antagonists with the chemicals used in the treatment of seeds. Such techniques have resulted in the effective control of plant pathogens through the use of biocontrol agents.

Sclerotinia sclerotiorum, the causal agent of white mold, is one of the most important soil-borne fungi under intensive cultivation of several crops, such as soybeans, beans, and cotton, reducing productivity. For the control of this pathogen, seed treatments or in-furrow application, spraying in the first stages of crop development, considering sclerotia as the biological target, which are found in the superficial layer of soil, inside the crop debris, and under the mulch cover in a no-tillage system. However, several farmers also apply the antagonists after the

soybean harvest in a second crop, which has a positive effect in reducing the initial inoculum for the next crop season. Another important factor of success in this kind of cultivation is the continued applications of biologicals due to the low conditions of establishment and permanence of the bioagents in the soil. *Trichoderma* and *Bacillus* are the main bioagents used in this system to control *S. sclerotiorum.*

Cropping systems that adopt crop rotation and cover crop species diversity present more adequate soil quality for the establishment of biocontrol agents. An example is the sowing of seed mixture of soil cover crops, such as vetch, oats, turnip, ervilhacaes, and rye. Afterwards, it returns to the cultivation of the main crop, with the use of biocontrol agents. This succession of crops interspersing cultures that produce mass for soil cover and diversity of root exploitation, provide the physical and biological improvement of the soil, and improve biocontrol's effectiveness. Associated with this, it also allows the recruitment of beneficial organisms by plants (Faria et al. 2020).

29.4 Potential for the Control of Plant Pathogens with Antagonistic Fungi

In a recent review covering the main research carried out during the last 50 years to assess the interactions between antagonistic fungi and plant pathogenic fungi, Thambugala et al. (2020) presented approximately 300 antagonistic fungi belonging to 13 classes and 113 genera, along with the target pathogens and the diseases. *Trichoderma* has been identified as the genus with the greatest potential, comprising 25 species that have been reported to be effective against various pathogenic plant fungi. Bettiol et al. (2019b) presented the details of 246 commercial products based on *Trichoderma* marketed, containing trade name, company, countries where they were registered, formulations, mode of action, targets and methods of application. In a previous publication, Bettiol et al. (2012) presented information on 133 commercial products based on biocontrol agents, both based on fungi and bacteria. In addition to *Trichoderma*, Thambugala et al. (2020) have considered species of the genera *Alternaria, Aspergillus, Candida, Fusarium, Penicillium, Pichia, Pythium, Talaromyces,* and *Verticillium* as important antagonists. However, considering the biodiversity, other genera of fungi should be sought for the development of bioprotectants. In Tables 29.1 and 29.2, there are several commercial products based on antagonistic fungi.

In recent years, the potential of entomopathogenic fungi (*Metarhizium anisopliae, M. robertsii, Beauveria bassiana*) has been demonstrated for the control of plant diseases caused by plant pathogenic fungi and for the growth promotion of plants (Ownley et al. 2008, 2010; Elena et al. 2011; Sasan and Bidochka 2012, 2013; Ravindran et al. 2014; Dutta et al. 2015; Liu et al. 2017; Raad et al. 2019; Barelli et al. 2020; Sarven et al. 2020; Tomilova et al. 2020; Zhang et al. 2020a), and also for mitigating salt stress (Khan et al. 2012a, b). For instance, *B. bassiana* has been reported to reduce severity and *M. Roberts* decrease disease caused by *Rhizoctonia solani* in potato and *S. sclerotiorum* in *Arabidopsis thaliana* (Raad et al. 2019;

Tomilova et al. 2020). Furthermore, this fungus is also reported to induce systemic resistance in cotton against bacterial blight caused by *Xanthomonas axonopodis* P.V. *malvacearum* (Ownley et al. 2008). Tomilova et al. (2020) reported the effect of *M. robertsii* on *Rhizoctonia solani* in potato similar to *B. bassians*. Sarven et al. (2020) observed that *M. anisopliae* showed a significant control of tomato gray mold caused by *B. cinerea*. *Trichoderma* spp. also present potential for the control of insect pest (Rodríguez-Gonzáles et al. 2018; Coppola et al. 2019). This new knowledge about the effects of antagonistic fungi on plant pathogens controlling insects, of entomopathogenic fungi controlling plant pathogens and promoting plant growth allows mixtures of these biocontrol agents to be used to control pests and diseases well as to promote the growth of plants.

Fungi antagonistic to plant pathogens exhibit mechanisms of action such as antibiosis, competition, parasitism, predation, hypovirulence and induction of defense of the plant, the latter with direct action on the host (Elad 1996; van der Boogert 1996; Woo et al. 2014; Monte et al. 2019; Thambugala et al. 2020). Monte et al. (2019) have given a detailed account of these mechanisms. However, the importance in the world market, the actions mechanisms of *Trichoderma* is the most studied and known.

In Brazil, *Trichoderma* is responsible for changing the scenario of biological control of plant diseases. The first product based on *Trichoderma* in Brazil was developed in 1987 by Embrapa Clima Temperado, consisting of a formulation of *Trichoderma viride* for the control of *Phytophthora cactorum* (Valdebenito-Sanhueza 1991). On the other hand, the first commercial product based on *Trichoderma* was registered in 2006 (Bettiol et al. 2014a, b).

The greatest success cases in Brazil are the use of *Trichoderma* to control white mold (*S. sclerotiorum*) in various crops, such as soybeans, beans, cotton, among others. The degradation of sclerotia, which are resistant structures of the pathogen to survive in soil, represents a special challenge to hyperparasites during attack. In the soybean crop, the efficiency of *Trichoderma* has been proven since 2012, through a nationwide network of cooperative assays of biological control of white mold, conducted by researchers from several institutions in Brazil. The management of white mold requires the joint adoption of cultural measures, the use of fungicides and biological control agents, to prevent and control disease in plants and reduce the amount of inoculum (Meyer et al. 2016). The reduction of the sclerotia population (source of the initial infection) in the soil is a key for the integrated management of the disease. The *Trichoderma* formulations are mainly used to reduce the viability of sclerotia of *S. sclerotiorum* as the part of biological control strategy. According to Meyer et al. (2019), the biological control efficiency depends on specific environmental conditions. For better establishment of the biocontrol agents in the soil, conditions that favor the carpogenic germination of sclerotia (high soil moisture, air temperature between 15 and 25 °C and low incidence of sunlight) are required. In this sense, the presence of straw covering the soil, formed through the cultivation of monocot cover crops in a no-tillage system, has been essential for establishing biological control agents and improving biocontrol efficiency. Figure 29.1 details the schedule for the use of biocontrol agents to the control of *S. sclerotiorum* in

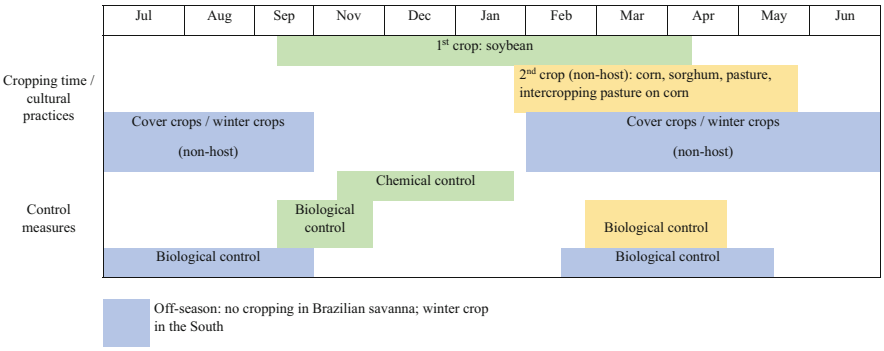

Fig. 29.1 Timeline indicating the use of biocontrol agents for the control of *Sclerotinia sclerotiorum* in soybean culture in Brazil. Note: biological control is usually applied once or twice in each cropping time, just when the weather conditions became ideal (rainy days)

soybean, which, according to Bueno et al. (2020) is responsible for the use of *Trichoderma* in 5.5 million ha; however, this area has increased in recent years, possibly reaching nine million ha. The parasitism of sclerotia and apothecia in *S. sclerotiorum* by *Trichoderma* is presented in Fig. 29.2.

29.5 Potential for Plant Nematodes Control with Antagonistic Fungi

More than a hundred species of fungi, with nematicidal activity, have been reported and Moosavi and Zare (2020) have reviewed their action mechanisms. Most of the research related to nematophagous fungi has been carried out in greenhouse experiments or in vitro conditions. This fact is due to the natural competitiveness of the soils (fungistatic, antibiosis, competition, among others), which reduces the efficiency of the introduced organisms in this environment.

Nematophagous fungi are classified as endoparasites, predators, producers of toxic metabolites and opportunists (Stirling 1991; Ferraz et al. 2010). Endoparasites, for example, species of the genus *Hirsutella, Catenaria,* and *Nematoctonus,* are characterized by their growth inside the nematode, with few hyphae found in the soil (Liu et al. 2009; Zhang et al. 2020b). In general, studies with endoparasitic fungi are limited to controlled conditions. Their survival due to low saprophytic activities depends on the presence of the nematodes in the soil, which compromises competition in the rhizosphere and the commercial use (Jaffee and Zher 1985; Ferraz et al. 2010).

Predatory fungi include saprophytic species, which form specialized structures that act as traps, aiming to capture nematodes in the soil (Ulzurrun and Hsueh 2018). Among these structures, hyphae, constrictive or non-constricting rings, and adhesive nodules, among others, are observed. *Monacrosporium, Dactylaria*, and *Arthrobotrys* are predatory fungi (Ahrén and Tunlid 2003), and *Arthrobotrys*

Fig. 29.2 Parasitism of *Sclerotinia sclerotiorum* sclerotia and aphothecia by *Trichoderma*, observed in network assays evaluating the biological control of white mold in soybeans in Brazil. Healthy sclerotia and apothecia (**a**, **b**); sclerotia and apothecia parasitized by *Trichoderma* (**c**–**g**); sclerotia and apothecia parasitized by *Trichoderma* and *Fusarium* (**h**). Photos: MC Meyer

being the most studied. *Arthrobotrys oligospora* is considered the most commonly isolated trap-forming fungus in nature (Wachira et al. 2009). In addition to three-dimensional adhesive hyphae, the fungus forms conidial traps and hyphal coils (Nordbring-Hertz 2004). Furthermore, chemical substances that can be harmful to nematodes, including alkaloids, quinone terpenoids, peptides, macrolides, aliphatic and aromatic compounds, and sterols, are produced (Li et al. 2007). Secondary metabolites such as oligosporol, oligosporon, flagranone, arthrosporol, and arthrobotrisine produced by *Arthrobotrys* spp. exhibit nematicidal activities (Degenkolb and Vilcinskas 2016).

France was a pioneer in producing bio nematicides based on *Arthrobotrys* spp. with the Royal 300® and Royal 350® products (Cayrol et al. 1978; Cayrol and Frankowski 1979). Nematus® (*A. conoides*), Nematofagin-BL® (*A. oligospora*) and Nemout® (*A. oligospora* and *A. botryospora*) are marketed in other parts of the world (Al-Hazmi et al. 1993; Vladimirovna 2016). In Brazil, there is no report of any registered product based on *Arthrobotrys*.

Another predatory species studied in Brazil with potential for use as a bionematicide is *Duddingtonia flagrans* (syn. *Arthrobotrys flagrans*), whose action mechanism involves capturing nematodes through the production of three-dimensional adhesive hyphae networks. Monteiro et al. (2020), in addition to several characteristics, have also described other advantages of this fungus, including the production of chlamydospores, endophytic association with plant roots, and plant growth promotion. More than 200 nematicide compounds, synthesized by fungi, have been described, including alkaloids, terpenoids, quinones, peptides, fatty acids, and aromatic compounds (Li and Zang 2014). Filtrates of *Fusarium, Paecilomyces, Aspergillus,* and *Penicillium* have been evaluated, and some inhibit the hatching of eggs and cause juvenile mortality, in vitro, more than 80% (Kimura et al. 1996; Zareen et al. 2001; Nakahara et al. 2004; Sharma et al. 2014; Jang et al. 2016; Kim et al. 2016). These metabolites also interfere with nematode mobility and infectious capacity (Zareen et al. 2001; Meyer et al. 2004; Jang et al. 2016). So far, little is known about the stability and effects of these metabolites when applied under field conditions, stability in different edaphoclimatic conditions, post-infection effects, among others.

Opportunistic nematophagous fungi are generally saprophytic and easily grow in culture medium, which are important characteristics from their application point of view. *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*) and *Pochonia chlamydospores* are the most studied species. Such fungi have similarities in their mechanisms of action, as they are considered chitinolytic organisms, with a high affinity for nematode eggs (Lopez-Llorca et al. 2002; Gortari and Hours 2008; Ferraz et al. 2010). This characteristic makes this group more efficient in controlling nematodes that deposit aggregated eggs, that is, sedentary species, emphasizing *Meloidogyne* spp. (Kerry and Jaffee 1997; Lopez-Llorca et al. 2002; Ferraz et al. 2010; Dallemole-Giaretta et al. 2012). Due to the commercial importance of these fungi, an extended description presented below.

Control by opportunistic nematophagous fungi occurs by direct infection of eggs and sedentary females. The infection starts with the formation of the appressorium

on the nematode and secretion of enzymes, such as proteases and chitinases, considered as virulence factors of these species (Yang et al. 2015). After overcoming the barriers imposed by the shell of the eggs or by the body of the females, the fungi have access to the necessary nutrients for the development of new hyphae, which grow abundantly in the soil, being able to feed on new eggs or, in the absence of nematodes, of matter decomposing organic (Mauchline et al. 2004; Dong et al. 2007; Dallemole-Giaretta et al. 2012).

Despite the efficiency of these fungi in controlling sedentary nematodes, the benefits can be extended to migratory species. *Purpureocillium lilacinum* can directly colonize mobile forms and produce toxic substances capable of immobilizing the migratory nematode infective stages in the soil (Khan et al. 2006; Li et al. 2020). *Pogonia chlamydosporia* can release several chemical compounds in the soil with nematicidal potential (Khambay et al. 2000; Wang et al. 2015; Lacatena et al. 2019). Dias-Arieira et al. (2018) observed that *P. platinum*, applied to soybean seeds, controlled *Pratylenchus brachyurus* between 26% and 65% and increased productivity by 14.6%. Infield conditions, *P. chlamydosporia* reduced the reproduction of *P. brachyurus* by more than 70%, and increased soybean productivity of 1170 kg ha-1 (Abreu et al. 2017).

Purpureocillium lilacinum causes deformities in the nematode eggs (Khan et al. 2012a, b, c), since the eggs are sensitive to serine proteases produced by the fungus (Yang et al. 2011). In addition, the release of collagenases and chitinases allows the fungus to penetrate the female cuticle and promote cell degradation (Jatala 1996; Ahman et al. 2002; Huang et al. 2004). *Paecilomyces* is recognized as a producer of compounds with potential action against pests and diseases. Li et al. (2020) reported the production of 148 compounds by this bioagent. Moreno-Gavíra et al. (2020) have discussed the versatility of the uses of different species of *Paecilomyces,* including *P. platinum*, not only in the control of nematodes but also in the control of nematodes fungi, bacteria, and arthropods.

In addition to the direct effect on the nematode, *Purpureocillium* and *Pochonia* can interact with the plant in an endophytic way or by association with the rhizosphere. These interactions have beneficial effects, such as promoting growth and inducing resistance against these parasites in plants. Medeiros et al. (2015) observed that *P. chlamydosporia i*ncreased the concentrations of polyphenoloxidases and peroxidases in tomato roots, modulating the pathways of salicylic jasmonic acids, which are associated with the activation of proteins that act in the defense of plants against *Meloidogyne* spp. (Ghaheremani et al. 2019). In addition, endophytic activity promotes the production of hormones, especially gibberellins and indoleacetic acid, which promote development and reduce abiotic stresses (Cabanillas et al. 1988; Khan et al. 2012a, b, c; Baron et al. 2020).

Some strains of *P. platinum* and *P. chlamydosporia* also help in the absorption of nutrients by the roots, especially P and N (Bhat and Mahmood 2000; Khan et al. 2012a, b, c; Dallemole-Giaretta et al. 2015; Monteiro et al. 2018; Gouveia et al. 2019). This fact explains the better development of plants treated with these fungi, even in the absence of nematodes (Bhat and Mahmood 2000; Monfort et al. 2005; Dallemole-Giaretta et al. 2015).

Pochonia chlamydosporia strains colonize plant root cells (Escudero and Lopez-Llorca et al. 2012; Manzanilla-Lopez et al. 2013; Dallemole-Giaretta et al. 2015) and form hyphal tangles similar to arbuscular mycorrhizae (Dallemole-Giaretta et al. 2015). Furthermore, colonization of plants leads to the expression of genes related to endophytic activity, including the production of hydrolytic enzymes, proteases, chitinases, and secondary metabolites (Larriba et al. 2014). Additionally, this fungus also has the advantage of forming chlamydospores, which survive in the soil under unfavorable conditions.

The commercial use of *P. platinum* in Brazil was marked by selecting isolates efficient in controlling *Meloidogyne paranaensis* (Santiago et al. 2006; Cadioli et al. 2007). The selection of *P. chamydosporia* was carried out by Dallemole-Giaretta (2008) enabled the development of the first bionematicide based on this fungus.

Trichoderma, the most important fungal antagonist for controlling plant diseases, has also been reported to act against nematodes (Medeiros et al. 2017). In addition to being the best-selling fungus for the control of plant pathogenic fungi inhabiting the soil (Bettiol et al. 2019b), different *Trichoderma* spp. have been promising for the management of sedentary and migrating nematodes. Several commercial products based on *Trichoderma* are marketable (Bettiol et al. 2019b; Agrofit 2021). *Trichoderma* spp. exhibit multiple mechanisms of action, including antibiosis, parasitism, competition, resistance induction, and promotion of plant growth, whose nematicidal activity can occur by the specific action of each mechanism or by the association between them. The action mechanisms are discussed by Monte et al. (2019).

Strains of *Trichoderma* directly infect nematodes by producing chitinase that cleaves glycosidic bonds in the egg wall and promotes the death of juveniles. Genes-*chi18–5* and *chi18–12* are essential for this to occur (Szabó et al. 2012). Parasitism plays a vital role in the efficiency of *Trichoderma.* Eggs and cysts of nematodes of the genus *Heterodera* and *Globodera* are sensitive to enzymes produced by *Trichoderma*. Zhang et al. (2014) observed that *T. longibrachiatum* parasitized 90% of the eggs of *Heterodera avenae*, 18 days after exposure to the fungus. Proteinases, such as serine protease, are also important in nematicidal action, as they promote physiological disturbances in juveniles (Zhang et al. 2015). The action of *Trichoderma* has also been observed against migrating nematodes, such as *Helicotylenchus* and *Scutellonema* (Chanu et al. 2015). These authors observed that *T. viride, T. harzianum, T. longibrachiatum, T. kiningii,* and *T. hamatum* adhered to the surface of the nematodes, germinated and penetrated the cuticle, causing their immobilization by enzymatic action. Dias-Arieira et al. (2018) observed that *T. harzianum*, applied via soybean seed treatment, reduced *P. brachyurus* reproduction and increased productivity by 21%. In addition to the nematicidal effect, *Trichoderma* colonizes roots and promotes plant growth. They also increase the resistance of plants to pathogens, by activating defense responses mediated by ethylene and jasmonic acid (Harman et al. 2004; Salas-Marina et al. 2011; Kath et al. 2017; Zhang et al. 2017).

Medeiros et al. (2017) observed that tomato plants treated with *Trichoderma atroviride* IMI352941 reduced the numbers of galls and egg masses of *Meloidogyne*

javanica inside the roots. A significant result described by the authors was that the progeny of tomato plants subjected to the "*Trichoderma* effect" inherited resistance to the pathogen and their parents' growth promotion.

Despite the selection of hundreds of strains of different nematophagous fungi, few reach commercial scale. In Brazil, *P. platinum, P. chlamydospores, T. harzianum,* and *T. koningiopsis* are marketed as bio nematicides, totaling 16 products registered (Agrofit 2021). In the Brazilian market, fungi-based bio nematicides are also commercialized in a mixture with *Bacillus*.

All fungi sold in Brazil for the control of nematodes are opportunistic (Table 29.2). Thus, the application associated with the presence of organic matter in the soil has benefits. Dias-Arieira and collaborators, while working with the soybean, observed that the association of *P. chlamydosporia* with brachiaria straw reduced the population of *M. javanica* by 23% compared to plants grown only on straw. On the other hand, the reduction was 62% compared to plants treated only with the bionematicide. When oat straw was used, the reductions were 58 and 78%, respectively. Associated with brachiaria straw, the reduction in reproduction by *P. lilacinum* was 72% in relation to plants grown on brachiaria straw and 98% in relation to the control. In the association with oats straw, the reductions were 55% and 66%, respectively. When associated with the cultivation of *Crotalaria spectabilis* and *Crotalaria ochroleuca* to biocontrol agents, the results indicate incompatibility. The hypothesis of the authors is that the harmful substances produced by *Crotalaria* to the nematode also have a negative effect on bionematidicides fungi (Dias-Arieira personal communication).

29.6 Compatibility of Use in Mixtures with Chemical Pesticides and Biopesticides

The incompatibility of biological control agents with chemical pesticides is recognized (Silva et al. 2018; Ons et al. 2020). Thus, biopesticides should preferably not be mixture with fungicides, bactericides, insecticides, and herbicides. However, the combination of biocontrol agents with chemical fungicides for the integrated management of plant diseases is of great practical importance (Ons et al. 2020). Ons et al. (2020) discuss combinations of chemical fungicides with biological antagonists, combinations of chemical fungicides with biological inducers of disease resistance, combinations of resistance-inducing fungi with chemical fungicides, and other combinations. Understanding these combinations helps the integration of cultural tracts.

Yildririm et al. (2020) showed that the fungicides boscalid+kresoxim methyl, fluopyram + tebuconazole, sulfur, and tetraconazole reduced the mycelial growth, spore germination and germ-tube elongation of *Trichoderma harzianum, T. hamatum, T. atroviride,* and *T. asperellum* isolates. Silva et al. (2018) evaluating the compatibility of *Trichoderma asperellum* (IBLF 897, IBLF 904, and IBLF 914) and *T. asperelloides* (IBLF 908) isolates, antagonists to *Sclerotinia minor* and *S. sclerotiorum,* observed that procymidone reduced the mycelial growth of

Trichoderma isolates at a concentration of 10 μg L-1 fungicide. Azoxystrobin reduced the conidial germination of *Trichoderma*, showing LD50 between 0.36 and 0.42 μg L-1 fungicide. These authors observed that the fungicides pencycuron and mandipropamid and the insecticide imidacloprid did not affect the mycelial growth of *Trichoderma* isolates. However, these authors also observed in the experiment carried out in soil substrate, none of the pesticides reduced the parasitism of baits and sclerotia or reduced the control of *S. minor* and *S. sclerotiorum* in lettuce seedlings. This result suggests that compatibility studies in disease management using a mixture of biopesticides and chemical pesticides should also be conducted directly on the soil and not only in vitro.

Due to the costs involved and the integrated control systems for pest and plant disease problems, it is essential to know the compatibility of antagonistic fungi with the main chemical pesticides used in crop management. Dalacosta et al. (2019), evaluating the compatibility of *Trichoderma* associated with different chemical pesticides, suggested that the biological agent should be used separately from the chemicals. Compatibility or incompatibility is related to the active ingredients and formulation, but there is also a diversity of response depending on the species of the biocontrol agents; the time of exposure to the active ingredient is directly related to the incompatibility. Investigations are needed, considering the different interactions, the variability of the biological agent, the wide availability of active ingredients of different classes, the conditions of applications and the edaphoclimatic diversity.

The biocontrol agent's impact on native or introduced microbiota can be beneficial, harmful or neutral, and these interactions determine the success of biological control agents (Medeiros et al. 2019). Thus, in studies on compatibility between biological control agents, it is crucial to consider that the organism is not being introduced into an environment free of any microorganisms.

In the association between microorganisms, the biological activity of each one, in order to avoid incompatibility between them, must be considered. Random mixtures present risks, especially when they involve microorganisms with antibiosis activity since the metabolites produced by one organism can inhibit another biocontrol agent. Another possibility of failure is related to the use of *Trichoderma* with other nematophagous fungi, since it may have an action of mycoparasitism. In addition, the mixture of biocontrol agents at the time of application must be considered, as several products contain metabolites from bioagents, such as Serenade® based on *Bacillus subtilis,* which may be incompatible with isolates sensitive to the metabolites in the product. However, there are also positive interactions as described by Medeiros et al. (2019). The co-inoculation of nitrogen-fixing bacteria with *Trichoderma* promoted synergy in protecting against plant pathogens and promoting the growth of plants. Thus, in advance, the associations must be evaluated to avoid problems of chemical or biological incompatibilities.

According to Ons et al. (2020), since fungicide inhibits the development and growth of biocontrol agents, it should separate the applications of these products in time or space to avoid the chemical acting directly on the antagonist. However, obtaining a biocontrol agent insensitive or resistant to a particular chemical active ingredient of a fungicide will allow the products to be used in a mixture without

incompatibility problems. This strategy has been used for several antagonists (Tronsmo 1991; Mukherjee et al. 1997; Khan and Shahzad 2007; Chaparro et al. 2011; Mutawila et al. 2015). However, selecting antagonistic fungal strains resistant to fungicides is not always possible, as they may lose characteristics that are suitable for a biocontrol agent. Therefore, we must consider all care with combinations.

29.7 On-farm or Homemade Production of Antagonistic Fungi for Biological Control

Before the development of the biological control industry, users of antagonistic and entomopathogenic fungi produced these agents independently. In Brazil, the primary example has been the use of the entomopathogenic fungus *Metarhizium anisopliae* that has been used in the sugarcane for the control *Mahanarva posticata, M. fimbriolata, M. liturata,* and *M. spectabilis* since 1970. In both the Northeast and the Southeast of Brazil, the beginning of large-scale use of this entomopathogen was based on its own production of fungus via solid fermentation using rice grains as a base. Even today, several sugarcane companies continue producing *Metarhizium* and with high quality.

However, with the explosion of problems caused by the outbreak of *Helicoverpa armigera* in Brazil in 2013, coinciding with the low supply of biological products for its control, producers started their own or on-farm production, on a large scale, of *Bacillus thuringiensis*. This fact triggered, in the last 7 years, a strong growth of on-farm production in Brazil. However, on-farm production is focused on producing biological control bacterial agents, such as the different species of *Bacillus*. This production is based on liquid fermentation and uses commercial products as inoculum. The most diverse types of fermenters and culture media have been used with the need for studies to evaluate their quality and efficiency. Currently, legislation on this production is being discussed with a view to its regulation.

Until now, on-farm production of biocontrol fungal agents via liquid fermentation is limited. However, there are important examples of the production of *Trichoderma* for the control of soil-borne plant pathogens within the farm itself, but through solid fermentation.

Considering the diversity of climatic conditions in Brazil and that antagonists need to survive local temperatures and withstand large temperature ranges in some regions of the country, there is an indication that, for greater efficiency, the antagonist should be isolated under the conditions in which it will be used (Bettiol et al. 2021). Thus, the isolation and selection of antagonistic fungi for use in biological control from the same environment are essential for developing biological products to be used in these regions.

Halfeld-Vieira (Personal communication) isolated and selected *Trichoderma harzianum* and *Trichoderma erinaceum* from soils collected in a bean production area with a substantial occurrence of *Sclerotinia* and *Fusarium* in order to control these pathogens. These antagonists have been multiplied via solid fermentation in cereal grains on the farm itself in a laboratory built for this purpose. The production

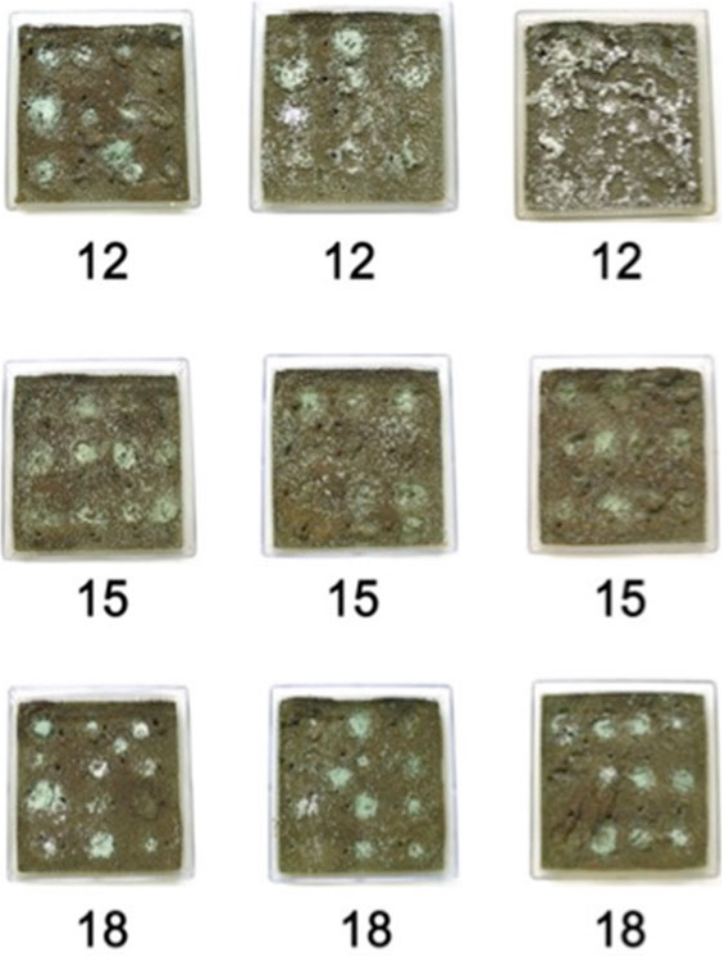

Fig. 29.3 *Trichoderma harzianum* (15) and *Trichoderma erinaceum* (12 & 18) colonizing sclerotia of *Sclerotinia sclerotia* in in vitro in soil ass

is carried out according to the farm's need since the objective is its use. The efficiency in colonizing *Sclerotinia* sclerotia and soil colonization with these isolates are shown in Fig. 29.3. This principle can be developed in regions with extreme climate conditions where there are difficulties in adapting commercial antagonistic fungi originating from other environmental conditions. However, there is a cost to carry out the isolation and selection of antagonistic fungi adapted to the site. In addition, the limited market for the control of certain diseases will also allow the use of this principle, such as the use of *Acremonium ultimatum* and *A. persicinum* for the control of tar spot of coconut (*Catacauma torrendiella* and *Coccostroma palmicola*) (Sudo 1989; Bettiol 1996).

Production on the farm has undergone constant conceptual transformations in the past 3 years. It was previously considered artisanal production, without quality control and with little care to asepsis and contamination. Now it is being replaced by production in scale, with a diversity of microorganisms and laboratories to guarantee quality control. Even so, it is worth highlighting some important factors that must be considered for the success of this production system, among them the use of biological agents (preferably obtained at the place of use) selected through appropriate methods; mastery of production techniques that allow contaminant-free production and the achievement of adequate concentrations. To this end, the current regulation will allow for adequate inspection and quality control.

Acknowledgments Wagner Bettiol (CNPq 307855/2019-8) and Claudia R. Dias-Arieira (CNPq 303269/2020-0) acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq for the productivity fellowship CNPq.

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