

Microbial Biopesticides: An Ecofriendly Plant Protection Measures

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Received 11 October 2023, Accepted 2 September 2024, Published on 18 October 2024

ABSTRACT

Microbial pesticides possess active microbes capable of controlling plant pests in agriculture, horticulture, and forests. Microbes benefit plants through metagenomics, metabolomics, and strain enhancement efforts, keeping the loss below the economic threshold. The study looked at over 50 years of literature from various sources. Other investigations combined the factors used in this review study. They are biologically effective in controlling plant disease and insect pests.

Since the modern agroecosystem depends more on chemical-based pesticides for pest control, microbial biopesticides are gaining popularity in terms of their natural, eco-friendly, and cost-effectiveness. Furthermore, the higher demand for organic food products further propels the future market for microbial pesticides. This review updates the mechanisms of controlling insect pests and plant diseases through biological control using biopesticides.

Keywords Microbial biopesticides, Agroecosystem, Microorganism, Biotechnology, Biocontrol.

INTRODUCTION

Disease and insects are the natural enemies to the agroecosystem associated with yield loss and diminishing return. Application of chemical pesticides is the immediate solution in modern crop management strategies to control the damage caused by the various pathogens and pests. However, the use of chemical and synthetic pesticides poses long-term impacts and a degradative impact on overall environmental health. Since the green revolution, the pesticide burden has increased in all areas of plant production and agrosystems due to their strong inhibitory action against different pests, resulting in chemical pesticides dominating the market (Liu *et al.* 2021). Although modern chemical crop protection chemicals and solutions have unique modes of action based on scientific advances and are designed to target noxious pests with minimal effects on human health

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or non-target species, the overuse of these synthetic chemicals has negative consequences. These chemicals are deleterious for soil microbes, amphibians, and birds and also pollute the water and aquatic ecosystems (Majumdar *et al.* 2021). Now, the pesticides have also been noticed in human breast milk, which affects children's health. Many of the chemical pesticides contain highly toxic ingredients, and if exposed through skin, ingestion, or inhaling, they can cause cancer and foetal impairment (Dewangan 2018). Other risks connected with chemical pesticides comprises dermatological, neurological, gastrointestinal, respiratory, carcinogenic and endocrine disturbances on human and animal health (Pimentel and Burgess 2014, Kumar and Chandra 2021). Even accidental or intentional exposition to pesticides can cause hospitalization and death (Chandra 2014a) as antidotes available for such incidences. The residue of pesticides has been percolated in every day foods and beverages, which has made the ecosystem toxic and increased environmental risk (Witczak and Abdel-Gawad 2014). These are the reasons why many countries have banned the use of some health hazardous agrochemicals to avoid their adverse effects on the environment, human and animal health. It urgently and increasingly needs to identify ideal alternatives to chemical pesticides for effective plant protection mechanisms without sacrificing the productivity and profitability of agriculture, horticulture and forestry.

Microbial biopesticides contain a high concentration of living microorganisms such as bacteria, fungi, nematodes, viruses, protozoans produced in biolaboratories to control disease and insects in different plants. The most typical and successful microbial pesticide is *Bacillus thuringiensis* (Bt), a naturally occurring bacterium, that has been used globally to control several important pests of pulse crops, vegetables, orchards, and forest species. Among biofungicides, *Trichoderma* is widely used as a disease control measure through seed, soil, and plant treatments. The other microbiopesticides that have a greater acceptance among farmers are *Bacillus sphaericus*, *Pseudomonas fluorescens*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lacani*, Baculovirus, and Nucleopolyhedrosis. The active components of a microbial pesticide is common the microorganism, which suppresses pests and plant

pathogens either by producing toxic metabolites, preventing the establishment of disease-causing microorganisms through competition, or other specific modes of action. As productivity is directly hampered by pest infestations and affects the income levels of farmers, crop protection solutions exploiting microbial pesticides play a vital role in protecting the crop from pests and increasing farm productivity. However, the adoption of microbial pesticides is critical due to their low specificity on target pests, low viability, and higher costs, which are expected to constrain the market. Currently, world-wide biopesticide share is just around 5% of the total crop defence market value of about \$3 billion (Olson 2015). However, bacterial biopesticides dominate and contribute 74%, fungal biopesticides 10%, predator biopesticides 8%, viral biopesticides 5%, and "other" biopesticides 3% of total marketable microbial pesticides.

History and current status of microbial pesticides

Plant and microbial biopesticides have been used for centuries in crop protection. The historic records depict that the nicotine extracts were some of the primitive biopesticides used against plum beetles. After that, *Beauveria bassiana* was demonstrated experimentally for controlling lepidopteran pests in 1835. In the early 20th century, with the growth of agricultural research, the bacterium *Bacillus thuringiensis* (Bt) was used as a microbial pesticide (Dara 2018) Shigetane Ishowata, a Japanese scientist, discovered Bt from a diseased silkworm in 1901, and after a decade, German biologist Ernst Berliner rediscovered Bt from a disased flour moth caterpillar (Limanpure and Dewangan 2018). During the 1980s and 1990s, *Agrobacterium radiobacter* was used to protect crown gall on woody species and *Pseudomonas fluorescens* for the avoidance of blight in orchards (Tiwari *et al.* 2018, Kumar *et al.* 2021a). The trend towards the use and adoption of biopesticides has grown stronger in the past decade, driven by factors such as the rapid expansion of organic agriculture, the increasing cost of chemical pesticides that do no harm to birds, animals, or human beings, and higher yields (Dara 2018).

The intensifying efforts by the government and market participants to incite the use of environmentally, cost-effective, and efficient products in crop

protection are likely to increase market development in the forthcoming years. The market has also been segmented geographically into North America, Europe, Asia Pacific, Latin America, Africa and the Middle East, which hold the largest share of the market. Asia-Pacific holds the second-largest market share next to North America due to the rise in demand for chemical-free products, sustainable agroecosystems, and the evergreen revolution. Bt products were first used in Argentina in 1950 against *Colias lesbia* in alfalfa (Chandra *et al.* 2022). The use of biopesticides has risen in Brazil (Kumar *et al.* 2021a). Approximately 40 commercial mycoinsecticides are available on the Brazilian market. More than twenty laboratories operated by sugar and ethanol mills develop *M. anisopliae* for their utilization to control cercopids in cane fields (Bhardwaj *et al.* 2023, Kumar *et al.* 2023). In Africa, the application of fungal-based *M. anisopliae* products has proven effective in pest management. China has been producing biopesticides since 1960, most of them in the form of unformulated dried cultures (Tijjani *et al.* 2017). There were 327 biopesticides registered in China. Japan is at the forefront of the use of biopesticide applications. In the last several years, Japanese research in biocontrol has identified and characterized few new insect pathogens (Chand and Chandra 2014 Kumar *et al.* 2024b). Companies have promoted the biopesticides *Bacillus thuringiensis* (BT), *T. harzianum*, entomopathogenic fungi, and NPV in Thailand. In South Korea, measures for microbial pest control were initiated during 1970 and by 2009, 34 microbial pesticide products were registered to protect plant diseases and insect pests in Korea (Rajak *et al.* 2022a, Kumar *et al.* 2022b).

The current biopesticide market is expected to reach USD 6.77 billion by 2016 from USD 3.14 billion in 2021, at a CAGR of 16.6%. (MDF 2021). The major players in the biopesticide markets are Parry America, Valent Biosciences, Certis USA LLC, Agbitech Pvt Limited, Andermatt Biocontrol, Marrone Bio Innovation Inc, Som Phytopharma Limited, Becker Underwood Inc, Graquest Inc, Biocare. Over 200 microbial pesticides are currently being prepared and traded in the United States, 60 products are prevailing in the European Union. About 225 biopesticides are presently produced within 30 countries, of which the

US, Canada, and Mexico share 45%, whereas Asia uses only 5% of biopesticides sold globally (Kumar and Singh 2016).

Microbial-based pesticides emerged in India as a response to the failure of chemical insecticides to control *S. litura*, *Helicoverpa armigera*, and other cotton pests (Darro *et al.* 2019a). In the last few years, microbes have exhibited high biocontrol potential that scientists throughout the world have reported thirteen products based on bacteria (*Bacillus thuringiensis* and *Pseudomonas fluorescens*), fungi (*Beuveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Verticillium lecanii*, *Trichoderma harzianum/viride*), and viruses (NPV of *Helicoverpa armigera* and *Spodoptera litura*) have been registered for use in India (Kumar and Chandra 2018a). Biopesticide consumption has increased in India from 219 metric tonnes (MT) to 683 MT between 1996 and 2001. Biopesticides represent only 2.89% of India's overall pesticide market and are expected to increase drastically in the recent years. In Chhattisgarh, the state biocontrol laboratory (SBCL) under Indira Gandhi Krishi Vishwavidyalaya has been operating since 2013. In 2014, SBCL produced 15–16 MT of biopesticides, i.e., *Trichoderma harzianum* and *Pseudomonas fluorescens*, and supplied them to the farmers of Chhattisgarh.

Classification of microbial-based biopesticides

Bacterial biopesticides

When used to control pathogenic bacteria or fungi, bacterial biopesticides colonize the plant and crowd out the pathogenic species (Sahu *et al.* 2018a, Kumar *et al.* 2018b). Around 90% of the biopesticide market in the United States is made up of *B. thuringiensis* (BT) subspecies and strains, which are the most commonly used microbial pesticides (Darro *et al.* 2019b, Pandey *et al.* 2018). Its main feature is that during sporulation, crystalline inclusions are made that contain proteins called δ endotoxins, or cry proteins, which can kill insects (Chandra and Bhardwaj 2016, Singh *et al.* 2018). Ruiu (2018) reported the development of over one hundred *Bacillus* spp. bio-insecticides, bio-pesticides, and bio-fungicides. Certain strains of *B. subtilis* work effectively against

a differ of plant pathogens that cause damping-off and soft rots.

Pseudomonads are also being studied extensively in agriculture as a way to control pathogens because they can break down a wide differ of substances and quickly colonise roots (Kumar *et al.* 2018c, Bhardwaj and Chandra 2016). *Udomona entomophila* has a toxin secretion system, both acting by ingestion (Darro *et al.* 2022, Rajkumar *et al.* 2022). They enhance plant growth and yield, reduce the severity of many diseases, and are among the most prolific PGPRs. Researchers demonstrated the role of *Pseudomonas fluorescens* in biologically suppressing fungal plant pathogens such as *Aspergillus*, *Alternaria*, *Fusarium*, *Macrophomina*, *Pythium*, *Ralstonia solanacearum*, *Rhizoctonia*, *Sclerotium rolfsii*, and *Sclerotinia* in India (Kumar *et al.* 2019). When made from *Pseudomonas fluorescens*, bioformulations, biopesticides, and bioinoculants can help plants grow, clean up the environment, and fight diseases (Chandra 2014b).

Fungal biopesticides

Fungal biopesticides exhibit varied modes of action, influenced by both the pesticide fungus and the target pests and pathogens. Biocontrol agents like *Trichoderma* are acclaimed as effective, eco-friendly, and cheap. *Trichoderma* is a fungal biocontrol agent used worldwide for unified management of various foliar and soil borne plant pathogens like *Ceratobasidium*, *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, *Pythium* and *Phytophthora* spp. (Kumar *et al.* 2022b). Roughly 750 species of entomopathogenic fungi belonging to 85 genera were identified from fungi (Litwin *et al.* 2020). Among fungal entomopathogens, *Beauveria bassiana*, *Verticillium lecani*, and *Metarhizium anisopliae* are naturally occurring entomopathogenic fungi that infect insect pests, i.e., whiteflies, aphids, thrips, mealybugs, leafhoppers, and weevils (Bhardwaj and Chandra 2017). *B. bassiana* and *B. brongniartii* strains exhibit varying levels of virulence against diverse targets and are used in biological control applications (McKinnon *et al.* 2017). *Metarhizium anisopliae* represents another well exploited fungal species that protects against diverse targets (Darro *et al.* 2022) through the secretion of a variety of toxins and virulence factors

(Rajak *et al.* 2022b).

Viral biopesticides

A leading company in the USA, Omnilytics, has envolved a range of phage products to control *Xanthomonas campestris* pv. *Vasicatoria* for the control of bacterial spots on peppers and tomatoes and *P. syringae* pv. *tomato*, the causative agent of bacterial specks on tomatoes (Sahu *et al.* 2019b). Baculoviruses are parted into two main groups: Nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) (Haase *et al.* 2015). Baculoviruses develop in the nuclei of the host insect cells. Upon ingestion by the host insect, infectious virus particles are internally liberated and become active. In a few days, the host larvae cannot digest food, so they weaken and die (Williams *et al.* 2017). Over the years, registered baculovirus products have treated millions of hectares, but their market share is limited to 6% of all microbial pesticides (Bhardwaj *et al.* 2023). In spite of many years of use and testing against non-target organisms, no adverse effects were observed on baculoviruses (Chandra 2014b).

Mechanism of biological control of plant diseases

Induction of host resistance

Pseudomonas and *Trichoderma* biocontrol strains have the ability to significantly stimulate plant host defences. Strains of *Pseudomonas fluorescens* are known to induce systemic acquired resistance (SAR) and induced systemic resistance (ISR) in radish, tomatoes, beans, and other crops (Kumar *et al.* 2019). *Bacillus subtilis* has utilized induced systemic resistance in sugar beet. After inoculation, the PGPR strains may release a variety of chemical elicitors of SAR and ISR, such as salicylic acid, siderophore, lipopolysaccharides, 2,3-butanediol, and other volatile compounds (Tables 1–2).

Antibiosis and lysis

Microbiological toxins known as antibiotics have the ability to poison or kill other microorganisms at low concentrations. The majority of microorganisms secrete one or more compounds that have antibiotic properties (Singh *et al.* 2018). Some types of *Pseudo-*

Table 1. Some of antibiotics produced by Biocontrol agents (Raaijmakers *et al.* 2002).

Antibiotic	Source	Target pathogen	Disease
Bacillomycin D	<i>Bacillus subtilis</i>	<i>Aspergillus flavus</i>	Aflatoxin contamination
Agrocin 84	<i>Agrobacterium radiobacter</i>	<i>Agrobacterium tumefaciens</i>	Crown gall
Iturin A	<i>B. subtilis</i>	<i>Rhizoctonia solani</i> , <i>Botrytis cinerea</i>	Damping-off
Mycosubtilin	<i>B. subtilis</i> BBG100	<i>Pythium aphanidermatum</i>	Damping-off
Pyoluteorin, pyrrolnitrin	<i>P. fluorescens</i>	<i>Rhizoctonia solani</i> , <i>Pythium ultimum</i>	Damping-off
Zwittermicin A	<i>Bacillus cereus</i>	<i>Pythium aphanidermatum</i>	Damping-off
2, 4-diacetyl-phloroglucinol	<i>Pseudomonas fluorescens</i>	<i>Sclerotium rolfsii</i> , <i>Pythium</i> spp., <i>Fusarium oxysporum</i> , <i>Rhizoctonia</i> <i>Solanacearum</i> , <i>Macrophomina</i> <i>phaseolina</i> , <i>Rhizoctonia solani</i> ,	Damping-off, Wilt disease
Gliotoxin	<i>Trichoderma virens</i>	<i>Rhizoctonia solani</i>	Root rots
Phenazines	<i>P. fluorescens</i> 2-79 and 30-84	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Take-all
Bacillomycin, fengycin	<i>Bacillus amyloliquefaciens</i> ,	<i>Fusarium graminearum</i> , <i>F. oxysporum</i>	Wilt

monas fluorescens were able to kill *Pythium* spp. that caused damping-off by making 2, 4-diacetyl-phloroglucinol and Pyoluteorin in vegetables. In the same way, *Bacillus subtilis* makes Bacillomycin D, which kills *Aspergillus flavus*, and Iturin A, which kills *R. solani* and *Botrytis cinerea* and stops growth (Table 3).

Lysis is a general term for the destruction, disintegration, and decomposition of biological material. For example, *Trichoderma harzianum* secretes cell wall lysis enzymes like chitinase and glucanase that can breakdown a wide various of polymeric compounds, including chitin, cellulose, proteins, and hemicelluloses. It is known that *Lysobacter* and *Myxobacteria* make lytic enzymes, and few isolates have been showed to be effective at stopping fungal

plant pathogens (Kenis *et al.* 2017). *Serratia marcescens* showed up to control *Sclerotium rolfsii* through chitinase expression (Padey *et al.* 2018).

Competition

Pathogens that spread through mycelial contact, like *Fusarium*, *Pythium*, *Rhizoctonia*, and *Sclerotium*, are usually more easily killed by other microbes that live in the soil and on plants (Litwin *et al.* 2020). *Trichoderma viride/ harzianum* is an example of space competition. These microbes also create metabolites that inhibit pathogens. Siderophore production is a mechanism used by few plant-growth-encouraging *Pseudomonas fluorescens* strains for biological control of *Erwinia carotovora*. There is a direct

Table 2. Bacterial determinants and types of host resistance induced by biocontrol agents (Bonaterra *et al.* 2022).

Bacterial strain	Plant species	Bacterial determinant	Type
<i>Bacillus mycoides</i> strain Bac J	Sugar beet Wheat	Chitinase, β -1,3-glucanase and Peroxidase	ISR
Bac J			
<i>Bacillus subtilis</i> GB03 and IN937a	<i>Arabidopsis</i>	2,3-butanediol	ISR
<i>Pseudomonas fluorescens</i>			
PF Strain CHA0	<i>Arabidopsis</i>	Antibiotics (DAPG)	ISR
<i>Pseudomonas putida</i>	<i>Arabidopsis</i>	Lipopolysaccharide	ISR
<i>Pseudomonas putida</i> WCS 358	<i>Arabidopsis</i>	Lipopolysaccharide	ISR
<i>Pseudomonas putida</i> WCS 358	<i>Arabidopsis</i>	Siderophore	ISR
<i>Pseudomonas putida</i> BTP1	Bean	Z,3-hexenal	ISR
PF Strain WCS 417	Carnation	Lipopolysaccharide	ISR
PF Strain WCS 374	Radish	Lipopolysaccharide	ISR
PF Strain CHA0	Tobacco	Siderophore	SAR

Table 3. Types of interspecies antagonisms leading to biological control of plant pathogens (Pal *et al.* 2006).

Type	Mechanism	Examples
Direct antagonism Mixed-path antagonism	Hyper-parasitism/ predation	<i>Trichoderma virens</i>
	Antibiotics	Cyclic lipopeptides, 2, 4 diacetylphloroglucinol Phenazines
	Lytic enzymes	Chitinases, Glucanases, Proteases
	Unregulated waste products	Ammonia, Carbon-dioxide, Hydrogen cyanide
Indirect antagonism	Competition	Exudates/leachates consumption, Physical niche occupation Siderophore scavenging,
	Induction of host resistance	Contact with fungal cell walls Molecular patterns, Phytohormone-mediated induction Detection of pathogen-associated

correlation in between siderophore synthesis in *Pseudomonas fluorescens* and their ability to inhibit *F. oxysporum chlamydospora* germination (Table 3).

Hyperparasitism and predation

The most common example of hyperparasitism is *Trichoderma* spp., which attacks a great variety of phytopathogenic fungi responsible for the most critical diseases suffered by crops of paramount economic importance worldwide. Plant-pathogenic nematodes are attacked by other hyperparasites at various phases of their life cycles (e.g., *Dactylella oviparasitica* and *Paecilomyces lilacinus*). *Trichoderma* produces a differ of enzymes that direct against the cell walls of fungi (Kumar, Bhardwaj *et al.* 2024c).

Mechanism of biological control of insect-pests

Entomopathogenic fungi

The virulence of fungal entomopathogens involves four steps: Adhesion, germination, differentiation, and penetration. The spore germination and behavior of

the fungi are affected by factors like as water, nutrients, and the physiological state of the host (Lacey 2017). The specificity and pathogenicity of entomopathogenic fungi are determined by epicuticular compounds like fatty acids, amino acids, and glucosamine (Vidal and Jaber 2015). Several cuticle-degrading enzymes synthesise during penetration of the host, including proteases, lipases, and chitinases (Tiwari *et al.* 2018).

Fungal spores, or conidia, that are produced asexually are typically the cause of infection and spread throughout the surroundings of their insect host. Conidia of hyphomycetes such as *Metarhizium* and *Beauveria* spp. are hydrophobic and passively disperse from infected cadavers. On the insect cuticle, entomopathogenic fungi reproduce by first germination and penetration of their spores, then by rapidly proliferating fungal cells, which ultimately cause the host to die. Penetration is both a mechanical and an enzymatic process (Darro *et al.* 2019a). The penetration of entomopathogenic fungi into the cuticle is accomplished by the germ tube itself or by forming an appressorium that attaches to the cuticle and gives rise to a narrow penetration peg (Luca 2018). Proteases, lipases and chitinases are the most important enzymes that entomopathogenic fungi release. They are made in a certain order to match the substrates they come across (Rajkumar *et al.* 2022). In many species of fungi, the production of conidia is highly dependent on moisture (Chandra 2014c).

Entomopathogenic bacteria and nematodes

Bacillus thuringiensis is primarily a gram-positive, aerobic soil bacterium that forms spores. During sporulation, it demonstrates an extraordinary capacity to generate various endogenous forms of crystal protein inclusions. The bacterium *B. thuringiensis*, also referred to as “Bt,” is insecticidal. One or more crystal (Cry) and cytolytic (Cyt) toxins, also known as δ -endotoxins or insecticidal crystal proteins, make up the inclusions of crystal proteins. Scholars have traditionally attributed the toxicity of Cry proteins to the creation of ion channels or transmembrane pores, which cause osmotic cell lysis (Kumar, Singh *et al.* 2024a). Furthermore, it appears that crytoxin monomers induce cell death in insect cells via an adenyllyl

cyclase/PKA signalling pathway mechanism.

The crystals are consumed by the insect while it feeds on the foliage, and in its midgut, they are hydrolyzed to create an active endotoxin. The active toxin causes an imbalance in the ionic composition of gut epithelial cells by binding to receptor sites on those cells. The result of osmotic shock causes the cells to swell and burst. The insect's mouth and stomach become paralyzed as a result of the subsequent symptoms. The toxin actively inhibits the feeding process, according to Asela (2020).

Furthermore, significant economic benefits have resulted from the successful transgenic technology used to transfer the genes encoding for the insecticidal crystal proteins into various crop plants. In the current transgenic era, *Bacillus thuringiensis* (Bt) insecticidal toxins play a major role in creating insect-resistant crops like rice, cotton, maize, potatoes, and so forth.

The most often used nematodes to kill insects are *Steinernema feltiae* (also known as *Neoaplectana carpocapsae*), *Riobravis*, *Scapteriscae*, *Heterorhabditis heliothidis* and *S. carpocapsae*. The mouth, anus, and spiracles (breathing pores) are among the bodily openings through which *Steinernema* species infect their insect hosts. Juveniles of *Heterorhabditis* also enter host insects through bodily apertures and, in certain cases, can also break through the cuticle of an insect. Nematodes finish their life cycle inside the infected insect if the surrounding conditions are warm and moist. Viral juveniles undergo a moult to become adults, and these adults then give birth to new generations inside of the same host. The young leave the dead insect and look for a new host when they reach the J3 stage of development.

CONCLUSION

Microbial pesticides are helping to control pests in agriculture, horticulture, and forest plants in a positive way. Microbes benefit plants via metabolomics, metagenomics and strain enhancement, keeping losses below the economic threshold. Microbial pesticides function as biofungicides and bioinsecticides, containing ingredients like fungal, bacterial,

and other products in liquid and dry formulations. Because the modern agroecosystem relies heavily on chemical-based pesticides for pest control, microbial biopesticides are gaining popularity as a natural, environmentally friendly, and cost-effective alternative. In addition, the growing required for organic food products will fuel the future market for microbial pesticides.

ACKNOWLEDGMENT

We would like to express our special gratitude to the staff of the Department of forestry, wildlife & environment science, Guru Ghasidas Vishwavidyalaya, Bilaspur Chhattisgarh, India.

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