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Microbes in insect pest management: An overview

Pankaj Sood, S.S. Kanwar*, P.K. Mehta and Amit Choudhary
Departments of Entomology, College of Agriculture
* Department of Microbiology, College of Basic Sciences
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

Abstract

Insect-pests and diseases are one of the major constraints in achieving higher crop productivity making usage of pesticides inimical. Indiscriminate and injudicious use of synthetic pesticides to suppress the pests and diseases during the post green revolution had led to the environmental and human health hazards. Consequently, the alternative strategies for pest management were looked for which make microbial pesticides in particular as attractive candidates. Several microbial pesticides have been developed, of which baculoviruses (NPV and GV), bacteria (*Bacillus thuringiensis*), fungi (*Beauveria*, *Metarhizium*, *Verticilium*), nematode (*Steinernema* and *Heterorhabditis*) are being utilized successfully for the management of insect-pests. The market for microbial pesticides is growing but is still less than one per cent of total; most of which is accounted for by Bt based products. In spite, the increasing gap between the demand and supply of biopesticides globally is a matter of concern. Slow action, restricted host range and limited persistence of microbial pesticides are some of the major drawbacks in their large scale usage. Efforts for identifying potent microbial agents in the rich biodiversity and molecular techniques have been applied extensively to alter the genetic make-up of these microbes. Apart from the development of transgenics in many crops against biotic stresses, the recombinant DNA technology in microbes has resulted in accomplishment of many achievements. In order to increase the uptake of microbial agents, it is necessary to develop robust biocontrol techniques utilizing the applications of molecular biology to enhance food production for the nutritional and health security of growing population.

Key words: Microbial control, insect-pests, entomopathogenic viruses, entomopathogenic bacteria, entomopathogenic nematodes.

Abbreviations: *Bt, Bacillus thuringiensis*; *Bp, Bacillus popillae*; CPV, cytoplasmic polyhedrosis viruses; EPN, entomopathogenic nematodes; GV, granulosis virus; NPV, nuclear polyhedrosis virus; ULV, ultra low volume

The green revolution in India reflected quantum jump in agricultural production, however, the growing population pressure and diminishing per capita available land, demands further increase in productivity. Insect-pests and diseases are one of the major constraints in achieving higher productivity. Indiscriminate and injudicious use of chemical pesticides to suppress the pests during the post green revolution era resulted in five fold increase in pesticide usage until 1990-91 (75063 metric tones technical grade) over the quantity used in 1965-66. Insecticides alone contributed for 62 per cent of the total pesticides used in India (Table 1). This had led to the environmental pollution, toxic hazards to human beings and other non-target species, apart from pest resurgence and resistance. Consequently, the plant protection researchers were forced to curtail the use of insecticides and look for safer and environmental friendly alternative strategies. Though with increasing awareness, the present quantity of pesticides applied per unit area has come down to 0.28 kg a.i. /ha as compared to 0.5- 1.0 kg a.i. /ha during 1955-64, it is likely to go down further with current ongoing efforts. However, intensive agriculture still depends on eco-destabilizing chemicals. Currently about 41350 MT (technical grade) of pesticides are used in the country. Ironically the pesticide consumption in India is far low as compared to some developed countries like USA (2.5 kg/ha), Japan (12.0 kg/ha) and Taiwan (17.0 kg/ha).

In 1962, Rachel Carson in her book "Silent Spring" aroused worldwide concern about the extensive use of pesticides which eventually led the development of the concept of Integrated Pest Management (IPM) as an environmentally sound alternative. In 1967, panel of experts of Food and Agriculture Organization (FAO) of United Nations defined IPM as "*A pest management system that, in the context of associated environment and the population dynamics of the pest species, utilizes all suitable techniques in as compatible manner as possible and maintains the pest population at levels below those causing economic injury*".

Table 1. Consumption levels of pesticides (MT Technical Grade) in India

Pesticide group	2000-01	2001-02	2002-03	2003-04	2004-05	Percentage of total (2004-05)
Insecticides	26756	29839	28197	25627	25929	62.70
Fungicides	8307	9222	10712	9087	6397.4	15.47
Weedicides	7299	6979	7857	5610	7364	17.81
Other	1222	1308	1398	438	1660	4.02
Total	43584	47348	48146	40762	41350.4	100.00

In India, the concept of IPM was first coined by Dr. S. Pradhan, who first proposed the integrated pest control utilizing the combination of different methods of pest control. Later, the understanding of the concepts of economic threshold (ETL) and economic injury level (EIL) replaced the term "**control**" with "**management**". The aim was to keep the pests below ETL and manage them without impairing the yields. The strategy to "**kill all**" paved the way to the concept of "**live and let live**".

For successful IPM, various components/ subsystems *viz.* host plant resistance, cultural practices, regulatory measures, chemical control, biological control and other measures *etc.*, need due consideration i.e. A to Z approach. Modern concepts of organic farming and natural resource management have further necessitated minimum or no use of harmful pesticides. Successful development and utilization of several microbial pesticides for the management of important pests have shown a promise in this direction. Rich diversity of microbial wealth endowed in the country and the successes achieved in pest management have made microbial control as an important and potential component of IPM programmes. The safety of microbial control to the environment and non-target species is the major advantage. In present scenario, the term Biointensive Insect Pest Management (BIPM) with more reliance on biological or microbial approaches has become more appropriate.

History of microbial control

First mention of diseases in insects dates back to the time of Aristotle (384- 322 B.C.), who mentioned diseases in honey bees. In the modern era, Bassi (1835) demonstrated the infectious nature of the fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill. in silk worm, *Bombyx mori* (L.) However, the idea of using diseases in pest control was coined by Le Conte in 1873 and consequently Metchnikoff (1879) used muscardine fungus, *Metarhizium anisopliae* (Matsch.) for the control of scarab beetle, *Anisoplia austriaca* (Hbst.). It was Steinhaus (1949), who coined the term microbial control and defined it as "the use of microbial organisms or their products (toxins) by man for the control of pests". Many attempts during 1920-1960 were made to utilize the insect pathogens for pest control and a number of bacteria, viruses and nematodes were identified with a potential to control the pests. The role of insect pathogens in pest management is becoming more evident with the advancement of knowledge.

Principal groups of insect pathogens

Bacteria, fungi, nematodes, viruses, rickettsia and protozoa are the principal groups of insect pathogens found suitable for pest management. Broadly the insect pathogens can be placed into two categories:

i) Ingested Microbes: Bacteria, viruses, rickettsia and protozoa (stomach insecticides)
ii) Penetrating Microbes: Fungi, nematodes (contact insecticides)

Viruses

Insect pathogenic viruses have been found in families Ascoviridae, Baculoviridae, Birnaviridae, Caliciviridae, Entomopoxviridae, Iridoviridae, Nodaviridae, Picornaviridae and Rhabdoviridae. However, the most studied and promising groups are the nuclear polyhedrosis (NPV) and granulosis viruses (GV) belonging to family Baculoviridae and cytoplasmic polyhedrosis viruses (CPV) belonging to family Reoviridae. The family Baculoviridae is characterized by the presence of double stranded DNA (dsDNA) genome, whereas Reoviridae represents double stranded RNA (dsRNA) genome.

Insect viruses can be grouped into two categories: Inclusion or occluded viruses (IV) producing inclusion bodies of crystalline protein and non-inclusion or non-occluded viruses (NIV). Based upon the shape of inclusion bodies, occluded viruses can further be divided into polyhedral viruses (NPV and CPV) and granulosis viruses (GV). The inclusion viruses are more

promising as insect control agents than the NIV's. Viruses are reported from virtually every insect order and are the smallest among entomopathogens. Virions of non-occluded forms range in size from 0.01 to 0.3 µm whereas, the polyhedra and other inclusion bodies which occlude the virions of the occluded viruses range from 1.0 to 1.5 µm in size (Lacey, 1997).

Nuclear polyhedrosis viruses (NPV)

Of the various types of viruses, the NPV have shown the greatest potential, because they are more virulent and kill the insects much faster than GV and CPV. The NPV are rod shaped 40-140 nm broad and 250-400 nm long. They are enclosed in two proteinaceous membranes which together with the virus particles constitute the polyhedral body or polyhedron. A polyhedron may contain one or more (upto 100) virus particles. A polyhedron is usually hexagonal but could be of other shapes. On ingestion, the polyhedra dissolve in the alkaline medium of the gut and the virions get entry into the body. The virions penetrate the gut epithelium and infect haemocytes, tracheal matrix or epidermal cells. Inside cells they enter the nuclei, attach to the chromatin and multiply. As multiplication goes on, chromatin is consumed. Eventually, the nuclear and cell membranes rupture, releasing the polyhedra into the body cavity to invade other cells. Symptoms of NPV infection appear only at a late stage when insect is near to its death. The insects stop feeding, become sluggish and pale in colour. The integument becomes fragile, ruptures easily to emit a turbid fluid with disintegrated tissues and polyhedra. Prior to death, insects climb to higher positions. The virus infected larvae die in about a week's time. The dead larvae are seen hanging upside down through pro-thoracic legs and turn black in colour.

Work on insect viruses in India was initiated as early as 1968-69 with the report of NPV from *Helicoverpa armigera* (Hb.) (Patel *et al.* 1968) and *Spodoptera litura* (F.) (Ramakrishnan and Tiwari 1969). Since then studies on insect viruses have progressed rapidly and several viruses are reported to occur in insect-pests, most of them from order Lepidoptera (Table 2). The NPV of *H. armigera* (HaNPV) have been studied very extensively (Rabindra and Subramanian 1974; Rabindra *et al.* 1986; Rabindra *et al.* 1990; Muthiah and Rabindra 1991; Rabindra *et al.* 1991; Rabindra *et al.* 1997a; Mote and Satpute, 2003). *S. litura* on banana, cotton, tobacco and cabbage (Santharam *et al.* 1978; Jayaraj *et al.* 1980; Hussain *et al.* 2003), *Ammsacta albistriga* (Walker) in sesamum (Basanth *et al.*, 2008) and *Crocidolomia binotalis* (Zell.) on cabbage (Narayanan, 2004) have been successfully controlled with NPV. NPV (Helivax + Spodovax) are highly effective in combination with nimbecidine for the management of *H. armigera* (Singh *et al.* 2004). The safety of these viruses to beneficial organisms like *B. mori* and honey bees is well established (Santharam *et al.* 1982; Muthiah and Rabindra, 1991).

Table 2. Nuclear polyhedrosis viruses recorded from some important pests

Pest	Crop	References
Adisura atkinsoni (Moore)	Pulses	Narayanan (2003)
Agrotis ipsilon (Hufnagel) and *A. segetum* (Denis and Schiffermuller)	Potato	Rabindra *et al.* (1990)
Galleria mellonella (L.)	Bee wax combs	Rabindra *et al.* (1997b)
Helicoverpa armigera (Hb.)	Tomato Chickpea Cotton	Mohan *et al.* (1996) Rabindra and Jayaraj (1988) Dhandapani *et al.* (1987)
Phthorimaea operculella (Zell.)	Potato	Sudeep *et al.* (2005)
Plutella xylostella (L.)	Cabbage	Padmavathamma and Veeresh (1991)
Hellula undalis (Fab.)		Ignacimuthu (2002)
Spodoptera litura (F.)	Tobacco Castor Cotton	Bhaskaran *et al.* (1996) Ramakrishnan *et al.* (1981) Jayaraj *et al.* (1980)
Thysanoplusia orichalcea (L.)	Sunflower	Men and Thakare (1998)
Maruca vitrata (F.)	Legumes	Lee *et al.* (2007)

Granulosis viruses (GV)

Virus particles of GV range from 30-60 nm x 260-360 nm in size. They largely inhabit the fat body, epidermis, blood cells and tracheal matrix. The infected tissues eventually disintegrate and the body fluid is filled with granulin inclusions. GV have been reported to be effectively used in the management of *Chilo infuscatellus* (Snell.) (Easwaramoorthy and David 1979; Mala and Solayappan 2001), *S. litura* (Narayanan 1985), *Chilo sacchariphagus* (Bojer)

(Easwaramoorthy and Jayaraj 1993; Easwaramoorthy and Santhalakshmi 2000), *P. operculella* (Pokharkar *et al.* 1999; Chandla and Verma 2000) and *P. xylostella* (Subramanian 2002; Kennedy *et al.* 2003). GV infecting *Pieris brassicae* L. have been reported for the first time from Himachal Pradesh by Sood (2004) and Narayanan and Sood (2006). Several granulosis viruses have been reported from India and abroad (Table 3) but only a few have been studied in detail.

Table 3. Granulosis viruses reported from pests of crops

Pest	Crop	Reference
A. segetum	Carrots, beets	Zethner (1980)
A. ipsilon	Tobacco	Choudhry *et al.* (1976)
C. infuscatellus	Sugarcane	Easwaramoorthy and David (1979)
Cnaphalocrocis medinalis (Guenee)	Rice	Jacob *et al.* (1971)
H. armigera	Cotton, chickpea, maize	Gitay and Polson (1971)
P. brassicae	Cabbage	Billotti *et al.* 1956; Sood (2004) Narayanan and Sood (2006)
P. operculella	Potato	Chandla and Verma (2000)
Pieris rapae (L.)	Brassica	Jaques (1973)
S. litura	Castor	Pawar (1977)
S. exigua	Castor	Narayanan (2003)

Cytoplasmic polyhedrosis viruses (CPV)

The virions of CPV (68-69 nm) have a characteristic 12 spikes on the icosahedral particles within the IB's. CPV infection is restricted to the columnar cells of midgut epithelium which may become white or yellow. Other symptoms include extended developments and reduced feeding leading to structural malformations eg. smaller sized larvae, disproportionate head & body, and ultimately lower longevity. CPV have been reported from *H. armigera*, *Maruca testulalis* (Gey.), *Papilio demoleus* (L.), *Euproctis fraternae* (Moore), *Spilarctia obliqua* (Walker) and *Sphenarches anisodactylus* (Walker) in India.

Genetic improvement of viruses

Novel techniques of biotechnology have been used to increase the host range, virulence, killing speed, persistence and storage stability. NPV's were the prime targets for genetic improvement in the past and strains with increased virulence have been isolated and characterized (Shapiro and Ignoffo 1970; Somasekar *et al.* 1993). Enhancement of their activity by inserting a number of insecticidal genes is well documented (Stewart *et al.* 1991; Watkins *et al.* 1997). Deletion of ecdysteroid UDP-glucosyl transferase (egt) gene is a very novel approach to enhance the efficacy of NPV's. Recombinant viruses with scorpion venom gene (Aalt) have been cloned in *Helicoverpa zea* (Boddie) NPV and successfully demonstrated under field conditions against *Heliothis virescens* (F.) (Gard 1997). Development of *in vitro* systems for replication of baculoviruses on insect cell lines has further opened new avenues for genetic improvement as well as genetic engineering of NPV's.

Bacteria

Many different types of bacteria are known to acutely or chronically infect insects which include both spore formers and non spore formers. They vary in their shape (cocci, pleomorphic, rods and spiral) size (0.5 to 50 µm) and occur singly or in chains. They are gram negative or gram positive and are aerobic or anaerobic. However, members of only two genera of the order Eubacteriales i.e. *Bacillus* (*Bacillaceae*) and *Serratia* (*Enterobacteriaceae*) have been registered for pest management. Spores (endospores) of sporulating bacteria are highly resistant to environmental changes and help them to persist in a dormant condition outside the host. On the basis of sporulation, bacterial pathogens of the insects can be classified as

i) Obligate pathogens-spore forming, non toxin producing
ii) Crystalliferous- spore forming with crystal protein
iii) Non crystalliferous- spore forming and without crystal
iv) Facultative pathogens- spore forming with phospholipase-C
v) Non spore forming- no spores and infest in extraordinary circumstances

Entomobacterial pathogens enter the body cavity through contaminated feed/food (oral); however, they are also known to invade through

injured integument of insects. The endospores germinate in the gut (if pH is < 9) and produce the bacterial cells. In case of *Bacillus*, the bacterial cells migrate into the haemocoel where they multiply rapidly and destroy certain tissues and result in filling up of much of the haemocoel (Septicemia). Whereas, in case of *Clostridium*, the cells don't invade the haemocoel but multiplies in the gut itself, and the host cadaver gets mummified instead of disintegrating. The death of the insect may either occur due to intoxication or a sudden lack of oxygen, chemical changes in the gut or by the toxins or crystals produced in the bacterial cells.

Several species of bacteria associated extracellularly with insects have been reported (Table 4). But the *Bacillus thuringiensis* (Berliner) (*Bt*) and *B. popillae* (Dutky) (*Bp*), are the true entomopathogenic bacteria that have been used most successfully as insect pathogens in pest management.

Table 4. Entomophathogenic bacteria recorded from India

Species	Number of hosts
B. thuringiensis (Berliner)	68
B. cereus (Frankland and Frankland)	7
B. subtilis (Ehrenberg)	1
Pseudomonas aeruginosa (Schroeter)	6
Serratia marcescens (Bizio)	28
Streptococcus faecalis (Orla- Jensen)	4

Bt was first isolated in 1902 from the diseased larvae of silkworm in Japan by Ishiwata, however, the name *Bt* was first given in 1915 by Berliner who discovered it to be a pathogen of flour moth in the province of Thuringia (Germany). It was first used as a commercial insecticide in France in 1938 and then in USA in 1950. However, these early products were replaced by more effective ones in 1960's, when various highly pathogenic strains were discovered with specific activity against different types of insects. For many years *Bt* was available only for the control of lepidopteron pests (using a highly potent strain, *Bt var kurstaki*), but now many strains with a wide host range (Coleoptera and Diptera) are also available. Based on types of toxins produced *Bt* can be classifled as under:

Gene	Crystal shape	Protein size kDA	Insect activity
Cry I (several subgroups A (a), A(b), A(c), B, C, D, E, F, G)	Bipyramidal	130-138	Lepidoptera
Cry II (subgroups A, B,C)	Cuboidal	69-71	Lepidoptera and Diptera
Cry III (subgroups A, B, B(b))	Flat/irregular	73-74	Coleoptera
Cry IV (subgroups A, B, C, D)	Bipyramidal/ round	72-124	Diptera
Cry V-IX	Various	35-129	Various

In India, Mazumdar *et al.* (1955) for the first time isolated a strain of *Bt* var *kurstaki* from *H. armigera*. More than fifty strains of *Bt* with vast differences in spectrum of activity have been reported thereafter. A sporeless mutant of *Btk* has been identified at Bangalore which is relatively safe to silk worm but highly effective against *P. xylostella* and *H. armigera* (Singh, 1999). *Bt* has been found effective against number of pests of various crops (Table 5) and IPM modules comprising *Bt* in combination with NSKE, endosulfan and bird perches has been found effective against *H. armigera*, *Exelastis atomosa* (Fab.) and *Melanagromyza obtusa* (Malloch) on pigeon pea (Siddhabhatti *et al.* 2003). Besides *B. thuringiensis*, another bacterium; *B. cereus* was used for the management of *A. janata* by Basappa and Lingappa (2004). It is commercially produced through fermentation technology whereas, *B. popillae*. is produced by infecting live white grubs. Several commercial products like Bactospeine, Doom (*Bp*), Dipel, Delfin, Novabit and Thuricide (*Bt*) are commercially available.

Genetic improvement of *Bt*

Plasmid curing and conjugal transfer and recombinant DNA technology have been used for the strain improvement of *Bt* to enhance their efficacy and host range (Carlton and Bruke 1993, Asokan *et al.*, 2008). These techniques are useful in deriving the strains which produce more than one toxin and are effective against a wide host range.

Table 5. ***Baccillus thuringiensis*-recorded from some important pests**

Pest	Crop	Reference
A. janata	Castor	Misra *et al.* 1993
Earias vitella (Fab.)	Cotton Okra	Sarag and Satpute, 1988; Sathpathy and Panda, 1997
H. armigera	Cotton Gram Tomato	Butter *et al.* 1995 Dabi *et al.* 1988 Gupta *et al.* 1998
Leucinodes orbonalis (Guenee)	Brinjal	Sasikala *et al.* 1999
P. operculella	Potato	Debnath *et al.* 1998
P. xylostella,	Crucifers	Justin *et al.* 1990
Crocidolomio binotalis (Zell.), *P. rapae*	Crucifers	Singh *et al.* 2000
S. litura	Cole crops	Prasad and Kushwaha, 1990
H. armigera	Sunflower	Visalakshmi *et al.* 2000

Apart from the use of *Bt* as a microbial pesticide, transgenic plants have been created by incorporating crystal gene of *Bt* into their DNA (Table 6). So far transgenic cotton, maize, tomato, rice, tobacco, cauliflower, cabbage *etc.* have been developed and approved for commercialization in many countries. The list of transgenic plants is growing rapidly and many countries are commercializing the transgenic crops. In India also, transgenic cotton is in cultivation and many more are in queue.

Fungi

Some of the most spectacular infections in insects are produced by fungal entomopathogens and many result in colourful and/or striking outgrowth of the fungus. Some fungal species that forcibly discharge spores from the host or grow onto the substrate from the host may produce a distinct halo around the infected insect. The entomopathogenic fungi are a broad and diverse biological and taxonomical group which infects virtually every insect order. The majority of them belong to divisions Ascomycotina, Deuteromycotina, Mastigomycotina and Zygomycotina. Due to the mode of entry through the host cuticle by most species of entomopathogenic fungi, they are the only entomopathogens found in sucking insects belonging to orders Homoptera and Hemiptera (Lacey and Brooks 1997). The infective unit in most fungi is a spore, usually a conidium. Under favourable conditions, the conidium germinates into a short germ tube which gives out small swellings called appressoria. The appressorium attaches itself to the cuticle and sends out an infection peg which provides a firm attachment that the fungus needs to physically force its way into the host. The hyphae then penetrate the layers of the integument by enzymatic dissolution of chitin and protein. It first ramifies in the cuticle and then infects the haemocoel and internal organs.

Table 6. Transgenic plants with pest resistance

Crop	Gene	Pest
Cauliflower	Cry 1A(b)	*P. xylostella*
Cotton	*Bt* toxin Cry 1A(b)	Boll worm
Maize	Syn Cry 1A(b)	*Ostrinia nubilalis* (Hbn.)
Rice (Indica)	Syn Cry 1A(b)	*Scripophaga incertulus* (Walker) *C. medinalis* *Chilo suppressalis* (Walker)
Rice (Japonica)	Modified Cry 1A(b)	Stripped stem borer and *C. medinalis*
Tobacco	Cry 1A(a)	
Tomato	Cry 1A(c)	Fruit borer

Infection propagule come in a broad array of shapes and sizes (5 µm to several centimeters) and may be motile, projected from host cadavers, wind borne, dispersed by water or by the insect host themselves. Entomopathogenic fungi kill their host soon after infection. Following death, infectious spores are usually produced on the surface of the host. Larger thick walled resting spores of many fungal species can also be found in the host. Insect cadavers are often mummified due to mycoses and may persist in the environment for several weeks, enabling isolation of the pathogen long after the death of host. In India, a number of entomopathogenic fungi have been reported (Table 7). However, only *M. anisopliae*, *B. bassiana*, *Nomuraea rileyi* (Farlow) Samson and *Verticillium lecanii* (Zimmermann) have been found effective against insects (Chavan and Kadam, 2008; Bhattacharya *et al.*, 2008; Irulandi *et al.*, 2008; Rajan and Muthukrishnan, 2008) and commercialized till date. Mycopesticides have also been found compatible with the synthetic pesticides and are helpful in reducing pesticide

Table 7. Entomofungal pathogens recorded from some important pests

Important species	Pest	References
B. bassiana	*H. armigera*	Prasad *et al.* (1990)
	S. litura	Gopalakrishnan and Narayanan (1990), Anand *et al.* (2009)
	C. infuscatellus	
	Holotrichia consanguinea (Blanchard)	Easwaramoorthy and Santhalakshmi (1987) Yadav *et al.* (2004)
Fusarium oxysporum (Schltdl.)	Culex sp.	Hasan and Vago (1972)
Erynia radicans (Brefeld) Batko	*P. xylostella*	Gopalakrishnan *et al.* (1999)
Hirsutella thompsonii (Fisher)	*Aceria guerreronis* (Keifer)	Kumar and Singh (2001)
M. anisopliae	*Oryctes rhinoceros* (L.)	Sundara Babu *et al.* (1983)
	H. consanguinea	Vyas *et al.* (1997)
P. farinosus	*P. xylostella*	Gopalakrishnan *et al.* (1999)
V. lecanii	*Idioscopus nitidulus* (Walker)	Viraktamath *et al.* (1994)
V. lecanii	*Coccus viridis* (Green)	Easwaramoorthy and Jayaraj (1978)

pressure (Bhattachary *et al.* 2004). *Paecilomyces farinosus* (Holmskiold) was used by the Govindappa *et al.* (2005) for the management of *B. tabaci*, a vector of tomato leaf curl virus (ToLCV).

Culture conditions have been manipulated to increase the efficiency of fungal pathogens. Blastospores of *B. bassiana* from nitrogen limited cultures are more virulent to rice green leafhopper owing to higher carbohydrate and lipid concentrations than blastospores from carbon limited cultures (Lane *et al.* 1991). Genetic improvement of the entomopathogenic fungi is complicated because majority of them belong to *Deuteromycetes* with no known sexual stage. However, mutants of some fungi are found more virulent (Couteaudier *et al.* 1996).

Nematodes

Biological control of insects with entomopathogenic nematodes (EPN's) progressed since 1935 with the discovery of *Steinernema glaseri* (Steiner) infecting the Japanese beetle (Glaser and Farrel 1935). Though forty nematode families have been found associated with insects (ranging in size from 1mm to several centimeters), only two families *Heterorhabtidae* and *Steinernematidae* have widely been used as biocontrol agents. EPN's in the genera *Steinernema* and *Heterorhabditis* are potential insect control agents. The free living, non-feeding infective juveniles are motile, highly virulent, possess chemoreceptors and have high reproductive potential and ability to seek out their hosts. The colouration of the host insect that are attacked by *Heterorhabditis* and *Steinernematids* changes to red, orange to honey or creamish due to the presence of bacterial symbionts. Apart from distinctive colouration, luminescent symbiontes (*Photorhabdus* sp.) have also been found associated with *Heterorhabditids*. EPN's are one of the valuable pesticides for pest management as these can even be effectively used against indoor pests (Kaushal *et al.* 2000), besides major field crop pests (Karunakar *et al.* 2000; Jothi and Mehta 2003; Prabhuraj *et al.* 2003; Hussani *et al.* 2005; Pilz *et al.* 2009).

In India, work on EPN's started as early as in 1966. Rao and Mazumdar (1966) used *Steinernema carpocapsae* (Weiser) (DD-136) for the control of pests of rice, sugarcane and apple. Thereafter, a number of reports are traceable on the use of EPN's against pests of various crops (Table 8).

Table 8. Entomopathogenic nematodes used for some important pests

Nematode species	Crop	Pest	Reference
Steinernema carpocapsae, Heterorhabditis indica (Poinar)	Brinjal	*L. orbonalis*	Hussaini *et al.* (2002a)
Steinernema spp.	Citrus	*Papilio* sp.	Singh (1993)
S. carpocapsae, H. indica, H. bacteriophora	Groundnut	*A. albistriga*	Bhaskaran *et al.* (1994)
S. carpocapsae	Potato	*Anomala sp.*	Sundrababu *et al.* (1984)
S. carpocapsae	Rice	*T. incertulus*	Israel *et al.* (1969)
S. carpocapsae	Rice	*T. incertulus*	Rao *et al.* (1971)
S. carpocapsae	Rice	*C. medianalis*	Srinivas and Prasad (1991)
S. carpocapsae	Rice, Sugarcane	*Tryporyza incertulus* (Walker)	Rao and Mazumdar (1966)
S. carpocapsae	Tobacco	*S. litura*	Sitaramaiah *et al.* (2002)
S. carpocapsae, S.abbasi,, H. indica	Tomato	*A. ipsilon*	Hussaini *et al.* (2002b)
S. bicornutum	Tomato	*A. ipsilon*	Hussaini *et al.* (2001)
S. carpocapsae	Vegetables	*Athalia proxima* (Klug.), *Aulacophora foveicollis* (Lucas), *Diacrisia obliqua* (Walker), *Dysdercus cingulatus* (F.), *H. armigera, L. orbonalis, S. litura*	Singh and Bardhan (1974)

Protozoans

Unlike other entomopathogens, protozoa usually produce chronic infections manifested by symptoms like irregular growth, sluggishness, loss of appetite, malformed pupae or adults with reduced vigor, fecundity and longevity. Infections of protozoans in insects are generally caused by feeding contaminated food (oral), and less frequently it could be through transovum transmission or by infected parasites. Insects are parasitized by several protozoa but only *Sporozoa* and *Cnidospora* cause death of the parasitized hosts. They multiply inter or intra- cellularly in the host. Their virulence lies in their fast reproduction abilities. Some of the important protozoans of insect pests are listed in Table 9 (Santharam 2001).

Rickettsia

Rickettsia are small (0.2-0.6μm), pleomorphic, gram negative bacteria which live and multiply in arthropods. They are regarded as an intermediate form between viruses and bacteria. Like bacteria, they have both the nucleic acids (DNA and RNA), enzymes showing metabolism, a bacterium like structure and binary fission. Majority of them are intracellular parasites like viruses and cannot be cultured in artificial medium. Members of genera *Entrella* living in the epithelial cells of the gut and *Rickettsiella* growing intracellularly in the fat body cells and haemocytes are highly pathogenic to the insects. Species in these genera are pathogenic to Coleoptera, Diptera, Lepidoptera and Orthoptera. Most common species of *Rickettsiella* is *R. melolonthae* which causes Lorsch disease in the grubs of the beetle, *Melolontha melolontha* (L.). Bluish green iridescent colour is often produced due to infections similar to blue iridescent viruses, hence termed as blue disease (Kreig 1963). Due to their low host specificity and pathogenicity to vertebrates their use as microbial control agents is limited. Rickettsias like organisms (RLO's) contribute very little towards effective management of insect-pests. It was used by Men and Thakare (1998) for the management of *T. orichalcea* on sunflower. Ansari *et al.* (2000) also reported the potential of RLO's for the biocontrol of nematodes.

Table 9. Protozoan pathogens recorded from some important pestss

Species	Pests
Bacillidium sp.	*Thrips flavus* (Schrank)
Farinocystis tribolii (Weiser)	*Tribolium castaneum* (Herbst)
Mattesia dispora (Nav.)	*Ephestia cautella* (Walker)
Microsporidium sp.	*S. litura, S. exigua, Utetheisa pulchella* (L.)
Nosema sp.	*Plusia peponis* (F.), *Spilarctia obliqua* (Walker), *A. atkinsoni, C.binotalis*
Tetrahymena sp.	*Chilo partellus* (Swinhoe)
Vairimorpha sp.	*H. armigera, S. litura, S. exigua* (Hbn.)

Role of microbial control in insect pest management programmes

In pest management programmes, a single method is usually not sufficient and one has to consider various insect control methods as tools. Microbial control, if used as a tool in pest management must confirm the following objectives:

i) Microbial agents should be relatively host specific and should not upset other biotic systems
ii) should be safe to human beings and the environment
iii) should be compatible with most other control methods

Many insect pathogens are currently being used and have the potential of becoming microbial insecticides; however, it is important to recognize them as naturally occurring pathogens (Maddox 1992). Insect pathogens can be used in pest management at least in four ways:

i) by utilizing naturally occurring diseases
ii) by the introduction of insect pathogens into insect pest population as permanent mortality factor
iii) by application of insect pathogens as microbial insecticide
iv) by novel manipulation of pathogen genes *i.e.* transgenic

Naturally occurring epizootics in certain instances check the insect population to a greater extent, but these are often sporadic and unpredictable. The knowledge of the damage threshold of a pest and insect pathogens of a locality is very important in utilizing the natural epizootics in management of pests. Insecticidal application(s) can be avoided or delayed or minimized if the threshold of the pest and the possibility of a disease epizootic are carefully monitored. Natural epizootics have generally been more successful against insect pests of high economic thresholds and in a more stable ecosystem (Tanada and Kaya 1993). The diseases caused by viruses and fungi are easily recognized than the diseases caused by other microbes.

The strategies for initiating an early epizootic generally fall into two categories, augmentation and environmental manipulation (Harper 1987). Augmentation is application of additional inputs of pathogens into pest populations (usually inoculative release of pathogens). For example, light traps attract adults of corn earworms which fall into containers that allowed passive contamination of moths with NPV. The moths fly back to spread the disease in wild populations of the pest (Jackson *et al.* 1992). Environmental manipulation implies to any practice (usually cultural) that enhances the development of an epizootic. For example, trap crops attract large insect populations in a smaller area, promoting faster development of a disease. Varying row spacing, minimum tillage, strip cropping, timing irrigation schedules may promote earlier epizootics (Allen *et al.* 1978).

When an insect pathogen is applied repeatedly in manner similar to chemical insecticides for temporary suppression of a pest, it can be considered as a microbial pesticide. The market for microbial pesticides is growing, but still represents only a fraction of pesticide market (most of which is accounted for by *B. thuringiensis*). The number of biopesticides registered for use in pest suppression is increasing and there are about 409 producers of biocontrol agents, but still the availability of these biopesticides is far less than the assumed demand.

Resistance in plants produced by expressions of an insecticidal protein has become a widely used strategy. The technology of insect resistant transgenic plants is expanding very rapidly, with considerable research activity in both public and private sectors. The only commercialized transgenic plants till date express genes derived from the bacterium, *B. thuringiensis*. A wide range of genes from higher plants have been transferred into crop cultivars, especially genes encoding inhibitors of digestive enzymes, chitins and lectins (Table 10). The use of resistant genes from other micro organisms has so for been limited.

Table 10. Genes deployed for development of transgenics against biotic stresses

Transgene(s)	Target
Bt genes	All major insect orders
Horn worm proteinase inhibitor (PI)	Whitefly
Cowpea serine PI	Stem borer, tobacco budworm
Potato serine PI	Tobacco hornworm
Insect chitinases	*H. virescens*
Lectins	Potato aphid, brown plant hopper
Oryzasystatins	Potato beetle
α-amylase inhibitors (bean α- AI)	Bruchids
Plant metabolic enzymes	White fly
Bacterial and fungal chitinase	Fungal diseases
Glucanase	Fungal diseases
Thaumatin encoding genes	Fungal diseases
Ribosome inactivating proteins (RIP,s)	Fungal diseases
Viral coat protein genes	Viral resistance

Transgenic crops are useful in pest management programmes as they have following advantages:

i) season long protection
ii) protection is independent of weather
iii) only crop eating insects are exposed
iv) insects are always treated at the most sensitive stage
v) affords protection of plant tissues which are difficult to treat using insecticides
vi) the active factor is biodegradable and choice of suitable genes/ gene products can ensure it is not toxic to man and animals
vii) material is confined to the plant tissues in which it is being expressed and therefore does not leach into the environment
viii) considerable financial savings

Interaction with other control measures

Microbial control is often more effective when combined with other means of control such as resistant varieties, botanicals, chemical insecticides and entomophages and hence, been successfully used in insecticide resistant management. Such combinations may produce acceptable levels of control, when the use of two methods independently does not. Moreover due to host specificity, the insect pathogens are more capable of suppressing specific pests without disturbing other non-target species.

Management of European corn borer, *Ostrinia nubilalis* (Hbn.) has been achieved with the integration of *Bt* and resistant corn hybrids, which formed a viable alternative to pesticides (Bollin *et al.* 1996). Enhanced mortality of pod borer, *H. armigera* due to NPV has been recorded in susceptible chickpea varieties (Sithanathan 1986; Sathiah 1987). Microbial pesticides have been considered together with chemical pesticides because of their added effectiveness (Hassan and Graham Smith 1995). The efficacy of *Bt* could be enhanced by mixing adjuvants such as boric acid (0.025 %) or neem seed kernel extract (2.0%). NPV has been proved safer to *Chrysoperla carnea* (Stephens) (Rabindra *et al.* 1994) and more effective in combination with *Bt* (Pyne *et al.* 1996), microsporidia (Novonti 1988), CPV, botanicals (Rabindra *et al.* 1991; Senthil Kumar *et al.* 2008). Similarly, *Bt* is more effective when integrated with fungus, *M. anisopliae* (Wernicke and Funke 1995) and nosema (Bauer and Nordin 1989) and has been found safer to entomophages (parasites and predators) like *Trichogramma*, *Coccinella, Apantales* and *Chrysoperla* (Mercy Jayarani 1995; Loganathan *et al.* 1999; Mohan *et al.* 2007). Dent (1997) has reported the interaction matrix for five commercially used entomopathogens with other control measures which implicates their usefulness in IPM programmes.

Microbial control is often associated with certain disadvantages like

i) Correct timing of application is very important which vary with stage and type of the insect
ii) Spectrum of the effectiveness against pest complex is narrowed down due to host specificity
iii) Success of microbial agents is weather dependent
iv) Insect cadavers remains on edible plant parts
v) Mass production in case of some insect pathogens like protozoa is very difficult
vi) Maintenance of virulence and viability of insect pathogens till its use is necessary

With the growing public concern over the consequences of chemical pesticides, microbial control has a tremendous scope in ecofriendly pest management, in spite of some disadvantages.

There is a need to give special emphasis at insti utional levels on development of bio-rational pest management techniques using microbial agents. With the enforcement of Dunkel draft and Patent requirements, a special care to preserve our entomopathogenic microbial wealth is needed so that these could be used in pest management programmes more effectively.

In conclusion, rich diversity of micro fauna in the continent provides excellent opportunities, for basic and applied research in the field of microbial control of pests affecting agricultural production and public health. A better understanding of insect pathogens and their effective utilization in pest management will then go a long way in providing "**Food for All**" and "**Health for All**".

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