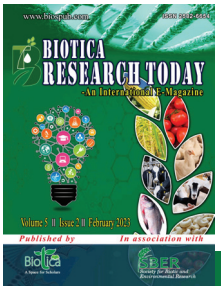
Bio. Res. Today 2023, 5(2):189-193



Biotica Research Today

e-ISSN: 2582-6654

February, 2023

Article ID: RT1255 **Popular Article**

Entomopathogenic Fungi: Mode of Action and Application Methods

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Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Prasad and Singh, 2023. Entomopathogenic Fungi: Mode of Action and Application Methods. *Biotica Research Today* 5(2), 189-193.

Abstract

Agriculture plays a vital role in Indian economy. Insect infestation significantly reduces the productivity of agricultural crop. In general, chemical pesticides are used to protect plants from insect-pests. Chemical pesticides have a negative influence on non-target species, resulting in biodiversity loss, food safety issues, insect resistance, and revival in novel locations. Over the last few decades, natural and biological pest and disease control of cultivated plants has been given more attention in the effort to minimize agricultural production's dependency on chemical products. Applying biological agents, predators, parasitoids, and microorganisms such as viruses, bacteria, and fungus to control pests has shown to be a successful and sustainable pest management strategy. Entomopathogens as bio-pesticides offer a safe, natural, and cost-effective substitute to detrimental chemical pesticides. This article covers the current status of entomopathogenic fungi in agriculture industry, its mode of action and application methods for management of insect-pests.

Keywords: Beauveria, Entomopathogenic fungi, EPF, Myco-insecticides

Introduction

The term entomogenous is derived from two Greek words: "entomon" (insects) and "genous" (arising in). Entomogenous microorganism is "microorganisms that grow in insects" and directly involved in natural or microbiological management of insect pests. Pests including fungi, oomycetes, bacteria, viruses, and nematodes, weeds, arthropods, mites, mollusks and a few vertebrates are prevalent worldwide. They have a significant influence on agricultural production, which has resulted in a considerable drop in crop yields. Agriculturists rely significantly on chemical pesticides and inorganic fertilisers to tackle these challenges in order to assure maximum output of produced plants. Another constraint for agriculturists is their overdependence on synthetic fertilisers to improve plant growth. This is due to the fact that overuse of these compounds has a number of negative consequences for users, non-target organisms, and the environment. As the world's population is predicted to reach 9.1 billion by 2050 (Liu *et al.*, 2017), efforts are being made to maintain sustainable agricultural production. However, excessive use and reliance on synthetic pesticides and fertilisers, climate changes, poor land management, and widespread urbanisation are some of the problems hurting these efforts. The potential use of entomopathogenic fungi (EPF) as bio-control agents against herbivores is an environmentally viable alternative insect pest management strategy. EPF are known for their capacity to infect insects and cause sickness by entering their cuticles. More than 700 species from around 90 distinct genera have been identified as insect-pathogenic fungus till date. The most well-known EPF are belonging to genera Beauveria, Metarhizium, Isaria, Hirsutella, and Lecanicillium. The most widely used EPF are *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium anisopliae*, and *Lecanicillium lecanii* (Chen *et al.*, 2015). Biological plant protection with entomopathogenic fungi is an important component of a long-term pest management strategy. When compared to conventional pesticides, entomopathogens offer significant benefits as biocontrol agents. Low prices, great efficiency, safety for beneficial creatures, residue reduction in the environment, and

Article History

RECEIVED on 17th February 2023 RECEIVED in revised form 22nd February 2023 ACCEPTED in final form 23rd February 2023

improved biodiversity in human-managed ecosystems are some of the benefits of EPF. This article covers the details of EPF including mode of action, target pests, trade name, manufacturer/ country and usage (Table 1).

EPF used in Pest Management

EPF is an essential component of myco-insecticides used in horticulture, forestry, and agriculture. It is a vital component of integrated pest management strategies for biological control of insect pests and other arthropods. The important genera of EPF are given under.

Genus - *Beauveria*

It is an entomopathogenic fungus used as a bio-pesticide in crop pest management. It lives in the soil saprophytically and frequently produces large epizootics that kill out insect populations on crops. The two more important species are in this group *i.e.*, *Beauveria bassiana* and *Beauveria brongniartii*. It creates poisons such as beauvericin, bassianocide, and others inside the host body, causing paralysis and eventually killing the insects within four or five days. They are particularly useful in controlling sucking pests and caterpillars that infest agricultural plants. These EPF are used to manage caterpillars such as the yellow stem borer and leaf folder of rice, the white grub of groundnut, the coconut rhinoceros beetle, sugarcane pyrilla, caterpillars of pulses, tomato, and cotton, diamond back moth and leaf eating caterpillars of tobacco & sunflower.

Genus - *Verticillium*

It is a naturally occurring entomopathogenic fungus which can be used as a bio-pesticide. The two most important species in this genus are *Verticillium chlamydosporium* and *Verticillium lecanii*. The fungus *V. lecanii* is widely spread and can produce massive outbreaks in tropical and subtropical climates, as well as in warm and humid conditions. *Verticillium lecanii* is most effective to manage the whitefly, thrips, mealy bug and several aphid species of vegetables and ornamentals *etc*.

Genus - *Metarhizium*

It is a fungal insecticide widely used against a wide range of insects including soil insects, caterpillars, sucking pests and locusts. *Metarhizium anisopliae*, *Metarhizium album* and *Metarhizium flavoviride* are the three most significant species in the genus. It is known to attack over 200 species of insects covering seven orders. This pathogenic fungus is mostly used to manage the coconut rhinoceros beetle, groundnut cut worm, rice brown plant hopper, diamond back moth, and sugarcane early shoot borer, top shoot borer, and internode borer.

Genus - *Nomuraea*

Table 1: EPF formulations available globally for management of insect-pests

EPF	Brand Name	Formulation	Target Pests	Crop	Producer/ Country
Metarhizium anisopliae	BIO 1020	G	Black vine weevil	Glasshouse Ornamental crops, Nursery stock	Bayer, Germany
	Metarhizium	G/WC	Sucking pests	Several crops	Multiplex, India
	Bio-Blast™	WP	Termites	Houses	Ecoscience, USA, Brazil, Colombia
	Metrocid	WP	Root grubs, BPH, Termite	Groundnut, Paddy	Sri Biotech Laboratories India Ltd.
	Jasmeta	WP	Termite and Weevils	Field crops	Shri Ram Solvent Extraction, India
Metarhizium flavoviride	Bio Green	G	Red-headed cockchafer	Pasture/ Turf	Bio-Care Technology, Australia
Metarhizium anisopliae var. *acridum*	Green Muscle	WP, OF	Locusts, Grasshoppers	Several crops	Biological control products SA (Pty) Ltd. (under license from UK) South Africa
Hirsutella thompsonii	Mycohit	WP	Mites, *Aphis craccivora* Koch	Citrus, Cowpea	-
Cladossporium oxysporium	-	WP	*Aphis craccivora* Koch	Cowpea	-
Nomuraea rileyi	Numoraea 50	WP	Lepidoptera	Several crops	Colombia
Lagenidium giganteum	Laginex	AS	Larvae of most pest mosquito species	-	USA
Conidiobolus thromboides	Vector 25 SL	WP	Thrips, Aphid, Whiteflies	Several crops	Mycolab, South Africa

Table 1: Continue...

EPF	Brand Name	Formulation	Target Pests	Crop	Producer/ Country
Paecilomyces fumosoroseus (*Isaria fumosoroseus*)	PFR-97TM	WDG	Whiteflies/ Thrips	Glasshouse crops	ThermoTrilogy, USA Eco-tech, USA
	Prioroty	WP	Mites	Wide range of crops	T. Stanes, India
	Bemisin	WP	Whiteflies	Tomato, Chilli	Probioagro, Venezuela
Beauveria bassiana	Botani Gard	ES	Whiteflies, thrips, aphids, psyllids, mealybugs	Several crops	LAM International, USA
	Bauveril	WP	Beetles, Butterflies and Moths	Several crops	Laverlam S.A., Colombia
	Bio Expert	WP	Whiteflies, Thrips	Tomato, Okra, Chilli	Live Systems Technology (Colombia)
	Biowonder	WP	Rice pests	Rice	Indore Biotech, India
	Mycojaal	WP	Diamond blackmoth	Cabbage	Pest Control India (Pvt) Ltd, India
	Bassicore	SC	Whiteflies	Tomato, Chilli	Core Biotechnology (Colombia)
	Racer BB	WP	Lepidoptera (Noctuidae)	Several crops	SOM Phytopharma, India
	Bea-Sin	WP	Paper weevil, Boll weevil, Whiteflies	Field crop	Agrobiologicos Noroeste (Agrobionsa), Mexico
Beauveria brongniartii	Betel	G	Scarab beetle Larvae	Sugarcane	NPP (Calioppe), France
	Melocont	G/WC	Scarab beetle Larvae	Pasture	Kwizda, Austria
	Engerlings pilz	G/WC	Scarab beetle Larvae	Pasture	Andermatt, Switzerland
	Biolisa	WC	Cerambycid beetles	Inga and Ficus	Nitto Denco, Japan
Lecanicillium longisporum (Old name: *Verticillum lecanii*)	Vertalec	WP	Aphids, Whiteflies and Thrips	Tomato, Chilli and Glasshouse crops	Koppert, Holland
	Verticare	WP	Mealybugs & Scales	Citrus	Viswamitra BioAgro, India
	Biocatch	WP	Whiteflies	Cotton	T. Stanes, India
	Inovert	WP	Aphids, Scales, Mealybugs	Glasshouse crops	Inora, India

Abbreviations: WP = Wettable powder, G = Granular, OF = Oil flowable, WC = Whole culture, WDG = Water-dispersible granular, AS = Aqueous suspension, EC = Emulsifiable concentrate, SC = suspension concentrate

It is also an EPF used as a bio-pesticide against *Sopdoptera litura*, *Helicoverpa armigera* of groundnut, sorghum and chickpea *etc*. *Nomuraea rileyi* is a dimorphic hyphomycete that may induce epizootic death in various insects. *N. rileyi*'s host specificity and environmentally favourable characteristics support its application in insect pest management. This biological control is effective against a variety of insect hosts, including *Trichoplusia* sp., *Heliothis zea*, *Bombyx mori*, *Plathypena scabra*, and others.

Genus - *Paecilomyces*

Paecilomyces fumosoroseus is a major bio-control agent against whiteflies that causes "yellow muscardine." The ability of this fungus to grow extensively over the leaf surface under humid conditions is a characteristic that certainly enhances its ability to spread rapidly through whitefly populations. *P. fumosoroseus* is best for controlling the nymphs of whitefly. This fungus is used to manage yellow and red mites, whiteflies, and other insects in both field and greenhouse environments.

Genus - *Hirsutella*

H. thompsonii, *H. gigantea*, and *H. citriformis* are the three most significant species in the genus Hirsutella. *H. thompsonii* is used to control the citrus rust mite. This bio-control is also effective against the Acarida, Lepidoptera, and Hemiptera insect families.

Mode of Action

EPF kill insects in a variety of ways, including starving to toxin production. It produces several toxins and extracellular enzymes such as proteases and chitinases. Cuticle is the principal barrier to infection in insects since it is the primary avenue of fungal penetration. As a result, it requires either physical or enzymatic techniques to breach the impenetrable cuticle. The infection process begins with spore contact with the host cuticle. Most fungi have an infective unit that is a spore, commonly a conidium. Conidia are usually sticky to the cuticle or exude adhesive mucus when they enlarge during pre-germination. In favourable conditions, the conidium germinates into a short germ tube that produces tiny swellings called appressoria. The appressorium adheres to the cuticle and sends out an infection peg, which gives the fungus with the solid connection it requires to physically push its way into the host. The hyphae then penetrate the insect cuticle by enzymatic chitin and protein disintegration, first dissolving the cuticle and then entering the insect's haemocoel and internal organs. The infectious fungal mycelium invades the insect until it is completely filled with the fungus and becomes quite solid to the touch. Following that, conidiophores are generated, which erupt through the cuticle and create spores on the exterior of the fly, infecting surrounding healthy insects as well. The fungus produces poisons that kill the host by mechanically obstructing the tissues. Toxins are generated by several EPF (Table 2), and many of them help in pathogenesis and serve an insecticidal role. These metabolites are harmful to invertebrates as well.

Methods of Application

1. Soil Drenching

1-1.5 kg EPF culture formulation mix in 200 litre of water and drench the soil in one acre area or @ 250 g/ 5 litre of water in soil from time to time for management of white grub and other soil pests. Maintain optimum soil moisture while applying. Repeat application at 2-3 week interval as and when required.

2. Soil Application

Table 2: Toxins produced by the various entomopathogenic fungi

Toxins	Fungus that produces toxins	Mode of general infection
Bassionolid	*Beauveria bassiana*	Ionophore is soluble in lipid layers and increases membrane permeability for specific ions. In this way, it damages the cell organelles and their functions.
Siklosporin A	*Beauveria bassiana, Tolypocladium* sp., *Verticillum* sp., *Fusarium* sp.	Blocks a step in Ca^{++} dependent signal transduction in vertebrate T cells. This causes immunosuppression. It can also suppress insect defence cells.
Bassianolide	*B. bassiana, Verticillium lecanii*	Acts as ionophore, toxic effect on insects.
Beauvericin	*Beauveria bassiana, Isaria* sp., *Fusarium* sp.	Cytotoxic effect and insecticidal properties. Ionophore is soluble in lipid layers and increases membrane permeability for specific ions. In this way, it damages the cell organelles and their functions.
Dipicolinic acid and Didepsi peptide (protein) Bassianolide	*Verticillium lecanii*	It causes atony. Interfere, the process of chitin formation and exposure the insect easily to the attack of natural enemy.
Swainsonin	*Metarhizum anisapliae*	Indolizidine alkaloid.
Destruxins	*Metarhizium anisopliae*	Immunodepressant activity in insect and cytotoxic effect.
Leucinostatins	*Paecilomyces lilacinus, Paecilomyces marquandii*	Insecticidal activity by interfering with oxidative phosphorylation.
Efrapeptins	*Tolypocladium niveum*	Inhibitors of mitochondrial oxidative phosphorylation and ATPase activity.
Sitokhalasin	*Metarhizum anisapliae*	Blocks the elongation of the actin filament.
Hirsutellin A	*Hirsutella thompsonii*	The ribosomal inhibitory protein (RIP) causes a specific cleavage of the rRNA and inhibits protein synthesis.

While preparing the field, @ 250-300 g m^{-2} or 1-2 kg $acre^{-1}$ (powder formulation) is added in 80-100 kg well decomposed farm yard manure (FYM). Mixed thoroughly, cover with jute bag/ sugarcane leaves/ paddy straw and kept for 1-2 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Apply well mixed/ decomposed EPF-based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/ pit/ pot and at the time of transplanting/ sowing for termite, white grub and other soil pest management. It should be repeated after 2-3 week interval.

3. Spraying

Mixing 1.0-1.5 kg of EPF culture in 200 litres of water with sticker should be sprayed in morning or evening for pest management (sucking pests, bug and beetles *etc.*) in one acre area. Mix the culture in water at in appropriate quantity and stir well till creamy, leave it for 3-4 hours. Empty the cream into spray tank together with required amount of water and agitate well. Spray immediately after preparation. Apply with high volume spraying equipment @ 2.5-5 g l^{-1} of water.

Conclusion

EPF are reflected to play a vital role as biocontrol agent of insect-pests. It is a very diverse range of fungal species from different groups and infect to insects. It infests the host insects via digestion, respiration and through integument. In infestation from integument which is one of the most common infestation methods, fungi grow hyphae to penetrate epicuticle and progresses into hypodermis to achieve the infestation. In certain circumstances, EPF have a high host selectivity for arthropod pest management. As a result, they have no effect on beneficial insect populations. Microbial pesticide residues provide no danger to humans or other beneficial creatures. Farmers can use them even when a crop is nearly ready for harvest. They persist in the environment for a long period after application, resulting in the rapid mortality of the arthropod hosts and provide long-term control.

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