REVIEW ARTICLE

Significance of Microbes and their Role in Pest Management in Tea Ecosystem

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ABSTRACT

Tea crop damage is caused by mites and insect pests, and each year a significant amount of crop loss is occurring due to their damage. Synthetic pesticides' efficiency has allowed them to be widely used as a control tool for several decades. Synthetic pesticides, on the other hand, have resulted in the development of insect pest resistance, pollution, and pesticide residues in the finished product, among many other issues, forcing the planting community to look for an alternative strategy. Microbial pesticides have been used to combat mite and insect pest-damaging tendencies, and a substantial portion of scientific evidence indicates that their actions are both desirable and environmentally beneficial. In recent years, there has been a lot more emphasis on the use of natural enemies such as entomopathogens for pest control. entomopathogenic microorganisms (EM) expands the range of pest control possibilities. Eco-friendly alternatives to existing agricultural pesticides that are employed to manage insect pests and improve agricultural sustainability are being developed. The study summarises current knowledge on EM (entomopathogenic fungi, nematodes, viruses, bacteria, etc) as it relates to their present application as biological pest management.

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

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INTRODUCTION

Tea is one of the most popular non-alcoholic beverages derived from *Camellia sinensis* (L.) O. Kuntze. C. sinensis is a perennial monoculture plantation crop that contributes to the economies of many countries,[1-2] including India. Many insect and mite pests endanger tea production around the world (Table 1). Because the tea ecology is changing rapidly, outbreaks of emerging pests occur from time to time as a result of global climate change.[2] Although these pests are managed through cultural and chemical practises, chemical insecticides have limitations in that they cause several ill effects as continuous application of synthetic pesticides causes different health hazards not only to tea workers but also to consumers, as well as environmental pollution.[3] In recent years, researchers have been interested in natural insecticides based on botanicals or bio-control agents to treat insect pests of tea plants,[4] and entomopathogens are no exception.

Biological control agents (BCAs) such as *Beauveria*, *Metarhizium*, and other species have been demonstrated to be safe and promising components of IPM strategies used in a variety of crops, including tea.[5] While the available research on this area is limited,[6] much of the data on the commercialization of beneficial fungi as microbial pesticides consists of case studies and success stories for the use of tea crops. Microorganisms are the most active and crucial component of many agroecosystems, including the tea ecosystem. They are found in soil, air, and phylloplane, and have various known and unknown interactions with plants, which greatly influence crop productivity. These bacteria interact with the ecosystem in various ways, resulting in both negative (the development of various illnesses of the tea crop) and beneficial effects (helping to keep the population of certain phytopathogens and insect pests below the economic threshold level ETL) (Table 2). These biological control agents (BCAs) are crucial in the management of many insect pests and diseases associated with various agricultural and plantation crops.[7] Since the 1970s, 72 viral species and around 40 fungal and bacterial species that are effective against insect and mite pests of tea plants have been identified as possible biocontrol agents for tea plants, and other viruses and fungus have been studied to identify others.[8] Table 3 contains a summary of mite and insect pest management strategies, including some effective microbial pesticides. This mini-review discusses various microbial pesticides with potential efficacy for tea pest management.

Bacteria

Biological pest management approaches, such as bacterial entomopathogens, are regarded to be safer than chemical pesticides and offer a number of advantages. For example, their mode of action is often more complex than standard pesticides, targeting at a variety of locations where resistant bugs are more likely to evolve.[22] While entomopathic bacteria can be used as a stand-alone pest management technique, for optimal efficacy and environmental sustainability, they are best used in rotation or in combination with insecticides. Numerous investigations have found that enteropathogenic bacteria and chemical substances are compatible and synergistic.[23] Other advantages include worker safety, fewer crop residues, harvest flexibility as a result of a short or no pre-harvest time, and the use of biopesticides in pest-management programmes. The majority of insect bacterial infections are caused by bacteria from the *Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae*, and *Micrococcaceae* families. The majority of these bacteria are moderate pathogens that infect insects under stress, but a few are quite virulent. Bacillaceae have gotten the greatest attention.

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Table 1: Different pests of tea crop[9-11]

Common name	*Scientific name*
Major pests of tea	
Tea mosquito bug:	*Helopeltis theivora* Waterhouse (Miridae: Hemiptera)
Thrips	*Scirtothrips dorsalis* Hood (Thripidae: Thysanoptera)
Jassid	*Empoasca flavescens* Fab. (Cicadellidae: Hemiptera)
Aphids	*Toxoptera aurantii* Boyer de Fonscolombe (Aphididae: Hemiptera)
Bunch caterpillar:	*Andraca bipunctata* Walker (Bombycidae: Lepidoptera)
Red spider mite	*Oligonychus coffeae* Nietner (Tetranychidae: Acari)
Tea looper complex	*Buzura suppressaria* Guen (Geometridae: Lepidoptera), *Hyposidra talaca* (Walker), *H. infixaria* (Walker) (Geometridae: Lepidoptera)
Shot hole borer	*Euwallacea fornicates* Eichhoff (Scolytidae: Coleoptera)
Live wood eating termite	*Microcerotermes sp.* (Isoptera:Termitidae)
Scavenging termites	*Odontermes sp.* (Isoptera:Termitidae)
Minor pests of tea	
Flush worm	*Cydia leucostoma* Meyrick (Tortricidae: Lepidoptera)
Pink and Purple mite	*Acaphylla theae* Watt and *Calacarus carinatus* Green (Eriophyidae: Acarina)
Scarlet mite	*Brevipalpus phoenicis* Geijskes (Tenuipalpidae :Acarina)
Yellow mite	*Polyphagotarsonemus latus* Banks (Tarsonemidae: Acarina)
Leaf roller	*Caloptilia theivora* Walsingham (Gracillariidae: Lepidoptera)
Scales	*Saissetia formicarii* Takahashi, *S. coffeae* Walker, *Eriochiton theae* Green, *Coccus viridis* Green (Coccidae: Hemiptera)
Tea tortrix	*Homona coffearia* Nietner (Tortricide: Lepidoptera)

Table 2: Economic Threshold Level (ETL) of major pests of tea[12]

Name of the Pest	*Economic Threshold Level (ETL)*
Tea Mosquito Bug	5% infestation
Aphids	20% infestation
Thrips	3 Thrips per shoot
Jassids	50 nymphs per 100 leaves
Looper caterpillar	4–5 Lopper per plant
Flush worm, Leaf Rollers	5 infested rolls per bush
Red Spider Mites, Pink and Purple Mites	4 mites per leaf
Termites	10% infestation
Nematodes	6 numbers of nematode/10 gm of soil

Bacillus popillae causes milky sickness in scarabaeids, but *Bacillus sphaericus* (Bacillales: Bacillaceae) is transmitted by mosquitos. *Bacillus thuringiensis* (Bacillales: Bacillaceae) (Bt) is a widely used entomopathogenic agent for the control of caterpillars and beetles. Bt is a spore-producing bacteria. Sporulation is frequently associated with the development of an insecticidal proteinaceous protoxin crystal. Ingested crystals breakdown in the stomach and are broken down by host proteases to produce endotoxin, an active toxin. This bacterium creates parasporal bodies (crystals) containing unique insecticidal endotoxins (Cry proteins) that act through ingestions via a pore-forming mechanism that harms the insect gut epithelium.[24] Several research have been carried out to evaluate the impact of Bt toxins on both target and non-target species.[25] The mechanism of action of these novel entomopathogenic bacteria is complex, and the metabolites recently related to insecticidal properties are diverse.[26] Cry toxins (-endotoxins) are now commercially accessible for use against a wide variety of insect pests such as Lepidoptera, Coleoptera, and Diptera species. Commercial cry poisons, which are used to control lepidopteran pests, have little direct effect on non-target creatures.[27] A study of five weekly administrations of low and high label rates of a genetically altered strain of Bt for control of *Leptinotarsa decemlineata* S. (Coleoptera: Chrysomelidae) discovered that the beetle was effectively controlled with no discernible impact on non-target organisms such as predatory Hemiptera.[28]

Applications of Bt bioinsecticides to agro-ecosystems and other habitats frequently do not result in spore accumulation, and the viability of spores, particularly those exposed to sunlight, has been shown to decrease.[29] Bt is a microbiological pest control product that is sprayed on insect infestations and causes the target insects to die swiftly, usually without recycling. In comparison to previous ecosystem interventions, the safety and environmental impact of EPBs should be evaluated while keeping non-target species in mind. Plants can produce the genes that code for the Bt-endotoxin, rendering them resistant to a variety of insect pests. According to farmer surveys, growing Bt crops can result in a significant decrease in the usage of traditional pesticides. Mirid bugs have caused secondary pest issues on Bt cotton grown in China. These pests, which were formerly controlled by a variety of insecticides, are no longer controlled by Bt cotton. Mirid issues did not develop in China until after Bt cotton had been widely employed for a few years. Genetically modified (GM) maize and cotton crops expressing lepidopteran active endotoxins have been available for some time, and they have changed farming in the countries where they are grown. GM crops are currently grown in eight countries (India, the

United States, Canada, China, South Africa, Paraguay, Argentina, and Brazil), and they have detrimental impacts.

Viruses

Entomopathogenic viruses (EPV), which destroy insects, have developed in recent years. Many viruses were tested for the management of insect pests all over the world in the early 1900s, but the first virus-based insecticide was only registered in the United States in 1970 to control the cotton bollworm.[30] Several viruses have been approved for use in insect pest control, and more research is being conducted to define and evaluate novel viruses.[31] Viruses infect and kill a wide variety of insects. EPVs are viruses that have been found in a wide range of insect orders. Because some insect pests are susceptible to viral infections, viruses can be used as a biological control agent. Insect viruses can be double-stranded or single-stranded DNA (dsDNA and ssDNA), as well as RNA (dsRNA and ssRNA). EPVs have been linked to sickness since the 16th century. Several viruses in worldwide agro-ecosystems targeted various plant pests. *A grasserie* (jaundice) disease was discovered in silkworms (*Bombyx mori* L. Lepidoptera: Bombycidae), as well as another viral ailment in honeybees (*Apis mellifera* L.) (Hymenoptera: Apidae). In virus partials, a nucleic acid is encased in a protein coat known as capsid, which plays an important role in the host cell infection process. When a virus enters a cell partially, its nucleic acid takes over the host metabolic system and multiplies many times until the cell dies. The virus is a mandatory parasite that cannot replicate *in-vitro*." EPVs are classified into 12 viral families by the International Committee on Virus Taxonomy (ICTV).[32] Viruses are extremely specific to their hosts and can result in significant host population declines. The viruses of three insect-specific families (Baculoviridae, Polydnaviridae, and Ascoviridae) are extremely host-specific and non-pathogenic to beneficial insects and other non-target creatures, including mammals; the baculoviridae has long been regarded as a potentially environmentally benign alternative to chemical pesticides. Baculovirus (ds DNA) is usually investigated to determine which virus in this family has the best possibility of controlling lepidopteran pests on crops, and it is classified into two groups: Granulovirus (GV) and Nucleopolyhedrovirus (NPV). Both populations have a circular double-stranded DNA genome.[33] The baculovirus has been widely used in insect cells and humans to produce a wide range of recombinant proteins.[34] In terms of outward symptoms, EPVs infection manifests differently in each population. The first observable symptoms appear when the insect slows down in its activities, stops eating, and ceases growing. The aetiology and reproduction of EPVs varied according on the family; nonetheless, infection is virtually always transmitted through ingestion. The virus particles bind to stomach receptors and pass past epithelial cells. The infection spreads to the haemocoel and then to critical organs and tissues, most notably fat bodies. Acute infections cause the host to die within 5 to 14 days. Baculovirus-infected insects appear white due to a significant infection of the fat body, which is visible through a more translucent integument (exoskeleton) that thins as the illness develops until it ruptures. A greyish to creamy liquid is expelled after the larva climbs up and hangs head down from its crochets in an inverted "V" form, including billions of occlusion bodies (OBs), which aid in the dissemination of inocula in the field.[35]

Table 3: Microbial pesticides used in controlling insect and mite pests of tea plants[13-21]

Microbial pesticide	*Insect/mite pest*
Ectropis *obliqua* nuclear polyhedrosisvirus (EcobNPV)	Ectropis *obliqua*
Ectropis *obliqua* single nucleocapsid nucleo-polyhedro- virus (EcobSNPV)	Ectropis *obliqua*
Pseudomonas fluorescens	O. coffeae
Bacillus thuringiensis (Bt)	E. obliqua
B. bassiana	E. onukii
V. lecanii, P. fumosoroseus, Hirsutella thompsonii,V. lecanii, P. fumosoroseus and H. thompsonii	O. coffeae
Entomopthora sp. and Verticillium sp.	O. coffeae
Metarhizium anisopliae	O. coffeae
Paecilomyces lilacinus	O. coffeae
A. niger and A. flavus	O. coffeae
Fusarium, A. flavus, A. niger, Cladosporium sp., Curvularia sp., Acremonium,and Trichoderma	H. theivora
B. bassiana	H. theivora

Caballero *et al.* (1992) investigated four nuclear polyhedrosis virus isolates from the beet armyworm, *Spodoptera exigua* H. (Lepidoptera: Noctuidae). The isolates were from three different countries: the United States, Thailand, and Spain (SeNPVSP1 and SeNPVSP2). There was very minimal restriction fragment length polymorphism in the viral genomes, indicating a large number of related but distinct genotypes (variants). The BglII fragment of each isolate can be utilised as a restriction fragment length polymorphism marker. The genome of SeNPVs is 134 kbp in size. The blocked virion polypeptide and polyhedrin mobility patterns of the four SeNPV isolates were very comparable. *Staphylococcus aureus* V8 digested the polyhedrin from SeNPVUS and discovered it to be unique. The SeNPVTH showed the lowest LD_{50} in the second instar *S. exigua* larvae bioassays, with just 1.5 polyhedra per second instar larva.[36]

Cotton pests such as *S. exigua* and *Pectinophora gossypiella* S. (Lepidoptera: Gelechiidae), as well as the *Heliothis/ Helicoverpa* complex, have been significantly reduced following the introduction of EPVs. Baculoviruses have a narrow host range that is largely restricted to the order and family of the host of origin, and commercial baculovirus biopesticides are regarded to pose little risk to humans and wildlife. Baculoviruses can only be created *in-vivo*, but they are commercially viable in larger hosts such as Lepidoptera.

Fungi

Entomopathogenic fungi (EPF) are important in biological pest management all over the world. EPF are microorganisms that reproduce sexually, asexually, or both and produce a variety of infective propagules.[37] Environmental variables such as UV radiation, temperature, and humidity can all have an impact on EPF efficiency in the field. The orders containing the most EPF (Entomophthoromycota) are the Hypocreales, Onygenales (Ascosphaera genus), Entomophthorales, and Neozygitales.[38] Entomopathogenic taxa present in most taxonomic groups include *Metarhizium, Beauveria, Verticillium, Nomuraea, Entomophthora,* and *Neozygites*.[39] EPF can attack insects from the orders Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, and Hymenoptera. Some fungi (such as those in the Hypocreales family) can infect a broad variety of insects, whereas Entomophthorales are diseases that only affect one type of insect. They have been shown to infect a wide range of insect pests and mite species, including lepidopterous larvae, aphids, and thrips, all of which are major agricultural pests worldwide.[40] In nature, EPF produces lethal illnesses and manages bug and mite populations. Because they are host-specific, they pose little risk of targeting non-target species. The fungus produces spores (conidia and blastospores), which infect their host by germinating on its surface and spreading through the external cuticle. The infection process includes spore attachment to the insect cuticle, germ tube penetration of the cuticle, fungus development inside the insect body, and fungal hyphae colonisation of the hemocoel. The spores of the EPF are usually covered with a mucus layer of proteins and glucans, which aids in adherence to the insect cuticle and the production of specialised structures known as appressoria (attachment of germinating spore). The mechanical pressure and hydrolytic enzymatic activity of the germ tube (lipases, proteases, and chitinases) culminate in the penetration of the insect cuticle.[41] The majority of EPF develop vegetatively in the insect hemocoel.[40] The most prevalent causes of insect death are mechanical damage caused by developing mycelia inside the insect (mummification) or toxins produced and released by the disease. Beauveria, Metarhizium, and Tolypocladium release toxins such as destruxin, bavericin, and efrapeptins, and their activities and participation in the pathogenesis process are well characterised.[42] After death, the fungus produces hundreds of new spores on the deceased corpse, which disperse and continue the fungus's life cycle on new hosts. 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, Santiago-lvarez *et al.*[43] discovered that the pathogenicity of The host plant on which the nymphs were raised had a significant impact on the mortality caused by *B. bassiana*, as did the creation of freshly produced conidia.

EPF-based biological pest management is a desirable and successful strategy that involves the use of natural microorganisms that impede their activity and can be used as a substitute to chemical insecticides. Pesticides for agricultural, greenhouse, woodland, storage, and residential pests are found in some EPF genera. EPF plants include *Beauveria, Metarhizium, Isaria, Lecanicillium*, and *Hirsutella*.[44] Many of these species are target selective and infect a wide variety of insects. EPF have various biological features that are significant in the biocontrol of insect pests, including target selectivity, strong reproductive ability, short generation time, and extended survival.[44]

EPF plays important roles as plant disease antagonists, rhizosphere colonisers, insect-pest biocontrol agents, plant growth-promoting fungi, and fungal endophytes. Biological control entails the use of naturally occurring or created fungi or bacteria that are antagonists of plant diseases. A pathogen's ability to survive or cause disease is reduced by the production of metabolites such as antibiotics, bioactive volatile compounds (e.g., ammonia, hydrogen cyanide, alkyl pyrones, alcohols, acids, esters, ketones, and lipids), and enzymes. Additional mechanisms at action include competition, antibiosis, hypovirulence, parasitism, and induced systemic resistance.[45] EPFs, like as *B. bassiana* and *Lecanicillium spp.*, are not only pests but also plant pathogens.[46] *B. bassiana's* antagonism methods include antibiosis, competition, and induced systemic resistance.[47] Fungal infections are gaining popularity as a biological control agent for a wide range of insect pests, and this method has been shown to be effective, cost-effective, and environmentally acceptable.[48] The properties of EPF make it a potential candidate for use in the IPM programme.

Nematodes

Entomopathogenic nematode (EPN) worms are soft-bodied, non-segmented roundworms that are obligatory parasites of insects and measure roughly 0.5 mm in length.[49] Species from two families (Heterorhabditidae and Steinernematidae) have been used successfully as biological pesticides in pest management programmes.[49] EPNs are naturally found in soil and identify their hosts in response to carbon dioxide and other chemical signals.[50] EPNs produced commercially are used as biological control agents against a number of soil insect pests and insects.[51] EPNs are soil organisms that have a symbiotic-mutualistic relationship with bacteria and can biologically regulate insect pests. EPNs are easily mass-produced and sprayed using ordinary spray equipment. They can live in a variety of situations and are eco-friendly. Infectious juveniles enter the hemocoel and release a symbiotic bacterium that the nematode keeps in its intestines.[52] Occasionally, *insects* and mites often suffer from lethal *disease called* Septicemia, which is caused by the invasion bacteria into the heamolymph and kills the host in 24 to 48 hours. Infectious juveniles eat on germs that multiply rapidly and disintegrate host tissues. The nematode completes 2-3 generations within the host corpse. The symbiotic interaction between EPN and bacteria boosts nematode proliferation (bacteria serve as food) and virulence. Nematodes work as vectors, transporting bacteria into a host where they can develop, and the bacteria offer the necessary conditions for nematode survival and reproduction within the insect carcass. EPNs, which are members of the families Steinernematidae and Heterorhabditidae, are well-known for their potential as a biological control agent in plant protection.[53] All Steinernema species are associated with the Xenorhabdus bacteria, whereas all Heterorhabditis nematode species are associated with the Photorhabdus bacteria.[51] Steinernematids and heterorhabditids are widespread and have been discovered in soils around the world.[54] Their usefulness against a wide range of pest insects has been widely investigated.[55] EPNs can be mass-produced using *in-vivo* or *in-vitro* techniques. *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae are frequently used to raise worms, as is the liquid fermentation method for large-scale nematode production.[56] EPNs can currently be produced *in-vivo* or *in-vitro* in a variety of methods.[57] Trays, shelves, and white traps were used *in-vivo* with surrogate host larvae of *G. mellonella*.[58] EPNs are cultured *in-vitro* by exposing worms to a pure culture of their symbiont in a nutritive media, and massive fermenters are used to manufacture massive volumes of EPNs for commercial application. Nematode virulence and viability tests, as well as age and the ratio of viable to non-viable worms, can all be used to evaluate the nematode product's quality.[59] EPNs enter the hemocoel after parasitizing their host insect through the spiracles, mouth, anus, or, in some species, intersegmental cuticle membranes.[60] They then introduce symbiotic bacteria, which multiply rapidly and cause septicaemia, which can kill the host in 48 hours. If heterorhabditids kill the insects, the cadaver turns red; if steinernematids kill the insects, the body turns brown or tan.[50] The bacteria eat the body of the insect, giving food for the nematodes. After the insect has died, the juvenile nematodes grow into adults and reproduce. A fresh generation of infective juveniles emerges after 8-14 days. The only free-living stage of EPNs is the infective juvenile stage. Heterorhabditis and Steinernema are mutually related to Photorhabdus and Xenorhabdus bacteria, respectively.[61] The intestines of the juvenile stage secrete symbiotic bacteria cells into the hemocoel. The bacteria multiply in the infected insect's hemolymph, and the infected host dies within 24 to 48 hours. After the host has died, nematodes continue to feed on host tissue, develop, and reproduce. The offspring's nematodes travel through four juvenile stages before reaching adulthood. The reproduction of heteroorhabditid and steinernematid nematodes differs. Infectious juveniles of heterorhabditid nematodes develop into hermaphroditic adults, but the next generation produces both males and females, whereas steinernematid worms generate both males and females in all generations.[59] EPNs grow best in sandy soil with a pH of 4 to 8, and they are susceptible to cold, high heat, dehydration, and UV radiation.

Researchers researched the distribution and biodiversity of EPNs in various Italian regions from 1990 to 2010 and discovered two significant species, *Steinernema feltiae* F. (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* P. (Rhabditida: Heterorhabditidae).[62] Garci' *et al.*[63] examined the effects of five distinct EPF strains on the newborn larvae of *Capnodis tenebrionis* E. (Coleoptera: Buprestidae). The mortality varied from 60 to 100% when exposed to 10 and 150 infective juveniles per larva (equivalent to 3 and 48 IJs/cm^2). At 150 IJs/larva, all nematode strains were pathogenic. Garcia *et al.*[64] investigated three native EPNs against *Tuta absoluta* M. (Lepidoptera: Gelechiidae) larvae, pupae, and adults: *Steinernema carpocapsae* W. (Rhabditida: Steinernematidae), *S. feltiae*, and *H. bacteriophora*. When these species' larvae were nested in the soil to pupate, a high majority of them died. Adult mortality rates for *S. carpocapsae* were 79.1% and 0.50%, respectively. When the effects of three regularly used pesticides against *T. absoluta* on these nematodes were investigated, it was discovered that insecticides had no effect on the entomopathogens.[64] Shamseldin *et al.*[65] discovered that inoculating Washington navel orange with *Pseudomonas flourescence* F. (Pseudomonadales: Pseudomonadaceae) strain 843 not only increased production and fruit quality under Egyptian soil conditions, but also prevented nematode survival.

CONCLUSIONS

To preserve plants, chemical insecticides are frequently used. This has led to increased resistance development in insects against a variety of chemical compounds included in plant protection formulations. In recent years, there has been a greater emphasis placed on the possibility of using natural enemies, such as entomopathogens, to manage insect infestations. Microorganisms that kill insect pests are known as entomopathogens and could open up new avenues for reducing pest infestations with reduced use of synthetic chemicals. Entomopathogens are being developed as environmentally friendly alternatives for use in agricultural crops. They can be employed as biological control agents to manage insect pests and promote agro-sustainability. One of the ecologically acknowledged ways is biological management of insect pests on agricultural crops. Microbial pesticides offer an unique chance to perform prospective and predictive research in the field of pesticides and insect pest management.

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