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Entomopathogenic Fungi: Need of Sustainable Agriculture

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Abstract

Entomopathogenic fungi are a major component of integrated pest management techniques as biological control agents against insect pests and other arthropods in agriculture, horticulture and forestry. Use of microbial agents particularly entomopathogenic fungi, have been investigated for the control of a wide range of orchard, field crop pests and are widespread component of most terrestrial ecosystems. Insect control using entomopathogenic fungi is achieved when sufficient infective propagules, conidia contact a susceptible host and conditions are suitable for a lethal mycosis to develop. Most of these fungi, along with a range of bacteria, can grow on artificial media *in vitro*. During the last four decades, numbers of companies worldwide have developed mycoinsecticides and myco-acaricides. Use of mycoinsecticides is likely to rise if research is focus on; improving its performance under challenging environmental conditions, formulations that will increase persistence, longer shelf life, ease of application, pathogen virulence and wider spectrum of action.

Introduction

Entomopathogenic fungi are a group of fungi living in soil that infect insects by penetrating their cuticle to penetrate their bodies to eventually kill them and feeding on them. Entomopathogenic fungi are microorganisms that specifically infect and often kill insects and other arthropods. Most are nonpathogenic to plants, and relatively non-toxic to humans and animals. These fungal pathogens are being identified as important contributors to insect population dynamics in soil. Derived from many years of study and about 800 fungi species identified as pathogenic for insects many commercial mycoinsecticides are being developed. A multi-faceted approach combining all available integrated pest management (IPM) tools into a comprehensive strategy has a better potential to suppress pests below economically damaging levels. With increased commercialization, entomopathogenic fungi are assured to become a significant component of IPM.

History

- **Bassi** in **1835,** first time formulated the germ theory by using white muscardine fungus on the silkworm that was then named in his honor as *Beauveria bassiana*.
- **In 1980s,** the first insect pathogenic studies were carried out and their focus was to find the methods of disease management of the silkworm.

Some Important Entomopathogenic Fungi

Genus: Beauveria

- *Beauveria* sp. is a filamentous fungus, belonging to the Phylum Ascomycota.

• Some important species of this genus are *Beauveria bassiana, Beauveria brongniartii, Beauveria amorpha* and *Beauveria caledonica*.

• *Beauveria bassiana* is a fungus that grows naturally in soil throughout the world and acts as a pathogen on various insect species, causing white muscardine disease.

• It acts against Cotton bollworms, Coffee berryborer (Coleoptera, Lepidoptera, Hemiptera and few in Diptera, Hymenoptera).

Genus: Metarhizium

• The genus Metarhizium has three important species, *Metarhizium anisopliae, Metarhizium album* and *Metarhizium flavoviride. Metarhizium anisopliae* is a potential entomopathogenic fungus.

• Pathogenicity of *M. anisopliae* has been tested on teak skeletonizer, *Eutectona machaeralis*, and found to be a potential myco-biocontrol agent of teak pest. And also against Sugarcane pyrilla, rhinoceros beetle (Coleoptera, Lepidoptera, Hemiptera, Diptera and Hymenoptera).

Genus: Lecanicillium

• *Lecanicillium lecanii* and *Lecanicillium chlamydosporium* are the important species of this genus.

• It is an effective biocontrol agent against *Trialeurodes vaporariorum* in greenhouses.

• *L. lecanii* is considered to control whitefly and several aphid species.

Genus: Nomuraea

• Few important species of this genus are *Nomuraea rileyi* and *Nomuraea atypicola. Nomuraea rileyi*, another potential insect-infecting fungus, is a dimorphic hyphomycete that can cause epizootic death in various insects such as *H. armigera, Achaea janata, S. litura* (Lepidoptera).

• Its mode of infection, and development have been reported for several insect hosts such as *Trichoplusia* sp., *Heliothis zea*, *Plathypena scabra*, *Bombyx mori*, *etc*.

Genus: Purpureocillium

• Purpureocillium is a genus of phylum Deuteromycota with important species, *Purpureocillium lilacinus* (*Paecilomyces lilacinus*).

• Purpureocillium is a genus of nematophagous fungus that kills harmful nematodes like root knot nematode by pathogenesis causing disease in nematodes.

Genus: Isaria

• *Isaria fumosoroseus* (*Paecilomyces fumosoroseus*) is the important species of this genus.

• *Isaria fumosoroseus* is one of the most important natural enemies of whiteflies worldwide and causes the sickness.

Transmission and Conidia Dispersal

• High humidity and moisture necessary for germination and sporulation are depending sources for transmission of conidia.

• Abundant spores are transmitted by: (1) Wind, (2) Rain, (3) Invertebrates, (4) Non-target insects, (5) Hyphae growing out of insect cadaver.

• *E.g.*, in some of the Hyphomycetes *viz*., *Metarhizium* spp. and *Beauveria* spp. conidia are hydrophobic and are passively spread from infected cadavers.

• The entomopathogenic fungi produces some Enzymes such as Chitinase, Chitosanase, Chitobiase, Lipases, Phospholipase, Proteases, Peptidases. The successfulness of infection was directly proportional to secretion of exoenzymes.

Table 1: Toxins Produced by Different Entomopathogenic Fungi

Toxin	Fungi	Function
Efrapeptins	*Tolypocladium niveum Syn., Beauveria nivea*	Inhibitors of mitochondrial oxidative phosphorylation and ATPase activity.
Destruxins	*Metarhizium anisopliae*	Immunodepressant activity in insect and cytotoxic effect.
Beauvericin	*Beauveria bassiana*	Cytotoxic effect and insecticidal properties.
Bassianolide	*Beauveria bassiana*	Acts as ionophore, toxic effect on insects.
Leucinostatins	*Purpureocillium lilacinus, Purpureocillium marquandii*	Insecticidal activity by interfering with oxidative phosphorylation.

(Source: Sinha *et al*., 2016)

Tritrophic Interaction

Tritrophic effects are the reason behind the success of entomopathogens (Cory and Ericsson, 2009).

1. Plant-mediated effects on fungal entomopathogens.

2. Plants could affect fungal entomopathogens either directly or indirectly-

•Direct effects mean anything produced by the plant that influences fungal infection of the insect.

• Indirect effects could occur before or after exposure of the insect to fungus, and would include factors that alter insect condition.

Symptoms Shown by Insects on Infestation by Fungus

- Loss of appetite.
- General/ partial paralysis.
- Discolored patches on integuments.
- Body hardens and the insect is in upright on its leg at the time of death.
- Specifically we uses the term "Mycoses" for such changes in insects and can be seen in - Lepidoptera, Hemiptera, Hymenoptera, Coleoptera and Diptera.

Defense Systems in Insects

Insects have evolved a number of mechanisms to prevent the invasion of fungi. The defense arsenal of insects contains both passive structural barrier (cuticle) and a cascade of active responses to keep the pathogens at bay. The active responses include melanization, cellular reactions to recognize the nonself pathogen, production of protease inhibitors, and symbiotic and behavioral defenses against microbes.

- Structural defenses (cuticle): chitin, protein, melanization, toxic lipids (hydrocarbons, fatty acids, esters, epoxides, *etc*.
- Microbial defenses: antimicrobial peptides, alkaloids, terpenes, quinones, β-13 glucanases, proteinase, Chitinase inhibitors.
- Insect behavioral defenses: grooming, burrowing, corpse removal, sunbathing, antifungal compounds from microbial symbionts.
- Developmental molting (Sinha *et al.*, 2016).

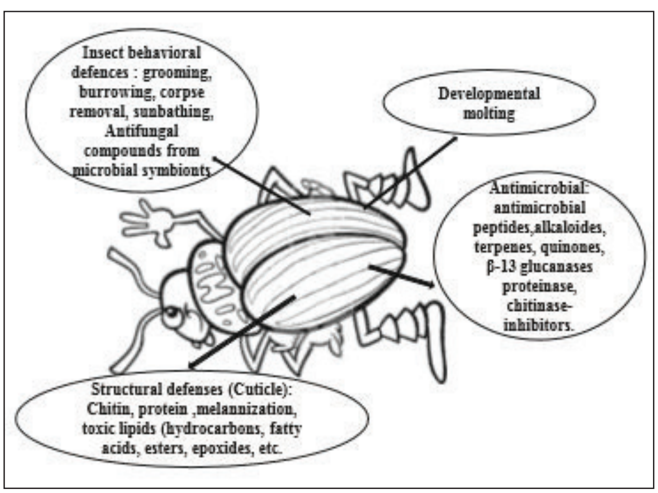

Figure 1: Possible defense mechanisms in insects against entomopathogens (Sinha *et al.*, 2016)

Isolation of Entomopathogenic Fungi from Insect Cadavers

- Collection of diseased caterpillar/ larvae.
- Surface sterilization.
- Removal of excess water (by Whatmann filter paper no. 1).
- Cutting of diseased larvae into small pieces.
- Transferred on SMYA (Sabourand Maltose Yeast Agar).
- Incubate at 25±1 °C.
- Observe the growth of fungi.
- Purification & Identification.

Isolation of Entomopathogenic Fungi from Soil by using Galleria Bait Technique

- Collection of soil sample (1.5 kg soil sample).
- *G. mellonella* larvae distribution in cups with soil @ 3 larvae cup^{-1} and wet it upto 15% water content.
- Incubate at 25 °C for 7 days.
- Transfer the infected larvae to petri dish furnished with wetted filter paper.
- Observe for sporulation.

Mass Production of Entomopathogenic Fungi

- A millet-based fungal production system.
- A vegetable materials as the medium, *e.g.*, rice or wheat bran, cracked barley, *etc*. even the peat soil has been used with success in the People's Republic of China (PRC).
- Cadavers of infected insects have been collected and introduced into fields.
- Production of the mycelium in submerged culture or by using liquid fermenter.

Effectiveness of Fungi as Biocontrol Agent

The effectiveness of fungi as biocontrol agent depends upon improved product shelf life, biocontrol efficacy.

Physical characteristics of the product for application:

- Spore distribution and coverage to ensure that the pest comes in contact with the fungus.
- The climatic conditions that favor survival and germination of the fungus before, during, and after application).

Formulation of Fungal Pesticides

They are being used in different forms like:

• Wettable powders.

• Liquid formulations.

• Oil formulations.

Best method: Application of active ingredient with oil.

Methods of Application

Four main methods of fungal applications are:

• Dipping the plant or roots,

• Spraying the foliage,

• Treating the soil, and

• Indirect transmission by vectors.

Mode of Action of Entomopathogenic Fungi

• The infection process starts with the adhesion of spores to arthropod shells and has two stages: the first depends on the action of hydrophobic and electrostatic forces and the second requires the activity of enzymes and low-molecular-weight proteins called hydrophobins (Skinner *et al.*, 2014).

• Spore germination occurs in the presence of carbon and energy sources on the insect's cuticle at sufficient humidity and temperature. The optimum temperature for the growth and germination of EPF is between 20 and 30 °C. Spores can also germinate at temperatures outside this range and this is a characteristic feature of the particular fungal strain (Skinner *et al.*, 2014).

• Subsequently, appressoria emerge, causing strong mechanical pressure on the cuticle and the production of lytic enzymes (proteo-, lipo- and chitinolytic) that disintegrate the insect's body shells (Skinner *et al.*, 2014).

• After penetrating the arthropod's body cavity (hemocel), the fungal hyphae start to grow. Some EPF can produce blastospores that enter host's hemolymph and produce secondary hyphae that inhabit the host's tissues. At this stage, the fungi produce secondary metabolites that cause paralysis and disrupt the host's physiological processes, mainly its immune responses (Donzelli and Krasnoff, 2016).

• Because of the developing infection, the insect's body is destroyed by both mechanical damages to the internal organs by the developing hyphae and nutrient depletion (Donzelli and Krasnoff, 2016).

• As a result of the progressive infection, the insect's body, initially soft, becomes stiff due to fluid absorption by the fungus. Cadavers of insects attacked by fungi of the genus Beauveria may initially take on a dark red color.

• The entire infection process is relatively long and takes approximately 14 days after infection, but first symptoms of infection usually occur about 7 days post infection (or even earlier, depending on fungal species).

• After killing the insect and using all nutrition, the hyphae of the fungus emerge from the cadaver of the host through holes in its body (mouth hole, anus) and through intersegmental areas. Then, resting or infective spores are produced, which allows the fungus to spread and infect other individuals (Skinner *et al.*, 2014).

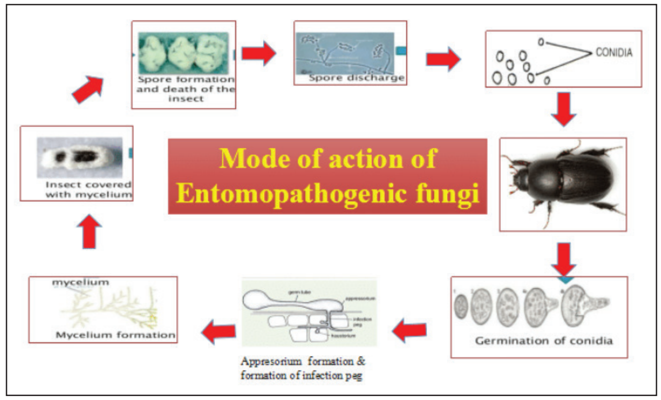

Figure 2: Mode of action of entomopathogenic fungi (Courtesy: Tanada and Kaya, 1993)

Advantages

• Nontoxic.

• Nonpathogenic.

• Specific.

• No residual toxicity.

• Can also apply at harvest stage.

Disadvantages

• No immediate action.

• Only effective to a specific group of insects.

• Each application may control part of the insect pest.

• If other species may present they may continue to cause damage.

Conclusion

Biopesticides which are based on entomopathogenic fungi are eco-friendly and advantageous in many different ways for controlling the insect pests. There is a lot of work conducted on the identification and characterization of the entomopathogenic fungi nowadays. However, it is crucial to look for novel and more potential EPF strains to produce commercial products rather than already known strains that have already shown the effectiveness against various insects. Biotechnology is doing immense efforts in this field. The combined efforts of various scientists and other stakeholders can provide a bright future for EPF-based biopesticides.

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