Chapter 19
Biocontrol Agents: Potential of Biopesticides for Integrated Pest Management

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Abstract Active compounds of biological origin and their synthetic derivatives are in high demand for crop protection over conventional pesticides since synthetic chemicals have reduced availability, adverse toxicological effects, and resistance and pest resurgence issues. Insecticides of biological origin (biopesticides) are less toxic and effective in small quantities and decompose quickly, leaving not much burden on environment. These are mostly target-specific and do not affect nontarget organisms much. Many of the bacteria, fungi, viruses, nematodes, protozoans, plants or plant-derived products (botanicals), pathogen/predator systems, insect pheromones, and plant-incorporated protectants (PIPs) are widely used as biological control agents for insect pest management (IPM). Among all, *Bacillus thuringiensis*-based biological insecticide has been primarily developed and commercialized. Biotechnological approaches such as transgenic technology and nanotechnology have recently come up that have potential to enhance expression and delivery mechanisms of biopesticide. Though the list is huge, only a limited number of living system-derived compounds have been used commercially, which are amenable to mass production and affordable to the growers. This chapter addresses the recent status of microbial control agents as biopesticides, which is used to improve agricultural productivity by restricting pest infestation.

Keywords Microbial pesticides · *Bacillus thuringiensis* · IPM · Bacterial · Fungal · Viral pesticides

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19.1 Introduction

Since ancient time, agriculture has been facing devastating harm caused by weeds, viruses, nematodes, fungi, insect pests, animals, and birds which has led to the decline in crop production. It has been evaluated that there has been a great loss of crop yield due to insects, diseases and weeds. To overcome this problem, various strategies were employed. One of the most commonly used methods to get rid of the pests is to use chemicals/synthetic pesticides (e.g. chlorinated hydrocarbons, carbamates, organophosphates, etc.). In spite of the success gained by the use of chemical pesticides, there are prospective health and environmental hazards/risks related with them. These chemical pesticides have long persistence period. Moreover, undiscerning and continuous application of these chemical products resulted in escalated residual problems, resistance among the pests and loss of some beneficial species. To overcome the hazards related to chemical pesticides, there is a need to adopt a coherent and eco-friendly approach. One such improvement in pest control tactic is to develop biopesticides which are derived from naturally occurring material such as plants, animals, microorganisms or their products. These are effective and biodegradable and pose less impact on the environment. The term 'biopesticide' is misleading in the sense it is not necessary that microbial agent for pest control will completely eradicate the pest, rather it suppresses and allow the crop to adequately develop some deleterious effect on the pest so that crop produce is not affected (Crump et al. 1999; Hynes and Boyetchko 2006).

Now a days, pesticides of biological origins are gaining popularity because of their low environmental impact and as a possible substitute to conventional synthetic pesticides, and a decline in the rate of usage of synthetic insecticides, occurrence of resistance to traditional synthetic pesticide, and increased public awareness about impact of synthetic pesticide on environment and humans have been observed. Some popular IPM strategies employ a combination of chemical and biological crop protection. Use of biological product at an appropriate time can reduce the total need for synthetic pesticides (Sara 2015). New biorational pesticides are also being developed which comprises pest control agents, chemical analogues of biochemicals such as pheromones, insect growth regulators, etc. These are more environment-friendly than synthetic chemical pesticides. The use of microbial control agents offers more realistic approach compared to chemical pesticides since it is an ecologically compatible IPM method (Koul and Cuperus 2007; Koul et al. 2008).

Biopesticides are broadly classified into several classes: microbial pesticides consisting of entomopathogenic bacteria (e.g., *Bacillus thuringiensis*), fungi (e.g., *B. bassiana*), or viruses (e.g., *Baculovirus*) including their metabolites, entomopathogenic nematodes, and protozoa. The member of *Bacillaceae* family, *Bacillus thuringiensis*, is widely used as biopesticide, since it produces a toxin that is active against many classes of insects (Fisher and Garczynski 2012). In addition, herbal/botanical pesticides provide coherent protection from pests and microbial diseases and can be used as plant-incorporated protectant (i.e., genetically modified crops like transgenic *Bt* cotton) though their use as food items is debatable (Sarwar 2015). Further, in order to improve the delivery methods of pesticide, nanomaterials have been designed as a carrier system that has potential to reduce the concentration of pesticide to be used (De Oliveira et al. 2014).

Improvements have been made in the production and formulation technology of microbial pesticides. But at the same time, the use of biopesticides has been restricted due to various constraints at developmental, registration, and production level. Although there are many developments in terms of novel discoveries of microbial isolates and increase in the ability of genetic manipulation, but concerns related to pest resistance, environmental issues, and human welfare still remain. In the current chapter, we focus on the use of biocontrol agents to control pest attack in order to improve crop production, and we attempt to provide the recent information on it.

19.2 Microbial Pesticides

The largest group of broad-spectrum biopesticides is derived from wide range of microorganisms such as bacteria, viruses, fungi, and nematodes. They are effective against pests and do not have much deleterious effect on nontarget pests and are safe for the environment. Microorganisms growing in the close proximity of plants can be either harmful or beneficial. Plant diseases caused by harmful microorganisms have caused serious loss to crop productivity. On the other hand, beneficial microorganisms increase soil fertility and help in pest control. Therefore, useful microorganisms are encouraged to be utilized in agriculture. Different types of useful microorganisms can be isolated, tested, and commercialized so that they can be used at larger scale (Fig. 19.1). Based on their origin, microbial pesticides have been broadly categorized as bacterial, fungal, viral and nematodal biopesticides.

19.2.1 Bacterial Biopesticides

They are the most widely used and inexpensive means of pest bioregulation (Sarwar 2015). A huge number of bacterial species have been reported with insecticidal properties, but only few could reach the stage of commercialization (Table 19.1).

19.2.1.1 Bt as Microbial Pesticide

The most well-known example of microbial pesticide is the bacterium *Bacillus thuringiensis* or *Bt* which is a Gram-positive, facultative, and spore-forming bacterium. There are nearly 100 well-known subspecies of *Bt* which have been reported to control certain insect pests (Schnepf et al. 1998; Jurat-Fuentes and Jackson 2012). They have wide host range, and they are active against Lepidoptera, Diptera

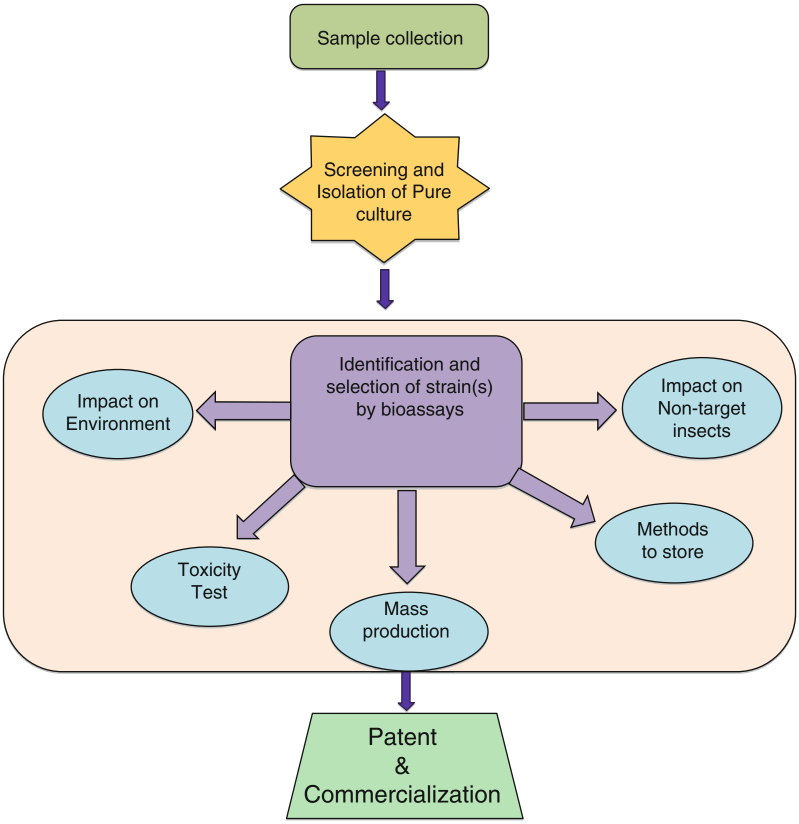

Fig. 19.1 A flowchart to depict the steps that are followed for screening and development of microbial pesticides

Table 19.1 Bacterial biopesticides developed to control pest attack on various crop plants

Name of the bacteria	Target pest
Bacillus popilliae	Members of Coleoptera
Paenibacillus popilliae	Coleoptera: Scarabaeidae: *Popillia japonica*
Bacillus thuringinesis var. kurstaki	Members of Lepidoptera and Coleoptera
B thuringinesis var. aizawal	Lepidoptera
B thuringinesis var. galleriae	*Helicoverpa armigera* and *Plutella xylostella*
B thuringinesis var. israelensis	Diptera: Culicidae, Simuliidae
B. thuringiensis subspecies *japonensis* strain Buibui	Coleoptera: Scarabaeidae
B. thuringiensis subspecies *tenebrionis*	Coleoptera: Chrysomelidae, predominantly *Leptinotarsa*
Lysinibacillus sphaericus	Diptera: Culicidae
Serratia entomophila	*Costelytra zealandica*
Chromobacterium subtsugae	*Leptinotarsa decemlineata*, Hemiptera, Acarina

(Nematocera), and Coleoptera (Chrysomelidae and Scarabaeidae) (Wei et al. 2003; van Frankenhuyzen 2009). Bt possesses the beneficial characteristics of both chemical pesticides and biopesticides, and, therefore, it is the most widely used microbial pesticide. Similar to synthetic pesticide, it is not expensive, can be easily formulated, acts quickly, and has an elongated shelf life; but unlike synthetic pesticides, they do not show much hazardous effect on environment and are specific to target organisms (Birch et al. 2011). The only disadvantage of *Bt* is its sensitivity toward sunlight; therefore, frequent applications are needed. *Bt* pesticides are available as formulated sprayable products of bacterial spores and endotoxin crystals and are used on broad acre crops. High level of selectivity and safety are required, when they are sprayed on fruits and vegetables. *Bt* formulations are not harmful to humans, vertebrates, beneficial organisms, and the environment (Chandler et al. 2011). A continuous monitoring of microbial pesticide is done so that it does not harm any nontarget organism including humans (Gupta and Dikshit 2010). In order to check the attack by lepidopteran insects (leaf rollers and defoliators) in orchards, two subspecies of *Bt*, *B. thuringiensis* subsp. *kurstaki* (Btk, Dipel) and *B. thurinigiensis* subsp. have been used (Glare et al. 2012). The above-mentioned subspecies are also utilized to control lepidopteran pests of crucifers, cucurbits, corn, legumes, cotton, and solanaceous vegetables. Btk is also applied to control the insect pests (*Plodia interpunctella* and *P. operculella*) of stored products such as grain, fruits and potato (Kroschel and Lacey 2009). Among coleopterans, Colorado potato beetle, *Leptinotarsa decemlineata*, is the main target of a subspecies of Bt, *B. thuringiensis* subsp. *tenebrionis* (Btt) (Wraight et al. 2007, 2009).

19.2.1.2 Mode of Action

Bacillus thuringiensis produce pesticidal toxins, namely Cry family of crystalline proteins that are encoded by the cry genes (Mazid et al. 2011). These are responsible for feeding cessation and death of the insect (Khachatourians 2009). Cry proteins possess three specific domains attached together by a single linker (Bravo et al. 2007). They are produced as protoxins of different length of which the longer C-terminal protoxins are involved in crystal formation and causing toxicity (de Maagd et al. 2001). When Cry proteins are ingested by the insects, after solubilization, biologically active endotoxins are released that are resistant to insect proteases (Schnepf et al. 1998; Whalon and Wingerd 2003). The C-terminal domain of this endotoxin binds to the receptors present on the cell membrane of the bush border of midgut after which the hydrophobic region of the toxin also gets linked to the membrane (Rodrigo-Simón et al. 2008). This linkage causes osmotic imbalance and formation of transmembrane pores leading to leakage of gut content and cell lysis in the gut wall (Fig. 19.2).

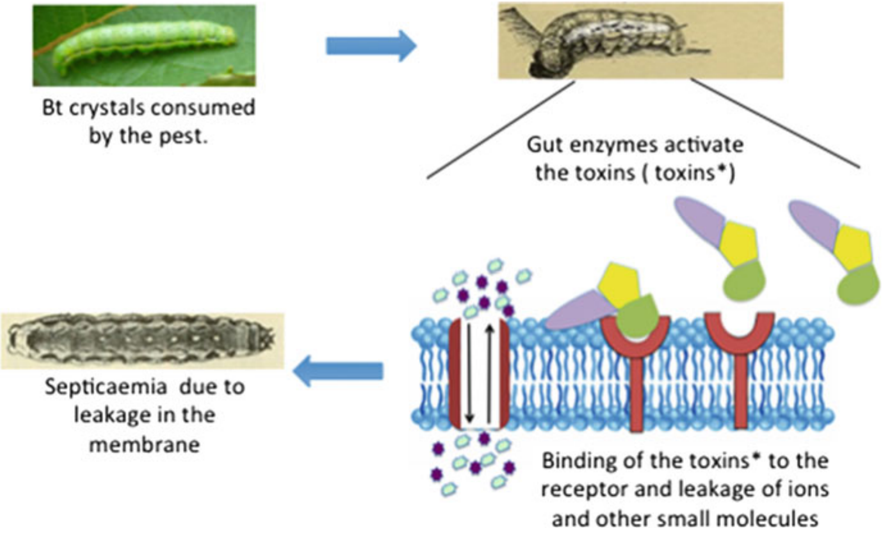

Fig. 19.2 Effects of *Bacillus thuringiensis* (*Bt gene* and *Cry protein*) on insect larvae

19.2.1.3 Bt-Crops

Bt-crops, a Bt product different from microbial pesticides, has been largely used in the last two decades. Genes coding for crystal proteins and vegetative storage proteins (VIPs) have been successfully transferred into different crop plants to form *Bt* transgenic crop varieties. In spite of huge controversy, Bt crops have been widely adopted due to its high efficacy and specificity. Moreover, they are safe for consumers and do not pollute the environment. There is availability of diversity of toxin genes from different strains that can be easily cloned, expressed and transformed to produce *Bt* crops (Kennedy 2008). Currently, approximately 75 classes of Cry toxins and 125 different VIPs are known (Crickmore et al. 2014). Transgenic 'Spunta' potato lines with the *Cry1Ia1* has been a great success providing complete resistance to potato tuberworm in laboratory and field tests (Douches et al. 2002). Another transgenic line of potato expressing Cry3Aa toxin shows significant resistance against *L. decemlineata.* In the last few decades, the area growing *Bt*-crops has increased at high rate. A growing interest in the use of *Bt-Brinjal*, *Bt*-cotton and *Bt*-maize has caused drastic decrease in the usage of chemical insecticides (Brookes and Barfoot 2012) as well as microbial pesticides. Due to high cost for generating GM crops, it is not possible to have transgenic variety for each crop. Therefore, other conventional but eco-friendly methods such as sprayable *Bt* formulations still have a great potential in the coming decades.

Table 19.2 Fungal biopesticides

Name of the fungus	Target pest
Aschersonia aleyrodis	Hemiptera (Aleyrodidae)
B. bassiana sensu lato	Acari, Diptera, Lepidoptera, Hemiptera, Isoptera Coleoptera, Diplopoda, Hymenoptera, Lepidoptera, Orthoptera, Siphonoptera, Thysanoptera
B. bassiana	Coleoptera, Acari, Diptera, Orthoptera, Thysanoptera, Hymenoptera, Hemiptera.
Beauveria brongniartii	Coleoptera (Scarabaeidae)
Conidiobolus thromboides	Acari Hemiptera, Thysanoptera
Hirsutella thompsonii	Acari
Isaria fumosorosea	Acari, Diptera, Coleoptera, Hemiptera, Thysanoptera
Lagenidium giganteum	Diptera (Culicidae)
Lecanicillium longisporum	Hemiptera
Lecanicillium muscarium	Acari, Hemiptera, Thysanoptera
Metarhizium anisopliae sensu lato	Acari, Blattoidea, Coleoptera, Diptera, Hemiptera, Isoptera, Lepidoptera, Orthoptera
Metarhizium acridum	Orthoptera
Nomuraea rileyi	Lepidoptera
Paecliomyces fumosoroseus	Hemiptera

19.2.2 Fungal Biopesticides

Another class of microbial insecticides, mycoinsecticides, are products of entomopathogenic fungi, which are natural pathogens of diverse agricultural pests both insects and acari. There are many suitable characteristic features of fungi, which make them suitable for use as biocontrol agents. They are pathogenic to pests but do not harm nontarget insects such as bees and parasites and predators of pests. They neither cause any risk on growth and development of beneficial organisms such as earthworms and collembola. Therefore, mycopesticides are potential agent for IPM and also useful for long-term agriculture and crop production by safeguarding biodiversity (Goettel et al. 2008; Kim et al. 2010; Koike et al. 2011).

Fungi-based biopesticides were considered for IPM by industrial methods of mass production and formulation for application with the use of few specific mycopathogens (Chandler et al. 2008). IPM using fungi utilizes ecological approaches, and appropriate environmental conditions are maintained to promote infection and spread of the pathogen within the pest (Lacey et al. 2015). Commercially available fungi-based biopesticides (Table 19.2) are mainly derived from *Beauveria* spp., *Metarhizium* spp., *Isaria fumosorosea*, and *Lecanicillium* spp.

Specifically, *Beauveria bassiana* and *Metarhizium anisopliae* are the two ascomycetes that are most commonly used as commercial mycoinsecticide. They are usually applied in the form of conidia or mycelium which sporulates after their application. Insect-pathogenic fungus *M. anisopliae* has been reported to be used against adult *Aedes aegypti* and *A. albopictus* mosquitoes (Driesche et Al. 2008). Entomopathogenic fungi alone or in combined application of insecticide with fungal entomopathogen could be a useful strategy in IPM (Sarwar 2015). Some mycoinsecticide has been developed for control of locust and grasshopper pests in Africa and Australia (Chandler et al. 2011). It has been observed that when *B. bassiana* have been applied along with sublethal concentration of insecticide, there is high insect mortality in potato beetle (*Leptinotarsa decemlineata*). A combination of *B. bassiana* and *neem* (*Azadirachta indica*) has also been explored, and their compatibility yielded highest mortalities *of B. tabaci* eggs and nymphs.

19.2.2.1 Mode of Action of Mycoinsecticides

The process of infecting pests includes gaining the access to host's hemolymph, producing toxins and growing up by using nutrients present in haemocoel. In some cases, species of pathogenic fungus such as *B. bassiana* and *M. anisopliae* cause muscardine insect disease; in which after killing the host, cadavers become mummified by mycelial growth (Miranpuri and Khachatourians 1995) (Fig. 19.3). Entomopathogenic fungi are the most effective against sucking insect pests such as aphids, thrips, scale insects, mealy bugs, whiteflies, mosquitoes and all kind of

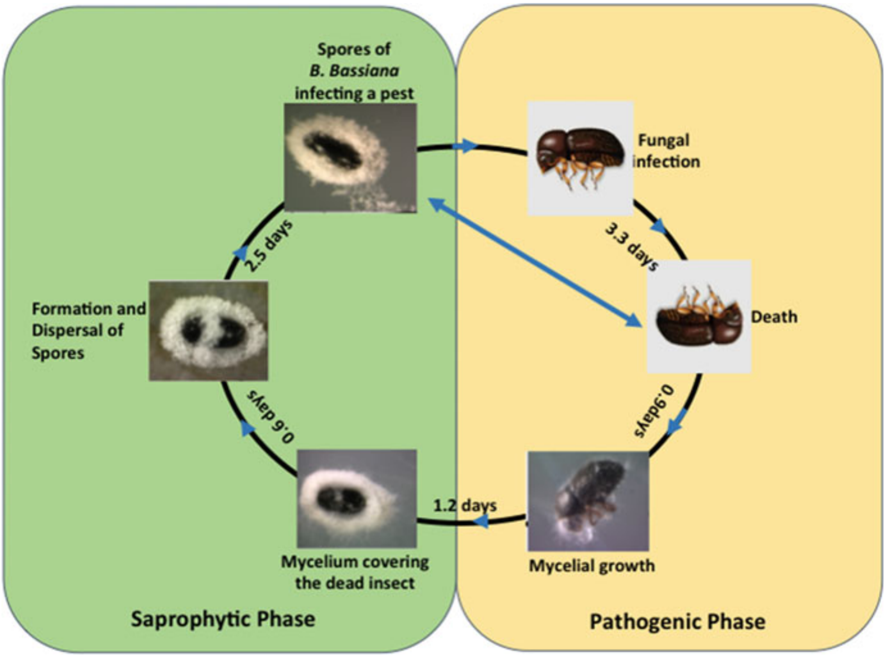

Fig. 19.3 *Beauveria bassiana* targeting coffee berry borer

mites (Barbara and Clewes 2003; Pineda et al. 2007). Certain fungal species, primarily Streptomycetes, are known to produce toxins against insect pest species belonging to Lepidoptera, Homoptera, Coleoptera, Orthoptera, and mites (Cole and Rolinson 1972). Examples of some most active toxins are actinomycin A, cycloheximide and novobiocin.

19.2.3 Mycoinsecticide: A Case Study

Solenopsis invicta Buren, a Hymenopteran, is native to South America and an aggressive ant species (Lowe et al. 2000). They are highly resistant to pathogens due to development of defensive alkaloids (Storey 1990), necrophoric behaviors (Qiu et al. 2014, 2015), trophallactic behavior (De Souza et al. 2008; Qiu et al. 2016), generation of volatiles (Wang et al. 2015), as oral transfer of chemical cues, growth proteins and hormones (Leboeuf et al. 2016). As a result, most of the biological control mediating organisms are not active against this invasive insect. Further, a combination of two species of fungi, *Metarhizium brunneum* and *Beauveria bassiana*, were used to manage *Solenopsis invicta* Buren. Results showed 51.35 and 56.68% of mortality in workers during day 1 and 2 with *M. brunneum* and *B. bassiana* GHA treatments. However, only 9.47 and 35.96% of the mortality could be explained by fungal infection. In *B. bassiana* NI8 treatment 84.48% of mortality was observed within 4–6 days. Mortality occurring in these two treatments can be explained. *M. brunneum* produces a toxin, destruxins (Strasser et al. 2000; Schrank and Vainstein 2010), and releases certain enzymes including lipases, proteases, and chitinases that attack the cuticle of the insects. Field study also showed positive results, and several fire ants were killed by *M. brunneum* and *B. bassiana* (Rojas et al. 2018).

19.2.4 Viral Biopesticides

Virus pesticides act on specific target and are mostly effective against lepidopteran pests of cotton, rice, and vegetables and plant-chewing insects. *Heliothis zea* nucleopolyhedrosis is the first viral insecticide with broad range. There are different groups of entomopathogenic viruses: baculoviruses (BVs), nucleopolyhedrosis viruses (NPVs), granuloviruses (GVs), acoviruses, iridoviruses, parvoviruses, polydnaviruses, poxviruses, reoviruses, cytoplasmic polyhedrosis viruses, nodaviruses, picorna-like viruses, and tetraviruses. Among them, baculovirus (BV) has received maximum focus for biopesticide development at commercial level (Moscardi et al. 2011). Non-BV (Tetraviruses, Cypovirus etc.) viruses have also been used for crop protection but only up to a limited extent (Ramle et al. 2005; Jackson et al. 2005). Baculovirus infects many species belonging to genera *Helicoverpa* or *Heliothis*. *HzSNPV* is efficacious against pests belonging to the genera soybean, sorghum, maize, tomato and beans (Sarwar 2015). A type of baculovirus namely *HaSNPV* has been reported from India which has been exclusively used in cotton field (Srinivasa et al. 2008).

19.2.4.1 BV as Viral Biopesticides

There are many beneficial aspects of BV because of which it has been picked for commercialization. There is significant information about pathology and ecology of BV, which is helpful in registration and product development. BV has widespread distribution allowing collaborative research and interaction between pesticide companies. It possesses high levels of virulence against pests. Moreover, BV shows great levels of replication, which is of commercial interest. The robust infective stage is the occlusion body (OB), which contains rod-shaped nucleocapsids and circular and double-stranded DNA. The OBs are made up of tough crystalline proteins making it ideal for product formulation, application, and commercialization. There is no requirement of keeping intervals between spray timings, and it is safe for human and nontarget insects. Moreover, OBs are large enough to be visualized and quantified by phase-contrast microscopy. The only limitation in its use is its degradation by sunlight because of which frequent applications are needed (Lacey et al. 2015).

BVs are active against world's most devastating agricultural pests, *Helicoverpa* spp. and *Spodoptera* spp. (Mazid et al. 2011). Two well-known commercial formulations based on *Spodoptera* NPV are available in the United States and Europe. BV-based biopesticides have been widely adopted in many different places including China, India, Thailand, Vietnam, Brazil, Mexico, and Guatemala Southeast Asia, Australia, and South America. Virus-based products are available against cabbage moths, corn earworms, cotton leafworms and bollworms, beet armyworms, celery loopers, tobacco budworms and many other pests (Table 19.3). Recombination technology has also lead to development of potential economical substitutes such as recombinant baculovirus, vEV-Tox34, expressing the gene Tox-34 from a mite *Pyemotes tritici* enhance the rate of killing of the corn earworm, *Helicoverpa zea* (Tomalski and Miller 1991).

19.2.4.2 Mode of Action

Viral infection involves entry of the virus to a target cell via replication in the nuclei or in the cytoplasm. Postinfection, virus exists in three phases: 0–6 h is designated as early phase, 6–24 h is called as second phase, and 24–72 h is labeled as very late phase. OBs/virions are formed during late phase of their life cycle. Infected nuclei per cell can produce hundreds of polyhedra (example in NPVs) or thousands of granules as in GVs. It may cause enzootics leading to the decrease in pest populations. It has been reported in baculovirus, occlusion bodies gets inactivated rapidly when exposed to solar ultraviolet radiations (280–320 nm) (Killick 1990). UV inactivation can be controlled by using plastic greenhouse structures which can

Table 19.3 Viral biopesticides

Name of virus	Target pest
Nudiviruses	
NPV for *Anagrapha falcifera*	*Anagrapha falcifera*
NPV for *A. gemmatalis*	*Mucuna pruriens* and *Diatraea saccharalis*
NPV for *Autographa californica*	*Autographa californica*
NPV for *H. zea* and *H. virescens*	*Helicoverpa zea* and *Helicoverpa virescens*
NPV for *Mamestra brassicae*	*Mamestra brassicae*
NPV for *Orgyia pseudotsugata*	*Orgyia pseudotsugata*
Corn earworm NPV (HezeSNPV)	*Helicoverpa zea*, *Helicoverpa armigera*, and *Heliothis virescens*
Cotton bollworm NPV (HearNPV)	*Helicoverpa armigera*
NPV for *Spodoptera exigua*	*Spodoptera exigua* and *Paradrina clavipalpis*
Unbarred *Spodoptera* moth NPV (SdalNPV)	*Spodoptera albula* (*sunia*)
Beet armyworm NPV (SpexMNPV)	*Spodoptera exigua*
Tobacco armyworm NPV (SpltNPV)	*Spodoptera exigua*
Egyptian cotton leafworm NPV (SpliNPV)	*Spodoptera littoralis*
SeMNPV	*Spodoptera exigua*
Gypsy moth, NPV (LydiMNPV)	*Spodoptera exigua*
Velvetbean caterpillar, NPV (AngeMNPV)	*Anticarsia gemmatalis*
Redheaded pine sawfly NPV (NeleNPV)	*Neodiprion lecontei*
Douglas fir tussock moth NPV (OrpsNPV)	*Orygia pseudotsugata*
Balsam fir sawfly NPV (NeabNPV)	*Neodiprion abietis*
Codling moth GV (CpGV)	*Cydia pomonella*
False codling moth GV	*Cryptophlebia*
CrleGV	*Leucotreta*
AdorGV	*Adoxophyes orana*
Potato tuber moth GV (PhopGV)	*Phthorimaea operculella*
Summer fruit tortrix GV (AdorGV)	*Adoxophyes orana*
Tea tortrix (HomaGV)	*Homona magnanima*
Smaller tea tortrix GV (AdhoGV)	*Adoxophyes honmai*
Alfalfa looper NPV (AucaMNPV)	*Autographa calofornica*
Cabbage looper (TrniSNPV)	*Trichoplusia ni*
Tea moth (BuzuNPV)	*Buzura suppressaria*
Tea tussock moth (Eups NPV)	*Euproctis pseudoconspersa*
Tea geomotrid EcobNPV	*Extropic obliqua*
Teak defoliator (HypeNPV)	*Hyblea peura*
CpGV	*Cydia pomonella*
Imported cabbageworm (PiraGV)	*Artogeia (Pieris) rapae*
Oriental armyworm (LeseNPV)	*Leucania (Mythimna) separata*
Diamond back moth GV (PlxyGV)	*Plutella xylostella*
Reoviridae	
Masson pine moth cypovirus (CPV)	*Dendrolimus punctatus*
Parvoviridae	

(continued)

Table 19.3 (continued)

Name of virus	Target pest
Cockroach densonucleosis virus (DNV)	*Periplaneta fuliginosa*
Others	
Oryctes virus	*Oryctes rhinoceros*
Granulosis virus	Lepidoptera

reduce the intensity of incident UV-B radiations reading by >90% compared with external readings leading to an increase in the prevalence of infection in larvae (Lasa et al. 2007).

The use of formulations such as stilbene can increase susceptibility to NPV infection either by disrupting the peritrophic membrane (Okuno et al. 2003) or by inhibiting shedding or by virus-induced apoptosis of insect midgut cells (Dougherty et al. 2006). Two genetically enhanced isolates of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) from the spider *Diguetia canities* and *Tegenaria agrestis* designated vAcTaITX-1 and vAcDTX9.2 have been commercially evaluated as potential biopesticide against lepidopteran insects (Hughes et al. 1997). Viral pesticides have numerous advantages over chemical pesticides, but their large-scale production, cost-effective methods for producing recombinants, intensive labor, and time-consuming transinfection pose certain difficulties. They are being produced on small scale by various IPM centers and state agricultural departments (Gupta and Dikshit 2010; Lacey et al. 2015).

19.2.4.3 A Case Study on the Use of *Oryctes nudivirus* for the Control of Invasive Coconut Palm Rhinoceros Beetle

Indigenous to Asia/West Pacific areas, *Oryctes rhinoceros* or coconut palm rhinoceros beetle was coincidentally established into Samoa and eventually extended to islands of southwest Pacific regions (Bedford 1980; Jackson 2009). These beetles are key pest of palm and coconut. They minimize the produce by ingesting the vegetation mainly the crown and its destruction, leading to the death of the whole tree (Bedford 1980). Larvae of *Oryctes rhinoceros* has diverse habitat such as inside rotting palm wood, dead tops of living trees, and organic content-rich sites (Bedford 1980). *Oryctes* virus was intensely established in the pest-infested regions of Samoa and other southwest Pacific islands to overcome the devastation produced by the beetles (Bedford 1980; Hüger 2005; Jackson 2009). These viruses were originally collected from Malaysia (Hüger 1966). Remarkable consequences were observed by using this virus as a biological control agent. It regulated and lowered the population of coconut palm rhinoceros beetle and their larvae. Infected adults served as reserves for virus. In beetle populations, virus spread from infected to noninfected larvae through feeding, mating, sites of larval breeding, etc. Larvae with severe infection die within 9–25 days after virus consumption (Hüger 1966; Zelazny 1972). Continuous reviews were conducted in the recent years, which suggest more fatal and pernicious strains of virus are required to reduce the problem of less efficacy of *Oryctes* virus on some beetle-infected islands (Jackson et al. 2005; Jackson 2009).

19.2.5 Nematode Biopesticides

Entomopathogenic nematodes (EPNs) are one of the most astonishing organisms as they repress insects in their perplexing habitats (such as soil-borne pests and stem borers). They have become an important microbial tool for biotic control.

19.2.5.1 *Steinernema* and *Heterorhabditis*: *EPNs as* Biopesticides

Steinernema and *Heterorhabditis* are the two widely used genera as EPNs in pest management. They are mostly present in all forest and agricultural land. They have an aggregated distribution, which depends upon their behavior, restricted dispersal ability, and changeability in spatial and temporal distribution of their natural enemies (Atwa 2014). EPNs are very often used as biological control agents since they are environment-friendly and do not harm human and nontarget organisms (Akhurst and Smith 2002; Ehlers and Shapiro-Ilan 2005). They are suitable for mass production, and it is easy to register and commercialize EPNs as biopesticide. They have a wide host range including 5–6 orders of insects (Poinar 1979; Klein 1990).

There are more than 10 industries which are involved in the production of EPNs as biocontrol agent, and approximately 15 species have reached up to the level of commercialization (Table 19.4). The efficacy of EPNs as biopesticide depends on environmental factors (biotic and abiotic). Biotic factors include the species of nematode that has been selected and number of times it has been applied. Abiotic factors include desiccation, ultraviolet light, type of habitat, and time of application. EPNs are sensitive to desiccation and ultraviolet light, and it works better if applied early morning or in evening.

Although the basic research on EPN involves figuring out its usage as biopesticide, the recent advanced research focuses on understanding how host attraction and infection can be improved for better efficacy. During this course, it has been concluded that vibration and electromagnetic stimuli can improve attraction toward the host (Torr et al. 2004; Ilan et al. 2013). These discoveries are certainly going to improve the suitability of EPNs as biocontrol agents.

19.2.5.2 Mode of Action

EPNs infect their host via spiracles or cuticle, mouth and anus opening as infective juveniles (IJs) (Kaya and Gaugler 1993; Koppenhöfer et al. 2003). EPNs carry mutualistic symbiotic bacteria such as *Xenorhabdus* spp. and *Photorhabdus* spp. for Steinernematids and Heterorhabditids, respectively (Poinar 1990). They liberate

Table 19.4 Nematode biopesticides

Name of nematode	Target pest
Heterorhabditis bacteriophora	Lepidoptera, cutworms, corn root worms, turf and Japanese beetles, flea beetles, soil insects, white grubs (scarabs), black vine weevils, and citrus root weevils
H. indica	*Galleria mellonella*, root mealybugs, grubs
H. marelata	White grubs (scarabs), cutworms, black vine weevils
H. megidis	Weevils
H. zealandica	Scarab grubs
H. megidis	Coleoptera (Scarabaeidae)
P. hermaphrodita	Slugs
Steinernema glaseri	Root weevils, cutworms, fleas, banana root borers and fungal gnats, white grubs (scarabs, especially Japanese beetle, *Popillia* sp.)
S. kraussei	Black vine weevil, *Otiorhynchus sulcatus*
S. carpocapsae	Lepidoptera, Coleoptera, Diptera, Hymenoptera, and Hemiptera
S. feltiae	Coleoptera, Lepidoptera, and others
S. longicaudum	Lepidopteran and Coleopteran
S. riobrave	*Diaprepes* spp. (citrus root weevils), *Scapteriscus* spp. (mole crickets)
S. scapterisci	*Scapteriscus* spp. (mole crickets)
Deladenus siricidicola	*Sirex noctilio* (Sirex wood wasp)

their bacterial symbionts into the haemocoel of the host, which are mainly responsible for the death of the host within 24–48 h (Dowds and Peters 2002). Entomopathogenic nematodes at most can have three cohorts in IJs and leave the body to infect a new one (Kaya and Gaugler 1993) (Fig. 19.4). EPNs can be produced under in situ or ex situ conditions in solid media or by liquid fermentation (Grewal and Georgis 1999; Shapiro-Ilan et al. 2006). Some successfully produced nematodes in fermenters are *Steinernema carpocapsae*, *S. riobrave*, *Steinernema glaseri*, *Steinernema scapterisci*, and *Heterorhabditis bacteriophora*.

19.2.5.3 A Case Study on *Steinernema scapterisci* for Controlling Invasive Mole Crickets in Florida

Scapteriscus species are key serious pest and known to cause acute destruction to turf especially reported in Florida (Frank 2009). For regulating their growing population several biological control methods were adopted. One such strategy made use of EPNs and parasitoids in Florida. In 1985, nematode species from Uruguay were introduced in Florida to manage and check the population of encroaching mole cricket (*S. scapterisci*). At the beginning, they helped in regulating the pest (Parkman et al. 1993). In Florida, Uruguay's nematode species were released, and they got established into *S. vicinus*, *S. borelli*, and *S. abbreviatus* populations (Hudson et al. 1988; Parkman et al. 1993). Further, two parasitoids (from South America) became established all over Florida. With the help of these three natural adversaries, *Scapteriscus* populations diminished by 95% (Frank and

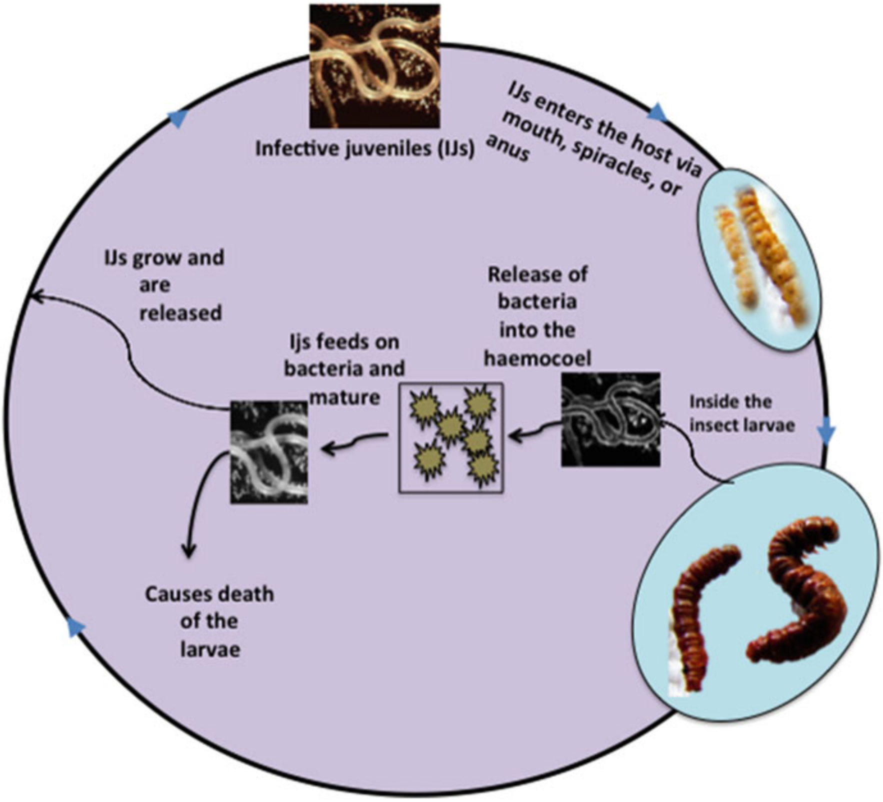

Fig. 19.4 Life cycle of entomopathogenic nematodes

Walker 2006). These EPNs with high successful rate are now applied at various infestation sites in Florida (Frank 2009).

Advantages of Microbial Pesticides over Chemical Pesticides

(a) They are safe to applicators (human) and nonpathogenic to nontarget organisms. They are not even harmful to beneficial organisms like predators and parasitoids.
(b) They are safe to be used in food supply.
(c) They do not persist in the environment.
(d) There are no/very little chances of development of resistance in the pests.
(e) They do not cause any lethal effect or risk to the environment.
(f) Most of them possess good shelf life.
(g) They are easy and inexpensive to mass produce.

(h) They are easy for application as well and do not need any specific equipment.
(i) They are adaptable for genetic modifications.
(j) They are suitable to be used in different types of habitat where use of chemical pesticides might be restricted.

Disadvantages of Microbial Pesticides

(a) Since they target a specific group of microbes, crop plants are still at risk and may be attacked by other pests.
(b) They show slower killing of pests as compared to chemical pesticides.
(c) They need precise timing for application so that they can attack early instars of pests and show better efficacy.
(d) Due to less persistence, many rounds of application may be needed.
(e) Microbial pesticides are sensitive to heat, UV radiation, desiccation, etc.
(f) Some have short shelf life.
(g) There are few constrains in their mass production, formulations, registration, and commercialization.
(h) Its cost of production may be higher except for high-value crops.

19.3 Increasing Trends in Production of Biopesticides

Outburst of secondary pests; growing pest resistance; toxicity of soil, air, water, and food; detrimental effect on humans; and ecological imbalance are some unacceptable effects of continuous and excessive use of chemical-based pesticides. Such emerging issues are of great concern and have led many countries to amend their policies on limiting the use of chemical pesticides and switch over to better biological control methods. Application of new environmentally friendly biopesticides is a better option than conventional chemical control techniques. Under integrated pest management, biopesticides have shown better effectuality compared to synthetic products (Mazid et al. 2011). Growing organic demand and residue free crop product are some of the decisive instigator for biopesticide demand. Eventually, the need for bioinsecticides, fungicides, and bionematicides is increasing exponentially. The US biopesticides market has anticipated that it may rise to approximately $300 million by 2020. In India, only 4.2% of overall pesticide market consists of biopesticide. It is expected to show expansion with annual growth rate about 10% in the near future. Till now, only 20–30 biopesticides have been registered under the Insecticide Act 1968. Considerable biopesticides manufactured and used in India are *Bacillus thuringiensis*, neem-based pesticide, *Trichoderma*, and nuclear polyhedrosis virus (Kumar 2012).

19.4 Policy Measures

Biopesticides do not produce any risk factor; therefore, the Environmental Protection Agency (EPA), USA, promotes its growth and utilization. EPA can register any new biopesticide within a year based on its virulence, constituents and data availability. Regular and continuous inspections are made to regulate the potency of current biopesticide. India has also adopted IPM strategies and considered the use of various biopesticides as its major component. Here, the Ministry of Agriculture employs the usage of pesticides under the Pesticides Management Bill 2008. As a substitute to regular synthetic pesticides, biopesticides do face innumerable challenges such as in their manufacturing, development and application issues.

19.5 Suggestions

Microbial pesticides have been widely used as biopesticides to check pest infestation and improve crop production. Further, the below-mentioned recommendations can be considered for the effective utilization of microbes to restrict pest infestation:

- Efforts should be made for advertisement and acceptance of biocontrol strategies by all the participants in the marketing chain from producer to consumer.
- Outreach activities such as demonstration, promotion, and training programs can be conducted in order to popularize biopesticides among the consumers.
- Further research is needed to figure out what new methods can be applied to overcome limitations that are faced while using microbial pesticides such as their sensitivity to UV light, desiccation, etc.
- Search for new biocontrol agents needs to be continued for future usage in different types of habitats and climates.
- Newer methods of production, formulation, storage, and application need to be established for better efficacy, user friendliness, and cost-effectivity.
- Transgenic plants with microbial genes can be generated for major crops.
- Further research is needed to find out ecology of pest pathogens for their sustainable use.

References

Akhurst R, Smith K (2002) 15 Regulation and safety. In: Gaugler R (ed) Entomopathogenic nematology. CABI, New York, pp 311–332

Atwa AA (2014) Entomopathogenic nematodes as biopesticides. In: Sahayraj K (ed) Basic and applied aspects of biopesticides. Springer, New Delhi, pp 69–98

Barbara DJ, Clewes E (2003) Plant pathogenic *Verticillium* species: how many of them are there? Mol Plant Pathol 4(4):297–305

Bedford GO (1980) Biology, ecology, and control of palm rhinoceros beetles. Annu Rev Entomol 25(1):309–339

Birch ANE, Begg GS, Squire GR (2011) How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. J Exp Botany 62(10):3251–3261

Bravo A, Gill SS, Soberon M (2007) Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49(4):423–435

Brookes G, Barfoot P (2012) GM crops: global socio-economic and environmental impacts 1996–2010. PG Economics Ltd. http://www.pgeconomics.co.uk/page/33/global-impact-2012

Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Technol 19(5):275–283

Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc Lond B Biol Sci 366(1573):1987–1998

Cole M, Rolinson GN (1972) Microbial metabolites with insecticidal properties. Appl Microbiol 24 (4):660–662

Crickmore, N., et al. (2014) Bacillus thuringiensis toxin nomenclature. Available in: http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/. Accessed 14 2015

Crump NS, Cother EJ, Ash GJ (1999) Clarifying the nomenclature in microbial weed control. Biocontrol Sci Tech 9(1):89–97

de Maagd RA, Bravo A, Crickmore N (2001) How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet 17(4):193–199

De Oliveira JL, Campos EV, Bakshi M, Abhilash PC, Fraceto LF (2014) Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises. Biotechnol Adv 32(8):1550–1561

De Souza DJ, Van Vlaenderen J, Moret Y, Lenoir A (2008) Immune response affects ant trophallactic behaviour. J Insect Physiol 54(5):828–832

Douches DS, Li W, Zarka K, Coombs J, Pett W, Grafius E, El-Nasr T (2002) Development of Bt-cry5 insect-resistant potato lines ‘Spunta-G2’ and ‘Spunta-G3’. Hort Sci 37(7):1103–1107

Dougherty EM, Narang N, Loeb M, Lynn DE, Shapiro M (2006) Fluorescent brightener inhibits apoptosis in baculovirus-infected gypsy moth larval midgut cells in vitro. Biocontrol Sci Tech 16(2):157–168

Dowds BC, Peters AR (2002) Virulence mechanisms. In: Gaugler R (ed) Entomopathogenic nematology. CABI, New York, pp 79–98

Ehlers RU, Shapiro-Ilan DI (2005) Mass production. Nematodes as biocontrol agents. In: Grewal P (ed) Nematodes as biological control agents. CABI, Wallingford, pp 65–78

Fisher TW, Garczynski SF (2012) Isolation, culture, preservation, and identification of entomopathogenic bacteria of the Bacilli. In: Lacey LA (ed) Manual of techniques in invertebrate pathology. Academic Press, London, pp 75–98

Frank JH (2009) *Steinernema scapterisci* as a biological control agent of Scapteriscus mole crickets. In: Hajek AE, Glare TR, O’Callaghan M (eds) Use of microbes for control and eradication of invasive arthropods. Springer, Dordrecht, pp 115–131

Frank JH, Walker TJ (2006) Permanent control of pest mole crickets (Orthoptera: Gryllotalpidae: Scapteriscus) in Florida. Am Entomol 52(3):138–144

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30:250–258

Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008) Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J Invertebr Pathol 98(3):256–261

Grewal P, Georgis R (1999) Entomopathogenic nematodes. In: Hall FR, Menn JJ (eds) Biopesticides: use and delivery. Humana Press, Totowa, pp 271–299

Gupta S, Dikshit AK (2010) Biopesticides: an ecofriendly approach for pest control. J Biopest 3 (1):186–188

Hudson WG, Frank JH, Castner JL (1988) Biological control of *Scapteriscus* spp. mole crickets (Orthoptera: Gryllotalpidae) in Florida. Bull Entomol Soc Am 34:192–198

Huger AM (1966) A virus disease of the Indian rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen. n., sp. n. J Invertebr Pathol 8 (1):38–51

Huger AM (2005) The *Oryctes* virus: its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). J Invertebr Pathol 89(1):78–84

Hughes PR, Wood HA, Breen JP, Simpson SF, Duggan AJ, Dybas JA (1997) Enhanced bioactivity of recombinant baculoviruses expressing insect-specific spider toxins in lepidopteran crop pests. J Invertebr Pathol 69(2):112–118

Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. Soil Biol Biochem 38:45–849

Ilan T, Kim-Shapiro DB, Bock CH, Shapiro-Ilan DI (2013) Magnetic and electric fields induce directional responses in *Steinernema carpocapsae*. Int J Parasitol 43:781–784

Jackson TA (2009) The use of *Oryctes* virus for control of rhinoceros beetle in the Pacific Islands. In: Hajek AE, Glare TR, O'Callaghan M (eds) Use of microbes for control and eradication of invasive arthropods. Springer, Dordrecht, pp 133–140

Jackson TA, Crawford AM, Glare TR (2005) *Oryctes* virus—time for a new look at a useful biocontrol agent. J Invertebr Pathol 89(1):91–94

Jurat-Fuentes JL, Jackson TA (2012) Bacterial entomopathogens. In: Insect pathology. Academic Press, San Diego, pp 265–349

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38(1):181–206

Kennedy GG (2008) Integration of insect-resistant genetically modified crops within IPM programs. In: Romeis J, Shelton A, Kennedy GG (eds) Integration of insect-resistant genetically modified crops within IPM programs. Springer, Dordrecht, pp 1–26

Khachatourians GG (2009) Insecticides, microbials. Applied Microbiology: Agro/Food 95–109

Killick HJ (1990) Influence of droplet size, solar ultraviolet light and protectants, and other factors on the efficacy of baculovirus sprays against *Panolis flammea* (Schiff.) (Lepidoptera: Noctuidae). Crop Prot 9(1):21–28

Kim JJ, Goettel MS, Gillespie DR (2010) Evaluation of *Lecanicillium longisporum*, Vertalec® against the cotton aphid, *Aphis gossypii*, and cucumber powdery mildew, *Sphaerotheca fuliginea* in a greenhouse environment. Crop Prot 29(6):540–544

Klein M (1990) Efficacy against soil-inhabiting insect pests. ln: Gaugler, R and Kaya HK (ed) Entomopathogenic nema-lOdes in biological control. CRC Press, Boca Raton, FL, pp 365

Koike M, Shinya R, Aiuchi D, Mori M, Ogino R, Shinomiya H, Tani M, Goettel M (2011) Future biological control for soybean cyst nematode. In: El-Shemy HA (ed) Soybean physiology and biochemistry. Intech Open Access, Croatia, pp 193–208

Koppenhöfer AM et al. (2003) Effect of neonicotinoid synergists on entomopathogenic nematode fitness. Entomol Exp Appl 106(1):7–18

Koul O, Cuperus GW, Elliott N (eds) (2008) Areawide pest management: theory and implementation. CABI, Oxfordshire

Koul O, Cuperus GW (2007) Ecologically based integrated pest management: present concept and new solutions. In: Koul O, Cuperus GW, Norman E (eds) Ecologically based integrated pest management. CABI, Wallingford, pp 1–17

Kroschel J, Lacey LA (2009) Integrated pest management for the potato tuber moth, *Phthorimaea operculella* (Zeller) – a potato pest of global importance. In: Kroschel J, Lacey LA (eds) Tropical agriculture 20, advances in crop research 10. Margraf Publishers, Weikersheim, p 147

Kumar S (2012) Biopesticides: a need for food and environmental safety. J Biofertil Biopestic 3 (4):1–3

Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41

Lasa R, Ruiz-Portero C, Alcázar MD, Belda JE, Caballero P, Williams T (2007) Efficacy of optical brightener formulations of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as a biological insecticide in greenhouses in southern Spain. Biol Control 40(1):89–96

LeBoeuf AC, Waridel P, Brent CS, Gonçalves AN, Menin L, Ortiz D, Riba-Grognuz O, Koto A, Soares ZG, Privman E, Miska EA (2016) Oral transfer of chemical cues, growth proteins and hormones in social insects. elife 5:e20375

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database (Vol. 12). Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), Auckland pp 12. www.issg.org/booklet.pdf

Mazid S, Kalita JC, Rajkhowa RC (2011) A review on the use of biopesticides in insect pest management. Int J Sci Adv Technol 1(7):169–178

Miranpuri GS, Khachatourians GG (1995) Entomopathogenicity of *Beauveria bassiana* toward flea beetles, *Phyllotreta cruciferae* Goeze (Col., Chrysomelidae). J Appl Entomol 119:167–170

Moscardi F, de Souza ML, de Castro MEB, Moscardi ML, Szewczyk B (2011) Baculovirus pesticides: present state and future perspectives. In: Ahmad I, Ahmad F, Pichtel J (eds) Microbes and microbial technology. Springer, New York, pp 415–445

Okuno S, Takatsuka J, Nakai M, Ototake S, Masui A, Kunimi Y (2003) Viral-enhancing activity of various stilbene-derived brighteners for a *Spodoptera litura* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. Biol Control 26(2):146–152

Parkman JP, Hudson WG, Frank JH, Nguyen KB, Smart GC Jr (1993) Establishment and persistence of *Steinernema scapterisci* (Rhabditida: Steinernematidae) in field populations of *Scapteriscus* spp. mole crickets (Orthoptera: Gryllotalpidae). J Entomol Sci 28(2):182–190

Pineda S, Alatorre R, Schneider ML, Martinez AM (2007) Pathogenicity of two entomopathogenic fungi on *Trialeurodes vaporariorum* and field evaluation of a *Paecilomyces fumosoroseus* isolate. Southwest Entomol 32(1):43–52

Poinar GO Jr (1979) Nematode groups. In: Poinar GO Jr (ed) Nematodes for biological control of insects. CRC Press, Boca Raton, FL, pp 277–289

Poinar GO Jr (1990) Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Guagler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, FL, pp 23–61

Qiu HL, Lu LH, Shi QX, He YR (2014) Fungus exposed *Solenopsis invicta* ants benefit from grooming. J Insect Behav 27(5):678–691

Qiu HL, Lu LH, Shi QX, Tu CC, Lin T, He YR (2015) Differential necrophoric behaviour of the ant *Solenopsis invicta* towards fungal-infected corpses of workers and pupae. Bull Entomol Res 105 (5):607–614

Qiu HL, Lu LH, Zalucki MP, He YR (2016) *Metarhizium anisopliae* infection alters feeding and trophallactic behavior in the ant *Solenopsis invicta*. J Invertebr Pathol 138:24–29

Ramle M, Wahid MB, Norman K, Glare TR, Jackson TA (2005) The incidence and use of *Oryctes* virus for control of rhinoceros beetle in oil palm plantations in Malaysia. J Invertebr Pathol 89 (1):85–90

Rodrigo-Simón A, Caccia S, Ferré J (2008) Bacillus thuringiensis Cry1Ac toxin-binding and pore-forming activity in brush border membrane vesicles prepared from anterior and posterior midgut regions of lepidopteran larvae. Appl Environ Microbiol 74(6):1710–1716

Rojas MG, Elliott RB, Morales-Ramos JA (2018) Mortality of *Solenopsis invicta* workers (Hymenoptera: Formicidae) after indirect exposure to spores of three entomopathogenic fungi. J Insect Sci 18(3):20

Sarwar M (2015) Biopesticides: an effective and environmental friendly insect-pests inhibitor line of action. Int J Eng Adv Res Tech 1(2):10–15

Schnepf E, Crickmore NV, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev 62 (3):775–806

Schrank A, Vainstein MH (2010) *Metarhizium anisopliae* enzymes and toxins. Toxicon 56 (7):1267–1274

Shapiro-Ilan DI, Gouge DH, Piggott SJ, Fife JP (2006) Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biol Control 38 (1):124–133

Srinivasa M, Jagadeesh Babu CS, Anitha CN, Girish G (2008) Laboratory evaluation of available commercial formulations of HaNPV against *Helicoverpa armigera* (Hub.). J Biopest 1 (2):138–139

Storey GK (1990) Chemical defenses of the fire ant, *Solenopsis invicta* Buren, against infection by the fungus, *Beauveria bassiana* (Balsamo) Vuill. Doctoral dissertation, University of Florida, Gainseville

Strasser H, Vey A, Butt TM (2000) Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? Biocontrol Sci Tech 10(6):717–735

Tomalski MD, Miller LK (1991) Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. Nature 352(6330):82

Torr P, Heritage S, Wilson MJ (2004) Vibrations as a novel signal for host location by parasitic nematodes. Int J Parasitol 34(9):997–999

Van Frankenhuyzen K (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101(1):1–16

Wang L, Elliott B, Jin X, Zeng L, Chen J (2015) Antimicrobial properties of nest volatiles in red imported fire ants, *Solenopsis invicta* (hymenoptera: formicidae). Sci Nat 102(11–12):66

Wei JZ, Hale K, Carta L, Platzer E, Wong C, Fang SC, Aroian RV (2003) *Bacillus thuringiensis* crystal proteins that target nematodes. Proc Natl Acad Sci 100(5):2760–2765

Whalon ME, Wingerd BA (2003) Bt: mode of action and use. Arch Insect Biochem Physiol: Published in Collaboration with the Entomological Society of America 54(4):200–211

Wraight SP, Sporleder M, Poprawski TJ, Lacey LA (2007) Application and evaluation of entomopathogens in potato. In: Lacey LA, Kaya HK (eds) Field manual of techniques in invertebrate pathology. Springer, Dordrecht, pp 329–359

Wraight SP, Hajek AE, Radcliffe EB (2009) Manipulation of arthropod pathogens for IPM. In: Radcliffe EB, Hutchison WD, Cancelado RE (eds) Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press, Cambridge, pp 131–150

Zelazny B (1972) Studies on *Rhabdionvirus oryctes*: I. Effect on larvae of *Oryctes rhinoceros* and inactivation of the virus. J Invertebr Pathol 20(3):235–241