Efficacy of Entomopathogenic Fungi as Green Pesticides: Current and Future Prospects

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Abstract

The growing commercialization all over the world has led to a boost in the widespread use of chemical pesticides for crop protection in agricultural fields. It has not only contributed to an increase in food production, but its toxic and non-biodegradable character has also resulted in adverse effects on environment and nontarget organisms. Moreover, most of the pests have developed resistance against them. These drawbacks of conventional pesticides have led to an increase in the need for the search of some novel, non-harmful, eco-friendly pesticides. Natural pest control materials commonly known as biocontrol agents are the most promising of them. Biocontrol agents include macroorganisms as well as microorganisms. The microorganisms used are bacteria, fungi, viruses, nematodes and protozoan. The exploitation of these natural and renewable resources is essential for a successful biocontrol strategy. The present review focuses on the use of fungi as potential biocontrol agent for insect pest management. Different fungal formulations and metabolites that have been successfully implemented for pest control and some of the recent patents in this field are also discussed here.

Keywords

Entomopathogenic Fungi • Biocontrol • Pesticide • Insect pest • Patents • Pathogenicity • Green pesticides

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D.G. Panpatte et al. (eds.), *Microorganisms for Green Revolution*,
Microorganisms for Sustainability 6, https://doi.org/10.1007/978-981-10-6241-4_17

17.1 Introduction

Majority of agricultural products are destroyed by plant pests, thus making them the most important biotic agents (Heydari and Mohammad 2010). Insect pests are most harmful of all the pests, since they cause about 42% of the total crop damage (Oerke and Dehne 2004). A variety of diseases are caused by these pests which are generally controlled by chemical pesticides (Cook 1993). But the repeated use of these chemical pesticides for the control of insect pests has caused various hazardous effects on the environment, animals, humans and other nontarget organisms over many years (Mahr et al. 2001). It is estimated that the total usage of chemical pesticides for agriculture is about 2.5 million tons per annum resulting in a loss of about $100 billion annually (Koul et al. 2008). The main reasons for the undesirable effects of chemical pesticides include their toxic and non-biodegradable character and increasing resistance among insects towards them (French-Constant et al. 2004). As a consequence of these drawbacks associated with the use of chemical pesticides, there is a growing interest among the agriculturist to search for some novel and eco-friendly strategies for pest control. A considerable number of effective pest control methods have been developed and are presently in use (Cook 1993; Benhamon 2004; Islam et al. 2005; Heydari 2007). Use of biological control agents is the most attractive and nonhazardous alternative method for insect pest management (Nicholson 2007). Therefore, realizing the need and importance of biocontrol methods, the current chapter has been written on the use of entomopathogenic fungi as biocontrol agent for insect pest management and some recent patents on the same.

17.1.1 What Is Biological Control?

It is a method in which the pest population is regulated by the use of natural enemies against them, thus reducing the damage caused by them. It is defined as "The action of parasites, predators and pathogen in maintaining another organism's density at a lower average than would occur in their absence" (De Bach 1964). These natural enemies are called as biological control agents (BCAs). They can also be referred to as green pesticides since they reduce the pest population and increase food production in a safe and eco-friendly way (Koul et al. 2008). The natural enemies used for biocontrol of insect pests are divided into two main classes (Kibata 1996,) namely, the microbials and the macrobials. The former class includes microorganisms such as viruses, bacteria, fungi, nematodes, protozoa and rickettsia, while the latter includes macroorganisms such as parasitoids, predators, invertebrates and vertebrates (birds and mammals). Microbial biocontrol agents are more efficient than others since they have complex mode of action as a result of which insect pest does not easily develop resistance against them (Khan et al. 2012). Major microbial biocontrol agents being used include viruses, bacteria, nematodes and fungi.

17.2 Myco-Biocontrol: Fungi as Biocontrol Agent

Myco-biocontrol is the process of controlling insect population by using fungi with the aim of reducing infestation and consequently crop damage caused by them (Chet et al. 1993). It is an eco-friendly and efficient means of reducing insect pests. There is an increasing interest in exploiting the use of fungi as biopesticides from various fungal taxonomic groups to control agricultural pests, because of their diversity, easy engineering and delivery techniques, variety of intracellular as well as extracellular toxic metabolites, etc. (Butt et al. 2001; St Leger and Wang 2009). Besides these their broad spectrum nature in terms of disease control and yield makes them widely accepted biological control agents (Pandya and Saraf 2010). The biodiversity of fungi is enormous including 1.5 million species out of which 70,000 species are known (Zain et al. 2013). The use of fungus as microbial control agents is experimentally being tested by several researchers since late nineteenth century (Lacey et al. 2001). Their complex metabolic pathways, large amount of secreted enzymes and secondary metabolites have been exploited since many years for preparation of various natural products (Moore et al. 2011; Hawksworth 2001; Turner 2000). The bioactivity of the fungal secondary metabolites and genes responsible for their synthesis has drawn attention of microbiologists and pharmacologists towards them (Yu and Keller 2005; Zain et al. 2009; Awaad et al. 2012). Insecticidal activity of fungal secondary metabolites has been studied by a number of researchers. Recently insecticidal activity of five fungal strains *Acremonium cephalosporium*, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viridae* and *Verticillum albo-atrum* was tested against house fly, *Musca domestica* (Al-Olayan 2013). The highest percentage of mortality to adults of house flies, 97.3 ± 3.52, was produced by 10^7 conidia ml^{-1} of *A. niger* with LT_{50} 3.49. While the lowest mortality percentage, 59.1 ± 2.38 with LT_{50} 6.91, was produced by 10^5 conidia ml^{-1} of *V. albo-atrum.* Fungi showing insecticidal activity mostly belong to *Hyphomycetes* group. *Beauveria bassiana* is most prominent member of the group and is used for preparation of various commercially available biopesticides such as Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G) and Naturalis-L (Troy BioSciences, Inc.) (Jim McNeil 2011).

17.2.1 Entomopathogenic Fungi as Biocontrol Agent

The term entomopathogenic fungi refer to fungi which induce disease symptoms in host insect. This domain does include the range of fungi from quick killers to absolute parasites that cause disease symptoms in the host and benefit at the host expense but does not diminish host's life span. Out of 700 species of fungi, about 90 genera are entomopathogenic (Khachatourians and Sohail 2008). Because of their wide range activity against a variety of sap sucking as well as chewing insect pests, entomopathogenic fungi are the first choice of fungal biocontrol agents (Butt 2002; Qazi and Khachatourians 2005; Fan et al. 2007; De Faria and Wraight 2007). Since these fungi are biological agents and do not produce any harmful effects on the environment, i.e. they are eco-friendly in nature, they could be referred to as green pesticides. Entomopathogenic fungi such as *Verticillium lecanii*, *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces sp., Acremonium* sp. and *Fusarium* sp. are strongest natural enemies of insect pests and hence are most commonly used mycobiocotrol agents (Sandhu 1993; Roberts and St. Leger 2004; Wang et al. 2004; Thomas and Read 2007; Li and Sheng 2007). The bioactivities of these entomopathogenic fungi have been experimentally tested since many years. The activity of *Metarhizium anisopliae* against *Eutectona machaeralis* larva, a serious pest of teak, was analysed (Sandhu et al. 2000). Maximum mortality ca. 97.5 and 95% occurred in I and II instar larvae with LT_{50} of 72 h and 96 h, respectively. The larval mortality was rapid with the higher conidial concentrations. Similarly, *Nomuraea rileyi* caused 90% mortality in second instar larvae of *Spilosoma obliqua*, a cosmopolitan polyphagous pest damaging different cereals, fibres, pulses oilseeds, vegetables and ornamental plants in various parts of India. The mortality was caused by 8.97×10^7 conidia/ml with LT_{50} as 144 h (Mathew et al. 1998). In another study the honey bee-mediated delivery of *Metarhizium anisopliae* increased pollen beetle control (*Meligethes* spp.) in oilseed rape (Butt et al. 1998). Recently, activity of the entomopathogenic fungus, *Paecilomyces lilacinus* was evaluated against the adults of melon flies (*Bactrocera cucurbitae*). After spraying four different spore concentrations of the fungus, highest percentage mortality in adult flies was recorded at a spore concentration of 2.4×10^9 spores ml^{-1} (Amala et al. 2013). The high virulence and epizootic efficiency of entomopathogenic fungi towards insect pests (Agarwal et al. 1990), high sporulation rate and ability to adapt to changing environmental conditions make them more beneficial organisms for biopesticide production (Sharififard et al. 2011; Mwamburi et al. 2010; Lecouna et al. 2005; Kaufman et al. 2005). In another study the extraction conditions of bioactive metabolite from *Cordyceps militaris* 3936 were optimized (Tuli et al. 2014a, b). *Cordyceps militaris* is an entomopathogenic fungus that grows parasitically on lepidopteron larvae and insect pupae. The secondary metabolite of this fungus contains a novel bio-metabolite called cordycepin which has numerous pharmacological and therapeutic potentials. Some of the commonly developed mycoinsecticides used for control of various insect pests include *Beauveria bassiana* (Balsamo) Vuillemin (Babu et al. 2001; Sharma 2004), *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Alter and Vandenberg 2000; Avery et al. 2004) and *Verticillium lecanii* (Zimm.) Viegas (Butt et al. 2001). About 95% of migratory alate aphids are infected by *Beauveria bassiana* (Balsamo) Vuillemin (Chen et al. 2008). Some fungi developed for insect pest control are depicted in Table 17.1. These formulations of entomopathogenic fungi for the regulation of different types of insect pests are not only being commercialized but are also being patented by their inventors. For example, a formulation containing strains of *Beauveria bassiana* was prepared for controlling cockroaches, carpenter ants and pharaoh ants and patented (Stimac et al. 1997). Similarly, a composition containing strain of entomopathogenic fungus *Isaria fumosorosea* ccm 8367 (ccefo.011.pfr) for controlling insect and mite pests was developed and patented (Prenerova et al. 2011).

Table 17.1 Mycoinsecticides developed by using some entomopathogenic fungi

Fungus	Product	Target	Producer
Beauveria bassiana	Conidia	Coffee berry borer	Live Systems Technology, Colombia
Beauveria bassiana	Ostrinil	Corn borer	Natural Plant Protection (NPP), France
Beauveria bassiana	Corn Guard	European corn borer	Mycotech, USA
Beauveria bassiana	Mycotrol GH	Grasshoppers, locusts	Mycotech, USA
Beauveria bassiana	Mycotrol WP and BotaniGard	Whitefly, aphids, thrips	Mycotech, USA
Beauveria bassiana	Naturalis-L	Cotton pests including bollworms	Troy Biosciences, USA
Beauveria bassiana	Naturalis	White flies, thrips, white grubs	Troy Biosciences, US
Beauveria bassiana	Proecol	Army worm	Probioagro, Venezuela
Beauveria bassiana	Boverin	Colorado beetle	Former USSR
Beauveria bassiana	Bio-power	Mite, coffee green bug	Stanes
Beauveria bassiana	Racer BB	Aphids spittle bug, sugarcane	SOM Phytopharma
Beauveria bassiana	Trichobass-L, Trichobass-P	Aphids spittle bug, sugarcane	AMC Chemical/Trichodex
Beauveria brongniartii	Engerlingspilz	Cockchafer(s)	Andermatt, Switzerland
Beauveria brongniartii	Schweizer Beauveria	Cockchafer(s)	Eric Schweizer, Switzerland
Hirsutella thompsonii	Mycar	Eriophyid mites	Abbott Laboratories, USA
Lagenidium giganteum	Laginex	Mosquito larvae	AgraQuest, USA
Metarhizium anisopliae	BIO 1020	Vine weevil	Licenced to Taensa, USA
Metarhizium anisopliae	Biogreen	Scarab larvae on pasture	Bio-care Technology, Australia
Metarhizium anisopliae	Metaquino	Spittle bugs	Brazil
Metarhizium anisopliae	Bio-Blast	Termites	EcoScience, USA
Metarhizium anisopliae	Cobican	Sugarcane spittle bug	Probioagro, Venezuela
Metarhizium anisopliae	Biologic	Black vine weevil	Bayer AG, Germany

(continued)

Table 17.1 (continued)

Fungus	Product	Target	Producer
Metarhizium flavoviride	Green Muscle	Locusts, grasshoppers	CABI BioScience, UK
Paecilomyces fumosoroseus	PFR-97	Whitefly	ECO-tek, USA
Paecilomyces fumosoroseus	PFR-21	Whitefly	W.R. Grace, USA
Paecilomyces fumosoroseus	Pae-Sin	Whitefly	Agrobionsa, Mexico
Verticillium lecanii	Mycotal	Whitefly and thrips	Koppert, the Netherlands
Verticillium lecanii	Vertalec	Aphids	Koppert, the Netherlands

Khachatourians (1986), Burges (1998), Butt and Copping (2000), Butt et al. (1999, 2001), Whright et al. (2001), Bhattacharyya et al. (2004), Copping (2004), Zimmermann (2007) and Khan et al. (2012)

17.2.2 Some Potential Candidates of Entomopathogenic Fungi

17.2.2.1 *Beauveria bassiana*

It is a ubiquitous, filamentous, soil-borne fungus possessing high host specificity. This is the most promising candidate of entomopathogenic fungi having a broad range of host such as termites, whitefly, malaria-transmitting mosquitoes, scarabs, weevil, etc. (Sandhu et al. 2012). It causes white muscardine disease of insects and has been developed as microbial insecticide against many major insects like lepidopterans, orthopterans, coleopterans, etc. (Mustafa and Kaur 2009). It produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short and ovoid and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium is produced, resulting in a long zigzag extension. The conidia are single-celled, haploid and hydrophobic (Fig. 17.1a). Spores produced by this fungus are resistant to extreme environmental conditions. It causes infection by attaching to the cuticle of the host insect and then germinating upon arrival of favourable conditions. The hypha arising from spore then penetrates the cuticle by secreting cuticle degrading enzymes and grows inside the insect body (Baskar and Ignacimuthu 2011). Thereafter, it suppresses the host immune system by producing a toxin called beauvericin. In a recent study, strain GHA of *B. bassiana* was found to be more potent against a wood boring insect *Xyleborus glabratus* (Carrillo et al. 2015) than two strains of *Isaria fumosorosea* (Ifr 3581 and PFR). Similarly, higher mortality rate was observed in *Polyphylla fullo* larvae infected with *B. bassiana* formulation (Erler and Ates 2015) as compared to those infected with formulations containing *Metarhizium anisopliae.* These studies indicate about the better insecticidal efficiency of *B. bassiana* over other entomopathogenic fungi.

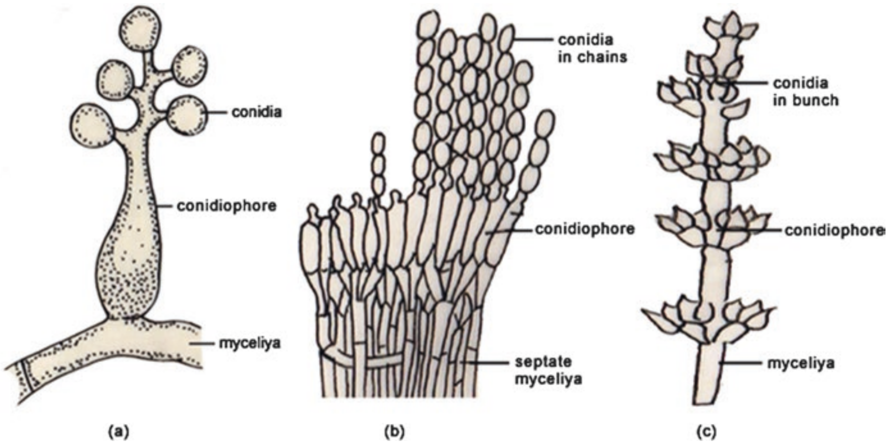

Fig. 17.1 Conidiophore with conidia: (**a**) *Beauveria bassiana* (**b**) *Metarhizium anisopliae* (**c**) *Nomuraea rileyi*

17.2.2.2 *Metarhizium Anisopliae*

It occurs naturally in soil and infects about 200 species of insects. It produces green cylindrical spores in chains from infected insects hence is the causative agent of "green muscardine" disease of insects (Cheraghi et al. 2013). The conidiophores are of variable length, penicillicate, in candle- or palisade-like arrangement, apically forming a sporulation layer, often aggregating into sporodochia (Fig. 17.1b). Its conidia are in long chains, often aggregated into prismatic columns, broadly ellipsoidal to cylindrical (Tzean et al. 1997). Like *B. bassiana* it also has a wide host range and infects some beneficial insects, for example, lady beetles and the teak pest *Eutectona machaeralis* (Sandhu et al. 2012).

17.2.2.3 *Nomuraea Rileyi*

It is yet another important entomopathogenic insect. It attacks mostly the larvae of rice insect. Other host insects of this fungus are leaffolder, stem border larva, green hairy caterpillar, army worm and caseworm (Rombach et al. 1994). It is composed of pale green to grey green conidiophores on a white basal felt of mycelium. The conidia are broadly ellipsoid and in dry chains (Padanad and Krishnaraj 2009). They are 3.5–4.5 × 2–3 μm long (Fig. 17.1c). The conidiophores have branches. Each branch contains 2–5 phialides or conidial chains (Humber 1997).

17.2.3 Beneficial Properties of Entomopathogenic Fungi

Some properties of entomopathogenic fungi which make them beneficial as compared to others (Sandhu et al. 2012) are:

(a) They are specific for particular insect species and do not infect other animals or plants.

(b) They have considerable epizootic potential and can spread quickly through an insect population and cause their collapse.
(c) They penetrate the insect body and infect sucking insects such as aphids and whiteflies that are not susceptible to bacteria and viruses.

17.3 Large-Scale Production of Fungi for Commercialization

The commercial use of entomopathogenic fungi for microbial control of insect pests require understanding of physiological aspect of growth, metabolic activity, genetic basis of virulence and host specificity. Several techniques for the mass production of entomopathogenic fungi are available, mostly designed to yield infective conidia; the conidia are harvested and formulated for storage and field use. Solid-state and liquid-state fermentation has gained significant importance in recent years for the same. Production of fungal conidiospores on large-scale trough is done by solid-state fermentation (Desgranges et al. 1993). Spore of *Metarhizium anisopliae* (ENT-12) has been produced on large scale by solid-state fermentation (Hasan et al. 2002). One of the major advantages of solid-state fermentation is the use of cheaper, easily available, agricultural-based and biodegradable substrate. Recently conidia of *Beauveria bassiana* Bb-202 were produced on rice by solid-state fermentation for the control of coleopteran pests (Xie et al. 2013). On the other hand, liquid-state fermentation is also beneficial in which mass production is carried out under controlled conditions. It has been successfully used for mass production of *Paecilomyces fumosoroseus* (Lozano-Contreras et al. 2007).

17.4 Mode of Action of Entomopathogenic Fungi

The different steps of fungal infection process are influenced by various intrinsic (fungal) and extrinsic (host, environmental) factors. These steps can be summarized as follows (Fig. 17.2):

17.4.1 Adhesion of Spore to the Host Cuticle

Adhesion is the most important prerequisite to infection. It involves the chemical and physical interactions of the insect epicuticle and the spore. For example, the airborne spores of some entomopathogenic fungi make contact where they land on insect surface, whereas zygospores of *Coelomycetes* locate their host by chemotaxis. Adhesion is normally achieved through secretion of cuticle degrading enzymes with the mucilage which interacts with and modifies epicuticular waxes. It also helps in host recognition and acts as cementing substance for pathogen and its substratum.

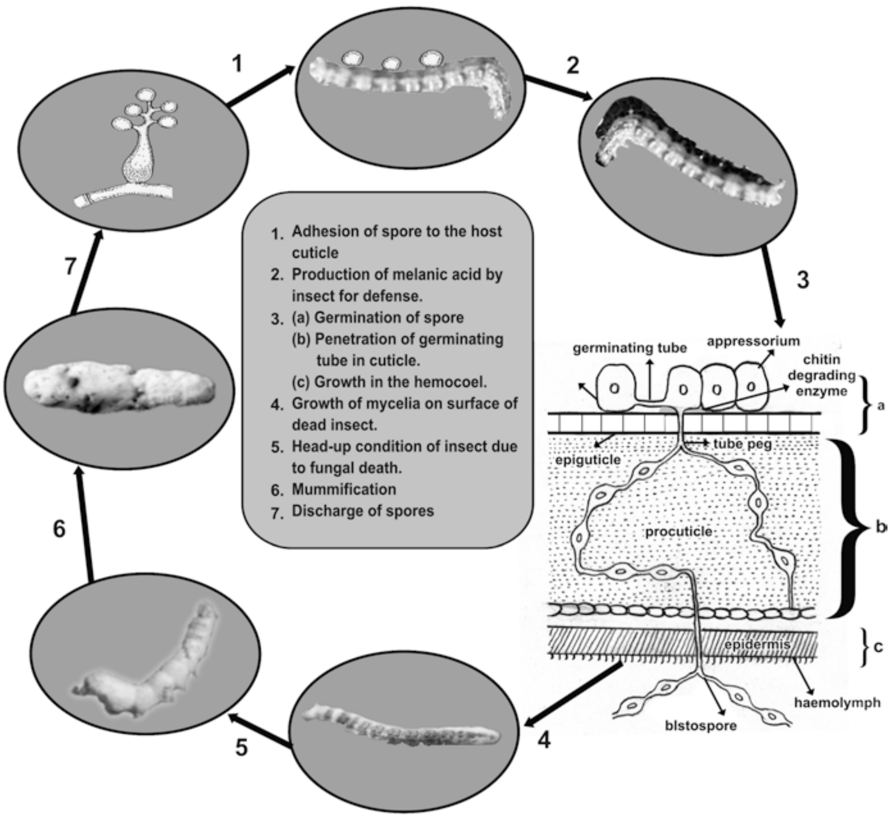

Fig. 17.2 Mechanism of action of entomopathogenic fungi

17.4.2 Defence Mechanism in the Host

Insect has several defence mechanisms which prevent the penetration and growth of the entomopathogenic fungus. One of the most common mechanisms of them is melanisation of the cuticle at the infection site. But it is initiated very late and thus is not able to stop the penetrating hyphae quickly (St. Leger et al. 1991).

17.4.3 Germination of Spore

A number of compounds have been found on the cuticle, which stimulate or inhibit germination (Latge et al. 1987). Nutrients accelerate germination, growth and development (Hassan et al. 1989). Fatty acids have a profound effect on the spore germination and differentiation either being toxic, fungistatic or stimulatory (Sandhu 1995).

17.4.4 Penetration of Cuticle

After adhesion, pathogenic fungi penetrate into the insect, the exact mechanism of which varies from species to species. A range of cuticle degrading enzymes are produced during penetration. Three most important classes of such enzymes are lipases, proteases and chitinases, which degrade the epicuticular waxes, followed by protein-chitin matrix (Smith et al. 1981). Besides this trypsin, chymotrypsin, elastases, collagenases and chymoelastases also play a role in penetration process (St. Leger et al. 1988; Bidochka and Khachatourians 1988).

17.4.5 Growth in the Haemocoel

Following penetration the fungus retaliates by rapid reproduction and tries to overcome the immune response in the haemocoel of insect body by various mechanisms:

(a) Formation of separate hyphal bodies by hyphal fission which are not as antigenic as hyphae (Pendland and Boucias 1986)
(b) Production of toxins by some members of *Deuteromycetes* such as *Beauveria* and *Metarhizium* (Roberts 1996)
(c) Development of wall less protoplast by *Coelomycetes* and members of the *Entomophthorales* in the haemocoel which are unrecognizable by the host (Sandhu 1993)

17.4.6 Death and Saprophytic Feeding

The toxin producers are quick killers and consequently secrete antibiotics so that they can continue their feeding saprobicly. Eventually all organs of the insect are consumed and replaced with hyphae. On the other hand, *Entomophthorales* are almost parasitic. The symptoms at the later stages of mycosis include physiological symptoms such as convulsions, lack of coordination, assuming lofty positions and outstretching of infected wings. These behavioural alterations are followed by death of the insect.

17.4.7 Hyphal Re-emergence and Sporulation

Upon favourable conditions hyphae repenetrate the cuticle and produce conidiophores on the outside of the insect producing spores both inside and outside of the cadaver (Sandhu 1995).

17.4.8 Mummification

After the death of insects infected with entomopathogenic fungi, fungal outgrowth from the insect body and coincidently the production and dispersal of spores to new host and environment occur (Hajek and Soper 1992).

17.5 Secondary Metabolites of Entomopathogenic Fungi as Potent Insecticidal Agent

Secondary metabolites are organic compounds which do not play a direct role in the growth and metabolism of organisms (Andersson 2012). Entomopathogenic fungi have been investigated as a source of a wide range of secondary metabolites possessing immense bioactivities against a broad range of insect pests. Diverse toxic metabolites have been described which display insecticidal properties against insect pests (Khan et al. 2012). Destruxins (A&B) produced by *Metarhizium anisopliae,* beauvercins, beauverolides, destruxins (dtx), bassianolides, bassianin and oosporein produced by *Beauveria bassiana* and *Nomuraea rileyi* are some examples of insecticidal metabolites of entomopathogenic fungi (Kodaira 1961). Table 17.2 below illustrates some commonly used insecticidal secondary metabolites of entomopathogenic fungi. Besides these compounds several extracellular enzymes produced by entomopathogenic fungi also play a significant role in their pathogenicity. Some important enzymes are chitinase, protease and lipase. Production of these enzymes has been reported in entomopathogenic fungi like *B. bassiana, Nomuraea rileyi* and *M. anisopliae* (Ali et al. 2011). Thus, special attention has been focussed on the isolation and purification of such enzymes from their respective entomopathogenic fungal strains and their use in biopesticide formulations. A few of these formulations have also been patented by their inventors. For example, an enzyme preparation comprising at least one protease derived from *Metarhizium*, *Beauveria*, *Verticillium* and *Aschersonia* was formulated and patented (US4987077) (Charnley et al. 1991). Similarly, a technology of controlling insect pest prepared with chitinolytic enzymes were patented (US6069299) (Broadway et al. 2000). Another invention which got patented involved an innovative combination of dormant spore of naturally occurring *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* fungus with enzymes, fats and growth-promoting molecules for controlling various foliage pest and soil-borne insect (WO2011099022 A1) (Patel 2011). Similarly, a combination of biopesticide and at least one exogenous cuticle degrading enzymes (e.g., a protease, chitinase, lipase and/or cutinase) was patented (US 20130156740A) (Leland 2013).

Table 17.2 List of metabolites having insecticidal activity produced by entomopathogenic fungi

S. No.	Fungi	Metabolite	Target Insect	Reference
1.	*Metarhizium anisopliae*	Destruxins (A & B)	*Spodoptera litura* (leafworm moth)	Kodaira (1961) and Hu et al. (2007)
2.	*Beauveria Bassiana* and *Nomuraea rileyi*	Beauvercins, beauverolides, destruxins (dtx), bassianolides, bassianin and oosporein	Various insects	Strasser et al. (2000)
3.	*Beauveria bassiana* and other species	Beauvericin (type A and B)	Various insects	Gupta et al. (1995)
		Bassianolide	Silkworm larva	Suzuki et al. (1977)
		Beauverolides	Unknown	Namatame et al. (1999)
		Bassianin, tenellin	Unknown	Mochizuki et al. (1993) and Jeffs and Khachatourians (1997)
4.	*Beauveria* spp. and other soil fungi	Oosporein (dibenzuoquinone)	Various insects	Eyal et al. (1994) and Wilson (1971)
5.	*Tolypocladium cylindrosporum*	Linear peptidic efrapeptins (types C to G)	Mites, beetle, budworm, moth	Weiser and Matha (1988) and Bandani et al. (2000)
6.	*Verticillium lecanii*	Vertilecanin A, B and C and their methyl ester	*Helicoverpa zea* (Corn earworm)	Soman et al. (2001)
7.	*Hirsutella thompsonii*	Hirsutellin A	*Galleria mellonella* (Wax-moth larvae)	Liu et al. (1995)
8.	Unidentified fungus (HF1)		*Oligonychus coffeae* (Tea Red Spider Mites)	Amarasena et al. (2011)
9.	*Hypocrella raciborskii* Zimm.	Ergosterol, dustanin (15α, 22 dihydroxyhopane) and 3β-acetoxy-15α,22-dihydroxyhopane	Spider Mite (*Tetranychus urticae* Koch)	Buttachon et al. (2013)

Hu et al. (2007), Strasser et al. (2000), Gupta et al. (1995), Suzuki et al. (1977), Namatame et al. (1999), Mochizuki et al. (1993), Jeffs and Khachatourians (1997), Eyal et al. (1994), Wilson (1971), Weiser and Matha (1988), Bandani et al. (2000), Soman et al. (2001), Liu et al. (1995), Amarasena et al. (2011), Buttachon et al. (2013)

17.6 Role of Biotechnology in Pest Management by Entomopathogenic Fungi

Recent developments in the field of genetic engineering provides new opportunities for the isolation of genes encoding pathogenicity and virulence and identification of markers for characterizing genome, thus allowing the genetic variation for strain improvement of entomopathogenic fungal population. The successful application of gene cloning technology to fungi of industrial and agricultural importance could be done by:

17.6.1 Development of an Efficient Transformation System

Nitrate reductase gene *(niaD)* of *Aspergillus niger* has been used for development of heterologous transformation system for entomopathogenic fungi *Beauveria bassiana* (Sandhu et al. 2001). Likewise, a heterologous transformation system for entomopathogenic fungus *Metarhizium anisopliae* was developed using the *cnx*-gene (cofactor for nitrate and xanthine dehydrogenase) of *Aspergillus nidulans* (Thakur and Sandhu 2003).

17.6.2 Protoplast Fusion Technique

It is yet another technique of biotechnology for production of potent entomopathogenic hybrid fungal strain. An intergeneric protoplast fusion of *Tolypocladium inflatum* and *Beauveria bassiana* was successfully accomplished to develop industrially as well as agriculturally important strain (Silawat et al. 2002).

17.6.3 Genetic Manipulation to Increase the Efficacy

Molecular biological studies on entomopathogenic fungi infection process have revealed that several genes are involved in the pathogenicity (Cho et al. 2007). Overexpression of such genes has resulted in the enhanced virulence of entomopathogenic fungal strains. Examples of some of these genes are subtilisin protease *PR1A* (St. Leger et al. 1996), subtilisin protease *PII* gene (Ahman et al. 2002) and chitinase gene *Bbchit1* (Fang et al. 2005). Besides these overexpression of genes for guanine nucleotide-binding proteins and its regulator (Fang et al. 2007, 2008), adhesin which helps in attachment of spore (Wang and St. Leger 2007), a perilipin-like protein that regulates appressorium turgor pressure and differentiation (Wang and St. Leger 2007) and a cell-protective coat protein helping in escaping the pathogen from the host immunity recognition have also increased the potency and ecological fitness of the engineered entomopathogenic fungal strains.

17.6.4 Development of Molecular Markers

Molecular markers are important tools for the identification and monitoring of specific fungal strains. Recently five microsatellite markers (Simple sequence repeats SSRs) were developed to monitor a commercialized isolate of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. in complex environmental samples such as bulk soil or plant DNA. Discriminatory power of these SSR markers was assessed in two commercialized *B. bassiana* isolates as well as in 16 *B. bassiana* isolates from a worldwide collection, and three of the five SSR markers were estimated to allow a confident discrimination among the given isolates (Reineke et al. 2014).

17.6.5 Development of Biochemical Markers

Such markers could be used for screening virulent strains of entomopathogenic fungi and then selecting the most promising candidates for biocontrol. In a recent study, the subtilisin-like protease *Pr1* activity of five *Metarhizium anisopliae* s.l. isolates was used as a biochemical tool to evaluate their virulence against *Rhipicephalus microplus* females. The isolates CG 629, CG 148 and CG 32 having higher protease activity showed higher virulence against *R. microplus* as compared to the isolates CG 112 or CG 347 with lower protease activity (Perinotto et al. 2014).

17.7 Recent Patents on Mycobiocontrol

With the increasing demand of new and eco-friendly biocontrol methods, several researchers have developed and patented their novel biocontrol strategies. Some of these employing the use of entomopathogenic fungi as biocontrol agents are listed in the Table 17.3.

17.8 Conclusion

Crop protection has relied mostly on synthetic chemical pesticides over many years. But their use is now declining owing to a number of factors like serious health problems caused due to their application, development of heritable resistance in pests and withdrawal of pesticide products by new health and safety legislation. Over 500 arthropod species now show resistance to one or more types of chemicals (Mota-Sanchez et al. 2002). This has forced the researchers to seek for some new pest control agents. Biological control agents have emerged as eco-friendly option for the management of insect pests. Numerous microbial candidates have been developed into biocontrol agents, but very few of these have been successful and persisted in the market place. This chapter clearly reveals the increasingly important role of entomopathogenic fungal biological control agents in the management of insect pest. The use of these agents will contribute in significant reduction of chemical pesticides usage in

Table 17.3 List of patents on use of entomopathogenic fungi for biocontrol of insect pest

Patent No.	Country	Inventor	Issue Date	Title
US2927060	United States	Oringer K	01 Mar. 1960	Refining of proteolytic enzymes
US3657414	United States	Paul et al.	18 Apr. 1972	Formulation of a boll weevil feeding stimulant mixture
US4027420	United States	McKibben et al.	07 Jun. 1977	Air dropped bait dispensers for attracting and killing the cotton boll weevil
US4293552	United States	Miesel JL	06 Oct. 1981	Novel 1-(mono-o-substituted benzoyl)-3-(substituted pyrazinyl) ureas
US4337271	United States	Jacobson M	29 Jun. 1982	Erythro-9,10-Dihydroxyoctadecan-1-ol acetate a boll weevil antifeedant
US4348385	United States	Synek J	07 Sep. 1982	Flowable pesticides
US4751082	United States	Schaerffenberg et al.	14 Jun. 1988	Insecticide and method for its distribution
US4797276	United States	Herrnstadt et al.	10 Jun. 1989	Control of cotton boll weevil, alfalfa weevil and corn rootworm via contact with a strain of *Bacillus thuringiensis*
US4908977	United States	Foster JP	20 Mar. 1990	Device for killing arthropods
US4925663	United States	Stimac JL	15 May 1990	Biological control of imported fire ants with a fungal pathogen
US4942030	United States	Osborne LS	17 Jul. 1990	Biological control of whiteflies and other pests with a fungal pathogen
US4987077	United States	Charnley et al.	22 Jan. 1991	Preparations of protease enzymes derived from entomopathogenic fungi
US5057316	United States	Gunner et al.	15 Oct. 1991	Method and device for the biological control of insects
US005360607A	United States	Eyal et al.	01 Nov. 1994	Method for production and use of pathogenic fungal preparation for pest control
US005413784A	United States	Wright et al.	09 May 1995	Biopesticide composition and process for controlling insect pests
US5516513	United States	Wright JC	14 May 1996	Biological ovicide for control of lepidopterous insects

(continued)

Table 17.3 (continued)

Patent No.	Country	Inventor	Issue Date	Title
EP0738317 A1	European	Clifford et al.	23 Oct. 1996	Formulations of entomopathogenic fungi for use as biological insecticides
USOO5683689A	United States	Stimac et al.	04 Nov. 1997	Controlling cockroaches, carpenter ants and pharaoh ants using strains of *Beauveria bassiana*
US005730973A	United States	Morales et al.	24 Mar. 1998	Water-dispersible granules of spores or live *Beauveria bassiana*
US006183733B1	United States	McKibben GH	13 Apr. 1999	Mycoinsecticide activity against grasshoppers produced by *Beauveria bassiana*
US005968504A	United States	Tahvonen et al.	19 Oct. 1999	Fungus *Gliocladium catenulatum* for biological control of plant diseases
US006069299A	United States	Broadway et al.	30 May 2000	Fungus and insect control with chitinolytic enzymes
US6254864 B1	United States	Stimac et al.	03 Jul. 2001	Method and formulations for control of pests
US006274157B1	United States	Lai et al.	14 Aug. 2001	Strains of *Streptomyces* and relevant uses thereof
US6280723 B2	United States	Stimac et al.	28 Aug. 2001	Methods and materials for control of termites
US006306386B1	United States	Cole et al.	23 Oct. 2001	Biological control formulations containing spores of nontoxigenic strains of fungi for toxin control of food crops
US006403085B1	United States	Stimac et al.	11 Jun. 2002	Method and formulations for control of pests
US006660290B1	United States	Stamets PE	09 Dec. 2003	Mycopesticides
US007037494B2	United States	Mattingly et al.	02 May 2006	Formulations and methods for insect control
US007402302B2	United States	Plato et al.	22 Jul. 2008	Composition of grandlure and dichlorvos for attracting and killing boll weevils in boll weevil traps
WO2009093261A2	India	Satyasayee et al.	30 Jul. 2009	Formulation of entomopathogenic fungus for use a biopesticide
EP2096926 A2	European	Samantha B	09 Sep. 2009	Use of entomopathogenic fungi as a means for the biological control of *Paysandisia archon*

(continued)

Table 17.3 (continued)

Patent No.	Country	Inventor	Issue Date	Title
EP2313488 A1	European	Prenerova et al.	27 Apr. 2011	Strain of entomopathogenic fungus *Isaria fumosorosea* ccm 8367 (ccefo.011.pfr) and the method for controlling insect and mite pests
US7943160 B2	United States	Borchert et al.	17 May 2011	Pest control methods
WO2011099022A1		Patel CS	18 Aug. 2011	Composition and method of preparation of fungal based bio insecticide from combination of *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* fungus with enzymes, fats and growth-promoting molecules for controlling various foliage pest and soil-borne insect
US008227224 B2	United States	Kalisz et al.	24 Jul. 2012	Method of making moulded part comprising Mycelium coupled to mechanical device
US8226938 B1	United States	Miekle et al.	24 Jul. 2012	Biocontrol of Varroa mites with *Beauveria bassiana*
US20130156740A1	United States	Leland JE	20 Jun. 2013	Biopesticide methods and compositions
US8501207B2	United States	Stamets P	06 Aug. 2013	Mycoattractants and mycopesticides

agriculture, horticulture and forest systems (Lacey and Goettel 1995). The pest control efficacy of these fungi is increased by the secondary metabolites produced by them (Vurro et al. 2001). Different extracellular enzymes are one of the most important secondary metabolites which could be used for the development of mycopesticides. Various entomopathogenic fungal strains have been exploited for their proteolytic enzymes. Recently, in a study a strain of *Verticillium lecanii* was found to be a good source of proteolytic as well as amylolytic and lipolytic enzymes, and their use as mycopesticide was rationally advocated (Hasan et al. 2013). With the development of modern techniques in the field of biotechnology now, it is possible to increase the efficacy of the entomopathogenic fungal strains by manipulating their desired traits. But still the research, development and final commercialization of fungal biological control agents continue to confront a number of obstacles which are needed to be removed for advancements in the field of myco-biocontrol of insect pests.

Acknowledgment The authors of this review would like to thank the Vice-Chancellor, R.D. University Jabalpur for his kind support and help. We would also like to thank the Head, Department of Biological Sciences, R.D. University, Jabalpur, India, for technical and linguistic assistance.

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