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# Entomopathogenic fungi: A comprehensive review of an ever-enduring biocontrol agent

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**Abstract**

Global food security is threatened by various agricultural pests which cause significant losses to economically important crops. Chemical pesticides have traditionally been used to control these pests worldwide. However, the adverse effects of chemical pesticides, such as harm to non-target organisms, environmental contamination, and residue problems, are well known. Therefore, there is a growing need to develop environmentally friendly pest management practices with minimal impact on non-target organisms. Mycobiocontrol, which involves using Entomopathogenic Fungi (EPF) to reduce the density of insect pests and minimize crop damage, is a key component of sustainable pest management. Currently, there are more than 750 species of EPF known to attack various insects and mites across more than 20 insect orders and all developmental stages. Unlike bacteria and viruses, EPF do not need to be ingested; mere contact with the host is sufficient to start the infection process. Various media and techniques have been used in the past, and newer formulations of EPF are being developed to increase efficacy under varied environmental conditions. This review focuses on the infection process of these EPFs, the toxins they produce, and the various media and methods used for mass multiplication of these EPFs at a commercial scale.

**Keywords:** Entomopathogenic fungus, Insect pest, pest management and eco-friendly, commercial production

## Introduction

Agriculture is the main source of food and a major contributor to the global economy. Throughout history, diseases and insect pests have caused significant losses in agricultural output. Chemical pesticides have helped increase agricultural production, but they also come with various disadvantages (Gupta & Dikshit, 2010) [9]. The historical over-reliance on synthetic chemicals has caused harm to the soil, nontarget creatures, and people who come into contact with them (Aktar *et al.*, 2009) [1]. A range of issues such as pest resurgence, insecticide resistance, and harm to biodiversity have prompted scientists to conduct various investigations to develop more effective and sophisticated pest management plans.

The successful introduction of the Myna bird (*Acridotheres tristis*) from India into Mauritius in 1762 to control the sugarcane red locust *Nomadacris septemfasciata* is a well-known example of biocontrol. For many years, biopesticides have been recognized as having potential in sustainable agriculture. The concept of microbial control has been around for many millennia. Agostino Bassi (1773-1856) was a pioneer in the modern study of entomopathogenic fungi. Based on his observations of fungal-mediated mal de segno, or white muscardine sickness, in silkworms, Bassi developed one of the earliest germ theories of disease (Porter, 1973) [21]. Several publications, such as studies on the diseases of the silkworm *Bombyx mori* (Rajula *et al.*, 2021) [22], have made a significant contribution to this concept. Among the microbial biopesticides, entomopathogenic fungi are the second-highest selling, accounting for around 9% of all microbial biopesticides sold globally (Glare *et al.*, 2012) [7]. Among the earliest species to be employed for pest biocontrol are Entomopathogenic Fungus (EPF). These entomopathogens are favored for killing insects at different phases of their life cycles because of their ecofriendliness and biopersistence. There is a wide variety of fungal species from several types that infect insects. These obligatory and facultative pathogens are among the many different adaptations and infectious powers that these insect pathogenic species possess (Sinha *et al.*, 2016) [27].

Entomopathogenic fungi (EPF) are naturally occurring in the soil (Litwin *et al.*, 2020) [15], but they are mainly isolated from insect cadavers. These microbes play a crucial role in regulating the number of insects and are effective in managing insect pests. Fungal infections can impact a wide variety of insects, and there are around 750 species of fungi that cause infection in insects and mites (Shapiro *et al.*, 2017). The high reproduction rate, ability to target specific environments, short generation times, and capacity to produce saprobic phases or resting stages make fungi promising biocontrol agents. EPFs are known to infect a wide range of insect stages, from larvae to adult hosts. They also target a total of 31 insect orders, significantly impacting twenty of them, and infect all developmental stages, including eggs, larvae, pupae, nymphs, and adults (A. Ortiz-Urquiza and N.O. Keyhani, 2013) [18]. Entomopathogenic fungi (EPF) are parasitic microorganisms that infect and kill arthropods, according to Litwin *et al.* (2020) [15]. They belong to various taxa including Oomycetes, Chytridiomycota, Microsporidia, Entomophtoromycota, Basidiomycota, and Ascomycota. These fungi possess different adaptations and infectious powers and can be both obligatory and facultative pathogens. Entomopathogenic fungi are found in the divisions Zygomycota, Ascomycota, and Deuteromycota as described by Samson *et al.* (2016) [23]. Many intriguing genera of entomopathogenic fungi are members of the Hyphomycetes class in Deuteromycota or the Entomophthorales class in Zygomycota.

## Entomopathogens of Different Phylum

### Phylum Oomycota

These fungi have biflagellate zoospores and cellulose in their coenocytic hyphae (without chitin). Sexual reproduction occurs between gametangia, either on separate or identical hyphae. While some species are saprophytes, they are also parasites on both plants and animals. For example, *Lagenidium giganteum* parasitizes mosquito larvae, and certain Lagenidium species are a threat to crabs and other aquatic crustaceans (Hatai *et al.*, 2000) [10].

### Phylum Chytridiomycota

This particular group of fungi has coenocytic hyphae, chitin-based cell walls, and zoospores with a single flagellum. Based on rRNA phylogenetic comparisons, this fungal group is considered to be basal. The genus Coelomomyces, which belongs to the order Blastocladiales and includes the insect-infecting Chytridiomycetes (Barr, 2001) [3], is known to have about 70 insect pathogenic species. These fungi mainly infect Hemipterans and Dipterans. Coelomycidium (Blastocladiales) targets mosquitoes, while Myriophagus (Chytridiales) affects Dipteran pupae.

### Phylum Zygomycota

Mycelium is made up of nonseptate, multicellular gametangia that fuse to generate zygospores. Trichomycetes are a group of species in the Zygomycota class that are primarily related to insects. There are more than 200 species in the order Entomophthorales that can infect insects.

### Phylum Ascomycota and Dueteromycota

Ascospores, or sexual spores, are generated in the ascus, the fruiting body, and mycelia are septate, haploid. Every ascus typically produces eight ascospores. Cordyceps contains more than 300 entomopathogenic species. *Aspergillus, Metarhizium, Hirsutella, Beauveria, Aschersonia, Culicinomyces, Lecanicillium, Paecilomyces, Tolypocladium*, and others are the most prevalent among them.

### Phylum Basidiomycota

There are very few reports of pathogenic Basidiomycetes for insects. According to Samson *et al.* (2016) [23], *Uredinella* and *Septobasidium* are entomopathogenic.

## Life Cycle of Entomopathogens

Entomopathogenic fungi synchronize their life cycles with insect host stages and environmental conditions. These fungi can be found in the environment as spores or resting spores. They infect insects when the spores come into contact with the insects on plant surfaces, in soil, in the air as windborne particles, or on deceased insects' bodies. Adhesive processes involve both physical and chemical interactions. Once an insect is infected, the spores germinate on its cuticle. The process of infection may vary between different genera, species, and isolates within a species. Factors such as insect host range, infection levels, germination rates, and temperature optima can vary and influence the infection process (Shaw *et al.*, 2002) [26]. Spores enter the insects through cracks or folds in the cuticle, and once germinated, they form a germ tube and enter the haemocoel (Baverstock *et al.*, 2010) [4]. The fungi then proliferate in the host and produce toxins that eventually cause the death of the insect. After the insects die, the fungus spreads through their exoskeleton, usually in thinner spots like seams or joints, and begins to produce spores. Many species develop resting spores that can cause infection when environmental conditions are suitable. The spores spread passively through the action of wind, rain, or contact with other hosts or animals in the environment. Insects killed by fungi often have a "fuzzy" appearance due to the way their exoskeletons protrude. If the spores do not find a host, they either die in the soil or remain in crop plants. Most spores are viable for only one growing season. However, some fungal species develop dormant spores that, under the right circumstances, can become infectious.

## Mode of Action of EPF

### Encounter with the Host

The host can play two different roles for the fungus: passive (serving as the main source of sustenance for the fungus) or active (fungal contact with the host is necessary under certain conditions) (Stefanini, 2018) [29]. In most cases, high humidity is needed for all developmental phases of fungi, while for infection, specific, limited ranges of temperature are required. The humidity requirement varies among different species; for example, *A. lecanii* (*L. lecanii*) (hyphomycete) at leaf level requires 100% humidity for 16 hours to kill whiteflies. Some entomopathogenic fungi (EPFs) can tolerate low humidity (Steinkraus and Kramer, 1987). Soil characteristics such as pH, moisture content, and organic matter significantly influence the pathogenicity of fungi. Wind and sunshine are additional abiotic elements that impact the relationship between infection and the host. The susceptibility of different strains and species to sunlight and ultraviolet (UV) rays varies significantly, as both can impair the ability of conidia to survive and the persistence of infection in different types of fungi (Fernandes *et al.*, 2015) [6]. Humidity is not a limiting factor for aquatic species of insect diseases; instead, temperature, salt, and organic pollutants are crucial for these species (Goettel and Glare, 2010) [8].

**Adhesion and Germination of Conidia**

Fungi have a unique and complex way of infecting insects by either penetrating their outer covering or entering through their mouthparts (Ortiz-Urquiza and N.O. Keyhani 2013) [18]. Unlike viruses, nematodes, and bacteria, which need specific entry points, entomopathogenic fungi can infect insects by penetrating almost anywhere on their outer covering. The fungus starts the infection by attaching its spores, such as conidia or blastospores, to the insect's outer covering before eventually entering the insect's body through the outer covering or possibly through the mouthparts.

The outer covering of insects, called the cuticle, is the first barrier that the fungus needs to overcome to infect the insect. The cuticle has a complex structure that changes as the insect goes through different life stages. It consists of several layers, including the epicuticle, which is the outermost layer, and the procuticle, which is further divided into exo-, meso-, and endo-cuticular layers. Beneath the cuticle, there is the innermost layer called the epidermis, which encloses the internal systems of the insect.

For the fungus to adhere to the epicuticular layer, two stages are involved: the initial passive attachment of fungal cells to the surface of the insect and the firm attachment that follows, although the details of this process are not yet clear. Studies on *B. bassiana* adhesion to different surfaces showed that conidia strongly bound to hydrophobic surfaces but weakly to hydrophilic surfaces. Additionally, *B. bassiana* forms a rodlet layer with the help of two hydrophobins (Hyd1 and Hyd2), which also contribute to cell surface hydrophobicity, adhesion to hydrophobic surfaces, and pathogenicity. Several genes responsible for infection have been identified in both Metarhizium and Beauveria, such as Mad1 and Mad2 in *M. anisopliae*. Homologs of these genes have also been found in the *B. bassiana* genome, although their specific functions are yet to be determined.

**Infection Structure Formation**

When a fungal spore comes into contact with the host's cuticle, it triggers the expression of enzymes such as protease, chitinase, lipase, and other hydrolytic enzymes. These enzymes help the fungus to germinate and grow on the host's surface, as well as penetrate the cuticular layers. The fungus forms a germ tube or an appressorium, which attaches to the cuticle and allows for a narrow penetration peg, enabling the fungus to penetrate the cuticle. The formation of the appressorium is an adaptation that efficiently concentrates chemical and physical energy in a small area to aid in the penetration process. The topography of the host surface, as well as the intracellular second messengers Ca2+ and cyclic AMP (cAMP), all play a role in the formation of the appressorium.

**Penetration of Host Cuticle**

For an organism to enter, both mechanical pressure and enzymatic degradation are required. Entry points are typically seen as dark, melanotic lesions in the outer layer. Insects and entomopathogenic fungi are known to produce various cuticle-degrading proteases (Samuels and Paterson, 1995) [24]. Proteases such as collagenase, chymotrypsin, trypsin, and chymoelastase have been identified. Hepburn (1985) [11] has noted that endoproteases (PR1 and PR2) and aminopeptidase are among the earliest enzymes produced on the cuticle and are closely linked to the development of appressoria. These endoproteases are crucial for the penetration process, as approximately 70% of the cuticle is made up of protein. The insect cuticle is a complex structure and requires the action of various molecules working together. Studies have linked eight cytochrome P450 (CYP) genes, four catalases, three lipases/esterases, long-chain alcohol and aldehyde dehydrogenases, and a potential hydrocarbon carrier protein to the degradation of cuticular lipids in B. bassiana (Mannino, M.C., *et al.,* 2021) [16]. After gaining entry, the fungus spreads into the insect's bloodstream by producing blastospores or by forming a structure that looks like yeast. It then enters the insect's respiratory system to absorb nutrients. Several factors contribute to the insect's death, including mechanical injury from tissue invasion, depletion of food, and the production of toxins in the insect's body (toxicosis).

**Toxin Production**

"Various harmful mycotoxins are produced by Deuteromycetes fungi and have detrimental effects on insect health. These mycotoxins can cause cellular disruption and then penetrate the hyphae. They are known to affect insect health by causing changes in behavior such as partial or general paralysis, sluggishness, and decreased irritability. Eyal *et al.* isolated several toxins including brassinolide, isarolides, beauverolides, and beauvericin from *B. bassiana* in 1994. It has been observed that infected insects of Metarhizium anisopliae produce cytochalasins and destruxins (DTXs). DTX depolarizes the muscle membrane of lepidopterans and alters the activity of insect hemocytes by opening calcium channels. These are some of the toxins produced by the EPFs."

**Destruxins**

Destruxins are cyclic depsipeptides that were first identified in M. anisopliae (Kodaira, 1961). They consist of five amino acids and a D-α hydroxyl acid. Currently, 28 destruxins have been isolated and identified from Metarhizium species, exhibiting various levels of activity. The quantity of destruxin has been associated with both host specificity and pathogenicity (Al-Aidroos and Roberts, 1978) [2]. These compounds are known to modulate the host cellular immune system, preventing the formation of nodules in infected insects and inhibiting their ability to phagocytose. Additionally, destruxins act as immune suppressants. Destruxin E was reported to have cytotoxic and cytostatic effects on mouse leukemia cells. In insect muscles, destruxins can open calcium channels, possibly aiding in the pathogen's establishment within the host. Following a fungus infection, these insect poisons may contribute to insect mortality.

**Beauvericin**

Beauvericin is a cyclic lactone trimer composed of d-α-hydroxyisovaleric acid and N-methyl l-phenylalanine amide. It is likely that beauvericin acts as an ionophore, forming complexes with divalent cations. Beauvericin is produced by

*Fusarium semitectum, F. moniliforme, C. fumosorosea* (*I. fumosorosea*), *B. bassiana*, and various plant pathogenic fungi belonging to the phylum Basidiomycota (Wang and Xu, 2012). It has been observed to have insecticidal effects on blowflies, Colorado potato beetles, and mosquito larvae, as well as exhibiting cytotoxic properties.

**Bassianolide**

Bassianolide is a cyclic polymer of d-α-hydroxyisovaleryl L-N-methylleucinol. It is extracted from strains of B. bassiana and *V. lecanii*, which were entomogenous on the pupae cadavers of *B. mori*. Bassianolide is toxic to insects (Eyal *et al.*, 1994) [5].

**Leucinostatins**

leucinostatin consists of l-threo-b-hydroxy leucine, 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD), and cis-4-methyl-l-proline. Leucinostatin possesses antibacterial properties that target a wide range of fungi and gram-positive bacteria. The main sources of leucinostatin isolation are *Purpureocillium lilacinus, Paecilomyces marquandii* (*Verticillium marquandii*), and *C. farinosa* (*I. farinosus*). In mice, this toxin is lethal when administered orally or intraperitoneally. Leucinostatins, mainly derived from an extract of *P. farinosus*, were discovered to have insecticidal effects against the Colorado potato beetle, Additionally, this toxin disrupts mitochondrial oxidative phosphorylation.

**Efrapeptins**

Efrapeptins are a group of peptide antibiotics produced by *Beauveria nivea*, a soil fungus, and *Tolypocladium niveum* (formerly known as *Tolypocladium inflatum*). When tested against preparations from entomopathogenic fungi (*M. anisopliae* and *T. niveum*), these peptides significantly inhibit mitochondrial oxidative phosphorylation and ATPase function. They bind to the soluble F′ portion of mitochondrial ATPase and are likely competitive inhibitors of catalysis.

**Mass production of EPF**

Chemical pesticides have been widely used for over 60 years to control weeds, insects, and plant diseases. However, in the 1970s and 1980s, there was a renewed interest in microbial agents due to the growing awareness of the environmental impact of chemical pesticides. This led to the introduction of a considerable number of EPF (entomopathogenic fungi) products, especially in the Indian subcontinent, China, and Latin America. In India, 104 biopesticides have been registered, with 56 of them being fungi-based biopesticides. EPF have been effectively used to control various pests of agricultural importance (Idrees *et al.*, 2023) [12], as well as mites (Parveen, S.S. and Rashtrapal, P.S., 2024) [20], due to their wide distribution, high virulence, ease of application, inexpensive mass production, and long shelf life (Khetan, 2001) [13]. Furthermore, they are safer for fish, birds, mammals, and other non-target organisms (Zimmermann, 2007) [32]. However, successful field use of entomopathogenic fungi requires effective mass production techniques. Sure, here is a revised version of the text for clarity:" The formulation of Additionally, they can be mass-cultured *in vitro* Fungal spores into a product with a long shelf life and suitable application techniques is essential for successful large-scale production at an economical and efficient rate. In an industrial setting, selecting strains for mass production that can sporulate on low-nutrient media and grow quickly is crucial. To maximize the production of conidia, it is important to select the right medium for mass multiplication of entomopathogenic fungi. The chosen medium will define the quantifiable pathogenicity of the fungi. The dietary components and the amount of accessible nutrients in entomopathogenic fungi can impact their growth, sporulation, and culture morphology. Evaluating the various substrates available in terms of conidial biomass, viability, stability, and disease-causing ability of the spores produced on different media is crucial.

**Commercial-scale production of entomopathogenic fungi**

EPF can be multiplied using methods such as solid-state fermentation or submerged fermentation. In solid-state fermentation, the fungus grows on solid substrates, while in submerged fermentation, it grows on liquid substrates. These processes require specific temperature, pH, and aeration conditions. Typically, the process involves inoculating a starter culture into the chosen medium, allowing it to grow, and then transferring it to a fresh medium to continue the multiplication process.

**Multiplication of EPF species on liquid fermenter**

The multiplication of EPF species usually involves cultivating them in a suitable growth medium under controlled conditions. The process includes inoculating the medium with a starter culture of EPF, providing optimal temperature, oxygen, pH, and nutrient conditions for growth, and allowing the fungus to proliferate. After a certain incubation period, the biomass can be harvested and formulated for further use in various applications.

- On Shaker-Small scale production
- On Growth Bags-Medium Scale Production
- Bioreactor/Fermenter-Commercial Scale Production

**Submerged fermentation:** EPF can be cultivated in a liquid medium in bioreactors or fermenters. The fungus is inoculated into a nutrient-rich liquid medium. With proper control of parameters like temperature, pH, oxygenation, and agitation, it multiplies in the liquid.

**Merits & Demerits of liquid fermentation**

- Easy to scale up, Lower production cost, less time per batch, commercially viable due to lower investment, lower batch variation.
- Chance of contamination, lower yield when compared to SSF, Low stability of CFU

**Solid state fermentation of EPF**

- On small flask-Small scale production
- On Growth Bags-Medium Scale Production
- Solid State Fermenter (SSF)-Commercial Scale Production

In solid-state fermentation (SSF), a cultivation method in which fungus is grown on a solid substrate is utilized. For the cultivation of entomopathogenic fungi (EPF), this method typically involves using solid materials such as grains and wheat bran.

In SSF, the solid substrates are moistened to a specific water content, then additional nutrients are added, and the substrates are inoculated with EPF spores or mycelium. The growth conditions are optimized for factors such as temperature, humidity, aeration, and sometimes pH. As a result, EPF grows and produces enzymes and secondary metabolites, which can be harvested after a designated fermentation period.

**Merits & Demerits of SSF**

- The method is favoured due to its cost-effectiveness, high product yields, feasible for different kind of formulation and potential for using waste materials as substrates.
- Isolation and selection of high-yielding strains: Identifying and using strains of EPF that exhibit faster growth and higher yields can contribute to mass multiplication efforts.
- Maintaining sterile conditions, monitoring growth parameters, and regular checks for contamination are crucial during the mass multiplication process.

**Substrates Used in mass production.**

Several attempts have been made to screen low-cost components for the mass production of industrialized biological insecticides. To increase mass production and expedite commercialization, a comprehensive analysis of fungal nutrition consumption is crucial. Yeast extract (SDAY)-supplemented Sabouraud dextrose agar (SDA) is widely used by insect pathologists for successful isolation and mass multiplication of the EPF. However, these media are expensive and not economically feasible for large-scale production units.

Various organic materials have been considered for use as substrates for Ascomycetes. While barley and rice are commonly used substrates in the Northern Hemisphere and the tropics, respectively, efforts have been made to identify inexpensive agricultural materials, especially waste and byproducts, as ideal substrates.

Some studies have quantitatively examined basic nutritional aspects, such as C:N ratio (Ortiz-Urquiza *et al.*, 2010) [19] or controlled levels of carbohydrates.

Among the various EPF, Metarhizium and Beauveria are highly potential groups used for managing major agricultural pests. Selecting a medium conducive to their growth and sporulation is essential for large-scale mass production and field use of entomopathogenic fungi.

When isolates of B. bassiana (Bb-BH1 and Bb-BH2) and M. anisopliae (Ma-PO1) were mass multiplied using solid substrates (rice, wheat, and oats) and liquid substrates (PDA, SDA, and OMA), the highest conidial count per unit area for B. bassiana isolates was obtained from PDA and rice grains, while wheat and sorghum resulted in maximum sporulation for *M. anisopliae*.

In Himachal Pradesh, Brahmina coriacea (Hope) is the predominant species of white grubs, posing a significant threat to vegetables like potatoes and causing substantial losses (40-90 per cent). *Beauveria brongiartii* is a successful biocontrol agent for the management of this pest, and cowpea (Soni *et al.*, 2017) [28] and sorghum are known for their ability to support high conidial mass and spore count during the mass multiplication of this species.

A critical aspect of biocontrol is the production of an ample supply of high-quality inoculum. To mass-produce three entomopathogenic fungi—*Beauveria bassiana*, *Paecilomyces fumosoroseus*, and *Verticillium lecanii*—a variety of agricultural materials and byproducts were investigated. These materials include grains, vegetable wastes, seeds, rice husk, sawdust, and liquid media such as coconut water, rice-washed water, and rice-cooked water. Among the grains, sorghum yielded the highest spore production for *P. fumosoroseus* and *V. lecanii*, while wheat supported the most spores for *B. bassiana*. Additionally, all three fungi displayed robust growth and sporulation when supported by carrot, jack seeds, and okra. Coconut water was found to be particularly beneficial for sporulation and maximal. Fishery waste, along with dextrose, coconut water, rice wash water, rice boiling water, and various minerals and glucose, collectively support the growth and spore production of fungi.

A study has confirmed that rice bran enriched with cricket powder is one of the most suitable media for the mass production of virulent *B. bassiana* among the various agricultural waste used. Not only does the influence of nutrients contained in rice bran affect this, but the chitin content also influences mortality toward Spodoptera litura larvae in rice bran enriched with cricket powder. The use of agricultural waste, such as Farmyard Manure (FYM) and sugarcane bagasse, was studied in the mass production of *B. bassiana*, *M. anisopliae*, and *V. lecanii*. The highest spore yield was achieved when using FYM, followed by SDB, and the lowest yield was obtained from sugarcane bagasse. Among the different media produced *in vitro*, FYM was found to be the most effective and low-cost substrate for spore production, followed by a mixture of Pressmud and FYM in a 1:1 ratio with 1.0g of dextrose for *B. bassiana*, SDB for *M. anisopliae*, and Crushed jowar grain with 1.0g of dextrose for *V. lecanii*.

At present, the most commonly used technique for cultivating *M. anisopliae*, *M. flavoviridae*, and *B. bassiana* involves using rice in polypropylene bags. Another method involves inoculating blastospore suspension into plastic bags containing parboiled, autoclaved rice. Even carrots are the most cost-effective and suitable medium for large-scale cultivation of deuteromycete fungus.

**Table 1:** Solid substrates used for the production of Entomopathogenic Ascomycetes

| Sl. No | Substrates used | Entomo-pathogenic Fungi |
|---|---|---|
| 1. | Agricultural byproducts | *Metarhizium* |
| 2. | Rice flour | *Metarhizium* |
| 3. | Rice bran ± 2% dextrose | *Beauveria, Metarhizium* |
| 4. | Rice + saccharomyces | *Beauveria, Metarhizium* |
| 5. | Press mud ± 2% dextrose | *Beauveria* |
| 6. | Rice | *Metarhizium* |
| 7. | Potato tubers | *Beauveria* |
| 8. | Prawn waste | *Beauveria* |
| 9. | Pearl millet | *Beauveria* |
| 10. | Maize bran ± 2% dextrose | *Beauveria* |
| 11. | Maize | *Beauveria, Metarhizium* |
| 12. | Kodo millet | *Metarhizium* |
| 13. | Groundnut cake | *Beauveria* |
| 14. | Mijo grains + organic nitrogen | *Nomuraea* |
| 15. | Chickpea | *Metarhizium* |
| 16. | Broken rice | *Metarhizium* |
| 17. | Rice husk ± 2% dextrose | *Beauveria* |
| 18. | Sugarcane bagasse ± yeast, molasses | *Beauveria, Metarhizium* |
| 19. | Wheat | *Beauveria, Metarhizium* |
| 20. | Wheat bran + organic nitrogen | *Nomuraea* |
| 21. | Wheat bran ± 2% dextrose | *Beauveria* |

**Table 2:** List of major used EPF products registered under CIBRC

| EPF Species | Strain | Formulation | Pest |
|---|---|---|---|
| *Beauveria bassiana* | - | 1.15% WP | Cotton bollworm, Rice leaf folder, |
| | BB-ICAR-RJP | 1.15% WP | Rice leaf folder |
| | BB-5372 | 1.15% WP | Rice leaf folder |
| | ICAR | 1.15% WP | Rice leaf folder |
| | NBAIM | 1.15% WP | Rice leaf folder |
| | BCRL | 1.15% WP | DBM |
| | NBRI-9947 | 1.0% WP | *Helicoverpa armigera* |
| | IPL/BB/MI/01 | 1.0% WP | Fruit borer (Okra), Spotted bollworm |
| | SVBPU/CSP/Bb-10 | 1.0% WP | *Helicoverpa armigera* |
| | IARI | 5.0% WP | DBM |
| | NBAII | 5.0% SC | Tomato fruit borer |
| | BB-AAU-RJP | 5.0% AS | Tomato fruit borer |
| | - | 10.00% SC | DBM |
| | - | 1.5% Liquid | Tomato fruit borer |
| *Metarhizium anisopliae* | - | 1.15% WP | Brown plant hopper (*Nilapavata lungens*) |
| | IPL/KC/44 | 1.0% WP | Shoot & Fruit borer (*Leucinodes orbonalis*) |
| | AAI | 1.15% WP | *Heliothis armigera* |
| | BCRL-Me | 10% GR | Potato white grub, |
| *Trichoderma harzianum* | IIHR-TH-2 | 1.0% WP | Root-knot nematodes (*Meloidogyne incognita*) (Tomato, Brinjal, Carrot, Okra, Gerbera, Carnation, Tuberose, Banana, Acid lime and Papaya) |
| | IIHR-TV-5 | 1.5% WP | Root-knot nematodes, (*Meloidogyne incognita*) (Tomato, Brinjal, Carrot and Okra) |
| *Verticillium chlamydosporium* | IIHR-VC-3 | 1.0% WP | Root-knot nematodes, (*Meloidogyne incognita*) (Tomato, Brinjal, Carrot and Okra) |
| *Verticillium lecanii* | AS MEGH-VL | 1.15%WP, | Cotton White flies, Citrus Mealybug (*Planococcus citri*) |
| | T Stanes VI-1 | 1.50% Liquid Formulation | Tomato White fly (*Bemisia tabaci*) |
| | MPKV/Biocontrol/RVN/VL-01 | 3.0% AS | Onion Thrips (*Thrips tabac*i) |
| | | 5.0% SC | DBM |
| | Own Red Isolate | 5.0% SC | Rice White backed plant hopper (*Sogotella furcifera*) |
| *Paecilomyces lilacinus* | T-Stanes PI-1 Strain | 01.15% WP | Brinjal Root Knot Nematode |

## Conclusion

Fungi and insects have co-evolved over thousands of years, leading to complex relationships involving both positive and negative interactions. These interactions have led to the development of biological control agents, such as entomopathogenic fungi (EPF), which can be used for integrated pest management to reduce reliance on chemical pesticides. However, in order to effectively use EPF as biological control agents, it is important to understand their susceptibility to environmental conditions and to develop formulations with longer shelf lives. Additionally, there is a need to improve the pathogenesis mechanism of EPF and to enhance their general field activity through molecular biology techniques. To further improve the efficacy and sustainability of integrated control techniques, it is important to explore synergistic combinations of EPF with other control strategies, such as chemical pesticides, other natural enemies, resistant plants, organic farming, and remote sensing.

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