International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 8.141
Peer Reviewed Bi-Monthly
Vol.12 No.2 November – December 2024

A Study on Entomopathogenic Microbes as Underground Allies for the Biocontrol of Soil Insect Pests

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DOI - 10.5281/zenodo.21642313

Introduction:

Due to the dependency of the market at a global scale to agricultural merchandise, leading-edge technology in agriculture is increasingly used that is required to develop new agricultural practices and has potential to alleviate the adverse negative effects on the ecosystem and consequently results in the production of valuable safe products for human consumption. However, there is a major constraint in increasing agricultural production yield due to loss caused by insect pests, plant diseases, weeds, *etc*. Pest issues are inevitable in evolutionary agriculture methods and are mostly caused by simplified agroecosystems as well as the development of less stable natural ecosystems. In many regions of the world, insect pests like locusts, grasshoppers, termites, and cattle ticks have been found to cause significant losses in agricultural and economic production roughly 40% (Chandler *et al*., 2011). Various industrialized nations are trying to shift their strategy to using transgenic plants that express particular traits like resistance to insects, fungi, or viruses for pest management. Several agrochemicals pesticides are still being used by farmers for the control of insect pests and diseases in their agricultural practices. They have been responsible for maintaining and increasing the quality and quantity of food and fiber worldwide. Due to the widespread use's detrimental effects on non-target organisms, deposition on edible food crops, groundwater pollution, insect resistance to the chemicals, and detrimental effects on human health, scientists are now forced to concentrate on creating dependable, affordable, and environmentally safe alternatives. An all-encompassing strategy for crop production is integrated pest management, or IPM. This is a change from the conventional individual pest-centered methods that mostly depended on chemical pesticides to a more comprehensive strategy that considers the entire crop production system as a whole and manages the pests rather than eliminating them. Accordingly, a number of microbial agents have been created to control insect infestations (Asi *et al*., 2013). Entomopathogenic fungus has been known as important inhibitory factor of plant bugs for more than a century and is continuously used in pest biocontrol management throughout the world. Entomopathogenic fungi fill an extremely important niche and are well suited for development as microbial biopesticides for control of the harmful insect pests. Fungal diseases can affect nearly every order of insects. Because of their excessive reproductive capacity, goal-specific activity, minimal effects on beneficial and other non-target organisms, short life span, and resting stage or saprobic phase generating competencies that can ensure their survival for an extended period of time when no host is present entomopathogenic fungi have the potential to be environmentally friendly biocontrol agents. Entomopathogenic fungi can be found in a wide range of environmental conditions (including arid to tropical settings, terrestrial to aquatic habitats, and arctic to temperate climates), possess potential to colonize a wide variety of plant species, and infect a broad array of insects. The orders Hypocreales and Entomophthorales contain the bulk of entomopathogenic fungi utilized for biological control of insects and mites. The majority of commercially produced biological control fungus belong to the Hypocreales family. Numerous additional fungi, including *Beauveria bassiana*, *Lecanicillium* spp., *Metarhizium* spp., and *Isaria fumosorosea* are employed to combat numerous pests in a range of crops. There are already about 170 fungal formulations made from at least 12 different species of fungi (Faria and Wraight 2007).

Classification and Type of Entomopathogenic Fungus:

With about 700 known entomopathogenic fungal species, which make up less than 1% of all fungal species, the kingdom Fungi is a significant category among eukaryotes (McLaughlin *et al*., 2009). Entomophthoromycota, Blastocladiomycota, and Microsporidia are the three major categories of entomopathogenic fungus, and there are twelve distinct classes among six phyla of fungi. The divisions Ascomycota, Zygomycota, Deuteromycota, Oomycota, and Chytridiomycota contain these deadly fungi. Many of the known genera of potentially entomopathogenic fungus are either members of the class Hyphomycetes in the Deuteromycota or the order Entomophthorales in the Zygomycota.

Beauveria Bassiana:

The genus *Beauveria* has at least 49 species of which about 22 are considered pathogenic. The most historically significant of the often-utilized fungi in this genus is *Beauveria bassiana*, a white muscardine fungus. This fungus, formerly known as *Tritirachium shiotae*, was renamed in honor of Agostino Bassi, an Italian scientist and lawyer who initially identified it as the cause of a white (later yellowish or occasionally reddish) muscardine sickness in domestic silkworms. *Beauveria bassiana*, a fungus that grows naturally in soils all over the world and causes white muscardine illness in a variety of insect species, is classified as an entomopathogenic fungus (Jain *et al*., 2008). The great host specificity of several isolates of *Beauveria* sp. is an intriguing characteristic. Hosts of agricultural and forest significance include the Colorado potato beetle, the codling moth, and several genera of termites, American bollworm, *Helicoverpa armigera*. Its host range spans to the insects of all orders and extends to ticks and mites. It causes white muscardine disease in the arthropod species when the respective host comes in contact with the infectious propagules. The infective propagules or conidia adheres to the host body, penetrates the cuticle where it proliferates, produces mycotoxin beauvericin that has been documented for its larvicidal properties.

Metarhizium Anisopliae:

The soil-dwelling entomopathogenic fungus *M. anisopliae* is frequently employed as a biocontrol agent against a variety of insect pests. It is a useful tool in IPM programs since it is a member of the phylum Ascomycota and has a wide host range. Through direct cuticle penetration, the fungus infects insects, causing illness that eventually results in the host's death. Greenish conidia, or spores, are produced by *M. anisopliae* and stick to the cuticle of the host insect. Under the right climatic circumstances, these conidia germinate and produce a germ tube that uses mechanical pressure and enzymatic degradation to pierce the insect cuticle (Madhu *et al*., 2010). Once within, the fungus multiplies in the bodily cavity, or hemocoel, creating blastospores that spread throughout the host. *M. anisopliae* is well known for its broad-spectrum effectiveness against a range of forestry and agricultural pests. It is an essential part of IPM methods because of its adaptability and eco-friendliness. Here, we examine how well it works against various pest species. *M. anisopliae* has also been successfully used to suppress lepidopteran pests, such as the autumn armyworm and the diamondback moth, especially when it comes to caterpillar and moth larval stages. Additionally, it has had a notable effect against hemipteran pests including the greenhouse whitefly and the green peach aphid, resulting in considerable pest population reductions and limiting crop loss. A study found that *M. anisopliae* effectively controlled wheat aphid species, including *Schizaphis graminum* and *Rhopalosiphum padi*, in field conditions. The highest concentration (1×10^8

CFU/mL) achieved 85 % mortality by the 5th day. This highlights *M. anisopliae* as a promising, sustainable biopesticide for managing wheat aphids in agriculture (Bhattacharyya *et al*., 2016). The efficiency of *M. anisopliae* in sustainable pest management in a variety of agricultural settings is demonstrated by case studies that emphasize its successful integration. In cotton farming, for example, it has been used in conjunction with crop rotation and pheromone traps to manage pests such as the cotton bollworm, leading to notable increases in productivity. *M. anisopliae* has been employed in conjunction with habitat management techniques in coffee plantations to target the coffee borer beetle and improve the general health of the plants.

Verticillium Lecanii:

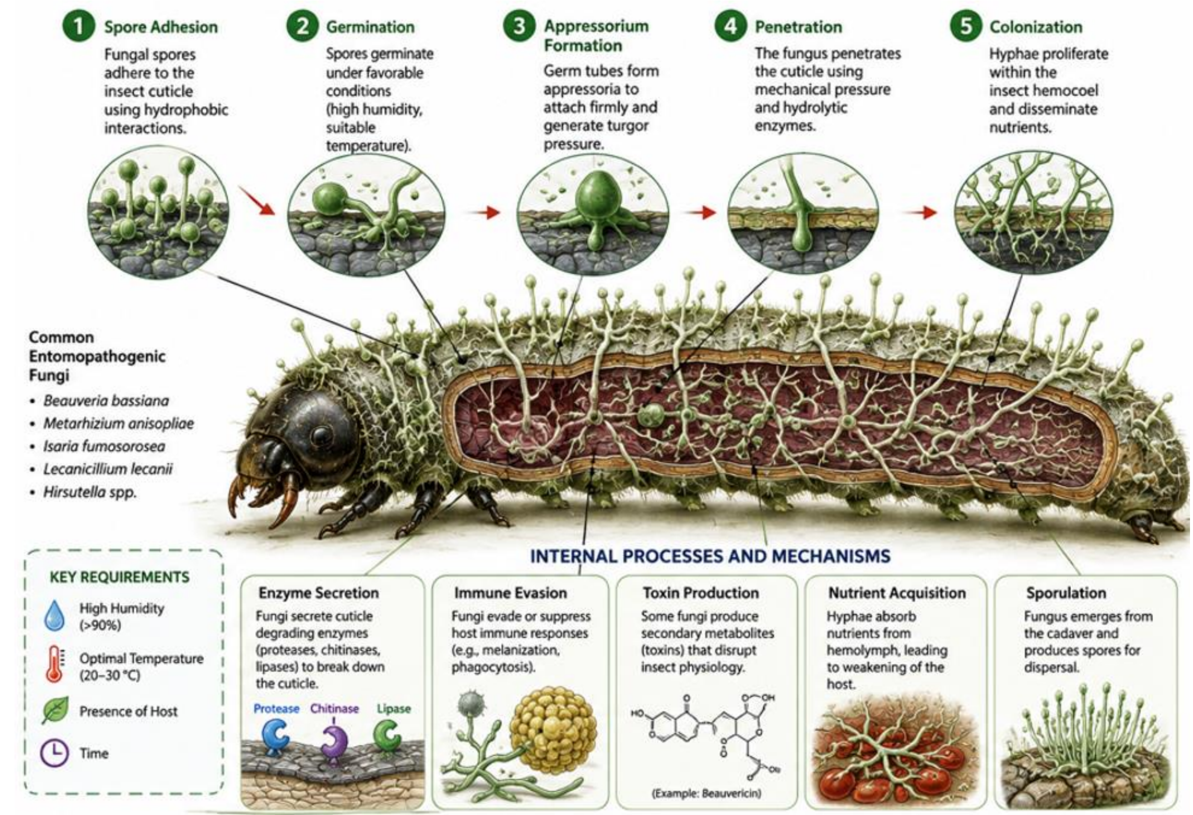
Verticillium lecanii (currently recognized as *Lecanicillium lecanii*) is an important entomopathogenic fungus widely used as a biological control agent against a variety of insect pests. It provides an eco-friendly alternative to chemical insecticides and plays a significant role in integrated pest management (IPM). The fungus is particularly effective against soft-bodied insects such as aphids, whiteflies, thrips, mealybugs, scales, and some species of mites. Due to its high efficacy, environmental safety, and compatibility with sustainable agriculture, *V. lecanii* has gained increasing attention in modern crop protection. The mode of action of *V. lecanii* begins when fungal spores (conidia) come into contact with the insect's cuticle. Under suitable environmental conditions, especially high humidity (above 80%) and temperatures ranging from 20–28°C, the spores germinate and produce germ tubes that penetrate the insect's body using mechanical pressure and enzymes such as chitinases, proteases, and lipases. Once inside the insect, the fungus multiplies rapidly, consuming nutrients and producing toxins that eventually kill the host within a few days. After the insect dies, white fungal growth emerges on the cadaver, producing new spores that spread the infection to nearby pest populations. The mode of action of *V. lecanii* begins when fungal spores (conidia) come into contact with the insect's cuticle. Under suitable environmental conditions, especially high humidity (above 80%) and temperatures ranging from 20-28°C, the spores germinate and produce germ tubes that penetrate the insect's body using mechanical pressure and enzymes such as chitinases, proteases, and lipases. Once inside the insect, the fungus multiplies rapidly, consuming nutrients and producing toxins that eventually kill the host within a few days. After the insect dies, white fungal growth emerges on the cadaver, producing new spores that spread the infection to nearby pest populations (Faria and Wraight, 2007).

***Nomuraea* sp.:**

Nomuraea species are entomopathogenic fungi that naturally infect and kill a wide range of insect pests. Among them, *Nomuraea rileyi* (currently reclassified as *Metarhizium rileyi*) is the most well-known species and is highly effective against several lepidopteran pests. These fungi play an important role in biological pest management by providing an environmentally friendly alternative to chemical insecticides. They are widely studied for their potential in integrated pest management (IPM) programs due to their host specificity and safety to beneficial organisms. *Nomuraea* species are particularly effective against caterpillar pests such as *Spodoptera litura, Helicoverpa armigera, Spodoptera frugiperda*, and other noctuid larvae that cause severe damage to agricultural crops. The fungus has been successfully evaluated in soybean, cotton, maize, pulses, and vegetable production systems. Its ability to naturally suppress pest outbreaks makes it a valuable component of sustainable agriculture.

Table 1: Entomopathogenic fungi effectiveness against agricultural insect pests

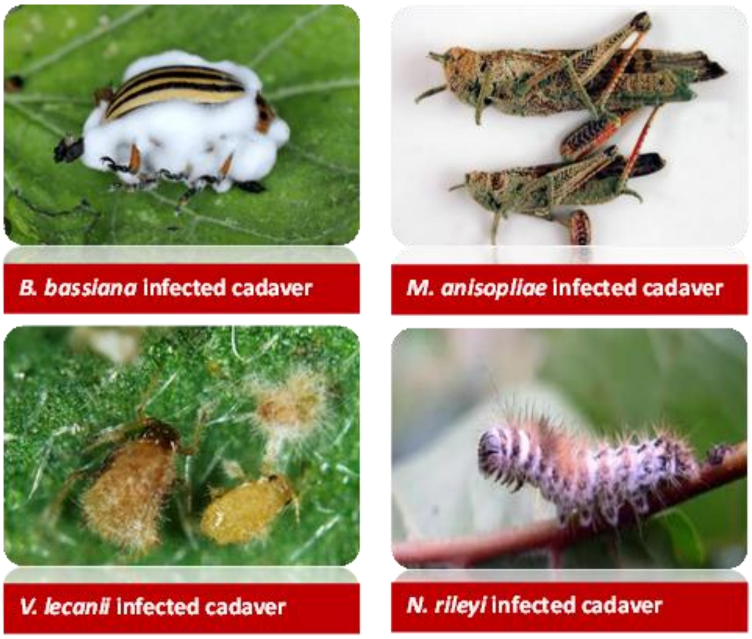
Fungus	**Target pest**	**References**
Beauveria bassiana PDRL1187	*Lipaphis erysimi, Aphis craccivora* Koch	Ujjan and Shahzad (2012)
Beauveria bassiana BB-01	*Schizaphis graminum, Rhopalosiphum padi, Brevicoryne brassicae* and *Lipaphis erysimi*	Palumbo (2001)
Verticillium lecanii	*Myzus persicae, Aphis craccivora* Koch	Michereff *et al*. (2011)
Paecilomyces fumosoroseus n32	*Lipaphis erysimi, Plutella xylostella*	Palumbo (2001)
Metarhizium anisopliae L6	*Lipaphis erysimi, Aphis gossypii, Aphis craccivora*	Ujjan and Shahzad (2012)
Peacilomyces lilcinus PDRL812	*Lipaphis erysimi*	Ujjan and Shahzad (2012)



Mode of Action of Entomopathogenic Fungi

Entomopathogenic Fungus Infection:

The mode of infection of entomopathogenic fungus is distinct. When insects come into contact with entomopathogenic fungal spores, many orders of insects (Lepidoptera, Coleoptera, Diptera, Hymenoptera, and Homoptera) become infected. Insect pathogenic fungus begin their life cycle when their spores germinate on the host's cuticle. Spores covered in a sticky or slimy material (such as *Lecanicillium* spp., *Entomophthorales* spp., and *Hirsutella* spp.) may exhibit passive attachment. Physical, chemical, and enzymatic interactions are all involved in adhesive processes. Final insect adaptation reactions provide a variety of hydrolytic and detoxifying enzymes, including protease, chitinase, lipase, catalyzes, cytochrome P450, and their secondary metabolite. Spore germination takes place in favorable environmental conditions, such as ideal temperature, humidity, nutritional and chemical cues, and cuticular secreted material of the host. The germinating spores create germ tubes through the penetration hook, which physically and enzymatically penetrate the cuticle and into the thinner, softer portion of the insect's body through sensory holes or wounds. Once within the body, the fungal spore begins to proliferate by consuming the insects' internal nutrient material, which causes nutrient deficiencies and disrupts biological processes. It also produces poisonous substances, invades the insect's tissues, blocks the vessels, and eventually kills the host. Depending on the fungal strain and species, but especially on the host's traits, the time it takes for an infected insect to die might range from two to fifteen days after infection. The fungus returns to its hyphal mode when the infection process is finished and the host dies. In comparatively humid conditions, the fungus then grows out of the corpse surface to form fresh, external, infectious conidial saprophytic growth. However, in extremely dry conditions, the fungus may continue to grow inside the cadaver at the hyphal stage, where the body produces conidia.



B. bassiana infected cadaver
M. anisopliae infected cadaver
V. lecanii infected cadaver
N. rileyi infected cadaver

Benefits of using Entomopathogenic Fungi:

- Their residues have no known adverse effects on the environment.
- Entomopathogenic fungi are little or non-toxic to non-target organisms.
- They have narrow area of toxic action, mostly specific to a single group or few species.
- They can be used in combination with synthetic chemical insecticides.
- They are self-perpetuating under ideal environmental conditions.
- Reduce chemical insecticide use.
- Protects biodiversity in managed ecosystem.
- Potential development of pest resistance to mycoinsecticde is less common or may develop more slowly due to unique mode of action.

Conclusions, Future Opportunities, and Challenges:

Myco-biocontrol of insects has been seen as a major alternative to synthetic chemical pesticides and an essential part of environmentally friendly pest management due to growing insect pest disease issues in agriculture and environmental concerns. Comprehending the ecological and environmental factors linked to insect-pathogenic fungi is crucial for both practical applications of biological management of insect pests and for improving our fundamental understanding of the development and maintenance of pathogenesis of these organisms. A distinct and highly specialized class of microorganisms, entomopathogenic fungi have a number of advantageous characteristics that support their development as biopesticides. Nevertheless, most commercially manufactured fungus are based only on a few species of Lecanicillium, Beauveria, Isaria, Metarhizium, etc. The availability of entomopathogen bio-processed products is still limited to specific agricultural pests, in addition to regulatory concerns about the premarket authorization of new active compounds. In fact, more commercially viable biopesticides based on fungal entomopathogens would be developed and adopted in mainstream farming if the ecology of these fungi were well understood. Furthermore, in addition to their genetically modified insecticidal efficiency, enhanced persistence, and improved delivery systems, microbial products with activity against diverse pests are required to promote the widespread adoption of fungal entomopathogen-based biopesticides.

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