Entomopathogenic Fungi

7

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Contents

Abstract

With the rising need of switching over to sustainable agricultural practices, utilization of entomopathogenic fungi (EPF) as biocontrol agents provides better and safe substitute against chemical insecticides, which are associated with

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several environmental and health hazards. Entomopathogenic fungi act as a parasite of insects and kill or critically disable the insects. These include different classes of fungi, viz., Oomycota, Chytridiomycota, Zygomycota, Ascomycota, Deuteromycota, Basidiomycota, and Entomophthoromycota that infect and kill the insects. Some of the merits related with the uses of entomopathogenic fungi as biocontrol agents are high host specificity, insignificant effect on the beneficial insects/nontarget organisms, and simple mass production. The EFP are reported to infect a very wide range of insects, such as lepidopterous larvae, aphids, and thrips, which are of enormous concern in the agriculture, globally. This approach of using EPF as biocontrol agent, instead of chemical pesticides, seems to be very effective and promising in the near future as it moves toward sustainable agricultural practices and protecting the environment, which is the need of the hour.

Keywords

Entomopathogenic fungi · Secondary metabolites · Biopesticides · Biocontrol agent · Insect pests

Learning Objectives

1. The entomopathogenic fungi (EPF) are amid pioneering microorganisms to be applied as biocontrol agent for pest management. EPF kill insect pests at several stages of life.
2. In addition, their incidences along with widespread occurrence make them suitable contenders for pesticides in integrated pest management strategies.
3. The knowledge of recent findings about EPF will signify new frontier contribution toward pathogenesis and multifunctional applications.
4. Therefore, in this chapter, we aim to foreground various aspects of EPF biology including their general characteristics, classification, mechanism of action, maintenance in culture, and some commercial formulations developed for field application.

7.1 Introduction

The fungi are eukaryotic organisms, ranging from unicellular to multicellular filamentous in form. They are chemo-organotrophic creatures without chlorophyll. They reproduce either asexually or sexually or by both means. Fungi often associate with other organisms, creating various biological associations. Entomopathogenic fungi (EPF) are parasitic microorganisms having the potential to cause infection and kill arthropods. Though they majorly belong to arthropod carcasses, yet they are found naturally occurring in soil (Behie and Bidochka 2014). The members of this

group are designated under six classes, viz., Oomycetes, Chytridiomycota, Entomophtoromycota, Microsporidia, Basidiomycota, and majorly Ascomycota. Because of their cosmopolitan occurrence and extensive diversity, EPF considerably contribute as biocontrol agents for sustainable management of insect populations as they own a unique model of infection in various orders of insects. Progressive findings concerning the genomic biology of EPF have revealed that genetic makeup of such microorganisms is advanced for fungal adaptation with plenty of insect hosts. Recent investigations reveal that they also work as endophytes as well as biocontrol mediators of plant pathogens (Behie et al. 2013); moreover, they endorse plant development as rhizosphere fungi. Entomopathogens as biocontrol weapons impart numerous assets over conventional insecticides including high efficiency, low costs, safety for beneficial organisms, lessening of its remains in the surrounding environment, along with amplified biodiversity in the human-controlled community (Asi et al. 2013; Ortiz-Urquiza and Keyhani 2013; Gul et al. 2014). Considering the high enzymatic pursuit, the potential to synthesize secondary metabolites, along with virtuous growth in culture media, their probable application in other fields of biotechnology, like biosynthesis of nanoparticles, making them economically significant (Kozłowska et al. 2019; Dou et al. 2019). The knowledge about their mechanism of virulence and level of tolerance toward adverse conditions, along with application of genetic engineering will potentiate cost-efficient products of mycoinsecticides for pest management in agricultural fields. Moreover, the recent findings concerning exploitation of their genetic diversity, vast ecological occurrence, and wide functional sphere make these fungi highly applicable for integrated pest management.

7.2 Groups of Entomopathogenic Fungi

The kingdom Fungi is the main eukaryotic group with about 700 well-recognized EPF species, accounting for less than 1% of whole fungal species (McLaughlin et al. 2009). EPF do not form a single monophyletic group. EPF are found in three major groupings, viz., Blastocladiomycota, Entomophthoromycota, and Microsporidia and 12 classes under 6 phyla of fungi. Ascomycota, Chytridiomycota, Deuteromycota, Oomycota, and Zygomycota (Humber 1997) are the main divisions harboring these pathogenic fungi. So far, 12 species of Oomycetes, 65 species of Chytridiomycota, 474 species of Entomophtoromycota, 339 species of Microsporidia, 476 species of Ascomycota, and 238 species of Basidiomycota have been described (Araújo and Hughes 2016; Jaber and Enkerli 2017). Amid the diverse phyla, the species belonging to genus *Beauveria, Hirsutella, Verticillium, Nomuraea,* and *Metarhizium* of diverse environmental groups are most conspicuously significant EPF, which are commercially applied effectively at field levels. Further, biological and ecological features of EPF have also been well reported (Steinhaus 1964; Samson et al. 1988; Balazy 1993).

Fig. 7.1 Some common Entomopathogenic fungi

7.2.1 Classification of the Entomopathogenic Fungi

EPF are vital biological control mediators, undergoing rigorous research for more than 100 years. Further, based on the characteristics of the hyphae, composition of cell wall, mode of nutrition and reproduction, entomopathogenic fungi can be classified into different groups. The details of the morphological view of EPF are being summarized as follows (Figs. 7.1 and 7.2):

Phylum Oomycota

The most distinctive feature of Oomycetes includes production of zoospores in sporangia. The coenocytic hyphae of these fungi possess cellulose (without chitin) and their zoospores are biflagellate. Sexual reproduction takes place between gametangia occurring either on the same hyphae or on different hyphae. They reproduce by oospores, which are thick-walled, and they have mitochondria with tubular cristae at the cellular level. They are parasitic on animals and plants, yet a few species are saprophytes. This phylum includes orders *Lagenidiales,* and *Leptomitales*. *Lagenidium giganteum* and *Leptolegnia chapmanii* are parasitic on mosquito larvae, arthropods, crabs, and some aquatic crustaceans (Hatai et al. 2000).

Phylum Chytridiomycota

The most distinctive feature of members of this phylum includes production of motile zoospores having solitary whiplash flagellum, inserted posteriorly. The cell wall of the members of this fungal group is predominantly made up of chitin, their hyphae are coenocytic. This group of fungus is regarded as basal, as per their comparative rRNA phylogenetic analysis. This phylum includes orders Blastocladiales, Chytridiales, and Blastocladiales. Some common genus belonging

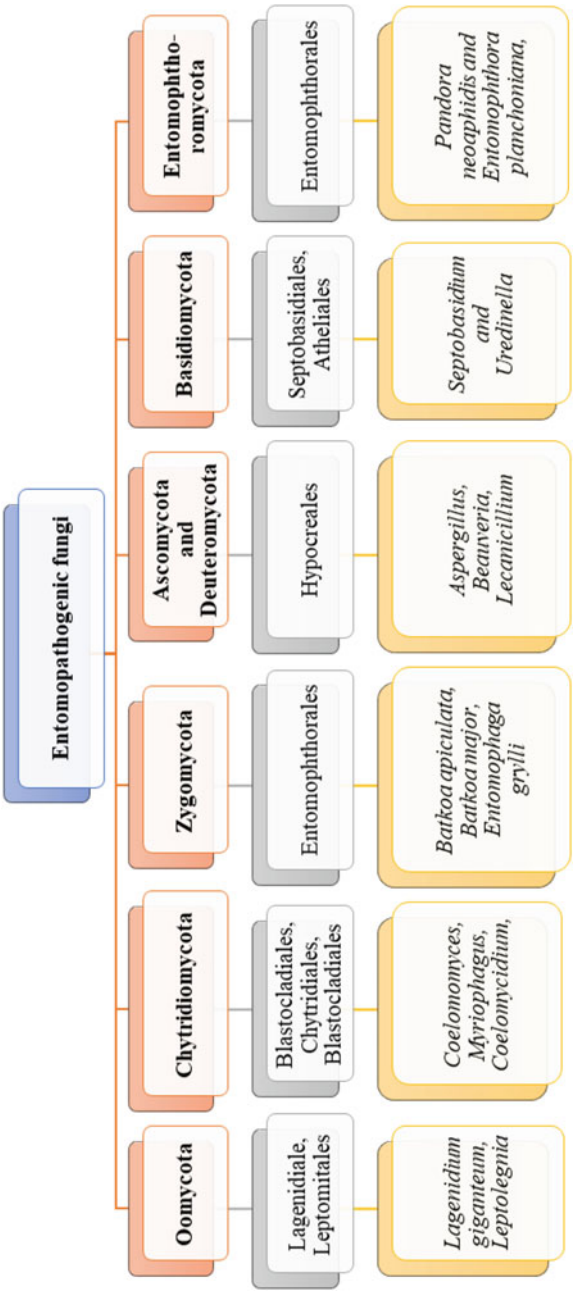

Fig. 7.2 Classification of the entomopathogenic fungus

to this phylum includes *Coelomomyces, Coelomycidium, Myriophagus, Coelomycidium (Blastocladiales),* and *Myriophagus* (Chytridiales). The most common insect hosts infected by them are members of Hemiptera and dipteran flies and mosquitoes.

Phylum Zygomycota

The members of this fungal group possess multicellular and nonseptate mycelium, and after fusion, the gametangia form zygospores (sexual stage), which is one of the most characteristic features of this group. Class Trichomycetes within the phylum consists mostly of species related to insects. The order Entomophthorales holds more than 200 insect-infecting species including *Batkoa apiculata, Entomophaga grylli, Entomophaga maimaiga, Conidiobolus thromboids, Pandora neoaphidis, Zoophthora radicans, Neozygites parvispora,* and various others. The most common insect hosts infected by them are hemipterans, homopterans, lepidopterans, grasshoppers, dipterans, leafhoppers Lepidoptera, gypsy moth larvae, psyllids, and others.

Phylum Ascomycota and Deuteromycota

The members of phylum Deuteromycota are well characterized by the presence of septate mycelium bearing conidiophores and they reproduce through conidia. However, members belonging to phylum Ascomycota bear distinctive feature "ascospores" that develop in the fruiting body, named ascus. Generally, there are eight ascospores produced per ascus formation of ascus bearing ascospores. The order Hypocreales belonging to Ascomycota includes various genera, including *Aspergillus, Aschersonia, Beauveria, Culicinomyces, Metarhizium, Hirsutella, Tolypocladium, Lecanicillium, Paecilomyces*, and others. Furthermore, more than 300 entomopathogenic species are present in Cordyceps. Among numerous insect hosts infected by them are whiteflies, grasshoppers, mosquitoes, potato beetles, Hymenoptera, Lepidoptera, coding moth, boll weevil, chinch bug, granary weevil, cowpea curculio, lygus bug, brown plant hopper, fire ants, termites, European cockchafer, sugarcane borer, and others.

Phylum Basidiomycota

One of the exclusive traits for the members includes "clamp connections." Their sexual spores called basidiospores are formed outside the reproductive cells known as basidia. The members of this group belong to orders Septobasidiales and Atheliales. Only few Basidiomycetes are reported to be pathogenic to insects. The common entomopathogenic genera include *Fibula rhizoctonia, Uredinella,* and *Septobasidium* (Samson et al. 1988), and the insect host infected by them includes termite eggs and scale insects (*Diaspididae, Hemiptera*).

Phylum Entomophthoromycota

The mycelium is well defined, coenocytic, or septate in a somatic state. The protoplast is changeable in shape, either amoeboid or hyphoid, and a few members form rhizoids or cystidia. Their conidiophores are branched or unbranched and the

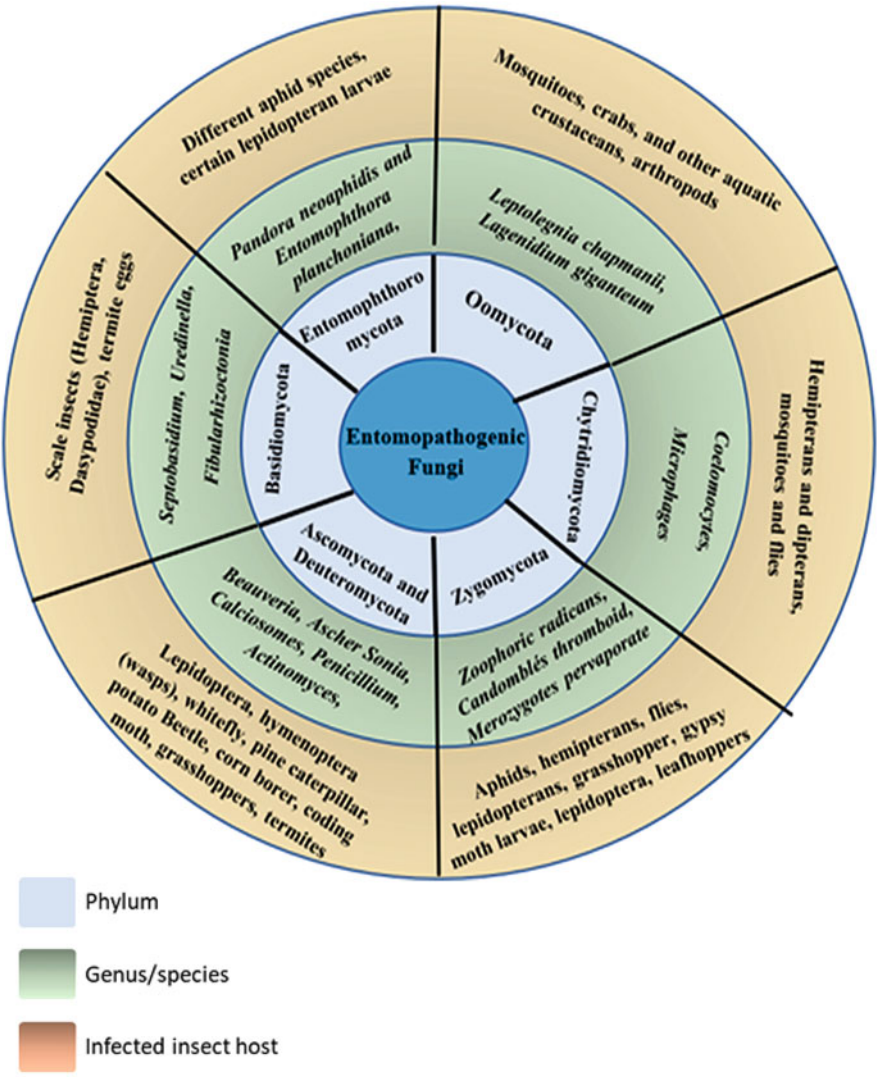

Fig. 7.3 Common Entomopathogenic fungi with their host insect

primary spores are true conidia with uni-, pluri-, or multinucleate conditions. The order Entomophthorales includes various species, e.g., *Entomophthora planchoniana, Pandora neoaphidis,* and *Entomophaga maimaiga,* which infect certain lepidopteran larvae and various aphid species (Fig. 7.3).

7.3 General Characteristics of Entomopathogenic Fungi

Entomopathogenic fungi are bioinsecticides having the potential to infect and kill arthropods by causing fatal diseases in them. EPF are heterogeneous microbes with huge ecological significance. For instance, various species of *Beauveria* and *Metarhizium*, commonly occurring in the soil are reported to form an endophytic relationship with plant leaves, stems, and roots (Jaber and Enkerli 2017) and control arthropod populations associated with them. However, in another report, *Metarhizium robertsii* and *Beaveria bassiana* support plant growth by supplying nitrogen to plants that assimilate at the time of insect parasitization (Behie and Bidochka 2014). Further, *Beauveria bassiana* is found to be endophytic in nearly 25 plant species, thereby aiding the control of their fungal plant pathogens and pests (Vega 2018). It inhabits shoots as well as leaves along with plant roots, imparting insect resistance to plants (Klieber and Reineke 2016; Ramakuwela et al. 2020), and also increases plant defense responses against their microbial pathogens, thus effectively suppressing disease-causing agents (Moonjely et al. 2016). Likewise, *Lecanicillium* reduces the incidence of fungal disease by growing on the surface of plant leaves, preventing nutrients availability and manufacturing antimicrobial compounds, and also induces plant responses while they colonize plant roots (Moonjely et al. 2016).

7.4 Mechanism of Infection of Entomopathogenic Fungi

Mode of action of entomopathogenic fungal infection is determined by various intrinsic and extrinsic factors. Major steps involved in infection process can be summarized as follows (Fig. 7.4):

- Adhesion of spores to cuticle of insect.
- Activation of some defensive biochemical process in insect foe defense.
- Germination of spore.
- Penetration of cuticle.
- Growth in the haemocoel.
- Death of insect and saprophytic feeding by fungi.
- Mummification.

Entomopathogenic fungi directly penetrate the cuticle of insects to cause infection. Rather, their ingestion by an insect is not necessary, as in the case of viruses or bacteria (Bilgo et al. 2018). During the initial phase of the infection process, the spores adhere to arthropod shells and then follow the two phases: the first one depending on the action of electrostatic and hydrophobic forces, while the other one involving the enzymatic activities and hydrophobins; the low-molecular-weight proteins (Skinner et al. 2014). Suitable environmental variables, such as satisfactory humidity and temperature, are necessary for spores to germinate on the insect's cuticle. Further, the presence of adequate energy and carbon sources and the optimal

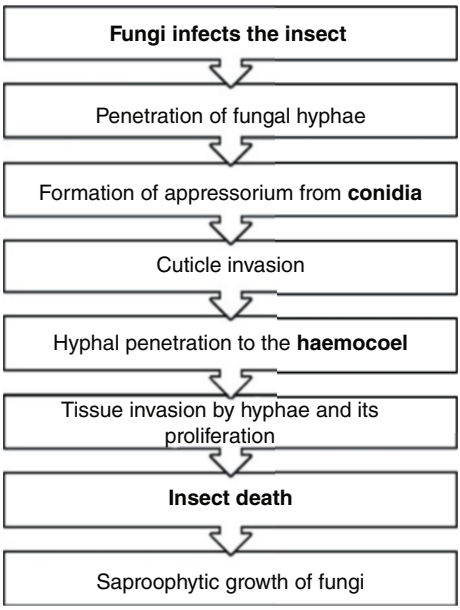

Fig. 7.4 Entomopathogenic fungi and it's mode of infestation

temperature (between 20 and 30 °C) are prerequisites for the growth and germination of EPF (Skinner et al. 2014). Thereafter, appressoria emerge by applying considerable mechanical pressure on the cuticle and start producing proteolytic, lipolytic, and chitinolytic enzymes that disrupt the insect's body shells (Lacey et al. 2015). The development of the fungal hyphae begins once inside the host's body cavity, i.e., hemocoel and in some cases, there is the production of blastospores that pass in the host's hemolymph where they begin to develop secondary hyphae inside the host's tissues. The process is followed by the synthesis and production of secondary metabolites by fungi soon after their commencement, thereby disrupting the host's physiological processes, immune responses, and causing paralysis (Donzelli and Krasnoff 2016). The development of infection leads to the destruction of the insect's body because it starts depleting the nutrient, even the internal organs are damaged by the developing hyphae (Fan et al. 2017). As the infection continues to develop, the insect's body subsequently becomes stiff because EPF continuously absorb the fluids from the initially soft insect's body. Sometimes, the corpse of insects destroyed by EPF may initially turn into a dark red color, as in the case of the genus *Beauveria*. The approximate duration of the entire infection process takes about 14 days after infection; however, the initial symptoms of the disease appear early, within 7 days after infection, which may differ depending on fungal species. Once the insect is dead and deprived of all nutrients, the hyphae of the EPF leave the cadaver of the insect body through intersegmental areas and openings, like mouth hole and anus. Formerly, infective spores or resting spores are formed, which promotes the spread and infection cycle of fungus, also known as mummification (Skinner et al. 2014) (Fig. 7.5).

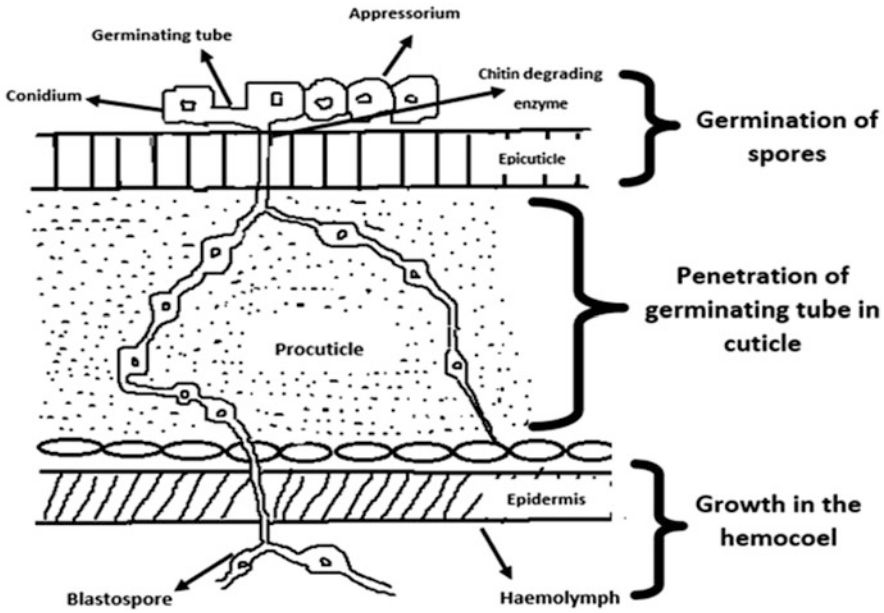

Fig. 7.5 Mechanism of action of Entomopathogenic fungi

Another significant progression involved in the process of infection by EPF is at biochemical levels involving infectious mediators. These **infectious agents** required by EPF for pathogenesis include several lytic enzymes, adhesion molecules, and secondary metabolites. A brief description of these agents is narrated below:

7.4.1 Adhesins

The phenomenon of adhesion of spores to the surface of the arthropod's body is the beginning of the spread of contagion. Two types of proteins, viz. adhesins (MAD1 and MAD2) and hydrophobins, aid in close union as well as recognition of the host by the fungal species (Greenfield et al. 2014).

7.4.2 Lytic Enzymes

Lytic enzymes are the utmost essential components of the infection process by EPF. These enzymes are synthesized and produced once the spore gets involved in the cuticle and starts to develop appressorium (Santi et al. 2010). The major function of these enzymes is the hydrolysis of the components of the insect cuticle and hence to promote the penetration of outer covers of arthropods by appressoria. Lipases, which hydrolyze the lipoproteins and lipids, are produced first, residing in the outer cuticle

of the insect (Pedrini et al. 2007). These enzymes function by breaking the ester bonds of triacylglycerols and allowing the subsequent release of free fatty acids, glycerol, monoacylglycerols, and diacylglycerols (Silva et al. 2009). Also, lipases improve hydrophobic interactions among the fungus and the cuticle surface, hence, promoting adherence of germinating spores to insect cuticles (Santi et al. 2010).

Some proteolytic enzymes hydrolyze peptide bonds of insect cuticles and play crucial roles in developing virulence of EPF. Subtilisin (Pr1) is a serine endoprotease that modifies the surface of the cuticle by degrading some cuticle proteins, so as to facilitate the adhesion of spores. It is reported to be found in *B. bassiana* (Donatti et al. 2008) and O. sinensis (Zhang et al. 2012). Some other proteases, like cysteine Pr4 proteinase, trypsin-like acid Pr2 protease, and metalloprotease reported from *M. anisopliae*; serine elastase found in *Conidiobolus coronatus* and *B. bassiana*, and Pr1- and Pr2- like serine proteases occurring in *Aschersonia aleyrodids, Metarhizium rileyi,* and *Beaveria brongniartii* also contribute to the process of pathogenesis (Zhao et al. 2016).

The chitinases, another group of lytic enzymes, are grouped on the basis of their site of action on the chitin molecule. Endochitinases are enzymes that hydrolyze the b-1,4-glycosidic bonds present inside the chitin molecules, and exochitinases are the ones hydrolyzing N-acetylglucosamine oligomers derived from the cleavage by endochitinases. The complete degradation of insect chitin requires the combined action of endo- and exochitinases. As reported by Duo-Chuan (2006), chitinolytic enzymes have been uncovered in several EPF.

EPF also produce additional enzymes, along with proteases, chitinases, and lipases that are not promptly involved in the collapse of the cuticle, still contribute significantly in pathogenesis. Acid trehalase (ATM1) is one of them, which hydrolyzes the main disaccharide of host hemolymph, i.e., trehalose and results in the release of two glucose molecules, thereby supplying a nutrient for EPF. As reported by Jin et al. (2015), disruption of the ATM1 gene in *M. acridum* causes a notable lessening in the virulence of EPF, which was confirmed by their failure to grow inside the host's body.

7.4.3 Role of Secondary Metabolites in Infection

Secondary metabolites are low-molecular weight organic compounds secreted abundantly by entomopathogenic fungi in response to environmental conditions. These compounds are crucial for affirming the vital functions of the host's system under stress conditions and efficiently protecting against infecting pathogens by reducing insect resistance (Donzelli and Krasnoff 2016). They can be classified on the basis of their chemical structures into the following groups, viz., cyclic depsipeptides (including cyclic tetradepsipeptides and cyclic hexadepsipeptides), amino acid derivatives, peptides (including dipeptides, octadepsipeptides, and depsipeptides), peptide hybrids, polyketides, and terpenoids (Donzelli and Krasnoff 2016; Wang et al. 2018; Fig. 7.6).

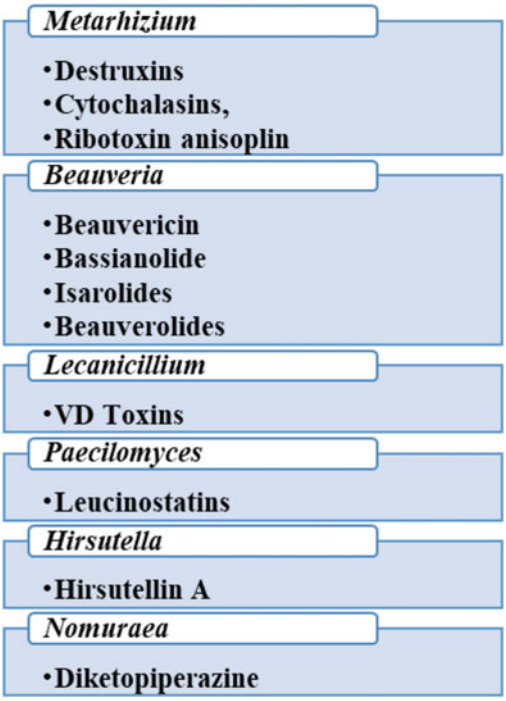

Fig. 7.6 Toxins produced by some Entomopathogenic fungi

Moreover, EPF belonging to deuteromycetes produce diverse fungal toxins that have numerous hostile effects in target insect at tissue level. Few vital toxins synthesized by EPF are summarized as follows:

Destruxins

Destruxins (dtxs), cyclic hexadepsipeptides found mainly associated with species of EPF genus *Metarhizium*. Dtxs are also found abundantly in potatoes (Carpio et al. 2016), strawberries, and maize (Taibon et al. 2015). Out of >40 types of dtxs reported so far, dtxs A, B, and E are of utmost significance for causing pathogenesis (Arroyo-Manzanares et al. 2017). Dtxs possess various biologically significant properties, such as antiproliferative, cytotoxic, antimicrobial, antiviral, and immunosuppressive properties. Dtxs are most prominently known for their phytotoxicity and insecticidal activity. Dtxs function by inhibiting the activity of V-ATPase proton pumps (Liu and Tzeng 2012).

Beauvericins

Beauvericins are commonly produced by EPF genera, such as *Isaria tenuipes, I. fumosorosea,* or *B. bassiana* (Weng et al. 2019), and possess various interesting properties. *Bassianolide* and other cyclic hexadepsipeptides are isolated from *Lecanicillum* sp. and *B. bassiana.* They hold robust cytotoxic abilities against

tumor cells and were reported to restrict the activity of inhibitors of the cholesterol acyltransferase. Another, secondary metabolite produced by *C. militaris* and *Isaria cicadae* is Cordycepine, which belongs to octadepsipeptides. Several reports have demonstrated the insecticidal potential of cordycepin since a long time agoback, including Kim et al. (2002), affirming the larvicidal activity of cordycepin from the fruiting body of *C. militaris* against *Plutella xylostella*. Further, Rosa et al. (2013) showed insecticidal activity of cordycepin against *Trypanosoma evansi*. VIu et al. (2014) also illustrated the iInsecticidal effects of cordycepine on larvae of the Colorado potato beetle Leptinotarsa decemlineata.

Oosporein

Oosporein is another secondary metabolite, isolated chiefly from B. brongniartii, or *B. bassiana* is a very reactive oktadepsipeptide having an antiviral and insecticidal activity (Feng et al. 2015). Further, McNamara et al. (2019) reported that oosporein obtained from *B. caledonica* contributed toward faster mortality of insects due to its greatest immunosuppressive properties.

Since EPF harbor a pool of highly valuable secondary metabolites with diverse activities, researchers are greatly concerned about exploring such compounds, studying their structures, and investigating their biosynthesis mechanisms in detail.

7.5 Culture of Entomopathogenic Fungi

7.5.1 Maintenance of Culture

Regarding this, a single colony isolation of a fungus from the insect (host) is required. A representative strategy is to first isolate the fungus from the host so as to make primary "mother culture" that is genetically uniform. The subsequent re-culturing of the fungi is to be made from this mother culture. Next, the removal of some part of the prime stock from stored one is to be made on annual or semi-annual basis to develop various subcultures on agar medium that can be applied in mass production for use in the future. Although cautions should be taken, still the preserved fungus cultures must not be sub-cultured several times on artificial media because successive passages through artificial media increase the risk of virulence attenuation (Shah et al. 2007) as well as variations in morphogenesis (Butt et al. 2006). The consistent sub-culturing of a fungus is not recommended as it increases the chances of genetic variations, attenuation in sporulation, or virulence abilities through frequent sub-culturing (Ansari and Butt 2011). Preferably, the primary culture should not be passaged in vitro more than four or five times from an insect host. It must be ensured that the fungus remains viable during storage and at the same time preservation method should be such that it inhibits genetic variations. Storage at low-temperature in liquid nitrogen, or under desiccation conditions, such as storage of dry spores with silica desiccant, freeze-drying is the norm. Further, the sporulated fungus in stock cultures can be preserved by means of small agar pieces positioned in 10% glycerol and kept at −80 °C. In some laboratories, a commercial form of this

practice (Microbank, Pro-Lab Diagnostics) is applied. Humber (2012) has described various methodologies for the appropriate preservation of such fungal cultures.

Some key information like site and date of collection and substratum or insect host as well as a notation of a code for every fungal isolate must be recorded and maintained related to fungal stock cultures.

7.5.2 Process Sterility

It is necessary to maintain sterility in order to prevent process contamination. Air, equipment, and the fermentation medium must be purified. Furthermore, it is compulsory to remove all the native microorganisms from raw materials and use apparatus at the beginning of the procedure. Various sterilization techniques used could be like the use of special filters, gamma-radiation, heat, and many more. If proper precautions are not taken, the contaminants could rapidly outstrip the desired strain, and the end product will be inadmissibly contaminated resulting in insignificant production.

7.5.3 Nutrients

Since nutrients are the building blocks, supporting fungal growth. They are key elements that provide co-factors and energy source for biochemical reactions. Depending upon the fungal species and strains under consideration, different concentrations of minerals, vitamins, oxygen, carbon, nitrogen, and hydrogen are required. As reported by Jackson (1997), fungal morphogenesis, specific growth rate, propagule formation, and propagule quality and fitness for application in biological control are all affected by the type and level of nutrient used.

Another key element under consideration is dissolved oxygen. During aerobic fermentation of the filamentous fungal entomopathogens, dissolved oxygen is often considered as the limiting factor. There is a specific requirement of an adequate supply of oxygen for the successful cultivation of EPF. The application of high-speed agitations in oxygen-enriched cultures is one of the means of improving the oxygenation of media and obtaining larger and faster biomass growth of EPF in liquid culture fermentation. Since cultures subjected to oxidative stress run the risk of limited growth and reduction in cell viability and biomass dry weight, therefore, high concentrations of oxygen in the atmosphere ($>21\%$ O_2) or dissolved oxygen in various cultures can be detrimental to fungal growth.

Different genotypes and strains of EPF are not equally receptive to the similar oxygen availability in the growing environment; therefore, specific studies on oxygen rate consumption are compulsory to obtain optimal oxygen requirements by a specific strain (Tlecuitl-Beristain et al. 2010; Garza-López et al. 2012). Recently established, response surface methodology (RSM) is variously used to proficiently determine the unsurpassed parameters.

7.6 Product Formulations

The substantial application of entomopathogenic fungi in diverse arenas make them of utmost importance to mankind. The myco-biocontrol of insects is among one of the best-applied fields of immense significance concerning environmental and food safety. Therefore, their mass production at low cost is among the most desirable targets. Numerous fungal-based products embracing *Metarhizium anisopliae, B. bassiana, Lecanicillium* spp., and *Isaria* spp. have been established for use contrary to a wide variety of pests of household, field, greenhouse, and forests. Currently, a wide range of commercial formulations obtained from such fungi is accessible to farmers in many parts of the world. Widespread research works have been conducted so far for improving fungal mass growth and production as well as to estimate the consequence of changes in additives, substrates, and additional aspects on the viability, virulence, and thermotolerance of spores of entomopathogenic fungal species (Machado et al. 2010; Kassa et al. 2004). Some commercial formulations of EPF used in different countries are given in Table 7.1 (Kaushal et al. 2016).

7.6.1 Mass Production

Miscellaneous approaches have been established for the practical application of EPF to control insect pests. Various approaches including direct application of collected cadavers of infected insects into fields in the same or upcoming season, or use of artificially infected insects in the laboratory and others are being practiced. However, a commonly used method includes the production of the EPF on artificial media. Vegetable materials, like cracked barley, rice or wheat bran, are the most exploited resources and used in the preparation of the medium for mass production schemes. Gouli et al. (2008) have established a comparatively simple millet-based fungal production system that provides nutritional support to growing fungi in the soil without an insect host. Another practice makes use of fungal-based baits to attract the target insects. Here, insects with a prominent feeding stage in the soil are used as targets for bait.

In Russia, large-scale production of conidia is being established by growing the EPF in a fermenter. The mycelium obtained is then placed in shallow pans of approximately 1.0 cm depth, and conidia are produced in the pans after some time. Production of the mycelium in submerged culture is another one. After culture, the fungus usually filtered from the medium to produce thin mats, approximately 3–6 mm thick, which are treated with a sugar solution (10% maltose or sucrose) as a desiccation protectant. The mat is air-dried at room temperature and then milled to particles of 2 mm size. The application of these particles into the field initiates production of conidia in presence of moisture. The particles of mycelium can produce new conidia for several consecutive days in the field, thereby, providing fresh inocula for a considerable period after the introduction of the fungus. Mass

Table 7.1 Mercantile formulations of entomopathogenic fungal pesticides

S. N.	Fungi	Formulation	Crop	Target pest	Product and company
1	*Aeschersorzia aleyrodis*	Wettable powder	Cucumber, potato	Whitefly	Koppert/ Holland
2	*B. Bassiana*	Suspendible granules	Coffee	Coffee berry borer	Conidia, AgroEvo, Germany, and Columbia
3	*Beauveria bassiana*	Liquid formulation	Cotton and glasshouse crop	Sucking insects	Naturalis™, tray bioscience, USA
4	*B. Bassiana*	Wettable powder	Field crops	Whiteflies/ aphids/Thrips	Mycontrol-WP/ Mycotech Corp, USA
5	*B. Bassiana*	Suspendible granules	Coffee	Coffee berry borer	Conidia, AgroEvo, Germany, and Columbia
6	*B. Bassiana*	Microgranules of mycelium	Maize	Corn borer	Ostrinil/natural plant protection/ France
7	*B. brongniartii*	Barley kernels colonized with fungus	Pasture	Scarab beetle larvae	Engerlingspilz/ Andermatt/ biocontrol
8	*Beauveria brongniartii*	Microgranules of mycelium	Sugarcane	Scarab beetle larvae	Betel/natural plant protection/ France
9	*Metarhizium anisopliae*	Conidia on a mycelium placed in trap/ chamber	Houses	Termites	Bio-path™/ EcoScience/ USA
10	*M. Anisopliae*	Granules of mycelium	Glasshouse ornamental crops, nursery stock houses	Black vine weevil	Biologie Bio 1020/Bayer AG, Germany
11	*M. Anisopliae*	Conidia produced on grains.	Pasture/turf	Locusts, grasshoppers and red-headed cockchafer	Biogreen/ biocare technology Pvt. ltd./Australia
12	*P. Fumosoroseus*	Wettable powder	Wide range of crops	Mites	Priority/T. Stanes, India
13	*Pacilomyces fumosoroseus*	Wettable powder	Glasshouse crops	Whiteflies/ Thrips	PFR-21™/WR grace USA
14	*V. Lecanii*	Wettable powder	Glasshouse crops	Aphids, whiteflies and Thrips	Vertatec/ Koppert/ Netherlands

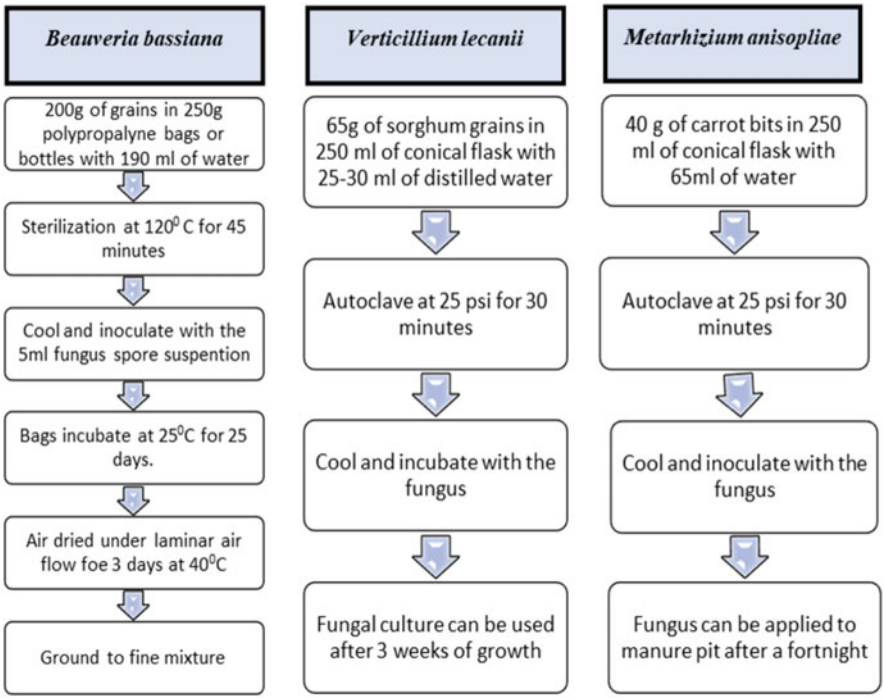

Fig. 7.7 Mass production of few Entomopathogenic fungi

production of some commercially useful entomopathogenic fungi are summarized in Fig. 7.7.

Mass-produced mycelium of EPF obtained after sieving from the medium is applied in making mycelial mats that are dried at room temperature. Mycopesticidal formulation is of major concern because of the brief half-life of the perishable conidia of entomopathogens in sunlight when applied on plant leaves. Another commonly exploited product is in the form of microencapsulation of dried mycelium, pregelatinized with starch. However, the utmost commercially established formulations of EPF are present in the form of wettable powders or emulsified oils.

7.6.2 Wettable Powders

The most commonly produced formulation of entomopathogenic fungi are wettable powder (WP). As reported by Burges (1998), WP is comprised 50–80% technical powder, 15–45% filler, 3–5% surfactant, and 1–10% dispersant. WP is assorted with water and applied to the verdure in the form of a standard insecticidal spray at ultra-low volume or hydraulic concentrations. Even these applications are efficient when applied to the soil as a drench. These formulations are being established using an extensive array of compounds, each with exclusive properties affecting particular aspects to enhance spore survival or efficacy. Additives, such as UV light

protectants, enhancing the capability of the spores to stick to the foliage or ones to increase humidity around the spore so as to promote germination under hostile environmental conditions are used extensively. Kim et al. (2010b) suggested that phloxine B (0.005 g/m), a photoactive dye protects from phytotoxicity. Since conidial viability in storage is greatly affected by moisture, makes it an important factor concerning shelf life. Therefore, the probable use of moisture absorbents, such as silica gel, calcium chloride, white carbon, sodium sulfate, or magnesium sulfate have been recommended in 10% WP conidial powder formulations.

7.6.3 Oil Formulations

Various oils are generally added to many fungal products for improving their shelf life and increasing the field efficacy of such formulations in a dry climate. When oil is used as a carrier, it helps to wet the plant leaves and waxy surface of insects. Moore et al. (1995a, b) reported that addition of oil to spore powder improves the survival rate and viability of conidia. Oils also stimulate the germination of spores and simplify the adhesion of spores to the insect, also facilitating the penetration by disrupting the waxy layer of the cuticle. Paraffin oil, mineral oil, and various other isoparaffinic hydrocarbon solvents are mostly used as carriers for oil-based formulations. Kim et al. (2011) have suggested the use of some additional oils, like methyl oleate, vegetable oils, corn, and cotton seed oil for production of more formulations based out of entomopathogenic fungi.

7.7 Patents Granted on Entomopathogenic Fungi Formulations

Various patents on formulations of entomopathogenic fungi have also been granted. Some of them are summarized in Table 7.2.

7.8 Conclusion

The findings clearly unveil the powerful application of EPF as a biocontrol agent, owing to great significance because of the sanctuary amendment and ecofriendly mode of action. Yet, there is a lot of scope for probable manipulations in desirable traits of EPF toward better overall field activity. Moreover, novel research is needed to develop techniques and formulations/nanoformulations with improved and rapid integrated pest management mechanisms. Their possession of enzymes capable of minimizing toxic anthropogenic compounds reveals their prodigious role as environment protectors. Nonetheless, despite their enormous uses in biocontrol processes, they are undervalued due to lack of information on their proficiencies. Therefore, there is need for additional researches and industrial interests concerning the usage of entomopathogenic fungi with maximized efficacy, amended compatibility, and pliant investments.

Table 7.2 Patents granted on entomopathogenic fungi formulations

S. No.	Patent No.	Country	Inventor	Tittle
1.	WO2008087294A3	WIPO patent	Samantha Besse, Antoine Bonhomme	Use of entomopathogenic fungi as a means for the biological control of paysandisia archon
2.	CA2699272C	Canada patent	Mark A. Jackson, Stefan T. Jaronski	Composition of entomopathogenic fungus and method of production and application for insect control
3.	EP0738317A1	European patent	Clifford A. Bradley, James H. Britton	Formulations of entomopathogenic fungi for use as biological insecticides
4.	WO2009035925A2	WIPO patent	Mark A. Jackson, Stefan T. Jaronski	Composition of entomopathogenic fungus and method of production and application for insect control
5.	US20050095259A1	United States patent	Chien Liu	Manufacturing method for entomopathogenic fungi
6.	WO1995010597A1	WIPO patent	Clifford A. Bradley, James H. Britton	Formulations of entomopathogenic fungi for use as biological insecticides
7.	US8501207B2	United States patent	Stamets P	Mycoattractants and mycopesticides
8.	US20130156740A1	United States patent	Leland JE	Biopesticide methods and compositions
9.	US8226938 B1	United States patent	Miekle et al.,	Biocontrol of Varroa mites with Beauveria bassiana
10.	US008227224 B2	United States patents	Kalisz et al	Method of making moulded part comprising mycelium coupled to mechanical device

7.9 Points to Remember

(i) Since last few decades, synthetics for crop protection were used for management of insect pests; however, use of such chemical pesticides is now declining very drastically due to various health and environmental hazards.
(ii) Further, development of pesticides resistance properties of the insects forced to develop an eco-friendly biological way to manage such problem. So far, more than 500 arthropods have shown to develop resistance to such chemicals.

(iii) Plentiful microbial species have been progressively used for successful control of infections, but very few of them are effective and persistent in the market.
(iv) Most commonly categorized EPF fall under divisions viz. Ascomycota, Deuteromycota, and Zygomycota, and they comprise good applicant to be explored for ecofriendly management of pest and diseases associated with agricultural crops.
(v) Diverse extracellular enzymes and various secondary metabolites produced by EPF could be used for the development of mycopesticide, viz., entomopathogenic fungi-*Verticillium lecanii* have been used for their proteolytic, amylolytic as well as lipolytic enzymes.
(vi) Recent expansion of contemporary techniques in the field of biotechnology has significantly improved the efficacy of the entomopathogenic fungal species, using their genetic and biochemical manipulations, but still there are many hindrances that impede the advancements of EPF in the field of mycopesticide that have to be resolved, for developing an alternative to the synthetics.

References

Ansari MA, Butt TM (2011) Effects of successive subculturing on stability, virulence, conidial yield, germination and shelf-life of entomopathogenic fungi. J Appl Microbiol 110:1460–1469

Araújo JPM, Hughes DP (2016) Diversity of Entomopathogenic fungi which groups conquered the insect body? In: Lovett B, Leger RJS (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 1–39. https://doi.org/10.1016/bs.adgen.2016.01.001

Arroyo-Manzanares N, Diana Di Mavungu J, Garrido-Jurado I et al (2017) Analytical strategy for determination of known and unknown destruxins using hybrid quadrupole-Orbitrap high-resolution mass spectrometry. Anal Bioanal Chem 409:3347–3357. https://doi.org/10.1007/s00216-017-0276-z

Asi MR, Bashir MH, Afzal M, Zia K, Akram M (2013) Potential of entomopathogenic fungi for biocontrol of Spodoptera litura Fabricius (Lepidoptera: Noctuidae). J Anim Plant Sci 23 (3):913–918

Balazy S (1993) Entomophthorales, Flora of Poland (Flora Polska), fungi (Mycota). Polish Acad Sci W Szafer Inst Botany, Krakow 24:1–356

Behie SW, Bidochka MJ (2014) Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. Appl Environ Microbiol 80:1553–1560. https://doi.org/10.1128/AEM.03338-13

Behie SW, Padilla-Guerrero IE, Bidochka MJ (2013) Nutrient transfer to plants by phylogenetically diverse fungi suggests convergent evolutionary strategies in rhizospheric symbionts. Commun Integr Biol 6:e22321. https://doi.org/10.4161/cib.22321

Bilgo E, Lovett B, Leger RJS et al (2018) Native entomopathogenic Metarhizium spp. from Burkina Faso and their virulence against the malaria vector anopheles coluzzii and non-target insects. Parasites Vectors 11:11–16. https://doi.org/10.1186/s13071-018-2796-6

Burges HD (1998) Formulation of mycoinsecticides. In: Burges HD (ed) Formulation of microbial pesticides: beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 132–185

Butt TM, Wang C, Shah FA, Hall R (2006) Degeneration of entomogenous fungi. In: Eilenberg J, Hokkanen (eds) An ecological and societal approach to biological control. Springer, Dordrecht, pp 213–226

Carpio A, Arroyo-Manzanares N, Ríos-Moreno A et al (2016) Development of a QuEChERS-based extraction method for the determination of destruxins in potato plants by UHPLC–MS/MS. Talanta 146:815–822. https://doi.org/10.1016/j.talanta.2015.06.008

Donatti AC, Furlaneto-Maia L, Fungaro MHP, Furlaneto MC (2008) Production and regulation of cuticle-degrading proteases from Beauveria bassiana in the presence of Rhammatocerus schistocercoides cuticle. Curr Microbiol 56:256–260. https://doi.org/10.1007/s00284-007-9071-y

Donzelli BGG, Krasnoff SB (2016) Molecular genetics of secondary chemistry in Metarhizium fungi. In: Lovett B, Leger RJS (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 365–436

Dou F, Wang Z, Li G, Dun B (2019) Microbial transformation of flavonoids by Isaria fumoso rosea ACCC 37814. Molecules 24:1028. https://doi.org/10.3390/molecules24061028

Duo-Chuan L (2006) Review of fungal chitinases. Mycopathologia 161:345–360. https://doi.org/10.1007/s11046-006-0024-y

Fan Y, Liu X, Keyhani NO et al (2017) Regulatory cascade and biological activity of Beauveria bassiana oosporein that limits bacterial growth after host death. Proc Natl Acad Sci U S A 114: E1578–E1586. https://doi.org/10.1073/pnas.1616543114

Feng P, Shang Y, Cen K, Wang C (2015) Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc Natl Acad Sci 112:11365–11370. https://doi.org/10.1073/pnas.1503200112

Garza-López PM, Konigsberg M, Gómez-Quiroz LH, Loera O (2012) Physiological and antioxidant response by Beauveria bassiana Bals (Vuill.) to different oxygen concentrations. World J Ind Microbiol Biotechnol 28:353–359

Greenfield BPJ, Lord AM, Dudley E, Butt TM (2014) Conidia of the insect pathogenic fungus, Metarhizium anisopliae, fail to adhere to mosquito larval cuticle. R Soc Open Sci 1(2):140193. https://doi.org/10.1098/rsos.140193

Gul HT, Saeed S, Khan FZA (2014) Entomopathogenic fungi as effective insect pest management tactic: a review. Appl Sci Bus Econ 1:10–18

Hatai K, Roza D, Nakayama T (2000) Identification of lower fungi isolated from larvae of mangrove crab *Scylla serrata*, in Indonesia. Mycoscience 41(6):565–572

Humber RA (1997) Fungi: identification. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic, London, pp 153–185

Humber RA (2012) Preservation of entomopathogenic fungal cultures. In: Lacey LA (ed) Manual of techniques in insect pathology, 2nd edn. Academic Press, San Diego, pp 317–327

Jaber LR, Enkerli J (2017) Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci Tech 27:28–41

Jackson MA (1997) Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. J Ind Microbiol Biotechnol 19:180–187

Jin K, Peng G, Liu Y, Xia Y (2015) The acid trehalase, ATM1, contributes to the in vivo growth and virulence of the entomopathogenic fungus, Metarhizium acridum. Fungal Genet Biol 77:61–67. https://doi.org/10.1016/j.fgb.2015.03.013

Kassa A, Vidal SD, Zimmermann G (2004) Production and processing of Metarhizium anisopliae var. acridum submerged conidia for locust and grasshopper control. Mycol Res 108:93–100

Kim JS, Je YH, Woo EO, Park JS (2011) Persistence of Isaria fumosorosea (Hypocreales: Cordycipitaceae) SFP-198 conidia in corn oil based suspension. Mycopathologia 171:67–75

Klieber J, Reineke A (2016) The entomopathogen Beauveria bassiana has epiphytic and endophytic activity against the tomato leaf miner Tuta absoluta. J Appl Entomol 140:580–589. https://doi.org/10.1111/jen.12287

Kozłowska E, Dymarska M, Kostrzewa-Susłow E, Janeczko T (2019) Cascade biotransformation of estrogens by Isaria fumosorosea KCh J2. Sci Rep 9:1–8. https://doi.org/10.1038/s41598-019-47225-1

Lacey LA, Grzywacz D, Shapiro-Ilan DI et al (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41. https://doi.org/10.1016/j.jip.2015.07.009

Liu BL, Tzeng YM (2012) Development and applications of destruxins: a review. Biotechnol Adv 30:1242–1254. https://doi.org/10.1016/j.biotechadv.2011.10.006

Mc Namara L, Dolan SK, Walsh JMD et al (2019) Oosporein, an abundant metabolite in Beauveria caledonica, with a feedback induction mechanism and a role in insect virulence. Fungal Biol 123:601–610. https://doi.org/10.1016/j.funbio.2019.01.004

McLaughlin DJ, Hibbett DS, Lutzoni F, Spatafora JW, Vilgalys R (2009) The search for the fungal tree of life. Trends Microbiol 17:488–497

Moonjely SS, Barelli L, Bidochka MJ (2016) Insect pathogenic fungi as endophytes. In: Lovett B, Leger RJS (eds) Advances in genetics. Elsevier, Amsterdam, pp 107–135

Moore O, Bateman RP, Carey M, Prior C (1995a) Long-ter m storage of Metarhizium flavoviride conidia in oil formulation for the control of locusts and grasshoppers. Biocontrol Sci Tech 5:193–199

Moore O, Bateman RP, Carey M, Prior C (1995b) Long-term storage of Metarhizium flavoviride conidia in oil formulation for the control of locusts and grasshoppers. Biocontrol Sci Tech 5:193–199

Ortiz-Urquiza A, Keyhani O (2013) Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 4:357–374

Pedrini N, Rosana C, Patricia Juarez M (2007) Biochemistry of insect epicuticule degradation by entomopathogenic fungi. Comp Biochem Physiol 146:124–137

Ramakuwela T, Hatting J, Bock C et al (2020) Establishment of Beauveria bassiana as a fungal endophyte in pecan (*Carya illinoinensis*) seedlings and its virulence against pecan insect pests. Biol Control 140:104102. https://doi.org/10.1016/j.biocontrol.2019.104102

Samson RA, Evans HC, Latg JP (1988) Atlas of Entomopathogenic fungi. Springer, Berlin Heidelberg, New York

Santi L, Beys da Silva WO, Berger M et al (2010) Conidial surface proteins of Metarhizium anisopliae: source of activities related with toxic effects, host penetration and pathogenesis. Toxicon 55:874–880. https://doi.org/10.1016/j.toxicon.2009.12.012

Shah FA, Allen N, Wright CJ, Butt TM (2007) Repeated in vitro subculturing alters spore surface properties and virulence of Metarhizium anisopliae. FEMS Microbiol 276:60–66

Silva WOB, Santi L, Berger M et al (2009) Characterization of a spore surface lipase from the biocontrol agent Metarhizium anisopliae. Process Biochem 44:829–834. https://doi.org/10.1016/j.procbio.2009.03.019

Skinner M, Parker BL, Kim JS (2014) Role of entomopathogenic fungi. In: Abrol DP (ed) Integrated pest management. Academic Press, Cambridge, pp 169–191

Steinhaus EA (1964) Microbial disease of insects. In: Debach P (ed) Biological control of insect pest and weeds. Chapman and Hall, London, pp 515–547

Tlecuitl-Beristain S, Viniegra-Gonzalez G, Diaz-Godinez G, Loera O (2010) Medium selection and effect of higher oxygen concentration pulses on Metarhizium anisopliae var. lepidiotum conidial production and quality. Mycopathologia 169:387–394

Vega FE (2018) The use of fungal entomopathogens as endophytes in biological control: a review. Mycologia 110(1):4–30

Wang X, Gong X, Li P et al (2018) Structural diversity and biological activities of cyclic depsipeptides from fungi. Molecules 23:169. https://doi.org/10.3390/molecules23010169

Weng Q, Zhang X, Chen W, Hu Q (2019) Secondary metabolites and the risks of Isaria fumosorosea and Isaria farinosa. Molecules 24(4):E664. https://doi.org/10.3390/molecules24040664

Zhang Y-J, Li E, Wang C, Li Y, Liu X (2012) Ophiocordyceps sinensis, the flagship fungus of China: terminology, life strategy and ecology. Mycology 3:2–10. https://doi.org/10.1080/21501203.2011.654354

Zhao H, Lovett B, Fang W (2016) Genetically engineering entomopathogenic fungi. In: Lovett B, Leger RJT (eds) Advances in genetics, vol 94. Elsevier, Amsterdam, pp 137–163