

# Chapter 6

## Biological Control Agents for Sustainable Agriculture, Safe Water and Soil Health

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**Abstract** Use of bio-agents must be encouraged in agriculture as the use of chemicals inversely impact population and natural resources. This chapter gives a wide variety of biological agents being used in India for various pests in different crops. Studies can further be stretched to use these bio-agents in turf and for ornamental pest control. Neem-based bio-insecticide is used at a concentration of 5% against Diamondback moth, *Plutella xylostella* in cabbage management. Farm yard manure (FYM) enriched with *Trichoderma harzianum* (4 g/kg) is used to control thrips, mites, and soil-borne diseases and *Pseudomonas fluorescens* is used (5 g/L) for inducing systemic resistance in hot peppers. *Beauveria bassiana* alone or in combination with BT have been used to control soil insects including potato beetles. The isolates of *Trichoderma* spp. have been characterized for biopriming, plant growth promotion characteristics, reduction of disease incidence, and corresponding yield increase in cabbage, cauliflower, mustard, and field pea at 5–10 g/kg seed. *T. harzianum* in the concentration of  $2 \times 10^8$  cfu/g of soil and *P. fluorescens*  $1 \times 10^{12}$  cfu/g of soil was said to be the best towards management of root knot nematode (*Meloidogyne incognita*). Application of *T. harzianum* (250 g) + *P. fluorescens* (250 mL), and FYM (25 kg) + *T. harzianum* (250 g) + *P. fluorescens* (250 mL) against fusarium wilt, mites, and root knot nematode has been found promising in cucumber. The above bio-agents can also be successfully used for the control of turf and ornamental plants by conducting field trials.

**Keywords** Bio-agents • Natural resources • Public health • Safe water • Soil health

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## 1 Introduction

The rigorous use of synthetic pesticides and their environmental and toxicological risks have generated augmented global interest to develop alternative sources of chemicals to be used in safe management of agricultural pests. The issue becomes important in the areas where large population is exposed agrochemicals. Recently, in different parts of the world, attention has been drawn towards exploitation of higher plant products as novel chemotherapeutics for plant protection because they are mostly non-phytotoxic and easily biodegradable. Different botanicals and microbes have been formulated for large-scale application as biopesticides in eco-friendly management of plant pests and are being used as alternatives to synthetic pesticides in crop protection. Annual Report (2010-11).

Environmental scientists throughout the world are searching for the alternatives of chemical pesticides for a healthy and fruitful tomorrow. Plants and their secondary metabolites are an important source for natural pesticides and the development of new pesticides. A number of plants are known to have insecticidal activity. Essential oils and other bioactive compounds have also been searched for potential insecticides Casida and Quistad (1998). The identification of the important role of these compounds has increased, particularly in terms of resistance to pests and diseases. Moreover, the purity of natural product is highly variable and is dependent upon the plant part, plant age, extraction method selected, geographical origin and location, climate, and overall growth and health of the plant from which the chemical is extracted. The rigorous use of synthetic pesticides and their environmental and toxicological risks have generated augmented global interest to develop alternative sources of chemicals to be used in safe management of agricultural pests. The excessive use of these chemicals has led to pesticides contaminating almost every part of the environment and poses a significant risk to non-target organisms (insects, plants, fish, and birds) (Aktar et al. 2009; Azmathullah et al. 2013).

Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutics for plant protection because they are mostly non-phytotoxic and easily biodegradable. Currently, different botanicals have been formulated for large-scale application as biopesticides in eco-friendly management of plant pests and are being used as alternatives to synthetic pesticides in crop protection. Despite these difficulties, research and development in plant-derived pesticides has increased considerably (Arthur 1996; Rahman and Talukder 2006; Rahman and Islam (2007); Murti et al. 2010; Panagiotakopulu et al. 1995).

Currently, the market is full with a variety of chemical, organic, and even some herbal pesticides, but the most commonly used are the chemical and organic pesticides which pose a threat to our environment when used on a large scale. Because of this reason, many plants and herbs are currently being researched for their insecticidal properties (Chauhan et al. 2016). One such plant is *Lantana camara*, also commonly known as wild sage or big sage; it is a species of flowering plant within

the verbena family, Verbenaceae that is native to the American tropics. Over time, it has spread to southern Asia, Europe, and Australia, making it an invasive species of weed. In the last decade, this plant has been extensively studied for its medicinal potential by using advanced scientific techniques (Ranjan et al. 2016; Jain et al. 1996; Giday et al. 2003).

Exposure of the general population to pesticides occurs primarily through eating food and drinking water contaminated with pesticide residues, whereas substantial exposure can also occur in or around the home (Damalas and Eleftherohorinos 2011). There are several factors that determine the toxicity of pesticides in the environment, including the measures taken during its application, the dosage applied, the adsorption on soil colloids, the weather conditions prevailing after application, and how long the pesticide persists in the environment. Thus, the need arises to look towards biological control of insects on plants.

Biopesticides include a wide range of microbial pesticides, biochemicals derived from microorganisms and other natural sources (including plants) and processes involving the genetic incorporation of DNA into agricultural commodities that confer protection against pest damage (Gupta and Dikshit 2010). Recently, the potential of products derived from higher plant products is being studied (Dubey et al. 2008) because of their phytotoxicity, easy biodegradability and stimulatory nature of host metabolism (Mishra and Dubey 1994), and low mammalian toxicity.

Botanical insecticides pose significantly less threat to the environment and non-target organisms. The increasing acceptance of their use is proven by the commercial production Pyrethrum and neem essential oils for use as insecticides (Isman 2006; Nauen and Bretschneider 2002). A major barrier to their limited commercial usage has been their relative cost and safety as compared to their chemical counterparts. Biopesticides tend to overcome many difficulties that are possessed by chemical or synthetic insecticides. They are inherently less harmful, have less environmental load, and are designed to affect only one specific pest or, in some cases, a few target organisms. The pesticides are often effective in very small quantities and are biodegradable, thereby resulting in lower exposures. Furthermore, the approach of Integrated Pest Management (IPM) can enable effective utilization of botanical insecticides (Gupta and Dikshit 2010).

Since ancient times, there have been efforts to protect harvest production against pests. The Egyptian and Indian farmers used to mix the stored grains with fire ashes (Bhargava 2009). The ancient Romans used false hellebore (*Veratrum album*) as a rodenticide, the Chinese are credited with discovering the insecticidal properties of *Derris* species, whereas *Pyrethrum* was used as an insecticide in Persia and China. In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation. Indian farmers use neem leaves and seed for the control of stored grain pests (Arthur 1996; Sharon et al. 2014).

## 2 Brief History of Biopesticides

Historically, nicotine was used to control plum beetles as early as the seventeenth century. A number of experiments were carried out in the nineteenth century by using plants and fungus as biological controls for insect pests in agriculture. The extensively used biopesticide included spores of the bacteria *Bacillus thuringiensis*. In 1938 (France), Sporeine, a first commercially available Bt product came in to picture. In 1977, *Bacillus thuringiensis* var. *israelensis* (toxic to flies) was discovered, and in 1983 the strain tenebrionis (toxic to beetles) was found. In 1979, the U.S. EPA registered the first insect pheromone for use in mass trapping of Japanese beetles. In the 1990s, researchers began testing kaolin clay as an insect repellent in organic fruit orchards. It was made commercially available, particularly for use in organic systems, in 1999. Throughout the early twentieth century, soil microbiology and ecology experiments had led to the identification and isolation of many soil-borne microorganisms that act as antagonists or hyper parasites of pathogens and insect pests. During 1980s and 1990s, several studies were done on the root cause of resistant pathogenic bacteria for the prevention of fire blight in orchards. Approximately 100 biopesticide active ingredients have been registered with the U.S. EPA Biopesticides Division since 1995 (Sources: University of Arkansas, the Ohio State University, U.S. EPA).

## 3 Biopesticides in India

In India, so far only 12 types of pesticides have been registered under the Insecticides Act, 1968 (Table 6.1). The pattern of pesticide usage in India is different from that for the world in general. The foremost use of pesticides in India is for cotton crops (45%), followed by paddy and wheat.

**Table 6.1** Biopesticides registered as insecticides Act, 1968

S. No.	Name of the biopesticide
1.	<i>Bacillus thuringiensis</i> var. <i>israelensis</i>
2.	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>
3.	<i>Bacillus thuringiensis</i> var. <i>galleriae</i>
4.	<i>Bacillus sphaericus</i>
5.	<i>Trichoderma viride</i>
6.	<i>Trichoderma harzianum</i>
7.	<i>Pseudomonas fluorescens</i>
8.	<i>Beauveria bassiana</i>
9.	NPV of <i>Helicoverpa armigera</i>
10.	NPV of <i>Spodoptera litura</i>
11.	Neem-based pesticides
12.	Cymbopogon

A rough estimate shows that about one third of the world's agricultural production is lost every year due to pests despite the pesticide consumption which totalled more than 2 million tons. In India, pests cause crop loss of more than Rs. 6000 crores annually, of which 33% is due to weeds, 26% by diseases, 20% by insects, 10% by birds and rodents, and the remaining (11%) is due to other factors (Bunch et al. 2003).

The advent of the green revolution in India in the 1960s to boost agricultural productivity using High Yielding Variety (HYV) crops led to an increased use of fertilizers (as nutrients) and pesticides (as insecticides) (Sebby 2010). The use of insecticides increased considerably as Government of India statistics reveal (Bunch et al. 2003). The research studies to assess the environmental impact of heavy use of pesticides in India have revealed that it is detrimental for us to look at alternates to chemically synthesized pesticides. The adverse environmental impact ranges from soil infertility, pollution of water bodies, and health impact on farmers, labourers as well as consumers (Bunch et al. 2003; Kandpal 2014).

## 4 Botanical Insecticides

Approximately 2400 plant species have bioactive compounds that possess pest control properties (Table 6.2). The activities of the extracts of such plants possess pest control properties, including killing activity, non-killing repellency activity, feeding deference, and growth inhibition (Bunch et al. 2003). In 1990, studies done by the World Health Organization reported that no segment of the population is protected against the hazardous exposure to pesticides and its adverse effects. The survey also noted that the risk of exposure detrimental to health is higher in developing countries (Aktar et al. 2009). The risk varies from direct impact on humans (Nigam et al. 1993), impact through food commodities (Kashyap et al. 1994), and impact on

**Table 6.2** Plant products registered as biopesticides (Kandpal 2014)

Plant product used as biopesticide	Target pests
Limonene and Linalool	Fleas, aphids, and mites, also kill fire ants, several types of flies, paper wasps, and house crickets
Neem	A variety of sucking and chewing insect
Pyrethrum/Pyrethrins	Ants, aphids, roaches, fleas, flies, and ticks
Rotenone	Leaf-feeding insects, such as aphids, certain beetles (asparagus beetle, bean leaf beetle, Colorado potato beetle, cucumber beetle, flea beetle, strawberry leaf beetle, and others), and caterpillars, as well as fleas and lice on animals
Ryania	Caterpillars (European corn borer, corn earworm, and others) and thrips
Sabadilla	Squash bugs, harlequin bugs, thrips, caterpillars, leaf hoppers, and stink bugs

environment, surface water contamination (Kubiak et al. 1989) ground water contamination, soil contamination, and effect on soil fertility (beneficial soil microorganisms), contamination of air, soil and non-target vegetation and non-target organisms (Reijnders 1986).

In northern Cameroon, cowpeas are traditionally mixed with sieved ash after threshing and the mixture put into mud granaries or clay jars. In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants. In Rwanda, farmers store edible beans in a traditional closed structure (imboho) and whole leaves of *Ocimum canum* are usually added to the stored foodstuff to prevent insect damage within these structures. Owusu suggested some natural and cheaper methods for the control of stored products from pests, with traditionally useful Ghanaian plant materials. In some south Asian countries, food grains such as rice or wheat are traditionally stored by mixing with 2% turmeric powder. The use of oils in stored products for pest control is also an ancient practice. Botanical insecticides such as *Pyrethrum*, derris, nicotine, oil of citronella, and other plant extracts have been used for centuries. More than 150 species of forest and roadside trees in India produce oilseeds, which have been mainly used for lighting, medicinal purposes, and also as insecticides from ancient times to early twentieth century. Turmeric, garlic, *Vitex negundo*, gliricidia, castor, *Aristolochia*, ginger, *Agave americana*, custard apple, *Datura*, *Calotropis*, *Ipomoea*, and coriander are some of the other widely used botanicals to control and repel crop pests (Rajashekar et al. 2012; Verma et al. 2010).

#### 4.1 Plant Pesticides/Bio-agents

Plant pesticides are pesticidal substances that plants produce from genetic material that have been added to the plant (Table 6.3). For example, scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plants own genetic

**Table 6.3** Potential biopesticides (from plant extract) (Kandpal 2014)

Plant extracts	Effective against
Adathoda kashayam and Pudhina kashayam	Leaf folder, bacterial leaf blight, Helminthosporium leaf spot
Thriphala kashayam	Bacterial leaf blight and Helminthosporium leaf spot
Andrographis kashayam and Sida kashayam	Aphids and borers in brinjal, ladies finger
Barley Sesamum Horsegram kashayam	Acts as fruit yield enhancer
Cow's urine arkam and Sweet flag arkam	Bacterial leaf blight, Helminthosporium leaf spot, vein clearing disease, fusarium wilt
Garlic arkam	Leaf folder, bacterial leaf blight, Helminthosporium leaf spot
Neem seed extract (for all crops)	Leaf folder, aphids, Jassids, fruit borer, and stem borer

material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. Both the protein and its genetic material are regulated by EPA; the plant itself is not regulated.

## 4.2 *Neem as Pesticides*

Derived from the neem tree, it contains several chemicals, including ‘azadirachtin’, which affects the reproductive and digestive process of a number of important pests. Recent research carried out in India and abroad has led to the development of effective formulations of neem, which are being commercially produced (Asogwa et al. 2010). As neem is non-toxic to birds and mammals and is non-carcinogenic, its demand is likely to increase. However, the present demand is very small. Neem-based pesticides are marketed in India in different trade names containing 300, 1500, 3000, 5000, 10,000, and 50,000 ppm of azadirachtin in it. Some of them are Ozoneem Trishul, Margocide OK, Godrej Achook, Nimbicidine, Bioneem, Neemark, Neem gold, Neemax, Rakshak, Econeem, Limnool, and Repelin containing 300 ppm of azadirachtin (Mishra 2014).

Almost all parts of neem tree, viz., leaves, drupes, barks, and seeds contain a pool of biologically active constituents, including the triterpenoids azadirachtin, salanin, and meliantriol. These compounds give protection against more than 100 species of insects, mites, and nematodes including economically important pests like desert and migratory locusts, rice and maize borers, plant hoppers of rice, pulse beetle and rice weevil, rootknot and reniform nematodes, and citrus red mite. Modes of pest control by neem include antifeedant, growth regulatory, repellent, hormonal or pesticidal action in larva, and/or adult stages of these pests. It is probably because of the pest control quality, idol of ‘Lord Jagannath’ is made up of neem tree trunk which will not be attacked by wood boring beetles, termites, and last long. That also proves the use of neem as a pest control agent in ancient India. Mishra in 2014 has described, *Pyrethrum*, Niotine Sulphate, *Parthenium hysterophorus*, *Vitex negundo* (Begunia), *Acorus calamus* L. (Bacha), *Adhatoda zeylanica* (Basanga), *Anacardium occidentale* (Cashew nut), *Ageratum conyzoides* (goat weed Pokasungha), *Chireita*, *Catharanthus roseus* (Sadabihari), *Clerodendron inerme* (Genguti), *Plumbago zeylanica* (Dhalachita): *Ipomeacarneae* (Amari) in details in his potential and comprehensive review.

## 5 Microbial Pesticides

Microorganisms such as bacteria, fungi, viruses, algae, and protozoan have been reported single or in combination as biopesticides. Microbial pesticides can control a variety of pests (Table 6.4). The most extensively known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or Bt, which can control certain

**Table 6.4** Microbial agents, crops, and target diseases

Microbial agent	Crop	Target disease
<i>Bacillus subtilis</i> DB1501	Turf grass	Brown leaf blight
<i>Paenibacillus polymyxa</i> AC-1	Pepper, cucumber	Phytophthora bright powdery mildew
<i>Streptomyces goshikiensis</i> WYE 325	Rice, turf grass	Sheath blight large patch
<i>Streptomyces eolombiensis</i> WYE 20	Turf grass, strawberry, cucumber	Grey mold, brown leaf bright powdery mildew
<i>Bacillus subtilis</i> KBC 1010	cucumber	Grey mold
<i>Bacillus subtilis</i> GB-365	Tomato	Bright grey mold
<i>Bacillus subtilis</i> GB-365	Turf grass	Phytophthora bright powdery mildew

insects in cabbage, potato, and other crops. Bt produces a protein that is harmful to specific insect pests. Certain other microbial pesticides act by out-competing pest organisms.

## 5.1 Bacteria

Several bacterial strains have been reported as entomopathogens but biopesticides that have been most successful commercially are based on spore forming bacterium *Bacillus thuringiensis* (Bt). Over 30 Bt subspecies have been discovered, but only half a dozen of them have been closely evaluated as pest control agents. Bt is known to infect at least four orders (Lepidoptera, Diptera, Coleoptera, Acarina) but lepidopteran larvae with gut pH of 9.0–10.5 are most susceptible. Bt is a crystalliferous spore former and in addition to endospores produces a parasporal crystal which contains delta endotoxin. Upon ingestion by susceptible individuals, the delta endotoxin crystal is digested into active toxins which kills the insects or weakens the host so that the bacteria can readily invade the haemocoel from the gut and produce lethal septicaemia (Mishra 2014).

## 5.2 Fungi

Approximately 750 fungal species belonging to 100 genera are entomopathogenic. Several strains of fungal pathogens have been used for the control of crop pests in India. The important genera are *Coelomomyces*, *Entomophthora*, *Massospora* belonging to *Mastigomycotina*; *Cordyceps*, *Podonectria*, *Torrubiella* belonging to *Ascomytina*; and *Aspergillus*, *Beauveria*, *Fusarium*, *Hirsutella*, *Metarhizium*, *Nomuraea*, *Paecilomyces*, etc. belonging to *Deuteromycotina*. The development of fungal infections in terrestrial insects is largely influenced by terrestrial conditions.



High humidity is vital for germination of fungal spores and transmission of the pathogen from one insect to another. Entomopathogenic fungi have several strains. They are known to produce toxins and nearly 33 toxins are known till date (Mishra 2014). Examples are *Metarhizium anisopliae* on *Oryctes rhinoceros* L., *Fusarium oxysporum* on BPH, *Verticillium lecanii* on *Coccus viridis* (Green), *Beauveria bassiana* on *Spodoptera litura*, and *Helicoverpa armigera*. Some of the trade products of *Beauveria bassiana* available in Indian market are Boverin, Biopower, Ankush, Daman, and Multiplex Beauveria.

### 5.3 Viruses

Approximately 60% of the 1200 known insect viruses belong to baculoviridae that can be used against 30% of all major pests of food and fibre crops. Majority of the baculoviridae, those have been developed as biopesticide are bacilliform or rod shaped and include nuclear polyhedrosis viruses (NPVs) and to a lesser extent granulosis viruses (GVs). Upon ingestion by the larvae, the protein coat dissolves in the mid gut and the virions enter the epithelial cells of mid gut. Later, they infect the fat bodies, epidermis, tracheal matrix, muscle, gonads, haemocytes, nervous and endocrine system. After an incubation period of 5–7 days (sometimes 20 days), the larvae become sluggish, yellowish, or pinkish in colour, swell slightly, and then become limp and flaccid. Shortly before death, the integument becomes very fragile. The dead larvae found hanging by their pro-legs from the top of the host plant. Finally, they dry up and look like a dark brown or black cadaver. Presently, NPVs for *Helicoverpa* (*Helicide*, *Heliocel*, Biovirus H) and *Spodoptera* (*Spodocide*, *Litucide*, Biovirus S) are available in India and used for control of these two polyphagous pests infesting tomato, tobacco, arhar, cotton, vegetables, oilseeds, etc. The need for propagating these organisms and costs involved in producing them have limited viruses as products of significant commercial importance. GV of *Chilo infuscatellus*, codling moth, potato tuber moth, and cabbage butterfly are widely used for control of vegetable and field pests in advanced countries and some parts of India. These are produced by the farmer's co-operatives or cottage industries (Mishra 2014).

### 5.4 Nematodes

Nematodes are known to parasitize insects. Notable among them are *Neoaplectana carpocapsae*, which infects ten different orders of insects. One of its strains DD-136 is used extensively for control of insect pests of orchards, vegetables, field crops, forests, and turf crops. Another nematode *Tetradonema plicans* is used against sciarid flies and pests of cultivable mushrooms. Similarly, *Romanomermis*

*culicivora* is marketed under the trade name ‘Skeeter’ and *Steinernema feltiae* as ‘Doom’, ‘Seek’, and ‘Spear’ is used for control of soil pests and termites. In India, *Rhabditis* sp. has been reported to be useful against *Holotrichia serrata* (white grub). (Mishra 2014).

## 5.5 Protozoa

Approximately 1000 species of protozoans pathogenic to insects have been described. Most of them are chronic debilitating agents, affecting host vigour, longevity, and fecundity. Most of the protozoa considered for use are microsporidia, and their spores enter the host by ingestion. Once in the gut, they exude a long tube that injects the pathogens into the host tissue where it multiplies vegetatively in the cytoplasm of cell, gradually spreading throughout the body and causing a chronic disease that may or may not kill the host. In India, *Farinocystis tribolii* has been found to be promising against *Tribolium castaneum*. ‘Noloc’ is the formulation based on *Nosema locustae* infecting grasshoppers and is regarded as safe to use. *Nosema* has been evaluated for control of grasshoppers, European corn borer, and spruce bud worm (Mishra 2014).

### Advantages

1. Microbials are naturally occurring.
2. These have a high degree of specificity to target pests.
3. No or little adverse effect on beneficial insects.
4. Potential development of pest resistance to microbials is less common or may develop more slowly due to unique mode of action.
5. No known environmental hazards.
6. Less residual activity. (Adopted from Mishra 2014)

### Limitations

1. Microbials have narrow spectrum of activity. They control only the target pest which is not economical when mixed populations are required to be controlled.
2. These are effective only when applied at specific development stage of target species.
3. Often slow acting.
4. Microbials have short residual toxicity, require frequent applications.
5. In order to be effective microbials require high application rate and thorough spray coverage.
6. Some of them require specific weather conditions to be effective (Adopted from Mishra 2014) (Table 6.5)

**Table 6.5** Benefits of biopesticides (Gupta and Dikshit 2010)

Factors	Benefits of biopesticides
Cost-effectiveness	Costlier but reduced number of applications
Persistence and residual effect	Low, mostly biodegradable and self-perpetuating
Knockdown effect	Delayed
Handling and bulkiness	Bulky: Carrier based Easy: Liquid formulation
Pest resurgence	Less
Resistance	Less prone
Effect on beneficial flora	Less harmful on beneficial pests
Target specificity	Mostly host specific
Waiting time	Almost nil
Nature of control	Preventive
Shelf life	Less

## 6 Conclusion

Rich source of Indian biodiversity is a potential source of all types of natural biopesticides which can be used at a large scale in agriculture. Biopesticides and natural enemies of pests are likely to play an important role in IPM in modern agriculture for controlling pests of vegetables and fruit crops in the near future besides grain crops, forest pests, and pests of domestic and public health. Because of their slow active nature, we need to develop effective strategies for using them in agriculture. Research and development of pest control methods must be given importance. Extension workers and farmers need to be educated on their use. The price of the commercial biopesticides has to be competitive with synthetic chemical pesticides or alternately the government has to provide subsidies for encouraging their use in agriculture. Related regulations do not go nearly far enough in evaluating the broader impacts of biopesticides. This will lead to an overall increased awareness and action about the benefits of biopesticides.

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