



Microbial Pesticides for Insect Pest Management: Success and Risk Analysis

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biotic stress is a major cause for pre and postharvest losses in agriculture. Food crops of the world are damaged by more than of 10,000 species of insects 30,000 species of weeds, 1,00, 000 types of diseases (due to fungi, viruses, bacteria and various microbes) and a 1,000 species of nematodes. Modern day management practices for the above specified stress factors largely depends on the utilization of synthetic pesticides. Pesticide misuse in numerous sectors of agriculture frequently has often linked to health issues and environmental pollution around the world. Thus, there is a growing interest in replacing or possibly supplementing the prevailing control strategies with new and safer techniques. One of the promising management tools in this new state of affairs for crop protection is microbial pesticides. At present, only 3% of plant protectants used globally are covered by bio pesticides, but their growth rate indicates an increasing trend in the past two decades. The discovery of insecticidal property of *Bacillus*

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thuringiensis (*Bt*) indicated a more extensive part of organism based natural control. Microbial pesticides comprise of a microorganisms (bacterium, fungus, virus or protozoan) or toxins produced by them as the active ingredient. The most commonly used microbial pesticides are entomopathogenic fungi (*Metarhizium*, *Beauveria* and *Verticillium*), entomopathogenic bacteria (*Bt*), entomopathogenic nematode (*Steinernema* and *Heterorhabditis*) and baculoviruses (NPV and GV) which able to cause disease in insects. Microbial insecticides are promising alternative to ecologically disruptive pest control measures as they are no longer harmful to the environment and non target organisms. If deployed appropriately, microbial insecticides have capability to bring sustainability to global agriculture for food and food safety.

Keywords: Environment; microbial pesticides; risk; bio-pesticides; bacillus; entomopathogens.

1. INTRODUCTION

Agricultural sector plays a strategic role in the process of economic development of India [1]. The increase in agricultural production and the increase in rural per capita income, together with industrialisation and urbanisation, are leading to an increase in demand for industrial production [2]. The agriculture has remained backbone of the Indian economy since independence and presently accounts for nearly 15% of the country's GDP. Around 58 % of the rural households count on agriculture as their capability of livelihood. The major constrains in the crop production is biotic and abiotic agents. Biotic agents encompass greater than 10,000 species of insects, 30,000 species of weeds, 1, 00,000 diseases (caused by fungi, viruses, microorganism and other microbes) and a 1,000 species of nematodes which are detrimental to the food plants in the course of the arena [3-4] However, the damage caused by insect pests is relatively high due to the prevalence of tropical climates in India. The yield loss estimation due to insect pests in Indian agriculture was predicted occasionally [5-6]. The Cotton crop continues to suffer the most losses (30%), followed by rice (25%), sugarcane and rapeseed-mustard (each 20%), Maize (18%), Groundnut and Pulses (15%), other oilseeds (12%), coarse cereals (8%) and wheat (5%) [7].

Indian agriculture suffers heavily due to insect pest damage with an estimated 16.8% annual yield loss amounting to US \$36 billion [8]. The expanded damage to food crops due to insect pests and following losses poses a severe threat to food security and also emphasises the significance of agrochemicals in Indian agriculture [9]. The Indian plant protection market is dominated with insecticides, which contributes nearly 60% of domestic crop protection chemical components market [10]. The foremost applications are observed in Rice and Cotton

growing belt. Fungicides and herbicides are the most important growing segments accounting for 18% and 16% of total crop protection chemicals market, respectively [11]. The aim of crop protection is to reduce the crop losses due to pests to acceptable level with least effect on different components of the environment [12]. Due to sole reliance on insecticides for the management of insect pest resulted in insecticide resistance, resurgence and accumulation of insecticide residue in different stage of food chain [13]. Hence, dependence on chemical pesticides should be reduced to produce healthy crop and at the same time prevent health hazards to the humans and environment [14-15]. Emphasis needs to be given to other eco-friendly approaches such as cultural practices, mechanical methods, biological control (use of predators, parasitoids and microbial agents) and transgenic crops [16]. There was an incredible development in the improvement of quite a number of bio-pesticide techniques over the last two decades. This assessment summarizes the fulfilment of presently evolved microbial insecticides and risks involved in its usage for the pest control.

2. MICROBIAL PESTICIDES

Deployment of microorganisms viz., such as bacteria, fungi, nematodes, viruses or protozoans, which capable of causing diseases in insects is another means of fighting crop pests [17]. The term "Microbial control" was coined by Steinhaus in 1949. These bio-pesticides consist of the spores and virions as the active ingredient. In India, 295 pesticides have been registered and out of which 15 are bio-pesticides under The Insecticide Act [18] (<http://www.cibrc.nic.in>). The popularity of bio-pesticides among the farming community has increased in recent years, as extensive and systematic research has greatly enhanced their effectiveness. Also, techniques for the mass production, storage, transport and

application of bio-pesticides have been improved in recent years [19].

2.1 Bio-pesticides in India: Current Status

The concept of biological control of insect pest and diseases has been in practice for a very long time [20]. The derivatives of neem tree, *Azadirachta indica* i.e. leaf extract, oil and seed cake have been using as seed grain protectant since long time [21]. However, the importance of bio-control in India, was realized when chemical insecticides failed to control *Helicoverpa armigera*, *Spodoptera litura*, and other pests of cotton [22]. It was understood that bio-control is the only alternative control method that can be utilized to control widespread insect pest and diseases, which developed resistance against chemical pesticides.

A total of 361 bio-control laboratories and units operate in India, according to the Directorate of Plant Protection, Quarantine and Storage (DPPQS), but only a few of them are involved in production (<http://ppqs.gov.in/divisions>). The data suggests that, the usage of bio-pesticides has increased in the past two decades. However, the share of bio-pesticides is only 2 percent of the overall pesticide market [19]. There are currently 970 bio-pesticide products registered with the Central Insecticides Board and Registration Committee (CIBRC), which is India's top governing body for all forms of bio-pesticides use. The industries are producing bacterial, fungal, viral and other (plant-based, pheromones) bio-pesticides with a percentage share of 29, 66, 4 and 1, respectively (<https://ppqs.gov.in/statistical-database>)

2.2 Entomopathogenic Fungi (EPF)

The fungi have distributed globally, and can cause diseases in many organisms. Among which, the entomopathogenic fungi (EPF) are insect parasites which cause death or seriously disable them. The first insect pathogenic research has been accomplished in the 1980's and their major focus was on to locate the strategies of disease management of the silkworm [23]. The germ theory was proposed by Bassi during 1835, while studying diseased silkworm larvae infected with white muscardine fungus, later called *Beauveria bassiana* in his honor. About 750 species of EPF described under all classes viz., Phycomycetes,

Ascomycetes, Basidiomycetes and Deuteromycetes [24]. Species which have been most intensively investigated within the crop pest control encompasses *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *M. Anisopliae* var. *acridium*, *Lecanicillium*, (previously *Verticillium lecanii*), *Hirsutella thompsonii*, *Nomuraea rileyi* and *Isaria fumosorosea* (previously *Paecilomyces fumosoroseus*). Fungi infect all the orders of insects, most commonly on Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera.

2.3 Mode of Action

In the infection and sporulation of EPF, environmental conditions, particularly relative humidity and temperature, play an important role [25]. High humidity (> 70%RH) is required for the germination of spores and sporulation outside the host. Most EPF in tropical and subtropical areas require an optimum temperature of 25-30°C for successful insect pest control. Firstly, the fungal spores settle on the insect cuticle and then the spores germinate and enter the cuticle by forming appressorium. The infective spore on germination penetrate the cuticle [26], either by mechanical pressure or enzymatic action [27-28]. Generally chitinase, protease and lipase play an important role in the pathogenicity by breaking down the insect cuticle for penetration of fungal germ tube into the insect body. Hyphae can also initiate infection. The mycelium develops in hypodermis and they continue to multiply in insect body and blood cells by continuously drawing nutrients from the insect body. The EPF typically cause insect mortality by means of nutritional deficiency, destruction of tissues and with the aid of release of toxins. Several mycotoxins like, Beauvericin, Beauverolides and Bassianolide (by *B. bassiana*, *V. lecanii*, *Paecilomyces spp.*) and Destruxins A, B, C, D, E, F (by *M. anisopliae*) are produced in the course of pathogenesis, which are toxic to the insects. The fungus breaks the integument after the death of the insects, and forms aerial mycelia and sporulates on the cadavers [29].

TNAU-Agrobiocide: A bio-pesticide formulations based on *Fusarium* sp. isolated from the dead mite has been released by TNAU, Coimbatore under the brand name "TNAU-Agrobiocide". It is used to control coconut mite as root feeding technique @ 30 ml/tree.

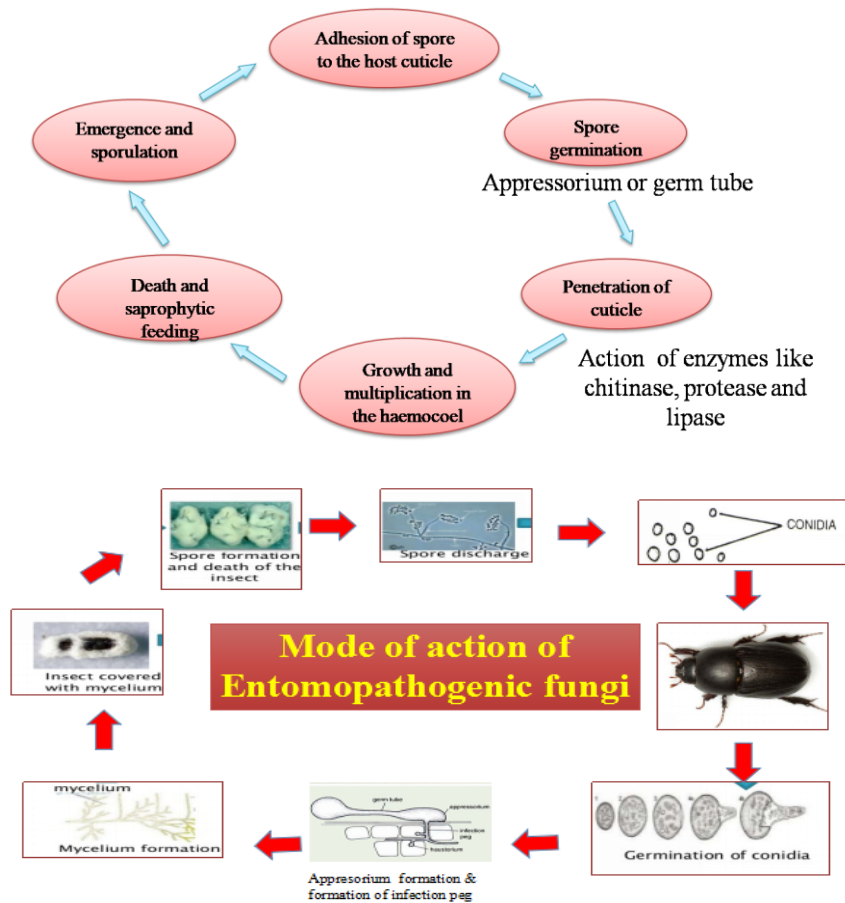


Fig. 1. Mode of action of entomopathogenic fungi (Courtesy from Tanada and Kaya, [29])

2.4 Entomopathogenic Bacteria(EPB)

Microbial bio-pesticides based on entomopathogenic bacteria are the commercially most successful among farmers. They dominate the bio-pesticide market globally due to their cost-effective mass production, specificity, persistence in the environment, and safety to non-target organism [31]. Most of these pathogenic bacteria belong to Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae and Micrococcaceae families. Members of Bacillaceae, in particular *Bacillus spp.*, received maximum attention as microbial control agents [29]. *Bacillus thuringiensis* is a gram-positive, rod-shaped, spore-forming, facultative entomopathogenic soil dwelling bacteria. *Bt* characterized by the production of a parasporal inclusion bodies upon sporulation and the crystal protein is toxic to insects and other invertebrates. Upon sporulation, *Bt* produces crystals of insecticidal protein *i.e.* δ -endotoxins (Cry proteins), which are encoded by cry genes.

Cry genes are found within the bacterial plasmid in most strains of *Bt*.

Commercial history and Uses: During an investigation into wilt disease in silk worms, this bacterium was isolated in 1901 by the Japanese biologist Shigetane Ishiwatari and he named it *Bacillus sotto* [32]. Ten years later, Ernst Berliner isolated the same bacterium from diseased larvae of Mediterranean flour moth, *Ephesia kuhniella* Zeller in the German province of Thuringia, and it was called *Bacillus thuringiensis* [33]. The first commercial product, Sporine produced in France in 1938 and then in the USA in the 1950s. *Bacillus thuringiensis* serovar *israelensis*, is widely used in the management of mosquito larvae, where it is considered as an environmentally friendly method of mosquito control. The spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s [34]. They are now used as specific insecticides under trade names such as Dipel, Halt, Delfin and Thuricide.

Table 1. Different entamopathogenic fungi manufacturing companies in India

Pesticide	Company	Formulation
<i>Beauveria bassiana</i>	M/s Agriland Biotech Ltd, Gujarat	5% WP
	M/s T. Stanes&Company Ltd., Coimbatore	Bio powder WP
	M/s International Panaacea, New Delhi	1*10 ⁹ cfu/gram
	M/s Sri Biotech, Hyderabad	1.15% WP
	M/s Pest Control (India) Pvt. Ltd	10% SC
	M/s Om Agro Organics, Yavatmal, Maharashtra	1.15% WP
	M/s Viswa Mithra Bio Agro P. Ltd., Guntur	1.15% WP
	M/s Amit Biotech, Kolkata	2.15% WP.
	M/s Varsha Bioscience & Technology, Hyderabad	1.15% WP
	M/s NirmalOrgano Bio Tech, Maharashtra	1.15% WP
<i>Metarhizium anisopliae</i>	M/s T. Stanes& Company Ltd., Coimbatore (T.N)	1.5% Liquid formulation
	M/s International Panaacea Ltd., New Delhi	2.0% AS
	M/s Microplex Biotech & Agrochem Pvt. Ltd., Wardha (MS)	1.15% WP
	M/s Pest Control (India) Pvt. Ltd., Bengaluru	10% Granular (ITCC 6911)
	M/s Govinda Agro Tech Ltd., Nagpur (Strain obtained from AAI, Allahabad, UP, Accession No. NACC-03037)	1.15% WP
<i>Verticillium lecanii</i>	M/s Parvara Bio Tech Ahmednagar, MS (Strain Designation: AS-MEGH-VL, Accession No. MCC-1028)	1.15% WP
	M/s Indian Institute of Horticultural Research, Bangalore	1% WP.
	M/s Agri Life, Secunderabad	1.15% WP
	M/s Varsha Bio Science & Technology, Hyderabad	1.15% WP
<i>Hirsutella thompsonii</i>	M/s International Panaacea Ltd., New Delhi	2.0% A.S

Table 2. Pests control using entomopathogenic fungi in India [30]

Fungus	Pest & Crop	Field efficacy
<i>Beauveria bassiana</i>	Coffee berry borer, <i>Hypothenemus hampei</i>	Spray of <i>B. bassiana</i> spore suspension (1×10^7 spores/ml) containing 0.1% sunflower oil and 0.1% wetting agent during monsoon reduced 50-60% berry borer incidence in Coorg, Karnataka
	Tea looper, <i>Buzura suppressaria</i>	Spray of <i>B. bassiana</i> spore suspension (2.5 g/l), gave 88% of pest reduction in West Bengal
	Sunflower: <i>Helicoverpa armigera</i>	Spray of oil suspension of <i>B. bassiana</i> (200mg/l) in Andhra Pradesh
	Green gram: White grubs	Soil application @ 5×10^{13} conidia/ha effective control achieved in Assam
<i>Beauveria brongniarti</i>	Sugarcane: white grub, <i>Holotrichia serrata</i>	Soil application @ 1kg/acre. Highest yield recorded
<i>Metarhizium anisopliae</i>	Coconut: Rhinoceros beetle, <i>Oryctes rhinoceros</i>	Spraying of spores in its breeding sites @ 5×10^{11} spores/m ³ to the compost pits and manure
	Pigeon pea: Pod borer, <i>Helicoverpa armigera</i>	<i>M. anisopliae</i> conidia in an oil formulation was effective in reducing <i>H. armigera</i> (66.74%) as compared to with Endosulfan in Maharastra(62.58%)
	Potato White grub, <i>Brahmina</i> sp.	Soil application @ 5×10^{13} conidia/ha along with chlorpyrifos 20 EC at 200 g a.i./ha resulted in the highest tuber yield (155 q/ha) in HP.
<i>Verticillium lecanii</i>	Coffee green scale, <i>Coccus viridis</i>	Spraying spores @ 16×10^6 spores/ml along with Tween-80 twice at 2 weeks interval caused 97.6 % mortality of the pest.
	Citrus green scale, <i>Coccus viridis</i>	Spraying of spore (2×10^6 spores/ml) along with 0.005% Quinalphos and 0.05% Teepol was found highly effective killing 95.58% and 97.55% scales in coffee and citrus respectively.

Table 3. Different entamopathogenic bacteria manufacturing companies in India

Pesticide (Strain/species)	Company/Institute	Formulation
<i>Bacillus thuringiensis</i>	M/s Rallis India Ltd., Bangalore	Bobit II WP
<i>Bacillus thuringiensis kurstaki</i> var. H 3a, 3b, 3c	Directorate of Oilseeds Research, Hyderabad	0.5% WP
<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	M/s Sandoz (i) Ltd.	Deflin WG
<i>Bacillus thuringiensis</i> var. <i>kurstaki</i> (Serotype 3a3b3c)	M/s Nitapol Industries, Kolkata	(Strain: DOR Bt-1) 0.5% WP
<i>Bacillus thuringiensis</i> var. <i>Kurstaki</i>	M/s Tuticorin Alkali chemicals & Fertilizers, Chennai	Tech & 2.5% Aqueous Suspension
<i>B.thuringiensis</i> var. <i>gallerine</i>	M/s Tuticorin alkali chemicals & fertilizers Ltd., Chennai (serotype 3a, 3b)	Tech (I), 0.5% WP
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	M/s Biotech International Ltd., Hyderabad	Tech (I) 1.15% WP
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	M/s Tuticorin Alkali Chemicals & Fertilizers Ltd., Chennai	Tech
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	M/s Aventis Crop Science India Ltd., Mumbai	Formulation For port
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	M/s tuticorin Alkali chemicals& fertilizers, Chennai	<i>Bti</i> (Aqueous Suspension)
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (H-14)	M/s Aventis Crop sci. Ltd	Vectobac 12 AS
<i>Bacillus sphaericus</i>	M/s Tuticorin alkali chemicals & fertilizers Ltd. (1543M serotype H 5a, 5b)	Tech
<i>B. sphaericus</i>	M/s Biotech International Ltd.	Tech (I) 1.15% WP
<i>Bacillus Subtilis</i>	M/s Biotech International Ltd., New Delhi	1.5 % AS
<i>Bacillus subtilis</i>	M/s Prathibha Biotech, Hyderabad (A)	2.0% WP
<i>Bacillus subtilis</i>	M/s Kan Biosys Pvt. Ltd., Pune (Strain: Designation: KTSB 1015, Strain Accession No.-MTCC-5786).	1.5% AS

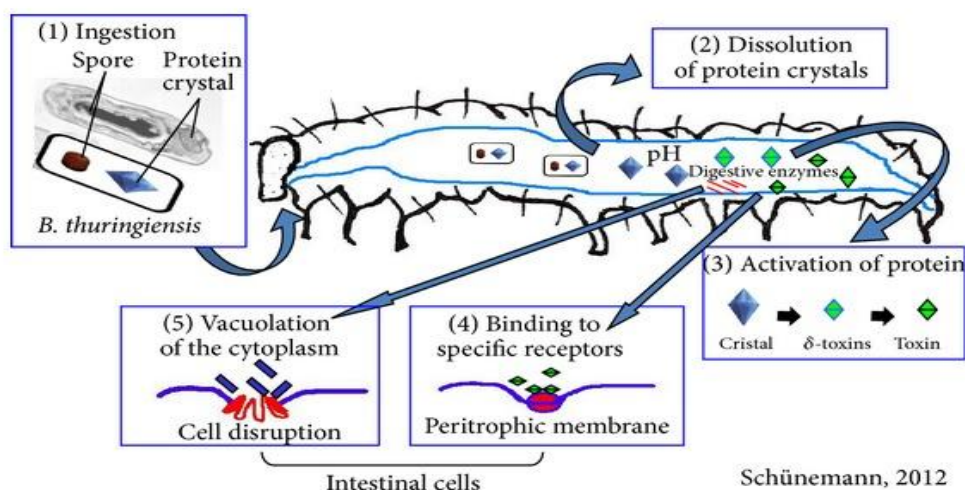


Fig. 2. Mode of action of *Bt* bio-pesticide [41]

Mode of action: Most of the insect pathogenic bacteria produce insecticidal toxins as the main mechanism for virulence. The toxin produced by bacteria mainly involves Cry, Cyt, Vip and Bin toxins [35-36]. The Cry toxins are the most extensively studied and characterised among these insecticidal toxins. After a susceptible insect has ingested the Cry protein, it is solubilised by the gut pH (8.5-11) and physicochemical conditions assisted by the proteases resulted in conversion of protoxin to an activated toxin in the digestive fluid of the insects. A solubilised and trypsinized forms of Bt proteins incorporated in artificial semi-synthetic diet of aphids showed the considerable mortality of Cotton aphid *Aphis gossypii* [37]. The crystalline toxin of the bacterium makes the gut permeable, the core of toxin travels through the peritrophic matrix and binds to the specific receptors on the brush border membrane like cadherin-like proteins, aminopeptidase-N [38], alkaline phosphatase [39]. Binding to these proteins facilitates accumulation of toxin oligomers on specialized membrane regions called lipid rafts [40] favouring insertion of the oligomer and formation of a toxin pore that leads to cell death by osmotic shock.

***Bt* Strains:** About 100 pathogenic bacteria described from *Bacillus thuringiensis* Berliner has been found to be most useful and it is the most commonly used commercial bio-pesticide worldwide [42]. The commercial formulations of *Bt* are used both for the control of agricultural and public health pests. The insecticides available are based on a number of *Bt* sub

species which commonly include *B. thuringiensis* var *kurstaki* and *aizawai*, which are selectively toxic to the lepidopteran larvae of various crops, while, *B. thuringiensis israelensis*, possess activity against mosquito larvae, black fly (Simuliid) and fungus gnats. Whereas, *B. thuringiensis tenebrionis*, acts against larvae and adults of Colorado potato beetle (*Leptinotarsa decemlineata*) and *B. thuringiensis japonensis* strain Buibui, has activity against soil-inhabiting beetles [43].

Commercial *Bt* bio-pesticide Composition: Insecticidal crystal proteins (ICP), viable spores, enzyme systems (proteases, chitinases, phospholipases), vegetative insecticidal proteins and many unknown virulent factors along with inert/ adjuvant. Commercial *Bt*-based products include powders, suspension, aqueous (flowable) or oil concentrate granules containing a combination of dried spores and crystal toxins. These are applied on leaves or other environments where the insect larvae feed. Toxin genes from *Bt* have been genetically engineered into several crops.

2.5 Entomopathogenic Viruses (EPV)

Insect viruses are the sub-microscopic, obligate, intracellular organisms capable of causing diseases in insects. More than 1100 viruses that infect insects belongs to 15 families, the largest number described to date, over 600, are from the family Baculoviridae [44] [45-46]. Baculoviruses are occluded DNA viruses that are lethal pathogens of larvae of Lepidopterans,

mosquitoes, and sawflies. Entomopathogenic viruses fall into two groups, *i.e.*, inclusion viruses (IV) that develop inclusion bodies in the host cells and non inclusion viruses (NIVs) that do not develop inclusion bodies. However, the inclusion viruses (IV) are subdivided into polyhedrosis viruses (PV) containing polyhedral bodies and granulosis viruses (GV) containing granular bodies. Polyhedrosis could inhabit the nucleus are called nuclear polyhedrosis viruses (NPV) or in the cytoplasm which are called cytoplasmic polyhedrosis virus (CPV) according to the site of its multiplication [47]. An individual polyhedral is about 0.5 to 15 microns in diameter, never sphere-like and is usually refractive and crystal like with many faces, it is made of concentric layers like an onion.

Mode of action: Infections occur after ingestion of Occlusion bodies (OBs) by a susceptible larva, usually during feeding on OB-contaminated plant foliage. Once ingested, the OB is carried to the alkaline insect midgut (pH 8-10) where the OB dissolves to release the occlusion derived virion

(ODV) within minutes. The host alkaline protease in the insect midgut also helps in the degradation of OB. In order to establish the infection, the released ODV must pass through the peritrophic membrane (PM), found in the midgut, to access the midgut epithelial cells. The baculoviruses established a special mechanism to disrupt the PM and to ease the movement of ODV. They contain metalloproteases called enhancins, which helps the movement of virions through the peritrophic membrane. The virus particles are released from polyhedral and are then bound to the midgut lining of the peritrophic membrane. The lipoprotein membrane that surrounds the virus fuses the gut wall cells with a plasma membrane and releases nucleocapsids into the cytoplasm. The nucleotide carries viral DNA into the nucleus of the host cell and this marks the beginning of the expression of virus gene. The virus quickly multiplies and eventually fills the host body with virus particles. Just before death, insects fly up to the tree's top and hang from its prolegs. This symptom is referred to as 'Tree Top disease'.

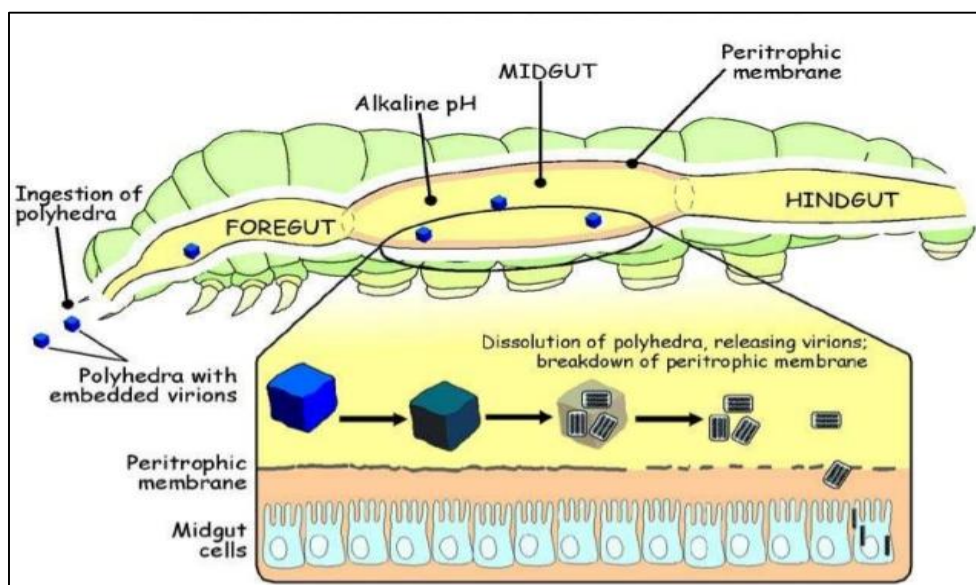


Fig. 3. Mode of action of NPV

Table 4. Nuclear polyhedrosis viruses recorded in India [48]

Pest	Crop
<i>Helicoverpa armigera</i>	Chickpea and others
<i>Spodoptera litura</i>	Tobacco and others
<i>S. exigua</i>	Tomato and others
<i>Amsacta moorei</i>	Pulses
<i>Agrotis ipsilon</i> , <i>A. segetum</i>	Potato and others
<i>Anadividia peponis</i>	Gourds
<i>Trichoplusia ni</i>	Potato and others

Table 5. Different NPV manufacturing companies in India

Pesticide	Company	Formulation
Nuclear Polyhydrosis Virus (NPV) of <i>Helicoverpa armigera</i>	M/s Pest control India Ltd., Bangalore	Helicide 0.50% AS
	M/s Biotech Industries Ltd. New Delhi	Biovirus-H
	M/s. Margo Biocontrols Pvt. Ltd.	64% AS
	M/s Om Agro Organics,	0.5% AS (POB count 1 x 10 ml or gm. Min.)
	M/s Biotech International Ltd., New Delhi	2.0% AS
	Dept. of Agriculture, Govt. of Uttar Pradesh, Lucknow	2.0% AS
	M/s Jyothiraditya Bio Solutions Ltd., Mysore	2.0% A.S.
NPV of <i>Spodoptera litura</i>	M/s Pest control India Ltd., Bangalore	Spodocide 0.50% AS
	M/s Ganesh Bio-control Systems, Gujarat	2% AS
	M/s Pest Control (India) Pvt. Ltd., Bangalore	0.5% AS
NPV of <i>Pseudomonas fluorescens</i>	M/s. Romvijay Bio Tech Pvt. Ltd.	0.5% WP

2.6 Entomopathogenic Nematodes (EPNs)

Entomopathogenic nematodes (EPNs) are soil-dwelling, obligate parasites of insect and are used as biological control agents of economically important insect pests. Even though 23 nematode families have been documented as EPNs [49], only two families such as Heterorhabditidae and Steinernematidae have generated considerable interest as potential biocontrol agents, as they carry lethal symbiotic bacteria such as *Photorhabdus* and *Xenorhabdus* in their guts respectively. The anterior part of the intestine of the infective juvenile (IJ) is modified as a bacterial chamber, which carries cells of a symbiotic bacterium. Due to variety of characteristics, the EPN can be considered as good candidates for integrated pest management and sustainable agriculture. It includes broad range target specificity, can actively ambush the host and is compatible with a wide range of chemical and biological pesticides used in IPM programs.

Life cycle: The members of the family, Steinernematidae and Heterorhabditidae have similar life cycles, and the only difference between the life cycles between them is occurred in the first generation. In Heterorhabditidae, all juveniles of the first generation will become hermaphrodites. In the second generation, males, females, and hermaphrodites develop. After mating, the females lays the eggs, which hatch into first-stage juveniles that moult successively to second, third and fourth-stage juveniles and then to males and females of the second generation. During late second juvenile stage, the nematode cease feeding and incorporate a pellet of bacteria in the bacterial chamber, and moult to the third stage (infective juvenile), retaining the cuticle of the second stage as a sheath, and leaves the cadaver in search of new hosts.

Mode of action: Infective juveniles (IJs), considered the only free-living stage of EPNs, enter the host insect through its natural apertures (oral cavity, anus, and spiracles) or through the cuticle only in *Heterorhabditis* [50]. The IJs release their symbiotic bacteria after penetrating the insect's haemocoel, which are the primary agents responsible for host death and also provide nematodes with nutrition and defence against secondary invaders [51]. Sometime the nematode also produces a toxin that is lethal to the insect. The nematode feeds on the bacteria and the digested insect tissues. The infected host usually dies within 24-48 hours by bacterial

toxins. The nematodes complete their development and live within their host for two or three generations. When food is exhausted, IJs leave the host cadaver to search for new hosts [52].

Commercial formulations: A major barrier in using EPNs as bio-insecticides is their shorter shelf life. This is partly due to their susceptibility to desiccation and UV radiation, and their poor tolerance of high temperature. Consequently, efforts were made to develop formulations that would overcome these limitations [54]. The novel technologies for EPN-application are, implanting *H. indica*-infected *Galleria mellonella* cadavers in the soil [55]. NemaGel, this is novel formulation, based on a newly formulated hydrogel, was found to increase the shelf-life of the indigenous nematode, *Steinernema thermophilum*. The nematode concentration in the NemaGel can be adjusted to a maximum of 1×10^5 infectious juveniles per gram. The survival of formulated nematodes was significantly better than that of aqueous suspensions after storage at 15°C for 9 months, and later 6, 2 and one weeks onward at 30, 35 and 40°C, respectively. More than 50 per cent survival of formulated infectious juveniles was recorded at 15°C even after 36 months of storage and at 30, 35 and 40 °C for 24, 16 and 8 weeks of storage.

2.7 Protozoan Pathogens

Protozoa are an extremely diverse group of single celled eukaryotic organisms. There are more than 1,000 species reported as pathogenic to insect, most commonly to the members of Lepidoptera, beetles, locusts and other Orthoptera. They are transmitted by fouled food, cannibalism and transovarially (transmitted to her progeny from infected females). They develop diseases that range from very pathogenic to chronic debilitating infections in insects. They can be important mortality factors which occur naturally. They are obligatory parasites which cannot complete their life cycles in artificial media. Entomopathogenic protozoans are highly diverse groups of organisms comprising around 1000 species attacking invertebrates and insect species, and are generally referred to as microsporidians [56]. Two genera, *Nosema* and *Vairimorpha*, have some potential as they attack Lepidopteran and Orthopteran insects and seem to kill hoppers more than any other insect [57]. They are generally host specific and slow acting, producing chronic infections with general debilitation of the host.

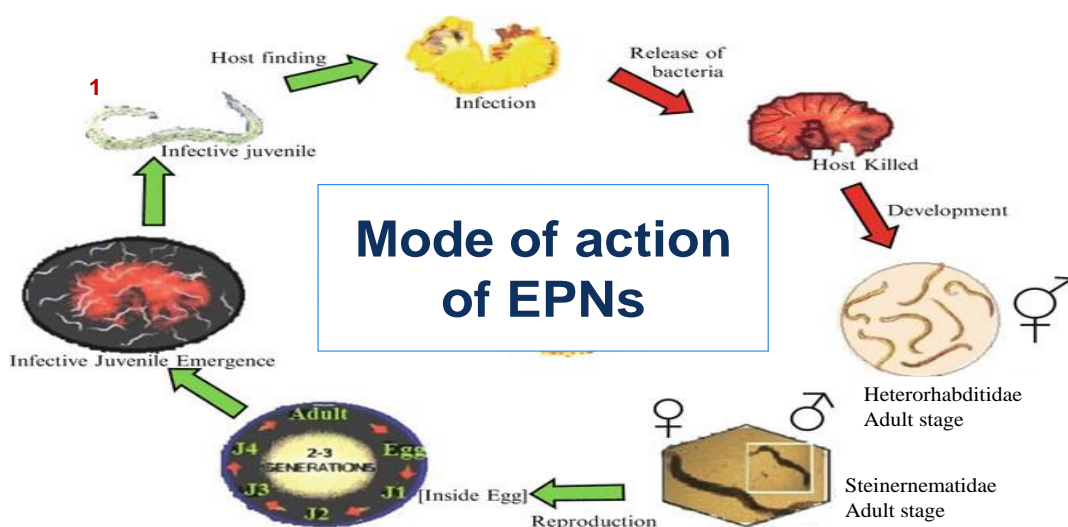


Fig. 4. Mode of action of entomopathogenic nematodes [53]

Mode of action: Typical genus *Nosema* spores (3 to 5 μm) germinate in the gut and live in cells of the gut, fat body and other tissue, gradually replicate and sporulate as organs destruction occurs. They often cause chronic, debilitating effects on the organisms. These are toxicity to vertebrates and slow speed of killing the host so its usage is very less as a bio-control agent (BCA).

Risk analysis: Risk assessment of BCAs should be conducted on a case-by-case basis and from a so-called 'remaining risk acceptance point of view' where appropriate. This means that micro-organisms should be used as BCAs when the control agent's benefits (efficacy and host specificity) exceed the risks, particularly when there is no danger of accumulation of toxins in the environment. A wide range of metabolites are secreted by fungi, some of which are important medicines or research tools [58]. While it is understood that some of these metabolites are essential determinants of pathogenicity or antagonistic factors, the function of others remains uncertain. Some metabolites are in vitro toxic to the animal cell lines [59]. Others have antibiotic, fungicidal, insecticidal or antiviral properties [60]. There is concern that toxic fungal metabolites may enter the food chain and create risks to humans and animals [61]. The persistence of *Bt* toxin was apparently the result of binding the soils to surface-active particles which reduced the toxin's biodegradation. The release of the toxin may enhance insect pest control or pose a threat to non-target species,

including soil microbiota, and increase the range of target insects that are toxin-resistant [62]. *Photorhabdus* a symbiotic mutualist with nematodes of the Heterorhabditidae family, which can cause fever and soft tissue infections [63].

3. CONCLUSION

The number and growth rate of microbial-pesticide showing an increasing marketing trend in past few decades. Microbial-pesticides are host specific and bio-degradable resulting in least persistency of residual toxicity so they can make vital contributions to IPM and can greatly reduce conventional pesticides, while crop yield remains high. Microbial-pesticides having lesser health hazard provides an important alternative in the search for an economically & environmentally sound and equitable solution to the problem of food security.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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