# Biocontrol based cost-effective and eco-friendly strategies for insect pest management in oilseed crops

**P. S. Vimala Devi**
**Principal Scientist (Entomology),**
**Directorate of Oilseeds Research, Rajendranagar, Hyderabad- 500 030**

In India, crops are affected by several pests including insects, diseases, weeds, rats, nematodes etc. causing 30% loss of the Indian crop yield potential amounting to more than 30 million tonnes of food grain valued at Rs.50,000 million, representing 18 % of the gross national agricultural production. Of this, 23 % loss is due to insect pests. Pest management was primarily dependant on chemical pesticides. Keeping in view the ill effects of chemical pesticides, the Govt. of India has adopted Integrated Pest Management (IPM) as the cardinal principle and main plank of plant protection strategy in the country. IPM is an eco-friendly approach aimed at minimum use of chemical pesticides by employing available alternative methods of pest control like cultural, mechanical and biological and use of bio-pesticides to maintain pest species at economically acceptable densities. Insect pathogens can have a major role in pest management but their effective integration into such systems depends on their compatibility with other components of this system such as chemical pesticides, other pathogens, parasitic and predaceous arthropods. Microbial control includes all aspects of utilization of microorganisms or their by-products for the control of insect pest species. Among the different microbial agents developed and tested, bacteria, viruses and fungi are considered promising for the control of insect pests. Microbial control agents are relatively host specific and do not upset other biotic systems. They are safe to humans, vertebrates, beneficial organisms and do not cause environmental pollution and are compatible with most other control methods. They are ideal for both short and long term pest suppression. Unlike chemical pesticides, they are harmless to humans and other non-target organisms, they do not leave chemical residues on crops, are easy and safe to dispose of and do not contaminate water systems.

With the ever-increasing awareness of the harmful effects of the chemical on man and his environment, the immediate need for sustainable, eco-friendly pest management has been felt very strongly providing an impetus to research and development of microbial pesticides. Microbial control includes all aspects of utilization of microorganisms or their by-products for the control of pest species. Among the different microbial agents developed and tested, bacteria, viruses and fungi are considered promising for the control of insect pests (Table 1). Microbial control agents are relatively host specific and do not upset other biotic systems. They are safe to humans, vertebrates, beneficial organisms and do not cause environmental pollution and are compatible with most other control methods. They are ideal for both short and long term pest suppression. Unlike chemical pesticides, they are harmless to humans and other non-target organisms, they do not leave chemical residues on crops, are easy and safe to dispose of and do not contaminate water systems. The microbial pesticides can be locally produced and used for pest management in Organic farming. Some of the promising microbial insecticides that can be employed in organic farming are discussed below.

### *Bacillus thuringiensis*

*Bacillus thuringiensis* (Bt) is a naturally occurring soil bacterium. The potential of this bacterium in the management of several caterpillars causing serious damage to the cultivated crops world over has been documented over the last fifty years. There are currently more than a hundred products of Bt registered for the management of important lepidopteran insect pests such as *Helicoverpa armigera* (Cotton bollworm), *Ostrinia nubilalis* (Europeon corn borer), *Plutella xylostella* (diamond back moth), *Trichoplusia ni* etc. Insecticides of microbial origin occupy only 1-2% of the global market for insecticides. However, Bt occupies 95% share of the microbial pesticide market. The production of Bt worldwide is dominated by the multinational companies and amounts to several thousand tonnes annually. Bt production by these companies involves high capital investment and is carried out traditionally through submerged fermentation in large fermenters. The commercial Bt products are powders containing a mixture of dried spores and toxin crystals. They are applied to leaves or other environments where the insect larvae feed. Today they are used mainly in viticulture, forestry, green spaces and in potato, fruit and vegetable cultivation. The toxin genes have also been genetically engineered into several crop plants.

When cultured under appropriate conditions, Bt sporulates (endospore) and forms a crystalline parasporal body which contains the insecticidal protein toxin called delta endotoxin. This body is usually referred to as the crystal. Bt can be produced in a liquid medium by deep fermentation. When sporulation is complete, the bacterial cell lyses and releases the spores and crystals into the surrounding medium. The crystal is a bipyramidal aggregate of protein molecules (about 130-140 kDa) that is actually a **protoxin** - it must be activated before it has any effect. The crystal protein is highly insoluble in normal conditions, so it is entirely safe to humans, higher animals and most insects. However, it is solubilised in reducing conditions of high pH (above pH 9.5) - the conditions commonly found in the mid-gut of lepidopteran larvae. For this reason, Bt is a highly specific insecticidal agent. When ingested by the lepidopterous caterpillars, the crystal is activated by alkali dissolution and digestion by gut proteases, releasing the smaller, potent endotoxin of about 60kDa which binds to the midgut epithelial cells, creating pores in the cell membranes and leading to equilibration of ions. As a result, the gut is rapidly immobilised, the epithelial cells lyse, the larva stops feeding, and the gut pH is lowered by equilibration with the blood pH. The length of time until death depends on the species of insect and the dosage but feeding stops rapidly. The lowered pH in the gut enables the bacterial spores to germinate, and the bacterium can then invade the host, causing a lethal septicaemia.

Bt can be effectively used for management all the major lepidopteran pests of oilseed crops *viz*., *Achaea janata*, *Helicoverpa armigera*, *Conogethes punctiferalis* etc. *Spodoptera litura* is less susceptible to Bt *kurstaki*. In spite of the proven efficacy against insects as well as safety to environment, prohibitive cost of commercial Bt formulations (Delfin, Dipel, Halt etc.) ranging Rs.1500–3600 per kg as well as the lack of ready availability limits the adoption of recommendation of Bt for insect pest management in India.

Work at the Directorate of Oilseeds Research, Hyderabad, on Bt with specific reference to cost-effective production, field testing, transfer of technology has met with success. A simple, cost-effective protocol based on on the principle of solid state fermentation for the multiplication of Bt was developed for which a patent application was filed by ICAR on 10/07/2002. One local isolate DOR Bt-1 isolated from a dead castor semilooper larva collected from farmers' fields at Kothakota has been identified for large scale field use. The formulation has been registered with the Central Insecticides Board. The technology is being offered on a non-exclusive basis for commercial exploitation and has been licensed to 40 firms till date. The formulation is a a wettable powder and can be effectively used for management of castor semilooper *Achaea janata* on castor @ 1.0 g/l. It is indigenously produced and is available commercially under several trade names like Cezar, VBt, Dipole, JAS Bt, Caterpillin, Beater. Mahastra etc. The strain has also been formulated as a suspension concentrate which can be used effectively for management of castor semilooper on castor and *Helicoverpa armigera* on sunflower @ 1.0 ml/l.

**Entomopathogenic fungi**

More than 700 species of fungi, mostly Deuteromycetes and Entomophthora from about 90 genera are pathogenic to insects. Genera that have been most intensively investigated for mycoinsecticides include *Beauveria, Metarhizium, Verticillium, Hirsutella, Erynia, Nomuraea, Aspergillus, Aschersonia, Paecilomyces, Tolypocladium, Leptolegnia, Coelomomyces* and *Lagenidium*. The first two genera have been used on a large scale over a number of years while others have been aimed mainly at glasshouse pests or disease vectors. There is a world wide resurgence of interest in the use of entomopathogenic fungi as biological control agents, and a significant advance in development and manufacturing of these agents in the future is expected with recent biotechnological innovations.

**Mode of Infection**: The fungus invades the host cuticle through the body wall and spiracles primarily and also through the mouth parts. The conidia germinate on the insect cuticle by producing germ tubes which penetrate the body wall. The penetration is both mechanical (pressure exerted by germ tube) and enzymatic through the action of proteinase, lipase and chitinase on the cuticle. Growth of the fungus after it reaches the haemocoel is by budding which produces hyphal bodies. These are transported through out the haemocoel and give rise to localised concentration of mycelia. A heavy growth of intertwining mycelia develops in the haemocoel 1-2 days later. The larval body is completely mummified and covered by a dense white mycelial mat from which conidiophores arise close together and produce conidia 1-2 days later. Under suitable environmental conditions (essentially high humidity), death is followed by external sporulation, which helps to spread the fungus and establish an epizootic which may result in very high levels of kill. Fungi, unlike bacteria or virus, do not require ingestion for infection, so sucking insects are also targets either by primary contact of a mycoinsecticide or by secondary uptake of the pathogen from sprayed vegetation.

Toxin production is reported for *Metarhizium anisopliae, Beauveria bassiana, Verticillium lecanii, Paecilomyces* etc. Several studies have revealed positive correlations between RH/rainfall with rate of fungal infections thus indicating that fungal survival and spread are assured under higher rainfall and humidity conditions.

Entomofungal pathogens recorded from India include *Beauveria bas*siana, *Metarhizium anisopliae*, *Verticillium lecanii*, *V. aphidicola*, *Nomuraea rileyi* from *H. armigera*, *S. litura* and *Trichoplusia* spp., *Hirsutella* spp., *Erynia ne*oaphidis, *Paecilomyces farinosus*, *Pandora delphacis*, *Entomophthora aulicae*. Several species of entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium an*isopliae, *Nomuraea rileyi*, *Hirsutella thompsonii* and *Verticillium lecanii* have been studied in detail for their efficacy in the laboratory and the field. Mass production techniques have been standardized. The entomopathogenic fungi have given promising results in humid and mild climate areas and seasons. In India, work with white halo fungus *V. lecanii* has brought down the coffee green bug *Coccus viridis* to almost negligible proportions in the Pulney hills. The green muscardine fungus *Metarhizium anisopliae* has also been useful for the control of rhinoceros beetle of coconut Oryctes rhinoceros.

At the Directorate of Oilseeds Research (DOR), studies with the fungal pathogen *Nomuraea rileyi* have generated the basic data pertaining to effective field dose, field persistence, instar susceptibility, identification of virulent isolates, cost-effective mass multiplication and formulation. This fungal pathogen infects a majority of noctuids viz., *Helicoverpa armigera*, *Spodoptera litura*, *Plusia* spp. etc. UAS Dharwad has undertaken mass production of the fungus and is making it available to farmers. Commercial formulations of *B. bassiana* are currently available in India. Commercial formulations of *Beauveria bassiana* are available under several tradenames *viz*., Daman, Toxin$^{M}$ etc. can be used effectively for management of *H. armigera* @5.0 g/l. A liquid formulation Mycojaal (10% SC) can be used effectively @ 2.0 ml/l. Sprays need to be undertaken when climatic conditions are congenical *ie*., high RH (70% and above) and low temperature (around $30^{0}$C or less).

A protocol for low cost mass production of *B. bassiana* as well as its formulation in oil as a suspension concentrate has been developed at DOR and commercialized. The technology has been licensed to 4 firms till date. This formulation is a low-volume formulation that can be used for management of Helicoverpa armigera on sunflower and pigeon pea @ 0.3 ml/l.

**BACULOVIRUSES**

Viruses of several types are known to infect insects but baculoviruses are found only in arthropods and have not been recorded from vertebrates/plants, safe to man and non-target organisms and hence have received priority consideration for development as insecticides. Insect baculoviruses are important natural regulating factors for insect populations particularly Lepidoptera. Their commercial development has been limited by the need to produce them *in vivo* and by their generally high specificity, which while desirable from an environmental point of view limits markets. Although baculoviruses have not had the commercial successes of *Bt*, they have significant potential for use in organic farming because they do not affect non-target insects (parasites and predators), safe to humans and environment. In pest management, it is important to have a selection of control agents when designing strategies. Viruses are not likely to evoke cross resistance to chemicals or to each other, so more attention must be given to viral pesticides.

**BACULOVIRUSES**

Non-occluded | Occluded Baculoviruses | Calyx virus (associated with the reproductive system of parasitic Hymenoptera)

Occluded Baculoviruses: NPV GV

Epizootics of baculovirus diseases are frequent in lepidoptera and sawflies with very high larval mortalities resulting in strong population depression.

Epizootic development requires:

a) several host generations
b) high virus infecting capacity
c) persistence of the virus inside and outside the host.

Baculoviruses survive long periods in soil. Reservoirs of baculovirus in soil have long term importance and initiate epizootics when insect populations resurge following a phase of low density.

***Symptoms of Viral infection***

Dead larvae may be found hanging from or lying on leaf or plant surfaces.

The cuticle may be very fragile, rupturing easily when touched, releasing the body contents which have become liquefied.

Porcelain white masses of fat body may be visible through the cuticle.

**Baculoviridae** are characterised by the presence of proteinaceous inclusion bodies within which either a single virus particle (GV) or many virus particles (NPV) are embedded. The matrix protein within which the virus particles (Virions) are embedded are called **polyhedrin** for NPV and **granulin** for GV. The polyhedral inclusion bodies (PIB) of NPV are upto 5Φm in diameter and may contain several hundred virus particles. Each virus particle may contain one or more than one nucleocapsid. The inclusion bodies of GV are called capsules or granules and measure 400 - 500 nm x 200 - 300nm. Each capsule contains a single virus particle.

**Mode of Infection:** Infection occurs most commonly via the midgut following ingestion of inclusion bodies. When infected, and under the alkaline conditions of the lepidopterous and sawfly midgut, the inclusions breakdown releasing virus particles. In lepidoptera, the virus particles pass through the midgut cells, sometimes with a replicative phase and enter the haemocoel. Major sites of replication are the nuclei of fat body, haemocytes and hypodermis.

All viral insecticides are currently produced in susceptible host insect larvae, most of which are reared on artificial diet. This is usually labor intensive and expensive but does not require much capital investment. Therefore, cost of crop protection basically depends on the number of larvae which have to be reared, infected and harvested to treat one hectare typically 100 - 1000. Labor is usually cheap and plentiful in developing countries and hence virus can be easily produced at a reasonable cost. Long term benefits can be achieved through the use of NPV since most of the dead larvae remain on the plant with their integument ruptured resulting in release of NPV laden haemolymph to persist in the soil and result in epizootic spread of the disease in the next crop.

Several bio-pesticide firms in India are producing and selling NPV formulations of *S. litura* and *H. armigera*. These NPVs can be used effectively for management of *S. litura* and *H. armigera* @ 250-500 LE/ha. It is essential to undertake these sprays during evening hours and should be directed against younger stages of larvae.

**Table 1: Candidate microbial pesticides available for pest control**

| **Pathogen** | **Major Target Groups** |
|---|---|
| *Bacillus thuringiensis* (Cry I A-G, Cry II A-C, Cry III A-D, Cry IV A-D, Cry II A) | Lepidoptera, Coleoptera, Diptera |
| *Bacillus sphaericus* | Diptera |
| Nuclear Polyhedrosis Viruses | Lepidoptera |
| Granulosis Viruses | Hymenoptera |
| *Beauveria bassiana* and *Metarhizium anisopliae* | Coleoptera, Lepidoptera, Hymenoptera, Orthoptera |

| | |
|---|---|
| *Beauveria brongniartii* | Coleoptera, Homoptera, Lepidoptera, Diptera |
| *Nomuraea rileyi* | Lepidoptera |
| *Verticillium lecanii* | Homoptera, Thysanoptera, Diptera |
| *Lagenidium giganteum* | Mosquito larvae |
| *Paecilomyces fumosoroseus* | Coleoptera, Homoptera, ematodes |

**References**:


Burges, H. D. (Ed.) 1998. Formulation of Microbial Biopesticides, beneficial microorganisms, nematodes and seed treatments. Publ. Kluwer Academic, Dordrecht, 412 pp.

Flexner, J. L. and Belnavis, D. L. 2000. Microbial Insecticides. In "Biological and Biotechnological Control of Insect Pests" (Eds. J. E. Rechcigl and N. A. Rechcigl), 35-56, Lewis Publishers, CRC Press, New York.

Lisansky, S. 1997. Microbial Biopesticides. In "Microbial Insecticides: Novelty or Necessity?" British Crop Protection Council Proceedings/Monograph Series No.68, 3-10

Copping, L. G. (Ed.) 2002. "The Biopesticide Manual" 2nd edition, British Crop Protection Council, Farnham, Surrey, UK, 528 pp.

Vimala Devi, P.S. and Sudhakar, R. 2006. Effectiveness of a local strain of *Bacillus thuringiensis* in the management of castor semilooper *Achaea janata* on castor (Ricinus communis). *Indian Journal of Agricultural Sciences*, 76(7), 447-449.

Vimala Devi, P.S. and Prashanth P. Hari. 2009. Identification of a virulent isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, its mass multiplication and formulation for development into a mycoinsecticide for management of *Helicoverpa armigera* (Hübner). *Journal of Biological Control*, 23(2), 137-144.

Vimala Devi, P.S. and Prashanth P. Hari. 2009. *Beauveria bassiana* suspension concentrate – A mycoinsecticide for the management of *Helicoverpa armigera* (Hübner). *Journal of Biological Control*, 23(4) –403-408.

Vimala Devi, P.S., and Duraimurugan, P. 2013. Exploitation of *Nomuraea rileyi* and *Beauveria bassiana* for the management of lepidopteran pests. *KAVAKA*, 41: 43-67.

Vimala Devi, P. S. and Vineela, V. 2015. "Suspension Concentrate Formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (*Helianthus annuus*)". Biocontrol Science and Technology. 25:3, 329-336, DOI.10.1080/09583157.2014.977846.