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Mycoinsecticide Fungi: A Sustainable Option for Insect-Pest Management

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Abstract

Agriculture is vital to India's economy. Insect infestation significantly reduces agricultural crop productivity. To satisfy the needs of India's and other emerging countries' growing populations, the Indian agricultural system need a sustainable agricultural production for future generations. Crop plants are susceptible to biotic and abiotic stresses caused by insect pests, plant diseases, and poor growth circumstances. These variables are to blame for massive plant productivity losses (up to 40% crop losses) globally. Chemical pesticides are frequently used to protect plants. This is due to an increase in insect resistance to a variety of chemical compounds included in plant protection solutions. 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. Employing biological creatures, predators, parasitoids, and microorganisms such as viruses, bacteria, and fungus to control pests has shown to be a successful and sustainable pest management strategy. Among the aforementioned, fungi, particularly insect-pathogenic species, have been used for more than 150 years. This article discusses the current state of Entomopathogens in

agriculture industry. Entomopathogens as bio-pesticides offer a safe, natural, and cost-effective alternative to harmful chemical pesticides. There are currently few entomopathogenic formulations on the market, which are insufficient to fulfil farmer demand due to a lack of new breakthroughs in research and legislation in India. When compared to manufactured chemical pesticides, the production and consumption of entomopathogens is restricted. In this light, this article discusses the possibility of EPF as alternatives or may be alternatives to chemical pesticides in plant protection.

Key words: *Entomopathogenic fungi, bio-insecticide, brand name, toxins, target insect-pets*

Introduction

Agricultural pests include plant pathogens (fungi, oomycetes, bacteria, viruses, and nematodes), weeds, arthropods (mostly insects and mites), mollusks (slugs and snails), and a few vertebrates. They impair crop output and quality by eating on them. Pest species are thought to number in the millions worldwide. They have a significant influence on agricultural production, which has resulted in a 40% drop in potential global 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. In the right setup, 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 to date. These strains include the most well-known members of the genera *Beauveria, Metarhizium, Isaria, Hirsutella*, and *Lecanicillium*. The most widely researched fungal species include *Beauveria bassiana*

(Balsamo-Crivelli) Vuillemin, *Isaria fumosorosea* Wize, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas (Chen *et al.*, 2015).

The term entomogenous is derived from two Greek words: "entomon" (insects) and "genous" (arising in). As a result, the etymological definition of entomogenous microorganism is "microorganisms that grow in insects." Entomogenous microorganisms are directly involved in natural or microbiological management of insect pests and are connected to human welfare, which has piqued the interest of microbiologists, molecular biologists, and entomologists in recent years. Interestingly, the idea of using microbes for pest management is not a new one. Agostino Bassi (1773-1856) identified and reported the first entomopathogenic fungus in 1835 that a fungus could cause a deliberately transmissible disease in silkworm (Lord, 2007), which caused white muscardine sickness in insects and was later termed *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales, Cordycipitaceae) (Rehner *et al.*, 2005). After a few years, Elias Metschnikoff (1845-1916) identified the green muscardine, a fungal disease that attacks insects and is caused by *Metarhizium anisopliae* Metschnikoff Sorokin (Hypocreales, Clavicipitaceae) (Zimmermann *et al.*, 1995).

Entomopathogenic fungi (EPF) with a vast and plentiful variety are a boon to sustainable pest management. Because of their environmental friendliness and bio persistence, entomopathogenic fungi prefer to kill insects at different phases of their life cycle. EPF species from numerous classes that infect insects have been found. These insect pathogens have a wide range of alterations and contaminating capacities employing facultative and obligate pathogens. These Entomopathogenic fungi are descended from the Deuteromycota, Ascomycota, and Zygomycota divisions. Mycoinsecticide/EPF is the employment of fungus in biological processes to minimise insect density and thereby crop damage. 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 improved biodiversity in human-managed ecosystems are some of the benefits. This article discusses the details of entomopathogenic fungi, mode of action, target pests, trade name, manufacturer/country and their detailed usage description in the current scenario (Table 1).

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Some important entomopathogenic fungi (EPF) using for pest management.

EPF are an essential component of myco-insecticides in horticulture, forestry, and agriculture, and are a vital component of integrated pest management strategies as biological control agents against insect pests and other arthropods. The important genera are illustrated in table 1 and details as given below-

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*. These parasites enter the host insect body by food or contact with the host cuticle, where they multiply. 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 entomopathogenic fungi 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, leaf eating caterpillars of tobacco and sunflower, and others in an environmentally friendly manner.

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-*Metarrhizium*

It is a fungal insecticide for use 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. *Metarhizium*

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multidisciplinary e-Newsletter

anisopliae is a fungus that may be harmful to insects. 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*

It is also an entomopathogenic fungus used as a bio-pesticide against *Sopdoptera litura,* Helicoverpa armigera of groungnut, 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. f*umosoroseus* is best for controlling the nymphs of whitefly. These fungi cover the whiteflies body with mycelial threads and stick them to the underside of the leaves. The nymphs show a “feathery” aspect and are surrounded by mycelia and conidia. 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

Table 1: Globally available Bio-insecticides/Mycoinsecticides/EPF formulations

EPF/ Mycoinsecticides	Brand Name	Formulation	Target Pests	Crop	Producer/ Country
Beauveria bassiana	Mycotrol	WP, ES, OF	Whiteflies, Thrips, Aphids	Field crops	Mycotech, USA
	Biowonder	WP	Rice pests	Rice	Indore Biotech, India

	Biosoft	WP	*Helocoverpa* and sucking pests	Tomato, Chickpea, Pigeon pea, Field pea and other several crops	Agri land Biotech, India
	Conidia	WDG	Coffee berry borer	Coffee	AgrEvo, Germany
	Naturalis	ES	Sucking insects	Cotton, Glasshouse crops	Troy BioScience, USA
	Ostrinol	G	Corn borer	Maize	NPP (Calioppe), France
	Mycojaal	WP	Diamond black moth	Cabbage	Pest Control India (Pvt) Ltd, India
	Beevicide	WP	Borer type pests	Several crops	-
	Botani Gard	ES	Whiteflies, thrips, aphids, psyllids, mealybugs	Several crops	LAM International, USA
	Botani Gard	WP	-do-	-do-	-do-
	Bauveril	WP	Beetles, Butterflies and Moths	Several crops	Laverlam S.A., Colombia
	Bio-Power	WP	Stem borers, cut worms, root grubs, leafhoppers, whiteflies, aphids, thrips and mealy bugs	Several crops	T. Stanes, India
	Boverin	WP	Colorado potato beetle; Leptinotarsa decemlineata, and the codling moth; Cydia pomonella	Several crops	Biodron, Russia
	Conidia	WDG	Coleoptera	-do-	Hoechst

			(Curculionidae)-Beetles		Schering Colombia and AgrEvo, Germany
	Naturalis-L	WP	Coleoptera (Chrysomelidae, Curculionidae), Hemiptera (Miridae, Cicadellidae, Aleyrodidae, Aphididae, Psyllidae), Lepidoptera, Thysanoptera (Thripidae)	-do-	Andermatt Biocontrol Troy Biosciences Inc., Switzerland, United States
	Racer BB	WP	Lepidoptera (Noctuidae)	-do-	SOM Phytopharma, India
	Proecol	WP	Army worm	Paddy, Maize etc.	Venezuela
	Bea-Sin	WP	Paper weevil. Boll weevil, Whiteflies	Field crop	Agrobiologicos Noroeste (Agrobionsa), Mexico
	Boverol-Spofa	WP	Colorado potato beetle	Potato	Czechoslovakia
	Ballvéria	WP	Whiteflies	Tomato, Chilli	Ballagro Agro Tecnologia (Brazil)
	Bovebio	WP	Whiteflies, Mites	Tomato, Chilli	Biofungi – Industriae Comerciode Defensivos Biológicose Inoculantes (Brazil)
	Bio Expert	WP	Whiteflies, Thrips	Tomato, Okra, Chilli	Live Systems Technology (Colombia)
	Adral	WP	Aphids	Mustard	Bio-Crop (Colombia)
	Broadband	WP	Whiteflies, Thrips, Mites, Diamondback	Cabbage, Tomato, Chilli	BASF South Africa

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			moth		
	Bassicore	SC	Whiteflies	Tomato, Chilli	Core Biotechnology (Colombia)
	BBC	WP	Bollworm, Cutworms, Root grub Termite, Whiteflies, Thrips, Mealy bug, Plutella	Several crops	Sri Biotech Laboratories India Ltd.
Beauveria bassiana PDRL1187	-	WP	Mustard Ahpid, *Lipaphis erysimi*, *Aphis craccivora* Koch	Mustard, Tomato, Wheat etc.	-
Beauveria brongniartii	Betel	G	Scarab beetle Larvae	Sugarcane	NPP (Calioppe), France
	Melocont	G/WC	Scarab beetle Larvae	Pasture	Kwizda, Austria
	Engerlingspilz	G/WC	Scarab beetle Larvae	Pasture	Andermatt, Switzerland
	Biolisa	WC	Cerambycid beetles	Inga and Ficus	Nitto Denco, Japan
Metarhizium anisopliae	BIO 1020	G	Black vine weevil	Glasshouse Ornamental crops, Nursery stock	Bayer, Germany
	Metarhizium	G/WC	Sucking pests	Several crops	Multiplex, India
	Multiplex	WP	Root grubs	Several crops	Multiplex, India
	Bio Magic	WP	Brown plant hopper	Rice	T. Stanes, India
	Bio-Blast™	WP	Termites	Houses	Ecoscience, USA, Brazil, Colombia
	Achieve	WP	Mites	Several crops	Real IPM (Kenya)
	Bio-Catch-M	WP	Hemiptera (Aleyrodidae, Aphididae)	-do-	T. Stanes, India

	Bio-Cane	WP	Sugarcane pest; grayback canegrub	Sugarcane	Granules Becker-Underwood, Australia
	Bio-Path	WC	Blattodea (Blattellidae, Blattidae)	Field crops	EcoScience, USA
	Campaign	WP	Mealybugs, Thrips, Fruit flies, Mealybugs	Field crops	Real IPM, Ghana, Uganda
	Cobican	WP	Sugarcane spittle bud	Sugarcane	Probioagro, Venezuela
	Metrocid	WP	Root grubs, BPH, Termite	Groundnut, Paddy	Sri Biotech Laboratories India Ltd.
	Kalichakra	WP	Root and beetle grubs, Cutworms, Aphids	Several field crops	Agrilife International Panacea, Ltd. India
	Jasmeta	WP	Termite and Weevils	Field crops	Shri Ram Solvent Extraction, India
	Biostorm	WP	Root weevils, Termite, Hoppers, White grub	Several field crops	Varsha Bio Science and Technology, India
Metarhizium flavoviride	Bio Green	G	Red-headed cockchafer	Pasture/Turf	Bio-Care Technology, Australia
	Green Muscle	WP, OF	Locusts, Grasshoppers	Several crops	CABI, UK
Metarhizium anisopliae Var. acridum	Green Muscle	WP, OF	Locusts, Grasshoppers	Several crops	Biological control products SA (Pty) Ltd. (under licence from UK) Soth Africa
Lecanicillium longisporum (Old name: *Verticillum lecanii*)	Vertalec	WP	Aphids, Whiteflies and Thrips	Tomato, Chilli and Glasshouse crops	Koppert, Holland,
	Mycotal	WP	Aphids, Whiteflies and	Glasshouse crops	Koppert, Holland,

			Thrips		
	Verticare	WP	Mealybugs & Scales	Citrus	Viswamitra Bio Agro, India
	Biocatch	WP	Whiteflies	Cotton	T. Stanes, India
	Inovert	WP	Aphids, Scales, Mealybugs	Glasshouse crops	Inora, India
	Verelac	WP	Sucking pests	Several crops	-
	Bioter	WP	Effective against termites	Several crops	-
Paecilomyces fumosoroseus (Isaria fumosoroseus)	PFR-97™	WDG	Whiteflies/ Thrips	Glasshouse crops	Thermo Trilogy, USA Eco-tech, USA
	Prioroty	WP	Mites	Wide range of crops	T. Stanes, India
	Pae-Sin	WP	Whiteflies	Tomato, Chilli	Agrobionsa, Mexico
	Bemisin	WP	Whiteflies	Tomato, Chilli	Probioagro, Venezuela
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
M. anisopliae + B. bassiana + I. fumosoroseus	Tri-Sin	WP	Psyllid	Citrus and other crops	Mexico
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

WP= Wettable powder @ 1 x 10^8 cfu's/gram or 5 x 10^{13} conidia/ha; G= Granular; OF= Oil flowable; WC= Whole culture; WDG=Water-dispersible granular; AS= Aqueous suspension EC= Emulsifiable concentrate; SC= suspension concentrate

Mode/mechanism of action of entomopathogenic fungi

Insect pathogenic fungus kill insects in a variety of ways, including starving to toxin production. These insects pathogenic fungus generate 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 appresoria. The appresorium 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 penetrates 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 insect pathogenic fungi (Table 2), and many of them help in pathogenesis and serve an insecticidal role. While certain other fungus create antibacterial compounds. Destruxins from Metarhizium species are among the fungal metabolites that promote the pathogenicity of the fungus. Beauvericins are generated by Beauveria species. These metabolites are harmful to invertebrates as well.

Table 2: Some important toxins produced by the various entomopathogenic fungi

Toxins	Fungus that produces toxins	Mode of general infection
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.
Destruxins	*Metarhizium anisopliae*	Immunodepressant activity in insect and cytotoxic effect.
Bassianolide	*B. bassiana, Verticillium lecanii*	Acts as ionophore, toxic effect on insects
Leucinostatins	*Paecilomyces lilacinus, Paecilomyces marquandii*	Insecticidal activity by interfering with oxidative phosphorylation
Efrapeptins	*Tolypocladium niveum*	Inhibitors of mitochondrial oxidative phosphorylation and ATPase activity.
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 vertabrate T cells. This causes immunosuppression. It can also suppress insect defence cells.
Hirsutellin A	*Hirsutella thompsonii*	The ribosomal inhibitory protein (RIP) causes a specific cleavage of the rRNA and inhibits protein synthesis.
Swainsonin	*Metarhizum anisapliae*	Indolizidine alkaloid
Sitokhalasin	*Metarhizum anisapliae*	Blocks the elongation of the actin filament.
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.

Source: Boucias and Pendland, 1998; Kumar *et al.*, 2020

Method of application of EPF/Mycoinsecticide:

1. **Soil application/treatment:** While preparing the field, @ 250-300 gram per square meters or 1-2 kg per acre (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.
2. **Spraying:** Mixing 1.0-1.5 kg of EPF culture in 200 liters 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 gram/liter of water.
3. **Soil drenching:** 1.0-1.5 kilogram EPF culture formulation mix in 200 litre of water and drench the soil in one acre area or @ 250 gm/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.

Advantages and disadvantages of Entomopathogenic Fungi

The practical application of EPF has various benefits and drawbacks. These are listed below.

Advantages

- In certain circumstances, EPF have a high host selectivity for arthropod pest management. As a result, they have no effect on beneficial insect populations. Some of them, however, may have a very broad host range.
- 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.

- They do not produce issues like as pesticide resistance while managing arthropod pests and hence provide long-term control.
- Microbial pesticides may become established in succeeding generations of pests or in their habitats, providing pest control throughout the following season or generation.
- They are accessible to development through biotechnological techniques.

Disadvantages

- The most significant drawback is that they are vulnerable to UV light, as well as low humidity and high temperatures.
- Because microbial insecticides are designed to inhibit certain pest species or groups, they may only control one pest in a field. Other pests that are present in the treated area continue to inflict damage.
- They are adversely affected by fungicides.
- Synthetic pesticides often kill arthropod pests quickly, while EPF take a longer time (sometimes 1-2 weeks).
- The expenses of production are often greater than those of many synthetic insecticides.

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