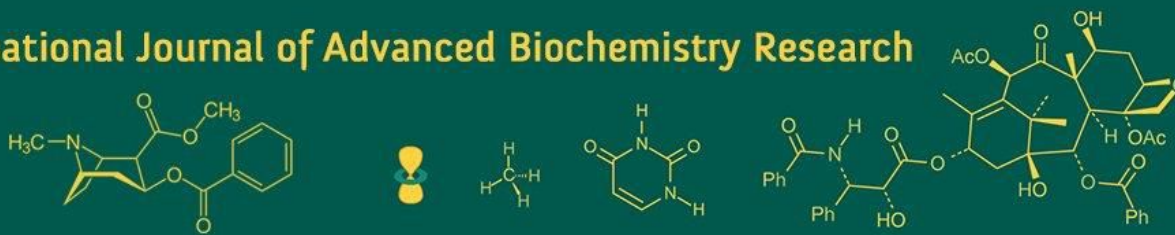


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Entomopathogenic fungi: Their role in controlling insect pests and disease suppression

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Abstract

Entomopathogenic fungi (EPF) represent a promising solution for sustainable pest management in agriculture by controlling insect pests and indirectly suppressing plant diseases. These fungi, including *Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii*, infect insects through direct contact, penetrating their cuticles, proliferating internally, and eventually killing the host. This process reduces pest populations and mitigates crop damage. Beyond pest control, EPF can colonize plant roots and contribute to disease suppression by forming protective barriers against soilborne pathogens and triggering induced systemic resistance (ISR) in plants. For example, *B. bassiana* effectively controls whitefly populations, *M. anisopliae* is utilized for locust management, and *L. lecanii* is applied to aphid control, reducing the spread of plant viruses. Recent research has enhanced the efficacy of EPF through improved formulations and integration with other beneficial microorganisms, increasing their field performance. Additionally, the development of EPF-based biopesticides, such as Botanigard® and Met52®, offers environmentally friendly alternatives to chemical pesticides, addressing concerns over pesticide resistance and environmental impact. As agriculture faces growing challenges, EPF play a critical role in integrated pest management (IPM) strategies, offering a sustainable path forward by reducing reliance on synthetic chemicals and enhancing crop resilience. This review explores the mechanisms, applications, and recent advancements of EPF, highlighting their potential to revolutionize pest and disease management in modern agriculture.

Keywords: Entomopathogenic fungi, plant diseases, biocontrol, disease suppression

Introduction

The growing demand for sustainable agriculture has led to an increased focus on biological control agents as alternatives to chemical pesticides. Among these agents, entomopathogenic fungi (EPF) have gained prominence for their ability to naturally infect and kill insect pests, offering a biological solution to pest management. EPF belong to a diverse group of fungi, including genera such as *Beauveria*, *Metarhizium*, *Lecanicillium*, and *Isaria*, which are effective against a wide range of insect pests. These fungi are known for their ability to penetrate the insect cuticle, proliferate within the host, and eventually kill the insect, reducing pest populations and minimizing crop damage (Shah and Pell, 2003; Butt *et al.*, 2016) [42, 11].

One of the key advantages of EPF is their dual role in pest control and plant disease suppression. Beyond killing insect pests, certain strains of EPF can colonize plant roots and induce systemic resistance, a mechanism by which plants enhance their defenses against pathogens (Ownley *et al.*, 2010) [38]. For instance, *Beauveria bassiana* has been shown to suppress root-knot nematodes (*Meloidogyne spp.*) in tomato plants, providing both pest control and disease suppression (Jaber and Ownley, 2018) [25]. Similarly, *Metarhizium anisopliae* has demonstrated potential in reducing the impact of soilborne diseases by forming a protective barrier around plant roots (Kabaluk and Gazdik, 2005) [27].

In addition to direct pest control, EPF contribute to plant health by reducing the transmission of plant viruses through insect vectors. Insects like aphids and whiteflies are known to spread viruses, but by controlling these insect populations, EPF can help limit the spread of viral diseases in crops (Cory and Ericsson, 2010) [13]. The role of *Lecanicillium lecanii* in managing aphid populations and reducing the incidence of viral infections in crops such as beans and potatoes is a notable example (Gottwald and Tedders, 1984) [21]. Despite their potential, the efficacy of EPF in field conditions can be influenced by environmental factors.

Recent advancements in the formulation and application of EPF have improved their stability and effectiveness, leading to the commercialization of EPF-based biopesticides such as Botanigard® (based on *Beauveria bassiana*) and Met52® (based on *Metarhizium anisopliae*) (Jackson *et al.*, 2010; Lacey *et al.*, 2015) [26, 31]. These biopesticides offer sustainable alternatives to chemical pesticides, addressing concerns about pesticide resistance and environmental contamination. This article explores the mechanisms, applications, and recent developments in the use of entomopathogenic fungi for controlling insect pests and suppressing plant diseases. Through detailed examples and recent research, the potential of EPF as integral components of sustainable agricultural practices is highlighted.

Mechanism of action

Entomopathogenic fungi (EPF) exhibit a complex and multi-faceted mechanism of action that makes them effective biological control agents against a variety of insect pests. The infection process begins when fungal spores (conidia) come into contact with the insect's cuticle. The initial step is the adhesion of the conidia to the insect's cuticle, which is facilitated by hydrophobic interactions and specific binding proteins on the spore surface (Holder & Keyhani, 2005) [22]. This is followed by germination of the spore, forming a germ tube that penetrates the insect cuticle through enzymatic degradation and mechanical pressure (Figure. 1).

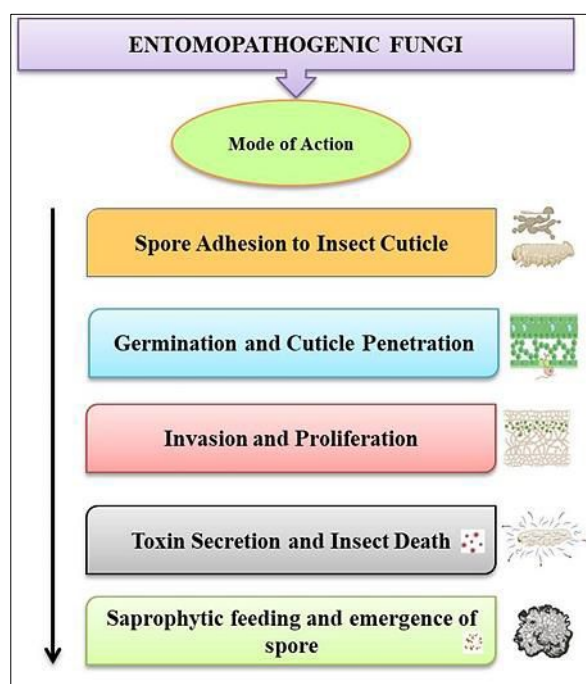


Fig 1: Mode of action of entomopathogenic fungi (created by PPT BioRender.com)

Key enzymes involved in the penetration process include cuticle-degrading proteases, chitinases, and lipases, which break down the structural components of the insect cuticle, such as proteins, chitin, and lipids (Leger *et al.*, 1986) [45]. These enzymes not only facilitate penetration but also allow the fungi to bypass the host's immune defenses. For instance, *Beauveria bassiana* produces proteases that degrade the insect's cuticle, enabling the fungus to enter the hemocoel (the insect's body cavity) where it proliferates (Charnley & Leger, 2011) [12].

Once inside the insect's hemocoel, the fungus undergoes rapid proliferation. The hyphae multiply, forming a mycelial network that invades the insect's internal tissues. During this phase, the fungus secretes a range of secondary metabolites, including toxins like beauvericin and destruxins, which disrupt cellular functions and contribute to the insect's death (Gillespie & Claydon, 1989) [18]. These toxins can cause physiological disruptions such as inhibition of immune responses, blocking ion channels, and inducing apoptosis (programmed cell death) in host cells (Wang *et al.*, 2016) [49].

The insect's death usually occurs within a few days to a week, depending on environmental conditions and the fungal strain. After the insect dies, the fungus emerges from the cadaver, producing new spores on the surface. These spores can then be dispersed to infect new hosts, completing the infection cycle (Butt *et al.*, 2016) [11]. Moreover, some EPF can also act as endophytes, colonizing plant tissues and enhancing plant resistance against both insect pests and pathogens. For example, *Metarhizium anisopliae* and *Beauveria bassiana* have been shown to induce systemic resistance in plants, enhancing their ability to fend off subsequent pathogen attacks (Vega *et al.*, 2009) [47]. This dual functionality - direct insecticidal activity and indirect promotion of plant health - makes EPF particularly valuable in integrated pest management (IPM) programs.

Significant Examples of Entomopathogenic Fungi in Pest Management

Entomopathogenic fungi (EPF) are widely used in pest management due to their ability to naturally control a variety of insect pests. Several species have demonstrated significant efficacy across different agricultural ecosystems, providing environmentally friendly alternatives to chemical pesticides. Below are some of the most notable examples of EPF and their applications in pest control:

The *Beauveria bassiana* is one of the most extensively studied and commercially used entomopathogenic fungi. It is effective against a wide range of insect pests, including whiteflies, aphids, beetles, and caterpillars. The fungus infects the host by penetrating the cuticle, proliferating inside the insect, and ultimately causing death (Butt *et al.*, 2016) [11]. *B. bassiana* has been successfully used in controlling the Colorado potato beetle (*Leptinotarsa decemlineata*) and the coffee borer beetle (*Hypothenemus hampei*), both of which are major pests in their respective crops (Lacey *et al.*, 2015) [31]. The commercial formulation Botanigard®, which contains *B. bassiana*, is widely applied in greenhouses and field crops as a biopesticide (Jackson *et al.*, 2010) [26].

The *Metarhizium anisopliae* is another widely used EPF, known for its effectiveness against soil-dwelling pests like termites, locusts, and grasshoppers. This fungus is particularly effective in controlling locust plagues, which can cause significant agricultural damage across large regions (Zimmermann, 2007) [51]. *M. anisopliae* has been used in locust control programs in Africa, where it has demonstrated the ability to reduce locust populations without harming non-target organisms (Kassa *et al.*, 2002) [29]. The commercial product Met52® is based on this fungus and is used to control root weevils, thrips, and ticks in various cropping systems (Lacey *et al.*, 2015) [31]. Formerly known as *Verticillium lecanii*, this fungus is effective against aphids, whiteflies, and thrips, which are

common pests in greenhouse environments. *Lecanicillium lecanii* works by adhering to the insect's cuticle, penetrating it, and proliferating within the host, leading to death. This fungus is particularly valuable in managing aphid populations that spread viral diseases in crops such as potatoes and beans (Gottwald & Tedders, 1984) [21]. The commercial product Mycotal®, based on *L. lecanii*, is commonly used in greenhouse settings to control whiteflies and thrips in ornamental plants and vegetables (Askary *et al.*, 2017) [4].

The *Isaria fumosorosea* is another versatile EPF that targets a range of pests, including whiteflies, thrips, aphids, and various lepidopteran larvae. It is particularly effective in controlling whiteflies (*Bemisia tabaci*), a notorious pest in cotton and vegetable crops. *I. fumosorosea* has been used in integrated pest management (IPM) programs to manage whitefly populations in greenhouses, often in combination with other biological control agents like parasitoids (Wraight *et al.*, 2016) [50]. The commercial product PFR-97®, which contains *I. fumosorosea*, is utilized in various agricultural systems to control soft-bodied insects (Faria & Wraight, 2007) [16]. The *Hirsutella thompsonii* is a specialized EPF used primarily against mite pests, such as the citrus rust mite (*Phyllocoptruta oleivora*). This fungus is applied in citrus orchards to control mite populations, which can cause significant yield losses in citrus production (McCoy *et al.*, 1988) [35]. *H. thompsonii* has been found to reduce mite infestations effectively without harming beneficial arthropods, making it a valuable tool in IPM programs (Vega *et al.*, 2009) [47].

The growing interest in EPF has led to increased research and development of new fungal strains and formulations. For example, studies have shown that combining EPF with other beneficial microorganisms, such as mycorrhizal fungi or rhizobacteria, can enhance their efficacy by improving plant health and stress tolerance (Goettel *et al.*, 2008) [19]. Moreover, advances in formulation technologies, such as encapsulation and liquid culture production, have improved the stability and shelf-life of EPF-based products, making them more accessible and reliable for growers (Mascarin & Jaronski, 2016) [34].

Interactions and Applications

Interactions between plants and entomopathogenic fungi

Entomopathogenic fungi (EPF) play a multifaceted role in pest control, extending beyond their primary function as pest regulators through epizootics. These fungi also serve as endophytes, colonizing internal plant tissues [Bing and Lewis, 1991; Vega, 2008] [8, 46], and rhizosphere colonizers [Hu and St. Leger, 2002; Bruck, 2005; Meyling and Eilenberg, 2007] [23, 10, 28, 36]. They promote plant growth and fitness [Kabaluk and Ericsson, 2007] [28], enhance drought tolerance, and act as biofertilizers [Vega, 2008] [46]. Additionally, EPF can antagonize plant pathogens by occupying vital nutrient space [Keller, 2009] and inducing systemic resistance, as observed with *Beauveria bassiana* in cotton against *Xanthomonas axonopodis* pv. *malvacearum* [Ownley *et al.*, 2008] [37].

B. bassiana is widespread across various plant hosts, though some fungal isolates struggle to colonize certain plants due to factors like plant genetics, fungal characteristics, and competition with other endophytes [Posada *et al.*, 2007; Akello *et al.*, 2007; Barta and Cagán, 2009] [40, 2, 6]. EPF have shown promise in reducing plant damage from

herbivores, such as *Ostrinia nubilalis* in maize [Bing and Lewis, 1992; Lewis *et al.*, 2001] [9, 32], *Tuta absoluta* in tomatoes [Garrido-Jurado *et al.*, 2016] [17], and *Cosmopolites sordidus* in bananas [Akello *et al.*, 2008] [3]. Foliar application, along with endophytic colonization, offers dual benefits by enhancing plant protection and minimizing exposure of conidia to adverse environmental conditions [Vega, 2008] [46].

Inoculating specific plant parts with EPF can minimize the risks posed by abiotic factors and provide lasting protection, as seen with *B. bassiana* in citrus [Bamisile *et al.*, 2018] [5]. EPF can establish systemic resistance against herbivores, even when only a single plant part is treated [Porras-Alfaro and Bayman, 2011] [39]. EPF persistence in the soil is influenced by soil type, plant species, and cultivation practices, with soil offering protection from UV light, high temperatures, and other environmental factors [St. Leger, 2008; Scheepmaker and Butt, 2010] [41].

EPF are environmentally safe, with low mammalian toxicity and long-term persistence, making them appealing in integrated pest management (IPM) strategies [Lacey *et al.*, 2015] [31]. While fungal infections account for 60% of insect diseases, naturally occurring infections in crops are often insufficient to prevent damage. Despite the potential of virulent fungi like *B. bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*, their use remains limited due to practical challenges such as high formulation costs, short inoculum shelf life, and the need for high humidity during application [Faria and Wraight, 2007] [16]. Nevertheless, EPF remain a crucial component of IPM, providing an eco-friendly alternative to chemical pesticides, which are increasingly burdening the environment and posing risks to consumer safety [Meyling and Eilenberg, 2007; Vega *et al.*, 2009] [36, 47].

Single-Agent and Combined-Agent Mechanisms in Pest Control with Entomopathogenic Fungi

Insect infection by entomopathogenic fungi (EPF) begins with fungal spore adhesion to the cuticle, followed by germination and enzyme production, enabling cuticle penetration. Once in the hemolymph, the fungus proliferates, causing insect death through nutrient depletion, toxin production, and organ disruption (Inglis *et al.*, 2001) [24]. Environmental factors, fungal virulence, and host susceptibility significantly influence infection outcomes. For instance, *Beauveria bassiana* induces a strong immune response in insects, ultimately leading to their death (Shang *et al.*, 2022) [43]. Fungal effectiveness varies based on species, host interaction, and environmental conditions, with high spore density correlating with increased insect mortality (Goettel *et al.*, 2000) [20]. *B. bassiana* shows broad temperature tolerance, thriving between 8 °C and 35 °C (Fargues *et al.*, 1997) [15].

Combining EPF with other agents enhances pest control. For example, *B. bassiana* effectively controls stored-grain pests like the rice weevil (*Sitophilus oryzae*) and the lesser grain borer (*Rhyzopertha dominica*) (Batta, 2005) [7]. Combining *B. bassiana* with *Chromolaena odorata* leaf powder or *Azadirachta indica* extracts increases mortality in pests like *Spodoptera litura* and reduces fungal spore viability (Afandhi *et al.*, 2020; Depieri *et al.*, 2005) [1, 14]. Synergistic effects have also been observed between *B. bassiana* and other fungi like *Trichoderma asperellum*, which enhances lethal activity against pests like the Asian

corn borer (*Ostrinia furnacalis*) (Mantzoukas *et al.*, 2019) [33]. Additionally, mixed infections of different EPFs can enhance or suppress entomopathogenic activity, often leading to synergistic effects in pest control (Mantzoukas *et al.*, 2019) [33]. For example, combining *Isaria fumosorosea* and *B. bassiana* increases mortality in both *S. oryzae* and *S. granarius* compared to single-agent applications (Mantzoukas *et al.*, 2019) [33].

Future prospects

Future research should focus on optimizing the application of EPF in diverse agricultural and storage environments. Advances in molecular techniques and genomics could enhance our understanding of fungal virulence factors and host-pathogen interactions, leading to the development of more effective fungal strains and formulations. Additionally, exploring the ecological impacts and long-term sustainability of EPF applications will be crucial for their widespread adoption. Investigating novel synergistic combinations of EPF with other biocontrol agents and evaluating their performance in real-world settings can provide valuable insights. Expanding research on the interaction between EPF and environmental factors, such as climate change, will help tailor pest control strategies to changing conditions. Overall, continued innovation and integration of EPF with other pest management practices hold the potential to improve pest control efficacy and sustainability in agriculture and beyond.

Conclusions

Entomopathogenic fungi (EPF) represent a promising tool for integrated pest management due to their specific mechanisms of action and ability to reduce reliance on chemical pesticides. Single-agent applications of EPF, such as *Beauveria bassiana* and *Metarhizium anisopliae*, involve spores adhering to the insect cuticle, germinating, and producing enzymes that penetrate the cuticle, leading to systemic infection and pest mortality. The effectiveness of these fungi depends on factors such as spore density, environmental conditions, and host susceptibility. *B. bassiana* and *M. anisopliae* exhibit broad temperature tolerances and can be effective across various climates, highlighting their versatility.

The combined use of EPF with other agents, such as plant extracts and diatomaceous earth, enhances pest control efficacy. Synergistic interactions between EPF and natural or chemical compounds can improve pest mortality rates and offer more sustainable pest management solutions. For example, combinations of *B. bassiana* with plant extracts or other fungi like *T. asperellum* have shown increased effectiveness against key agricultural pests. The incorporation of EPF into post-harvest pest management strategies also reduces the need for chemical fumigants, promoting environmentally friendly practices.

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Data availability

There is not hidden data, all data are presented in this manuscript.

Ethical statement

This study did not involve human or animal subjects; therefore, informed consent is not applicable.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have approved to influence the work reported in this paper.

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