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Contents

Trichoderma spp.: An eco-friendly approach for disease management

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Abstract

Agriculture is an indispensable part of any country to feed the millions of people but plant pathogens are the most important factors affecting the production of crops inflicting severe losses to agricultural products every year. To protect the crops from this huge yield loss recently, chemical pesticides are used. Long-term using of chemical pesticides contaminate water, cause atmosphere pollution, and some-times leave harmful residues which can lead to development of certain resistant organisms. Due to the side effects of chemical pesticides, sustainable crop production through eco-friendly management is essentially required in the current scenario. In biological control, genus *Trichoderma* serves as one of the best bioagents, which is found to be effective against a wide range of soil-borne, seed-borne and foliar pathogens. This species of fungi has been considered to be very beneficial for different levels of life.

Key words: *Trichoderma* strains, Mycoparasitism, Antibiosis, Competition, Bio-control agents

Introduction:

Agriculture is critically important for ensuring food security, alleviating poverty and conserving the vital natural resource on which the world's present and future generation will be entirely dependent upon for their survival and wellbeing. Agricultural crops are vulnerable to attack number of pests like bacteria, fungi, weed and insects, leading to reduced yield and poor quality of the produce. Most of the plant pathogens, which cause important diseases in cereals, oilseeds, pulses, vegetables, fibres and fruit crops, are seed and soil borne in nature. The continuous cultivation of a crop in the same piece of land resulted in heavy incidence of soil borne diseases due to build-up of a high inoculum of the pathogen. That forces the farmers to change either crop or land. Fungicides play an important role in management of seed borne and air borne pathogens. However, the soil borne plant pathogens are often difficult to manage with the fungicides and other ordinary methods due to their limitations to perform better against to pathogens. In recent years, indiscriminate and expensive use of pesticides has posed a serious problem of pollution in the ecosystem and development of resistance in the pathogens. While the farmer is exemplified by pesticide residues in soil, air, water, food etc., the latter includes phytotoxicity, physiological deformities, diseases, mortality, population changes, genetic disorders, gene erosion, etc. in plant, mammal, avian, insect and other organisms. Therefore, biological control of pathogens has gained importance as component of integrated disease (pest) management (IDM) for sustainable agriculture as it is a long lasting and eco-friendly.

Currently, several bio-control agents have been recognized and are available as fungal agents i.e., *Trichoderma* spp., *Gliocladium virens, G. roseum, Aspergillus niger, A. flavus, Chaetomiam globosum*, *Ampelomyces* spp., *Candida* spp., and *Coniothyrium* spp. and bacterial agents like *Bacillus subtilis, B. cereus, Pseudomonas fluorescens, Agrobacterium radiobacter* etc. It has been reported to work nicely as antagonists against many fungal plant pathogens *in vitro* and *in vivo* conditions. Among these biocontrol agents *Trichoderma* spp. is one of the most versatile bio-control agents which has long been used for managing plant pathogenic fungi.

Genus Trichoderma and their habitats:

Trichoderma is a fungal genus that was described in 1794, including anamorphic fungi isolated primarily from soil and decomposing organic matter (Persoon 1794). Trichoderma term has been derived from two words thrix (hair means thread like) and derma (skin). Genus *Trichoderma* is a soil inhabiting green filamentous fungus, which belongs to the division Ascomycota that reproduce asexually. In the early 1930s Trichoderma was introduced as possessing biocontrol ability (Weindling, 1934). Trichoderma is an opportunistic, avirulent plant symbiont fungus which acts as an antagonistic and parasitic fungus against many plant pathogenic fungi and offers protection from phytopathogenic plant diseases. It has been proven in numerous studies that *Trichoderma* spp. are effective biocontrol agents for managing plant disease (table 1), and currently commercial products of *Trichoderma* are available as biopesticides or soil amendments or as enhancers for plant growth (Papavizas, 1985; Vinale *et al.,* 2008). The efficacy of *Trichoderma* depends on many abiotic parameters such as soil pH, water retention, temperature and presence of heavy metals. The genus *Trichoderma* includes more than 80 species that can be used to control phytopathogenic fungi. Among them, *T. harzianum, T. viride T. hamatum, T. polysporum, T. pseudokoningii, T. deliquescens, T. aureoviride, T. koningii, T. lignorum, T. reesei, T. longibrachiatum* and *T. virens* (formerly *Gliocladium virens*) are considered as most potential biocontrol agents. *Trichoderma* occurrence is worldwide and is commonly found associated with roots, soil and plant debris, forest humus and orchids. Some of the commercially existing bio-control products available in the market are shown in table-2.

Mode of action: *Trichoderma* spp. are biocontrol agents, effective against fungal phytopathogens. The three most important antagonistic process of *Trichoderma* spp. include.

1. **Mycoparasitism/Hyper-parasitism:** The mechanism of mycoparasitism/hyper-parasitism includes different kind of interaction like coiling of hyphae around the target organism, penetration, production of haustoria and lysis of hyphae through secretion of intercellular lytic enzymes like glucanase, cellulase, chitinase, protease, lipase etc, which disintegrate the cell wall of pathogen.
2. **Antibiosis:** Liberation of an antibiotic like substances or other chemical metabolites by the antagonistic fungi viz. *Trichodermin, viridin* etc. that are harmful to the pathogen and inhibit or kill their growth.
3. **Competition:** It is a condition in which there is a suppression of one organism (target pathogen) as the two species struggle for limiting quantities of nutrients, oxygen, space or other requirements.

Method of application of *Trichoderma* spp.:

1. **Seed treatment:** Use of 8-10gram *Trichoderma* spp. (powder formulation $2x10^6$ cfu/g) with 50 ml of water (bigger seeds) while small seeds at the rate of 6-8 gram for the treatment of one kg seed before sowing. Apply 5-10 ml *Trichoderma* spp. (liquid formulation) per litre of cow dung slurry for treatment of one kg seed before sowing particularly for cereals, pulses and oilseeds. Shade dries the seeds for 20-30 minutes

before sowing is essential. Seed treatment is highly effective against seed and soil borne diseases.

2. **Seed biopriming:** Seed biopriming is treatment of seed with *Trichoderma* formulations (@ 5-10 gram/kg seed) and incubating under moist and warm conditions until just prior to radicle emergence. After radicle emergence sow the bioprimed seeds in the field. In bioprimed seeds, the germinating conidia of *Trichoderma* form a layer around the seeds. Such seeds tolerate adverse conditions of the soil better than the non-primed seeds. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. Seed biopriming is beneficial for tomato, brinjal, chickpea, soybean etc crops.
3. **Seed material treatment:** Apply at the rate of 8-10gram *Trichoderma* powder with one litre of water (30 minutes) for the treatment of seed material like sugarcane setts, banana suckers, turmeric, ginger rhizomes and potato tubers before sowing. Shade dries the seeds for 20-30 minutes before sowing is essential.
4. **Soil application:** 1-2 kg *Trichoderma* spp. (powder formulation) or 500-1000 ml (liquid formulation) is added in 25-50 kg farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 2-3 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Maintain optimum moisture for better multiplication of *Trichoderma* formulations. Apply well decomposed *Trichoderma* based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing. This mixture is sufficient for one acre of land.
5. **Cutting/Seedling's root dip application:** 20-25gram *Trichoderma* spp. (powder formulation) or 5-10 ml (liquid formulation) dissolve in one litre of water for about 30 minutes. Dip the cuttings and roots of seedlings in to this prepared suspension for half an hours and transplant immediately. Root dipping is effective against soil borne diseases.
6. **Nursery bed treatment:** 500gram *Trichoderma* spp. (powder formulation) mix in 10-15 kg well decomposed FYM/compost/vermicompost and broadcast in a one-acre area at evening time and at proper moisture conditions. 5-10 ml/litre of water *Trichoderma* spp. (liquid formulation) is sufficient for soil drenching.
7. **Soil drenching:** One-to-two-kilogram *Trichoderma* formulation mix in 200 litre of water and drench the soil in one acre area or 8-10 gm/litre of water in soil in the nurseries from time to time. Maintain optimum soil moisture while applying.
8. **Horticultural crops:** Fifty-to-hundred-gram *Trichoderma* formulation mix in sufficient quantity of FYM/compost/vermicompost/field soil and apply the mixture per plant in effective root zone of fruit tree. Doses will change in depending upon age of the plant.
9. **Foliar application:** 8-10 gram/litre of water *Trichoderma* spp. (powder formulation) or 3-5 ml/litre of water (liquid formulation) spray on diseased plants at cooler hours on 10-12 days intervals.

Benefits:

1. *Trichoderma* strains act against many plant pathogenic fungi (seed, soil-borne) and control the diseases by action of myco-parasitism and antibiosis.
2. *Trichoderma* strains decomposes organic farm wastes, solubilizes soil phosphorous and micronutrients, reclaims adverse soil, increases the absorption of nutrients, improve soil fertility and protects soil eco-system.
3. Reduces crop losses, increases plant growth, yield and source of income.
4. Reduces the need for harsh and expensive chemical fungicides.
5. It is compatible with organic manures and bio-fertilizers. like *Azospirillum*, *Rhizobiu*m, *Bacillus subtilis*, *Mycorrhizae*, phosphorus solubilizing bacteria and other bio-agents.
6. It increases the rate and percentage of seed germination, root and shoot growth as well as built systemic resistance of plants to diseases, pests and drought.
7. *Trichoderma* strains play an important role in the bio-remediation of soil that are contaminated with pesticides and herbicides. It has ability to breakdown the pesticides and herbicides residues in the soil. This process is called bio-remediation. Trichoderma spp. have the ability to degrade a wide range of insecticides groups like organochlorines, carbamates and organophosphates.
8. It is an eco-friendly, beneficial for environment, safe for users and farming communities. It is effective in organic farming for management of diseases.

Precautions in use of *Trichoderma* formulations:

1. Don’t use chemical fungicide after application of *Trichoderma* formulation for 4-5 days.
2. Don’t use *Trichoderma* in dry soil, moisture is essential for its growth and survivability.
3. Do not keep *Trichoderma* treated seeds in direct sun light.
4. Don’t put the treated FYM for a longer duration.
5. Do not use *Trichoderma* formulation without organic manure or slurry.

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Table 1: Disease management through *Trichoderma* spp.

Crop Name	Disease Name	Causative agent	Effective *Trichoderma* spp.	Mode of application
Cereal crops				
Rice	Sheath blight	*Rhizoctonia solani*	*Trichoderma viride, T. harzianum, T. virens*	Seed, soil, seedling treatment and foliar spray
	Brown spot	*Drechslera oryzae*	*Trichoderma viride*	Seed treatment
	Bunt	*Neovossia indica*	*Trichoderma viride, T. harzianum, T. virens*	Seed treatment
	Kernel smut	*Tilletia barclayana*	*Trichoderma viride, T. harzianum, T. virens*	Seed, soil, seedling treatment
Barley	Foot and root rot	*Sclerotium rolfsii, Fusarium, Curvularia, Pythium, Penicillium, Aspergillus*	*Trichoderma viride, T. pseudokoningii*	Seed treatment
Wheat	Root rot	*Sclerotium rolfsii, Fusarium oxysporum*	*Trichoderma harzianum*	Seed and soil treatment
	Loose smut	*Ustilago segatum tritici*	*Trichoderma viride, T. harzianum, T. virens, T. lignorum, T. koningii*	Seed treatment
	Spot blotch	*Drechslera sorokiniana*	*Trichoderma viride, T. reesei, T. pseudokoningii*	Foliar spray
	Take-all	*Gaeumanomyces graminis var. tritici*	*Trichoderma harzianum*	Seed treatment
	Karnal bunt	*Neovossia indica*	*Trichoderma viride, T. harzianum, T. virens, T. deliquescens, T. koningii*	Seed treatment
Maize	Charcoal rot, Banded blight	*Macrophomina phaseolina, R. solani*	*Trichoderma* spp.	Seed treatment and foliar spray
Pulse crops				
Chickpea	Wilt, seed rot, root rot	*Fusarium oxysporum* f. sp. *ciceris, R. bataticola, Pyhtium sp.*	*Trichoderma harzianum*	Seed and soil treatment
	Grey mould	*Botrytis cineria*	*Trichoderma* spp.	Foliar spray

	Stem rot	*Sclerotinia sclerotiorum*	*Trichoderma harzianum*	Seed treatment
Pigeon pea	Wilt	*Fusarium udum*	*Trichoderma viride, T. harzianum, T. koningii*	Seed and soil treatment
	Seed borne diseases	*Alternaria alternata, Curvularia lunata*	*Trichoderma viride*	Seed treatment
Mung bean	Root rot	*Macrophomina phaseolina*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Lentil	Wilt complex, Collar rot	*R. solani, F. oxysporum, S. rolfsii*	*Trichoderma virens, T. viride, T. harzianum*	Seed and soil treatment
Cowpea	Wilt	*F. oxysporum* f. sp. *ciceris*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
	Charcoal rot	*Macrophomina phaseolina,*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Moth bean	Blight	*Macrophomina phaseolina,*	*Trichoderma viride, T. harzianum*	Foliar spray
Oilseed crops				
Mustard	Damping off	*Pythium aphanidermatum*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Sesamum	Blight	*Phytophthora sp.*	*Trichoderma viride, T. harzianum*	Seed treatment
	Root rot	*Macrophomina phaseolina*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Safflower	Root rot	*Macrophomina phaseolina*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Sunflower	Blight	*Alternaria helianthii*	*Trichoderma virens*	Seed treatment
	Root rot, collar rot	*Sclerotium rolfsii, R. solani, Sclerotinia sclerotiorum*	*Trichoderma viride, T. harzianum*	Seed treatment
Groungnut	Wilt complex, seed rot, root rot, stem rot	*Sclerotium rolfsii, F. solani, F. oxysporum, R. solani,*	*Trichoderma viride, T. harzianum, T. virens*	Soil treatment
	Leaf rust	*Puccinia arachidis*	*Trichoderma harzianum*	Foliar spray
	Collar/root/crown/stem/pod rot	*Aspergillus flavus, S. rolfsii, A. niger*	*Trichoderma viride, T. harzianum*	Soil treatment
Fruit crops				
Mango	Fruit rot	*Lasiodiplodia theobromae, Rhizopus arrhinus*	*Trichoderma* spp.	Fruit treatment
Apple	White root rot	*Dematophora necatrix*	*Trichoderma viride, T. harzianum*	Soil treatment
Citrus group	Root rot	*Phytophthora nicotianae*	*Trichoderma viride, T. harzianum, T. virens*	Soil treatment
Banana	Wilt (Panama disease)	*F. oxysporum* f. sp. *cubense*	*Trichoderma viride*	Soil and Rhizome treatment

Orange	Blue mould	*Penicillium italicum*	*Trichoderma harzianum*	Fruit dip
Guava	Antharcnose	*Colletotrichum gloeosporioides, Pestalotia psidii*	*Trichoderma harzianum*	Foliar spray
	Wilt	*Fusarium oxysporum*	*Trichoderma viride, T. harzianum*	Soil treatment
Vegetable crops				
Tomato	Damping off	*Pythium indicum*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
	Seedling wilt	*Fusarium oxysporum* f. sp. *lycopersici*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Potato	Black scurf	*R. solani*	*Trichoderma viride*	Tuber treatment
Brinjal	Wilt, damping off	*F. solani, P. aphanidermatum*	*Trichoderma viride, T. harzianum, T. virens*	Seed and soil treatment
	Collar rot	*Sclerotinia sclerotiorum*	*Trichoderma viride, T. virens*	Soil treatment
Chilli	Root rot	*Sclerotium rolfsii*	*Trichoderma harzianum, T. viride*	Soil treatment
Radish	Seedling rot, damping off, seed rot	*Pythium sp., R. solani*	*Trichoderma harzianum, T. hamatum*	Seed treatment
Pea	Seed and collar rot	*Pythium sp., R. solani*	*Trichoderma harzianum, T. hamatum*	Seed treatment
	Wilt	*Fusarium oxysporum f. sp. pisi*	*Trichoderma harzianum, T. viride*	Soil treatment
Cauliflower	Damping off	*R. solani, P. aphanidermatum*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Cabbage	Damping off	*R. solani*	*Trichoderma harzianum, T. viride*	Seed treatment
Bean	Seedling rot	*Pythium sp., S. sclerotiorum, R. solani, B. cineria*	*Trichoderma koningii*	Seed treatment
Plantation crops				
Rubber	Brown rot	*Phellinus noxius*	*Trichoderma viride, T. harzianum, T. hamatum*	Soil treatment
Coffee	Collar rot	*R. solani*	*Trichoderma harzianum*	Seed and soil treatment
Mulberry	Cutting rot	*Fusarium solani*	*Trichoderma viride, T. virens, T. pseudokoningii*	Cutting and soil treatment

	Stem canker, die back	*Botryodiplodia spp.*	*Trichoderma viride, T. virens, T. pseudokoningii*	Cutting and soil treatment
Cash crops				
Sugarcane	Red rot	*Colletotrichum falcatum*	*Trichoderma viride, T. harzianum*	Soil treatment and spray
	Root rot, Seedling rot	*Pythium graminicola*	*Trichoderma viride, T. harzianum*	Soil treatment
	Wilt	*Fusarium monilifomae*	*Trichoderma viride, T. harzianum, T. longibrachiatum*	Sett treatment
Sugarbeet	Damping off	*P. aphanidermatum*	*Trichoderma harzianum*	Seed and soil treatment
	Wilt and root rot	*S. rolfsii*	*Trichoderma harzianum*	Soil treatment
Cotton	Root rot	*Rhizoctonia sp., M. phaseolina*	*Trichoderma viride, T. harzianum*	Soil treatment
Spices crops				
Ginger	Rhizome rot	*F. oxysporum* f. sp. *Zingiberi, Pythium myriotylum, F. solani*	*T. harzianum, G. virens*	Rhizome treatment
Coriander	Wilt	*Fusarium oxysporum* f. sp. *corianderii*	*Trichoderma viride, T. harzianum*	Seed and soil treatment
Pepper	Collar rot	*Phytophthora capsici*	*Trichoderma viride, T. harzianum*	Soil treatment, Dernching
Cardamum	Damping off	*F. moniliformae, Pythium vexans, P. aphanidermatum*	*Trichoderma viride, T. harzianum*	Soil treatment, Dernching
	Capsule rot	*Phytophthora meadii*	*Trichoderma viride, T. harzianum*	Soil treatment

Table 2: Common commercial *Trichoderma* formulation used in India.

Trade Name	*Trichoderma* strains/species	Manufacturer
Ecofit	*Trichoderma viride*	Hoechest and Schening Agro. Evo. Ltd. Mumbai, India
Funginil	*Trichoderma viride*	Crop Health Bioproduct Research Centre, Ghaziabad, Uttar Pradesh, India
Trichogourd	*Trichoderma viride*	Anu Biotech International Ltd., Bangalore, India
Defence SF	*Trichoderma viride*	Wockhrtd Life Science Ltd., Mumbai, India
Bioderma	*Trichoderma viride + T. harzianum*	Biotech International Ltd., New Delhi, India
Bio-fit	*Trichoderma viride*	Ajay Biotech (India) Ltd., Pune, India
Biocon	*Trichoderma viride*	Tocklai Experimental Station Tea Research Association, Jorhat (Assam), India
Antagon TV	*Trichoderma viride*	Green Tech, Agroproducts, Rajaji Road Coimbatore, India
Trichostar	*Trichoderma harzianum*	Green Tech, Agroproducts, Rajaji Road Coimbatore, India
Gliostar	*Trichoderma virens*	GBPUAT, Pantnagar, Uttarakhand, India
Monitor	*Trichoderma spp.*	Agricultural and Biotech Pvt. Ltd. Gujarat, Department of Plant Pathology, MPKV, Rahuri
Tricho-X	*Trichoderma viride*	Excel Industries Ltd., Mumbai, India
Biogourd	*Trichoderma viride*	Krishi Rasayan Export Pvt. Ltd., Solan (HP), India
Ecoderma	*Trichoderma viride + T. harzianum*	Morgo Biocontrol Pvt. Ltd., Bangalore, India
Trieco	*Trichoderma viride*	Ecosense labs, India
Tricon	*Trichoderma viride*	Green Max, India

Mycorrhiza culture: An invaluable gift of nature for organic and natural farming

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Abstract

Many microorganisms form symbiotic relationships with plants, on a continuous scale and from parasitic to mutualistic. Of the seven types of mycorrhizae described (Endomycorrhizae, Ectomycorrhizae, Ectendomycorrhizae, Arbutoid mycorrhizae, Monotropoid mycorrhizae, Eericoid mycorrhizae and orchidaceous mycorrhizae) on the basis of anatomical and morphological features. Among them, ectomycorrhizae are the most common among forest and ornamentals trees and the endomycorrhizal fungus is also known as arbuscular mycorrhizae. Arbuscular mycorrhizal fungi (AMF) are the major components of the rhizosphere of the most plants and play an important role in decreasing the plant disease caused by *Phytophthora, Rhizoctonia, Pythium, Aphanomyces, Verticillium, Fusarium, Macrophomina,* etc. and other soil borne diseases also. Disease severity are reduced by root colonization of AMF via several mechanisms including competing plant pathogens for nutrients, production of antibiotics, induction of hydrolytic enzymes, enhancing the PR-protein, induction of phytoalexins and by stimulating defense related enzymes. Plant species are benefited from mycorrhizal association because of superior effectiveness in nutrient and water uptake. Mycorrhizal fungi greatly enhance nutrient uptake especially phosphorus and improve plant growth and crop productivity. It helps to maximize ecological benefits and minimize environmental hazards.

Keywords: Mycorrhizae, Biocontrol, Nutrition uptake, Soil borne disease management

Organic and Natural Agriculture

Overview of Mycorrhizae:

Mycorrhizal fungi are a major component of the agricultural natural resource and they are members of the fungus kingdom. This fungus is commonly found in the soil, because this fungus cannot make its own food, so it makes a friendly relationship with the roots of higher plants. This friendly relationship is called ***"Symbiotic/Mutualism".*** More than 95% of the plant species are infected by the symbiotic fungi. The most preferred plant species for the symbiotic fungi are acacia, citrus, legume, sorghum, maize, onion and other grasses. German Botanist, Plant Pathologist and Mycologist **Albert Bernard Frank (Frank, 1885),** in his study of soil microbial-plant relationships (in forest tree such as pine), introduced/coined the Greek term 'mycorrhiza', which literally means ***"fungus roots"*** or the word mycorrhiza is made up of two words, myco means- fungus and rhiza means- roots. Mycorrhiza can be defined as the mycelial hyphae living in association with roots of higher plants. The nature of this association is believed to be symbiotic (mutualistic association/mutualism e.g. symbiosis- means both partners appears to benefit- a type of relationship between the host and a symbiont, where both organisms benefit and no one is harmed), non-pathogenic or weekly pathogenic. The term mutualism can be simply defined as a relationship in which both species are mutually benefited. This relationship can either be within the species or between the two different species. The species with this relationship is termed as symbionts. Mycorrhizal fungi form symbiotic relationships with plant roots in a fashion similar to that of root nodule bacteria within legumes. Mycorrhizal association is symbiotic association between plants and fungi, where both the partners are benefitted from each other. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue. A mycorrhiza (fungus-root) is a type of endophitic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystems. Based on anatomical and morphological features mycorrhizae are classified in to seven types *viz.*, Endomycorrhizae (vesiclular arbuscular mycorrhiza or VA mycorrhiza means VAM; now known as arbuscular mycorrhiza, AM *e.g. Acaulospora, Endogone, Gigaspora, Glomus, Glazialla*); Ectomycorrhizae *e.g. Amanita muscaria, Boletus, Scleroderma citrinum;* Ectendomycorrhizae e.g. *Wilcoxina;* Arbutoid mycorrhizae e.g. *Boletus, Scleroderma;* Monotropoid mycorrhizae e.g. *Monotropa hypopitys;* Eericoid mycorrhizae e.g. *Rhizoscyphus, Sebacina* and Orchidaceous mycorrhizae e.g. *Sesbania, Russula* (Siddiqui *et al.*, 2088). Among them, Ectomycorrhizae and endomycorrhizae are important in agriculture and forestry. But, endomycorrhizae (arbuscular mycorrhizae) are the most abundant and widespread of vascular flowering plants in natural and agricultural ecosystems (Siddiqui *et al.*, 2008; Kakraliya, *et al.*, 2020 and Dalavi *et al.*, 2020).

Types of Mycorrhizal Fungi

On the basis of morphological and anatomical features, mycorrhizal fungi are divided into three types i.e. Endomycorrhizae, Ectomycorrhizae and Ectendomycorrhizae. Endomycorrhizae and Ectomycorrhizae are important in agriculture and forestry.

1. **Endomycorrhizae:** It is a mycorrhizal association in which the fungal hyphae are present on root surface as individual threads that may penetrate directly into root, other epidermal cells and into cortical hairs. It is further classified in to five types:-

(i) VAM fungi (Vesicular Arbuscular Mycorrhizae): VAM fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants. Vesicular arbuscular mycorrhizae are characterized by the formation of unique structures such as arbuscules and vesicles by fungi of the phylum *Glomeromycota*; earlier known as *Endogonales* of *Zygomycetes.* VAM fungi help plants to capture nutrients such as phosphorus and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants (Siddiqui *et al.*, 2008, Kakraliya *et al.*, 2020). All VAM fungi are obligate biotrophic, as they are completely dependent on plants for their survival. Some important genera are *Glomus, Acaulospora, Gigaspora, Scutellospora, Enterophospora and Sclerocystis* (Ejersa, MT, 2021).

(ii) Ericoid mycorrhizae: Ericoid mycorrhizae are known to form between autotrophs in the *Ericaceae* and fungi in the *Ascomycota*. Intracellular penetration of root cells occurs and there is no mantle or hartig net development. The hyphae coils in outer cells of the narrow **"hair roots"** of plants in the plant order *Ericales*. These associations also occur in thicker roots of Australian members of the Epacridaceae (Siddiqui *et al.*, 2008, Kakraliya *et al.*, 2020).

(iii) Arbutoid mycorrhizae: Arbutoid mycorrhiza possess characteristics of both ECM and AM fungi, i.e., there is a well-developed mantle, a hartig net, and prolific extrametrical mycelium. Additionally, intra-cellular penetration occurs and hyphal coils are produced in autotrophic cells. A major differences between the arbutoid and ectomycorrhizal association is that the hyphae penetrate the outer cortical cells of plant roots and fill them with coils. These mycorrhizae are associated with members of the Ericales; namely, Arbutus and Arctostaphylos species. The fungal symbionts are exclusively Basidiomycete species, which may form ECM with other autotrophic hosts (Siddiqui *et al.*, 2008, Kakraliya *et al.*, 2020).

(iv) Orchideoidal mycorrhizae: Orchid mycorrhizae have only been found in association with Basidiomycetes species. This association is probably pseudomycorrhizal. It consist of coils of hyphae within roots of plants in the family Orchidaceae. Young orchid seedlings and some adult plants which lack chlorophyll are entirely dependent on mycorrhizal fungi for their survival (Siddiqui *et al.,* 2008, Kakraliya *et al.,* 2020).

(v) Monotropoid mycorrhizae: The fungi belongs to Basidiomycotina, colonizing achlorophyllous members of angiosperms belonging to family Monotropaceae and a specific subset of fungi in the Russulaceae or the Boletaceae family. These plants are heterotrophic or mixotrophic and derive their carbon from the fungus partner. This is thus a non-mutualistic parasitic type of mycorrhizal symbiosis (Siddiqui *et al.,* 2008, Kakraliya *et al.,* 2020).

2. **Ectomycorrhizae:** Ectomycorrhizal (ECM) fungi are also widespread in their distribution but associate with only the roots of around 10% of plant families, mostly woody plants including the birch, dipterocarp, eucalyptus, oak, pine, and rose families and fungi belonging to the Basidiomycota, Ascomycota and Zygomycota. Ectomycorrhizae are association, where fungi form a mantle around the roots. There is no hyphal penetration of cells. Fungal hypha is generally separate. A distinct Hartig's net is present between the cells (Siddiqui *et al.,* 2008, Kakraliya *et al.,* 2020).

3. **Ectendomycorrhizae:** It possess characteristics of both ECM and AM fungi. As with ECM, both a hartig net and mantle structures are produced in ectendomycorrhizae, although the mantle may be reduced compared with ECM. The fungi belongs to Basidiomycotina, which covers both gymnosperms and angiosperms plants. The formation of Ectendomycorrhizae begins with formation of a hartig's net, which grows behind the apical meristem of the growing root. The hartig net penetrates between the epidermal and outer cortical cells and later extends to the inner cortex. Intracellular penetration of healthy plant cells by these fungi also occurs, a characteristic unlike that of ECM but consistent with AM. In fact, the same fungal species can form either ECM or ectendomycorrhizae depending upon the plant species with which it is associated (Siddiqui *et al.,* 2008, Kakraliya *et al.,* 2020). *Alnus*sp, *Salix* spp, *Populus* spp and *Eucalyptus* spp. can have both endomycorrhizal and ectomycorrhizal associations (Ejersa, MT, 2021).

Mode/mechanism of action of Mycorrhizae:

Mycorrhizal fungi have attracted a lot of interest in recent years because they are useful as bio-control agents and have other agricultural applications. Mycorrhizal mode/mechanism of action working either separately or together. The limitations of using AM fungus for bio-control include their obligatory nature, a lack of knowledge of the processes involved, and the influence of environmental conditions in these interactions. The several mechanism in control of plant diseases are involved by mycorrhizal symbiosis and these are:-

1. **Mycorrhizae creating physical/mechanical barrier for the pathogen to penetrate:** VAM fungi act as a barrier/ creating a mechanical/physical barrier for the pathogen penetration and subsequent spread. Mechanical barrier creates a physical rather than a chemical hindrance to prevent the entrance or spread of a pathogen. Some of the fungi such as which forms ectomycorrhizae with more than 46 tree species belonging to at least eight genera, provide physical barrier in the form of fungal mantle of various thickness on the surface of the root thus protect from the infection of root pathogenic fungi and nematodes. *Phytophthora cinnamomi* (Pine root rot) control by EM fungi, because it creates a physical barrier in the in the form of mental covering the cortical cells, fungus do not penetrate the roots (Mohammad, IK, 2019).

2. **Secreting antibiotics/metabolites (Inhibition of pathogen by antibiosis):** Several Mycorrhizal fungi and other saprophytic fungi associated with mycorrhizosphere have been found to produce different types of antibiotics /metabolites exhibiting inhibition to pathogenic organisms and protecting the root systems. Several mycorrhizal fungi viz *Amanita spp., Boletinus spp., Cenococcum spp., Lactarius spp., Laccaria laccata, Leucopaxillus cerealis* var. *Piceina*, *Pisolithus tinctorius, Suillus lute*us and *Thelephora terrestris* have been found to produce antifungal, antibacterial and antiviral metabolites. The antifungal and antibacterial antibiotics produced by *Leucopaxillus cerealis* var. *picein*a has been identified as Diatretyne nitrile, Diatretyne amine and Diatretyne-3. These have been found inhibiting number of pathogens viz

nine species of *Phytophthora,* twenty four species of *Pythium,* five species of *Rhizoctonia, Sclerotium bataticola* and *Cylindrocladium scoparium* (Sharma *et al.,* 2014).

3. **Competing with the pathogens for the uptake of essential nutrients in the rhizosphere and the root surface (Host nutritional effects/Improved plant nutrition):** Mycorrhizal fungi are most efficient competitor with the other pathogens, because adequate supply of carbohydrates, amino acids, vitamins, minerals etc. from root exudates and protection afforded by the host roots. Mycorrhizal association utilize surplus carbohydrates from the root exudates and transform the sucrose or monosaccharide (hexose) to less soluble sugars like trehelose, mannitol, sorbitol etc. The propagules of infectious fungi like *Pythium, Phytophthora, Rhizoctonia, Sclerotium, Fusarium* etc. require nutrients for germination and these are less available in mycorrhizosphere thus discouraging the propagules germination and attractiveness of the roots to the pathogen infection. Stimulating the host roots to produce and accumulate sufficient concentrations of metabolities (terpenes, phenols etc.) which impart resistance to the host tissue against pathogen invasion. Mycorrhizal plants are generally able to tolerate pathogens and compensate for root damage and photosynthate drain by pathogens because AMF enhance host nutrition and overall plant growth (Roy, *et al.,* 2018).

4. **Anatomical and morphological changes in the root system:** The root morphology system can be changed as a result of AMF colonisation. Roots colonised by AMF are more branching than non-colonized plants, and adventitious root diameters are bigger, providing more infection sites for a pathogen. Fusarium wilt infection of tomato and cucumber may be slowed due to morphological changes in the root cells of the endodermis of AM plants, which include lignification and incensement. Raising lignifications may protect the roots from other pathogens while increasing phenolic metabolism in the host plant. Thickening of the cell wall by lignification and synthesis of additional polysaccharides, which inhibits root pathogen entry (Tahat *et al.,* 2010).

5. **Competition for colonization and infection sites:** The primary mechanism to explain the interaction between AMF and soil microorganism is physical completion between AMF and rhizosphere microorganism to occupy more space in the root architecture. Stimulating microbial activity and competition in the root zone (rhizosphere, rhizoplane), restricting pathogen access to the roots. Roots colonised by VAM/AM fungus may also harbour actinomycetes that become antagonistic to root pathogens (Tahat *et al.,* 2010).

6. **Check in leakage of nutrients from host cell (Physiological and biochemical alterations of the host):** During AMF colonisation, host root tissue phosphorous levels are often increased, which modifies the phospholipid composition and hence the root membrane permeability, resulting in a decrease in net sugar, carboxylic acid, and amino acid leakage into the rhizosphere. These changes reduce pathogen chemotactic effects on plant roots and hinder pathogen entry. Symbiotic associations have been found to enhance the concentration of these inhibitors many times greater than non-symbiotic roots. Pine (*Pinus sylvestris*) seedlings with variegatus symbiotic association have been found to produce eight times higher concentration of fungistatic compounds like terpenes and sesquiterpene. Similar compounds have been also reported in certain ectomycorrhizal fungi like *Amanita* and *Rhizopogon*. Several mycorrhizal fungi *i.e., Amanita rubescans, Boletus variegatus, Pisolithus tinctorious* and *Hebelomasarcophllum* and have been found to produce various volatile organic compounds like ethanol, isobutanol, Isoamylalcohol, acetoin and Isobutyric acid and volatile substances are inhibitory to many pathogenic fungi some of which are *Phytophthora cinnamomi, Rhizoctonia undulates, Phymatotrichum omnivorum, Fomes annosus, Pastalotia rhododendry etc* (Roy *et al.*, 2018).

Method of application of Mycorrhiza culture:

1. **Soil application/treatment:** While preparing the field, 4-5 kg Mycorrhiza culture (powder formulation) is added in 200-300 kg well decomposed farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 1-2 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Apply well mixed/decomposed Mycorrhiza culture based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing. This mixture is sufficient for one acre of land. Keep in mind that if the number of plants per acre is more than 40,000, then 8 to 10 kg of culture should be mixed with 400 to 500 kg of rotten cow dung/FYM.
2. **Root treatment:** Root treatment can be done in vegetables and other plantation crops. For this, prepare the suspension @ 20 grams of Mycorrhiza culture by mixing 15 to 20 grams of jaggery with 1 liter of water. Dip the roots of seedlings in to this prepared suspension for 15-20 minutes and transplant immediately. Root dipping is effective against soil borne diseases. At the time of sowing and planting of vegetable crops, the distance from plant to plant is being kept more, then sowing and transplanting should be done by making a small pit in the prepared field by spud and mixing @ 2 grams of culture in it.
3. **Use at the time of plantation:** At the time of plantation, apply Mycorrhiza culture @ 5 gram/plant/3x3 feet pit at 3-4 inches depth. In old orchards, while giving fertilizers in the month of February/July, it can be given at the rate of 5-10 grams per plant along with well decomposed farm yard manure in the pits.
4. **Nursery bed treatment:** 1-2 kg Mycorrhiza culture (powder formulation) mix in 40-50 kg well decomposed FYM/compost/vermicompost and broadcast in a one-acre area at evening time and at proper moisture conditions.
5. **Soil drenching:** Two-to-three-kilogram Mycorrhiza culture formulation mix in 200 litre of water and drench the soil in one acre area or 15-20 gm/litre of water in soil in the nurseries from time to time. Maintain optimum soil moisture while applying.

Benefits:

Mycorrhizal fungi enable plants to absorb more nutrients and water from soils. They also improve plant tolerance to various environmental conditions. Moreover, these fungi play a significant role in soil aggregation and boost soil microbial activity.

1. Mycorrhizal fungi convert insoluble phosphorus in the soil into a soluble form, making it more accessible to plants.
2. Micro elements such as manganese, sulphur, iron, zinc, molybdenum, nickel, cobalt, and others are made more easily available to plants by supplying them in soluble form.
3. This fungus also improves the performance of nitrogen-fixing bacteria such as Rhizobium and Azotobacter. It has been revealed that employing it increases yield by 20 to 25%.
4. Mycorrhizal fungi release hydrogen cyanide around plant roots, which kills pathogenic fungi, bacteria, nematodes, and other organisms in the soil.
5. Plants show resistance to diseases and pests as a result of mycorrhizal fungi.

6. It enhances water availability in non-irrigated locations where crops are rain-fed and increases drought resistance in plants.
7. Mycorrhizal benefits may include increased production, nutrient accumulation, and reproductive success.
8. Mycorrhizal fungi reduce agricultural losses both directly and indirectly by enhancing plant vigour against pests like nematodes and diseases while also increasing plant growth, output, and profitability.
9. Mycorrhizal fungi may protect their plant partners from excessive salt concentrations in salty soil.
10. The outside walls of the filaments release gluey substances, which induce fine earth particles to clump together, building soil structure and making the soil less prone to erosion.
11. Especially, Mycorrhizal fungi are extremely beneficial in agriculture because they act as bio-fertilizers by boosting in the absorption of phosphorus and other nutrients.
12. Mycorrhizal fungi may also directly or indirectly help to the stabilization of soil carbon in addition to promoting plant growth development by fixing additional carbon in the vegetation. First, fungus filaments contain a component that is rich in carbon and may stay in the soil for a very long time. It also offers the initial stage in the process of turning plant waste into stable soil carbon for other soil fungi.
13. Increase plant survival and establishment when sowing or transplanting. Mycorrhizal fungi increases blooming and fruiting also. Mycorrhizal fungus optimize fertilizer use, particularly phosphorus and also contribute to the maintenance of soil quality and nutrient cycling.
14. A single treatment can last the entire life of the plant. It minimize the fertilizers use, watering costs and plantation management costs also.
15. Plants gain non-nutritional advantages from changes in water relations, phytohormone levels, carbon absorption, and so on. Some ECM and ericoid fungi may break down phenolic substances in soils, which can interfere with nutrient absorption.
16. Mycorrhizae improve seed germination and seedling establishment due to break down of seed dormancy by avoiding environmental stresses and it increases the photosynthesis rate, N, P, and K content, and finally enhances the seedlings’ resistance to environmental stresses, including pathogens.
17. Mycorrhiza colonization of plant roots reduces translocation of heavy metals (Bioremediation) to shoots by binding of the heavy metals to the cell walls of the fungal hyphae in roots. In this way, Mycorrhiza can help higher plants to adapt and survive in contaminated habitats.

Precautions in use of *Mycorrhizae* formulations:

1. Don't utilise this culture in direct sunlight as it will be damaged by the warmth; therefore, be sure the area where it will be used has enough moisture.
2. Use of chemical fertilisers is not recommended in the fields where this culture will be employed before and after 10 to 15 days.

3. Only usage of this culture should take place 3 to 4 inches below the ground's surface, close to the root zone.
4. Rotten cow dung manure and organic manure should be added to this culture for it to function properly.
5. Mycorrhiza culture should be moistened with water after utilize; do not use too much water.
6. If there are more than 40,000 plants per acre, 8 to 10 kg of mycorrhiza culture should be applied.

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Entomopathogenic nematode: A potential bio-control agent for sustainable crop production

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Abstract

Agriculture is vital to India's economy. Insect infestation significantly reduces agricultural crop productivity. To satisfy the demands of India's and other emerging countries' growing populations, the Indian agricultural system requires sustainable agricultural production for future generations. This article discusses the current state of Entomopathogens in the agriculture sector. Synthetic chemical pesticides are routinely employed to manage insect pests, but they have a negative influence on our environment and the systems of non-targeted beneficial organisms, including humans. Entomopathogens as bio-pesticides are a safe, natural, and cost-effective alternative to replace those dangerous chemical pesticides. Due to a lack of innovative advancements in research and policies in India, there are currently few entomopathogenic formulations commercially available, which are insufficient to fulfil farmer demand. Particularly compared to synthetic chemical pesticides, entomopathogen production and usage are minimal. This article recommends improving technologically focused research and raising entomopathogenic bio-pesticide formulations to increase agricultural productivity in an environmentally responsible manner.

Key Words: Entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema carpocapsae*, mode of action, biological control

Introduction

The word "entomopathogenic nematodes" (EPNs) is derived from the Greek vocables, 'Εντομος' (entomos, 'insect') 'πάθη' (pathê, 'disease') and 'γ΄ενος' (guenos, 'producing'), means a group of nematodes which have the ability to cause disease in insects by suppressing the immune system of insects. Entomopathogenic nematodes (EPNs) were first identified in the 1920s, and their commercialization began in the 1980s. Entomopathogenic nematodes are beneficial nematodes attack soil-borne insect pests while being non-harmful to humans, animals, plants, or earthworms, and can thus be utilised as biological control organisms. Nematodes are worm-like unsegment invertebrates that are found all over the world. They have a wide variety of habitats, including soil, water (fresh and salt), plants, and animals. Entomopathogenic nematodes (EPNs) are the microscopic worms that have the ability to kill insects. Entomopathogenic nematodes belong to order Rhabditida and families Heterorhabditidae and Steinernematidae present naturally in soil habitat and find their host via signal responses (chemical and physical) (Shapiro-Ilan *et al*. 2012). Both of these families have species, which have been successfully used as bio-insecticides in pest management programs. Rao and Manjunath (1966) first demonstrated DD-136 strain of *Steinernema carpocapsae* in India for the management of lepidopteran insect pests of apple, rice and sugarcane. Farmers utilise species-specific EPN-based products such as Biovector, Sanoplant, Helix, Magnet, and Entonem, which are available in developed countries. Only two formulations, "Green Commandos" and "Soil Commandos," were developed in India utilising foreign EPN species, however these nematodes were ineffective against insects, most likely due to their poor adaption to Indian climatic conditions. Currently, Multiplex Biotech Pvt. Ltd.'s latest formulations are advertised as Soldier (contains *Heterorhabditis indica*) and Bouncer (contains *Steinernema carpocapsae*), although these formulations are not widely used by farmers. Currently, *Steinernema carpocapsae, Steinernema feltiae, Steinernema kraussei, Steinernema glaseri, Steinernema riobrave, Heterorhabditis bacteriophora* and *Heterorhabditis megidis* are the most commonly used and successfully applied nematodes due to the fact that they can easily be produced in liquid culture (Abate *et al.* 2017).

The indiscriminate use of chemical pesticides for insect control in various agro-ecosystems has produced several environmental problems, including ground water contamination, residue in food, resistance development, soil pollution, air pollution, secondary pest breakout, pest resurgence, and so on. Because of their environmentally favourable features, biological control agents like as entomopathogenic nematodes have become increasingly popular as a pesticide option. In most circumstances, no specialised application equipment is required. Most nematode species are suitable with sprayers that are pressurised, mist, electrostatic, fan, or aerial. Watering cans, hose-end sprayers, and pump sprayers are all excellent applicators. During crop growing seasons, nematodes can also be sprayed to agricultural fields through irrigation systems. Commercial use of EPNs reported by Prasad *et al.* (2007), Shapiro-Ilan and Gaugler (2010) and Gozel and Gozel (2016) against some insect-pests and other targets are given in Table 1, 2 and 3.

Mode of action

Only the third stage of EPNs, known as the infective juvenile (IJ) of both nematodes i.e. *Steinernema* and *Heterorhabditis*, is considered infective and pathogenic. *Xenorhabdus* and *Photorhabdus* are entomopathogenic bacteria (EPB) vectored by *Steinernema* and *Heterorhabditis* genera of EPNs, respectively. The sole free-living stage of EPNs is infective juveniles, while other developmental stages can only be seen inside infected insect hosts. Infective juveniles enter the homocoel of the host insect by natural apertures like as spiracles, mouth, and anus, or in certain species through inter-segmental membranes of the cuticle. In addition to natural holes, *Heterorhabditis* species can enter the insect host by abrading the skin. The infective juveniles enter the epidermis and release symbiotic bacterial cells from their alimentary canal (intestine) into the hemocoel. Apart from natural holes, *Heterorhabditis* species can also enter the insect host by abrading the skin. After piercing the epidermis, the IJs discharge symbiotic bacteria cells from their alimentary canal into the hemocoel. The bacteria proliferate in the insect hemolymph, release poisons and specific immune depressors, and the insect's immune system is suppressed, resulting in death within 24-48 hours. *Photorhabdus* and *Xenorhabdus*, two well-known bacterial symbionts of EPNs, are not only toxic to entomic fauna, but they also inhibit opportunistic bacteria and fungi from using the nutrient-rich carcass, effectively trapping.

Table 1: EPN formulations developed and tested in India

SN	EPN Species	Formulation/Tested/Products name
1.	*Heterorhabditis indica*	Spray formulations talk-based, Soldier,
2.	*Steinernema carpocapsae*	Spray formulations talk-based, Bouncer, Alginate capsule, Wheat bran pellets, Pearl (Sodium alginate-based),Vermiculite-based
3.	*Steinernema abbasi*	Talk based
4.	*Steinernema bicornutum*	Bait as alginate capsule
5.	*Steinernema riobrave*	Spray adjuvants
6.	*Steinernema abbasi*	Hydrogel

Table 2: EPN formulations developed by different countries

SN	EPN Species	Formulation/Products name	Country
1.	*Heterorhabditis bacteriophora*	E-Nema Gmbh	Germany
		Otinem	USA, Switzerland
		Soil commondos	India
		Nemopak HB	Italy
2.	*Steinernema carpocapsae*	ORTHO Biosafe, Biovector, X-GNAT, Ecomask, Heteromask	USA
		Biovector	Columbia
		Green commonda, Bouncer	India
		Helix	Canada
		CAPSANEM	Netherland
		Mioplant	Austria
		Sanoplant	Switzerland
		Nemastar	Italy
3.	*Steinernema feltiae*	Magnet	USA
		Entoname	Netherland
		Nemasys, Stealth	U. K.
		Exhibit	Switzerland
		Agrifutur	Italy
4.	*Steinernema kushidai*	SDS Biotech	Japan
5.	*Steinernema riobrave*	Vector MG, Biovector	USA
		Biovector	Columbia
6.	*Heterorhabditis indica*	Soldier	India
7.	*Heteroshabditis megidis*	Nemasya	UK
		Larvanem	Netherland
		NovoNem	Germany
8.	*Steinernema scapterisci*	Proactant Ss	USA
9.	*Phasmarhabditis hermaphrodita*	Nemaslug	UK

Table 3: Globally commercial use of EPN against some insect-pests as bio-control agents

Pests/Common Name	Scientific name	Crops/Targeted	Effective nematode species

Artichoke plume moth	*Platyptilia carduidactyla*	Artichokes	*Steinernema carpocapsae*
Black cutworm	*Agrotis ipsilon*	Turf, vegetables	*Steinernema carpocapsae*
Rice moth	*Corcyra cephalonica*	Rice	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Gram pod borer	*Helicoverpa armigera*	Chickpea, Pigeon pea, Tomato, Field pea	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Greater wax moth	*Galleria mellonella*	Worker honey bee	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
White grub	*Holotrichia consanguinea*	Potato, Sugarcane, Groundnut	*Steinernema carpocapsae, S. glaseri*
Blue butterfly	*Lampides boeticus*	Black and Green gram	*Steinernema carpocapsae, S. seemae, S. masoodi*
Diamond black moth	*Plutella xylostella*	Cabbage	*Steinernema carpocapsae*
Tobacco caterpillar	*Spodoptera litura*	Tobacco	*Steinernema carpocapsae, S. feltiae, S. abbasi, Heterorhabditis indica*
Armyworm	*Spodoptera frugiperda*	Vegetables	*Steinernema carpocapsae, S. feltiae, S. riobrave*
Banana root borer	*Cosmopolites sordidus*	Bananas	*Steinernema carpocapsae, S. feltiae, S. glaseri*
Banana moth	*Opogona sacchari*	Ornamentals, Banana	*Steinernema carpocapsae, Heterorhabditis bacteriophora*
Plum curculio	*Conotrachelus nenuphar*	Fruit trees	*S. riobrave*
Sweet potato weevil	*Cylasformic arius*	Sweet potato	*Steinernema carpocapsae, Heterorhabditis bacteriophora, S. feltiae*
Root weevil	*Otiorhynchus ovatus*	Berries strawberry	*Heterorhabditis marelata*
Leaf miner	*Liriomyza* spp. (dip: Agromyzidae)	Vegetables, Ornamentals	*Steinernema carpocapsae, S. feltiae*
Mole cricket	*Scapteriscus* spp.	Turf	*Steinernema carpocapsae, S. riobrave*

Grape root borer	*Vitace apolistiformis*	Grapes	*Heterorhabditis bacteriophora, H. zealandica*
Corn rootworm	*Diabrotica spp.*	Vegetables	*Steinernema carpocapsae, Heterorhabditis bacteriophora*
Corn earworm	*Helicoverpa zea*	Vegetables	*Steinernema carpocapsae, S. feltiae, S. riobrave*
Citrus root weevil	*Pachnaeus* spp.	Citrus, Ornamentals	*Heterorhabditis bacteriophora, S. riobrave*
Shore fly	*Scatella* spp.	Ornamentals	*Steinernema carpocapsae, S. feltiae*
Black vine weevil	*Otiorhynchus sulcatus*	Berries, Ornamentals	*Heterorhabditis bacteriophora, H. downesi, H. morelata, H. megidis, Steinernema carpocapsae, S. glaseri*
Navel orange worm	*Amyeloistran sitella*	Nut and fruit trees	*Steinernema carpocapsae*
Small hive beetle	*Aethina tumida*	Bee hives	*Heterorhabditis indica, S. riobrave*
Plum curculio	*Conotrachelus nenuphar*	Fruit trees	*S. riobrave*
Iris borer	*Macronoctua onusta*	Iris	*Heterorhabditis bacteriophora, Steinernema carpocapsae*
Fungus gnat	Dip: Sciaridae	Mushrooms	*Heterorhabditis bacteriophora, S. feltiae*
Codling moth	*Cydia pomonella*	Pome fruit	*Steinernema carpocapsae, S. feltiae*
Cranberry girdler	*Chrysoteuchia topiaria*	Cranberries	*Steinernema carpocapsae*
Billbug	*Sphenophorus* spp. (Col: Curculionidae)	Turf	*Heterorhabditis bacteriophora, Steinernema carpocapsae*
Cat flea	*Ctenocephalides felis*	Home yard, turf	*Steinernema carpocapsae*

Application Technology

The application of EPN in soil and plants requires additional caution in handling so that the negative effects of the environment are minimised and they perform efficiently. EPN effectiveness is reduced if post-application survival is poor. Several methods have been explored to solve this problem, including (i) desiccated cadavers (ii) capsules and (iii) baits.

EPN can be delivered along with pesticides or fertilizers. They can also be used with an irrigation system. Small pressured sprayers, mist blowers, and fan sprayers are utilized in such situations. The pressure in the spray tank should not be too high, otherwise the EPN will shred into small pieces. EPN can sustain pressures of up to 300 pounds per square inch and is compatible with all standard nozzle types, with openings as little as 50 microns in diameter.

1. **Soil Application:** In general, sandy loan soil is preferable than clay soil for nematode migration and survival. When using EPN, the temperature of the soil is also taken into consideration. If the soil temperature is higher than 28^0C, a mild pre-irrigation is normally recommended to bring the temperature down. A light irrigation is also often suggested after adding EPN to soil so that the nematode may move deeper into the soil and act efficiently (Prasad *et al.,* 2007). Also of paramount importance, to be effective, EPNs usually must be applied to soil at minimum rates of 2.5 x 10^9 IJs/ha (=25/cm^2) or higher. Before application of EPN, irrigate the field before 2-3 days to maintain the soil moisture or if required moisture level is already present in the field then EPN can be applied. While preparing the field, 2-5 kg/acre EPN culture (powder formulation) is added in 20-50 kg well decomposed farm yard manure (FYM) or vermin-compost/cocopit/sand. 5-25 gram EPN is required for plantation and fruit crops/plant.

2. **Soil Drenching:** Maintain the soil moisture and mix 10 gram/litre of clean water. Mix thoroughly. While drenching, remove the nozzle of Knapsack pump and apply EPN at the root zone area.

3. **Foliar application/Stem borer management:** One of the most prevalent techniques of EPN application is spraying in standing agricultural plants. The kind of nozzle utilised, droplet size, and spray distribution mechanism all influence EPN deposition on plant surfaces. Adjuvant addition promotes EPN deposition on foliage. To overcome the extremities of high temperature and UV radiation adjuvant are required. The addition of fluorescent brighteners such as Tinopal, Ujala, Ranipal (@ 0.01%) or Glycerine @ 1% and others can provide effective protection against these environmental influences (Prasad *et al.,* 2007). Spray of 1000 IJs per plant (equivalent to 125 million IJs/ha) or @ $1x10^6$ IJs/ml or $3x10^9$ IJs/ha as a prophylactic control measure. For stem borer/Pseudostem weevil/Banana stem weevil (*Odoiporous longicollis*) management, the holes along with frass material or jelly like exudations are observed on pseudostem, inject 20 ml of active infective juveniles(IJs) of EPN @1000 IJs/ml in to the holes as curative measure.

Compatibility with Pesticides

EPN are tolerant to most agrochemicals, including fungicides, insecticides, herbicides, and acaricides, and may therefore be blended in the tank. Many hazardous compounds only have a temperature impact because the nematodes recover fast after being exposed to them. Thus, when exposures are short, simultaneous use of nematode with pesticides and fertilizers should be feasible. *S. glaseri* nematode is compatible with carbofuran, quinalphos while *S. carpocapsae* is compatible with dimethoate, endosulfan, mancozeb, malathion and zineb. Mancozeb and neem are safe to all nematode populations except *H. indica.* Neem products like Achook and Neem Gold are compatible with *S. carpocapsae* (Prasad *et al.,* 2007).

Advantages:

1. Highly toxic to important insect pests with little off-target effects.

2. Humans and the environment are quite safe. There is no need for safety equipment, there are no residual effects, there is no groundwater contamination, and pollinators are not harmed.
3. Kills insects within 24-48 hours.
4. They are simple to mass produce and may be applied using normal agrochemical equipment and irrigation systems.

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Entomopathogenic Fungi: a sustainable option for insect pest management

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Abstract

Agriculture is vital to India's economy. Insect infestation significantly reduces agricultural crop produ ctivity. To satisfy the needs of India's and other emerging countries' growing populations, the Indian agricultural system need a sustainable agricultural production for future generations. Crop plants are susceptible to biotic and abiotic stresses caused by insect pests, plant diseases, and poor growth circumstances. These variables are to blame for massive plant productivity losses (up to 40% crop losses) globally. Chemical pesticides are frequently used to protect plants. This is due to an increase in insect resistance to a variety of chemical compounds included in plant protection solutions. Chemical pesticides have a negative influence on non-target species, resulting in biodiversity loss, food safety issues, insect resistance, and revival in novel locations. Over the last few decades, natural and biological pest and disease control of cultivated plants has been given more attention in the effort to minimize agricultural production's dependency on chemical products. Employing biological creatures, predators, parasitoids, and microorganisms such as viruses, bacteria, and fungus to control pests has shown to be a successful and sustainable pest management strategy. Among the aforementioned, fungi, particularly insect-pathogenic species, have been used for more than 150 years. This article discusses the current state of Entomopathogens in agriculture industry. Entomopathogens as bio-pesticides offer a safe, natural, and cost-effective alternative to harmful chemical pesticides. There are currently few entomopathogenic formulations on the market, which are insufficient to fulfil farmer demand due to a lack of new breakthroughs in research and legislation in India. When compared to manufactured chemical pesticides, the production and consumption of entomopathogens is restricted. In this light, this article discusses the possibility of EPF as alternatives or may be alternatives to chemical pesticides in plant protection.

Key words: Entomopathogenic fungi, bio-insecticide, brand name, toxins, target insect-pets

Introduction

Agricultural pests include plant pathogens (fungi, oomycetes, bacteria, viruses, and nematodes), weeds, arthropods (mostly insects and mites), mollusks (slugs and snails), and a few vertebrates. They impair crop output and quality by eating on them. Pest species are thought to number in the millions worldwide. They have a significant influence on agricultural production, which has resulted in a 40% drop in potential global crop yields. Agriculturists rely significantly on chemical pesticides and inorganic fertilisers to tackle these challenges in order to assure maximum output of produced plants. Another constraint for agriculturists is their overdependence on synthetic fertilisers to improve plant growth. This is due to the fact that overuse of these compounds has a number of negative consequences for users, non-target organisms, and the environment. As the world's population is predicted to reach 9.1 billion by 2050 (Liu *et al.*, 2017), efforts are being made to maintain sustainable agricultural production. However, excessive use and reliance on synthetic pesticides and fertilisers, climate changes, poor land management, and widespread urbanisation are some of the problems hurting these efforts. The potential use of entomopathogenic fungi (EPF) as bio-control agents against herbivores is an environmentally viable alternative insect pest management strategy. In the right setup, EPF are known for their capacity to infect insects and cause sickness by entering their cuticles. More than 700 species from around 90 distinct genera have been identified as insect-pathogenic fungus to date. These strains include the most well-known members of the genera *Beauveria, Metarhizium, Isaria, Hirsutella,* and *Lecanicillium*. The most widely researched fungal species include *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, *Isaria fumosorosea* Wize, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas (Chen *et al.,* 2015).

The term entomogenous is derived from two Greek words: "entomon" (insects) and "genous" (arising in). As a result, the etymological definition of entomogenous microorganism is "microorganisms that grow in insects." Entomogenous microorganisms are directly involved in natural or microbiological management of insect pests and are connected to human welfare, which has piqued the interest of microbiologists, molecular biologists, and entomologists in recent years. Interestingly, the idea of using microbes for pest management is not a new one. Agostino Bassi (1773-1856) identified and reported the first entomopathogenic fungus in 1835 that a fungus could cause a deliberately transmissible disease in silkworm (Lord, 2007), which caused white muscardine sickness in insects and was later termed *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales, Cordycipitaceae) (Rehner *et al.,* 2005). After a few years, Elias Metschnikoff (1845-1916) identified the green muscardine, a fungal disease that attacks insects and is caused by *Metarhizium anisopliae* Metschnikoff Sorokin (Hypocreales, Clavicipitaceae) (Zimmermann *et al.*, 1995).

Entomopathogenic fungi (EPF) with a vast and plentiful variety are a boon to sustainable pest management. Because of their environmental friendliness and bio persistence, entomopathogenic fungi prefer to kill insects at different phases of their life cycle. EPF species from numerous classes that infect insects have been found. These insect pathogens have a wide range of alterations and contaminating capacities employing facultative and obligate pathogens. These Entomopathogenic fungi are descended from the Deuteromycota, Ascomycota, and Zygomycota divisions. Mycoinsecticide/EPF is the employment of fungus in biological processes to minimise insect density and thereby crop damage. Biological plant protection with entomopathogenic fungi is an important component of a long-term pest management strategy. When compared to conventional pesticides, entomopathogens offer significant benefits as biocontrol agents. Low prices, great efficiency, safety for beneficial creatures, residue reduction in the environment, and improved biodiversity in human-managed ecosystems are some of the benefits. This article discusses the details of entomopathogenic fungi, mode of action, target pests, trade name, manufacturer/country and their detailed usage description in the current scenario (Table 1).

Some important entomopathogenic fungi (EPF) using for pest management

EPF are an essential component of myco-insecticides in horticulture, forestry, and agriculture, and are a vital component of integrated pest management strategies as biological control agents against insect pests and other arthropods. The important genera are illustrated in table 1 and details as given below:-

Genus –*Beauveria*

It is an entomopathogenic fungus used as a bio-pesticide in crop pest management. It lives in the soil saprophytically and frequently produces large epizootics that kill out insect populations on crops. The two more important species are in this group i.e. *Beauveria bassiana* and *Beauveria brongniartii*. These parasites enter the host insect body by food or contact with the host cuticle, where they multiply. It creates poisons such as beauvericin, bassianocide, and others inside the host body, causing paralysis and eventually killing the insects within four or five days. They are particularly useful in controlling sucking pests and caterpillars that infest agricultural plants. These entomopathogenic fungi are used to manage caterpillars such as the yellow stem borer and leaf folder of rice, the white grub of groundnut, the coconut rhinoceros beetle, sugarcane pyrilla, caterpillars of pulses, tomato, and cotton, diamond back moth, leaf eating caterpillars of tobacco and sunflower, and others in an environmentally friendly manner.

Genus-*Verticillium*

It is a naturally occurring entomopathogenic fungus which can be used as a bio-pesticide. The two most important species in this genus are *Verticillium chlamydosporium* and *Verticillium lecanii*. The fungus *V. lecanii* is widely spread and can produce massive outbreaks in tropical and subtropical climates, as well as in warm and humid conditions. *Verticillium lecanii* is most effective to manage the whitefly, thrips, mealy bug and several aphid species of vegetables and ornamentals etc.

Genus-*Metarrhizium*

It is a fungal insecticide for use against a wide range of insects including soil insects, caterpillars, sucking pests and locusts. *Metarhizium anisopliae, Metarhizium album* and *Metarhizium flavoviride* are the three most significant species in the genus. *Metarhizium anisopliae* is a fungus that may be harmful to insects. It is known to attack over 200 species of insects covering seven orders. This pathogenic fungus is mostly used to manage the coconut rhinoceros beetle, groundnut cut worm, rice brown plant hopper, diamond back moth, and sugarcane early shoot borer, top shoot borer, and internode borer.

Genus-*Nomuraea*

It is also an entomopathogenic fungus used as a bio-pesticide against *Sopdoptera litura*, Helicoverpa armigera of groungnut, sorghum and chickpea etc. *Nomuraea rileyi* is a dimorphic hyphomycete that may induce epizootic death in various insects. *N. rileyi's* host specificity and environmentally favourable characteristics support its application in insect pest management. This biological control is effective against a variety of insect hosts, including *Trichoplusia* sp., *Heliothis zea, Bombyx mori, Plathypena scabra*, and others.

Genus-*Paecilomyces*

Paecilomyces fumosoroseus is a major bio-control agent against whiteflies that causes "yellow muscardine." The ability of this fungus to grow extensively over the leaf surface under humid conditions is a characteristic that certainly enhances its ability to spread rapidly through whitefly populations. P. fumosoroseus is best for controlling the nymphs of whitefly. These fungi cover the whiteflies body with mycelial threads and stick them to the underside of the leaves. The nymphs show a “feathery” aspect and are surrounded by mycelia and conidia. This fungus is used to manage yellow and red mites, whiteflies, and other insects in both field and greenhouse environments.

Genus-*Hirsutella*

H. thompsonii, H. gigantea, and *H. citriformis* are the three most significant species in the genus *Hirsutella. H. thompsonii* is used to control the citrus rust mite. This bio-control is also effective against the Acarida, Lepidoptera, and Hemiptera insect families.

Table 1: Globally available Bio-insecticides/Mycoinsecticides/EPF formulations

EPF/ Mycoinsecticides	**Brand Name**	**Formulation**	**Target Pests**	**Crop**	**Producer/ Country**
Beauveria bassiana	Mycotrol	WP, ES, OF	Whiteflies, Thrips, Aphids	Field crops	Mycotech, USA
	Biowonder	WP	Rice pests	Rice	Indore Biotech, India

	Biosoft	WP	*Helocoverpa* and sucking pests	Tomato, Chickpea, Pigeon pea, Field pea and other several crops	Agri land Biotech, India
	Conidia	WDG	Coffee berry borer	Coffee	AgrEvo, Germany
	Naturalis	ES	Sucking insects	Cotton, Glasshouse crops	Troy BioScience, USA
	Ostrinol	G	Corn borer	Maize	NPP (Calioppe), France
	Mycojaal	WP	Diamond black moth	Cabbage	Pest Control India (Pvt) Ltd, India
	Beevicide	WP	Borer type pests	Several crops	-
	Botani Gard	ES	Whiteflies, thrips, aphids, psyllids, mealybugs	Several crops	LAM International, USA
	Botani Gard	WP	-do-	-do-	-do-
	Bauveril	WP	Beetles, Butterflies and Moths	Several crops	Laverlam S.A., Colombia
	Bio-Power	WP	Stem borers, cut worms, root grubs, leafhoppers, whiteflies, aphids, thrips and mealy bugs	Several crops	T. Stanes, India
	Boverin	WP	Colorado potato beetle; Leptinotarsa decemlineata, and the codling moth; Cydia pomonella	Several crops	Biodron, Russia
	Conidia	WDG	Coleoptera (Curculionidae)-Beetles	-do-	Hoechst Schering Colombia and AgrEvo, Germany
	Naturalis-L	WP	Coleoptera (Chrysomelidae, Curculionidae), Hemiptera (Miridae, Cicadellidae, Aleyrodidae, Aphididae,	-do-	Andermatt Biocontrol Troy Biosciences Inc., Switzerland, United States

			Psyllidae), Lepidoptera, Thysanoptera (Thripidae)		
	Racer BB	WP	Lepidoptera (Noctuidae)	-do-	SOM Phytopharma, India
	Proecol	WP	Army worm	Paddy, Maize etc.	Venezuela
	Bea-Sin	WP	Paper weevil. Boll weevil, Whiteflies	Field crop	Agrobiologicos Noroeste (Agrobionsa), Mexico
	Boverol-Spofa	WP	Colorado potato beetle	Potato	Czechoslovakia
	Ballvéria	WP	Whiteflies	Tomato, Chilli	Ballagro Agro Tecnologia (Brazil)
	Bovebio	WP	Whiteflies, Mites	Tomato, Chilli	Biofungi – Industriae Comerciode Defensivos Biológicose Inoculantes (Brazil)
	Bio Expert	WP	Whiteflies, Thrips	Tomato, Okra, Chilli	Live Systems Technology (Colombia)
	Adral	WP	Aphids	Mustard	Bio-Crop (Colombia)
	Broadband	WP	Whiteflies, Thrips, Mites, Diamondback moth	Cabbage, Tomato, Chilli	BASF South Africa
	Bassicore	SC	Whiteflies	Tomato, Chilli	Core Biotechnology (Colombia)
	BBC	WP	Bollworm, Cutworms, Root grub, Termite, Whiteflies, Thrips, Mealy bug, Plutella	Several crops	Sri Biotech Laboratories India Ltd.

Beauveria bassiana PDRL1187	-	WP	Mustard Ahpid, *Lipaphis erysimi*, *Aphis craccivora* Koch	Mustard, Tomato, Wheat etc.	-
Beauveria brongniartii	Betel	G	Scarab beetle Larvae	Sugarcane	NPP (Calioppe), France
	Melocont	G/WC	Scarab beetle Larvae	Pasture	Kwizda, Austria
	Engerlingspilz	G/WC	Scarab beetle Larvae	Pasture	Andermatt, Switzerland
	Biolisa	WC	Cerambycid beetles	Inga and Ficus	Nitto Denco, Japan
Metarhizium anisopliae	BIO 1020	G	Black vine weevil	Glasshouse Ornamental crops, Nursery stock	Bayer, Germany
	Metarhizium	G/WC	Sucking pests	Several crops	Multiplex, India
	Multiplex	WP	Root grubs	Several crops	Multiplex, India
	Bio Magic	WP	Brown plant hopper	Rice	T. Stanes, India
	Bio-Blast™	WP	Termites	Houses	Ecoscience, USA, Brazil, Colombia
	Achieve	WP	Mites	Several crops	Real IPM (Kenya)
	Bio-Catch-M	WP	Hemiptera (Aleyrodidae, Aphididae)	-do-	T. Stanes, India
	Bio-Cane	WP	Sugarcane pest; grayback canegrub	Sugarcane	Granules Becker-Underwood, Australia
	Bio-Path	WC	Blattodea (Blattellidae, Blattidae)	Field crops	EcoScience, USA
	Campaign	WP	Mealybugs, Thrips, Fruit flies, Mealybugs	Field crops	Real IPM, Ghana, Uganda
	Cobican	WP	Sugarcane spittle bud	Sugarcane	Probioagro, Venezuela
	Metrocid	WP	Root grubs, BPH, Termite	Groundnut, Paddy	Sri Biotech Laboratories India Ltd.

	Kalichakra	WP	Root and beetle grubs, Cutworms, Aphids	Several field crops	Agrilife International Panacea, Ltd. India
	Jasmeta	WP	Termite and Weevils	Field crops	Shri Ram Solvent Extraction, India
	Biostorm	WP	Root weevils, Termite, Hoppers, White grub	Several field crops	Varsha Bio Science and Technology, India
Metarhizium flavoviride	Bio Green	G	Red-headed cockchafer	Pasture/Turf	Bio-Care Technology, Australia
	Green Muscle	WP, OF	Locusts, Grasshoppers	Several crops	CABI, UK
Metarhizium anisopliae Var. acridum	Green Muscle	WP, OF	Locusts, Grasshoppers	Several crops	Biological control products SA (Pty) Ltd. (under licence from UK) Soth Africa
Lecanicillium longisporum (Old name: *Verticillum lecanii*)	Vertalec	WP	Aphids, Whiteflies and Thrips	Tomato, Chilli and Glasshouse crops	Koppert, Holland,
	Mycotal	WP	Aphids, Whiteflies and Thrips	Glasshouse crops	Koppert, Holland,
	Verticare	WP	Mealybugs & Scales	Citrus	Viswamitra Bio Agro, India
	Biocatch	WP	Whiteflies	Cotton	T. Stanes, India
	Inovert	WP	Aphids, Scales, Mealybugs	Glasshouse crops	Inora, India
	Verelac	WP	Sucking pests	Several crops	-
	Bioter	WP	Effective against termites	Several crops	-
Paecilomyces fumosoroseus (Isaria fumosoroseus)	PFR-97^{TM}	WDG	Whiteflies/ Thrips	Glasshouse crops	Thermo Trilogy, USA Eco-tech, USA
	Prioroty	WP	Mites	Wide range of crops	T. Stanes, India
	Pae-Sin	WP	Whiteflies	Tomato, Chilli	Agrobionsa, Mexico

	Bemisin	WP	Whiteflies	Tomato, Chilli	Probioagro, Venezuela
Hirsutella thompsonii	Mycohit	WP	Mites, *Aphis craccivora* Koch	Citrus, Cowpea	-
Cladossporium oxysporium	-	WP	*Aphis craccivora* Koch	Cowpea	-
Nomuraea rileyi	Numoraea 50	WP	Lepidoptera	Several crops	Colombia
M. anisopliae + *B. bassiana* + *I. fumosoroseus*	Tri-Sin	WP	Psyllid	Citrus and other crops	Mexico
Lagenidium giganteum	Laginex	AS	Larvae of most pest mosquito species	-	USA
Conidiobolus thromboides	Vector 25 SL	WP	Thrips, Aphid, Whiteflies	Several crops	Mycolab, South Africa

WP= Wettable powder @ 1 x 10^8 cfu's/gram or 5 x 10^{13} conidia/ha; G= Granular; OF= Oil flowable; WC= Whole culture; WDG=Water-dispersible granular; AS= Aqueous suspension

EC= Emulsifiable concentrate; SC= suspension concentrate

Mode/mechanism of action of entomopathogenic fungi

Insect pathogenic fungus kill insects in a variety of ways, including starving to toxin production. These insect pathogenic fungus generate several toxins and extracellular enzymes such as proteases and chitinases. Cuticle is the principal barrier to infection in insects since it is the primary avenue of fungal penetration. As a result, it requires either physical or enzymatic techniques to breach the impenetrable cuticle. The infection process begins with spore contact with the host cuticle. Most fungi have an infective unit that is a spore, commonly a conidium. Conidia are usually sticky to the cuticle or exude adhesive mucus when they enlarge during pre-germination. In favourable conditions, the conidium germinates into a short germ tube that produces tiny swellings called appresoria. The appresorium adheres to the cuticle and sends out an infection peg, which gives the fungus with the solid connection it requires to physically push its way into the host. The hyphae then penetrates the insect cuticle by enzymatic chitin and protein disintegration, first dissolving the cuticle and then entering the insect's haemocoel and internal organs. The infectious fungal mycelium invades the insect until it is completely filled with the fungus and becomes quite solid to the touch. Following that, conidiophores are generated, which erupt through the cuticle and create spores on the exterior of the fly, infecting surrounding healthy insects as well. The fungus produces poisons that kill the host by mechanically obstructing the tissues. Toxins are generated by several insect pathogenic fungi (Table 2), and many of them help in pathogenesis and serve an insecticidal role. While certain other fungus create antibacterial compounds. Destruxins from Metarhizium species are among the fungal metabolites that promote the pathogenicity of the fungus. Beauvericins are generated by Beauveria species. These metabolites are harmful to invertebrates as well.

Table 2: Some important toxins produced by the various entomopathogenic fungi

Toxins	**Fungus that produces toxins**	**Mode of general infection**
Beauvericin	*Beauveria bassiana, Isaria sp. Fusarium sp.*	Cytotoxic effect and insecticidal properties. Ionophore is soluble in lipid layers and increases membrane permeability for specific ions. In this way, it damages the cell organelles and their functions.
Destruxins	*Metarhizium anisopliae*	Immunodepressant activity in insect and cytotoxic effect.
Bassianolide	*B. bassiana, Verticillium lecanii*	Acts as ionophore, toxic effect on insects
Leucinostatins	*Paecilomyces lilacinus, Paecilomyces marquandii*	Insecticidal activity by interfering with oxidative phosphorylation
Efrapeptins	*Tolypocladium niveum*	Inhibitors of mitochondrial oxidative phosphorylation and ATPase activity.
Bassionolid	*Beauveria bassiana*	Ionophore is soluble in lipid layers and increases membrane permeability for specific ions. In this way, it damages the cell organelles and their functions.
Siklosporin A	*Beauveria bassiana, Tolypocladium sp., Verticillum sp., Fusarium sp.*	Blocks a step in Ca ++ dependent signal transduction in vertabrate T cells. This causes immunosuppression. It can also suppress insect defence cells.
Hirsutellin A	*Hirsutella thompsonii*	The ribosomal inhibitory protein (RIP) causes a specific cleavage of the rRNA and inhibits protein synthesis.
Swainsonin	*Metarhizum anisapliae*	Indolizidine alkaloid
Sitokhalasin	*Metarhizum anisapliae*	Blocks the elongation of the actin filament.
Dipicolinic acid and Didepsi peptide (protein) Bassianolide	*Verticillium lecanii*	It causes atony. Interfere, the process of chitin formation and exposure the insect easily to the attack of natural enemy.

Source: Boucias and Pendland, 1998; Kumar *et al.*, 2020

Method of application of EPF/Mycoinsecticide:

6. **Soil application/treatment:** While preparing the field, @ 250-300 gram per square meters or 1-2 kg per acre (powder formulation) is added in 80-100 kg well decomposed farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 1-2 week in shade for proper multiplication. Maintain

moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Apply well mixed/decomposed EPF based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing for termite, white grub and other soil pest management. It should be repeated after 2-3 week interval.

7. **Spraying:** Mixing 1.0-1.5 kg of EPF culture in 200 liters of water with sticker should be sprayed in morning or evening for pest management (sucking pests, bug and beetles etc.) in one acre area. Mix the culture in water at in appropriate quantity and stir well till creamy, leave it for 3-4 hours. Empty the cream into spray tank together with required amount of water and agitate well. Spray immediately after preparation. Apply with high volume spraying equipment @ 2.5-5 gram/liter of water.
8. **Soil drenching:** 1.0-1.5 kilogram EPF culture formulation mix in 200 litre of water and drench the soil in one acre area or @ 250 gm/5 litre of water in soil from time to time for management of white grub and other soil pests. Maintain optimum soil moisture while applying. Repeat application at 2-3 week interval as and when required.

Advantages and disadvantages of Entomopathogenic Fungi

The practical application of EPF has various benefits and drawbacks. These are listed below.

Advantages

- In certain circumstances, EPF have a high host selectivity for arthropod pest management. As a result, they have no effect on beneficial insect populations. Some of them, however, may have a very broad host range.
- Microbial pesticide residues provide no danger to humans or other beneficial creatures. Farmers can use them even when a crop is nearly ready for harvest.
- They persist in the environment for a long period after application, resulting in the rapid mortality of the arthropod hosts.
- They do not produce issues like as pesticide resistance while managing arthropod pests and hence provide long-term control.
- Microbial pesticides may become established in succeeding generations of pests or in their habitats, providing pest control throughout the following season or generation.
- They are accessible to development through biotechnological techniques.

Disadvantages

- The most significant drawback is that they are vulnerable to UV light, as well as low humidity and high temperatures.
- Because microbial insecticides are designed to inhibit certain pest species or groups, they may only control one pest in a field. Other pests that are present in the treated area continue to inflict damage.
- They are adversely affected by fungicides.

- Synthetic pesticides often kill arthropod pests quickly, while EPF take a longer time (sometimes 1-2 weeks).
- The expenses of production are often greater than those of many synthetic insecticides.

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Entomopathogenic bacteria: A potential biological weapons against insect-pests management

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Abstract

Food security and safety are major concerns in the world's ever-growing human population. Insect-pests have a huge impact on agricultural output every year. The majority of farmers utilised chemical pesticides to control them, which are harmful to the environment and human health. The loss in term of productivity and high cost of chemical pesticides enhance the production cost also. Biological management of agricultural pests and diseases has been discovered to have an important role in reducing dependency on chemical pesticides. The control of pests by entomopathogenic and plant associated bacteria is an alternative that may contribute to reduce or eliminate the pesticide use. Entomopathogenic bacteria, which cause illnesses in insects, kill the host through septicaemia and toxin production. Plant-associated bacteria, such as rhizospheric, endophytic, and phylloplane bacteria, play a role in biocontrol by producing defence compounds that induce systemic resistance. Among the entomopathogenic groups of bacteria *Bacillus thuringiensis*, *B. popilliae, B. Sphaericus* and *B. cereus* are used as biocontrol agents nowadays. Families of bacteria having the properties of pathogenesis comprise Bacillaceae, Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Streptococcaceae. Some of these bacterial families are highly lethal to the various insect pests. Among these virulent families, Bacillaceae contain the genus *Bacillus* having *B. popilliae* species, which is responsible for causing the milky spore disease in the scarab beetles. Even one more species *B. sphaericus* is highly infectious to mosquitoes. Pathogenicity of *B. thuringiensis* is also well known. They are virulent against a wide range of insect pests mainly lepidopterans and are world widely distributed as biocontrol agents. This article describes different types of bio-insecticides and the potential use for the control of insect-pests at global level.

Keywords: Entomopathogenic bacteria, mode of action, cry toxin, target pest management

Introduction

Indian agriculture sector accounts for 18% of India's gross domestic product (GDP), and about 58% population of India is dependent upon agriculture for its livelihood. The Indian population is expected to exceed 1.5 billion by 2050. There will be a huge pressure on the agricultural sector to feed such a gigantic population by increasing food production in an environmentally sustainable manner. Nowadays, agriculture supports two-thirds of the world's population, yet crop production is becoming increasingly susceptible owing to insect assault. Chemical pesticides are mostly used in agriculture to control insect pests. Farmers frequently use enormous volumes of chemical insecticides to reduce crop loss caused by pests. In spite of heavy insecticide application, crop loss rises due to a variety of factors such as resistance development, pest resurgence, and pest replacement, as well as negatively impacting the environment and human health by leaving harmful residues. Hence there is a need to evolve eco-friendly management strategies. Biological pest and disease control of cultivated plants has received a lot of attention during the last two decades as a strategy to reduce the use of conventional pesticides in agriculture. Natural enemies like as predators and parasitoids are used to manage pests, whereas helpful microorganisms such as insect pathogenic bacteria, fungi, viruses, protozoa, and nematodes are used to control pests.

Bacterial populations pathogenic to insect pests can cause major damage to the target insect population and are known as entomopathogenic bacteria. As the potential of entomopathogenic bacteria, particularly *Bacillus* species, was revealed, the biological control paradigm shifted. Initially, the species *Bacillus popilllae* Dutky was introduced for the management of the Japanese beetle *Popillia japonica* Newman, but more concrete results were achieved with the discovery of new *Bacillus thuringiensis* (*Bt*) strains showing high toxicity against specific insects at a competitive level in terms of efficacy and production costs. The bacteria *Bacillus thuringiensis* (*Bt*) was the first biological control agent developed for control of insects (Burges, 1982). Bt is a spore forming, entomocidal, gram-positive soil bacterium. The protein, delta toxin, formed during sporulation of the bacterium causes the insecticidal activity. These protein crystals dissolve in alkaline conditions of the insect midgut (Gill, 1992). Till now several bacterial insect pathogens and their insect hosts have been identified as bio-control agents and are highly pathogenic to arthropods (Table 1). The different strains of commercial formulations of bacterium are active against insect management (Table 2). Bacterial insecticides are the most common type of microbial pesticide. Bacterial-based bio-insecticides have been utilised to manage a variety of insect-pests. Both spore-forming and non-spore-forming bacteria are employed to manage insects. Bacteria disrupt the digestive system of insects by creating endotoxins (insecticidal proteins) that are particular to the insect. *Bacillus thurigiensis*, sometimes known as *Bt*, is the most widely utilised bacterium. *B. thuringiensis* has been shown to be effective against several insect pests in forestry, agriculture and other areas. Besides the entomopathogenic bacteria, there exist certain bacteria that can effectively colonise plants by occupying different sites such as roots, leaves or as inter or intracellular colonisers. Pest management with entomopathogenic and plant-associated bacteria is an option that may help to decrease or eliminate the usage of pesticides.

Major groups of entomopathogenic bacteria

Entomopathogenic bacteria are unicellular prokaryotic organisms (without nucleus) having size ranging from less than 1 μm to several μm in length. Bacteria with rigid cell walls are cocci, rod-shaped and spiral while bacteria without cell walls are pleomorphic. There are more than 100 species of bacteria, found to be pathogenic to insect pests. Among the most entomopathogenic groups of bacteria are both gram-negative as well as gram-positive and are soil-borne. Some important gram-negative entomopathogenic bacteria include *Photorhabdus* spp., *Xenorhabdus* spp., *Serratia* spp., *Yersinia entomophaga*, *Pseudomonas entomophila*, *Chromobacterium* spp., and *Burkholderia* spp. Gram-positive bacteria include *Bacillus thuringiensis*, *Lysinibacillus sphaericus*, *Paenibacillus* spp., *Brevibacillus laterosporus*, *Clostridium bifermentans*, *Saccharopolyspora spinosa*, and *Streptomyces* spp. The bacterial families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae have so far found vast majority of arthropod infections. The majority of these bacteria are mild pathogens that infect insects under stress, but a small number are very virulent. These families often contain epiphytes, however certain infections have been demonstrated to be extremely harmful to their hosts. A small number of entomopathogenic bacteria have been commercially developed for control of insect pests. These include several *Bacillus thuringiensis* sub-species, *Lysinibacillus* (*Bacillus*) *sphaericus*, *Paenibacillus* spp. and *Serratia entomophila*. *B. thuringiensis* sub-species *kurstaki* is the most widely used for control of insect-pests of crops and forests, and *B. thuringiensis* sub-species *israelensis* and *L. sphaericus* are the primary pathogens used for control of medically important pests (Sand flies, black flies, stable fly, Red imported fire ants, southern fire ants, Aedes, Anopheles, Culex, Honey bees, bumble bees, cicada killer wasps) including dipteran vectors. There is dozen recognize subspecies of *Bacillus thuringiensis* but most common species and their target pests are *Bacillus thuringiensis* sub-species *kurstaki* (moths and butterflies), *Bacillus thuringiensis* sub-species *israelensis* (flies and mosquitoes), *Bacillus thuringiensis* sub-species *aizawai* (wax moth larvae), *Bacillus thuringiensis* sub-species *tenebrionis* (potato beetle and cotton ball weevil) and *Paenibacillus popilliae* (Japanese beetle *Popillia japonica*).

Family Bacillaceae

Bacillaceae family bacteria are Gram-positive, heterotrophic, rod-shaped bacteria with the ability to produce endospores. Members of this family include *Bacillus thuringiensis, B. sphaericus, B. popillae, B. pumilus, Brevibacillus laterosporus* etc.

1. ***Bacillus thuringiensis***

Bacillus thuringiensis (Bt) is the most effective insect pathogen used for pest control, accounting for 2% of the overall insecticidal market. Bt is an aerobic spore forming, gram-positive, soil-dwelling bacteria known for its ability to produce crystalline inclusions (Cry poisons) containing insecticidal proteins known as δ-endotoxin (delta-endotoxin) during sporulation. These toxins damage the midgut of the pest causing septicaemia and death within 2-3 days (Ruiu, L., 2015, Mampallil *et al.,* 2017). *B. thuringiensis* subsp. *kurstaki* (Btk) is generally used against young Lepidopteran larvae and includes different strains with significant commercial interest like HD-1, SA-11, SA-12, PB 54, ABTS-351 and EG2348. Strains of *B. thuringiensis* subsp. *aizawai* (Bta) (i.e., ABTS-1857) are also used against armyworms and diamondback moth larvae. Besides, strains belonging to the *Bacillus thuringiensis* subsp. *israelensis* (Bti) and Bacillus *thuringiensis* subsp. *tenebrionis* (Btt) have been employed for the management of mosquitoes and simulids, and against coleoptera, respectively Glare and O'Callaghan, 2000).

2. ***Bacillus popilllae***

The spore-former *B. popillae* (Dutky) is the causal agent of milky disease in phytophagous coleopteran larvae. The production of parasporal inclusions within the sporangial cells has been observed in *B. popillae*, even if they are not directly responsible for the insecticidal action. After the spores are ingested by the host, they germinate in the midgut (Mampallil *et al.,* 2017).

3. ***Lysinibacillus sphaericus (Bacillus sphaericus)***

Entomopathogenic strains belonging to the *L. sphaericus* (formerly *Bacillus sphaericus*) species group are featured by the production of spherical endospores closely associated with parasporal crystals containing an equimolar ratio of binary protein toxins (BinA and BinB). The insecticidal mechanism of action involves midgut microvillar epithelial cell destruction similar to that seen with B. thuringiensis. Also, certain strains' vegetative cells release mosquitocidal poisons (Mtx proteins). The main targets of commercial formulations based on *L. sphaericus* strains are mosquitoes, blackflies and non-biting midges (Ruiu, L., 2015, Mampallil *et al.,* 2017).

4. ***Bacillus pumilus***

Bacillus pumilus strain 15.1 has recently been shown to be poisonous to larvae of the Mediterranean fruit fly, *Ceratitis capitata*, one of the most devastating pests to fruits and vegetables globally. During sporulation, this strain produces parasporal crystals that resemble those formed by *Bacillus thuringiensis* cry proteins. Several genes in the *B. pumilus* 15.1 genome encode well-known entomopathogenic factors such chitinases, metalloproteases, and cytolysins (Mampallil *et al.,* 2017).

5. ***Brevibacillus laterosporus***

So many *B. laterosporus* strains have been shown to be insecticidal against insects of many orders, including coleoptera, lepidoptera, and diptera. It has the ability to create a variety of poisons. Some strains that generate insecticidal secreted proteins (ISPs) that function as binary toxins in the insect midgut and have high similarity with *B. thuringiensis* vegetative insecticidal proteins cause toxicity against maize rootworms (*Diabrotrica* spp.) and other coleopteran larvae (Mampallil *et al.,* 2017).

Family Pseudomonadaceae

Pseudomonadaceae family members are strictly aerobic, gram-negative, straight or curved rods with polar flagella. Many species are pathogens, whereas others are frequent commensals in insect digestive systems. A variety of *Pseudomonas* species are present in the digestive tracts of insects, either as pathogens or as commensals. Despite the fact that *Pseudomonas aeruginosa* is one of the most regularly isolated bacteria from insects, it seldom causes epizootics in field populations (Mampallil *et al.,* 2017).

Family Enterobacteriaceae

Several of the more recognisable diseases, such as *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella,* and *Shigella,* are members of the Enterobacteriaceae family of gram-negative bacteria. *Serratia* and *Enterobacter* are the most commonly reported entomopathogenic bacteria in the Enterobacteriaceae. *Serratia marcescens* is a facultative anaerobe that grows rapidly in the guts of many insect species, producing septicaemia and death. It is frequently isolated from dead and sick insects. Some species, such as *S. entomophila,* cause illnesses in pest insects when the bacterium is consumed. *Serratia entomophila* and *Serratia proteamacula* are used as effective biological pesticides against Newzeland grass grubs, *Costelytra zealandica* (Hurst, M.R.H. 2000, Mampallil *et al.,* 2017).

Mode/mechanism of action of Entomopathogenic bacteria

Bacteria cause illnesses in insects when they are eaten. They have developed a variety of techniques to penetrate the host, defeat its immune defences, infect, and kill it. They infect the host through the midgut epithelial cells, causing septicaemia and the generation of toxins. Entomopathogenic bacteria comprise both spore-forming and non-spore-forming bacteria. Endospores are produced by all spore-forming bacteria, allowing them to survive in a latent state outside of the host. Spores germinate in the intestines after consumption. Crystalliferous spore formers create parasporal crystals in addition to endospores in the sporangium. Insecticidal action is exhibited by non-spore formers by the synthesis of insecticidal poisons (Mampallil *et al.,* 2017).

When larvae consumes foliage treated with Bt (spores and crystalline toxin) and it enters the gut, alkaline conditions (pH >8) activate the toxin protein (delta endotoxin), within a minutes, the toxin binds to receptor sites in the midgut wall and causes abrasions in midgut cells and the larvae/caterpillar stop feeding. Within hours, the gut wall breaks down. This results in ion loss due to leaching, midgut paralysis, and cell lysis. Midgut contents flow into insect blood (hemolymph) and body cavity (hemocoel), disturbing the pH equilibrium. Bacteria enter into the body cavity cause septicaemia and eventually death of the host insect (Yadav *et al.,* 2021). *Lysinibacillus sphaericus* cause injury to the epithelial microvilli in the midgut of the insect. *Clostridium bifermentans* is highly pathogenic against black flies and mosquitoes as it has Cbm71 protein showing a similar effect like delta endotoxins of *B. thuringiensis*.

Table 1: Bacterial insect pathogens and their insect hosts (Vallet-Gely *et al.,* 2008)

Entomopathogenic Bacteria	**Type of interaction**	**Mode of interaction**	**Insect hosts**
Erwinia aphidicola	Pathogen	Ingestion	Pea aphid
Dickeya dadantii	Pathogen	Ingestion	Pea aphid
Pseudomonas entomophila	Pathogen	Ingestion	Drosophila, Bombyx, galleria
Yersinia pestis	Pathogen	Ingestion	Rat flea

Serratia entomophila	Pathogen	Ingestion	Grass grub
Serratia marcescens	Pathogen	Ingestion	Drosophila
Photorhabdus sp.	Pathogen	Assisted entry	Lepidopteran
Xenorhabdus sp.	Pathogen	Assisted entry	Lepidopteran
Vibrio cholera	Pathogen	Ingestion	Drosophila
Melissococcus pluton	Pathogen	Ingestion	Honey bee
Bacillus thuringiensis	Pathogen	Ingestion	Different orders
Bacillus papillae	Pathogen	Ingestion	Scarab larvae
Paenibacillus lentimorbus	Pathogen	Ingestion	Scarab larvae
Paenibacillus larvae	Pathogen	Ingestion	Honey bee larvae
Bacillus sphaericus	Pathogen	Ingestion	Mosquito
Bacillus laterosporus	Pathogen	Ingestion	Bee larvae, dipteran
Pseudomonas aeruginosa	Opportunistic	Ingestion	Caterpillar
Pseudomonas aeruginosa	Opportunistic	Direct injection	Drosophila
Bacillus cereus	Opportunistic	Ingestion	*Galleria mellonella*
Erwinia carotovora	Infectious	Ingestion	Drosophila larvae
Shigella spp.	Passive	Ingestion	Vector house fly
Rickettsia spp.	Vector	Ingestion	Cat flea
Bartonella spp.	Vector	Ingestion	Cat flea

Table 2: Commercial Entomopathogenic bacterial formulations and target pest management

Entomopathogenic bacterial formulation	**Products name**	**Target pests**
Bacillus thuringiensis subsp. *israelensis* 5% WP, 5% AS	Tacibio/Vectobac®, Teknar®, Bactimos®, Skeetal®, and Mosquito attack®, VectoBac, Vectoba	Lepidopterous pests/Diptera pests/ Mosquito and black flies, house fly, stable fly or blow fly
B. thuringiensis subsp. *kurstaki* 5% WP, 7.5% WP	Bio-Dart/ Biolep/Halt/Taciobio-Btk, Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer®, Bactospeine® and SOK-Bt® Biobit, Cordalene, Costar-WG, Crymax-WDG, Deliver, Foray, Javelin-WG, Lepinox Plus, Lipel, Rapax	Lepidopteran pests/American ball worm in cotton, caster semilooper, diamond back moth on cruciferous vegetables, rice stem borer, *Heliothis armigera* on various crops

B. thuringiensis subsp. *Kurstaki* plus beta-exotoxin	Javelin	Armyworm and other moths
Bacillus thuringiensis subsp. *sphaericus* 1.3% FC	VectoLex, VectoMax	Diptera/Mosquito larvae
Bacillus thuringiensis subsp. *galleriae*	VectoLex, Spicturin	Lepidopteran larvae /Mosquito larvae, bollworms, Diamondback moth
Bacillus thuringiensis sub-species *aizawai*	Able-WG, Agree-WP, Florbac, XenTari, Certan, Agree	Lepidoptera/Wax moth larvae, Armyworms, diamondback moth
Bacillus thuringiensis sub-species *tenebrionsis*	M-One®, Novodor, Trident	Coleoptera/Potato beetle and cotton ball weevil
Paenibacillus popilliae and *B. lentimorbus*	Doom®, Japidemic®, Grub attack®	Japanese beetle *Popillia japonica*
Bacillus moritai	-	Diptera
Bacillus popilliae	-	Coleoptera
Pseudomonas fumosoroseus 0.5%, 1% WP	Nemato-Guard, Bio-Dart	Whitefly
Pseudomonas lilacinus	Yorker/ABTEC/Paceilomyces/Paecil/Pacihit/R OM biomite/Bio-Nematon	Whitefly
Saccharopolyspora spinosa	Tracer™ 120, Conserve	Insects
Chromobacterium subtsugae	Grandevo	Chewing and sucking insects and mites

Method of application of Bacterial bioagents:

1. **Foliar application:** 1.0-1.5 gram/litre of water bacterial formulation (powder formulation) or 1.0-1.5 ml/litre of water (liquid formulation) with sticker spray uniformly in evening or cloudy day condition for pest management (sucking pests, bug and beetles etc.), not during rains. 2-3 spray are required depending upon the intensity of the pests infestation at 5-10 days intervals. Scout fields 3-5 days after application to time of subsequent applications. The bacterial formulations are most effective when eaten by newly hatched or 1st and 2nd instar larvae. Scout for eggs and newly hatched larvae on terminals and squares to ensure proper application time.
2. **Soil application:** For control of soil inhabiting larvae, 2.0-2.5 kg bacterial formulation (powder formulation) or 500-1000 ml (liquid formulation) is added in 25-50 kg farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 2-3 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Maintain optimum moisture for better multiplication of bio-agents. Apply well decomposed bacterial based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing. This mixture is sufficient for one acre of land.

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Bio-pesticides: A Classic Weapons for Pest Management

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Abstract

Indiscriminate use of chemical pesticide in recent years has resulted in severe implications in the development of pesticide resistance in key pests and diseases, pesticides residues in food chain, disruption of ecosystem, environmental pollution affecting human health. Microbes such as fungi, bacteria, viruses, protozoa, nematodes and botanicals are the major bio-pesticides that are used mostly as alternatives to chemicals. Bio-pesticides are a cost-effective, eco-friendly and safe alternative to chemical pesticides. They are easily biodegradable, have a long shelf-life, and are less toxic to live organisms. This articles discusses the present status of some commonly used bio-pesticides and their role for pest management.

Introduction:

Pesticide use has undoubtedly helped to improve agricultural production, both in terms of yield and quality, hence increasing agricultural income, particularly in developed nations. However, the careless use of pesticides without following safety norms and recommended practices has posed serious health risks to humans, other living organisms, environment, from on-farm worker exposure and release of chemicals into the soil, air and water to pesticide-contaminated commodities and development of resistance, pest resurgence and outbreak of secondary pests. Therefore, there has been a growing demand for food safety and quality in recent decades, as reflected in the tight safety regulations on imports of products and strict regulations on the amount of pesticide residues on commodities. In recent years, public awareness of pesticides' negative impacts on food safety and the environment has grown, and the hunt for alternatives to commonly used chemical pesticides, including bio-pesticides, has become a priority. In light of this, it is critical to develop alternatives to chemical pesticides in crop protection without sacrificing agricultural productivity and profitability. Among various non-chemical options (host plant resistance, cultural, mechanical and biological management) bio-pesticides which are target specific, eco-friendly and biodegradable are potential alternatives to chemical pesticides in addressing various pest management issues.

The term “Bio-pesticides” is usually used for all biological materials and organism which can be formulated for use as a pesticides for pest management. These include microorganisms such as bacteria, fungi, virus and as well as materials of plant origin such as a neem. They are considered as minimal risk products safe to human and his environment. There are several types of bio-pesticides, including bio-insecticides, bio-fungicides, bio-nematicides, and bio-herbicides. The bio-pesticides fall into three major categories.

A. Microbial pesticides: Microbial pesticides are the most common pest-specific, broad-spectrum bio-pesticides. These contain microorganisms such as fungi, bacteria, virus, protozoa and nematodes or their derivatives with deleterious effects on insect-pest and diseases of different crops. They are reasonably exact for their intended species. Microbial bio-pesticides are self-sustaining, host-specific, and environmentally safe.

B. Biochemical/Botanical pesticides: These are naturally occurring plant materials that are used for managing the insect-pests and diseases of different crops. They are also called herbal pesticides (Pal *et al.*, 2013). They are naturally occurring chemicals and secondary metabolites that prevent or kill pests. The most extensively used biochemical pesticide is neem oil, neem seed kernel extract, and neem extract concentrates from bark and leaves, all of which are accessible in India. Biochemical pesticides also include essential oils from canola, tea tree, lemongrass (*Cymbopogon citrates*), and pyrethrin from *Chrysanthemum cinerariaefolium*. Diatomaceous earth (DE), formed from the fossilized sediments of several marine and freshwater siliceous species, particularly diatoms and other algae, is used to control a variety of field pests. It has a high absorption potential, which causes abrasion and desiccation in the insect cuticle, eventually leading to the insect's death.

Semiochemicals are classified as biochemical pesticides, which include insect attractants, repellents, sex pheromones, and kairomones. Semiochemicals are chemical signals produced by one organism, typically insects, which cause behavioural changes in individuals of the same or different species. The most common commercially available semiochemicals are insect sex pheromones. Sex pheromones are extremely effective in controlling adults because they disturb mating. Mass trapping with sex pheromones to control flying insects, particularly lepidopterans and coleopterans, is exceptionally effective in IPM operations.

C. Plant Incorporated Protectants (PIPs): Plants with the toxin-producing genes to fight against the pest are called plant-incorporated protectants or genetically modified crops or genetically engineered plants. These PIPs help the plants to resist bacteria, fungi or viruses. Plant-incorporated protectants are pesticidal compounds produced by plants, with the genetic material required to produce such substances introduced into the plants to provide resistance to pests. Pesticidal proteins isolated from bacteria or fungi are injected into the plant, making genetically modified plants resistant to specific pests. A common example is the use of Bt protein to create PIP by genetic engineering. Bt toxin is host-specific and kills pests quickly, typically within 48 hours (Siegel, 2001). No harmful effect on the ecosystem and it does not harm vertebrates (Lacey *et al.* 2015). Other examples of PIPs are soybeans, brinjal, cotton, corn, potatoes, and tomatoes. Transgenic plants are created by transferring the target gene via Agrobacterium-mediated transfer, gene gun or ballistic techniques. These approaches were employed on wheat, maize, rice, and corn.

Current status of bio-pesticides:

There are several bio-pesticides that are commercially available to farmers. According to the latest information, there were approximately 970 microbial formulations and 15 microbial species registered with the Central Insecticide Board and Registration Committee (CIBRC) in India. Here's some information about the status of bio-pesticides in 2023: The global bio-pesticides market was valued at $8,123.8 million in 2023. Bio-pesticides make up about 5% of the global crop protection market. In the fruit and vegetable sector, bio-pesticides account for more than 18% of the market. The use of bio-pesticides has been increasing, with a compound annual growth rate (CAGR) of 11% from 2018 to 2022. The global bio-pesticides market is expected to grow at a compound annual growth rate (CAGR) of 10.3% from 2024 to 2033. Europe and North America each have over 30% of the global bio-pesticide market share. Latin America has the fastest growing market. North America dominated the bio-pesticides market in 2023, with a market share of 36.6%. Bayer AG, DowDuPont, BASF.SE, Syngenta AG, Marrino Bio Innovations, and Certis USA L.L.C. hold an approximate share of 20-30% in the global bio-pesticides marketplace. The importance of some commonly used bio-pesticides and recent development in bio-pesticide research as follows:

A. Microbial pesticides:

Fungal Pathogens (Mycopesticides) as bio-pesticides:

Over the last few years, the use of entomopathogenic fungi/mycoinsecticide i.e. *Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, Metarhizium album, Metarhizium flavoviride, Verticillium chlamydosporium, Verticillium lecanii, Nomuraea rileyi, Paecilomyces fumosoroseus, Hirsutella thompsonii, H. gigantea*, and *H. citriformis*, and *Lecanicillium* and mycofungicides i.e., *Trichoderma viride, T. harzianum, Gliocladium virens, G. roseum, Aspergillus niger, A. flavus, Chaetomiam globosum, Ampelomyces* spp., *Candida* spp. and *Coniothyrium* spp. has become an area of growing interest in countries with intensive agriculture activities (Table 1). More than 700 fungal species from around 90 distinct genera have been identified as insect-pathogenic and more than 80 species of phytopathogenic fungi have been identified to date. The pathogenic action of fungi depends on the contact and subsequent infection to the target pests. Insect pathogenic fungus kill insects in a variety of ways, including starving to toxin production. These insect pathogenic fungus generate several toxins and extracellular enzymes such as proteases and chitinases that kill the host by mechanically obstructing the tissues. Fungal bio-agents control several fungal diseases by the mechanism of mycoparasitism/hyper-parasitism, antibiosis and competition. Soil application of mycoinsecticides generally recommended @ 1-2 kg per acre (powder formulation) with 80-100 kg well decomposed farm yard manure. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing for termite, white grub and other soil pest management. It should be repeated after 2-3 week interval. Mixing 1.0-1.5 kg of EPF culture in 200 liters of water with sticker should be sprayed in morning or evening for pest management (sucking pests, bug and beetles etc.) in one acre area. 1.0-1.5 kilogram EPF culture formulation mix in 200 litre of water and drench the soil in one acre area or @ 250 gm/5 litre of water in soil from time to time for management of white grub and other soil pests. Maintain optimum soil moisture while applying. Repeat application at 2-3 week interval as and when required. Mycofungicides generally used to control the diseases as seed treatment @ 8-10 gram *Trichoderma* spp. (powder formulation $2x10^6$ cfu/g) with 50 ml of water (bigger seeds) while small seeds at the rate of 6-8 gram for the treatment of one kg seed before sowing. Apply 5-10 ml *Trichoderma* spp. (liquid formulation) per litre of cow dung slurry for treatment of one kg seed before sowing particularly for cereals, pulses and oilseeds. Shade dries the seeds for 20-30 minutes before sowing is essential. Seed treatment is highly effective against seed and soil borne diseases. Seed biopriming is treatment of seed with *Trichoderma* formulations (@ 5-10 gram/kg seed) and incubating under moist and warm conditions until just prior to radicle emergence. Seed biopriming is beneficial for tomato, brinjal, chickpea, soybean etc. crops. For seed material treatment, Apply at the rate of 8-10gram *Trichoderma* powder with one litre of water (30 minutes) for the treatment of seed material like sugarcane setts, banana suckers, turmeric, ginger rhizomes and potato tubers before sowing. Shade dries the seeds for 20-30 minutes before sowing is essential.

Table 1: List of commercially available fungi-based bio-pesticides

Fungi	Host range	Products
Beauveria Bassiana	Effective against a variety of insects such as rickets, white grubs, fire ants, flea beetles, plant bugs, grasshoppers, thrips, mites, mosquito larvae and aphids, fungus gnats, whiteflies and to stored pest	BotaniGard, Mycotrol, Naturalis, Racer BB, Ostrinil, Brocari l, Mycotrol, Mycotrol-0 BotaniGard, Boverin, Naturalis-L, Naturalis-H&G Naturalis-T&O
Metarhizium anisopliae	Effective against a wide range of pests. Black vine weevil, locusts and grasshoppers	Bio-Blast, Bio-Path, Green Muscle, Met52 Metaquino Pacer MA Tick-Ex

Trichoderma viride	Effective against wilt, seedling rot, damping off, root rot, collar rot and cutting rot diseases in several crops	EcosomTV, Tricon, Trieco
Trichoderma harzianum	Effective against a variety of soil pathogens	Rootshield, BioTrek, Supresivit
Beauveria brongniartii	Scarab beetle Larvae in sugarcane and pasture	Betel, Melocont, Engerlingspilz
	Cerambycid beetles in Inga and Ficus	Biolisa
Metarhizium flavoviride	Red-headed cockchafer in Pasture/Turf	Bio Green
	Locusts, Grasshoppers in several crops	Green Muscle
Lecanicillium longisporum (Old name: *Verticillum lecanii*)	Aphids, Whiteflies and Thrips in Tomato, Chilli and Glasshouse crops	Vertalec, Mycotal, Inovert
	Mealybugs & Scales in citrus	Verticare
	Whiteflies in cotton	Biocatch
	Effective against termites	Bioter
	Sucking pests for several crops	Verelac
Paecilomyces fumosoroseus (Isaria fumosoroseus)	Whiteflies/Thrips in Glasshouse crops	PFR-97^{TM}
	Mites in wide range of crops	Prioroty
	Whiteflies in chilli, tomato	Pae-Sin, Bemisin
Hirsutella thompsonii	Mites, *Aphis craccivora* Koch in Citrus, Cowpea	Mycohit
Nomuraea rileyi	Lepidoptera of several crops	Numoraea 50
M. anisopliae + B. bassiana + P. fumosoroseus	Psyllid in citrus and other crops	Tri-Sin
Lagenidium giganteum	Larvae of most pest mosquito species	Laginex
Conidiobolus thromboides	Thrips, Aphid, Whiteflies in several crops	Vector 25 SL

Source: Usta (2013)

Bacterial pathogens as bio-pesticides:

Bacillus thuringiensis (Bt), *Bacillus thuringiensis* var. *kurstaki* is the most commonly used and commercially most successful bio-pesticide. It is primarily a pythogen of most damaging second instar larvae of lepidopteran pests such as American bollworm in cotton, caster semi looper, tobacco caterpillar, diamond black moth on cruciferous vegetables, rice stem borer and *Heliothis armigera* on various crops. Bt is an aerobic spore-forming bacterium off Coccobacilli group and its insecticidal property is due to crystals of insecticidal proteins produced during sporulation. When ingested by larvae

Bt releases toxins (delta-endotoxin) which damage the midgut of the pest causing septicaemia and death within 2 to 3 days. It is also safe to humans. General recommended dose of spraying is 1-1.5 ml/g per litre of water. It acts very well during afternoon hours and in cold climate. Several strains of Bt have been isolated and are now mass produced and sold as pest control agents. Each strain has different host specificity. *Bacillus popilliae:* cause milky disease (causing turbidity of body fluid) in the larvae of chafer grubs and kill. *Bacillus subtilis:* is an excellent antagonist for management of soil borne diseases of cotton. *Pseudomonas fluorescens:* this bio-fungicide controls the pathogen by substrate competition for nutrient available in the soil and phyllosphere. It can be used against *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Botrytis cinerea* etc. of different agriculture and horticultural crops (Table 2). It is commercially available as a talc besed WP product which is widely used as a seed treatment at the rate of 4 gram per kg seed and soil application @ 2 to 3 kg per hectare along with any organic manure before sowing and against the seed borne and soil borne diseases and also as foliar spray @ 10 gram per litre of water for rice blast, sheath blight, sheath rot brown spot and glume discussion management. It is also comfortable with fungicides and *Trichoderma viride*. A commercial formulation of *Trichoderma viride* + *Pseudomonas fluorescence* is also available in the market for soil application to manage soil borne pathogen causing seedling diseases.

Table 2: Commercial Entomopathogenic bacterial formulations and target pest management

Bacterial formulation	**Products name**	**Target pests**
Bacillus thuringiensis subsp. *israelensis* 5% WP, 5% AS	Tacibio/Vectobac®, Teknar®, Bactimos®, Skeetal®, and Mosquito attack®, VectoBac, Vectoba	Lepidopterous pests/Diptera pests/ Mosquito and black flies, house fly, stable fly or blow fly
B. thuringiensis subsp. *kurstaki* 5% WP, 7.5% WP	Bio-Dart/ Biolep/Halt/Taciobio-Btk, Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer®, Bactospeine® and SOK-Bt® Biobit, Cordalene, Costar-WG, Crymax-WDG, Deliver, Foray, Javelin-WG, Lepinox Plus, Lipel, Rapax	Lepidopteran pests/American ball worm in cotton, caster semilooper, diamond back moth on cruciferous vegetables, rice stem borer, *Heliothis armigera* on various crops
B. thuringiensis subsp. *Kurstaki* plus beta-exotoxin	Javelin	Armyworm and other moths
Bacillus thuringiensis subsp. *sphaericus* 1.3% FC	VectoLex, VectoMax	Diptera/Mosquito larvae
Bacillus thuringiensis subsp. *galleriae*	VectoLex, Spicturin	Lepidopteran larvae /Mosquito larvae, bollworms, Diamondback moth
Bacillus thuringiensis sub-species *aizawai*	Able-WG, Agree-WP, Florbac, XenTari, Certan, Agree	Lepidoptera/Wax moth larvae, Armyworms, diamondback moth
Bacillus thuringiensis sub-species *tenebrionsis*	M-One®, Novodor, Trident	Coleoptera/Potato beetle and cotton ball weevil
Paenibacillus popilliae and *B. lentimorbus*	Doom®, Japidemic®, Grub attack®	Japanese beetle *Popillia japonica*

Bacillus moritai	-	Diptera
Bacillus popilliae	-	Coleoptera
Pseudomonas fumosoroseus 0.5%, 1% WP	Nemato-Guard, Bio-Dart	Whitefly
Pseudomonas lilacinus	Yorker/ABTEC/Paceilomyces/Paecil /Pacihit/R OM biomite/Bio-Nematon	Whitefly
Saccharopolyspora spinosa	Tracer™ 120, Conserve	Insects
Chromobacterium subtsugae	Grandevo	Chewing and sucking insects and mites
Pseudomanas fluorescens	Plant Bio-control Agent-3, Conquer, Bioshield, Frostban, Bio-jet, Spot less,	Bacterial leaf blight, sheath blight and sheath rot in rice, Take-all disease in wheat & barley, Vascular wilt in raye, grey rot in caster, root rot & damping off in vegetable,
Bacillus subtilis	GB 34, Kodiac companion, Serenade, Avo Green, Bio-safe, Subtilex/Pro-Mix, Biotok, Larminar, Rhapsody, Subtilex	Wilt in cotton, cucumber, pigeon pea, chickpea, seed rot & root rot in chickpea, Sigatoka in banana,
Agrobacterium radiobacter K84	Galtrol, Dygall, Nagol, Nogall	Crown gall disease in stone fruits,

Source: Usta (2013)

Viruses as bio-pesticides:

Baculoviruses (BV) are frequently utilized as viral biopesticides. They are extremely harmful to insects and other arthropods. Baculoviruses are particularly effective against the lepidoptoran pests of cotton, rice and vegetables. Baculoviruses are new options for integrated pest management through the use of naturally occurring disease caused by viruses. BV are small, double-stranded DNA viruses that parasitically replicate minuscule components. Baculoviruses have three subgroups: nuclear polyhedrosis viruses (NPVs), cytoplasmic polyhedrosis viruses (CPVs), and granulosis viruses (GVs). They are classified as inclusion viruses, distinguished by the number of occlusion bodies and the shape of the protective protein coat. NPV and CPV create polyhedral entities made up of many viral particles. GV creates granular occlusion bodies that contain only one viral particle. Baculoviruses have unique processes for NPV, CPV, and GV virus uncoating. To infect the host, NPVs uncoat in the nucleus, CPVs uncoat in the cytoplasm, and GVs uncoat within the nuclear pore complex. The occlusion bodies allow the virus live outside of the host (Rajamani and Negi, 2021).

Viruses act similarly to bacteria and must be swallowed by insect larvae to cause infection. Immediately after ingestion, the virus enters the insect's body via the intestinal epithelium and infects the host cells. NPV travels past the intestinal epithelium to hemocoel, fat body, and other tissue cells, disintegrating the tissues and liquefying the cadavers. In the case of GV, infection is restricted to the insect midgut (Cory, 2000). The NPV and GV are most popular in recent years and farmers are using them as bio-pesticide in several crops as baculoviruses have no deleterious effect on useful insects, pollinators and non-target organism and human beings. NPV is used for management of Helicoverpa (HaNPV) and Spodoptera (SiNPV). GV is used against *Cydia pomonella* on apple, *Pthorimaea* *operculella* on potato and *Plutella xylostella* on cabbage. General recommended odse of spraying of NPV and BV is @ 1 ml/litre of water (i.e. 250 LE/ha; LE= Larval equivalent; 1 LE= 6 x 10^9 PIB; PIB= Polyhedral inclusion bodies). NPV and GV act as a true stomach poison. The effective lethal concentration (LC_{50}) of NPV is 1 x 10^9 /mL for *H. armigera* larvae. For gram, pigeon pea and cotton HNPV at 250–300 LE, 500 LE and 250 LE/ha should be used. Viruses are very host-specific and can cause significant reduction in host populations. Examples of some commercially available viruses include *Helicoverpa zea* single-enveloped Nuclear polyhedrosis virus (HzSNVP), *Spodoptera exigua* multi-enveloped nuclear polyhedrosis virus (SeMNPV) and *Cydia pomonella* granulovirus (CpGV) (Rajamani and Negi, 2021). The list of commercially available viral bio-pesticides are depicted in table 3.

Table 3: List of commercially available viral bio-pesticides

Target pest	**Host**	**Products available**
NPV of *H. armigera* (HaNPV)	*Helicoverpa zea H. armigera*	Biokill-H, BioVirus-H, Heli-Cide, Heliokill, Helimar-NPV, Helivax, Jas Viro–H, Helicop, Heligard, Somstar-Ha
NPV of *S. litura* (SlNPV)	*Spodoptera litura, S. exigua*	BioVirus-S, Jas Viro–S, Spodo-Cide, Spodopterin, Somsta-SL
Nuclear polyhedrosis for Gypsy moth	Gypsy moth caterpillars	Gypchek virus
Tussock moth NPV	Tussock moth caterpillars	TM Biocontrol-1
Pine sawfly NPV	Larvae of pine sawfly	Neochek-S
Granulosis virus for Codling moth (GV)	Codling moth caterpillars	Madex, Carpovirusine, CYD-X

Source: Usta (2013)

Protozoans as bio-pesticides:

Protozoans are sometimes called microsporidians. Microsporidia are ubiquitous obligate intracellular parasites that can attack lepidopteran and orthopteran insects and hence can be utilized as part of an integrated pest control program, such as *Nosema* sp. and *Vairimorpha* sp. Although protozoa are pest-specific, slow-acting, and have chronic and debilitating effects on targets, their usage as bio-pesticides is less effective than that of other species such as bacteria, viruses, and fungi. One notable exception is *Nosema locustae*, a microsporidian that has been mass-produced and marketed for management of grasshoppers. Microsporidia must be eaten by insects to get infected. Microsporidian infects European corn borer, *Ostrinia nubilalis*. The spores germinate in the midgut region and the sporoplasm is injected into the midgut cells (Rajamani and Negi, 2021). The spores spread to different tissues and organs, where they multiply resulting in tissue breakdown and septicaemia (Senthil-Nathan 2015). The list of commercially available viral bio-pesticides are depicted in table 4.

Table 4: Protozoa based successful commercial bio-pesticides

Protozoan	**Host Range**	**Products**
Nosema locustae	European corn borer caterpillars, grasshoppers and Mormon crickets	NOLO Bait, Grasshopper attack

Source: Usta (2013)

Entomopathogenic Nematodes (EPN) as bio-pesticides:

The word "entomopathogenic nematodes" (EPNs) is derived from the Greek vocables, 'Εντομος' (entomos, 'insect') 'πάθη' (pathê, 'disease') and 'γ΄ενος' (guenos, 'producing'), means a group of nematodes which have the ability to cause disease in insects by suppressing the immune system of insects. Entomopathogenic nematodes are soft-bodied, unsegmented roundworms that live in water film around soil particles. Nematodes are obligatory or sometimes facultative parasites that seek host insects using carbon dioxide, vibration, and other chemical cues. In commercial pest management programs, two families, Steinernematidae and Heterorhabditidae, have been successfully identified and employed as bionematicides. (Kachhawa 2017). *S. carpocapsae, S. thermophilum, H. bacteriophora, H. indica* products are registered and sold in India.

Only the third stage of EPNs, known as the infective juvenile (IJ) of both nematodes i.e. *Steinernema* and *Heterorhabditis*, is considered infective and pathogenic. *Xenorhabdus* and *Photorhabdus* are entomopathogenic bacteria (EPB) vectored by *Steinernema* and *Heterorhabditis* genera of EPNs, respectively. The sole free-living stage of EPNs is infective juveniles, while other developmental stages can only be seen inside infected insect hosts. Infective juveniles enter the homocoel of the host insect by natural apertures like as spiracles, mouth, and anus, or in certain species through inter-segmental membranes of the cuticle. In addition to natural holes, *Heterorhabditis* species can enter the insect host by abrading the skin. The infective juveniles enter the epidermis and release symbiotic bacterial cells from their alimentary canal (intestine) into the hemocoel. Apart from natural holes, *Heterorhabditis* species can also enter the insect host by abrading the skin. After piercing the epidermis, the IJs discharge symbiotic bacteria cells from their alimentary canal into the hemocoel. The bacteria proliferate in the insect hemolymph, release poisons and specific immune depressors, and the insect's immune system is suppressed, resulting in death within 24-48 hours. *Photorhabdus* and *Xenorhabdus*, two well-known bacterial symbionts of EPNs, are not only toxic to entomic fauna, but they also inhibit opportunistic bacteria and fungi from using the nutrient-rich carcass, effectively trapping (Rajamani and Negi, 2021). The list of commercially available viral bio-pesticides are depicted in table 5.

Table 5: List of commercially available EPN based bio-pesticides

Entomopathogenic nematodes	**Host range**	**Commercial products**
H. bacteriophora	Effective against root weevils, cutworms, fleas, borers and fungal gnats. Effective against black vine weevil populations by 56–100%	BioSafe, Larvanem, Nemaplant, NemaShield-HB, Nematop, Nematech-H NemaTrident-H, NemaTrident-C, Nema-green, Optinem-H
H. indica	*H. armigera, Conogethes punctiferalis, Athalia proxima*	Soldier, Nema power, GrubTerminator, Grubcure, Calterm, Aarmour
H. downesi	Black vine weevil	NemaTrident-CT

Steinernema feltiae, S. riobravis	Effective against black vine weevils, strawberry root weevils, cutworms, cranberry girdler and termites	Biosafe, Ecomask, Hortscan, Guardian, Millenium, Nematac C, NoFlea, Savior WG, Scanmask, Termask, Vector
S. carpocapsae	Borer beetles, caterpillars, crane fly, moth larvae, coconut rhinoceros beetle	Capsanem, Carpocapsae-system, Exhibitline SC, Optinem-C, NemaGard, Nemastar, NemaTrident-T, NemaRed, Nemasys-C, Palma-life

Source: Koul (2011), Gupta *et al.* (2010), Ruiu (2018)

B. Biochemical/Botanical pesticides:

Plant-derived compounds, sometimes known as phytochemicals or herbal insecticides. Each phytochemical has a distinct structure and serves multiple functions in plants, including protection, growth acceleration, and reproduction (Huang *et al.* 2014). Phytochemicals are found in a variety of plant parts, including fruits, vegetables, grains, legumes, nuts, seeds, and bark. It has many bioactive chemicals with antiparasitic, bactericidal, fungicidal, viricidal, and insecticidal effects, making it a possible alternative to inorganic pesticides. Terpenes contain phyto-volatiles and glycosides, whereas sterols contain phenolic components such as phenolic acids, lignin, tannins, and alkaloids. Secondary metabolites are essential components of the plant's insect defence system. It functions as a toxin, insect growth regulator, repellent, and antifeedant (Mossa, 2016). Phytochemicals from plants can be extracted via solvent extraction, microwave-assisted extraction, or ultrasonic-assisted extraction depending on the presence of a certain set of phytochemicals (Altemimi *et al.* 2017). Currently, only a few phytochemicals are employed as bio-pesticides, with the majority of them being neem-based. These are primarily 3% neem oil or 5% neem seed kernel extract concentrations that can be sprayed at a rate of 25 kg/ha to control important agricultural pests (Rajamani and Negi, 2021). The list of commercially available viral bio-pesticides are depicted in table 6.

Herbal pesticides function by absorption through the insect cuticle, inhalation, or ingestion, affecting the insects' physiology, metabolic pathway, or neurological system. It interferes with insect respiration by obstructing spiracles, causing asphyxia. At the cellular level, it will disrupt the ion channel and ion pump of the insect membrane. It also affects insects' neuroendocrine systems and causes juvenile hormonal imbalances, culminating in insect deformity. Other impacts include a reduction in adult mating time, ovulation, fecundity, and egg fertility. Monoterpenes included in essential oils operate as neurotoxins, inhibiting the acetylcholinesterase enzyme involved in nerve impulse transmission in insects, resulting in paralysis and death. It also hinders DNA, RNA, and protein synthesis (Rajamani and Negi, 2021).

Semiochemicals are chemical molecules created by organisms that facilitate and mediate communication among them. Semiochemicals are divided into two types: intraspecific (pheromones) and interspecific (allelochemicals). Pheromones are volatile chemical molecules produced by an organism to elicit a reaction or response from another member of the same species. The releaser pheromones induce the recipient's conduct to change. Pest management makes use of synthetic sex pheromone mimics. In fact, sex pheromones are employed to monitor pests, disrupt mating, and mass trap (Rajamani and Negi, 2021).

Table 6: List of commercially available biochemical pesticides

Plant source	**Target pest**	**Commercial name/compounds**

Azadirachta spp., *Nicotiana* spp.	Aphis craccivora, Bollworms, Aphids, Jassids, Thrips, Whitefly, Leaf folder, Pod borer, Fruit borer, Leafhopper, Diamondback moth	Essential oils, nicotine, azadirachtin, salanin, nimbin, (leaf, seed, kernel) nimbecidin, bionimbecidin
Chrysanthemum cinerariaefolium Lonchocarpus spp. *Ryania* spp.	Crawling and flying insects such as cockroaches, ants, mosquitoes, termites	Pyrethrium, Rotenone Ryanodine
Artemisia annua	*Helicoverpa armigera*	Essential oils from leaf and seed
Vinca rosea	*Helicoverpa armigera*	Essential oils from leaf and seed
Ocimum basilicum Salvia Officinalis	*Aphis craccivora* Koch, *Agrotis ipsilon*	Essential oils form aerial parts leaves
Citrus sp.	Fleas, aphids, mites, paper wasp, house cricket	D-limonene linalool
Schoenocaulon officinale	Bugs, blister beetles fly, caterpillars, potato leafhopper	Sabadilla dust
Adenium obesum	Cotton pests	Chacals Baobab (Senegal)

Source: Rajamani and Negi, 2021.

C. Plant Incorporated Protectants (PIPs):

Plants having toxin-producing genes to combat pests are known as plant-incorporated protectants, genetically modified, or genetically engineered plants. These PIPs aid plants in resisting bacteria, viruses, and fungi. pest-resistant crops are those that use PIPs to control a specific pest. Some of the examples of PIPs are soybeans, brinjal, cotton, corn, potatoes, and tomatoes. Transgenic plants are created by transferring the target gene via *Agrobacterium*-mediated transfer, gene gun or ballistic techniques. These approaches were employed on wheat, maize, rice, and corn (Rajamani and Negi, 2021).

Plant-incorporated protectants have various advantages, including higher yields with fewer pesticide application. As a result, they are cost-effective and preserve neighboring non-Bt crops. Some possible disadvantages are also connected with plant-incorporated protectants, including as the effects on non-target pests, the risk to human health and the environment, the likelihood of the PIP gene spreading to other plants, and the development of pesticide or insect resistance (Rajamani and Negi, 2021). The list of commercially available viral bio-pesticides are depicted in table 7.

Table 7: List of plant-incorporated protectants against crop pests

Plant	**Gene incorporated**	**Resistance to insect**	**Commercial name**
Cotton	Cry 1Ac toxins from *Bacillus thuringiensis*	Cotton and tobacco bollworm	Bollgard

Maize	Cry 1Ab,1F,9c toxins from *Bacillus thuringiensis*	European corn borer	Yieldgard, Knockout, Herculex I, Starlink, Maximizer
Rice	Cry1C from *Bacillus thuringiensis*	Leaf folders (Cnaphalocrocis medinalis) and stem borers	-
Tobacco	QB protein of photosystem II from mutant Amaranthus	Atrazine resistant	
Brinjal	Cry1Ac from the soil	Shoot and fruit borer, *Leucinodes orbonalis*	Bt Brinjal
Tomato	Cry1Ab gene of *Bacillus thuringiensis*	Second instar larvae of *H. armigera* and *S. litura*	Bt tomato

Rajamani and Negi, 2021.

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Application and recent trends of biotechnology in sustainable agriculture: A brief review

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Abstract

India has emerged as one of the world's fastest growing economies. Although agriculture and other sectors employ about 52% of the Indian population, the majority of farmers still rely on subsistence farming with average annual incomes below poverty levels. Despite the fact that we have achieved food self-sufficiency following the green revolution, the condition of small and marginal farmers remains unchanged. Climate change, biotic and abiotic pressures, tiny landholdings, land degradation, low market prices for products, and a lack of low input-high output technologies are the key difficulties we must address in order to boost farmer income. The use of innovative technologies is one of the most effective strategies for raising crop output and farmer revenue. Biotechnology is one of the most appealing crop improvement methods, and it is the most rapidly adopted technology worldwide. Plant tissue culture, molecular breeding, transgenic development, and animal cloning are all key methods in biotechnology. It has improved yield, tolerance to stress, quality, and introduced new features, such as bio-plastic manufacturing. Biotechnology in agriculture has increased farmer income and agricultural sustainability in both developed and developing countries. It has multiple applications, including crop development, livestock enhancement, and fisheries. To improve farmers' lifestyles and the status of Indian agriculture while preserving international standards of food quality and nutritional value, it is vital to promote the use of biotechnology in agriculture and related fields. The article begins by defining sustainability and highlights the importance of biotechnology in establishing sustainable agriculture. The goal of sustainable agriculture is to produce enough food and fibre to meet present demands while preserving and improving natural resources for future generations.

Key words: Agricultural biotechnology, crop and pests resistance, genetically modified crops

Introduction

Global population growth has become a major issue for food security. Population growth in developing countries requires a 70% increase in food production by 2050, emphasizing the importance of increasing agricultural productivity in the next decades (Chekol and Gebreyohannes, 2018). Biotechnology has primarily focused on developing technologies to boost crop production. Biotechnology is the use of scientific procedures to modify and improve plants, animals, and microbes to increase their value. Biotechnology, in the form of genetic engineering, is a branch of science that has the potential to deliver significant benefits if utilized responsibly and ethically. Society should be given a comprehensive understanding of the principles of biotechnology and genetic engineering, as well as the techniques employed in generating transgenic organisms, the sorts of genetic material used, and the new technology's benefits and risks. Agricultural biotechnology is the field of biotechnology that applies to agriculture. Modern biotechnology represents novel scientific applications that can be used to benefit society by developing crops with enhanced nutritional quality, resistance to pests and diseases, and lower production costs. In agriculture, biotechnology has become a powerful tool that has altered food production and increased crop yields. Biotechnology is transforming animal feeding techniques to increase nutrition and reduce environmental waste (James, 2002). Agricultural biotechnology can benefit the poor (Tanaka, *et al.,* 2010), but it is also employed for disease diagnosis and animal vaccine development. Cereal starch is commonly used in value-added biopolymers and biofuels, which are less environmentally hazardous than petrochemicals (Thitisaksakul *et al.,* 2012 and Kumar *et al.,* 2020).

The word "sustainable" comes from the verb "sustain," which means to maintain, uphold, and endure. The goal of sustainable agriculture is to satisfy society's needs for food and fiber while preserving the environment and natural processes. To create sustainable, climate-resilient agriculture, social and economic equality must be combined with economic prosperity and a healthy environment. With the potential to boost productivity, biotic and abiotic resistance, and nutrient-rich crops, biotechnology has become a promising technique in agricultural enhancement. Developing climate-resilient agriculture and combining social and economic equality with a healthy environment and financial success are the main prerequisites for sustainable agriculture. Economic profitability, environmental stewardship, and social responsibility must all be balanced in order to achieve agricultural sustainability. Particularly when dealing with biotic and abiotic stresses including pests, diseases, climate change, soil erosion, and water scarcity, this can be challenging. These problems can be solved by using biotechnology to produce crops that are resistant to diseases and pests. Lack of nutrients in the soil can lower agricultural production and plant health, which is another barrier to sustainable agriculture. More productive and nutrient-dense crops could be developed with the help of biotechnology. At the same time, though, it is crucial to make sure that new technologies are developed responsibly and that regions and people equally benefit from them. This article summarizes biotechnology's multiple applications in agriculture, highlighting its significant contributions to crop development, pest and disease management, and sustainable farming practices (Das *et al.,* 2023).

Historical background of agricultural biotechnology

Biotechnology has its origins in ancient times, when people started modifying biological systems for useful purposes. Around 10,000 years ago, early farmers domesticated plants and animals by selecting breeding them for desired qualities like improved flavor and larger yields. This set the stage for current plant breeding. By establishing the laws of inheritance through his studies with pea plants, he discovered the concepts of heredity, which became the scientific foundation for biotechnology. Advanced breeding methods were made possible by this genetic foundation (Smykal *et al.,* 2016). The production of food and beverages advanced as a result of Louis Pasteur's work on fermentation, which revealed the microbiological underpinning of this process (Katz, 2012). The 1928 discovery of penicillin transformed medicine and demonstrated the biotechnological potential of microbes (Kingston, 2000). When molecular biology emerged in the middle of the 20th century, the structure of DNA was discovered, laying the groundwork for genetic engineering. Genetically modified organisms (GMOs) were made possible by the advancement of recombinant DNA technology in the 1970s, revolutionizing agricultural science. The term "genetically modified organism" or "GMO" is commonly used, however genetic manipulation has been practiced for centuries. Crossing one variety with another results in offspring that are genetically modified relative to their parents. Foods developed from transgenic plants are referred to as "GMO foods," "GMPs" (genetically modified products), or "biotech foods." Genetically engineered foods might be referred to as "biotechnology-enhanced foods" or "frankenfoods” (Wieczorek, 2003).

Agriculture's Biotechnology Evolution

Developments in genetics, molecular biology, and plant breeding methods have propelled a substantial evolution in biotechnology in agriculture. Mendelian genetics improved conventional plant breeding techniques, resulting in hybrid crops that showed better resistance to pests and diseases and had larger yields. The introduction of high-yielding rice and wheat varieties during the Green Revolution greatly increased food production and prevented famines in emerging nations (Shiferaw *et al.,* 2013). Direct manipulation of plant genomes became possible with the introduction of genetic engineering in the 1970s and 1980s. Early in the 1980s, tobacco and petunia were among the first plants to be genetically modified. The commercialization of genetically modified crops began in the 1990s, with the FlavrSavr tomato bred for longer shelf life, followed by herbicide-resistant soybeans and insect-resistant Bt cotton, both of which soon gained popularity due to their agronomic benefits. The introduction of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 gene editing technology in the early twenty-first century transformed agricultural biotechnology by allowing for precise genome editing, which improved features such as nutritional content, pest resistance, and tolerance to environmental stress. Modern plant breeding approaches, such as marker-assisted selection (MAS) and genomic selection, use molecular markers to discover and select desired features, resulting in faster crop development and improved breeding efficiency. Tissue culture and micro-propagation techniques enable rapid replication of disease-free planting material while conserving genetic resources (Tegen and Mohammed, 2016).

Milestones in Indian Agricultural Biotechnology

India has a long history of agricultural innovation, and it was an early adopter of agricultural biotechnology. The creation of the Indian Agricultural Research Institute was a key milestone, as it developed and disseminated high-yielding wheat and rice varieties during the Green Revolution (Zeigler and Mohanty, 2010). Another significant milestone was the commercialization of Bt cotton in 2002, when it became the first GM crop in India. Bt cotton, resistant to the bollworm insect, enhanced yields, reduced pesticide use, and raised farmer profitability. By 2018, it covered over 90% of the cotton-growing area. While Bt cotton has been successful, other GM crops, such as Bt brinjal and GM mustard, have suffered regulatory and popular acceptance issues. Bt brinjal has not been commercialized because to biosafety issues and public opposition, while GM mustard has been pending approval since 2016. India has made great advances in biotechnology, including increasing the use of bio-fertilizers and insecticides to improve soil fertility and reduce chemical inputs. The National Organic Farming Project promotes the use of bio-fertilizers and organic farming practices. The Indian Council of Agricultural Research (ICAR) has created superior crop varieties using MAS and genomic selection, thereby speeding up the breeding process and increasing attributes such as yield and disease resistance (Chakraborti *et al.,* 2021). The Department of Biotechnology (DBT), as well as institutes such as the National Agri-Food Biotechnology Institute (NABI) and the Biotechnology Industry Research Assistance Council (BIRAC), have bolstered India's agricultural biotechnology research and innovation capabilities. Recent research focuses on using CRISPR-Cas9 technology to improve crop features such as disease resistance and nutritional value. In India, the Genetic Engineering Appraisal Committee (GEAC) oversees agricultural biotechnology regulations, assuring the safety of genetically modified crops through risk assessments, field experiments, and public participation. Public opinion and acceptance, regulatory barriers, and approval process delays are all significant challenges. Addressing these needs efficient communication, transparent regulatory processes, and rigorous biosafety assessments (Cornish *et al.,* 2021, Kumar *et al.,* 2024).

Advancements in Agricultural Biotechnology

Genetically modified (GM) crops are a ground breaking advancement in agricultural science. Crops are developed with specific features that improve growth, yield, and resilience. The method involves adding genes from diverse species into the target crop's genome to improve traits such as pest resistance, herbicide tolerance, and nutritional content. The first genetically modified crops were introduced in the mid-1990s, and their acceptance has since risen tremendously around the world. Soybean, maize, cotton, and canola are the most frequently produced genetically modified crops, with features such as herbicide tolerance and insect resistance built in. Herbicide-tolerant crops, such as

Roundup Ready soybeans and maize, enable farmers to control weeds more effectively while minimizing crop damage, resulting in better weed management methods and reduced tillage, which enhances soil health. *Bacillus thuringiensis* genes are found in insect-resistant crops like Bt cotton and Bt maize. These crops produce proteins that are harmful to particular insect pests, lowering the need for chemical insecticides and safeguarding beneficial insect populations. The safety of genetically modified crops for human consumption and the environment is guaranteed by extensive study and regulatory inspection; multiple studies have confirmed that GM crops are just as safe as their conventional counterparts and provide environmental benefits like decreased greenhouse gas emissions and pesticide use. Bt cotton adoption has resulted in significant production gains and reduced pesticide use. According to studies, Bt cotton growers achieve 30-60% higher yields than non-Bt cotton farmers, with a 40-70% reduction in insecticide applications. Smallholder farmers saw a 24% rise in cotton income as a result of these benefits, leading to higher profitability and improved rural livelihoods. Despite the success of Bt cotton, commercialization of other GM crops in India, like as Bt brinjal and GM mustard, has experienced regulatory and public acceptability problems due to biosafety concerns and opposition. Building trust in genetically modified (GM) technology involves transparent and science-based regulatory systems, as well as effective communication with stakeholders (Kumar *et al.,* 2024).

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology is a ground breaking genetic engineering tool that enables precise genome editing (Loureiro and Silva, 2019). The CRISPR-Cas9 system, derived from bacterial immunity, employs a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, resulting in the addition, removal, or change of genetic material. This approach has advantages over traditional genetic editing procedures, including increased precision, efficiency, and versatility. CRISPR can be used to create crop types with better features such as increased yield, higher nutritional content, resistance to pests and diseases, and tolerance to environmental challenges (Jaganathan *et al.,* 2018). CRISPR technology has great potential for Indian agriculture, addressing issues such as food security, climate change, and sustainability. CRISPR has been utilized by Indian researchers to generate disease-resistant agricultural varieties, such as tomato plants resistant to the tomato yellow leaf curl virus, as well as rice and wheat with improved fungal disease resistance. CRISPR technology is being utilized to enhance agricultural nutrition, including bio-fortified rice with added iron and zinc to treat micronutrient inadequacies (Kumar *et al.,* 2019). Investing in research and capacity building for scientists and farmers is crucial for realizing CRISPR's potential in improving Indian agriculture (Munawar *et al.,* 2024).

Marker-assisted selection (MAS) is a molecular breeding strategy that uses DNA markers to identify and select plants with desirable features early in the breeding process. MAS is commonly used to create crop varieties with higher yields, resistance to diseases and pests, and tolerance to abiotic conditions including drought and salinity. In India, MAS has been used to create multiple high-yielding and disease-resistant crop types, including rice cultivars resistant to bacterial blight and blast, as well as wheat varieties immune to rust disease. MAS has also been used to boost the nutritional value of crops, such as bio-fortified pearl millet with high amounts of iron and zinc, so treating micronutrient inadequacies (Srivastava, *et al.,* 2021). Genomic selection (GS) is an advanced molecular breeding strategy that utilizes genome-wide markers to predict breeding line performance. GS uses high-density markers distributed across the genome to estimate breeding value. This enables for more precise selection of plants with complex properties regulated by numerous genes, such as yield, drought tolerance, and disease resistance. In India, GS has been used on major crops including as rice, wheat, and maize. Collaborative work with institutes such as the International Rice Research Institute have employed GS to generate high-yielding rice cultivars that are more resilient to environmental challenges (Pathak *et al.,* 2018).

Bio-fertilizers and bio-pesticides are crucial components of sustainable agriculture, providing ecologically benign alternatives to chemical fertilizers and insecticides. Bio-fertilizers use microorganisms to improve plant nutrient availability, whereas bio-pesticides use natural materials such as plants, bacteria, and minerals to promote soil health, reduce pollution, and increase crop yields (Kawalekar, 2013). Bio-fertilizers such as Rhizobium, Azotobacter, Azospirillum, and phosphate-solubilizing bacteria (PSB) are frequently utilized in diverse cropping systems to improve nitrogen availability and phosphorus nutrition. Bio-pesticides, such as neem-based solutions and *Bacillus thuringiensis* (Bt) formulations, successfully manage a variety of pests while lowering the need for chemical pesticides. Bio-fertilizers boost nutrient availability and uptake, resulting in better plant growth and yields. Rhizobium inoculants can boost nitrogen fixation and yields by 20-30%, whereas PSB enhances phosphorus availability and crop yields. Bio-pesticides help to achieve sustainable pest management by reducing the demand for chemical pesticides, lowering environmental impact, and increasing yields (Khan *et al,* 2022).

Biotechnology application for sustainable agriculture

Agricultural biotechnology is a major component of the research instruments that scientists employ to comprehend and modify the genetic composition of organisms for use in crops, forestry, fisheries and livestock. Micro-propagation, tissue culture, cloning, artificial insemination, embryo transfer, markers-assisted selection, genomics and bioinformatics, and other technologies are all part of the broad range of applications that biotechnology offers beyond genetic engineering. Scientists can use a variety of methods in modern agricultural biotechnology to comprehend and modify an organism's genetic composition for use in the production or processing of agricultural products. Problems in every aspect of agriculture production and processing are being solved by biotechnology.

Today's agricultural scientists face several challenges, including the world's population growth, the depletion of natural resources, and the loss of arable land, climate change, and environmental degradation. Biotechnology will offer alternatives to existing strategies for enhancing the agricultural system and environment. Biotechnology has the potential to decrease the use of pesticides and fertilizers in the current agricultural production system. The quality of the soil, air, and water can be enhanced by using fewer inorganic fertilizers and pesticides. Biotechnology can be an effective strategic approach to developing different high-yielding and stress-tolerant crop varieties. The main goal of recent developments in biotechnology research has been to clarify the molecular principles underlying various metabolic processes and use this understanding to increase crop and animal productivity. Gene transfer by genetic engineering is faster and more accurate than traditional crossbreeding. Herbicide, pest, and disease-resistant crop varieties can be created by genetic alteration, which can also improve the nutritional profile of crops. Agricultural productivity is severely hampered by abiotic stress, and farmers can make use of previously unsuitable land by cultivating plants with stress tolerance traits including resistance to cold, drought, and salinity. The use of scientific instruments and methods, including genetic engineering, molecular biology, and micro-propagation, to alter plants, animals, and microbes is known as agricultural biotechnology. The development of sustainable agriculture may be aided by agricultural biotechnology in addressing key issues like producing enough food in a limited amount of space (loss of usable lands), with limited resources (water scarcity), under various environmental stresses (high temperatures, salinity, and drought), and with fewer synthetic fertilizers and pesticides.

Biotechnology also has the ability to improve soil quality through phytoremediation, in addition to crop production. By creating hardier crops that can survive in challenging conditions with little fuel, labour, fertilizer, or water input, biotechnology can also help conserve natural resources, improve plant nutrient uptake, decrease nutrient runoff, increase soil organic carbon sequestration, and satisfy rising food and land demands. Crops resistant to severe illnesses have been developed thanks to the application of biotechnology in agriculture. Biotechnology is essential to sustainable agriculture because it makes it possible to create crops with improved qualities like drought tolerance, pest resistance, and nutritional value. This results in higher crop yields, less use of pesticides, and ultimately more financial success for farmers while having a smaller negative impact on the environment. Recent advances in biotechnology have made it possible to create molecular tools that can be used to detect important qualities like higher yield, resistance to pests, drought, and herbicides. These traits can then be modified in crops to provide the desired results. Das *et al.,* 2023 reported different biotic and abiotic stress resistant crops i.e. insect resistant plants-poplar 741, virus resistant- plant Rainbow papaya, SunUP papaya, herbicide resistance plant-Petunia hybrida (glyphosate tolerant), drought tolerant plant-apple, tomato, potato, salt tolerant plant- Apple, tomato, biofortified- rice, maize, potato and high yielding-maize. Biotechnology also holds promise for the development of novel medicines using genetically altered crops (plant-based vaccines and antibodies), resulting in a new sustainable plant-based pharmaceutical industry with lower production costs.

1. **Impact on Agricultural Productivity**

Yield improvements

The use of agricultural biotechnology has resulted in significant yield increases across a variety of crops. Transgenic crops with improved photosynthetic efficiency, lower height, greater harvest index, and better nutrient utilization can increase agricultural output potential. New genome technology and bioinformatics tools will transform Indian agriculture, improving photosynthetic efficiency and agricultural yields. A significant example is India's adoption of genetically modified (GM) Bt cotton. *Bacillus thuringiensis*-derived genes in Bt cotton produce proteins that are poisonous to specific insect pests. Since its launch in 2002, it has been widely accepted by Indian farmers. Numerous studies have shown that Bt cotton producers can increase their yields by 30% to 60% when compared to non-Bt cotton farmers. Controlling the bollworm pest has greatly reduced crop damage, leading to increased yields. A study studied data from 1,500 cotton farmers in four main cotton-growing states in India and found that adopting Bt cotton resulted in a 24% increase in yields and 50% increase in revenues. Aside from cotton, other GM crops, such as GM maize, have seen considerable yield improvements. A meta-analysis of 76 research on GM maize revealed a 25% increase in yield compared to conventional maize. GM crops provide much higher yields compared to traditional farming practices that use conventional breeding and chemicals (Kumar *et al.,* 2024). Pesticide reduction also has environmental benefits, such as lowering the chemical load on ecosystems and encouraging beneficial insect populations (Boudh and Singh, 2019). Similarly, GM maize with insect resistance and herbicide tolerance addresses pest infestation and weed competition, resulting in higher and more stable yields, demonstrating biotechnology's potential to overcome key limitations of traditional farming and contribute to sustainable agricultural productivity. A recent study found that overexpressing the gene OsDREB1C can dramatically enhance rice production by 41.3% to 68.3% when compared to wild types (Wei *et al.,* 2022). Transgenic plants have made a substantial contribution to yield increases. For example, one study found that overexpressing the zmm28 gene resulted in increased maize production with no detrimental effects (Wu *et al.* 2019). Another study discovered that a transgenic wheat line had a 6% higher yield and 9.4% higher water use efficiency than its control under stress (Gonza´lez *et al.,* 2019).

Nutrient use efficiency improvement

Nutrient usage efficiency (NUE) is defined as the ratio of production outputs to inputs or the rate at which input nutrients are recovered. For nitrogen (N), the NUE is defined as grain yield in relation to the quantity of N available to the crop from all sources, including fertilizer, soil organic matter mineralization, and atmospheric deposition. NUE is determined by various factors, including the plant's photosynthetic efficiency. Maximizing NUE is crucial for sustainable agriculture since it reduces fertilizer input, increases output, and lowers environmental losses. Environmental losses, particularly for nutrients such as nitrogen, are concerning in terms of water and air quality, as well as climate change. Genetic modification of key genes governing the rate-limiting processes in nutrient uptake and utilization efficiency would be an ideal method for developing superior crop varieties. Several important genes that are involved in N-metabolisms include glutamine synthetase, glutamate synthase, nitrate transport, and ammonium transport (Das *et al.,* 2023). Recently, Wei *et al.* (2022) reported that overexpressing the gene OsDREB1C can dramatically enhance rice production as compared to wild types. The expression of the OsDREB1C gene in rice reduced growth length, increased nitrogen usage efficiency, and promoted efficient resource allocation.

2. Impact on biotic and abiotic stress resistance/climate resilience

Insect resistance

Both public and commercial sector institutes have conducted substantial research on producing insect-resistant transgenic plants, making it a remarkable feat in agricultural biotechnology. The most widely commercialized transgenic plant contains cry genes from the *Bacillus thuringenesis* bacterium (Tabashnik *et al,* 2008 & 2013), but other genes, including API (arrowhead proteinase inhibitor), OC-I (cysteine proteinase inhibitor- oryzacystatin-I), Vgb (Vitreoscilla hemoglobin), SacB (levansucrase-encoding gene), JERF-36 (Jasmonic ethylene responsive factor), BADH (betadine aldehyde dehydrogenase gene), and NTHK1 (Wand *et al.,* 2018). Transgenic cotton and maize plants have shown resistance to lepidopteran and coleopteran larvae, resulting in lower pesticide costs and increased crop yields. According to studies, Bt cotton growers use between 40% and 70% less insecticide than non-Bt cotton farmers. This cuts production costs while also reducing farmers' and agricultural workers' exposure to dangerous chemicals. Reduced pesticide use benefits the environment by reducing chemical runoff, soil contamination, and dangers to non-target creatures such as beneficial insects and mammals. Bt cotton's success in reducing pesticide use has sparked interest in developing other GM crops with similar pest resistance traits. Bt brinjal, while not yet commercialized in India, has shown effectiveness in controlling the fruit and shoot borer pest, potentially reducing the need for chemical insecticides in brinjal cultivation.

Disease resistance

The Indian Council of Agricultural Research has created various rice cultivars that are resistant to major diseases like bacterial blight and blast. Rice variety IR64, selected for resistance to bacterial blight through marker-assisted selection, has much reduced disease incidence and higher yields than susceptible varieties. Wheat, maize, and pulse crops have benefited from biotechnological interventions, with resistant varieties reducing disease and pest yield losses and leading to enhanced production (Pathak *et al.,* 2018).

Controlling viral infections is a major concern in modern agriculture. Traditional management strategies aim to eradicate viral vectors and sick plants, but have low success rates. Plants can be engineered with viral resistance using biotechnological methods such as RNA-mediated expression, homology-dependent gene silencing, and microRNA-mediated resistance (Wilson, 1993). The University of Hawaii and Cornell University, reported successful example, generated two types of papaya by transmitting a virus gene to papaya plants that are resistant to papaya ring spot virus by transferring one of the virus' genes to papaya. Since 1998, papaya growers have received seeds from the two kinds, known as 'SunUp' and 'Rainbow', under licensing arrangements (Gonsalves, 1998).

Abiotic stress tolerance/climate resilience impact and sustainability

Climate change presents considerable obstacles to agricultural output, particularly in areas prone to drought and water scarcity. Plants face severe abiotic stress in their natural habitat. Abiotic stresses include drought, flooding, waterlogging, severe temperatures (cold, chilling, frost, and heat), salinity, mineral deficiency, and toxicity can negatively impact plant metabolism, growth, and development, even leading to plant mortality. These pressures can reduce productivity and generate economic losses. Extreme abiotic stresses harm 70% of agricultural productivity around the world. However, biotechnological technologies such as marker-assisted selection (MAS), tissue culture, in vitro mutagenesis, and genetic transformation have resulted in the creation of various abiotic stress-tolerant plant cultivars. In recent years, the introduction of "omics" technologies and the establishment of various model plants, such as *Arabidopsis thaliana, Medicago truncatula,* and *Lotus japonicus*, has begun promising tactics for studying the molecular and genetic basis of stress tolerance (Das *et al.*, 2023). For example, the drought-tolerant rice variety Sahbhagi Dhan, which was produced using marker-assisted selection, performs better under drought circumstances than traditional varieties (Majumder, *et al.,* 2023). Another example, drought-tolerant maize varieties have been developed through conventional breeding and genetic engineering. Flood-tolerant rice varieties have greatly improved climate resilience. Swarna-Sub1, created by the International Rice Research Institute (IRRI) in partnership with Indian research institutions, has the Sub1 gene, which confers resistance to submergence for up to two weeks (Mackill, *et al.,* 2012). Flood-tolerant rice cultivars, such as Samba Mahsuri-Sub1 and CR1009-Sub1, have been adopted in India's flood-prone regions, assisting farmers in mitigating flooding impacts and sustaining production (Dar, *et al.,* 2018).

Soil Health and Fertility

Biotechnology is an exciting tool for enhancing soil health and nutrient cycling in sustainable agriculture. Microorganisms with certain qualities can be introduced into soil to boost plant development, control plant diseases, and improve soil structure and fertility. Bio-fertilizers and bio-pesticides are two examples of using natural resources to improve soil health, plant health, and productivity. Bio-fertilizers, such as nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and mycorrhizal fungi, improve soil fertility and reduce the need for chemical fertilizers, which can harm soil over time. Rhizobium inoculants in leguminous crops fix atmospheric nitrogen, increasing soil nitrogen levels without the negative environmental impact of synthetic fertilizers. Genetically engineered cover crops can enhance soil health by reducing erosion, increasing organic matter, and improving structure. Long-term research on the impact of biotechnology on soil quality have yielded varied results, requiring careful management and monitoring. A meta-analysis indicated that GM crops, particularly Bt crops, led to improved soil microbial diversity and activity by reducing pesticide use (Belousova, *et al.,* 2021). Biotechnology has enormous potential for sustainable agriculture, offering numerous chances to improve agricultural output, quality, and sustainability.

Water Use Efficiency

Biotechnology has brought novel ideas for improving water use efficiency in agriculture, hence addressing increasing water constraint. A fundamental goal is to develop drought-tolerant GM crops that can maintain productivity in water-limited settings. These crops have characteristics that promote water intake, minimize transpiration, and increase stress tolerance. For example, GM maize cultivars with enhanced drought tolerance improve root growth, water retention, and cellular stress responses, resulting in greater yields in water-limited situations than conventional varieties. Genetic alterations that improve physiological features like stomatal conductance and photosynthetic efficiency also help to increase water use efficiency. GM rice varieties with altered stomatal density and behaviour improve water efficiency while retaining high photosynthetic rates and yields (Blum, 2009). In India, drought-tolerant rice cultivars such as Sahbhagi Dhan, which were produced using marker-assisted selection, have had a considerable impact. According to field trials and farmer experiences, Sahbhagi Dhan retains higher yields and grain quality when exposed to water stress than older varieties.

Ecosystem balance and biodiversity

The relationship between agricultural biotechnology and local biodiversity is complex and disputed. Genetically modified crops raise concerns about effects on non-target organisms, gene flow to wild relatives, and changes in agricultural practices impacting biodiversity. Bt crops, which produce insecticidal proteins, have been shown to reduce pest populations while having negligible effects on non-target organisms. For example, Bt cotton effectively controls bollworm populations without adversely affecting natural predators and pollinators (Tian, *et al.*, 2015). Research indicates that gene flow can occur, but its ecological influence is frequently limited by reproductive barriers and environmental constraints. Minimizing the risk of gene flow and its impact on biodiversity requires effective containment tactics and regulatory measures. Implementing best management methods like integrated pest management (IPM) and crop rotation improves the sustainability of genetically modified crops. IPM uses biological, cultural, and chemical management strategies to successfully manage pests while reducing environmental damage.

3. Herbicide tolerance and resistance

Farmers frequently use chemical pesticides to regulate weed development because weeds compete with crops for critical resources such as soil nutrients, water, and sunlight, resulting in lower agricultural yields. Moreover, weeds act as vectors for a variety of insects and dangerous pathogens. Uncontrolled weed growth can significantly reduce agricultural productivity, requiring farmers to use herbicides, tilling, and manual weeding to control their spread. Herbicides are used directly on crops in modern agriculture to suppress weed development. Farmers use mixtures of several herbicides once plants begin to grow since these herbicides typically have selective effects, attacking just specific plants while conserving the crop. To simplify weed control and promote the use of safer chemicals, scientists have looked into genetically engineering crops to be resistant to a wide spectrum of herbicides. This genetic modification has been used in a variety of crops, including corn, soybeans, cotton, canola, sugar beets, rice, and flax, and some of these genetically modified cultivars are marketed in numerous countries.

Herbicide-resistant crop varieties, such as glyphosate and glufosinate-tolerant crops, have been developed as a result of biotechnological developments. Glyphosate herbicides reduce plant development by inhibiting the EPSPS enzyme (5-enolpyruvylshikimate-3-phosphate synthase), which is necessary for the production of aromatic amino acids, vitamins, and other plant metabolites. Plants modified with genes like CP4-EPSP synthase and GOX (glyphosate oxidoreductase) produce glyphosate-tolerant EPSPS and glyphosate-degrading enzymes, respectively (Shaner, 2000 and Owen, 2005).

4. Quality and nutritional value improvement

The quality of agricultural products is the primary factor of their market price. The use of poorer planting material, a lack of nutrients, inadequate storage facilities, and a variety of biotic and abiotic stresses all have a negative impact on crop quality. This ultimately reduces farmers' income. Biotechnology can improve both the quality and nutritional content of agricultural products. Genetic engineering and plant tissue culture have been employed to extend the shelf life and keeping properties of food crops, as well as to enhance their colours and aromas (Matas, 2009). Fruit juices have a short shelf life. Tomatoes are widely consumed globally. Harvest tomatoes in the ripe green stage for transportation. Exposure to ethylene promotes early ripening and subsequent harvesting. Tomatoes ripen faster at higher temperatures, but their flavor may be compromised at lower temps. Calgene, a California-based business, developed genetically modified tomatoes to address the issue. They developed the Flavr Savr tomato variety to address the issue. Polygalacturonase is an enzyme that breaks down pectin to ensure that it ripens properly. Scientists genetically engineered tomatoes to reduce the amount of enzyme. Antisense RNA is utilized for this specific reason (Adenle, 2011). Low levels of the enzyme cause cell wall and pectin breakdown in stronger tomatoes. These Flavr Savr tomatoes have a firmer texture, a longer shelf life, and better transportability (Dawkins B, 2010).

Malnutrition is a major issue in underdeveloped nations, especially in Asia, where thousands of children die each year due to a lack of availability of balanced diets. Bio-fortification, which raises crop micronutrient and macronutrient values by conventional breeding or biotechnology approaches, is a promising prospective option. Crops can be genetically modified to contain more vitamins, hence increasing their nutritional value. Three genes have been added to genetically modify "golden rice," allowing plants to produce beta-carotene, which the human body turns into vitamin A (Ye *et al.*, 2000). Vitamin A insufficiency is the primary cause of blindness in children, affecting up to 250 million of them worldwide. Golden rice can biosynthesize beta-carotene, a precursor to vitamin A. It could be used as a nutrient supplement in South and Southeast Asia, where rice accounts for about two-thirds of daily calorie intake (Black, 2008). Biotechnology has also been used to modify the composition of specific oil crops in order to improve the amount of oil produced or to alter the type of oil produced. Crops with high nutritional quality features, such as iron-rich rice and vitamin A-rich rapeseed oil, as well as the development of edible vaccines and other pharmaceutical proteins, can benefit human and livestock health.

Biotechnology is being used to create more flavourful fruits. GM foods with increased taste include eggplant, cherries, peppers, seedless watermelon, and tomatoes. Removing the seeds from fruits leads to increased sugar content in soluble form, resulting in sweeter fruits (Falk *et al., 200*2). Biotechnology alters fermentation processes to enhance wine flavor and aroma (Haroon and Ghazanfar, 2016).

Utilizing gene-editing methods, biotechnology plays a key role in producing new varieties with distinct colours, scents, sizes, and blooms. Utilizing biotechnological methods such breeding, micro-propagation, tissue culture, mutation, and creation of polyploidy. There are many different kinds of decorative plants. More than 50 ornamental plants are now being changed using particle bombardment and Agrobacterium-mediated transformation techniques.

5. Biofuels development

Future prosperity is heavily reliant on the availability of egalitarian, secure, sustainable, and inexpensive energy. Production of biofuel is one of the emerged trends in recent years. Biotechnology is also being utilized in agriculture for the production of biofuels. Biofuels are fuels made from natural components such as algae, maize stover, and sugarcane bagasse, which reduce dependency on petroleum products. Biofuels are carbon-free and help reduce greenhouse gas emissions. Moreover, biofuel production does not compete with food production because certain inputs, such as algae, can be grown using wastewater or on non-arable land. Expanding fuel sources increases access and competition, potentially lowering prices. Six microalgae strains were photosynthesised in a photobioreactor. The Chlorella vulgaris strain is the most prominent of the six microalgae used in biodiesel production. *Chlorella vulgaris* was used as a feedstock. The quality of biofuel and lipid production could be used as criteria for selecting biodiesel-producing species. Modern biotechnological techniques allow for the development of biofuels, which have the potential to reduce greenhouse gas emissions and provide a more reliable fuel supply (Francisco, 2010).

6. Social and Economic Effects

Agricultural biotechnology improves crop yields and reduces production costs, leading to increased income and profitability for farmers. Bt cotton, resistant to the bollworm pest, minimizes the need for chemical pesticides, resulting in lower input costs and higher net earnings. A meta-analysis found that GM crops boost yields by an average of 21%, leading to increased farmer incomes. Bt cotton has been a success in India, with adoption increasing since 2002 and covering more than 90% of the cotton-growing region by 2018, resulting in a 24% increase in yields and a 50% increase in revenues. Higher and more consistent yields allow farmers to diversify their revenue streams by investing in livestock, secondary crops, or small companies, resulting in a more resilient economic base and less sensitivity to crop failure and market changes. Agricultural biotechnology's economic benefits vary by location in India, reflecting varying agro-climatic conditions and farming techniques. Bt cotton adoption in Maharashtra, a major cotton-growing state, has resulted in increased yields and incomes. According to an IFPRI study, Bt cotton farmers in Maharashtra had an average yield increase of 34%, resulting in an increased income of INR 18,000 per hectare compared to non-Bt cotton producers (Venugopalan and Reddy, 2017). The expansion of the biotechnology sector has been a driver of employment creation, contributing to overall economic development. The biotechnology sector encompasses research and development (R&D), production, processing, and distribution of biotech goods, producing employment in a variety of roles, ranging from highly qualified research scientists to field workers and technicians. The socioeconomic impact of agricultural biotechnology goes beyond individual farmers and into rural development as a whole. Increased profitability of GM crops has led to infrastructural improvements, such as irrigation systems, storage facilities, and transportation networks. Farmers in Maharashtra and Gujarat have leveraged greater income from Bt cotton to implement modern irrigation methods, resulting in improved crop yield and water efficiency (Birthal, 2013).

Conclusions

Agriculture biotechnology applications help to sustain food production. Biotechnology is a supplement, not a replacement, for many aspects of traditional agricultural research. It provides a number of tools to help us better understand and manage genetic resources for food and agriculture. Biotechnology has transformed the agriculture economy, offering numerous benefits to both farmers and consumers. Biotechnology is the use of live organisms to develop new goods, methods, and strategies to solve agricultural difficulties. One of the most notable effects of biotechnology on agriculture, has transformed crop development by creating transgenic crops with improved yield, pest and disease resistance, and environmental tolerance. Genetically modified crops have improved farmer productivity and profitability while reducing the need for chemicals, leading to more sustainable and environmentally friendly farming practices. Biotechnology has the potential to be a game changer in meeting the world's rising food demand while minimizing its negative environmental impact, resulting in a more resilient and sustainable agricultural system.

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Application of Biotechnological Approaches in Insect-Pest Management

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Abstract

Global agricultural pest management incurs substantial annual expenditures exceeding billions of dollars. For many decades, broad-spectrum synthetic insecticides were regarded as the most effective option for pest suppression. However, their indiscriminate and excessive use has accelerated the evolution of resistance in pest populations, disrupted natural enemies, and posed serious threats to human health and environmental safety. Therefore, sustainable pest management requires minimizing chemical pesticide use and conserving beneficial organisms to maintain ecological balance. Modern biotechnology provides promising and environmentally sound alternatives for pest control and has significantly reshaped present-day agriculture. Techniques such as genetic transformation and genetic engineering for crop protection—including *Bt* crops, recombinant DNA technology, gene pyramiding, RNA interference (RNAi), and the sterile insect technique—are now widely adopted for eco-friendly pest management. Emerging gene-editing tools, especially CRISPR-Cas9, offer new opportunities for precise control of insect pests. RNA interference strategies utilizing double-stranded RNA enable targeted elimination of pest species while preserving non-target organisms. Likewise, gene-drive systems modify specific gene inheritance patterns, providing effective population management capabilities. The integration of agricultural biotechnology thus provides innovative, sustainable, and effective solutions to rising pest challenges while supporting long-term food security for the growing global population.

Key words: Biotechnology approaches, pest management, genetically modified crops

Introduction

Projections indicate the global population will approach ten billion by 2050 (Nishimoto, 2019), necessitating approximately fifty percent greater food production to meet demand (Kumari *et al*., 2022). Escalating threats from pests and diseases underscore the urgency of developing innovative agricultural methodologies (Sundstrom *et al.,* 2014; Ristaino *et al.,* 2021). Research by Joshi *et al.* (2020) and Kumari *et al.* (2022) attributes an estimated twenty-five to thirty percent reduction in agricultural productivity to insect pests. Global crop losses from field to consumption range between thirty and forty percent due to pest and disease pressure (Kumara and Thiruchchelvan, 2022). Viral pathogens cause approximately three to seven percent losses, while bacterial and fungal diseases account for roughly fifteen percent (Iksat, *et al.,* 2023). Phytopathogens significantly diminish crop yields and quality worldwide, severely constraining agricultural productivity. Conventional chemical insecticide applications have demonstrated inadequate efficacy (Dhananjayan *et al*., 2020), prompting increased research focus on biotechnology-based alternatives.

Advanced biotechnological methods including gene transformation and genetic engineering provide novel, effective strategies for pest and disease management. Developments in gene editing technologies such as CRISPR-Cas9 create opportunities for controlling insect infestations. RNA interference approaches employing double-stranded RNA can specifically eliminate pest species while leaving nontarget organisms unaffected. Gene-drive systems alter inheritance patterns of targeted genes, establishing effective pest management capabilities. This review examines biotechnological applications for insect pest and disease control, including gene editing, RNAi, and gene-drive technologies. We address successful gene editing implementations in insects including fruit flies and migratory locusts, along with potential applications for developing pest-resistant crops using CRISPR-

Cas9. Agricultural biotechnology integration delivers innovative approaches to address increasing pest prevalence while ensuring sustainable nutrition for the growing global population.

Role of Biotechnology in Insect-Pest Management

Biotechnology significantly enhances bioinsecticide efficacy and cost-effectiveness while expanding market opportunities. Insect pest management employs biotechnological approaches including genetic engineering, genome editing, RNA interference, and complementary techniques. These methodologies support sustainable agriculture while reducing dependence on toxic pesticides. Detailed examination of these approaches follows.

1. Genetic Engineering for Pest Management

Genetic engineering, alternatively termed gene transformation, develops insect resistance in crops through insertion of specific DNA sequences or genes into plant genomes. These introduced sequences typically encode instructions for producing proteins with insecticidal properties. Plants expressing these proteins acquire resistance to targeted insect pests (Gatehouse, 2013). This strategy has demonstrated effectiveness against diverse pests from Lepidoptera, Coleoptera, and Diptera orders (Birkett and Pickett, 2014). Global agriculture has utilized genetically modified crops producing insecticidal proteins from the soil bacterium *Bacillus thuringiensis* (Bt) since 1996 (Abbas, 2018). The cry gene transformation methodology employs particle bombardment or Agrobacterium-mediated techniques to deliver insecticidal genes into crop plants (Juturu *et al*., 2015). *Bacillus thuringiensis* cry genes predominate in commercial transgenic crops (Tabashnik *et al.*, 2008; 2013). Additional genes including OC-I (oryzacystatin-I cysteine proteinase inhibitor), *Vgb* (*Vitreoscilla* hemoglobin), *NTHK1*, *SacB* (levansucrase), JERF-36 (jasmonic ethylene responsive factor), *BADH* (betaine aldehyde dehydrogenase), and API (arrowhead proteinase inhibitor) have undergone investigation for enhancing resistance and stress tolerance (Wang *et al*., 2018). Transgenic cotton and maize incorporating these genes demonstrate substantial resistance to lepidopteran and coleopteran pests, resulting in reduced insecticide application and enhanced crop yields. Research indicates Bt cotton producers utilize forty to seventy percent less insecticide compared to conventional cotton cultivation. This reduction decreases production costs while minimizing farmer and agricultural worker exposure to hazardous chemicals. Reduced pesticide application benefits environmental quality through decreased chemical runoff, diminished soil contamination, and reduced risks to beneficial insects and vertebrates. Bt cotton's success in reducing insecticide use has stimulated interest in developing additional GM crops with similar pest resistance characteristics. Bt brinjal, though not yet commercialized in India, demonstrates effectiveness against fruit and shoot borer pests, potentially reducing chemical insecticide requirements in brinjal production.

Genetically Modified Crops/Plants/Transgenic Plants for Pest Resistance

Genetically modified microbial pesticides, classified as plant-incorporated protectants (PIPs), utilize microorganisms including bacteria, fungi, viruses, and protozoa modified to produce pesticidal substances, providing natural targeted pest control approaches. Plant-incorporated protectants represent genetically engineered plants containing genes encoding toxin production for pest suppression. These PIPs enable plants to defend against pathogenic microorganisms including fungi, viruses, and bacteria. Crops utilizing PIPs for targeted pest control are designated pest-resistant crops. Transgenic plant development involves inserting desired genes into plant genomes through techniques including particle bombardment, gene gun delivery, or Agrobacterium-mediated transformation. These methodologies have been applied to wheat, maize, rice, and corn (Rajamani and Negi, 2021). Plant-incorporated protectants offer multiple advantages including enhanced yields with reduced pesticide application. These genetically modified crops provide economic benefits while protecting neighboring non-Bt crops through local insect population reduction. Potential disadvantages associated with plant-incorporated protectants include effects on non-target pests, human and environmental risks, possible PIP gene transfer to other plants, and development of pesticide or insect resistance (Rajamani and Negi, 2021). Commercially available plant-incorporated protectant pesticides appear in table 1.

Protease inhibitors, which disrupt insect digestive processes by inhibiting enzymes required for protein digestion, have been introduced into plants through transgenic methods (Singh *et al*., 2020). Additionally, transgenic plants expressing alpha-amylase inhibitors have been developed to combat insect pests from multiple orders including Lepidoptera, Coleoptera, Diptera, and Hemiptera by interfering with starch digestion in insect guts. Insect chitinase and chitinase-like proteins represent another important strategy, degrading chitin—a major component of insect exoskeletons and gut linings—thereby compromising structural integrity and digestive capabilities. Lectins, naturally occurring in diverse plant species, bind to specific carbohydrates in insect midguts, impairing digestion and nutrient absorption. These lectins demonstrate strong insecticidal activity and have been successfully cloned into transgenic plants. Detailed examination of each strategy and their significance in insect pest management follows.

Cry Genes

The gram-positive soil bacterium *Bacillus thuringiensis* (Bt) produces insecticidal crystalline proteins (ICPs) toxic to insects. Genes encoding these proteins, specifically parasporal crystal protoxins, have been inserted into numerous crop species to confer insect resistance. These crystal proteins function as protoxins that dissolve and undergo proteolytic activation in the alkaline midgut environment of insect larvae. Activated toxins bind to specific receptors on midgut epithelial cells, forming membrane pores. This process causes gut paralysis within two to three days, terminating feeding and resulting in larval mortality (Kumari *et al*., 2022).

Table 1: Plant-Incorporated Protectants against crop pests (Transgenic Crops Carrying Bt Genes for Insect Resistance)

Plant	**Gene incorporated**	**Resistance to insect**	**Commercial name**
Cotton	Cry 1Ac toxins from *Bacillus thuringiensis*	Cotton and tobacco bollworm	Bollgard
Maize	Cry 1Ab,1F,9c toxins from *Bacillus thuringiensis*	European corn borer	Yieldgard, Knockout, Herculex I, Starlink, Maximizer
Rice	Cry1C from *Bacillus thuringiensis*	Leaf folders (*Cnaphalocrocis medinalis*) and stem borers	-
Tobacco	QB protein of photosystem II from mutant Amaranthus	Atrazine resistant	-
Brinjal	Cry1Ac from the soil	Shoot and fruit borer, *Leucinodes orbonalis*	Bt Brinjal
Tomato	Cry1Ab gene of *Bacillus thuringiensis*	Second instar larvae of *Helicoverpa armigera* (Fruit borer) and	Bt tomato

		Spodoptera litura (Cutworm)	
Pigeon pea	cry1Ac and cry2Aa	*Helicoverpa armigera* (Pod borer)	-
Sweet potatoes	Cry1Aa gene	Lepidopteran insect *i. e, Spodoptera litura*	-
Cotton	Cry2AX gene	*Helicoverpa armigera*	-
Rice	*Cry2AX1*	*Scirpophaga incertulas* (Yellow stem borer) *and Cnaphalocrocis medinalis* (Leaf folder)	-
Tomato	Cry1Ac	*Tuta absoluta* (Leaf minor)	
Chickpea	*CryIIAa*	*Helicoverpa armigera* (Pod borer)	-
Pigeon pea	Cry1AC + Cry2Aa	*Helicoverpa armigera* (Pod borer)	-
Soybean	Cry 8 like	*Holtrichia panallele* (Black chafer)	-
Caster	Cry1AC	*Achaea janata* (Caster semilooper) and *Spodoptera litura* (Cutworm)	-

Vegetative Insecticidal Protein (Vip) Genes

Bacillus thuringiensis (Bt), present in diverse natural habitats, possesses inherent insecticidal properties effective against numerous economically significant agricultural pests. This effectiveness stems from its capacity to produce various insecticidal proteins during different growth phases. Among these, **Vip proteins** (vegetative insecticidal proteins) generated during bacterial vegetative growth represent valuable insecticidal agents due to their distinct sequences and target receptor sites compared to conventional Cry proteins.

Vip proteins classify into three principal subfamilies: Vip1, Vip2, and Vip3. Vip1 and Vip2 function cooperatively as heterodimers, demonstrating particular effectiveness against Hemipteran and Coleopteran pests. Vip3, the most extensively investigated Vip family member, exhibits potent toxicity against lepidopteran pests. Designated as second-generation insecticidal proteins, they demonstrate effectiveness against diverse insect pests when administered individually or combined with cry proteins. Vip genes provide excellent pest control options due to enhanced resistance against tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa armigera*) (table 2). Transgenic cotton containing Vip3A alone and an alternate multitoxin line expressing both Vip3A and cry1Ab (VipCot) demonstrated enhanced resistance to *H. zea* and *H. virescens* respectively (Kumari *et al.*, 2022).

Table 2: Expression of vegetative insecticidal proteins (Vip) genes for insect resistance

Plant	Transgene	Resistance to insect
Cotton	*Vip3A + cry1Ab*	*Heliothis zea* (Corn earworm) and *H. virescens* (Tobacco bud worm)
Cowpea	*Vip3Ba1*	*Maruca vitrata* (Bean pod borer)
Cotton	*Vip3AcAaa (Vip3Aa1+Vip3Ac1)*	*Helicoverpa armigera* (Cotton bollworm)
Sugarcane	*Vip3A*	*Chilo infuscatellus* (Yellow top borer)

Lectins

Lectins comprise carbohydrate-binding proteins that, upon ingestion by insects, bind to chitin in the midgut peritrophic membrane, impeding nutrient assimilation. Transgenic tobacco expressing pea lectin gene demonstrates resistance to *H. virescens*. Numerous plant species naturally produce lectins—carbohydrate-binding proteins with insecticidal properties that protect plants against herbivory by adversely affecting consuming insects and animals. Leguminous seeds frequently contain substantial lectin concentrations, particularly abundant in plant families including Solanaceae, Fabaceae, and Poaceae. Plant lectins, functioning as storage proteins, play essential roles in protecting plants from phytophagous insects. Recent development of insect-resistant plants has highlighted the potential for employing plant lectins in pest management strategies. Lectin incorporation into transgenic crops has yielded promising results, particularly in enhancing resistance to sap-sucking insects, especially when combined with *Bacillus thuringiensis* (Bt) Cry toxins. Furthermore, lectins added to artificial diets or expressed in transgenic plants adversely affect growth and development of insect pests across multiple orders including Lepidoptera, Coleoptera, and Hemiptera. Plant lectins disrupt digestive and cellular processes through strong affinity for specific sugar residues present on insect cell membrane glycoproteins and glycolipids (table 3). Transgenic rice plants expressing *Galanthus nivalis* agglutinin (GNA) and *Allium sativum* leaf agglutinin (ASAL) demonstrate resistance to common sap-sucking pests including brown planthoppers, white-backed planthoppers, and green leafhoppers, providing valuable biological tools for sustainable pest control (Kumari *et al.*, 2022).

Table 3: Expression of lectin genes for insect resistance

Plant	Transgene	Resistance to insect/Target insects
Stored grains	*Polygonum persicaria* L. (PPA) Lectin	*Sitophilus oryzae* (Rice weevil)
Common bean	Arcelin-5, Leucoagglutinin, Erythroagglutinin	*Callosobruchus chinensis* (adzuki bean weevil, pulse beetle, chinese bruchid, or cowpea bruchid)
Common bean	Arcelin	-do-
Stored grains	*Microgramma vacciniifolia* rhizome lectin (MvRL)	*Sitophilus. Zeamais* (Maize weevil)

Cowpea	Arcl on APA locus from *Phaeselous vulgaris*	*Callosobruchus chinensis* (adzuki bean weevil, pulse beetle, chinese bruchid, or cowpea bruchid)
Wheat	*Pinellia pedatisecta* agglutinin (PPA)	*Metopolophium dirhodum* (Rose grass aphid), *Schizaphis graminum* (Green bug), *Rhopalosiphum padi* (Bird cherry-oat aphid) and *Sitobion avenae* (English grain aphid)
Mustard	*Colocasia esculenta* tuber agglutinin (CEA)+ *Galanthus nivalis* agglutinin (GNA)	*Lipaphis erysim*i (Musrad aphid/turnip aphid)
Potato	*Galanthus nivalis* agglutinin (GNA)	*Myzus persicae* (Potato aphid)
Potato	Hv1a/GNA	*Myzus persicae Myzus persicae* (Potato aphid) and *Sitobion avenae* (English grain aphid)
Rice	*Allium sativum* leaf agglutinin (ASAL) and *Galanthus nivalis* lectin (GNA)	*Nilaparvata lugens* (Brown planthopper), *Sogatella furcifera* (White-backed planthopper) *and Nephotettix nigropictus* (Rice green leaf hopper)
Rice, Sugarcane	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	*Eoreuma loftini* (Dyar)-Maxican rice borer and *Diatraea saccharalis* (Sugarcane borer)
Wheat	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	*Sitobion avenae* (English grain aphid)
Rice	Snowdrop lectin (*Galanthus nivalis* agglutinin; GNA)	Sap sucking pests

Fusion Proteins

Fusion proteins result from combining multiple insecticidal proteins into single genetic constructs. When expressed in transgenic plants, these fusion proteins form single polypeptide chains demonstrating enhanced efficacy against diverse phytophagous insect pests. Transgenic cotton lines developed by Dow AgroSciences containing Cry1Be + Cry1Fa fusion protein exhibited improved resistance to pests including *Spodoptera litura* and *Ostrinia nubialis*. Similarly, *Agrobacterium*-mediated transformation in *Brassica juncea* expressing lectin-protease inhibitor fusion protein conferred resistance against phytophagous aphids. Maize incorporating Cry1Ab/Cry2Aj fusion protein into kernels demonstrated increased mortality in both *Spodoptera exigua* (Lepidoptera) and *Harmonia axyridis* (Coleoptera). Transgenic rice lines expressing combined Cry1Ac and *Allium sativum* agglutinin (ASAL) provided broad-spectrum, sustained protection against significant insect pests including brown planthoppers, leaf folders, and yellow stem borer (Kumari *et al*., 2022). Table 4 summarizes studies employing fusion proteins for developing insect-resistant crop plants (Kumari *et al*., 2022).

Table 4: Fusion proteins for insect resistance in crop plants for pest management

Plant	Transgene	Resistance to insect/Target insects
Sugarcane	*Cry2Aa + Cry1Ca, Cry1Ab + Cry1Ac*	*Scirphophaga excerptalis* (Sugarcane top borer)
Cotton	*Cry2Ab + Cry1F + Cry1AC*	*Helicoverpa armigera* (Cotton bollworm) and *Spodoptera litura* (Leaf worm)
Rice	*Cry2Aa + Cry1Ca*	*Chilo suppressalis* (Rice stem borer)
Rice	*Cry1AC + ASAL*	*Scirpophaga incertulas* (Yellow stem borer), *Cnaphalocrocis medinalis* (Rice leaf folder), *Nilaparvata lugens* (Brown plant hopper)
Cotton	*Cry1Be + Cry1Fa*	*Spodoptera litura* (Tobacco cutworm), *Ostrinia nubialis* (Europian corn borer)
Brassica juncea-Mustard	Lentil lectin (LL) and chickpea protease inhibitor (CPPI) genes	*Lipaphis erysimi* (Mustard/turnip aphid)
Maize	*Cry1Ab/Cry2Aj*	*Spodoptera exigua* (Armyworm), *Harmonia axyridis* (Lady beetle)
Cotton	*Cry51Aa2*	*Lygus* spp. (Tarnished plant bug)
Rice	*Cry2AX1 (Cry2Aa + Cry2Ac)*	*Scirpophaga incertulas* (Yellow stem borer), *Cnaphalocrocis medinalis* (Rice leaf folder and rice leaf roller)

Protease Inhibitors

These natural plant compounds contribute to insect pest defense by interfering with digestive processes. They function through blocking digestive protease enzymes that insects utilize for protein breakdown, leading to reduced nutrient assimilation. This disruption results in diminished reproductive success, elevated mortality, and retarded growth due to amino acid deficiencies. Among extensively studied PIs are serpins and cystatins. Serpins, with molecular weights approximately 39-43 kDa, irreversibly inhibit serine proteases. These serine proteases commonly occur in insect orders including Diptera (flies), Orthoptera (grasshoppers and locusts), Lepidoptera (moths and butterflies), Coleoptera (beetles), and Hymenoptera (bees and wasps). Cystatins, smaller at 12-16 kDa, inhibit cysteine proteases—primary digestive enzymes in Coleopteran and Hemipteran insects. Research indicates certain plant-derived signaling compounds, notably methyl jasmonate essential for plant defense, can suppress gut protease activity in insects following tissue damage (Kumari *et al.*, 2022). Despite potential applications, protease inhibitor use in pest management has encountered limitations primarily due to incomplete understanding of insect physiology and biochemistry. Nevertheless, PIs remain promising tools for developing novel environmentally compatible pest control strategies.

α-Amylase Inhibitors

Insects utilize the digestive enzyme α-amylase for carbohydrate digestion. α-Amylase inhibitors disrupt insect digestion by suppressing enzyme activity. Plant seeds and vegetative organs contain α-amylase inhibitors that effectively regulate phytophagous insects. *Phaseolus vulgaris* seeds containing α-amylase inhibitors demonstrate reduced growth and development of bruchid pests including azuki bean weevil (*Callosobruchus chinensis*) and cowpea weevil (Kumari *et al*., 2022). Recent developments include tobacco engineered with wheat gene encoding α-amylase inhibitor, enhancing resistance to *Agrotis* and *Spodoptera* species (Jaiswal *et al*., 2018). Morton and colleagues (2000) demonstrated transgenic pea and azuki bean seeds expressing α-amylase inhibitor αAI-1 exhibited enhanced resistance to bruchid pests including azuki bean weevil (*C. chinensis*), cowpea weevil (*C. maculatus*), and pea weevil (*Bruchus pisorum*). Kaur *et al.* (2022) found maize genotypes with elevated α-amylase inhibitor activity in central whorl leaves and stems demonstrated enhanced resistance to *Chilo partellus* infestation.

Insect Chitinase

These hydrolytic enzymes degrade chitin, a fundamental structural component of insect exoskeletons and peritrophic membranes that provides protection against environmental stress, mechanical damage, and predators. Transgenic plants expressing chitinase genes have been developed to enhance crop resistance. Osman *et al*. (2016) found maize engineered to produce chitinase exhibited enhanced resistance to corn borer (*Sesamia cretica*). Chitinase roles in pest control have been investigated in multiple insect species including silkworm (*Bombyx mori*), rice brown planthopper (*Nilaparvata lugens*), cotton mealybug (*Phenacoccus solenopsis*), and rice striped stem borer (*Chilo suppressalis*) (Omar *et al*., 2019). Their inhibitory effects on insect development have led to applications as transgenes and biopesticides. However, limited research and insufficient structural data on certain insect chitinases have constrained targeted agrochemical development. Enhanced understanding of molecular structure and biological functions could accelerate biotechnological pest management applications (Kumari *et al.*, 2022).

2. Genome Editing for Pest Management

Insect resistance development to Bt traits presents significant challenges to agricultural productivity, stimulating demand for innovative, economical, environmentally responsible pest management approaches including strategies countering insect resistance. Recent focus has shifted toward gene editing—a more sophisticated and precise methodology (Anastacia Books, 2019). Gene editing enables scientists to insert, delete, or replace DNA bases at specific genomic locations, modifying gene function through natural organismal repair processes (Bortesi and Fischer, 2015). Recognized as globally applied contemporary scientific technique, gene editing permits targeted DNA modifications in living organisms (Belfort and Bonocora, 2014). In insect pest management, this technique gains momentum by expanding options for enhancing plant pest resistance. Genome editing typically employs nucleases for site-specific DNA cleavage, with transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) as widely utilized tools. The CRISPR/Cas9 system comprises two principal components: Cas9 protein and single-guide RNAs (sgRNAs), readily synthesized, whereas TALEN requires modification for each target locus. This approach offers advantages over conventional genetic editing procedures including enhanced precision, efficiency, and versatility. CRISPR enables development of crop varieties with improved characteristics such as increased yield, enhanced nutritional content, pest and disease resistance, and environmental stress tolerance (Jaganathan *et al.*, 2018). Disease-vector mosquitoes can be genetically modified to reduce pathogen transmission capacity. In this context, CRISPR/Cas9 has recently emerged as a powerful, user-friendly, highly efficient tool for developing insect-resistant crops (Table 5). This technique functions through specific gene deletion or missense mutation introduction in target genes, effectively blocking protein production in various crops (Gao, 2021). Many generalist insects depend on plant-specific signals including volatiles, taste, visual cues, and oviposition preferences for host location. Genome editing can modify these volatile compounds, offering novel pest control strategies while ensuring changes do not adversely affect beneficial insects including pollinators and natural predators.

Table 5: List of insect engineered for pest management using CRISPR/Cas9

Target insects	Target gene
Plutella xylostella	Abdominal-A homeotic gene (*Pxabd-A*)
Drosophila melanogaster	Chitin synthase 1
Agrotis ipsilo	*Yellow-Y* Gene
Spodoptera litura	Abdominal-A (*Slabd-A*) gene
Spodoptera littoralis	Olfactory receptor co-receptor (*Orco*) gene
Helicoverpa armigera	*HaCad*
Helicoverpa armigera	*Tetraspainin*
Spodoptera exigua	Ryanodine receptor
Ceratitis capitata	Eye Pigmentation Gene White Eye (*We*)
Helicoverpa armigera	α-6- nicotinic acetylcholine receptor (*nAchR*)
Plutella xylostella	*PxABCC2, PxABCC3*
Ostrinia furnacalis	*ABCC2*
Rhopalosiphum padi	*ß-1-3glucanase* in maize
Tribolium castaneum	E-cadherin gene, *EGFP*
Locusta migratoria	Odorant receptor co-receptor (*Orco*) gene

3. RNA Interference (RNAi) for Pest Management

RNA interference (RNAi) constitutes a technique reducing specific gene expression through targeted sequence suppression. Alternatively designated post-transcriptional gene silencing (PTGS), this process operates through introducing double-stranded RNA (dsRNA) into cells, triggering suppression of specific undesired genes (Kamthan *et al*., 2015). In pest control, RNAi functions through pest consumption of dsRNA, which subsequently distributes through their systems, disrupting essential biological processes (Katoch *et al*., 2013) (Table 6). While transgenic Bt toxins primarily target midguts of Lepidoptera and Coleoptera larvae, RNAi provides broader, more targeted approaches without affecting nontarget insect species. The RNAi approach demonstrated potential for controlling numerous insects, particularly sap-sucking species, while creating new opportunities for environmentally compatible pest management.

Table 6: List of transgenic crops for insect resistance through RNA interference

Target crop	Target insect	Silenced gene
Tobacco	*Spodoptera littoralis*	*Sl 102 immune* gene
Tobacco	*Bemisia tabaci*	Phenolic glucoside malonyltransferase
Soybean	*Aphis glycines*	*TREH, ATPD, ATPE, CHSI*
Tobacco	*Bemisia tabaci*	*BtACTB gene*
Rice	*Scirpophaga incertulas*	*AchE*-Acetylcholine esterase

Tobacco	*Manduca sexta*	v-*ATPaseA* gene
Potato	*Leptinotarsa decemlineata*	*ECR* gene
Cotton	*Helicoverpa armigera*	Juvenile hormone methyl transferase (JHMT)
Maize	D*iabrotica virgifera virgifera* LeConte	*Dvvgr, dvbol*
Soybean	*Leguminivora glycinivorella*	SpbP0-dsRNA
Rice	*Scirpophaga suppressalis*	Aminopeptidase N genes *APN1+APN2*
Tomato, Tobacco	*Helicoverpa armigera*	Chitinase gene-*HaCHI*
Maize	Lepidopteran	dsRNA-Spray
Potato	*Leptinotarsa decemlineata*	*β-actin* gene
Maize	*Diabrotica v. virgifera*	hunchback (hb) and brahma (brm) gene
Maize	D*iabrotica virgifera virgifera* LeConte	Suppression of target mRNA

4. Gene-Drive Approach for Pest Management

Gene drive technology represents a genetic engineering technique favoring inheritance of specific traits, enabling rapid spread of single genetic factors through populations for potential pest or disease management applications (DiCarlo *et al.*, 2015; Books, 2019). Gene drives build upon natural phenomena where specific genes (or genetic elements) demonstrate enhanced likelihood of transmission to offspring compared to expectations under standard Mendelian inheritance patterns. Scientists can employ gene drives to introduce particular traits into pest populations, reducing their agricultural impact or disease transmission capacity. Gene drive examples include population suppression—introducing genes inducing pest sterility or diminishing general fitness, resulting in pest population decline—and population modification—introducing genes altering pest behavior or reducing disease transmission capacity without necessarily reducing total population numbers. Gene drives can manage crop-damaging insect pests, potentially reducing pesticide requirements. Invasive organisms threatening agriculture or ecosystems can be managed or eliminated through gene drives (Kumari *et al.*, 2022).

5. Marker-Assisted Selection (MAS) for Pest Management

Marker-assisted selection (MAS) constitutes a crop improvement method employing DNA marker-based selection rather than visible trait observation. Plants are selected for breeding based on genetic composition rather than phenotypic appearance or field performance. Multiple molecular marker forms, including single-nucleotide polymorphisms (SNPs), have been identified demonstrating substantial potential for enhancing traditional breeding efficacy and precision. Molecular marker approaches represent the most recent methodology for introducing desired genes into optimal crop plant combinations (Kumari *et al.*, 2022). MAS research indicates *Bph14* and *Bph15* were introduced into Minghui 63 and related hybrids through molecular marker-assisted selection to enhance BPH resistance (Hu *et al.*, 2012). Using marker-assisted backcrossing (MABC) with phenotypic selection, Yuehui9113 and its F_1 hybrids acquired resistance to both brown planthopper (BPH) and bacterial blight (BB) through gene pyramiding combining one BB resistance gene (*Xa21*) with two BPH resistance genes (*Bph14* and *Bph15*) (He *et al.*, 2019). In another case, resistant rice line ASD7 carrying *bph2* gene crossed with susceptible variety C418 produced hybrids exhibiting significantly enhanced BPH resistance when evaluated using MAS (Li-Hong *et al*., 2006). Liu *et al.* (2016) reported rice cultivars with two stacked BPH resistance genes, *Bph3* and *Bph27*(t), demonstrated stronger resistance and experienced reduced yield loss from BPH.

Conclusions

Insects constitute primary agents reducing agricultural productivity. Farmers conventionally employ chemical insecticides for rapid pest infestation management. Alternative pest management strategies gain importance through public awareness of adverse effects from indiscriminate pesticide use on human, animal, and environmental health. Progress achieved in insect biotechnology derives primarily from logical advances in molecular research, particularly technological improvements enabling genetic control of living organisms and cells. Biotechnology applications for pest management have expanded significantly. Insect biotechnology provides valuable assets across diverse industries including development of novel catalysts, microbial insect toxins, and other substances. It enables genetic characterization and engineering of organisms and cells, along with recombinant DNA technology applications creating microbial agents with enhanced insecticidal potency. Additionally, RNA interference and genome editing with CRISPR/Cas9 offer new approaches for developing insect-resistant crops. Creating insect-resistant agricultural plants proves commercially viable, pesticide-resistant, and environmentally safe (Kamatham *et al*., 2021). Plants can achieve insect resistance through modern approaches including RNAi and CRISPR for sensitive gene elimination or modification.

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Bio-herbicides: An Eco-friendly Approach for Weed Management

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Introduction

Weed plant species have a considerable negative impact on agricultural crop output, and managing them is a serious challenge for the agricultural sector. These plants can quickly absorb scarce natural resources including water, light, soil nutrients, and space. They can multiply quicker than cultivated plants due to characteristics such as a deep root system, drought and frost resilience, and high nutrient utilization efficiency. Furthermore, weeds can leak allelopathic compounds into the soil, which promotes the growth of pests and crop pathogens. These features make them competitive with arable crops, typically resulting in a decrease in agricultural output while increasing cultivation costs. Unfortunately, the excessive and frequently improper use of chemical herbicides has resulted in a variety of negative side effects, including plant resistance, soil and groundwater contamination, and harm to non-target organisms. There is a need requirement for suitable alternatives of chemical herbicides. Bio-herbicides are an eco-friendly alternative to chemical herbicides, reducing environmental impact and health risk.

Bio-herbicides are substances that suppress weed populations by the use of bio-organisms like as fungi, bacteria, viruses and plant based products including plant extract and essential oils and natural metabolites. Bio-herbicides are a new strategy for addressing the inadequacies of traditional conventional herbicides. The bio-herbicide inhibit of physiological activities like nutrient uptake, photosynthesis etc and disrupt cellular functions like cell wall and cell membrane, hormone and toxic production etc. Microbes can overcome weed plant resistance and infect the target plant due to their virulence factors. There are two major groups of virulence factors. The first category contains enzymes that break down cell walls, lipid membranes, and weed proteins. Amylases, pectinases, cellulases, enzymes that change lignin, proteases, peptidases, and phospholipases are all members of this class. The second group of virulence factors consists of peptides and secondary metabolites with phytotoxic characteristics that disrupt weed physiological and metabolic systems. Different types of formulation have been developed to enhance the shelf life of different bio-herbicides for successful commercialization.

Fungal pathogens as bio-herbicides (Mycoherbicides)

Many fungi have been shown to exhibit broad spectrum weed control ranges. Fungal pathogens are the most promising alternative of synthetic chemical herbicides for weed management systems. Fungal pathogens weed control is based on their phytotoxic metabolites .These metabolites inhibit the plant pathways and toxic to weed plant cells. *Alternaria, Phytophthora, Colletotrichum, Fusarium, Pseudocercospora, Cochliobolus, Ascochyta, Drechslera, Phoma, Phyllostictica, Pyrenophora, Septoria, Stagonospora, Amphobotrys, Myrothecium* are the most common fungal pathogens for the bio-control of weeds. Fungi also suppress weed growth and development by creating specific types of secondary metabolites. Fungal phytotoxins attack plants, disrupt the proper functioning of their systems, and ultimately kill their hosts. Soil-borne fungi are potentially important bio-herbicide candidates because they may be applied directly to soils to reduce weed populations through the decay of seeds before emergence or kill seedlings shortly after emergence. Some important commercially available mycoherbicides are listed in table-1.

Table 1: Commercial use of mycoherbicides, target weed and ecosystems

Bio-control agent/Fungal Pathogens	**Target weeds**	**Formulation/Carrier**	**Ecosystem**
Colletotrichum gloesporiodes f. sp. aeschynomene	Northern joint vetch (*Aeschynomene virginica*)	Collego, LockDown	Rice, soybean
Colletotrichum gloesporiodes f. sp. malvae	Roundleaf mallow (*Malva pusilla*)	Biomal, Mallet	Flax, lentils, horticultural crops
Colletotrichum gloesporiodes f. sp. cuscutae	Dodders (*Cuscuta* spp.)	Product in use in China "Lubao 1-S22"	Soybean
Colletotrichum acutatum f. sp. hakeae	Hakea (*Hakea sericea*)	Hakatak- available - South African market	Mountain meadows
Colletotrichum truncatum	Hemp sesbania (*Sesbania exaltata*)	Coltru	Soybean, cotton, rice
Colletotrichum coccodes	Velvetleaf (*Abutilon theophrasti*)	Velgo	Maize, soybean
Phytophthora palmivora	Strangler or milkweed vine (*Morrenia odorata*)	DeVine	Citrus groves
Alternaria cassia	Sicklepod (*Cassia obtusifolia*)	CASST	Soybean
Alternaria destruans	Dodders	Smolder	Cranberry
Puccinia candiculata	Yellow nutsedge (*Cyperus esculentus*)	Dr. BioSedge	Horticultural crops
Puccinia thlaspeos	Dyer's woad (*Isatis tinctoria*)	Woad Warrior	Rangelands, non-crop areas
Chondrostereum pupureum	Black cherry (*Prunus seratina*), Red alder (*Alnus rubra*), *Populus* spp.	Biochon, Chontrol-Canada	Forest, rangelands
Cylindrobasidium	*Acacia* spp.	Stumpout-South Africa	Forest, rangelands
Nectria ditissim	Red alder (*Alnus rubra*)	PFC-Alderkill- use in Canada	Forest
Phoma macrostoma	Various broadleaf weeds	Bio-Phoma	Pastures, grasslands
Lasiodiplodia, *Macrophomina*, *Neoscytalidium* combination	Parkinsonia, Jerusalem thorn (*Parkinsonia aculata*)	Di-Bak- use in Australia	Grassland, rangeland riparian areas

Sclerotinia minor	Dandelion (*Taraxacum officinale*)	Sarritor- Canada	Lawns, recreational areas
Fusarium oxysporum f. sp. orthoceras and F. oxysporum	Broomrape (*Orobanche cumana* and *O. crenata*)	"FOO"- Formulation trials, commercialization underway-Egypt	Sunflower, faba bean, tomato
Trichoderma koningiopsis	Wild poinsettia (*Euphorbia heterophylla*)	Field evaluation-Brazil	Row crops and noncrop areas

Source: Kremer, 2023

Bacterial pathogens as bio-herbicides

Many bacteria have been proved as potential bio-control agent for weeds. Plant pathogenic bacteria, including *Xanthomonas campestris pv poae* (Xcp) and *Pseudomonas syringae pv tagetis* (Pst), were developed as foliar-applied bio-herbicides for the control of annual bluegrass (*Poa annua*) and Asteraceae (composite) weeds, respectively. Two highly effective non-pathogenic isolates, *P. fluorescens* D7 and *P. fluorescens* ACK55 have been commercialized, applied as liquid suspensions or in encapsulated clay for use in cereal crops and rangelands (Table 2).

Table 2: Different bacterial target weed and ecosystems

Bio-control agent/Bacterial Pathogens	**Target weeds**	**Formulation/Carrier**	**Ecosystem**
Xanthomonas campestris pv. poae	Annual bluegrass (*Poa annua*)	Camperico- Used in Japan	Turf, lawns
Pseudomonas syringae pv. phaseolicola	Kudzu (*Pueraria lobata*)	Formulated, field tested	Non cropland, pastures
P. syringae pv. tagetis	Asteraceae weeds (composites)	Formulated, field tested	Maize, soybean, pastures
Ralstonia solanacearum	Tropical soda apple (*Solanum viarum*)	Formulated, field tested	Pastures, noncrop areas
Pseudomonas fluorescens D7	Downy brome (*Bromus tectorum*)	Commercialized- "D7"	Cereal crops
Pseudomonas fluorescens ACK55	Downy brome (*Bromus tectorum*) Jointed goatgrass (*Aegilops cylindrica*), Medusahead (*Taeniatherum caput-medusae*)	Commercialized- "Battalion Pro"	Cereal crops, rangelands
Lactobacillus spp. strains; *Cremoris* sp.	Clovers (*Trifolium* spp.), birdsfoot trefoil, (*Lotus corniculatus*)	Commercialized- "Organo-Sol," "Kona," "Bioprotec"-Canada	Lawns, turf

Source: Kremer, 2023

Viruses as bio-herbicides

Viruses can also be employed as bio-herbicides to suppress certain weeds, however due to limitations, they are ineffective as fungal pathogens. Viruses have lots of genetic variability and are not target specific. The tobacco mild green mosaic virus (TMGMV) is a patented bio-herbicide used to control tropical soda apple (*Solanum viarum*), an invasive perennial weed found in pastures and non-cropland areas in the south-eastern and mid-southern United States (table 3).

Table 3: Viral pathogens, targeted weed and ecosystems

Bio-control agent/Bacterial Pathogens	**Target weeds**	**Formulation/Carrier**	**Ecosystem**
Tobacco mild green tobamovirus	Tropical soda apple	Commercialized-“SolviNix”	Pastures, non-crop areas

Source: Kremer, 2023

Natural products as bio-herbicides

Many plant-based products can also be used as natural weed control agents. Plants produce secondary metabolites or other photochemicals that hinder seed germination and other growth activities. Plant products can be used as weed control agents in three forms i.e. plant extracts, essential oils, and allelochemicals. These three plant components have been used as potential bio-herbicides for several decades. The major method of action of plant-based products is to impede the germination of weeds and reduce plant development. Plant extracts contain many elements such as peptides, secondary metabolites, alkaloids, terpenoids and tetraterpenoids etc. These compounds, grouped as “biochemical bio-herbicides”. Table 4 shows examples of natural compounds with bio-herbicidal action, including those commercialized or in development for practical application.

Table 4: Representative natural products as bio-herbicides

Bio-herbicide	**Target weeds**	**Mode of action/Comments**
Plant Extracts		
Corn gluten meal extract (hydrolysate)	Broad-spectrum PRE control of weeds in turf	Complex of phytotoxic dipeptides
Mustard seed meal extract	Green foxtail (*Setaria viridis*); Powell amaranth (*Amaranthus powellii*)	Sinalbin (4-hydroxybenzyl glucosinolate) active substance in extract
Rapeseed oil	Broad spectrum weeds; potato vine desiccant	Commercialized as ‘Beloukha’-Europe
Dried distillers grains extracts (DDGSs)	Annual bluegrass (*Poa annua*); common chickweed (*Stelaria media*)	Methanolic extracts of DDGS

Saturated fatty acids, i.e., Pelargonic acid	Broad-spectrum annual weeds	Geranium (*Pelargonium roseum*) leaf extract; many preparations marketed
Aglaia odorata leaf extract-PORGANIC(™)	Inhibited root and shoot growth of garden cress (*Lepidum sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), timothy (*Phleum pratense*), ryegrass (*Lolium multiflorum*) and *Echinochloa crus-galli*	Inhibit growth of weed plant
Ammi visnaga (L.) Lam. Khellin and Visnagin- plant extract	lettuce (*Lactuca sativa*) and duckweed (*Lemna paucicostata*) velvetleaf (*Abutilon theophrasti*), crabgrass (*Digitaria sanguinalis*)	Inhibit seed germination, photosynthesis, and cellular activities.
Lichen (*Cladonia verticillaris)*- Phenolics extracts	Lettuce	Change the cellular structure of leaves and roots of lettuce seedlings
Essential oils		
Manuka oil	Crabgrass (*Digitaria* spp.) and broad-spectrum of dicotyledonous weeds	Distilled from plant parts of manuka tree (*Leptospermum scoparium*)
Artemisia oil	Coffee senna (*Cassia occidentalis*), Ageratum (*Ageratum conyzoides*); Barnyardgrass	Volatile oil (1,8 ceneole) of wormwood or sage brush (*Artemisia scoparia*)
Savory oil	Redroot pigweed (*Amaranthus retroflexus*), Common lambsquarters (*Chenopodium album*)	Carvacrol and terpinene oils; prepared oil/water nanoemulsion for application
Monoterpene cineoles	Broad-spectrum	Numerous aromatic herbs, i.e., Eucalyptus, Salvia
Eucalypt (*Eucalyptus nicholii*), Rosemary (*Rosmarinus officinalis* L.), Lawson cypress (*Chamaecyparis lawsoniana*) and White cedar (*Thuja occidentalis*) plants	Amaranth, Purslane and Knapweed	Amaranth, Purslane and Knapwee germination inhibitors species
Allelochemical-based		
Sorgoleone	Barnyard grass (*Echinochloa crus-galli*), velvetleaf (*Abutilon theophrasti*), Bent grass (*Agrostis stolonifera*)	Exuded from roots of sorghum (*Sorghum bicolor*)

Rice hull extracts	Barnyard grass	Inhibition of germination, seedling growth, and weight in barnyard grass
Black walnut (*Juglans nigra*) from walnut- Plant extract	Horseweed (*Conyza canadensis*) and hairy fleabane (*Conyza bonariensis*)	Inhibit growth of horse weed (*Conyza canadensis*) and hairy fleabane (*Conyza bonariensis*) act as a pre- and post-emergent bio-herbicide- Commercialized as “NaturCur”.
Rice var. OM5930 shoot þ root extracts	Barnyardgrass, fimbry (*Fimbristylis miliacea*), red sprangletop (*Leptochloa chinensis*)	Crystalized formulation of aqueous extracts; suppress seedling growth
Barley (*Hordeum vulgare*) extract	Filamentous algae in aquatic environments	Extract released during decomposition in water
Wild tomato (*Solanum habrochaites*) extracts	Broad-spectrum weeds	Burndown (knockdown) use; commercialized as “WeedLock” in Malaysia

Source: Nusrat *et al.*, 2018; Kremer, 2023

References

Kremer, R. J. (2023). Bioherbicide development and commercialization: Challenges and benefits. https://doi.org/10.1016/B978-0-323-95290-3.00016-9.

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