Chapter 7

Insect Pathogens as Potential Biocontrol Agents

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Abstract

Insect pest threat is a major factor that causes diseases in human beings and disrupts crop productivity in agro ecosystems. The middle era of the last century is the keynote witness of chemical insecticides augmentation for the management of insect pests and their backlashes. These chemical insecticides are the silent killers, and are responsible for the development of resistance in pests, environmental persistence and toxicity. A striking substitute to chemical insecticides is the use of Microbial Biocontrol Agents (MBCAs). These entomopathogens are natural enemies of insect pests devastating their populations with no hazardous effects on environment and human health. The MBCAs are not only good alternatives to hazardous chemical pesticides but their immense selectivity by infecting very narrow range of host pests is the key features of their effective utility.

Keywords: Entomopathogenic fungi, bacteria, viruses, nematodes, biological control

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7.1. Introduction

The science of invertebrate pathology provides the scientific fundamentals of microbial control. Insects and microorganisms have ancient and old complex relationship well described by the insects conserved in amber before 15 to 20 million years ago. The collection of several insect cadavers dressed with entomopathogens like neucleopolyhedrovirus (NPV), nematode and trypanosomes is reported (Poinar and Poinar 2005). It has been an old profession, however, its roots can be traced to ancient history from the Aristotle's times (2700 BC), who observed the diseased silk worm with whitish growth on the dead larvae of silk worm during 335 BC in China. It was not until the work of Agostino Bassi (1773-1856) an Italian Lawyer and Scientist, reported the fungus *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin on the larvae with a whitish sooty growth. Thus enunciating the germ theory of disease and named "calcinaccio" disease because the dead larvae exhibit whitish calcium powder like coverings (Steinhaus 1956, 1975).

Agostino Bassi observed that the causal agent of the disease is "vegetable parasite" - a fungus now called *B. bassiana* which may be transferred through inoculation, contact or by the ingestion of the leaves by the caterpillar. This was the first research of Bassi which confirmed that microorganisms can cause disease and also it was the important contribution towards disproving the idea of spontaneous generation. Calcinaccio disease was found plaguing the silk industry first time in Italy (1805) and then in France (1841). Bassi conducted the scientific study on Calcinaccio disease in 1807. After a long term and comprehensive observation, during the year 1835 Bassi confirmed that this disease causing entity is a living organism which may produce whitish growth on the dead larvae. He was honored with rescuing the precious and economically important industry of silk by providing his valuable suggestions for the use of separating the rows of caterpillars feeding on the mulberry leaves, disinfection process, destroying dead cadavers, keeping the rearing room clean and infection free. His findings were translated and distributed throughout the Europe which greatly helps Louis Pasteur (1822-1895) to study the cause and potential cure of the disease in Europe (Porter 1973). In the same year, famous Italian naturalist Giuseppe Gabriel Balsamo-Crivelli studied and named the fungus, *Botrytis bassiana*, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin in the honor of Bassi (Steinhaus 1949; Müller-Kögler 1965; Rehner 2005). The species *B. bassiana* came into existence when in 1911 Beauverie studied the fungus again and Vuillemin created the new genus *Beauveria* in honor of Beauverie in 1912, since then the species *B. bassiana* became the type.

In 1865, French silk industry was badly devastated and Louis Pasteur was asked to identify the disease. He was not reluctant to accept the offer as he was not fully aware about silk worms, although he was persuaded by his teacher and friend Senator Jean-Baptist Dumas to move and consult to famous entomologist Jean Henri Fabre (1823-1915) in Alés Village in the south of France. After the efforts of several years he came to the result that two silk worm diseases "pébrine" and "flacherie" (thought to be caused by bacterium) are responsible for the decline of silk industry. He proposed that pébrine is characterized by tiny black spots on the surface of dead larvae of silk worm caused by the microorganism *Nosema bombycis*, previously described by

Nägeli (1857). For the potential elimination of the disease, he proposed that careful handling, segregating healthy from diseased larvae and well maintained sanitation may be helpful in disease prevention (Debré 1998).

During the study it was found that disease can be transmitted by contaminated food, contact with the infected caterpillar and even from mother to the offspring. This is the first study demonstrating the vertical transmission of the disease (Pasteur 1874). He published his findings in two series to make aware the people about silk worm disease and its protection (Pasteur 1874). This work laid the foundation of advance sericulture in Japan dealing with the molecular and biochemical biology of the silk worm. The scientists like, Agostino Bassi, Louis Pasteur and Elie Metchnikoff, the 19th century pioneers also proposed that these microorganisms can be a good solution for controlling economically important insect pests (Steinhaus 1956, 1975). Number of developments in invertebrate pathology took place in the first half and late of the 19th century, it wasn't until the discovery of *Bacillus thuringiensis* (*Bt*) Berliner, when practical and commercial use of microbial control began on a large scale (Lacey and Goettel 1995).

In 1879, Metchnikoff discovered the diseased larvae of wheat cockchafer and later on *Cleonus punctiventris* Schonherr near Odessa (Ukraine). He named this fungus as green muscardine fungus. The genus *Metarhizium* was first described by Sorokin (1883). For this fungus, he first proposed the name *Entomophthora anisopliae* Metchnikoff and later renamed as *Isaria destructor* Metchnikoff. The history of the description, discovery, the scientific research and on the use of fungus in biological control is described in detail by Steinhaus (1949) and Müller-Kögler (1965). In the start of 20th century *B. thuringiensis* was first isolated, from infected silk worm larvae by a Japanese bacteriologist (Ishiwata 1901) and subsequently in 1911 German biologist Berliner re-discovered the disease. He isolated the bacterium from infected larvae of Mediterranean flour moth, collected from a mill in Thuringe Province (Berliner 1915), so named this bacterium *B. thuringiensis.* Because of high and knock down mortality effects with small amount of *B. thuringiensis* preparations, the agronomist get aware about the insecticidal properties of this bacterium. The first *B. thuringiensis* based commercial formulation "Sporéine" was developed in France in 1938, but the 1st well documented record of commercial procedure for producing *Bt*-based product dates from 1959 by the "Bactospéine" under the 1st French patent as a bio-pesticide formulation. Since after, a vast array of microorganisms like fungi, bacteria, viruses and protozoans has been identified as potential biocontrol agent against insect pests (Riba and Silvy 1989). So far, even though more than 100 species of entomopathogenic bacteria have been identified, only few *Bacillus* species have met with commercial success, *B. thuringiensis* in particular (Starnes et al. 1993).

Today a variety of microbes are used for the control of arthropod pests of field crops, glasshouse, row crops, ornamentals, stored products, orchards, range, turf and lawn, forestry and for the abatement of insect vectors and pests of medical and veterinary importance (Tanada and Kaya 1993; Lacey and Kaya 2007). Entomopathogenic microbes used as a microbial control include viruses, bacteria, protozoa, fungi, and nematodes. Considering the adverse effects of conventional chemical insecticides, environmental and public health issues in tropical countries, these biopesticides which exert only a minor impact on the environment have occupied the stable

position, although modest in the insecticide market. The share of biopesticide in crop protection market is about 600 million US$ which accounts for only 2% of the total pesticides, with about 90% of all bio-pesticide sales involving products based on *B. thuringiensis*.

The comparison of microbial pesticides with chemical pesticides is usually exclusive from the view point of their cost effectiveness. These microbial insecticides offer unique advantages when there are environmental and human safety concerns along with the increasing need of enriched biodiversity in an ecosystem and increased activity of natural enemies (Shahid et al. 2012). Furthermore, easy application method, production on artificial medium and long term storage are further distinct features of these insecticides over other insect control tactics.

7.2. Entomopathogenic fungi

The ancient surveillance history and research of mycopathogens invading insect pests is long standing thousands of years. Before the invention of microscopes fungi can be seen with naked eye and this observation helped in invention of invertebrate pathology as a modern study. Fungi are categorized in a number of taxa that exhibit greater diversity in properties, requirements and found in all arthropod habitats. As a result, great attention was diverted in using fungi as microbial control of insect pests. The fungi are heterotrophic, eukaryotic, absorptive individuals which may develop in different patterns like diffuse, branched, or tubular body that can reproduce sexually as well as asexually (Kendrick 2000).

The primitive studies regarding entomopathogenic fungi (EPFs) were conducted during start of 18th century with an aim to develop control strategies for managing muscardine disease of silk worm (Steinhaus 1975). Bassi (1835 as cited by Steinhaus 1975) proposed the germ theory using silkworm and invading fungus, later this was named as *B. bassiana* in the honor of Bassi. His studies on silkworm disease assisted him to introduce the fungal biocontrol agents for the control of insect vector that elicit disease in human beings. The silkworm diseases provided gross root foundations for the control of insect pests by employing entomopathogens. Nevertheless, the major efforts were attempted in deploying EPFs for the control of insect pests carried out during 1950's when chemical insecticides were invented. There are many fungal based products commercially available worldwide now-a-days (Shah and Goettel 1999; Copping 2001).

7.2.1. History

EPFs have a long primordial historic recognition; their illustrated descriptions can be seen centuries back, infection of *B. bassiana* and *Cordyceps* sp. (L.) Fr. to silk worm described in ancient Japanese paintings infections of insects date from the 19th century (Samson et al. 1988). As a vocation, invertebrate pathology is an organized discipline. Historic stories can be drawn from the solution of silk worm and honey bee diseases prevention from entomopathogens (Steinhaus 1956, 1975). Very first reports of managing insect pests in insect pathology with entomopathogenic fungi

were proposed by the legend pioneers like Louis Pasteur, Elie Metchnikoff and Agostino Bassi (Steinhaus 1975).

Currently numerous entomopathogens are deploying for managing insect pests in lawn and turf, orchards, glasshouse, ornamentals, row crops, forestry, range lands, stored products, pest and insect vectors of medical and veterinary importance (Tanada and Kaya 1993; Lacey and Kaya 2007).

7.2.2. Classification

Among different fungal divisions, entomopathogenic fungi are found in the Ascomycota, Zygomycota and Deuteromycota (Samson et al. 1988), Chytridiomycota and Oomycota (previously classified within Fungi). Many of the genera of entomopathogenic fungi currently under research belong either to the class Hyphomycetes in the Deuteromycota or to the class Entomophthorales in the Zygomycota. The general classification of entomopathogenic fungi is given in Table 1.

Table 1 Classification of entomopathogenic fungi

Division	Class	Order	Family	Genus
Zygomycota	Zygomycetes	Entomophthorales	Entomophthoraceae	Entomophaga
				Entomophthora
				Erynia
				Eryniopsis
				Furia
				Massospora
				Strongwellsea
				Pandora
				Tarichium
				Zoophthora
			Neozygitaceae	Neozygites
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	Beauveriaa
				Cordyceps
				Cordycepioideus
				Lecanicilliuma
				Metarhiziuma
				Nomuraea

Source: (Roy et al. 2006)

7.2.3. Host range

Fungal infections to the most insect orders with all life stages have been observed, while infection to the immatures of holometabolous insects have been reported more commonly (Tanada and Kaya 1993). The host range may differ significantly among different species of EPFs and even among different strains of the same single species. For obligate pathogens, specifically restricted to a narrow host range and complicated life cycles associated to their insect host like *Strongwellsea castrans* Batko and Weiser, restricted to flies like anthomyiid (Eilenberg and Michelsen 1999) and entomophthorales, *Massospora* sp. are restricted to a single genus belonging to

cicadas (Soper 1974). In contrast, deuteromycetes, particularly *B. bassiana*, have wide host range including numerous genera of insects (McCoy et al. 1988). It must be kept under consideration that description of host range to some extent mainly relies on laboratory studies which do not reflect the true picture in nature. Some factors like insect host, fungal biology and ecology may be responsible for reducing infection in insect host. It is important to mention that fungi are capable of infecting several other arthropods, insects and the species which are not pests of cultivated crops (*Gibellula* species infect spiders and *Cordyceps* sp. and *Erynia* infect ants) (Shah and Pell 2003).

7.2.4. Mode of infection

The fungal infection to insect host is a complex process, involving chemical and physical procedures starting from spore attachment to the host death. Following steps are undertaken during infection process: (1) attachment of the spore to the host cuticle, (2) germination of fungal spore, (3) penetration into the host cuticle, (4) overcoming the immune defense mechanism, (5) formation and proliferation of hyphal bodies into the haemocoel, (6) saprophytic outgrowth from the dead host, production and dissemination of new conidia. For the successful attachment, mainly hydrophobicity of the spore and cuticular surface play significant role.

Furthermore, the germination and successful infection is affected by a number of factors e.g., susceptible host stage, humidity, optimal temperature and cuticular lipids, such as aldehydes, ketones, short-chain fatty acids, wax, esters and alcohols which may exhibit antimicrobial activity. Generally, fungal spores breach through the non-sclerotised areas of the cuticle such as joints, between segments or the mouthparts. The conidial germination starts after 10 h of attachment and may complete by 20 h at 20-25^{o}C. Before infection process, germ tube produce appressorium or penetration pegs which is accompanied by mechanical and chemical processes by the production of several enzymes (proteases, chitinases and lipases etc.) (Ortiz-Urquiza and Keyhani 2013).

The enzymes responsible for pathogenesis are generally grouped as chitinases, proteases, peptidases, and lipases as follows:

7.2.4.1. Chitinases

The chitin is a major constituent of the insect cuticle, therefore, endo- and exo-chitanses are important enzymes for the breakdown of N-acetylglucosamine polymer of insect cuticle into monomers and a key factor determining the fungal virulence (Khachatourians 1991). Endochitinases, N-acetyl-β-D-glucosaminidases and chitinolytic enzymes from *B. bassiana*, *M. anisopliae* (Metchnikoff) Sorokin and *Metarhizium flavovirid* Gams and Roszypal were presented in broth culture nourished with insect cuticles.

7.2.4.2. Proteases and peptidases

Chitin and protein are the main constituents of insect cuticle; hence proteases and peptidases of EPFs is considered key degrading enzymes of insect cuticle, saprophytic growth, initiation of prophenol oxidase in insect haemolymph,

furthermore they are also responsible for virulence in EPFs. Some genes of overlapping response with a unique expiration pattern were observed when encountered with the cuticle of *Lymantria dispar* (Linnaeus), *Blaberus giganteus* (Linnaeus) and *Popilla japonica* Newman using cDNA counted gene expression responses to the cuticles of number of host insects and constructed microarrays from expressed sequence tags, clone of 837 genes (Freimoser et al. 2005).

7.2.4.3. Lipases

The epicuticle of the insects is chiefly composed of non-polar lipids which play an important role in chemical signaling between insect host and EPFs (Blomquist and Vogt 2003). It keeps cuticular outer surface dry which aids to avoid the penetration of chemicals and insecticides (Blomquist et al. 1987; Juárez 1994). They are chemically stable with high molecular mass, mainly due to the presence of specific physicochemical characteristics, like number of carbons, length of the chain and the kind and position of double bond and the functional groups. The long chain HC, free fatty acids, fatty alcohols and wax esters are ample components of the insect epicuticle. It also contains fats, waxy layers and lipoproteins which act as a barrier to the action of lipoxygenases and lipases of eontomopathogenic fungus. Among these compounds some have anti-fungal activities (Khachatourians 1996) while some other possess saturated fatty acids which can inhibit the fungal growth.

7.2.5. Toxins

The biochemical properties and structure of some major fungal metabolites have been investigated in detail (Vey et al. 2001), but very few studies have been conducted regarding the metabolite production under field conditions (Bandani et al. 2000; Strasser et al. 2000). One major problem to fungal toxins is that one type of fungi can produce variety of bioactive metabolites and risk assessment to these entire compounds would be enormous. Furthermore, fate of their toxins is little known in the environment, which would be the key question for their registration.

7.2.5.1. Destruxins

Destruxins are moderately dissimilar compounds which occur as isomers. Basically destruxins contain 5 amino acids and α-hydroxy acid which may be found in many different forms. So far 28 different but structurally similar destruxins have been isolated from different EPFs mostly were discovered from *M. anisopliae* isolates (Vey et al. 2001). Insects exhibit varying susceptibility levels to destruxins and lepidopterans have been reported as the most susceptible amongst the all studied insect orders (Samuels et al. 1988; Kershaw et al. 1999). The toxicosis symptoms also vary among insect pests - the most peculiar symptom is an immediate tetanus; which at low concentrations develops for up to three minutes period, while brief or no paralysis is depicted at high dose rates (Abalis 1981; Samuels et al. 1988).

7.2.5.2. Oosporein

Oosporein is produced mainly from the soil inhibiting fungi like *Beauveria* spp. which contain red colored di-benzoquinone (Eyal et al. 1994). It reacts with amino acids and proteins through redox reaction by altering the SH-groups and results

malfunctioning in enzymes (Wilson 1971). Like bassianin and tenellin, oosporein also inhibit the activity of erythrocyte membrane ATPase which is directly proportional to the dose rate of oosporein. Upto 50% activity can be ceased at 200g/ml. All these pigments greatly influenced Ca^{2+}-ATPases compared to the activity of Na^{+}/K^{+}-ATPase. Antibiotic effect of oosporein against gram-positive bacteria has also been observed with no or little effect on gram negative bacteria (Taniguchi et al. 1984; Wainwright et al. 1986).

7.2.5.3. Beauvericin and beauveriolide

Beauvericin is also an important toxin isolated from *Beauveria*, *Paecilomyces* sp. Samson, the plant pathogenic fungi *Polyporus fumosoroseus* and *Fusarium* sp. (Gupta et al. 1991; Plattner and Nelson 1994). Gupta et al. (1995) described two different forms of these toxicants Beauvericin A and B forms K^{+} and Na^{+} complexes, which increase the membranes permeability (Ovchinnikov et al. 1971). It also exhibits antibiotic activity against a number of bacteria like, *Escherichia coli* Migula, *Mycobacterium phlei* Lehmann and Neumann, *Bacillus subtilis* Ehrenberg, *Sarcinea lutea*, *Streptococcus faecalis* and *Staphylococcus aureus* Rosenbach (Ovchinnikov et al. 1971).

7.2.5.4. Bassianolide

Another toxin cyclo-octadepsipeptide also called bassianolide is secreted by *B. bassiana* (Suzuki *et al.* 1977). Bassianolide is also an ionophore which exhibits different reactions with different hosts (Kanaoka et al. 1978). Very little is known about the toxic nature of bassianolide against plants and animals, the synergistic interaction with the structurally associated mycotoxin moniliformin may be possible.

7.2.5.5. Beauveriolide

Beauveriolide were isolated from *Beauveria* spp. which is structurally related to bassianolide and beauvericin (Namatame et al. 1999). The toxic effect of beauveriolid towards plants and animals is still unknown except beauveriolide I (Mochizuki et al. 1993). Over all these cyclodepsipeptides may still have an unreported health hazard effects is common. Except the above mentioned metabolites these *B. bassiana* also produce bassianin, tenellin and two non-peptide toxins isolated from *Beauveria* spp. which aid in inhibiting the erythrocyte membrane ATPases (Jeffs and Khachatourians 1997).

7.3. Bacteria

Existence of bacteria is as old as the history of life on earth. Evidence of rocked bacterial fossils date back to Devonian period (416-359.2 million years ago) and considerable signs depict their presence from Precambrian time, about 3.5 billion years ago. The fossils found in north-west Australia's Pilbara region are thought to be nearly 3.5 billion years old and considered the oldest ones on earth planet. In Proterozoic Eon (about 1.5 billion years ago), when the activity of cyanobacteria resulted in oxygen production, bacteria became widespread (*Anonymous 2013)*. The gradual evolution of the bacteria made them able to survive under wide range of environment with several descendent forms. As a result of this, today an uncountable

and immeasurable diversity in morphology, physiology and taxonomy of bacteria prevails. Bacteria have been found living very close to other living organisms particularly human beings. Both beneficial and harmful forms of bacteria have been thriving in various climates like soil, water, air and hot water springs etc.

Within the preview of Prokaryotes, bacteria are the microorganisms that lack the nuclear membrane which separates genetic material from cytoplasmic contents and other membrane bounded organelles. Bacteria surround us all around and thus, can be isolated from any environment and hence their enriched flora can be given the name of metabolic strategy which they use to earn energy such as *phototrophs* (gain energy from sunlight), *lytotrophs* (obtain energy form inorganic material) and *organotrophs* (receive energy from organic material). The variation in their size is from one to few microns, and depending upon the morphologies, they can be grouped as *cocci* (spherical), *bacilli* (rod shaped) and *spirochetes* (spiral shaped). Propagation in bacteria is carried out through binary fission, a mode of asexual reproduction in which daughter cells is produced from mother cell as clonal copies (Jurat-Fuentes and Jackson 2012).

7.3.1. Classification

The recognized factor for classifying bacteria involves the sequence of 16S ribosomal RNA. Two important groups of bacteria are *Eubacteria* (true bacteria) and *Archaea* containing bacteria having similar features of DNA replication, transcription and translation as exhibited by eukaryotes. Three major divisions within Eubacteria are primarily based on the presence or structure of cell wall: *Gracilicutes* Gibbons and Murray (gram negative typed cell wall bacteria), *Firmicutes* Gibbons and Murray (gram positive typed cell wall bacteria) and *Tenericutes* Murray (Eubacteria which are devoid of cell wall). Most recent classification within Eubacteria mostly relies on the use of polyphasic taxonomy that includes analysis of nucleotide sequence of RNA (16S rDNA), DNA-DNA hybridization, genotypic, phenotypic and phylogenetic aspects (Brenner et al. 2005). Entomopathogenic bacteria; greatly concerned with entomological studies are grouped in Eubacteria.

The cell wall of bacteria greatly serves the purpose to classify, support molecules and organelles. In gram-positive bacteria, the cell wall is formed of cross-linked peptidoglycan. On the other hand, cell wall in gram-negative bacteria is formed of rather complex thin layer of peptidoglycan, lipoproteins and an outer polysaccharide membrane. Gram-negative bacteria are distinguished from gram-positive bacteria by lacking the ability to retain crystal violet dyes. Gram-positive are endospore forming, rod and cocci shaped bacteria often undergoing sporulation. Gram-negative bacteria on the other hand appear to be in rod or cocciform. They are much more diverse in their distribution, also can be isolated from diseased and dead insect specimens (Jurat-Fuentes and Jackson 2012).

7.3.2. Entomopathogenic bacteria

7.3.2.1. History

Confirmatory evidences of using entomopathogens for the control of insect pests are not known, however, human interest in exploiting microbes particularly bacteria rose to its extreme after the discovery and the commercial availability of microscope in late 19th and early 20th century. Scientific efforts for the survival of famous Japanese silk industry against sudden death of caterpillars proved fruitful resulting in the discovery of spore forming bacterium *Bacillus sotto* by Sigetane Ishiwata (1868-1941) (Aizawa 2001). This discovery lead to the world's first ever demonstration of toxin when many other scientists including Aoki and Chigasaki (1915) and Mitani and Watari (1916) found enhanced lethal action of bacterial culture on silk worms when it was dissolved in alkaline solution (Aizawa 2001). Doors of discoveries were opened for man and a German scientist Ernst Berliner in 1909 isolated a bacterium named by him as *B. thuringiensis* that killed Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

7.3.2.2. Mode of infection

The ingestion of *B. thuringiensis* compounds by insects follows the route of mid gut to expose it to alkaline environment of gut (pH >9.5). Here the higher pH of the gut solublizes the inactive, otherwise insoluble proteins resulting in the release of crystal proteins that produces δ-endotoxins. This proteolytic activation of δ-endotoxins offers an extraordinary insecticidal activity to insects and this activated toxin readily gets bound to specific receptors present at apical brush border of the midgut microvillae in target insects (Hofmann et al. 1988). The toxic action of proteins is attributed to N-terminal half consisting of seven anti-parallels α-helices. These α-helices offers potential gradient by penetrating the membrane and forming an ion channel in apical brush border membrane allowing rapid flux of ions. Loss of integrity of insect's gut is the outcome of *B. thuringiensis* activity causes starvation and septicemia which leads to the death of insects (Kumar et al. 2013).

The penetration of α-helices in the apical brush border membrane forms an ion channel (Knowles and Dow, 1993). As a result, rapid flux of ions takes place because of toxin-induced pores formation (Wolfersberger 1989). Consequently the gut integrity gets lost that resultant starvation and/or septicemia leads to insect death.

A wide array of *B. thuringiensis* products formulated for commercial uses have an extended spectrum of action effective to secure food crops, forest trees, stored grains and ornamentals (Meadows 1993). Contrary to hazards associated to chemical pesticides, *B. thuringiensis* formulation offers a wide range of benefits. Although it is highly virulent to target insects, yet it is harmless to non-target insects due to its specificity. In spite of decades of use in field, *B. thuringiensis* toxins are still reported as non-hazardous to animals, human beings and other non-target pests. All these characteristics render it highly suited to include IPM programs (Nester et al. 2002). Besides these benefits, *B. thuringiensis* formulations have some associated limitations (McGaughey and Whalon 1992). One of the limitations is its effectiveness against specific stage of insect especially immature stage. For this reason, an effective control of targeted insect requires repeated application. *B.*

thuringiensis products perform excellently against insect pests exposed to environment than insects concealed within plant parts or some other structures. But the expression of *B. thuringiensis* gene(s) using transgenic cultivars (Krattiger 1997) can come up with all such concerns.

7.3.3. Important entomopathogenic bacteria

7.3.3.1. Paenibacillus popilliae

Paenibacillus popilliae Dutky formerly known as *Bacillus popilliae* Dutky is a gram-positive, spore-forming bacterium which was initially isolated from infected Japanese beetle (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae) larvae in the late 1930s and then named after the name of its first host. The spore forming capability of bacterium protects it from heat, cold, drying and other harsh environmental regimes.

Paenibacillus popilliae plays a major role in biologically managing scarabs, particularly Japanese beetle (Petterson et al. 1999). *Bacillus popilliae* has been reported from at least 29 scarabs, mostly from Melolonthinae and Rutelinae. *Paenibacillus popilliae causes milky spore disease in P. japonica* and it is the first pathogen registered as insect biological control in USA.

7.3.3.2. Brevibacillus laterosporus

Brevibacillus laterosporus Laubach is a gram-positive, rod-shaped, endospore-forming bacterium and is considered an important entomopathogenic and antimicrobial agent. It is morphologically distinguished by producing characteristic canoe-shaped parasporal body (CSPB) firmly attached at one end of the spore imparting it lateral position in the sporangium. Ubiquitous existence of this bacterium has enabled its isolation from various reservoirs particularly soils, insect bodies, fresh and sea water, leaf surfaces, compost, milk, honey, factory effluents, animal hide, wool and many other materials (Ruiu 2013). It was discovered by White (1912) during 20th century associated with honey bees determined during an investigation on European foulbrood.

7.3.3.3. Bacillus subtilis

German botanist Ferdinand Cohn in 1877, while working on hay *Bacillus*, discovered two new forms of *Bacillus* strain named *Bacillus subtilis*; one of them was heat sensitive (without endospore) while other was heat tolerant (endospore). A significant genomic diversity in the bacterium has been publicized using genomics analysis based on microarray-based techniques. It is competent for growth in many environmental conditions and is often considered as soil dweller. Most common sources of its isolation are air, soil, water and decomposing plants. However in most of the cases, it is not found naturally in biologically active but occurs in spore forms. *Bacillus subtilis* is scientifically fabulous for its ability to produce a number of antibiotics especially bacitracin and iturin. It regulates the development of adult mosquitoes by inhibiting their growth (Ramathilaga et al. 2012).

7.3.3.4. Bacillus sphaericus

Bacillus sphaericus is a naturally occurring spore-forming gram positive bacterium that exhibits strong insecticidal properties. It possesses efficient larvicidal properties against mosquito by producing delta-endotoxins via sporulation that binds strongly to receptors in midgut epithelial lining of mosquito larvae. The bacterium has narrow spectrum and quite specific activity that sometimes decreases its demand for use in field. Enhanced time of lethal action against some mosquito species and recycling of toxin within dead mosquito sometimes works as limiting factors for its use. One of the advantages exhibits over *B. thuringiensis* var. *israelensis* is its longer persistence that provides long lasting control (Filha et al. 2008).

7.3.3.5. Wolbachia

Wolbachia are inherited from α-protobacteria, litreally the members of the order Rickettsiales; a varied group of intracellular bacteria that comprises species exhibiting parasitic, mutualistic and commensal associations with their hosts. With its pathogenic nature extended to arthropods and filarial nematodes, it is regarded as the most common endosymbiotic bacterial species on the globe. The only member contained with genus *Wolbachia* in family Anaplasmataceae and order Rickettsiales is *Wolbachia pipientis* Hertig; rest of species; *W. melophagi* Noller, Philip and *W. persica* Suitor and Weiss have been recently declared as unrelated (Dumler et al. 2001). An insight into the intracellular life study of the bacterium ensures its obligate nature of infection to hosts and it has been found successfully infesting about 66% of the insect species (Hilgenboecker et al. 2008). *Wolbachi*a being intracellular bacterium get vertically transmitted through egg. The outcome of this cellular transmission is the manipulation of reproduction by invading bacteria that mostly appears in the form of cytoplasmic incompatibility. One of the vital reasons behind the successful propagation of *Wolbachia* in arthropods is its inherent ability to take control of the host's reproductive cycle by providing nutrients and protecting host from other pathogens (Hosokawa et al. 2010).

The genera quite related to *Wolbachia*; *Anaplasma*, *Ehrlichia* and *Neorickettsia* during their life stages include an invertebrate 'vector' and mammalian 'host' and in some cases invertebrate associations in some species have also been found. But contrary to unlike members, *Wolbachia* not necessarily involves or infects vertebrates. One of the important reasons behind increased interest for *Wolbachia* is their immense diversity, interesting phenomena shown while infecting their hosts such as reproductive manipulation, and their possible exploitations for pest and disease vector control (Bourtzis 2008).

7.3.3.6. *Bacillus thuringiensis*

Bacillus thuringiensis (*Bt*) holds a prominent position among commercial chemical compounds important for agricultural insect pests. It is a naturally occurring spore forming, gram-positive bacterium. It has been found as a source and reservoir of several important insecticidal proteins like *δ*-endotoxins, vegetative insecticidal proteins (vip) and cytolytic proteins etc. Among these proteins, *δ*-endotoxins have a vital role in protecting number of important crops from various insect pests. *Bacillus thuringiensis* insecticides have proved their worth as a biopesticide to protect food

crops, cash crops, ornamentals, forest trees and stored commodities (Meadows 1993).

For convenience, life cycle of *B. thuringiensis* can be divided into different phases; Phase-I: vegetative growth; Phase-II: transition to sporulation; Phase-III: sporulation; and Phase-IV: spore maturation and cell lysis (Berbert-Molina et al. 2008). More than 150 genes of exhibiting insecticidal nature have been identified from *Bt* δ-endotoxins family of proteins (Crickmore et al. 1998). These crystalline (cry) proteins remain inactive until the exposure to alkaline contents (pH >9.5) of insect mid gut, solubilize them (Milne and Kaplan 1993) and ultimately liberating δ-endotoxins proteins.

7.3.4. Host range of *B. thuringiensis*

Different commercial products of *B. thuringiens*is in use for crops, forests and aquatic system not necessarily contain β-exotoxin. Most of the *B. thuringiensis* products registered against insect pests contain Cry toxins (also known as δ-endotoxins). Normally, a single Cry protein works perfectly against a single order and sometimes against several families within an order. The Cry2 is an exception to this fact as it exhibits insecticidal nature against several families of Diptera and Lepidoptera (Schnepf et al. 1998).

Most of the commercial *B. thuringiensis* products or purified Cry toxins formulated for lepidopterous insects are non-hazardous to a vast variety of non-target organisms (Sims, 1997). However, non-target lepidopterans are mostly at risk in *B. thuringiensis* treated plants particularly in forests (Herms et al. 1997). For instance, the aerial spray of *B. thuringiensis* subsp. *kurstaki* (*Btk*) to control gypsy moth was found to be lethal to non-target Lepidoptera 3000 m away from treated site (Whaley et al. 1998). However, no or negligible effect was found for aquatic habitats in *Bt* treated sites when Kreutzweiser et al. (1992) demonstrated high concentrations of *Btk* on drift and mortality of Ephemeroptera, Plecoptera, and Trichoptera. Predators that preyed upon *B. thuringiensis* treated hosts were not found susceptible except the *Chrysoperla carnea* (Stephens). So in this regards, it would rather be justified statement to declare *B. thuringiensis* toxin rather safe, specific in action and compatible to non-target individuals.

7.4. Viruses

The name virus is basically derived from the Latin word ‘venome’ meaning poisonous fluid. When litreally defined, it is an infectious entity with non cellular features exhibiting either RNA or DNA, encased in a proteinaceous coat and capable of reproduction in living cells only. Generally viruses make use of host’s biosynthetic machinery for their replication to get transferred themselves to other cells of their host’s body.

7.4.1. Entomopathogenic viruses

Viruses particularly the baculoviruses also constitute an important component of microbial control program of insects. They comprise the most diverse group of entomopathogenic viruses which have been found exclusively on insect populations, especially within the insect order Hymenoptera, Coleoptera and Lepidoptera.

7.4.2. History

Diseases related to entomopathogenic viruses are known since the 16th and 17th century when Vida in 1557 discovered the Nuclear polyhedrosis virus (NPV) causing *Jaundice or grasserie* disease in silk worm moths *Bombyx mori* L. (Lepidoptera: Bombycidae) (Steinhaus 1975). In 1856, two Italian scientists Maestri and Corline described the refractive occlusion bodies in jaundiced larvae of silk moth. Bolle (1906) demonstrated that these refractive occlusion bodies were the causative agent of viral transmission in healthy animals and mentioned if refractive bodies were removed before inoculum the jaundice did not occur. Glaser (1918) was first who mentioned the nuclear polyhedrosis as a filterable virus and later published his work in Science. By 1926, French investigator Andre Paillot (Paillot 1926) discovered first time granuloviruses which were found in cabbage butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae) and was characterized by a large number of small granules. Likewise, Ishimori (1934) described another disease in which host replication site of occlusion bodies were cytoplasm rather than nucleus (Cypoviruses). The invention of electron microscope makes many invisible objects visible and allowing Bergold and Suter (1959) to observe the occlusion bodies with in the crystalline matrix and they published an article on electron micrograph of NPV in the first volume of Journal of Invertebrate Pathology (Arif 2005). During 1970s and 1980s significant advances were made on the genetics of entomopathogenic viruses, especially baculoviruses.

7.4.3. Classification

The classification of viruses is based on several factors such as the type of nucleic acid (i.e. single stranded or double standard RNA or DNA), nucleocapsid symmetry and size (i.e. isosederal or oval etc.), presence or absence of occlusion body around the viron, host range, replication site and many others. The naming of entomopathogenic viruses followed the same criteria set by International Committee on Taxonomy of Viruses (ICTV). In general, viruses are classified on the basis of nucleotide sequence which not only distinguish the viral species, but also establish their evolutionary relationship among the viruses with in the same group. Insect viruses are named in acronym, according to their host and viral group to which they belong, for example *Autographa californica* (Speyer) multi-nucleopolyhedrovirus (AcMNPV), *Cydia pomonella* (Linnaeus) granulovirus (CpGV) and *Orycetes rhinoceros* (Linnaeus) nudivirus (OrNV).

7.4.4. Some important groups of entomopathogenic viruses

7.4.4.1. Nudiviruses

Nudiviruses are single stranded RNA viruses formed as rod shaped non occluded viron which was previously classified in single genus (baculovirus) and family baculoviridae. But after the sixth report of International Committee on Taxonomy of Viruses (ICTV), taxonomy status of non occluded viruses has been changed and placed in a separate family Nudaviridae (Murphy et al. 1995). This group included three viruses *Orycetes rhinoceros* nucleovirus (OrNV), *Helicoverpa zea* (Boddie) nucleovirse (HZNV-1) and *Gryllus bimaculatus* De Geer nucleovirus (GbNV) (Huger et al. 1985). All these viruses are similar in terms of replication which takes place in the cytoplasm and later spread into other tissues and organs (Huger et al. 1985; Huger and Krieg 1991), but different in mode of transmission. Both OrNV and GbNV inoculum transmitted orally (Huger *et al.* 1985) but HZNV-1 transferred through the reproductive system of both infected male and female of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae).

7.4.4.2. Cypoviruses

Members of the family Reoviridae have wide range of hosts including vertebrate and invertebrates. Insect specific cyproviruses isolated from more than 250 different species of insects belonged to order Lepidoptera, Diptera and Hymenoptera (Hukuhara and Bonami 1991). They contain a double stranded linear RNA molecule consisting of 12 lateral projections on non-enveloped isohedral viron, occluded with in the polyhedrin protein which has different amino acid sequence from polyhedrin and granulin protein of Baculoviruses (Arella et al. 1988). Cypovirus infection start when larvae consume the virus infected leaf, viron enter into the midgut columnar cell through plasma membrane and replicate only within these cells (Tan et al. 2003). Infections by cypoviruses are chronic in nature and often lead to larval retardation, un-matured adult and transfer of disease from infected to healthy ones (Nagata et al. 2003; Bellonick and Mori 1998).

7.4.4.3. Entomopoxyviruses

Entomopoxyviruses were discovered by Vago (1963). Early research shows its close association with orthoviruses on the basis of morphology but differentiated being progeny virons occluded in proteinaceous matrix after replication (Arif 2005). Entomopoxyviruses belong to family poxyviridae, have double stranded DNA molecule, formed brick shaped virons occluded in spheroid shaped occlusion bodies. Due to the wide host range, *entomopoxyviruses* are divided into two sub-families i.e., Chordopoxvirinae (vertebrate viruses) and Entomopoxvirinae (insect viruses) (Faulkner et al. 1997). Entomopoxyvirinae is further divided into three genera: Alphaentomopoxyvirus (specific to Coleoptera), Betaentomopoxyvirus (specific to Lepidoptera and Coleoptera) and Gammentomopoxyvirus (specific to Diptera).

7.4.4.4. Baculoviruses

The potential role of Baculoviruses in pest management has stemmed after their frequent isolation from insect host. Baculoviruses have two distinct phenotypes on the basis of structure; the occlusion derived viron (ODV) and budded viron (BV)

(Henderson et al. 1974; Summerson and Volkman 1976). The occlusion derived viron are embedded inside the matrix, mostly initiate the infection processes of baculoviruses from cell to cell and having single or multiple nucleocapsid per viron in parallel pattern (Adams and McClintock 1991). On the other hand, the budded viron transfer Baculovirus infection from insect to insect having single nucleocapsid per viron (Summerson and Volkman 1976). The family baculoviridae has double stranded circular DNA molecule, after removing the non-occluded DNA viruses from occluded DNA baculovirus, baculoviridae comprises of two genera Polyhedrovirus and Granuloviruses (Murphy et al. 1995). The cuboidal shaped Nucleopolyhedrosisviruses (NPVs) have occlusion body of 0.4 to 2.5 μm in size visible under electron microscope (Moser et al. 2001; Shapiro et al. 2004). In contrast, granuloviruses have ovocylindrical shape, occlusion body of 0.13 to 0.50 μm in size and cannot be seen under electron microscope (Tanada and Hess 1991). However, sequences of NPVs isolated from different hosts showed that they are polyphyletic (Herniou et al. 2004) which subdivide the family baculoviridae into four genera (Jehle et al. 2006): alpha baculoviruse (Lepidopteran specific genus NPV), beta baculoviruse (Lepidopteran specific genus GV), gamma baculovirses (Hymenopteran specific genus NPV) and delta baculoviruses (Diptera specific genus NPV).

7.4.5. Mode of infection

7.4.5.1. Primary infection

Primary infection of baculovirus is initiated when occlusion body (OB) are ingested by the susceptible larvae during feeding on contaminated foliage. The occlusion body (OB) desiccated in the high pH (<7) of midgut lumen and liberate the occlusion derived virion (ODVs) (Vega and Kaya 2012; Rohrmann 2008). The encapsulated viron enter into peritrophic membrane of midgut columnar cells and bind to the tip of microvilli (brush boarder membrane of cells). After fusion between cytoplasm and virus membrane (Faulkner et al. 1997; Haas-Stapleton et al. 2004), nucleocapsid get released into the cytoplasm and move by means of actin polymerization. Some nucleocapsids are transported into the nucleus and uncoat the viral DNA which express the early gene glycoprotein encoding the enveloped budded viron (BV) (Rodriguez et al. 2012).

7.4.5.2. Secondary infection

Systematic infection from cell to cell is accomplished by budded viron (BV) which are produced in midgut (Rodriguez et al. 2012). Budded viron of baculovirus enter in to insect cells through Clathrin-Mediated Endocytosis pathway, followed by internalization of BV into low pH endosome prompting the fusion between BV and cellular membrane (IJkel et al. 2000). The released nucleocapsid move into cytoplasm through actin polymerization and are ultimately transported to the nucleus. The electron dense structure formed in the nuclei called virogenic stroma where viral DNA transcription and translation can occur and the progeny nucleocapsid is released (Kawasaki et al. 2004; Vega and Kaya 2012). The nucleocapsid gets enveloped in the peritomial region to form the occlusion derived viron (ODV). In most of pathogenic viruses, break down of host tissue occurs from the expression of

cathepsin proteins and virus-encoded chitinases (Ohkawa et al. 1994; Slack et al. 1995). As a result of expression of viron encoded chitinase and cathepsin proteins tissue degradation of host occurs. OB is released in to the environment after the breakdown of insect cuticle and approximately 1×10^9 OB are released from the single host larvae and remain in the environmental reservoir over a long period of time, until eaten by any other susceptible larvae to resume the infection (Evans and Harrap 1982).

7.5. Nematodes

Nematodes belong to the kingdom Animalia. These are non-segmented animals generally referred as roundworms, eelworms or threadworms due to their usually cylindrical and elongated body. Their size generally ranges from few mm (0.1 mm) to several meters in length. The female of some species, however become swollen at maturity and have pear shaped or spheroid bodies. The nematode body is more or less transparent. It is covered by a colorless cuticle, which is usually marked by striations or other marking. The cuticle molt when a nematode goes through the successive juvenile stage. The cuticle is produced by the hypodermis which contains living cells and extends into the body cavity as four chord separating four bands of longitudinal muscles. Numerous species of nematode attack and parasitize human and animals in which they cause various diseases. Several species are characterized of feeding on living plants, obtaining their food with spear or stylet and become a reason for numerous diseases of plants worldwide.

7.5.1. Entomopathogenic nematodes

The widely used term entomopathogen both in parasitology and pathology referred to microorganisms which have the ability to cause diseases in insect host (Onstad et al. 2006). Among the seven families of insect parasitic nematode, the nematode in the family Steinernematidae and Heterorhabditidae have evolved the symbiotic association with insect pathogenic bacteria. Entomopathogenic bacteria are those who have the LD_{50} of 10000 cells are less. This means that an inoculum of 10,000 bacterial cells required to kill the half population of tested insect (Bucher 1960). The bacteria in the genus *Xenorhabdus* makes mutualistic interaction with Steinernematidae and Photorhabdus with Heterorhabditidae.

7.5.2. History

Gotthold Steiner was the first who described the entomopathogenic nematode as *Aplectana kraussei* (now *Steinernema kraussei*) (Poinar 1983). Glaser and Fox (1930) isolated the second nematode *Neoaplectana glaseri* (now *Steinernema glaseri*) from Japaneese beetle, *Popillia japonica* and Steiner placed in the family Oxyuridae. The importance of bacteria in the life cycle of nematode was described by Gaugler et al. (1992). He showed that when trying to develop axenic (without any organism) culture of *S. glaseri* for the control of insect pest, the bacterial contamination was tolerated by nematodes (Lewis and Clarke 2012). Probably the contaminated bacteria were *Xenorhabdus poinari* a symbiont of *S. glaseri.* Later he

developed the axenic culture of *S. glaseri* to control the Japanese beetle, but success was limited (Lewis and Clarke 2012).

The first symbiotic relationship between nematode and bacteria was described by Dutky (1937) and he noticed the antibiotic properties of bacterium associated with *Steinernema carpocapsae* (Nemata: Steinernematidae), which explained how it could destroy foreign bacteria that invaded the insect cadaver containing the developing nematodes. Since then several antibiotics, including xenorhabdins, xenocaumacins, hydroxystilbenes, indole derivatives, and anthraquinone derivatives, were recovered from cultures of *Xenorhabdus* and *Photorhabdus* (Walsh and Webster 2003).

7.5.3. Classification

Out of 23 insect parasitic nematode families seven have the ability to serve as the potential biocontrol agent. These are, Allantonematidae, Heterorhabditidae, Mermithidae, Phaenopsitylenchidae, Sphaerulariidae (***Order:*** Tylenchida); and Steinernematidae (***Order:*** Rhabditida) and Tetradonematidae (***Order:*** Stichosomida). Recently only few of them are in commercial production as microbial insecticide particularly Heterorhabditidae and Steinernematidae. The spectrum of biocontrol potential of most of nematodes is limited because of problems associated with their culturing except the Tylenchid, Deladenus (Beddingia) and Siricidicola, which are still in convention for inoculative control of wood wasp species in Australia (Bedding 1993), the microbial control potential of other nematode species is rather limited because of problems with their culture.

7.5.4. Mode of infection

The infective juvenile stage (IJs) is the only free living stage in entomopathogenic nematodes (EPNs) which have the ability to infect new host. Mostly nematode based commercial formulations have this IJs stage to control insect pest but some formulations contain infected insect host. There are six life stages in EPNs, first egg stage, four juvenile stages and last one is adult stage. The third stage is IJs which is similar to dauer stage of *Caenorhabditis elegans* (Maupas) (Rhabditida: Rhabditidae). The term dauer meaning “enduring” and in dauer stage nematode survive external harsh condition without nourishment. The IJs enter the host through anus, mouth and spiracles. But in some cases both Heterorhabditidae and Steinernematidae makes a thin hole in host cuticle. After successful penetration the IJS release the bacteria in host haemolymph. The bacteria produced toxin which kill the host (Lewis and Clarke 2012). The main difference between Heterorhabditidae and Steinernematidae is that first IJs in Heterorhabditidae become hermaphrodites adult but amphimictic in the following generation. But in case of Steinernematidae all adults are amphimictic except in *Steinernema hermaphroditum* in which adult are hermaphrodites in first generation (Koppenhöffer 2007).

7.5.5. Host range

The main reason for their failure is poor understanding of nematode ecology. A significant reduction in pest population has been achieved by corresponding biological and ecological studies of nematode and target pests. The similarity in biological and ecological consideration becomes essential for nematode and their hosts if some output has to be brought out in nematological applications. The most important considerations that have to be met to make nematode applications as efficient control strategies are the foraging efficiency and temperature dependency of nematode species. Further the ease of access to its host and suitability to host is another factor required by nematodes.

Applications of EPN have been made against soil insects, cryptic insect, aquatic insect and on foliage insect (Koppenhöffer 2007). Mostly success with nematode has been achieved against soil insects because soil is the natural habitat of nematode which acts as buffer for its IJ stages.

The IJ stages of nematode enter the host through mouth, anus and spiracles but in some insect's nematodes are not accessible to these openings (Koppenhöffer 2007). For example in case of wire worm, the blockage of mouth by oral filters, in sucking and young instars of chewing insects, narrow passage of mouth parts, constriction of anus in wireworm, covering of spiracles with septa or sieves in scarab beetle or very narrow passage in some dipterous and lepidopterous insects. The restriction to nematode entrance could be due to some other obstacles in intersegmental membranes, fore and hindgut cuticular linings, or the peritrophic membrane. Aggressive grooming or evasion behavior may also hinder nematode infections in many cases (Gaugler et al. 1994). The formation of impenetrable cocoons or soil cells seems to be another limitation in the use of nematodes for insect control (Eidt and Thurston 1995). Social insects as a whole appear to be susceptible host to nematode infections but they cater these pathogenic invasions by isolation, removal of infected individuals, social grooming, and translocation of colony (Klein 1990; Gouge 2005).

7.6. Conclusion

Arthropods are dominant creature in the world which has millions of described species. Among them a vast majority is beneficial to human beings. While small percentage is considered pest and vector species which cause number of diseases in human beings and devastate the crops, cause losses about 18% by cutting down the world annual crop production, contribution of losses in stored grains appears to be 20%, with total devastation of around 100 billion US$ every year. Worldwide inter-dependence of markets for agricultural produces have gradually brought to forefront the need to develop agricultural practices that moderate the hazards of insecticide residues on the environment and that result in bio-rational products that are safe for human consumption. Frantic efforts are being focused towards non-chemical and sustainable plant protection methodologies. The greater concerns of synthetic chemicals to human, its belongings and environment have highlighted the importance of "entomopathogenic" microorganisms as a reality. Amongst the virus, bacteria, nematode and fungi are applicable, well studied and widely used in pest control. They

have shown meritorious results in the management of various insect pest populations. Numerous entomopathogens are employing for the control of insect pests in various agricultural systems. New research suggests that they deploy various mechanisms and their effectiveness is affected by plenty of biotic and abiotic factors. Naturally occurring non-pathogenic endophytic and epiphytic microorganisms may also affect the effectiveness of entomopathogenic fungi against plant pathogens. Detecting deleterious and beneficial relationships with other microbes may permit for the manipulation of agricultural ecosystems to enhance the synergistic interactions. Research has also been focused to examine the spore surface chemistry of entomopathogenic fungi solving problems of fungal formulations to improve the shelf life, physical characters and biocontrol efficacy of the product.

The greater concern of future research regarding commercial *Bt* products is to develop newer products with increased host range, effective infection cycle, improved shelf life, enhanced persistence and decreased cost of production. Studies at molecular level are assisting to identify and isolate the toxins and virulent factors responsible for host infection.

Expressing fusion proteins has successfully broadened the host range of entomopathogenic viruses is another area of future research. Gene silencing method RNA interference (RNAi) is another new and promising approach to control the pest population.

In vitro mass production of nematode can be increased by understanding the nutritional contribution with their symbiotic bacteria *Photorhabdus* and *Xenorhabdus*. Different rational based techniques could be used to increase the nutrients requirement of nematode for efficient recovery of IJs. Research should also be focused on the mechanisms of suppression of plant pathogenic nematodes by EPN, which will emphasize the need on the use of EPN in integrated disease and pest management systems.

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