Role of Entomopathogenic Fungus in Pest Management

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Abstract

Insect pests cause enormous damage to our crops. However, insects like other living things have their enemies in nature. Microorganisms like fungi, bacteria, and viruses also play a vital role in checking the insect population. Among which fungi parasitize the insects and cause severe epizootics than bacteria and viruses, and it is distributed worldwide. During the late nineteenth and early twentieth centuries, many entomopathogenic fungi were examined as possible control agents for various insect pests. Entomopathogenic fungi belong to 750 species that cause infections in insects or mites. Fungus is very specific and belongs to the superkingdom Eukaryota. During the recent years, there has been a resurgence of interest in entomopathogenic fungi caused by factors such as increasing insecticide resistance and environmental concerns over pesticide use. This has led to the isolation and identification of native isolates of fungi from a wide range of hosts. Many entomopathogenic fungi are relatively common and often induce epizootics and are therefore an important factor in regulating insect populations. The research has to be focused in mass multiplication and formulations of fungi using locally available cheap and cost-effective materials.

Keywords

Entomopathogenic fungus • Distribution and diversity • Pest management

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3.1 Introduction

Insect pests cause enormous damage to our crops. There are a majority of insects like beetles, aphids, hoppers, scale insects, white flies, thrips, plant bugs, moths, borers, etc., that are inimical when they reach a high population level. However, insects like other living things have their

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enemies in nature. Microorganisms like fungi, bacteria, and viruses also play a vital role in checking the insect's population. Among which fungi parasitize the insects and cause severe epizootics than bacteria and viruses. During the late nineteenth and early twentieth centuries, many entomopathogenic fungi were examined as possible control agents for various insect pests. During the recent years, there has been a resurgence of interest in entomopathogenic fungi caused by factors such as increasing insecticide resistance and environmental concerns over pesticide use. This has led to the isolation and identification of native isolates of fungi from a wide range of hosts. Increasing awareness about abuse of chemical pesticides had stimulated renewed interest in the development of alternative and environmentally compatible materials for insect pest control. In this regard, entomopathogenic fungi have been considered as one of the components of IPM. Before the advent of the "Green Revolution", our farmers largely relied on organic manure and cultural method of pest management, which were helpful in promoting biocontrol agents. Diversified fungal species occur on insect pests in different ecosystem, thus maintaining a biotic tolerance to keep the pest population below economic injury level.

Fungi have been suggested as agents for the biological control of insects for over a century, but their use remains extremely limited. Under natural conditions, fungi are a frequent and often important natural mortality factor in insect population. Some species are facultative generalist pathogens, such as *Aspergillus* and *Fusarium*. However, most species are obligate pathogens; they are quite specific. These fungi often have a wide host range although there is considerable genetic diversity within species (Driver et al. 2000). Many entomopathogenic fungi are relatively common and often induce epizootics and are therefore an important factor in regulating insect populations. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their hosts but invade directly through the cuticle so that they can be effectively used for the control of all the insects including sucking insects.

Over 100 different fungal genera have been shown to parasitize living insects (Roberts and Yendol 1971). Both saprotrophs and primary pathogens have been isolated from dead or diseased insects in most ecological niches. They tend to be more common in tropical areas where temperature and humidity favour their growth, but they are also important in the regulation of insect number in temperate areas. Fungal pathogens flourish abundantly in the rice ecosystem with its prevailing high humidity and infection occurs naturally (Narayanasamy 1994). Epizootics of fungus *Fusarium pallidoroseum* (Cooke) Sacc. (Manisegarane and Letchoumanane 1996) and *Beauveria bassiana* (Bals.) Vuill. (Ambethgar 1996) were noticed at Karaikal, India. Epizootics caused by naturally occurring viral and fungal pathogens are often responsible for spectacular crashes of insect pest populations (Evans 1986). Introduction of fungal pathogens into the host population initiates epizootics and prevents or reduces damage by the pest. The initiation of artificial epizootics has been accomplished for long-term control especially in areas where high humidity condition prevails (Sandhu et al. 2012). To date, the use of entomopathogenic fungi has been considered as one of the potential tools in biological control. This chapter outlines the current state of knowledge of insect fungal pathogens as it relates to their present use and future potential as mycoinsecticides.

3.2 Distribution and Pathogenicity of Entomopathogenic Fungi

The entomopathogenic fungi include taxa from several of the main fungal groups. Many common and/or important entomopathogenic fungi are in the order Hypocreales of the Ascomycota: the asexual (anamorpha) phases (e.g. *Beauveria*, *Metarhizium, Nomuraea*, *Paecilomyces* = *Isaria*, *Hirsutella*) and the sexual (teleomorph) state (e.g. *Cordyceps*, *Entomophthora*, *Zoophthora*, *Pandora*, *Entomophaga*) which belong in the order Entomophthorales. Entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems. Furthermore,

entomopathogenic fungi are distributed in a wide range of habitats including aquatic forest, agricultural, pasture, desert, and urban habitats. It seems they are not only in places where there are no victims – insects nor other arthropods. Of course, the spread of individual species of entomopathogenic fungi is different. However, some of them can be found practically throughout the world. The effects of factors such as geographical location, climatic conditions, habitat type, cropping system, and soil properties, as well as the effects of biotic factors on the occurrence and distribution of entomopathogenic fungi, have been broadly studied elsewhere by many microbiologists.

Entomopathogenic fungi have been also recorded north of the Arctic Circle. They have been *Tolypocladium cylindrosporum*, *B. bassiana*, and *Metarhizium anisopliae* in Norway (Klingen et al. 2002) and *B. bassiana*, *M. anisopliae*, and *Isaria farinosa* (=*Paecilomyces farinosus*) in Finland (Vänninen 1995). In addition, entomopathogenic fungi have been reported also from Arctic Greenland (Eilenberg et al. 2007) and Antarctica. In the latter location including endemic Antarctic species, *Paecilomyces antarctica* isolated from the Antarctic springtail *Cryptopygus antarcticus* in the peninsular Antarctic (Bridge et al. 2005). It is associated in variety of habitats. India is bestowed with a rich biodiversity of entomopathogens, and the exploitation of these natural and renewable resources is essential in a successful biocontrol strategy. Performance of a bait matrix treated with the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin, Strain Saravan (DEMI 001), against termite *Microcerotermes diversus* Silvestri was evaluated. The highest rate of mortality occurred at concentrations of 3.7×10^7 and 3.5×10^8 conidia per mL (Cheraghi et al. 2013).

Entomogenous fungi are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics. These fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. The most important fungal pathogens are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi, Verticillium lecanii*, and *Hirsutella* spp. (Table 3.1).

Table 3.1 Occurrence and pathogenicity of entomopathogenic fungi in different insect hosts in India and various countries

Crop	Common name	Scientific name	Location	Entomopathogenic fungi
Different localities in India				
Rice	Green leafhopper (GLH)	*Nephotettix virescens* Dist.	Ludhiana	*Penicillium* spp., *Alternaria tenuis*, *Curvularia* spp., *Fusarium oxysporum* Schlecht
	Brown plant hopper (BPH)	*Nilaparvata lugens* (Stal.)	Karaikal	*Pandora delphacis* (Hori) Humber
	BPH and GLH	*N. lugens* and *N. virescens*	Annamalai Nagar, Chidambaram	*P. delphacis*
	BPH and WBPH	*N. lugens* and *Sogatella furcifera* (Horv.)	Coimbatore	*Metarhizium anisopliae* (Metsch.) Sor.
	BPH, GLH, and WBPH	*N. lugens, N. virescens* and *S. furcifera*	Ludhiana	*Hirsutella* spp.
	BPH, GLH, and WBPH	*N. lugens*, *N. virescens* and *S. furcifera*	Ludhiana	*M. anisopliae*
	BPH and GLH	*N. lugens* and *N. virescens*	Ludhiana	*Aspergillus flavus* Link, *A. niger, Mucor* spp., *Rhizopus* spp.

(continued)

Table 3.1 (continued)

Crop	Common name	Scientific name	Location	Entomopathogenic fungi
	BPH and GLH	*N. lugens* and *N. virescens*	Ludhiana	*Beauveria bassiana* (Bals.) Vuill.
	Stem borer	*Chilo auricilius* (Dudgn)	Ludhiana	*B. bassiana*
	Yellow rice stem borer, leaf folder, yellow hairy caterpillar, and cutworm	*Scirpophaga incertulas* (Walk.), C*naphalocrocis medinalis* (Guenee), and *Psalis pennatula* (Fab.)	Annamalai Nagar, Chidambaram	*M. hiemalis* Wehmer., *F. moniliforme* Shield, and *Scopulariopsis* spp.
	Leaf folder	*C. medinalis*	Karaikal	*B. bassiana*
	Leaf folder	*C. medinalis*	Karaikal	*Aspergillus* spp.
	Leaf folder	*C. medinalis*	Karaikal	*Fusarium pallidoroseum* (Cooke) Sacc.
	Leaf folder	*C. medinalis*	Karaikal	*Fusarium pallidoroseum* (Cooke) Sacc.
	Leaf folder	*C. medinalis*	Karaikal	*Zoophthora radicans* (Brefeld) Batko
	Leaf folder and stem borer	*C. medinalis* and *S. incertulas*	Amritsar	*B. bassiana*
				A. parasiticus Speare
	Leaf folder	*C. medinalis*	Ludhiana	*B. bassiana*
				Penicillium spp.
				A. niger var. Tieghem
	Leaf folder	*C. medinalis*	Karaikal	*B. bassiana*
	Leaf folder	*C. medinalis*	Karaikal	*F. pallidoroseum*
	Leaf folder	*C. medinalis*	Karaikal	*Z. radicans*
	Leaf folder	*C. medinalis*	Punjab	*Nomuraea* spp.
	Leaf folder	*C. medinalis*	Arunachal Pradesh	*B. velata* Samson and Evans
	Leaf folder and skipper	*C. medinalis* and *Pelopidas mathias* (Fab.)	Ludhiana	*B. bassiana*
	Leaf folder and ear head bug	*C. medinalis* and *Leptocorisa acuta* (Thumb)	Coimbatore	*B. bassiana*
	Skipper	*Marasmia patnalis* Bradley	Karaikal	*B. bassiana*
	Skipper	*P. mathias*	Cuttack	*B. bronbniartii(= tenella)*
	Hispa	*D. armigera* Oliv.	Assam	*B. bassiana*
	Hispa	*D. armigera*	Ludhiana	*B. bassiana*
	Green horned caterpillar	*Melanitis leda ismene* Cramer	Ludhiana	*F. oxysporum*
	Grasshopper	*Oxya nitidula* (Walk.)	Karaikal	*B. bassiana*
	Grasshopper	*O. nitidula*	Karaikal	*Aspergillus* spp.
	Cutworm	*Pseudaletia unipuncta* (Haworth)	Karaikal	*N. rileyi* (Farlow) Samson
	Ladybird beetle	*Coccinella septempunctata* L.	Lucknow	*B. bassiana*
	Grass hopper	*Hieroglyphus banian*	Coimbatore	*M. anisopliae*
	Grass hopper	*Oxya nitidula*	Coimbatore	*M. anisopliae*
Sorghum	Ear head caterpillar	*Helicoverpa armigera* Hubner	Bangalore	*N. rileyi*
Red gram	American boll worm	*H. armigera*	Bapatla	*B. bassiana*
	American boll worm	*H. armigera*	Bangalore	*N. rileyi*
Soybean	Tobacco caterpillar	*Spodoptera litura* (Fab.)	Vridhachalam	*N. rileyi*
Pea	Aphids	*Aphis craccivora* Koch	Vellayani	*F. pallidoroseum*
Black gram	Spotted pod borer	*Maruca testulalis*	Karaikal	*B. bassiana*

(continued)

Table 3.1 (continued)

Crop	Common name	Scientific name	Location	Entomopathogenic fungi
Groundnut	Yellow hairy caterpillar	*Amsacta albistriga* Walker	Karnataka	*B. bassiana*
	Tobacco caterpillar and American boll worm	*S. litura* and *H. armigera*	Guntur	*N. rileyi*
	Tobacco caterpillar and American boll worm	*S. litura* and *H. armigera*	Hyderabad	*N. rileyi*
	Groundnut pests	*S. litura*	Palayamkottai	*B. bassiana*
		Aproaerema modicella (Deventer)		*Paecilomyces fumosoroseus (Wize)*
		Aphis craccivora (Koch)		*Verticillium lecanii (Zimm.)*
		Mylabris pustulata Faust		
Sugarcane	Shoot borer	*Chilo infuscatellus* Snell	Coimbatore	*B. bassiana*
	White grub	*Holotrichia serrata* (Fab.)	Coimbatore	*M. anisopliae*
Cotton	Red cotton bug	*Dysdercus cingulatus*	Thoothukudi	*M. anisopliae*
Mango	Leaf webber	*Idioscopus* spp.	Basti (UP)	*B. bassiana*
Coconut	Rhinoceros beetle	*Oryctes rhinoceros* L.	New Delhi	*M. anisopliae*
	Rhinoceros beetle	*O. rhinoceros*	Coimbatore	*M. anisopliae*
Castor	Hairy caterpillar	*Pericallia ricini* Fab.	Palayamkottai	*B. bassiana*
				P. fumosoroseus
				V. lecanii
Teak	Leaf skeletonizer	*Eutectona machaeralis* (Walker)	Jabalpur	*B. bassiana*
				F. oxysporum Schlecht
Other parts of the world				
Rice	GLH	*N. bipunctatus*	China	*B. bassiana*
	GLH	*N. virescens*	Taiwan	*B. bassiana*
	BPH	*N. lugens*	Manila	*B. bassiana* and *M. anisopliae*
	WBPH	*S. furcifera*	China	*B. bassiana*
	Black bug	*Scotinophara lurida* B.	Philippines	*B. bassiana*
	Black bug	*S. coarctata* B.	Korea	*B. bassiana*
	Leaf folder	*C. medinalis*	USA	*Z. radicans*
	Leaf folder	*C. medinalis*	Philippines	*N. rileyi*
	Leaf folder	*C. medinalis*	Philippines	*Paecilomyces farinosus* (Holm ex S.F. Gray)
	Leaf folder	*C. medinalis*	Philippines	*B. bassiana*
	Leaf folder	*C. medinalis*	Philippines	*B. bassiana*
Rice	Leaf folder	*C. medinalis*	Fiji	*M. anisopliae*
	Leaf folder	*C. medinalis*	Fiji	*Spicaria rileyi* (Farlow) Charles
	Zigzag leafhopper	*Recilia dorsalis* (Mot.)	Philippines	*B. bassiana*
	Small plant hopper	*Laodelphax striatellus*	China	*B. bassiana*
Maize	Stem borer	*Chilo partellus* (Swinhoe)	Kenya	*B. bassiana*
	Stem borer	*Eldana saccharina* W.	Ghana	*Verticillium aboatrum*
				Aspergillus flavus
				Fusarium oxysporum
	Western corn Rootworm	*Diabrotica virgifera* LeConte	Hungary	*Metarhizium anisopliae*
				Beauveria bassiana

(continued)

Table 3.1 (continued)

Crop	Common name	Scientific name	Location	Entomopathogenic fungi
Soybean	Cabbage looper	*Trichoplusia ni* Hubner	Columbia	*N. rileyi*
Crucifers	Diamond back moth	*Plutella xylostella L.*	England	*Z. radicans*
	Cabbage looper	*T. ni*	USA	*S. rileyi* (Farlow) Charles
Coconut	Rhinoceros beetle	*O. rhinoceros*	France	*M. anisopliae*
	Rhinoceros beetle	*O. rhinoceros*	New Zealand	*M. anisopliae*
Guava	Scale	*Icerya seychellarum*	Egypt	*V. lecanii*
				M. anisopliae
				B. bassiana
Potato	Ladybird beetle and Colorado potato beetle	*Coleomegilla maculata lengi, Leptinotarsa decemlineata* (Say)	Canada	*B. bassiana*
–	Aphids	*Myzus persicae* Sulzer	Britain	*Verticillium lecanii* (Zimm.) Viegas
–	–	Lepidoptera, Coleoptera	UK	*B. velata, B. amorpha*
Elm	Bark beetle	*Scolytus scolytus* (Fab.)	England	*B. bassiana, M. anisopliae, P. farinosus*
Peas	Aphid	*Acyrthosiphon pisum* Harris	England	*Entomophthora* sp.
–	Bug	*Lygus hesperus* (Knight)	California	*B. bassiana*
–	Grasshopper	–	Argentina	*Fusarium verticillioides*
Pine	Bark beetle	*Ips* spp.	Bulgaria	*Beauveria bassiana*
				Isaria farinosa

3.3 Isolation and Identification of Entomopathogenic Fungi

Most of the entomopathogenic fungi are found within the deuteromycetes and entomophthorales. Entomopathogens such as *M. anisopliae* and *B. bassiana* are well characterized in respect to pathogenicity to several insects, and they have been used as agents for the biological control of agriculture pests worldwide. The entomopathogenic fungi can be collected from naturally infested dead cadavers and also from soil.

3.3.1 Dead Cadavers

The insects were found either sticking to the leaf sheath or floating on standing water, being overgrown by a chalky white mass of conidia. The cadavers were collected in sterile glass tube for isolating the causal organism in the laboratory. The insect cadavers were surface sterilized with 0.5 % sodium hypochlorite in 75 % alcohol and rinsed in three changes of sterile water for 1–2 min. Washed specimen was cut into pieces and placed on Sabouraud's dextrose agar (SDA) with yeast extract medium. The fungal colonies appeared were observed. Tip of the mycelial growth was transferred aseptically into the SDA slants. The cultures were purified and maintained in SDA slants (Dayakar and Kanaujia 2001).

3.3.2 Soil

Sixteen rice moth *Corcyra cephalonica* (Stainton) larvae were placed in a plastic container with the soil collected from four layers of soil at four larvae per layer. Baited plastic container was left at room temperature for 3–5 days (Zimmermann 1986). In this technique, *Corcyra* was used instead of *Galleria*. After 3 days, the dead larvae were examined for the fungal

infection under microscope. The fungus associated with mycosed larvae were isolated as mentioned above. Sahayaraj and Borgio (2009) isolated *Metarhizium anisopliae* from the soils collected from various regions in Tirunelveli district of Southern Tamil Nadu, India. Very recently, Sánchez-Peña et al. (2011) used *Tenebrio molitor* (Coleoptera: Tenebrionidae) larval baits for the collection of selective entomopathogenic fungi from soil in four adjacent habitats (oak forest, agricultural soil, pine reforestation, and chaparral habitat) in Saltillo, México. The persistence of fungal pathogens was found to be higher in soil than the phyllosphere indicating that they can be naturally favoured for the control of pests in groundnut (Sahayaraj and Karthick Raja 2011). The major entomopathogenic fungi recovered from samples were *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces* spp., *B. brongniartii*, *P. chlamydosporia*, and *Lecanicillium attenuatum*. The diversity of entomopathogenic fungi was greater in soil samples from forests compared to crop fields, vegetables, and fruits, respectively, in Pakistan (Waqas et al. 2013).

3.4 Identification

Entomogenous fungi can be identified by ARS Collection of Entomopathogenic Fungal Cultures, US Department of Agriculture, Agricultural Research Service, US Plant, Soil, and Nutrition Laboratory, Tower Road, Ithaca, NY.

3.4.1 *Beauveria* sp.

Beauveria bassiana, a filamentous fungus, belongs to a class of insect pathogenic deuteromycetes also known as imperfect fungus. An example of such species may be *Beauveria bassiana* which is reported from tropical rainforest (Aung et al. 2008) and has been found in Canada as far north as latitude 75° (Widden and Parkinson 1979). Strains of *Beauveria* are highly adapted to particular host insects. Broad ranges of *B. bassiana* spp. have been isolated from a variety of insects worldwide which are of medicinal or agricultural importance. *Beauveria bassiana* is a fungus that grows naturally in soils throughout the world and acts as a pathogen on various insect species, causing white muscardine disease. The colonies of the fungus are white in colour with cottony aerial mycelium. Conidiophores are single, or branched, oblong, cylindrical, or flask shape bearing laterally or at extremity vesicles giving rise to sporogenous cells (phialides). Phialides generally globose, sometimes cylindrical, flask like, and curved or straight. Conidia are globose (1–4 μ) to oval shape (1.5–5.5 × 1–3 μ).

3.4.2 *Metarhizium anisopliae*

This is commonly called as green muscardine fungus. *Metarhizium anisopliae* author family *is* also a very potential pathogen on insect pests and is explored for mycobiocontrol of notorious insect pests. This fungus is widely distributed and recorded in more than 300 hosts. The affected cadavers show typical green aerial mycelia. Initially the colony appears white in colour and later turns to green in colour. Mycelium is composed of hyaline, septate, branched hyphae. Conidiophores are short, erect, hyaline, septate, simple, or branched, terminating in single or a cluster of phialides. Conidia are single celled, hyaline, smooth, and long ovoid to cylindrical (4.8 × 1.6 μ). Based on the size of the conidia, *M. anisopliae* is divided into two varieties, namely, *M. anisopliae var. anisopliae* and *M. anisopliae var. major*. The former has short conidia, of 3–9 μ length, and the latter has long spores, of 9–18 μ length. Scanning electron microscope studies revealed that naturally fungi *B. bassiana* and *M. anisopliae* infection begins when conidia (asexual spores, the seeds of a fungus) attach to insect's cuticle, the spores germinate and penetrate the insect's skin and enter the host. Once the fungus penetrates the host; it produces toxins that overcome the insect immune system. Thereafter, the hyphae penetrate through the cuticle to the outside and cause white (*B. bassiana*) or green (*M. anisopliae*) sporulation on the insect's body (Kabarty et al. 2014).

3.4.3 *Verticillium lecanii (=Lecanicillium lecanii)*

Another entomopathogenic fungus *Verticillium lecanii* is a widely distributed fungus, which can cause large epizootics in tropical and subtropical regions, as well as in warm and humid environments. It is known primarily as a pathogen of aphids, scales, white flies, thrips, and red spider mites. It is also called as white halo fungus. The fungus is characterized by the presence of conidiophores in verticillate whorls and on which conidia are borne in slime or mucus balls.

3.4.4 *Nomuraea* sp.

Nomuraea rileyi, another potential entomopathogenic fungus, is a dimorphic hyphomycete that can cause epizootic death in various lepidopteran and coleopteran insects. It has been shown that *Spodoptera litura* and some belonging to *Coleoptera* are susceptible to *N. rileyi*. The host specificity of *N. rileyi* and its ecofriendly nature encourages its use in insect pest management. The colonies of the fungus are white initially and later turn to green in colour (malachite green). Hyphae are 2–3 μ in diameter, smooth, septate, hyaline, and slightly pigmented. Conidiophores are long (160 μ) and consist of dense compacted clusters of phialides and branches in whorls on the upper section. The branches are short and swollen. Phialides are short and cylindrical to globose, with very swollen base tapering abruptly to a narrow neck. The conidia are 3–4 × 2.5 μ and pale green in colour.

3.4.5 *Paecilomyces* sp.

Paecilomyces is a genus of nematophagous fungus which kills harmful nematodes by pathogenesis, causing disease in the nematodes. Thus, the fungus can be used as a bio-nematicide to control nematodes by applying it to soil. *Paecilomyces lilacinus* principally infects and assimilates eggs of root-knot and cyst nematodes. The fungus has been the subject of considerable biological control research following its discovery as a biological control agent in 1979. *Paecilomyces fumosoroseus* (Wize) *P. farinosus* causes a sickness called "yellow muscardine" in white flies. The colonies of these fungi are white, red, or yellow in colour, and phialides occur as divergent loose groups.

3.4.6 *Hirsutella thompsonii*

Hirsutella thompsonii Fisher is a well-known fungal pathogen which is commonly associated with the acarines. This parasitic fungus was originally described by Fisher, who isolated it from the citrus rust mite, *Phyllocoptruta oleivora*, in Florida. It has a grey, fluffy mycelial growth and a brownish to greyish-green substratum colour. The hyphae are 1.5–2.0 μm wide and smooth. The conidiogenous cells arise singly at intervals from the vegetative hyphae, mono- or polyphialide, unevenly verrucose, with a conical to flask-shaped base and a narrow neck. The neck may be unbranched or branched, bearing enteroblastic conidia singly at the tip of the branch.

3.5 Mode of Action of Entomopathogenic Fungus (EPF)

In contrast to bacteria and viruses that pass through the gut wall from contaminated food, fungi have a unique mode of infection. They reach the haemocoel through the cuticle. Fungi invade insects by penetrating their cuticle or "skin". Once inside the insect, the fungus rapidly multiplies throughout the body. Death is caused by tissue destruction and, occasionally, by toxins produced by the fungus. The fungus frequently emerges from the insect's body to produce spores that, when spread by wind and rain or contact with other insects, can spread infection. Attachment of a fungal spore to the cuticle surface of a susceptible host represents the initial event in the establishment of mycosis. When the pathogen reaches and adheres to the host surface, it proceeds with rapid germination and growth which are profoundly influenced by the availability of water, nutrients, oxygen as well as pH, and temperature and by the effects of toxic host-surface compound. Fungi invade their hosts by

penetration of the host cuticle or put pressure on cuticle by making appressorium and then penetrate by penetration peg (Sandhu 1995).

In many areas of the cuticle, the chitin is organized helically giving rise to a laminate structure. Conidia germinate on the host surface and differentiate an infection structure termed appressorium (St Leger 1991). Entomopathogenic fungi need to penetrate through the cuticle into the insect body to obtain nutrients for their growth and reproduction. Entry into the host involves both enzymatic degradation and mechanical pressure as evidenced by the physical separation of lamellae by penetrated hyphae. A range of extracellular enzymes that can degrade the major components of insect cuticle, including chitinases, lipases, esterases, and at least four different classes of proteases, have been suggested to function during the fungal pathogenesis. The fungi begin their infective process when spores are retained on the integument surface, where the formation of the germinative tube initiates; the fungi starts excreting enzymes such as proteases, chitinases, quitobiases, lipases, and lipoxygenases after the successful penetration; and the fungus is then distributed into the haemolymph by formation of blastospores (Bhattacharyya et al. 2004). The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are ubiquitously distributed in soils. As insect pathogens, they adhere to the insect cuticle and penetrate through to the insect haemocoel using a variety of cuticle-hydrolysing enzymes. Once in the insect haemocoel, they are able to survive and replicate within, and/or evade, phagocytic haemocyte cells circulating in the haemolymph (Michael et al. 2010; Sahayaraj et al. 2013). Shaukat Ali et al. (2014) reported that lipases play an important role in the infection process of entomopathogenic fungi by hydrolyzing the ester bonds of lipo proteins, fats and waxes present on the insect surface and in the body. This was studied in the scale insect *Isaria fumosorosea* and confirmed that extra cellular lipase enzyme produced by this insect can be exploited for enzyme based insect management.

3.6 Mass Multiplication

Culture conditions influence the virulence of any fungi (Ortiz-Urquiza et al. 2013); hence, it is imperative to study about the culture of entomopathogenic fungi. Hussey and Tinsley (1981) observed that wheat bran was considered to be a suitable substrate for mass production of *B. bassiana*. Silva and Loch (1987) reported that *N. rileyi* had been multiplied on polished rice grain. Soybean chunk (Pandit and Som 1988), carrot medium (Deva Prasad 1989), and rice grain (Rama Mohan Rao 1989) were recommended for the mass production of *B. bassiana*. Recently grains, vegetable wastes, seeds, rice husk, sawdust, and liquid media such as coconut water, rice and wheat washed water, and rice cooked water recommended for the mass production of *B. bassiana* (Sahayaraj and Karthick Raja 2008). However, par-boiling, water, and washing regimes of the rice substrate were recommended by Taylor et al. (2013). Mathai et al. (1998) studied the use of different locally available cheaper substrates for the mass multiplication of *F. pallidoroseum* and found that wheat bran alone and rice bran with tapioca bits gave maximum spore count followed by wheat bran with straw bits. Vyas et al. (1989) showed that crushed maize was shown to be an ideal substrate for culturing *B. brongniartii*. Liu et al. (1990) observed that chitin-supplemented rice grain medium gave the highest germination of *B. bassiana* in the laboratory. Devi (1994) reported that crushed sorghum was effective for the conidia production in *N. rileyi*. Narayanasamy (1994) observed that *P. delphacis* was mass produced on the cereal grain of *Sorghum vulgare* and *Pennisetum typhoides* and it sporulated enormously in few days.

Mazumder et al. (1995) evaluated the locally available industrial waste substrates for culturing *B. bassiana* and revealed that husk supplementation with 2 % dextrose was found to be more suitable. Udayaprabhakar (1995) tested various natural substrates and noticed sorghum-impregnated media was found to be efficient for the mass production of the fungi *P. delphacis* and

M. hiemalis. Faizal and Mathai (1996) suggested that wheat bran and rice bran were found to be suitable substrates for the mass production of *F. pallidoroseum* for the control of aphids. Puzari et al. (1997) reported that *B. bassiana* could be mass multiplied with cellulose wastes, namely, rice hull, sawdust, and rice bran either alone or in combination. Parthasarathy (1997) reported that sorghum and maize grain media excelled among the various diets evaluated with respect to biomass production, radial growth, and spore germination of *Z. radicans*. Reji Rani et al. (2000) found that the fungus *F. pallidoroseum* can be mass multiplied well on the naturally available substrates like cowpea leaves and cotton seed cake and gave maximum spore count and mycelial growth, followed by rice bran, gingelly cake, and fallen *Pongamia* leaves. Among the liquid media tried, coconut water was found to yield maximum spore count.

Green muscardine fungus *M. anisopliae* could be mass multiplied using tapioca chips method and coconut water method (Mohan and Gopal 2002). Sharma et al. (2002) observed that *M. anisopliae* produced maximum sporulation on molasses yeast broth; the highest conidia of *Beauveria* species were obtained with molasses yeast broth and bajra followed by sorghum and maize grains for *M. anisopliae* and cowpea grains for *B. bassiana* and *B. brongniartii*. Several techniques for the mass production of entomogenous fungi were mostly designed to yield infective conidia in large numbers. Wadyalkar et al. (2003) reported that potato dextrose broth was found to be the best in spore production for *M. anisopliae*. Sahayaraj and Karthick Raja (2008) reported that wheat grains supported maximum spore production for *B. bassiana*, while sorghum recorded maximum spore production in *P. fumosoroseus* and *V. lecanii*. Similarly carrot, jack seeds, and lady's finger also supported good growth and sporulation of all the three tested fungi. Coconut water supported maximum growth and sporulation. Paddy chaffy grains support maximum growth for all the three *Fusarium* isolates. Paddy chaffy grains support maximum sporulation for *B. bassiana* isolates and *F. moniliforme* GLH isolate. Similarly, rice bran recorded maximum sporulation for *F. pallidoroseum* leaf folder isolate (Senthamizhlselvan and Alice 2008). Jagadeesh Babu et al. (2008) reported that the highest spore count was recorded in the broken rice and followed by that broken jowar.

Nomuraea rileyi is a well-known fungal pathogen thriving in a range of obligate hosts, particularly the polyphagous species of *Heliothis*, *Spodoptera*, *Pseudoplusia*, *Trichoplusia*, *Plutella*, and *Rachiplusia* (Boucias et al. 1984; Chen et al. 2012). However, the lack of reliable cost-effective protocols for mass production of this entomopathogenic fungus limits its commercialization. Thakre et al. (2011) studied various agricultural products like rice, sorghum, and wheat and agro wastes, namely, refuse raw potatoes and refuse raw bananas for mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi*. Among the grains, rice (5.53×10^7 spore/g) supported the maximum spore production of fungus followed by refuse raw bananas (4.2×10^7 spores/g) and sorghum (4.01×10^7 spores/g) on the 11th day after inoculation of spore suspension.

3.7 Formulations

Next to isolation of virulent fungi, the important step is to identify the use of appropriate formulations against insect pests. Formulations can greatly improve the efficacy of entomopathogens. A substantial number of mycoinsecticides and mycoacaricides have been developed worldwide since the 1960s. Products based on *Beauveria bassiana* (33.9 %), *Metarhizium anisopliae* (33.9 %), *Isaria fumosorosea* (5.8 %), and *B. brongniartii* (4.1 %) are the most common among the 171 products. Examples of few commercial formulations are given in Table 3.2 (de Faria and Wraight 2007). The key components were active ingredient, carrier, stickers, surfactants, UV protectants, etc. The principal ingredients in most biopesticide products based on insect pathogenic fungi (mycopesticides) are dry spores called conidia, and viability of these conidia is

Table 3.2 Examples of some of the fungal formulations used for the management of various pests in different localities

Fungus	Country	Product name	Target insect pests
Beauveria bassiana	Czech Republic	Boverol	Coleoptera (Chrysomelidae)
	Czech Republic	Boverosil	Coleoptera (Curculionidae) and stored pests
	France	Ostrinil	Lepidoptera (Crambidae)
	Hawaii	BotaniGard ES	Coleoptera
		Mycotrol	
	Spain	Trichobass	Coleoptera (Curculionidae, Scarabaeidae), Lepidoptera (Castniidae, Pieridae), Hemiptera (Aleyrodidae), Thysanoptera (Thripidae) + Acari (Tetranychidae)
	South Africa	Bb Plus	Hemiptera (Aphididae) + Acari (Tetranychidae)
	South Africa	Bb Weevil	Coleoptera (Curculionidae)
	India	BioGuard	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera (Aleyrodidae, Aphididae), Lepidoptera (Crambidae), Thysanoptera (Thripidae)
		Rich	
	India	Bio-Power	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera: Auchenorrhyncha (Cicadellidae, Delphacidae), Lepidoptera (Plutellidae)
	India	Racer	Lepidoptera (Noctuidae) +
	Russia	Boverin	Hemiptera (Aleyrodidae), Thysanoptera (Thripidae) + Acari (Tetranychidae)
	Mexico	Bea-Sin	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera (Aleyrodidae)
	Mexico	Bio-Fung	Orthoptera
	USA	Balence	Diptera (Muscidae)
	USA, Mexico, Denmark, Italy, Spain, Sweden, Japan	BotaniGard 22 WP	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera (Miridae, Cicadellidae, Fulgoridae, Aleyrodidae, Aphididae, Pseudococcidae, Psyllidae), Thysanoptera (Thripidae)
	USA, Mexico, Denmark, Italy, Sweden	Mycotrol ES	Coleoptera (Chrysomelidae, Curculionidae, Scarabaeidae), Hemiptera (Miridae, Cicadellidae, Fulgoridae, Aleyrodidae, Aphididae, Pseudococcidae, Psyllidae), Lepidoptera (Crambidae), Orthoptera (Acrididae, Tettigoniidae), Thysanoptera (Thripidae)
	USA, Mexico, Greece, Italy, Spain, Switzerland	Naturalis L	Coleoptera, Diptera Lepidoptera, Hemiptera, Hymenoptera, Orthoptera, Thysanoptera and Acari
	USA	Organigard	Lepidoptera, Hemiptera,, Orthoptera, Thysanoptera
	Costa Rica, Panama	Beauvedieca	Coleoptera (Curculionidae)
	Costa Rica	Nativo 2 SC	Coleoptera (Curculionidae)
	Egypt	Bio-flay Biosect	Hemiptera
	Brazil	Boveriol	Isoptera (Rhinotermitidae, Termitidae)
	Colombia	Ago Biocontrol Bassiana 50	Coleoptera, Diptera, Hemiptera, Lepidoptera
	Colombia, Dominican Republic	Bauveril	Coleoptera (Curculionidae, Scarabaeidae), Lepidoptera (Castniidae)
	Colombia	Conidia	Coleoptera (Curculionidae)

(continued)

Table 3.2 (continued)

Fungus	Country	Product name	Target insect pests
Beauveria brongniartii	Austria, Italy	Melocont-Pilzgerste	Coleoptera (Scarabaeidae)
	Switzerland	*Beauveria brongniartii*	Coleoptera (Scarabaeidae)
		Myzel	
	Reunion Island	Betel	Coleoptera (Scarabaeidae)
	Japan	Biolisa Kamikiri	Coleoptera (Cerambycidae)
Hirsutella thompsonii	India	MeteHit	Acari
	India	Mycohit	Acari (Eriophyidae)
	USA	Mycar	Acari (Eriophyidae)
Isaria fumosorosea (formerly *Paecilomyces fumosoroseus*)	Europe	PreFeRa	Hemiptera (Aleyrodidae)
	India	Priority	Acari (Eriophyidae,Tetranychidae)
	Mexico	Pae-Sin	Hemiptera (Aleyrodidae)
	Mexico	*P. fumosoroseus*	Hemiptera (Aleyrodidae)
	USA, Mexico	PFR-97 20 % WDG	Hemiptera (Aleyrodidae, Aphididae), Thysanoptera (Thripidae) + Acari (Tetranychidae)
	Colombia	Ago Biocontrol *Paecilomyces* 50	Coleoptera + Nematoda
	Venezuela	Bemisin	Hemiptera (Aleyrodidae)
	India	PaciHit Rich	Hemiptera (Aleyrodidae), Thysanoptera (Thripidae) + Nematoda
Lecanicillium sp. (formerly *V. lecanii*)	Spain	Trichovert	–
	India	Bio-Catch	Hemiptera (Aleyrodidae, Aphididae, Pseudococcidae)
	India	Biovert	"Insects" + Nematoda
		Rich	
	India	Mealikil	Hemiptera: Sternorrhyncha ("scales") + others (not specified)
	Colombia	Ago Biocontrol *Verticillium*50	Hemiptera, Diptera
	Scandinavia	MicroGermin	Hemiptera (Aleyrodidae, Aphididae)
Metarhizium anisopliae	Germany, Switzerland	BIO 1020	Coleoptera (Curculionidae)
	Switzerland	*Metarhizium*	Coleoptera (Scarabaeidae)
		Andermatt	
		C + H/TK	
		Metarhizium	
		Schweizer	
		C + H/TK	
	India	Pacer	Isoptera
	Australia	BioCane	Coleoptera (Scarabaeidae)
		Granules	
		Biological	
		Insecticide	

(continued)

Table 3.2 (continued)

Fungus	Country	Product name	Target insect pests
	Mexico	Fitosan-M	Coleoptera (Scarabaeidae), Orthoptera
	Mexico	Meta-Sin	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera (Cercopidae), Orthoptera
	USA, Mexico	Bio-Blast	Isoptera (Kalotermitidae, Rhinotermitidae, Termopsidae)
		Biological	
		Termiticide	
	USA	Bio-Path	Blattodea (Blattellidae, Blattidae)
		Cockroach	
		Control	
		Chamber	
	USA	MET 52	Thysanoptera
			Hemiptera(Aleyrodidae)
	Guatemala	Salivase	Hemiptera (Cercopidae)
	Brazil	Biocontrol	Hemiptera (Cercopidae)
	Brazil, Panama	Biotech	Hemiptera (Cercopidae)
	Brazil	Metaquino	Hemiptera (Cercopidae)
	Colombia	Ago Biocontrol *Metarhizium* 50	Coleoptera, Hemiptera, Lepidoptera, Orthoptera
	Venezuela	Cobican	Coleoptera (Scarabaeidae), Hemiptera (Cercopidae, Aphididae)
Nomuraea rileyi	Colombia	Ago Biocontrol *Nomuraea* 50	Lepidoptera

one of the most commonly reported indicators of product quality (de Faria et al. 2010). The first attempt to control a pest with a fungal agent was carried out in Russia in 1888, when the fungus now known as *Metarhizium anisopliae* (Metschn.) Sorokın was mass produced on beer mash and sprayed in the field for control of the beet weevil *Cleonus punctiventris* (Germar) (Lord 2005). Recently a new mycoinsecticide was developed from *M. anisopliae* for greenhouse and vegetable growers in the USA (Thomas Ford 2013). Boverin, a *Beauveria bassiana*-based mycoinsecticide for control of the Colorado potato beetle and codling moth in the former USSR, was developed in 1965 (Kendrick 2000). The common formulation that must be ready to use is wettable powder, "A powder formulation to be applied as a suspension after dispersion in water". Another one is suspension concentrate, "A stable suspension of active ingredient in water, intended for dilution with water before use". Oil-miscible flowable concentrate is also becoming popular everywhere during recent time. Effectiveness of oil-based conidia formulation of three indigenous fungal isolates such as *Beauveria bassiana*, *Verticillium lecanii*, and *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) was evaluated against fourth instar larvae of *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) under laboratory conditions and found that oil-based formulations of *V. lecanii* caused the highest mortality of 97.33 % at 3.9×10^9 spores per mL^{-1}(Sahayaraj and Borgio 2012).

3.8 Future Thrust Area

Selection of virulent strains through genetic manipulation will be the most important advancement in the use of entomopathogenic fungi. Use of agricultural wastes can be employed for the cultivation of fungus in large

scale. Awareness must be created among the farming community to prepare cheaply available, inexpensive substrates for their use. Sufficient funding can be directed to carry out research in this line. Further, hands-on training can also be initiated in this area of research to scientists and farmers. The main bottleneck in this line of research is lack of quality products. Hence, this area can be strengthened.

3.9 Conclusion

At present this area of research is least exploited, even though the entomopathogenic fungi are considered to be one of the important, potential candidates as there are several advantages of using these fungal pathogens as pest control agent. Most of these are host specific and less toxic to mammals having a wide host range, and moreover these mycoinsecticides are ecofriendly too. Survey work should be undertaken in different geographical locations to collect and identify virulent fungal pathogens. The use of mycoinsecticides has to be popularized.

References

Ambethgar V (1996) Occurrence of the white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill on the rice leaf folder, *Cnaphalocrocis medinalis* (Guenee). Ann Plant Prot Sci 4(2):165–188

Aung OM, Soytong K, Hyde KD (2008) Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. Fungal Divers 30:15–22

Bhattacharyya A, Samal AC, Kar S (2004) Entomophagous fungus in pest management. News Lett 5:12

Boucias DG, Bradford DL, Barfield CS (1984) Susceptibility of the velvetbean caterpillar and soybean looper (Lepidoptera: Noctuidae) to *Nomuraea rileyi*: effects of pathotype, dosage, temperature and host age. J Econ Entomol 77:247–253

Bridge ES, Andrew WJ, Allan JB (2005) A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. Mol Phylogenet Evol 35:459–469

Chen H, Yin Y, Li Y, Mahmud MS, Wang ZK (2012) Identification and analysis of gene differentially expressed in the *Spodoptera litura* fat body in response to the biocontrol fungus, *Nomuraea rileyi*. Comp Biochem Physiol B Biochem Mol Biol 163 (2):203–210

Cheraghi A, Habibpour B, Mossadegh MS (2013) Application of bait treated with the entomopathogenic Fungus *Metarhizium anisopliae* (Metsch.) Sorokin for the Control of *Microcerotermes diversus* Silv. J Entomol 1:1–5

Dayakar S, Kanaujia KR (2001) Susceptibility of tobacco caterpillar, S. litura (F.) (Lepidoptera: Noctuidae) to different isolates of entomopathogenic fungi (Deuteromycotina: Hyphomycetes). Indian J Plant Prot 29(182):62–67

de Faria MR, Wraight SP (2007) Mycoinsecticides and Mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43(237–256):247

de Faria MR, Hotchkiss JH, Hajek AE, Wraight SP (2010) Debilitation in conidia of the entomopathogenic fungi *Beauveria* and *Metarhizium anisopliae* and implications with respect to viability determinations and mycopesticide quality assessments. J Invertebr Pathol 105(1):74–83

Deva Prasad V (1989) Studies on certain entomopathogens of *Heliothis armigera* Hb. and *Spodoptera litura* F. and their interaction with some botanicals. PhD thesis, Tamil Nadu Agriculture University, Coimbatore, India, p 147

Devi PSV (1994) Conidia production of the entomopathogenic fungus, *Nomuraea rileyi* and its evaluation for the control of *Spodoptera litura* (Fab.) on *Ricinus communis*. J Invertebr Pathol 63:145–150

Driver F, Milner RJ, Trveman JWH (2000) A taxonomic revision of *Metarhizium* based on sequence analysis of ribosomal DNA. Mycol Res 104:135–151

Eilenberg J, Schmidt NM, Meyling N, Wolsted C (2007) Preliminary survey for insect pathogenic fungi in Arctic Greenland. IOBC/WPRS Bull 30(1):1027–3115

Evans HF (1986) Ecology and epizootiology of Baculoviruses. In: Granados RR, Federici BA (eds) The biology of baculoviruses. Vol. II "Practical application for insect control". CRC Press, Boca Raton, pp 89–132

Faizal MH, Mathai S (1996) Evaluation of different substrates for mass production of *Fusarium pallidoroseum* an entomogenous fungus. J Entomol 58(2):99–102

Hussey NW, Tinsley JW (1981) Improvement of insect pathology in People's Republic of China. In: Burges HD (ed) Microbial control of pests and plant disease 1970–1980. Academic, New York, pp 785–795

Jagadeesh Babu CS, Venkatachalapathy CM, Anitha CN (2008) Evaluation of locally available substrates for mass multiplication of entomopathogenic fungi, *Metarhizium anisopliae* (Metch.) Sorokin. J Biopest 1(2):146–147

Kabarty A, Salem HM, Fouda MA, Abas AA, Ibrahim AA (2014) Pathogenicity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn). J Radiat Res Appl Sci 7(1):95–100

Kendrick M (2000) The fifth kingdom, 3rd edn. Mycologue Publications, Sidney

Klingen I, Eilenberg J, Meadow R (2002) Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric Ecosyst Environ 91(2–3):191–198

Liu SD, Chang YC, Huang YS (1990) Application of entomopathogenic fungi as biological control of *Leucaena psyllid*, *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) in Taiwan plant. Plant Prot Bull Taipei 32:49–58

Lord JC (2005) From Metchnikoff to Monsanto and beyond: the path of microbial control. J Invertebr Pathol 89:19–29

Manisegarane S, Letchoumanane S (1996) *Fusarium pallidoroseum* on rice leaf folder, *Cnaphalocrocis medinalis*. Indian J Entomol 58(4):364–368

Mathai S, Geetha D, Mohandas N (1998) Use of different locally available and cheaper substrates for the mass multiplication of *Fusarium pallidoroseum* (Cooke) Sacc. An effective fungal pathogen against pea aphid, *Aphis craccivora* Koch. In: Subbarao NS, Rajagopalan C, Ramakrishnan SV (eds) New trends in biotechnology, 1992, pp 249–251

Mazumder D, Puzari KC, Hazarika LK (1995) Mass production of *Beauveria bassiana* on rice hispa. Indian Phytopathol 48(3):275–278

Michael JB, David C, Clark MW, Nemat OK (2010) Could insect phagocytic avoidance by entomogenous fungi have evolved via selection against soil amoeboid predators. Microbiology 156:2164–2217

Mohan C, Gopal M (2002) Mass production and use of microbial for the management of Oryctes rhinoceros. In: Rabindra RJ, Natarajan N, Balagurunathan R, Durairaj C, Ramaraju K, Srinivasan MR (eds) Modern trends in integrated pest management. TNAU Press, Coimbatore, pp 127–129

Narayanasamy P (1994) Final report of a project titled development and use of mycoinsecticide from indigenous fungal pathogen against the brown plant hopper *Nilaparvata lugens* (Stal.) problem in rice. Sponsored by the Ministry of Environment and Forestry, Government of India, New Delhi, p 88

Ortiz-Urquiza A, Keyhani NO, Quesada-Moraga E (2013) Culture conditions affect virulence and production of insect toxic proteins in the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci Technol. doi:10.1080/09583157.2013.822474

Pandit NC, Som D (1988) Culture of *Beauveria bassiana* and its pathogenicity to insect pest of jute (*Corchorus capsularis* and *C. olitorius*) and mesta (*Hibiscus cannabinus* and *H. sabdariffa*). Indian J Agric Sci 58:75–76

Parthasarathy R (1997) Biological suppression of rice leaf folder, *C. medinalis* (Guenee) with mycoparasite. M.Sc. (Ag.) thesis, Annamalai University, Annamalainagar, Tamil Nadu, India, p 152

Puzari KC, Sarmah D, Hazarika LK (1997) Medium for mass production of *Beauveria bassiana* (Bals.) Vuill. J Biol Control 11:97–100

Rama Mohan Rao P (1989) Studies on culture techniques. Safety and control potential of certain entomopathogenic fungi of rice pests. PhD thesis, Tamil Nadu Agricultural University, Coimbatore, p 132

Reji Rani OP, Mathai S, Peethambaran CK (2000) Evaluation of different naturally available substrates for mass production of the entomogenous fungus *Fusarium pallidoroseum* (Cooke) Sacc. In: Proceedings of the 12th Kerala Science Congress, Thiruvananthapuram, pp 27–29

Roberts DW, Yendol WG (1971) Use of fungi for microbial control of insects. In: Burges HD, Hussey NW (eds) Microbial control of insects and mites. Academic, London/New York, pp 125–149

Sahayaraj K, Borgio JF (2009) Distribution of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) in Tamil Nadu, India, its biocontrol potential on *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). Arch Phytopathol Plant Prot 42(5):424–435

Sahayaraj K, Borgio JF (2012) Screening of some mycoinsecticides for the managing hairy caterpillar, *Pericallia ricini* Fab. (Lepidoptera: Arctiidae) in castor. J Entomol 9(2):89–97

Sahayaraj K, Karthick Raja NS (2011) Field evaluation of three entomopathogenic fungi on groundnut pests. Tropicultura 29(3):143–147

Sahayaraj K, Karthick Raja NS (2008) Mass production of entomopathogenic fungi using agricultural products and by products. Afr J Biotechnol 7(12): 1907–1910

Sahayaraj K, Francis Borgio J, Lucini L (2013) Route of infection and haematological effect of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) on *Dysdercus cingulatus* (Fab.) adult (Hemiptera: Pyrrhocoridae). J Basic Microbiol 53:1–12. doi:10.1002/jobm.201200258

Sánchez-Peña SR, Lara JSJ, Medina RF (2011) Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, Mexico, and their virulence towards thrips and whiteflies. J Insect Sci 11:1

Sandhu SS (1995) Effect of physical factors on germination of entomopathogenic fungus *Beauveria bassiana* conidia. Nat Acad Sci Lett 18(1–2):1–5

Sandhu SS, Anil KS, Vikas B, Gunjan GPB, Anil Kumar Sundeep J, Sharma AK, Sonal Malhotra (2012) Mycobiocontrol of insect pest factors involved mechanism, and regulation. J Pathog 1–10

Senthamizhlselvan P, Alice J (2008) Biodiversity of entomogenous fungi in the cropped ecosystem of Karaikal region. M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, India, p 110

Sharma S, Gupta RBL, Yadava CPS (2002) Selection of a suitable medium for mass multiplication of entomofungal pathogens. Indian J Entomol 64(3):2254–2261

Shaukat Ali, Shunxiang Ren, Zhen Huang (2014) Extracellular lipase of an entomopathogenic fungus effecting larvae of a scale insect. J Basic Microbiol, On line Journal, John Wiley and Sons. 032014

Silva LD, Loch LC (1987) Sporulation of the entomogenous fungus *Nomuraea rileyi* (Farlow) Samson on polished rice grain media. Entomol Soc Braz 16:213–222

St Leger RJ (1991) A model to explain differentiation of appressoria by germlings of *Metarhizium anisopliae*. J Invertebr Pathol 57:299–310

Taylor B, Edgington S, Luke B, Moore D (2013) Yield and germination of the entomopathogenic fungus *Beauveria bassiana* when grown on different rice preparations. J Stored Pro Res 53:23–26

Thakre M, Thakur M, Malik N, Ganger S (2011) Mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi* using agricultural products and agro wastes. J Biopest 4(2):176–179

Thomas Ford (2013) A new mycoinsecticide for green house and vegetable growers. Penn State Extension

Udayaprabhakar L (1995) Investigation on collection, identification and formulation of Pandora delphacis (Hori.) Humber as mycoinsecticide against the rice brown plant hopper, Nilaparvata lugens (Stal). Ph.D. thesis, Annamalai University, Annamalainagar, India, pp 127

Vänninen I (1995) Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycol Res 100(1):1469–8102

Vyas RV, Yadava DN, Patel RJ (1989) Mass production entomopathogenic fungus, *Beauveria brongniartii* on ideal substrates. Indian J Exp Biol 29:795–797

Wadyalkar SR, Wasule DL, Bhoyte SJ, Wadaskar RM (2003) Mass multiplication and formulation of *Metarhizium anisopliae* (Metsch.) Sorokin. J Biol Control 7(2):141–146

Waqas W, Usman Ghazanfar M, Riasat T, Qayyum MA, Yasin M (2013) Occurrence and diversity of entomopathogenic fungi in cultivated and uncultivated soils in Pakistan. Entomol Res 43(1):70–78

Widden P, Parkinson D (1979) Populations of fungi in a high arctic ecosystem. Can J Bot 57(21):2408–2417

Zimmermann G (1986) The *Galleria* bait method for detection of entomopathogenic fungi in soil. J Appl Entomol 102(3):213–215