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Integrated Pest Management Strategies Using Endophytic Entomopathogenic Fungi

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Abstract

Insect pests pose a significant threat to crops, causing extensive damage and facilitating the spread of various diseases transmitted by insects. The widespread use of chemical pesticides has been a common approach for managing these pests. However, due to continuous and prolonged usage over the years, insect pests have developed increasing resistance to virtually all types of chemical pesticides. This resistance has prompted a growing demand for alternative methods of pest control. In the quest for effective and environmentally safe insect pest control, insect pathogenic fungi emerge as a promising alternative to conventional pesticides. However, the feasibility of this strategy faces limitations due to a slow death rate and the need for high conidial concentrations. While the ability of these fungi to regulate insects has been explored previously, recent research has shifted focus towards their potential as plant endophytes. This role involves protecting plants from phytopathogens and enhancing various elements of agricultural output. This article delves into the significance of entomopathogenic fungi as endophytes in the realm of biological control. Our research for this review centers on identifying local strains of entomopathogenic fungi capable of colonizing endophytes and explores their potential utility in managing disease-causing pests.

Keywords

Biocontrol; Entomopathogenic fungi; Environmental friendly; Pest control

Introduction

Half of the world's population, amounting to three billion people, resides in rural areas, with 2.5 billion individuals relying on agriculture as their primary source of livelihood. The United Nations (UN) projects that by 2050, 70% of the existing global food supply will be required to meet the growing demand for food, necessitating the intensification and expansion of agricultural practices. Nevertheless, agricultural production faces threats such as climatic changes, an increase in insect pests, and the spread of diseases. Food crops are believed to suffer damage from over 10,000 different insect species, leading to an annual loss of 14% and incurring a cost exceeding USD 100 billion (1).

Agrochemical crop protection has been acknowledged for its role in sustaining and enhancing crop yields globally. However, the widespread and often irresponsible use of these chemicals has diminished the effectiveness of natural control mechanisms. This decline is attributed to issues such as pest resistance, the resurgence of secondary pests, and the disruption or destruction of natural enemy complexes. Growing concerns about the environmental and public safety implications of these factors have generated a demand for more reliable and cost-effective solutions that prioritize environmental safety (2).

One strategy for biocontrol involves utilizing bacteria, viruses, and fungi that naturally parasitize insects as agents. Examples include *Metarhizium anisopliae*, an increasingly popular pesticide substitute, and the insect-pathogenic fungus *Beauveria bassiana.* However, fungi have not performed as well as expected as biocontrol agents, primarily due to their longer death times compared to chemical pesticides, the requirement for large quantities of inocula, and inconsistent results compared to the chemicals they compete with. Pathogenic fungi infect insects by breaking through the host's cuticle, in contrast to bacteria and viruses, which need to be ingested to be contagious. The primary cuticle components (protein, chitin, and lipids) are broken down by the proteases, chitinases, and lipases produced by various insect illnesses, allowing hyphal penetration (3).

Integrated pest management (IPM), a comprehensive approach to crop production, integrates a diverse range of complementary techniques. These include sanitation, survey and detection, the use of resistant varieties, cultural manipulation, trap and companion cropping, biological control, and the judicious use of agricultural chemicals when necessary. The goal of IPM is to maintain pest populations below levels that cause economic damage (4). In contrast to traditional, individual, pest-centered strategies that heavily rely on chemical pesticides, IPM adopts a more holistic approach. It considers the entire agricultural production system, focusing on managing pests rather than attempting to eradicate them.

Endophytes – Role in Plants

Endophytes are commonly defined as microbes that inhabit the internal tissues of living plants without causing immediate, overt negative effects (5). Virtually every plant on the planet harbors endophytic bacteria. These microorganisms reside in the living tissues of the host plant and engage in a spectrum of interactions, ranging from symbiotic to potentially harmful. By definition, an endophytic fungus biologically interacts with a living plant during at least a portion of its time in mycelial form. Therefore, the presence of a fungus' hyphae in living tissue is a prerequisite for labeling it as an endophyte. An endophytic fungus is a type of fungal microbe that spends all or part of its life cycle colonizing the healthy tissues of the host plant while typically manifesting no outward signs of illness (6).

These fungi have been isolated from numerous types of grasses and woody plants, residing asymptomatically in plant tissues. Endophytic fungi, also known as acquired plant defenses, are hypothesized to engage in mutualistic interactions with their host plants, primarily by enhancing host resistance to herbivores. Endophytes initiate vital processes of nutrient cycling through the biodegradation of dead and dying host plant material. Endophytic fungi can now be reliably counted on to defend their hosts against herbivores. The activation of plant defenses through fungal endophytic colonization can directly impact herbivores and plant pathogens. Additionally, a crucial aspect of plant defense responses involves the release of volatile organic compounds, which function as an indirect defense mechanism by attracting the natural enemies of herbivores (7). Recently, the volatile compounds released by leaves of melon plants treated with various strains of entomopathogenic fungi, namely *Beauveria bassiana* (Bals.) Vuill. or *Metarhizum brunneum* (Petch) (Ascomycota:Hypocreales), were examined (8).

Various diseases affect animals that consume plants with endophyte infestations. Numerous publications describe the role of endophytes in defending host plants against insects by producing bioactive metabolites. It is well-acknowledged that fungi serve as a rich source of antibacterial compounds. Additionally, endophytes induce or activate the host's defense mechanisms (9).

Entomopathogenic Fungi:Green tool for pest management

One of the environmentally friendly techniques employed in integrated pest management programs is the use of entomopathogenic fungi. These fungi surpass other microbial pesticides derived from bacteria and viruses due to their distinct method of action and suitability for large-scale production (10). Fungi rank as the second most commonly used microbes for plant protection in the global biopesticide market (11). However, the efficacy of fungal entomopathogens is hindered by abiotic conditions that impede the viability of infectious propagules. One approach to address these challenges is to inoculate plants with these microbial populations. Fungal endophytes, a type of fungus, can live asymptomatically inside plants for all or part of their life cycles. In the past, endophytes were considered neutral entities that neither benefited nor harmed plants (12).

Later, fungal endophytes were thoroughly investigated for their imperceptible functions in plants. Some of these roles include protection from diseases and pests, as well as the enhancement of plant development. These fungal endophytes possess remarkable abilities to mitigate both abiotic and biotic stress factors, including drought, salinity, heavy metals, and other toxic compounds introduced by the environment. Moreover, they offer protection against floods, extreme temperatures, predators, and pathogens (13).A considerable number of naturally occurring fungal endophytes have been identified to date. The majority of these endophytes are found in members of the Gramineae plant family, along with entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillerain and *Metarhizium anisopliae* (Metchnikoff) Sorokin (14). Crop plants such as wheat, sorghum, coffee, and maize have all been discovered to contain *M. anisopliae* as endophytes. While few studies have revealed their mechanism of action, it seems to involve antibiosis or feeding deterrence induced by the toxins these organisms produce in plants (15).

Most plant species host endophytes, and these organisms have been found in all the environments studied so far (16). The taxonomic groups to which most fungal endophytes belong are Ascomycota, Basidiomycota, and Zygomycota. Common in nature, these endophytes are capable of producing a diverse array of secondary metabolites. These metabolites are commercially valuable and find extensive applications in biotechnology, agriculture, and human health (17).

Due to the advantageous traits conferred on their hosts, these fungi are extensively investigated in agriculture. Endophytes play a diverse array of symbiotic and ecological roles, stimulating plant development, preventing pathogenic organisms, eliminating soil toxins, and enhancing tolerance to adverse environmental factors such as temperature, water availability, and salinity. The interaction with the endophyte, in exchange for carbon-based resources, provides numerous benefits to the host plant (18).

Different endophyte species exhibit a spectrum of hosts, ranging from highly specific single species to those with many host species. The transmission of endophytes can occur either vertically (from one generation to the next) or horizontally (from one plant tissue to another) (19). They can spend a significant portion of their life cycle in plant tissues, providing protection to the host against pathogen invasion and avoiding external environmental changes that might jeopardize their survival and biocontrol efficacy (20).

Problems with Chemical Pesticides

Biological control and transgenic crops are increasingly being utilized, although chemical pesticides still constitute the majority of methods for eradicating arthropod pests. Despite the vast array of available pesticides, the majority of chemical insecticides operate by modifying one of the 6 molecular targets in insect synapses: Avermectins, glutamate, and gamma-aminobutyric acid (GABA) all bind to the glutamate receptor (21). Examples such as fipronil and cyclodienes can enter cation-gated chloride channels. Cation-gated sodium (NaV) channels are targeted by pyrethroids, dihydropyrazoles, and dichlorodiphenyltrichloroethane (DDT). Nicotinic acetylcholine receptors (nAChRs) function as ryanodine receptors. However, due to the extensive and prolonged use of these pesticides, more than 600 species of arthropods have developed resistance to one or more chemical insecticides (22) (Fig. 1).

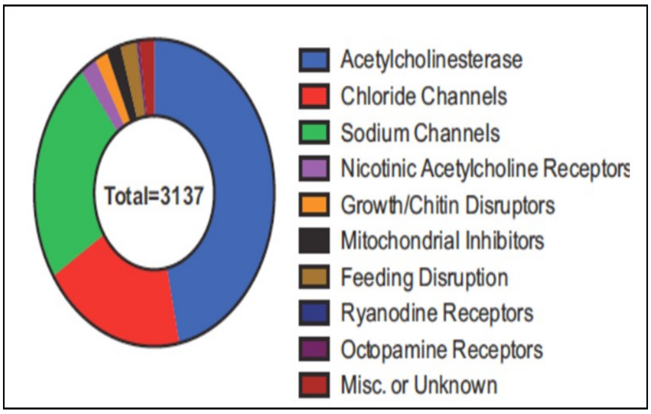

Fig. 1. Total number of insecticide resistance cases grouped according to molecular target (46)

Microbial control agents

The mentioned issues with chemical insecticides underscore the urgent need for the development of new, environmentally friendly insecticides. Biological control techniques for crop protection offer a potential and attractive alternative for managing insect pests. Microbial control agents consist of molecules with a biological origin, whether derived from the entire organism or its byproducts. They are considered less harmful to the environment due to their complex mechanism of action, making them less susceptible to resistance. Globally, North America leads in the use of biopesticides (44% of the total), followed by Europe, Latin America, and Asia (6% of the total). There have been significant successes in employing microorganisms such as viruses, bacteria, fungi, and nematodes as bio-control agents (23).

Entomopathogenic fungi as biocontrol agents

It has been observed that several other biological control agents, particularly the associated natural enemies of targeted pests, such as predators and parasitoids, can coexit with several Entomopathogenic Fungi (EPF) species. For instance, Jaber and Araj showed how the parasitoids *Aphidius colemani* Viereck (Hymenoptera:Braconidae) may be able to inhibit the green peach aphid *Myzus persicae* Sulzer (Hemiptera:Aphididae) in sweet pepper by working with *B. bassiana* and *M. brunneum*. Similarly, the pea leafminer, *Liriomyza huidobrensis* Blanchard (Diptera:Agromyzidae) can be controlled using two parasitoid species, *Diglyphus isaea* Walker (Hymenoptera:Eulophidae) and *Phaedrotoma scabriventris* Nixon (Hymenoptera:Braconidae), in combination with different fungal isolates, including *B. bassiana* and *H. lixii* (24).

The predatory mite *Phytoseiulus persimilis* Athias-Henrio (Acarina:Phytoseiidae) can be used in conjunction with 2 isolates of *B. bassiana* and *M. robertsii* to control the two-spotted spider mites *Tetranychus urticae* Koch (Acari:Tetranychidae) on strawberry plants in greenhouses and strawberry fields. Similarly, studies have showed that *L. lecanii* can be used in conjunction with an aphid alarm pheromone and sublethal doses of the insecticide imidacloprid as part of an auto-dissemination technique to enhance the effectiveness of the fungus for aphid biocontrol. Another typical generalist insect-pathogenic fungus species, *Zoophthora radicans* Brefeld (Zygomycetes:Entomophthorales), has undergone compatibility testing with various biocontrol agents (25).

The fungus was employed in an autodissemination method along with semiochemicals to manage the diamondback moth (*Plutella xylostella* Linnaeus) (Lepidoptera: Yponomeutidae). A host-specific semiochemical attracted the insects into an inoculation device, exposing the moths to *Z. radicans* conidia. When harvested strawberries were treated with *B. bassiana*, *M. anisopliae*, and various chemical fungicides, the treatment of *B. cinerea* and *Rhizopus* sp. was also found to be effective (26). It is crucial to apply specific biological control tactics in an integrated manner, in addition to other cultural or traditional interventions, as the majority of biological control strategies operate best when used in tandem. This approach can lead to a significant reduction in both biocontrol agents and pest populations. Utilizing classical and immunization methods alongside conservative practices can enhance the efficacy of both tactics (27).

Entomopathogenic fungal endophytes for plant disease control

Various plant species can host endophytes from entomopathogenic fungi, providing an opportunity for biological pest control along with their insect-controlling abilities. Entomopathogenic Fungal Endophytes (EFEs) might have the potential to simultaneously manage diseases and pests, as suggested by several researchers (28, 29). Additional information is available on EFE genera such as *Beauveria*, *Lecanicillium*, and *Metarhizium*, which have been associated with phytopathogenic activities. *B. bassiana* has been predominantly employed as an antagonistic endophyte to combat plant diseases caused by various pathogens, including fungi, bacteria, and viruses. Its ability to produce a diverse array of bioactive and antimicrobial metabolites, such as destruxins, oosporein, beauvericin, bassianolide, bassianin, beauveriolide, bassiacridin, cordycepin, and ciclosporin, may contribute to this capacity (30).

Pest control mode of action

Most fungal entomopathogens infect the insect directly through the cuticle, unlike bacteria and viruses, which typically infect their host through the gut (31). Under favorable conditions, fungi can adhere to an insect's cuticle, develop germ tubes, and penetrate the cuticle layer, forming swollen "holdfasts" known as appressoria to aid in penetration. The appressorium is responsible for tasks such as cuticle softening and breakdown by lipases, proteases, and chitinases, anchoring the cuticle for penetration, and concentrating penetration-related components (32).

The fungus undergoes a transformation from filamentous to multiplying into yeast-like hyphal structures. As a result, the insect succumbs to various causes, including physical obstruction, nutrient depletion, toxicosis, or organ invasion (33). The overall time until death is determined by the duration of the infection process and the virulence of the fungal isolate. Subsequently, hyphal bodies reemerge from the cadaver to release conidia. In contrast to bacteria and viruses, fungal infections do not require ingestion by the host; thus, they can be employed to control sucking insects such as mosquitoes and aphids (34).

Table 1. Entomopathogenic fungi produced commercially and experimentally.

Fungus	Product/ Trade name	Company/ Producer	Country/ Origin	Target pests	Reference
Culicinomyces clavisporus	-	-	Austria, Belgium, Czech	Mosquito larvae	(38)
Hirsutella thompsonii	Mycar	-	Austria, Belgium	Citrus rust mite	(39)
Metarhizium anisopliae	Meta-Sin®	-	-	Spittle bug; Sugarcane frog hopper	(40)
Nomuraea rileyi		-	-	Lepidopteran larvae	(41)
Verticillium lecanii	Vertalec	-	-	Aphids; Coffee green bug; Greenhouse whitefly thrips	(42)
Beauveria bassiana	Bio-Power	Stanes	India	Mite; Coffee green bug	(43)
	BotaniGard ES; Botani-Gard 22WP	Laverlam International (formerly Emerald BioAgriculture)	USA	-	-
	Boverol	Fytovita	Czech Republic	-	-
	Conidia	LST	Columbia	-	-
	Mycotrol ES; Myco-trol-O	Laverlam International (formerly Emerald BioAgriculture)	USA	-	-
	Naturalis	Intrachem	Italy	Aphids Spittle bug; Sugar-cane	(44)
	Naturalis-L Andermatt Biocontrol	Troy Biosciences Inc	Switzerland USA	-	-
	Ostrinil	Arysta (formerly NPP, Calliope)	France	-	-
	Proecol	Probioagro	Venezuela	-	-
	Racer BB	SOM Phytopharma	India	-	-
	Trichobass-L; Trichobass-P	AMC Chemical/Trichodex	Spain	-	-
	Beauveria Schweizer	Lbu (formerly Eric Schweizer Seeds)	Switzerland	Greenhouse whitefly thrips Mosquito larvae	(45)
B. brongniartii (*B. tenella*)	Betel	Arysta (formerly NPP, Calliope)	France	-	-
	Biolisa-Kamikiri	Nitto Denko	Japan	-	-
	Engerlingspilz	Andermatt Biocontrol AG	Switzerland	-	-
	Melocont-Pilzgerste	Agrifutur-Kwizda	Italy, Austria	-	-

Commercial development of fungal entomopathogens as bio-control agents

Since 1995, more than 100 fungus-based biocontrol products have received approval from the US Environmental Protection Agency (EPA). A commercially available product called Mycotrol, containing Beauveria, serves as a mycoinsecticide to eliminate aphids, grasshoppers, thrips, and whiteflies. "Green Muscle," produced commercially in Africa, is a conidial preparation of *M. anisopliae* var. *acridum* specifically harmful to grasshoppers and locusts. These conidia formulations can remain infectious for over a year when stored at 25–30°C without harming non-target organisms. As per the studies mentioned, fungal biopesticides are considered more environmentally friendly than chemical pesticides (35) (Table 1).

Problems with entomopathogenic fungi as bio-control

Because they infect insects more slowly than chemical pesticides and require a significant amount of inoculum, fungi have a very small market share. Occasionally, they also perform poorly in the field, reducing their efficiency as biological control agents. The susceptibility of fungal infections to environmental stress conditions accounts for their variable performance in the field. Even with more virulent strains, it takes 2–5 days before the host is destroyed (36). This delay may allow insects to cause substantial damage to crops even after the infestation. To achieve high mortality, the disease must find effective ways to increase its virulence. Consequently, both the median lethal dose (LD50) and median lethal time (LT50), representing the dose and duration needed to kill 50% of the test population, must be reduced (37).

Conclusion

Integrated Pest Management (IPM) strategies incorporating endophytic entomopathogenic fungi present a promising and environmentally friendly approach to pest control in agriculture. The use of these fungi not only enhances overall plant health but also provides sustainable, long-term solutions for pest management. By integrating these beneficial organisms into IPM programs, farmers can reduce their reliance on chemical pesticides, thereby decreasing the negative environmental impact associated with conventional pest control methods. Moreover, endophytic entomopathogenic fungi play a vital role in ecosystem functioning by contributing to the natural regulation of insect populations. Extensive research has been conducted to understand their mode of action and optimize their application techniques. Consequently, these unique organisms hold significant potential for revolutionizing the field of pest control and contributing to more sustainable agricultural practices globally.

Authors' contributions

SP collected the data and drafted the manuscript. PSR revised the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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