Entomopathogenic microorganisms: their role in insect pest management

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Abstract

Background: Entomopathogens are pathogenic to insect pests. Several types of naturally occurring, viz. fungus, bacteria, viruses, and nematodes, infect a range of insect pests and help manage crop growth. They offer several advantages over chemical pesticides, including being precise, safe, and ecologically sustainable. Agricultural systems are streamlined, and changes to natural ecosystems occur. Viruses, bacteria are host-specific, while fungi have a greater host range, and they may infect both soil-dwelling and aboveground pests.

Main body: The study highlights the current state of knowledge on entomopathogenic microorganisms (EM) (entomopathogenic fungi, nematodes, viruses, bacteria, etc.) as it relates to their current usage as biological pest management. It is essential to enhance our understanding of the ecology of EM and their role in nature to use a variety of biological control techniques against insect hosts. This article may help to comprehend their accomplishments in the significant field. Some recent researches indicated common patterns in interactions between insect pests and EM.

Conclusion: More focus has been placed on the use of natural enemies like entomopathogens for pest control in recent years. EM expands possibilities for insect control. Eco-friendly alternatives to existing agricultural pesticides are being developed which are utilized to control insect pests and support agricultural sustainability.

Keywords: Entomopathogenic fungi, Bacteria, Viruses, Nematodes, Pest management

Background

Plant pathogens (fungi, oomycetes, bacteria, viruses, and nematodes), weeds, arthropods (mainly insects and mites), molluscs (slugs and snails), and a few vertebrates are among the agricultural pests. By feeding on crops, they degrade the production and quality of a product. Pest species are believed to number in the millions globally. They have a major impact on a limitation on agricultural output that has resulted in a 40% reduction in potential world crop yields these setbacks (Mantzoukas and Eliopoulos 2020). Mites and insect pests host several naturally occurring bacteria, fungi, nematodes, and viruses that infect a wide range of organisms. Insect pests are considered important deterrents, accounting for an estimated 10.80% of worldwide agricultural losses in the post-green revolution age (Dhaliwal et al. 2015). In addition, an estimated 18–26% decrease in world yearly agricultural output, worth $470 billion, was recently recorded (Mantzoukas and Eliopoulos 2020). Insecticides are used to reduce these losses and, as a result, have become an essential method for controlling insect pest infestations due to their low application effort, high usefulness, and expediency (Sharma 2019). However, concentrated application of chemicals has resulted in the advancement of resistance to one or more classes of insecticides in as much as 80% of cases (Sharma 2019). As a result, entomopathogens, which include fungi, viruses, protozoa, and bacteria, are seen as regulatory operators of pest infestations. Entomopathogens that occur naturally are important control elements for insect populations (Roy and Cottrell 2008). The word entomopathogens was coined by Tanzini et al. (2001) to describe microorganisms that control the population of insect pests to levels...
that cause no economic harm to crop plants. Delgado and Murcia (2011) defined the term concerning the microbial population that can attack insect pests by incorporating them into their life cycle and using them as hosts, as well as classifying these microorganisms as either facultative or obligate parasites attacking insect pests with high survival potential. The use of entomopathogenic microorganisms (EM) as pest control agents is not only efficient against insect pests but is also ecologically friendly for both humans and nontarget creatures (lower pesticide residues). Natural enemies in an agro-ecosystem serve an important role in keeping pests from reaching dangerous levels. Biological control agents (BCAs) are utilized in different ways depending on the type of pest and the control agent’s biological properties, which have several appealing characteristics, including host specificity, absence of toxic residue, no phytotoxic effects, human safety, and the possibility for self-sustaining pest management are just a few of the benefits. However, successful use necessitates a thorough understanding of both the natural enemy and the pest's ecology. Entomopathogens (fungi, bacteria, viruses, and nematodes) must be developed as an efficient biological control agent, which necessitates a thorough understanding of bioassay procedures, as well as manufacturing, formulation, and application strategies.

**Main text**

**Entomopathogenic fungi**

Entomopathogenic fungi (EPF) play a significant role in biological pest management across the world. EPF are heterotrophic, eukaryotic, unicellular, or multicellular (filaments) microorganisms that reproduce sexually, asexually, or both, and generate a range of infective propagules (Bahadur 2018). The efficiency of EPF in the field can be influenced by environmental conditions such as UV light, temperature, and humidity. Hypocreales, Onygenales (Ascosphaera genus), Entomophthorales, and Neozygitales are the orders with the most EPF (Entomophthoromycota) (Sung et al. 2008). *Metarhizium, Beauveria, Verticillium, Nomuraea, Entomophthora*, and *Neozygites* are among the entomopathogenic taxa found in most taxonomic groupings (Deshpande 1999) (Table 1). Insects belonging to the orders Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, and Hymenoptera can be attacked by EPF. Some fungus (such as those in the Hypocreales family) has a wide range of possible victims, whereas Entomophthorales are diseases that affect only one type of insect. They have been documented to infect a broad variety of insect pests and mite species, including lepidopterous larvae, aphids, and thrips, all of which are major agricultural pests across the world (Roberts and Humber 1981). In nature, EPF produce deadly diseases and manage the population of insects and mites. They have a limited danger of targeting nontarget species since they are host-specific. The fungus generates spores (conidia and blastospore), which infect their host by germinating on its surface and then spreading into its body via the exterior cuticle. Spore attachment to the insect cuticle, germ tube penetration of the cuticle, fungus development inside the insect body, and fungal hyphae colonization of the hemocoel are all phases in the infection process. The EPF’s spores are typically covered with a mucus layer of proteins and glucans, which assists in their adhesion to the insect cuticle and the formation of specialized structures known as appressoria (attachment of germinating spore). The mechanical pressure and hydrolytic enzymatic activity (lipases, proteases, and chitinases) of the germ tube culminate in the penetration of the insect cuticle (Xiao et al. 2012). Most EPF develop vegetatively in the insect hemocoel (Roberts and Humber 1981). Mechanical damage induced by developing mycelia inside the insect (mummification) or poisons generated and released by the pathogen is the most common causes of insect death. Toxins such as destruxin, baerovicin, and efrapeptins are secreted by *Beauveria, Metarhizium*, and *Tolypocladium*, and their actions and participations in the pathogenesis process are well understood (Hajek and St. Leger 1994). The fungus generates hundreds of new spores on the deceased corpse after death, which spread and continues the fungus’s life cycle on new hosts. Santiago-Álvarez et al. (2006) while studying the fungus, *Beauveria bassiana* B. (Hypocreales: Cordycipitaceae) for pathogenicity to the sweet potato whitefly, *Bemisia tabaci* G. (Hemiptera: Aleyrodidae), after growing on cucumber, tomato, melon, green pepper, potato, eggplant, marrow, cabbage, bean, or cotton, found that the pathogenicity of *B. bassiana* to the *B. tabaci* was influenced by the host plant. The host plant on which the nymphs were grown had a major impact on the mortality caused by *B. bassiana*, whereas the production of newly formed conidia was also affected by the host plant.

Biological management of insect pests using EPF is a desirable and effective approach that involves the use of natural microorganisms that inhibit their activity and can be used as an alternative to chemical insecticides. Some EPF genera are pesticides for agricultural, greenhouse, forest, storage, and residential pests. *Beauveria, Metarhizium, Isaria, Lecanicillium*, and *Hirsutella*, for example, can be used as EPF (Sharma and Sharma 2021). Many species in these genera are target selective and infect a wide range of insects. EPF have several biological characteristics, including target selectivity, high reproductive
ability, quick generation time, and extended survival, all of which are important in the biocontrol of insect pests (Sharma and Sharma 2021).

Plant disease antagonists, rhizosphere colonizers, insect–pest biocontrol agents, plant growth-promoting fungi, and fungal endophytes are all significant roles for EPF. Biological control involves the employment of natural or engineered fungus or bacteria that are antagonists of plant diseases. The creation of different metabolites, such as antibiotics, bioactive volatile chemicals (e.g., ammonia, hydrogen cyanide, alkyl pyrones, alcohols, acids, esters, ketones, and lipids), and enzymes, reduces a pathogen’s survival or disease-causing ability. Competition, antibiotics, hypovirulence, parasitism, and induced systemic resistance are among the additional mechanisms at work (Ownley and Windham 2007). The EPF, such as *B. bassiana* and *Lecanicillium* spp., are not only hostile to insects but also to plant diseases (Kim et al. 2008). Antibiosis, competition, and induced systemic resistance are some of the antagonism mechanisms used by *B. bassiana* (Benhamou and Brodeur 2001). Fungal infections are gaining popularity as a biological control agent for a variety of insect pests, and this technique is proven to be successful, cost-efficient, and environmentally friendly (Wraight et al. 2001). EPF have various characteristics that make them a viable option for use in the IPM program.

### Table 1
Entomopathogenic microorganisms in crops and their host as potential target for pest management. Source: Klein (1990), Desphande (1999), Okano et al. (2006), van Oers and Flak (2007), Dara (2017)

| Entomopathogenic group | Entomopathogen species | Target pest as host |
|------------------------|-------------------------|---------------------|
| Bacteria               | *Paenibacillus popilliae* | Japanese beetle, *Popillia japonica* |
|                        | *Bacillus sphaericus*    | Diptera             |
|                        | *Bacillus popillae*      | Coleoptera          |
|                        | *Bacillus thuringenesis kurstaki* | Lepidoptera |
|                        | *Bacillus thuringenesis israelensis* | Diptera |
|                        | *Bacillus thuringenesis tenebrionis* | Coleoptera |
|                        | *Bacillus thuringenesis aizawai* | Lepidoptera |
| Viruses                | Nucleopolyhedrovirus (NPV): | Lepidoptera, Hymenoptera |
|                        | *Hyposidra talaca npv*  |                     |
|                        | *Helicoverpa zea NPV*    |                     |
|                        | *Spodoptera exigua NPV*  |                     |
|                        | *Granulovirus (GV):*     | Lepidoptera         |
|                        | *Cydia pomonella Granulovirus (CpGV)* |                     |
| Fungi                  | *Paecilomyces lilacinus* | Plant–parasitic nematodes |
|                        | *Verticillium lecanii*   | One or more pests of Coleoptera, Hymenoptera, *Acarina*, Hemiptera, Lepidoptera, Orthoptera, Thysanoptera, etc. |
|                        | *Lecanicillium longiosporun* |                     |
|                        | *Lecanicillium lecanii*  |                     |
|                        | *Metarhizium brunneum*   |                     |
|                        | *Metarhizium anisopliae* |                     |
|                        | *Entomophthora muscae*    |                     |
|                        | *Hirsutella thompsonii*  |                     |
|                        | *Beauveria bassiana*     |                     |
|                        | *Nomuraea rileyi*        |                     |
|                        | *Isaria fumosorosea*     |                     |
|                        | *Neozygites fresenii*    |                     |
| Nematodes              | *Heterorhabditis heliothidis* | Several orders of soilborne pests |
|                        | *Heterorhabditis bacteriophora* |                     |
|                        | *Steinernema feltiae*    |                     |
|                        | *Steinernema carpocapsae*|                     |
species from two families (Heterorhabditidae and Steinernematidae) have been successfully employed as biological insecticides (Koppenhöfer 2007). EPNs are found in soil settings naturally, and they identify their hosts in response to carbon dioxide and other chemical signals (Kaya and Gaugler 1993). Commercially generated EPNs are employed as biological control agents against a variety of soil insect pests and insects (Boemare 2002). EPNs are soil organisms that have a symbiotic–mutualistic interaction with bacteria that can manage insect pests biologically. EPNs may be mass-produced and sprayed using standard spray equipment with ease. They can live in a wide range of environments and are environmentally friendly. Infectious juveniles penetrate the hemocoel and release a symbiotic bacterium that is kept in the nematode's gut (Poinar 1990). The germs induce septicemia, which kills the host in 24–48 h. Infectious juveniles feed on bacteria that are quickly proliferating and disintegrating host tissues. Within the host corpse, the nematode completes around 2–3 generations. EPN's symbiotic relationship with bacteria improves nematode reproduction (bacteria serve as food) and pathogenicity. Nematodes operate as vectors, carrying bacteria into a host where they may grow, and the bacteria provide the required circumstances for nematode survival and reproduction within the insect carcass. EPNs, which belong to the Steinernematidae and Heterorhabditidae families, are well known for their potential as a biological control agent in plant protection (Klein 1990) (Table 1). All Steinernema species are linked to Xenorhabdus bacteria, while all Heterorhabditis nematode species are linked to Photorhabdus bacteria (Boemare et al. 1993). Steiner- nematids and heterorhabditids are widely distributed and have been found in soils all over the world (Hominick et al. 1996). Their effectiveness against many pest insects has been thoroughly researched (Ebsssa 2005). In vivo or in vitro procedures can be used to mass-produce EPNs. The wax moth larva, Galleria mellonella L. (Lepidoptera: Pyralidae) is often used to raise nematodes, as is the liquid fermentation approach for large-scale nematode production (Friedman 1990). EPNs are now created in vivo or in vitro using a variety of ways (Shapiro-Ilan and Gaugler 2012). Trays, shelves, and white traps were used in vivo with G. mellonella surrogate host larvae (White 1927). In vitro culturing of EPNs is based on exposing worms to a pure culture of their symbiont in a nutritive medium, and huge fermenters are employed to generate enormous amounts of EPNs for commercial application. Nematode virulence and viability tests, age, and the ratio of viable to non-viable worms may all be used to assess the quality of the nematode product (Grewal and Peters 2005). EPNs enter the hemocoel after parasitizing their host insect by the spiracles, mouth, anus, or in certain species, intersegmental membranes of the cuticle (Bedding and Molyneux 1982). They then introduce symbiotic bacteria, which proliferate quickly and produce septicaemia, which can kill the host in as little as 48 h. If the insects are killed by heterorhabditids, the cadaver turns red; if the insects are killed by steinerne- matids, the body turns brown or tan (Kaya and Gaugler 1993). The bacteria consume the insect's body, providing food for the nematodes. The juvenile nematodes mature into adults and reproduce after the insect has perished. After 8–14 days, a new generation of infective juveniles arises. The infective juvenile stage of EPNs is the sole free-living stage. Photorhabdus and Xenorhabdus bacteria are mutualistically related to Heterorhabditis and Steinernema, respectively (Ferreira and Malan 2014). The juvenile stage releases symbiotic bacteria cells into the hemocoel from their intestines. The bacteria proliferate in the hemolymph of the infected insect, and the infected host dies within 24–48 h. Nematodes continue to feed on host tissue after the host has died, develop, and breed. To reach adulthood, the offspring's nematodes go through four juvenile stages. Heterorhabditid and steinernematid nematodes reproduce differently. Infectious juveniles of heterorhabditid nematodes mature into hermaphroditic adults, but the following generation produces both males and females, whereas steinernematid worms produce men and females in all generations (Grewal and Peters 2005). EPNs thrive in sandy soil with a pH range of 4–8 and they are vulnerable to cold, extreme heat, dehydration, and UV radiation.

From 1990 to 2010, researchers studied EPNs distribution and biodiversity in different Italian regions and reported two major species, Steinernema feltiae F. (Rhabditida: Steinernematidae) and Heterorhabditis bacteriophora P. (Rhabditida: Heterorhabditidae) (Tarasco et al. 2015). García et al. (2005) tested five different EPFs strains on Capnodis tenebrionis E. (Coleoptera: Buprestidae) neonate larvae. As a result of exposure to 10 and 150 infective juveniles per larva (corresponding to 3 and 48 IJs/cm²), the mortality ranged from 60 to 100% was recorded. All nematode strains were pathogenic at 150 IJs/larva. García et al. (2013) tested 3 native EPNs, viz. Steinernema carpocapsae W. (Rhabditida: Steinernematidae), S. feltiae, and H. bacteriophora against Tuta absoluta M. (Lepidoptera: Gelechiidae) larvae, pupae, and adults. These species’ larvae died in large numbers when nested in the soil to pupate. Adults died at a rate of 79.1% for S. carpocapsae and 0.50% for S. feltiae. When studied the effects of 3 commonly used pesticides against T. absoluta on these nematodes, it was found that the entomopathogens were not affected by insecticides (Garcia et al. 2013). Shamseldin et al. (2010) reported that inoculation of Washington navel orange with
produce a variety of recombinant proteins (Condrey and humans, the baculovirus has been widely employed to populations (van Oers and Flak 2007). In insect cells and circular double-stranded DNA genome is found in both (GV) and Nucleopolyhedrovirus (NPV) (Table 1). The it is divided into two categories, namely: Granulovirus chance of controlling lepidopteran pests on crops, and ised to discover the virus in this family that has the best eficial insects and other nontarget creatures, including mammals; the baculoviridae has long been regarded as a potentially ecologically benign alternative to chemical pesticides and offer several benefits. Their mechanism of action, for example, is typically more complex than traditional pesticides, aiming at a range of places where resistant pests are more prone to emerge.

Entomopathogenic viruses
Insect-killing viruses, known as entomopathogenic viruses (EPV), have emerged in recent years. Many viruses were tested in the early 1900s for the management of insect pests all over the world, but the first virus-based insecticide was only registered in the USA in 1970 to control the cotton bollworm (Ignoffo 1973). Several viruses are approved for use in the control of insect pests and increased research is being undertaken to characterize and evaluate new viruses (López-Ferber 2020). Viruses attack and destroy a wide range of insects. EPVs are viruses that have been identified in a variety of insect orders. Viruses can be utilized as a biological control agent because some insect pests are sensitive to viral infections. Insect viruses can be made up of either double-stranded or single-stranded DNA (dsDNA and ssDNA), as well as RNA (dsRNA and ssRNA). EPVs have been recognized to cause diseases since the sixteenth century. Several viruses targeted several plant pests in the global agro-ecosystems. A disease grasserie (jaundice), now known as nucleopolyhedrosis, was discovered in silkworms (Bombyx mori L. Lepidoptera: Bombycidae), as well as another viral illness in honeybees, Apis mellifera L. (Hymenoptera: Apidae). A nucleic acid is wrapped in a protein coat known as capsid in virus partials, which plays a vital role in the host cell infection process. When a virus partially enters a cell, its nucleic acid takes control of the host metabolic system, producing many copies until the cell dies. The virus is an obligatory parasite that cannot multiply in vitro. The International Committee on Taxonomy of Viruses (ICTV) divides EPVs into 12 viral families (van Regenmortel et al. 2000). Viruses are very particular to their hosts and can lead to large reductions in host numbers. Three insect-specific families (Baculoviridae, Polydnaviridae, and Ascoviridae) viruses are extremely host-specific and nonpathogenic to beneficial insects and other nontarget creatures, including mammals; the baculoviridae has long been regarded as a potentially ecologically benign alternative to chemical pesticides. The baculovirus (ds DNA) is commonly examined to discover the virus in this family that has the best chance of controlling lepidopteran pests on crops, and it is divided into two categories, namely: Granulovirus (GV) and Nucleopolyhedrovirus (NPV) (Table 1). The circular double-stranded DNA genome is found in both populations (van Oers and Flak 2007). In insect cells and humans, the baculovirus has been widely employed to produce a variety of recombinant proteins (Condrey and Kost 2007). Each group’s EPVs infection manifests itself differently in terms of outward symptoms. When the insect slows down in its activities, stops eating, and stops growing, the first visible indicators arise. The route of pathogenesis and reproduction of EPVs differs per family; however, infection is almost always transmitted by ingestion. The viral particles attach to receptors in the stomach and pass through epithelial cells. Infection proceeds to the haemocoeel and subsequently to vital organs and tissues, notably fat bodies. Acute infections result in the death of the host in 5–14 days. Infected insects with baculoviruses appear white due to a major infection of the fat body, visible through a more transparent integument (exoskeleton) that thins as the illness progresses until it ruptures. After the larva climbed up and hung head down from its crochets in an inverted "V" shape, a grayish to creamy liquid is discharged, including billions of occlusion bodies (OBs), which aid in the spread of inocula in the field (Granados and Williams 1986).

Four nuclear polyhedrosis virus isolates from the beet armyworm, Spodoptera exigua H. (Lepidoptera: Noctui-dae) were studied by Caballero et al. (1992). The isolates came from three countries: the USA, Thailand, and Spain (SeNPVSP1 and SeNPVSP2). The viral genomes had only minor restriction fragment length polymorphism, indicating many related but distinct genotypes (variants). Each isolate’s BgIII fragment can be used as a restriction fragment length polymorphism marker. SeNPVs have a genome size of 134 kbp. The four SeNPV isolates had very similar occluded virion polypeptide and polyhedrin mobility patterns. The polyhedrin from SeNPVUS was digested by Staphylococcus aureus V8 and found to be unique. In the second instar S. exigua larvae bioassays; the SeNPVTH had the lowest LD50, only 1.5 polyhedra per second instar larva (Caballero et al. 1992).

Cotton pests include S. exigua and Pectinophora gossypiella S. (Lepidoptera: Gelechiidae), as well as the Heli-othis/Helicoverpa complex, which are under substantial control after the use of EPVs. Baculoviruses have a limited host range that is generally limited to the order and family of the host of origin, and commercial baculovirus biopesticides are thought to pose a low danger to people and wildlife. Baculoviruses can only be mass-produced in vivo; however, they are economically viable for bigger hosts like Lepidoptera.

Entomopathogenic bacteria
Biological control methods, such as bacterial entomopathogens, are generally thought to be safer than chemical pesticides and offer several benefits. Their mechanism of action, for example, is typically more complex than traditional pesticides, aiming at a range of places where resistant pests are more prone to emerge.
Bacillus - The Bacillaceae have received the most attention. Insects under stress, but a small number are very virulent. Most of these bacteria are mild pathogens that infect and other environments often do not result in a build-up of spores in the environment, and the vitality of spores, particularly those exposed to sunshine, is shown to reduce (Ignoffo 1992). Bt is a microbial control treatment that is sprayed to insect infestations that causes the target insects to die quickly, typically without recycling. In comparison to other ecosystem interventions, the safety and environmental effect of EPBs should be assessed considering the danger to nontarget species. Plants can express the genes that encode the Bt-endotoxin, making them resistant to various insect pests. According to farmer surveys, cultivating Bt crops can result in a large reduction in the use of conventional pesticides. Mirid bugs have generated secondary pest concerns on Bt cotton cultivated in China. Bt cotton does not control these pests, which were previously managed by a broad range of insecticides. Mirid problems did not appear in China until a few years after Bt cotton was widely used. For some years, genetically modified (GM) maize and cotton crops expressing lepidopteran active endotoxins have been available, and they have revolutionized farming in the nations where they are cultivated. Eight nations presently cultivate GM crops (India, the USA, Canada, China, South Africa, Paraguay, Argentina, and Brazil), and GM crops do have negative consequences.

Conclusions
Chemical pesticides are often employed to protect plants. This has the reason of increasing insect resistance to numerous chemical chemicals included in plant protection products. More emphasis has been made in recent years on the prospect of employing natural enemies, such as entomopathogens, to manage insect infestations. EM are microorganisms that kill insect pests. This might open new possibilities for controlling insect infestations. Entomopathogens are being developed for use in agriculture crops as ecologically favorable alternatives. They can be used to manage insect pests as biological control agents and increase agro-sustainability. The biological control mechanism against insect pests on agriculture crops is one of the ecologically recognized methods. In the realm of pesticides, the field of microbial pesticides provides a unique opportunity to conduct prospective and predictive research.

Abbreviations
BCAs: Biological control agents; EPF: Entomopathogenic fungi; EPNs: Entomopathogenic nematode; EPV: Entomopathogenic viruses; ICTV: International Committee on Taxonomy of Viruses; dsDNA: Double-stranded DNA; ssDNA: Single-stranded DNA; GV: Granulovirus; NPV: Nucleopolyhedrovirus; OBs: Occlusion bodies; GM: Genetically modified; Bt: Bacillus thuringiensis.

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Authors’ contributions
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