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Review Article

Management of Insect Pests by Microorganisms

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Insects, like other organisms, are susceptible to a variety of diseases caused by bacteria, viruses, fungi and protozoans, and these pathogens are exploited for biological control of insect pests through introductory or inundative applications. Microbial pathogens of insects are intensively investigated to develop environmental friendly pest management strategies in agriculture and forestry. In this paper, the scope for utilization of insect pathogens in pest management in the world and India is reviewed. The most successfully utilized insect pathogen is the bacterium, *Bacillus thuringiensis (Bt)* which is used extensively for management of certain lepidopteran pests. In India, mostly imported products of *Bt kurstaki* have been used, which are expensive and there is an urgent need to develop aggressive indigenous *Bt* strains against various pests. Baculoviruses comprising nuclear polyhedrosis virus (NPV) and granulosis virus (GV) have been successfully used as insect pathogens because of their high virulence and specificity. NPV and GV formulations are used for lepidopteran pests like *Helicoverpa armigera* (HaNPV) and *Spodoptera litura* (SlNPV) in India, besides *Anticarsia gemmatalis* NPV in Brazil, *Lymanttria disper* NPV, *Orgyia pseudotsugata* NPV in USA and GV of *Pieris rapae* in China. Lack of easy mass multiplication methods for the commercial production of baculoviruses calls for R&D to develop production in insect tissue cultures. Entomopathogenic fungi like *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, M. anisopliae* var. *acridium, Lecanicillium* spp., *Hirsutella thompsonii, Nomuraea rileyi* and *Isaria fumosorosea* are gaining importance in the crop pest control in recent years due to the simpler, easier and cheaper mass production techniques. Environmental humidity and temperature play an important role in the infection and sporulation of these fungi and as such they are highly suitable during cool and humid cropping seasons. Successful uses include *M. anisopliae* var. *acridium* for locust control in Africa, Australia and China, *M. anisopliae* in sugarcane spittle bug management in Brazil and pine moth (*Dendrolimus* spp.) control in China using *B. bassiana*. Since talc - based formulations of these fungi have limited shelf life of 3-4 months, alternative formulations with longer shelf life (12-18 months) have to be developed besides suitable oil based formulations for dry land agriculture. There is a scope to utilize the biodiversity of Entomophthorale group of fungi like *Entomophthora, Zoophthora, Neozygites etc*., which have potential for management of aphids, thrips and lepidopteran pests.

Key Words: Entomopathogens; Microbial Pesticides; Biological Control; *Bacillus thuringiensis*; Nuclear Polyhedrosis Virus; Granulosis Virus; Mycoinsecticides

Introduction

Research on microbial pathogens of insects is increasing considerably in recent times to find out environmental friendly alternatives to hazardous chemical insecticides. These microbial pesticides occupy around 1.3 per cent of the world's total pesticide market of which, 90 per cent of them are used as insecticides [1]. The pathogens that cause diseases in insects fall into four main groups, fungi, bacteria, viruses and protozoans. The most successful insect pathogen used for insect control is the bacterium, *Bacillus thuringiensis (Bt),* which presently occupies about 2 per cent of the total

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insecticidal market [2]. The most widely used bacterial pathogens include subspecies or strains of *Bacillus thuringiensis*. Each one of the strains produces different mix of toxins and specifically kills one or a few related species of insects (*Bt* subspecies *kurstaki* and *aizawai* for lepidopteran larvae) and (*Bt* subspecies *tenebrionis* for coleopteran larvae). Some of these strains are specific to mosquitoes (*Bt* subspecies *israelensis*). Among the insect viruses baculoviruses (Nuclear polyhedrosis virus, NPV and Granulosis virus, GV) are the most promising for insect control particularly of Lepidoptera and Diptera because of their specificity. NPVs have been successfully used for management of devastating pests like *Heliothis* spp. and *Spodoptera* spp. on cotton, fruit and vegetable crops in several countries [3]. Entomopathogenic fungi like *Beauveria* spp., *Metarhizium* spp., *Lecanicillium* spp. and *Isaria* spp. have been developed as successful mycoinsecticides for various groups of insect pests [4]. Several hundreds of commercial products of fungi, bacteria and viruses are available worldwide for the biological control of insect pests in agriculture and forestry.

The growth rate of the bio-pesticide industry has been forecasted in the next 10 years at 10-15 per cent per annum in contrast to 2-3 per cent for chemical pesticides [5]. Some of the successful and large-scale pest management programmes using microbial insecticides include, management of *Lymantria* spp. outbreak in forestry in Poland and North America with the products of *B. thuringiensis* subspecies *kurstaki (Btk)* [6], Pine caterpillar management with *B. bassiana* in China [7], locust control with *Metarhizium anisopliae* var. *acridium* in Africa, Australia and China [8], sugarcane spittle bug management by *Metarhizium anisopliae* in Brazil, rubber lace bug control with *Sporothrix insectorum*, corn borer management with *B. bassiana* in Europe and USA [3], and European pine sawfly management with NPV in Europe and North America [9]. The main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organism, they do not cause ill effects on environment and human health and can be used against pests which develop resistance to the conventional insecticides, they fit as ideal components in integrated pest management (IPM) and also in organic farming systems. However, these biocontrol agents are reported to be slow in action or kill rate and sensitive to the environment, which lead to the inconsistent and poor success rate in the field. To overcome these problems, attempts are made to identify highly efficient and aggressive strains to improve the field efficacy and to develop suitable formulation technologies for increased field persistence and to withstand harsh environmental factors of radiations and dry weather conditions. In this paper, a review is made on the prospects of utilization of insect pathogens in pest management worldwide and in India.

Bacteria as Insect Pathogens

The year 2011 was marked as a century of research and development of *Bacillus thuringiensis* (*Bt*). *Bt* is a Gram-positive, rod-shaped, spore-forming entomopathogenic bacterium characterized at the species level by the production upon sporulation of a parasporal inclusion body, the crystal which is toxic to insects and other invertebrates and is the most commonly used commercial biopesticide worldwide [2]. *Bt* protein toxins are highly selective to their target insect, are innocuous to humans, vertebrates, plants and are completely biodegradable. Therefore, *Bt* is a viable alternative for the control of insect pests in agriculture and disease spreading vectors in public health.

The primary sources of proteins were used in developing genetically engineered crops to protect them from insect damage. Transgenic crops based on insecticidal crystal proteins of *Bt* are now an international industry with revenues of several billion dollars per year [10]. Classification of *Bt* strains has been accomplished by H serotyping, the immunological reaction to the bacterial flagellar antigen. Specific flagellin amino acid sequences have been correlated to specific *Bt* H serotypes and at least 69 H serotypes and 82 serological varieties (serovars) of *Bt* have been characterized from around the world [11].

1. Mode of Action of *Bt*

A key feature of all Cry, Cyt and Vip proteins is that they are stomach poisons and must be ingested for toxicity. The *cry* genes code for proteins with a range of molecular masses from 50 to 140 kDa. Upon ingestion by the susceptible insect larvae, protoxins are solubilized in the high alkaline pH of midgut and proteolytically digested by midgut proteinases to release the toxic fragments of approximately 55-68 kDa. A generally accepted model for Cry toxin action is that it is a multistage process. First, the activated toxin binds to receptors located on the apical microvillus membrane of epithelial midgut cells. Two important insect proteins have been identified as receptors for Cry toxins *viz*., aminopeptidase N (APN) and Cadherins. Insect glycolipids were additionally suggested as a receptor in nematodes. Binding of the toxin to the receptor leads to change in the toxin's conformation, allowing toxin insertion into the membrane. Oligomerization of the toxin follows and this oligomer then forms a pore that leads to osmotic cell lysis and larval death [2].

Host Factors and Bt *Toxin Diversification*

Cry toxins are encoded by cry genes found mainly on large plasmids. Normally a Bt strain synthesizes one to five Cry toxins with varying toxicity and expression levels. Since the cloning and sequencing of the first *cry* genes, nucleotide sequences have been reported for more than 300 *cry* genes. The new nomenclature system for the Cry toxins are based on the degree of relatedness as determined by the nucleotide and deduced amino acid sequences of the encoding genes. Till date more than 240 holotype of Cry toxins have been reported under 68 major classes (Cry1 to Cry68). For example, Cry1, Cry2, Cry9 and Cry15 classes are lepidopteran specific. Cry4, Cry10, Cry11, Cry16, Cry17, Cry19 and Cry20 are dipteran specific. The coleopteran active classes are Cry3, Cry7 and Cry8. The nematicidal toxins belong to Cry6 class (http://www.lifesci.sussex.ac.uk).

Differences in physiological conditions of the gut such as pH, midgut proteases and toxin receptors, may have been important selective forces for the evolution of toxins [12]. Solubilization of long protoxins depends on the high pH in lepidopteran and dipteran midguts, contrasting with rather acidic coleopteran midgut [13]. The main digestive proteases of Lepidoptera and Diptera are serine proteases, whereas those of Coleoptera are cysteine and aspartic proteases [14]. Activation is a complex process; apart from the protoxin proteolysis at the N and C terminal, intramolecular processing within domain I and II can also take place [15, 16, 17]. Specificity is determined by the interaction of Cry toxins with specific high-affinity receptors on the gut epithelium and often insect-resistance to Cry toxins is correlated with alterations in receptor binding [18]. Coevolution of Cry toxins and insects has been hypothesized and interaction of Cry toxins with their receptors may play an important role in selection of new varieties [12].

Screening of cultures from *B. cereus* and *B. thuringiensis* has led to the discovery of novel toxins that are secreted during vegetative growth by many different strains. Although some *Bt* toxins are also produced during vegetative growth, these novel toxins do not share any homology to the known crystal proteins and hence not called Cry, but rather Vip toxins [12]. Sequence homology search suggests that Vip1/Vip2-complex is a typical binary toxin of the A+B type, where Vip2 is the cytotoxic A-domain and Vip1 contains the receptor binding domain; both are required together for activity against some coleopteran larvae [12]. The vip3A(a) gene of strain AB88 of *B. thuringiensis* encodes a 88.5-kDa protein active against several, but not all, lepidopteran larvae tested, without sequence homology to any known protein [19, 20]. In larval midgut fluid, it is processed by proteases at several sites, leaving active 33-kDa fragment of 200-455 amino acids. Vip3A binds to midgut epithelium of susceptible larvae but not to that of an insensitive species, causing cell death in a process resembling apoptosis [21]. Vip3A production by germinating spores is an important factor in the combined toxicity of spores and relatively inactive Cry toxins against several insects [22].

Hence, as summarized by de Maagd *et al*. [12] protein toxins produced by Gram-positive spore-forming entomopathogenic bacteria belong to a

number of homology groups containing diverse protein structures and modes of action. In many cases, the toxins consist of unique folds or novel combinations of domains having known protein folds. Some of the toxins display structure and mode of action similar to certain toxins of mammalian pathogens, suggesting a common evolutionary origin. Most of these toxins are produced in large amounts during sporulation and have the remarkable feature that they are localized in parasporal crystals. Localization of multiple toxin-encoding genes on plasmids together with mobilizable elements enables bacteria to shuffle their armoury of toxins. Recombination between toxin genes and sequence divergence has resulted in a wide range of host specificities**.**

Formulation

Biological based pesticides (bacteria, fungi, virus, pheromones, plant extracts) are currently being used for insect pest control. *B. thuringiensis* (*Bt*) based biopesticides are especially of utmost importance and occupy almost 97 per cent of the world biopesticide market [23, 24]. A biopesticide can be effective only if it has a potential major impact on the target pest, substantial market size, variability of field performance and cost effective [25].

The first commercial product of *Bt* ''Sporeine'' was introduced in France in 1938 and since then, there has been continuous rise in development of advanced products with high virulence and field persistence. Several core areas need to be addressed before the biopesticides can penetrate the international market. These include activity spectrum, persistence and recycling, and improvement of formulations by using conventional and simple adjuvants/additives, which are not cost intensive [26].

The final fermented *Bt* broth usually comprises of spores, cell debris, inclusion bodies, enzymes and other residual solids, these have to be recovered efficiently to be utilized in subsequent formulation step [27, 28]. Most commercial *Bt* products contain insecticidal crystal proteins (ICP), viable spores, enzyme systems (proteases, chitinases, phospholipases), vegetative insecticidal proteins and many unknown virulent factors along with inerts/ adjuvants. Earlier, lactose-acetone technique was used as a method to recover *Bt* spores with measurable losses [29]. However, use of advanced methods like ultracentrifugation, microfiltration and vacuum filtration to separate insoluble solids (active ingredients) from soluble liquid (inert) fraction of the harvest liquor, has resulted in efficient recovery of the active ingredient. Harvesting microorganisms from submerged fermentation is often difficult due to low concentration of products, their thermolabile nature and in some cases, poor stability [25]. Stabilizing adjuvants may have to be incorporated in postharvest operation to prevent spore mortality and/ or germination [25]. Rapid drying or addition of specific biocidal chemicals may be required to prevent growth of microbial contamination in the broth or centrifuge slurry [30].

Some authors have utilized spray-drying method for large broth volumes [31]. This drying can be preceded by thickening of the fermentation liquid by centrifugation and filtration using filter aids like celite, superfloc, etc., to reduce handling volume [32, 33]. In the spray drier, water is removed from the broth slurry as it passes through the heated inlet (150-200°C). The resulting powder coats the walls and collects in the spray dryer [34]. Although there was no physical loss, yet, measurable bioactivity diminished by spray drying process due to continuous exposure of the bioactive components to the high temperatures [35]. An efficient recovery of active spore-crystal complex of *Bt* was reported by Rojas *et al*. [36] by using either a disk-stack centrifuge or a rotary vacuum filter with spore recovery efficiency higher than 99 per cent [36]. Nevertheless, the concentration of dry solids produced by filtration (31.5 %) was superior to centrifugation (7.5 %). Similar study on *Bt* var. Berliner was carried out by using a continuous centrifuge with recovery rate of 85-90 per cent and decrease in separation efficiency with increase in flow rates [37]. At the final stage after fermentation, lactose (5%) was sometimes added as a cryoprotectant to prevent clumping during storage and lactose-acetone co-precipitation could be used as a sequential step to centrifugation to achieve higher δ-endotoxin recovery efficiency [38]. The final

products (powder/suspension) are suitably formulated as aqueous (flowable) or oil concentrate, spraying powder, or granulates. Nevertheless, literature on harvesting methods is very scarce as most of the *Bt* commercial production is carried out by industries and hence separation process is proprietary and secure. Although harvesting is considered as an important step that may augment or suppress biopesticidal activity, still current techniques based on centrifugation - conventional, differential and density gradient and spray drying suffer inefficient ä-endotoxin recovery and inherent losses [25]. For down steam processing, centrifugation appears to be a viable alternative and with further advances in design and speed, it could serve as better alternative [25].

Bt based biopesticidal formulations will find wider application in future by adopting simple harvesting methods and robust and economical choice of various additives for different formulations. *Bt* formulation trends have progressed in the direction of "maximum efficacy per drop" resulting in high potency concentrates requiring lower spray volumes. Wastewater and wastewater-sludge based formulations may hold the key to various problems encountered by commercial medium based formulations aiding in two ways-sustainable reuse of wastes and enhanced penetration of Bt biopesticides into global pesticide market. This will also greatly expand the repertoire of commercial *Bt* product types [10]. In India, mostly imported *Bt* based products based on *Bt kurstaki* (Delfin, Dipel, Thuricide, Halt, Biobit, Bactospeine, Agree *etc.*) have been successfully used to manage lepidopteran pests, *Plutella xylostella*, *Helicoverpa armigera and Achaea janata*. Cry gene profiling of indigenous *Bt* isolates is being carried out at various institutes like Indian Agricultural Research Institute, National Bureau of Agriculturally Important Insects, Indian Institute of Horticultural Research *etc*. to identify suitable indigenous strains and genes for management of various lepidopteran pests. Varying susceptibility to *cry* genes have been observed in the different populations of *H. armigera* collected from different insect hosts and geographical regions. At IIHR, Bangalore, coleopteran active genes like *cry9Da1*, *cry22A, cry23A, cry43* and *cry43B* and nematode active *cry* genes *cry5, cry12, cry13, cry14* and *cry 21* have been identified and cloned successfully. At DOR, Hyderabad, solid-state fermentation technology for indigenous *Bt* isolate (DORBT-5) has been developed and commercialized for the management of lepidopteran pests. At NBAII, Bangalore, liquid formulation technology was developed for indigenous *Bt* isolates (PDBCBT1 and NBAIIBTG4) that are effective against pigeon pea pod borer. Since most of the *Bt* products used in India are imported and expensive, there is an urgent need to develop aggressive indigenous *Bt* strains against various pests and suitable formulation technology for large-scale production and supply to the Indian farming community. Table 1 lists the commercially available *Bt* products worldwide and Table 2 lists *Bt* strains registered under Insecticide Act in India.

However, *Bt* based sprayable products have certain limitations in the usage in agriculture since Cry toxins are very specific to young larval stages, sensitive to solar radiation and have limited activity against borer insects. To overcome this problem, genes coding for Cry proteins in *Bt* and insecticidal toxins genes from other sources have been transferred and expressed in many crop plants to protect them against pests. Currently 14 insecticidal genes are employed in six crops like cotton, corn, potato, rice, soybean and tomato that are available for commercial cultivation in several countries like USA, Argentina, Mexico, China, Canada, South Africa, France, Australia, Spain, Ukraine and Portugal. In the year 2011, the stacked double and triple gene traits occupied 26 per cent of GM area, followed by insect resistant varieties (23.9 mi ha) at 15 per cent. In India, the cultivation of *Bt* cotton is expanded to the tune of more than 95 per cent of total cotton area and is offering great scope to minimize the pesticide usage [10].

The major threat to the use of *Bt* products or *Bt* crops is the appearance of insect resistance. Resistance to Cry toxin emerges by the mutation in the insect pest that affects the mode of action of Cry toxins [2]. In the field condition three lepidopteran insect pests, *Plodia interpunctella, Plutella xylostella*

Table 1: Commercial *Bacillus thuringiensis* formulations available worldwide

Manufacturer	Product	*Bt* strain employed	Toxic units	Target pest(s)
Valent Biosciences Corporation	Dipel WP (Wettable powder)	*Bacillus thuringiensis*	16000 BIU/mg	Spruce budworm; gypsy moth; bagworm; spring and fall cankerworms and cabbage looper
	Thuricide 48LV (Liquid suspension)	*Bacillus thuringiensis*	12.7 BIU/L	Bagworm; elm spanworm; fall spanworm; gypsy Moth; spring and fall cankerworm; spruce budworm
	Vectobac-200G Larvicide (Granules)	*berliner* ssp. *kurstaki*	200 ITU/mg	
	Teknar Granules Larvicide		260 AAU/mg	Mosquitoes
	Vectobac 200G (Granules) 200	*Bacillus thuringiensis israelensis*	ITU/mg 1986 2007	
	Vectobac 600L (aqueous suspension)		600 ITU/mg	Fungus gnats
	Teknar HP-D Larvicide (aqueous suspension)		3000 AAU/mg	Mosquitoes & Black flies
	Novodor Flowable Concentrate	*Bacillus thuringiensis* ssp. *tenebrionis*	3.6%	Colorado potato and elm leaf beetle
	Foray 48B		12.7 BIU/L	Spruce budworm (Eastern & Western); gypsy moth;
	Foray 48B A Low Volume		12.7 BIU/L	jackpine budworm; eastern hemlock looper; whitemarked tussock moth and forest tent caterpillar
	Foray 76B (aqueous xoncentrate)		20.0 BIU/L	whitemarked tussock moth forest tent caterpillar and satin moth
	Foray 96B		25.4 BIU/L	
	Dipel 2X DF (Dry Flowable (Wettable Granules)	*Bacillus thuringiensis berliner* ssp. *kurstaki*	32,000 IU/mg	spruce budworm; gypsy moth; bagworm; spring and fall cankerworm; fall webworms; elm spanworm; tent caterpillar; cabbage looper; leafroller and diamondback moth
AFA Environment Inc	Aquabac II XT (Liquid suspension)	*Bacillus thuringiensis israelensis*(*Bacillus thuringiensis* serotype H-14)	1.28 BITU/Kg 0.20 BITU/Kg	Mosquitoes
	Aquabac 200G (10/14) (Granules)		200 ITU/mg	
	Aquabac 200G (10/14) (5/8)			
AFA Environment Inc	Aquabac XT	*Bacillus thuringiensis israelensis*	1200 ITU/mg	Mosquitoes and blackflies
Abbott Lab. Ltd. 1988	Dipel 176 (Emulsifiable suspension)		16.9 BIU/L	Forest tent caterpillars; gypsy moth spruce budworms; hemlock looper
Woodstream Canada Corporation Safer's	BTK (Liquid concentrate)	*Bacillus thuringiensis Berliner* ssp *kurstaki*	12.7 BIU/L	Gypsy moth; tent caterpillar and cabbage looper
AEF Global Inc.	Bioprotec Aqueous Biological		12.7 BIU/L	Gypsy moth; eastern spruce budworm; western spruce budworm; jack pine budworm;
	(aqueous suspension)		12.7 BIU/L	forest tent caterpillar; eastern hemlock looper;
	Bioprotec CAF		17,5000 IU/mg	bagworm; elm spanworm; fall spanworm; spring
	Aqueous Bioprotec HP			& fall cankerworm; satin moth and white marked
	Bioprotec ECO		12.7 BIU/L	tussock moth

Source: Modified from S.K. Brar *et al*. [25]. ITU, International Toxic Units; IU, International Units; BITU, Billion International Toxic Units; AAU, *Aedes Aegypti* Units (1 ITU = 2.5 AAU). IU: it refers to standardized potency (by bioassay) of different marketed Bt products against Bt var. *thuringiensis* E-61 standard from Institut Pasteur, Paris, France, assigned a potency of 1000 International Units (IU) per mg. Bioassay is carried out against mediterranean flour moth (*Ephestia kuhniella*) in Europe and in US, a primary reference standard of Bt HD-1-S-1971 strain with an assigned potency of 18000 IU/mg is being used, bioassayed against cabbage looper (*Trichoplusia ni*).

Table 2: *Bt* strains registered under Insecticide Act in India

Bt subspecies	Strain/serotype	Formulation type
Bt kurstaki	i) Strain A-97, serotype H-3a	35 WP
	ii) Strain DOR-Bt-1, serotype-(3a, 3b 3c)	0.5% WP
	iii) Strain HD-1, serotype 3a, 3b,	3.5% ES
	iv) Serotype 3a, 3b, Strain Z-52	-
Bt galleriae	Strain R 1593m, serotype 3a,	1.3% FC
Bt israelensis	i) Strain 164, serotype H-14,	WP
	ii) Strain VCRC B-17, serotype H-14	Slow release
	iii) Strain VCRC B-17, serotype H-14,	granules WP
	iv) Serotype H-14,	12 AS
	v) Strain VCRC B-17, serotype H14	5 AS
	vi) Serotype H-14	5% WP

and *Trichoplusia ni* have evolved resistance to the formulated *Bt* products, [39, 40, 41]. Four cases of resistance to *Bt* crops have been documented, *H. zea* to *Bt* cotton expressing Cry1Ac in USA [42], *S. frugiperda* to *Bt*-corn expressing Cry1F in Puerto Rico [43], *Busseola fusca* to *Bt*-corn expressing Cry1Ab in South Africa [44] and *Pectinophora gossypiella* to *Bt*-cotton expressing Cry1Ac in India [45]. Development of resistance in insects can be delayed by planting a significant percentage of non-*Bt* plants in the proximity of *Bt*-crops that express a high dose of Cry toxin which allows breeding between susceptible and resistant populations emerging on the *Bt* and non-*Bt* plants [42]. Recently in Arizona State, USA, the release of sterile *P. gossypiella* females along with the use of *Bt*-cotton could efficiently slow down the frequency of resistance development in the pink bollworm [46]. Other strategy to cope up with the appearance of insect resistance is the use of the gene stacking of different Cry toxins with different mode of action in the same plant [47].

Baculoviruses as Insect Pathogens

Baculoviruses are a very diverse group of viruses with DNA double-stranded, circular, supercoiled genomes, with sizes varying from about 80 to over 180 kb, that encode between 90 and 180 genes. Members of the Baculoviridae are characterized by their presence in occlusion bodies called polyhedra for NPVs and granules or capsules for GVs. The occlusion body consists of a crystalline matrix composed of a protein called polyhedrin in NPVs and granulin in GVs. A prominent feature of the nucleocapsids within polyhedra is their organization into either single or multiple aggregates of nucleocapsids within an envelope [48]. For example, in some NPVs there can be from 1 to 15 nucleocapsids per envelope, with bundles of 5 to 15 predominating. In contrast, strains defined as having a single nucleocapsid per envelope rarely show more than one nucleocapsid per envelope. Because this feature is so distinctive and characteristic of specific isolates, it was incorporated into the early nomenclature such that NPVs were categorized as either MNPV or SNPVs (also previously called multiply or singly embedded virions (MEV and SEV). In addition, whereas MNPVs and SNPVs were both found in lepidopteran viruses, only SNPVs were observed in other insect orders. GVs were also categorized as singly enveloped; however, multiple GVs, although rare, have been described [49]. Infection with baculoviruses occurs when a susceptible host eats the polyhedra or granules, which are dissolved in the basic digestive gut juices. The virions are released when the protein matrices dissolve. The virions enter the nuclei of midgut cells and eventually infect many of the tissues and organs in the insect, primarily the fat body, epidermis, and blood cells. Nonoccluded baculoviruses (NOB), or nudiviruses, are not occluded in polyhedra and have recently been removed from the Baculoviridae.

Biopesticides based on baculoviruses are ideally suited in integrated pest management programme. Since, baculoviruses are highly specific, known to be completely safe to mankind, animals, and beneficial insects such as bees, predatory insects and parasitoids. Baculoviruses have been described in over 600 insect species. The family Baculoviridae consisting of four Genera: Alpha baculovirus (Lepidoptean specific NPVs), Betabaculoviruses (Lepidopteran specific GVs), Gammabaculoviruses (Hymenopteran specific NPVs) and Deltabaculoviruses (Dipteran specific NPVs) [50]. Over the years, baculoviruses have been reported from a variety of different species of invertebrates. However, the only well-documented hosts are Diptera, Hymenoptera and Lepidoptera. Further, baculoviruses exhibit enormous genetic variation within host insect species [51]. The NPV from *Autographica californica* (AcMNPV) is one of the most intensively studied species.

NPV and GV attracted the attention of researchers looking for an alternative to hazardous chemical pesticides. NPV infects and kills some of the most important crop pests such as *Helicoverpa armigera* and *Spodoptera litura*. The GV has been successfully used to manage diamondback moth, *Plutella xylostella*, apple codling moth, *Cydia pomenella,* sugarcane shoot borer, *Chilo infuscatellus* and coconut rhinoceros beetle, *Oryctes rhinoceros*. Insect larvae infected with baculoviruses usually die from 3 to 12 days after infection depending on viral dose, temperature and the larval instar at the time of infection. Insects killed by baculoviruses have a characteristic shiny-oily appearance and often crawl to the tops of plants or any other available structure where they die and decompose. The virus particles are attached to the peritrophic membrane lining the midgut. The lipoprotein membrane surrounding the virus fuses with plasma membrane of the gut wall cells and liberates nucleocapsids into the cytoplasm. The nucleotide transports virus DNA into the nucleus of the cell and virus gene expression begins. The virus multiplies rapidly and eventually fills the body of the host with virus particles. The infected larvae are extremely fragile to the touch, rupturing to release fluid filled with infective virus particles. This tendency to remain attached to foliage and then rupture is an important aspect of the virus life-cycle. Infection of other insects will only occur if they eat foliage that has been contaminated by virus-killed larvae. The polyhedra look like clear, irregular crystals of salt when viewed at 400x or 1000x. The fluid inside a dead insect is composed largely of virus polyhedra - many billions are produced inside one cadaver.

Despite their proven efficiency, large-scale commercial application of NPV is low as compared to *Bacillus thuringiensis* based biopesticides. NPVs must replicate in the nuclei of living host cells. They cannot be cultivated in microbial culture media without living cells. This means that the production of NPV isolates as microbial biopesticide, requires a colony of insects or a suitable insect tissue culture. This is more expensive than producing organisms such as the bacterium *Bacillus thuringiensis* in an artificial medium. This hinders its large-scale application in field.

Most of the research on virulence involves inserting genes that produce toxic substances into the polyhedral gene. For example, genes for insect specific toxins, inserted into the polyhedral gene locus are expressed at the time the polyhedral gene would have been expressed. The toxins kill the insect at an earlier stage than occurs in a normal infection. There are nearly 50 registered baculoviruses under different trade names in different countries. The first commercial formulation of NPV biopesticide Elkar[@] to control *Helicoverpa zea* was prepared and marketed by Sandoz Inc in 1975 [52]. It has broad-spectrum action against species of *Helicoverpa* and *Heliothis*.

NPVs are most commonly considered for development of microbial insecticides because of the inexpensive formulation technologies and relatively simple and standard pesticide application methods [53]. A number of NPVs are currently manufactured at a commercial scale and applied in large areas of different crops. NPV formulations are used extensively for management of lepidopteran pest like *Ha*NPV and *Spodoptera litura* (*Sl*NPV) in India, NPVs of *S. littoralis* and *S. exempta* in Egypt and

Kenya and *Anticarsia gemmatalis* NPV in Brazil, *Lymanttria dispar* NPV and *Orgyia pseudotsugata* NPV in USA. A few of the granulosis viruses like, GV of *Cydia pomonella* for the control of codling moth of apple and pears in Europe and GV of *Pieris rapae* for the control of *Pieris rapae* in China and GV of *Erinnyis ello* for the control of cassava horn worm in Brazil have been successfully used as biocontrol agents [53]. In India, GV of *Chilo infuscatellus* was used for control of sugarcane shoot borer in Tamil Nadu [54]. Since no fermentation technology is available for the NPV and GV production, the products are required to be produced on the respective insect hosts and formulated in small-scale cottage industries or medium sized commercial units. This has been one of the impediments for the large-scale commercial production of baculoviruses. Another limitation of the NPV or GV formulation is its rapid inactivation by the UV radiation in the fields. Therefore, formulations with suitable adjuvants (UV protectants) for field persistence and efficacy have to be developed for large scale adaption of these biocontrol agents. In the developing countries, NPV production is done mostly manually and there is a considerable scope for mechanization of the production process. Research has to be focused to develop NPV and GV production in insect tissue cultures so that large-scale production and commercialization can take place for increased uptake of these biocontrol agents.

Baculoviruses is supposed to play an integral role in the natural regulation of insect populations. Safety of the use of baculoviruses is still not clear. In USA, three baculoviruses have been registered as pesticides: *Helicoverpa zea nuclear polyhedrosis virus* (HzSNPV) in 1975, *Orgyia pseudotsugata* (Ot) MNPV in 1976, *Lymantria dispar* (Ld) MNPV in 1993. But the only privately produced and commercially available viral pesticide there, is the SeMNPV (Spod-X^{TM}).

In India, two companies namely M/s Biocontrol Research Laboratory (BCRL) and M/s Multiplex at Bangalore are involved in mass production of baculoviruses belonging to HaNPV and SlNPV. The HaNPV formulation of 0.43 per cent in cotton and tomato, HaNPV formulation of 2.0 per cent in pigeon pea, chickpea and tomato were approved for use to manage *H. armigera* larvae. Similarly, Sl-NPV formulation (0.5% AS) was registered for use against the larvae of *Spodoptera litura* on tobacco and other crops (http://ppqs.gov.in/Ipmpest_main.htm). The NPV based biopesticides are marketed under different trades names such as Spodo-Cide®, Spodopterin®, Heli-Cide®, Heliokill® *etc*., in the Indian market.

*Ha*NPV is multiplied in the fifth instar larvae of *H. armigera*. The dose of the inoculum used is 5 x 10^5 polyhedral occlusion bodies (POB) in 10 ml suspension. A viral dose of 5 x 10^5 polyocclusion bodies is mixed with the semisynthetic diet. Early fifth instar larvae are released and maintained singly under optimum laboratory conditions. The infected larvae collected, homogenized in sterile ice-cold distilled water at the ratio 1: 2.5 (w/v). The homogenate is filtered through double-layered muslin and repeatedly washed with distilled water. The filtrate is centrifuged for 30-60 sec at 500 rpm to remove debris. The supernatant is centrifuged for 15-20 min at 5,000 to 6000 rpm. Then the pellet containing the polyhedral occlusion bodies (POB) is suspended in sterile distilled water and washed three times by centrifuging the pellet in distilled water. The final pellet is suspended in distilled water and made up to a known volume, which is necessary to calculate the strength of the POB in the purified suspension. Similarly, the NPV of *Spodoptera litura* is mass-produced on the larvae of *S. litura*. Spraying of NPV late in the evening and addition of UV blockers are recommended to improve the effectiveness of NPV. For chickpea, pigeon pea and cotton, HaNPV should be used at 250-300LE, 500LE and 250 LE/ha, respectively.

Genetic engineering offers better scope for utilization of baculoviruses. Cloning of insect toxic genes into the viral genome and expression during replication will enable quicker killing. The toxic genes that have been inserted and expressed in baculovirus genome include the *Buthus eupeus* insect toxin-1, the *Manduca sexta* diuretic hormone, the *Bacillus thuringiensis* ssp. *kurstaki* HD-73 delta-endotoxin, the *Heliothis virescens* juvenile hormone

esterase, the *Pyemotes tritici* TxP-I toxin, *Androctonus australis* neurotoxin, Dol m V gene and T-urf 13 genes. The most effective gene inserts have been the neurotoxins and the T-urf 13 gene, which is responsible for cytoplasmic male sterility of maize. Several major pesticide companies are currently involved in the commercial development of these and other genetically enhanced viral pesticides.

2. Fungi as Insect Pathogens

Entomopathogenic fungi are gaining importance in the crop pest control in recent years, although *Bacillus thuringensis* (*Bt*) is the most widely used microbial agent at present. Fungal pathogens have certain advantages in pest control programmes over other insect pathogens like bacteria and viruses. Mass production techniques of fungi are much simpler, easier and cheaper than those used for *Bt* and NPVs. Fungi unlike bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection and so sucking insects are also infected by entomopathogenic fungi. Entomopathogenic fungi play an important role in the natural pest control in various crops through epizootics. More than 750 sp. of fungi, mostly from hyphomycetes and entomophthorales are pathogenic on insects, many of them offer great potential for pest management. Species that have been most intensively investigated for mycoinsecticides in the crop pest control include *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, M. anisopliae* var. *acridium, Lecanicillium* spp., (previously *Verticillium lecanii*), *Hirsutella thompsonii, Nomuraea rileyi* and *Isaria fumosorosea* (previously *Paecilomyces fumosoroseus*). Fungi infect insects of almost all orders, most common on Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera. In some insect orders, nymphal or larval stages are more often infected than the adult stages, in others the reverse may be the case. Some fungi have restricted host ranges, *e.g., Aschersonia aleyrodis* infects only whiteflies and *N. rileyi* infects only lepidopteran larvae, while others like *B. bassiana* and *M. anisopliae* infect more than 700 species in several insect orders and they have several pathotypes, which have high degree of host specificity.

Entomopathogenic fungi from hypomycetes group are opportunistic pathogens and usually cause insect mortality by nutritional deficiency, destruction of tissues and by release of toxins. Cuticular degrading enzymes of entomofungal pathogens like chitinase, protease and lipase play an important role in the pathogenicity of these organisms on insects in the breakdown of insect cuticle for penetration of fungal germ tube into the insect body. The entry of entomopathogenic fungi through the insect cuticle is considered to occur by a combination of mechanical pressure and enzymatic degradation. Several mycotoxins like, Beauvericin, Beauverolides Bassianolide (by *B. bassiana, V. lecanii, Paecilomyces* spp.) and Destruxins A, B, C, D, E, F (by *M. anisopliae*) are produced during pathogenesis and these act like poisons for the insects. After the death of the insects, the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers [55]. The fungi of entomopthorales group are obligate pathogens of insects and cause host death by tissue colonisation with little or no use of toxins [56].

Environmental conditions particularly humidity and temperature play an important role in the infection and sporulation of entomopathogenic fungi. Very high humidity (> 90% RH) is required for spore germination and sporulation outside the host. Most of the entomopathogenic fungi in tropical and subtropical areas require an optimum temperature of 25-30^{o}C for successful control of insect pests.

Currently, the largest single microbial control program using fungi involves the use of *M. anisopliae* for control of spittlebugs (Cercopidae) in South American sugarcane and pastures [7]. The application of *B. bassiana* for the control of the pine moth *Dendrolimus* spp. in China probably represents one of the largest uses of a biocontrol agent over one million hectares of pine forest [9]. *B. bassiana* strain Bb-147 is registered on maize in Europe for control of the European corn borer, *Ostrinia nubilalis* and the Asiatic corn borer, *Ostrinia furnacalis*. The strain GHA is registered in the US for control of the whitefly, thrips, aphids and mealybugs and strain ATCC 74040 is registered against many soft-bodied

insects of the orders Homoptera, Heteroptera and Coleoptera. *B. brongniartii* is registered on sugarcane and barley for control of white grubs and cockchafers. New formulations of *M. anisopliae* are being used in Africa, Australia and Brazil against locusts and grasshoppers. *M. anisopliae* (var. *acridum*) has been found effective against the brown locust, *Locustana pardalina* in Africa, *Locusta migratoria* in Madagascar and the Australian plague locust *Chortoicetes terminifera* and *L. migratoria* in Australia. With variable success, *M. flavoviride* has also been tested against the tree locust *Anacridium melanorhodon* in Sudan, the rice grasshopper *Hieroglyphus daganensis* in Benin, Mali and Senegal and the desert locust, *Schistocerca gregaria* in Mauritania [3].

Several commercial formulations of entomofungal pathogens have been developed for crop pest management. Among the 171 products of entomopathogenic fungi developed, products based on *B. bassiana* represent 33.9% of total products, *M. anisopliae* products were 33.9%, and *I. fumosorosea* and *B. brongniartii* products represented 5.8 and 4.1% respectively. [4]. A total of 28 products (all based on *Hirsutella thompsonii*) were exclusively developed as acaricides and claimed to control mites and ticks. Approximately 43 per cent of all products were developed by South American companies and institutions [4]. A list of commercially available fungi, the target pests and the current producers are presented in the Table 3.

Research on entomofungal pathogens in India is confined to a few institutes like, National Bureau of Agriculturally Important Insects (NBAII), Bangalore, Directorate of Oilseeds Research (DOR), Hyderabad, Central Plantation Crop Research Institute (CPCRI), Kayangulam, Assam Agricultural University (AAU), Guwahati and University of Agricultural Sciences (UAS), Dharwad. At NBAII, Bangalore, an excellent culture collection of entomofungal pathogens like, *B. bassiana* (77 isolates), *B. brongniartii* (3 isolates), *M.anisopliae* (39 isolates), *Lecanicillium* spp. (35 isolates), *N. rileyi* (37 isolates), *I. fumosorosea* (3 isolates) and *I. farinosa* (3 isolates) have been made from various insect hosts from different geographical regions of the country. All these isolates were characterized with regard to their morphology, ITS sequencing, cuticle degrading enzymes production capability and virulence to several lepidopteran and sucking pests. Based on ITS-1 and ITS-2 sequence analysis, the indigenous isolates of *V. lecanii* are now placed under *Lecanicillium* genus and grouped into four species, *viz*. *L. lecanii*, *L. attenuatum*, *L. longisporum* and *L. muscarium* [57]. Based on toxicity of extracellular crude soluble proteins (CSPs) against *Spodoptera litura*, virulent isolates of *B. bassiana* (Bb-11, 47 and 49) and *M. anisopliae* (NBAII Ma-4, & 42) were identified [58]. Based on the ability to produce cuticle degrading enzyme production ability, promising isolates with higher chitinase, protease and lipase activities were identified [59]. Promising strains of *B. bassiana*, *M. anisopliae*, *Lecanicillium* spp. and *N. rileyi* were identified against *Helicoverpa armigera, Spodoptera litura* [60], *Aphis craccivora*, *A. gossypii, Rhopalosiphum maidis* [61], *Brevocoryne brassicae* [62], *Myzus persicae* and *Bemisia tabaci* in the field level in different centres of AICRP on biological control. At DOR Hyderabad, mass production technology for *N. rileyi* and oil formulation of *B. bassiana* for management of pests of oilseed crops has been developed. Promising strains of *N. rileyi*, *B. bassiana* and *M. anisopliae* have been identified against pests of soybean, groundnut and sugarcane at UAS, Dharwad. *B. bassiana* based mycoinecticide for rice hispa at AAU, Guwahati and *M.anisopliae* based formulation for coconut rhinoceros beetle at CPCRI, Kayangulam were developed. A list of successfully tested entomofungal pathogens against various pests in different crops in India are listed in Table 4.

In India, microbial pesticides registered for management of insect pests include 17 products of *B. thuringiensis* var. *kurstaki*, 58 of *B. bassiana*, 49 of *V. lecanii*, 11 of *M. anisopliae*, 18 of HlNPV and 3 of SlNPV [86]. Surveys were conducted in 2010 by scientists of NBAII, Bangalore to ascertain the status of production of microbial pesticides in the country. Only 21 out of 125 companies responded to the queries. Considerable increase in the production of all microbial pesticides except SlNPV and *Bt* was

Table 3: Commercial Scale Production of Mycoinsecticides in different countries

Fungus	Brand name	Target pests	Crop	Producer/country
Beauveria bassiana	Mycotrol	Whiteflies/Aphids/Thrips	Field Crops	Mycotech, USA
	Naturalis	Sucking insects	Cotton, Glasshouse crops	Troy BioScience, USA
	Conidia	Coffee berry borer	Coffee	AgrEvo, Germany
	Ostrinol	Corn borer	Maize	NPP (Calioppe), France
	Myco-Jaal	Diamond backback moth	Cabbage	Pest Control India (Pvt) Ltd, India
	Biosoft	*Helocoverpa* & sucking pests	Several crops	Agriland Biotech, India
	Biowonder	Rice pests	Rice	Indore Biotech, India
B brongniartii	Betel	Scarab beetle Larvae	Sugarcane	NPP (Calioppe), France
	Engerlingspilz	Scarab beetle Larvae	Pasture	Andermatt, Switzerland
	Melocont	Scarab beetle Larvae	Pasture	Kwizda, Austria
Metarhizium anisopliae	BIO 1020	Black Vine weevil	Glasshouse Ornamental crops, Nursery stock	Bayer, Germany
	Bio-Blast™	Termites	Houses	EcoScience, USA
	Bio Magic	Brown plant hopper	Rice	T. Stanes, India
	Multiplex	Root grubs	Several crops	Multiplex, India
	Metarhizium	Sucking pests		
M. flavoviride	Green Muscle	Locusts, Grasshoppers	-	CABI, UK
	BioGreen	Red-headed cockchafer	Pasture/Turf	Australia
Verticillum lecanii	Vertalec	Aphids, Whiteflies	Glasshouse crops	Koppert, Holland
	Mycotal	and Thrips		
	Inovert	Aphids, Scales, Mealybugs	-	Inora, India
	Biocatch	Whiteflies	Cotton	T. Stanes, India
	Verticare	Mealybugs & Scales	Citrus	Viswamitra Bio Agro, India
Paecelomyces fumosoroseus	PFR-97™	Whiteflies/Thrips	Glasshouse crops	Thermo Trilogy, USA
	Prioroty	Mites	Wide range of crops	T. Stanes, India

observed during the year 2009-10 (Table 5) as compared to the year 2006-07. Notable changes were observed in the formulations including a shift from solid formulations to liquid formulations due to the high efficacy and long shelf life of the latter.

In India, talc based formulations of entomofungal pathogens are extensively marketed for pest management programs. These talc formulations have shelf life of 3-4 months. Hence, there is a need to develop formulations with longer shelf life of at least 18 months. Since the efficacy of entomofungal pathogens in the field is greatly dependent on existence of favourable climatic conditions (low temperature <30°C and high humidity >70%), there is great necessity for development of suitable oil based formulations for improving the field performance. At NBAII, Bangalore, quality analysis of the products of microbial pesticides produced in the country is being done regularly and it is observed that 50-70 per cent samples do not confirm the Central Insecticide Board standards (Table 6). The main drawbacks in the quality of the formulations are lesser cfu counts of the biocontrol agent than prescribed, more contamination levels than prescribed and either higher or lower moisture level in solid formulations than prescribed (Table 6). Since majority of the microbial pesticides produced at present are of inferior quality, the government agencies should interfere and enforce strict quality parameters. To ensure that the products of microbial BCAs do not affect the environment, human beings and other living organisms adversely and to prevent the sale of poor

Table 4: Biological Control of pests using entomopathogenic fungi in India

Fungus	Pest & Crop	Field efficacy	Reference
Beauveria bassiana	Rice Hispa, *Dicladispa armigera*	Spray of *B. bassiana* spore suspension 10 million spores/ml	Hazarika and Puzari (1997)
	Coffee berry borer, *Hypothenemus hampei*	Spray of *B. bassiana* spore suspension ($1X10^7$ spores/ml) containing 0.1% sunflower oil and 0.1per cent wetting agent during monsoon reduced 50-60per cent berry borer incidence in Coorg, Karnataka	Anon. (2001)
	Tea looper, *Buzura suppressaria*	Spray of *B. bassiana* spore suspension (2.5 g/l), gave 88per cent reduction in West Bengal	Ghatak and Reza (2007)
	Sunflower: *Helicoverpa armigera*	Spray of oil suspension of *B. bassiana* (200mg/l) in Andhra Pradesh	Devi and Hari (2009)
	Green gram: White grubs	Soil application @$5X10^{13}$ conidia/ha effective control achieved in Assam	Bhattacharyya *et al*. (2008)
Beauveria Brongniarti	Sugarcane: white grub, *Holotrichia serrata*	Soil application @ 1kg /acre. Highest yield recorded	Chelvi *et al*. (2010)
Metarhizium anisopliae	Coconut: Rhinoceros beetle, *Oryctes rhinoceros*	Spraying of Spores in its breeding sites @ $5X10^{11}$spores/m^3 to the compost pits and manure heaps	Anon. (2000)
	Sugarcane: White grub	*M. anisopliae* at $1x10^{13}$/ha gave yield of 91.18 q/ha as compared with chlorpyrifos (93.29 q/ha) in Karnataka	Rachappa *et al*. (2004)
	Pigeon pea: Pod borer, *Helicoverpa armigera*	*M. anisopliae* conidia in an oil formulation was effective in reducing 66.74per cent *H. armigera* as compared to 62.58per cent with endosulfan in Maharastra	Nahar *et al*. (2004)
	Potato White grub, *Brahmina* sp.	Soil application @ $5x10^{13}$ conidia/ha along with chlorpyrifos 20 EC at 200 g a.i./ha resulted in the highest tuber yield (155 q/ha) in HP.	Bhagat *et al*. (2003)
	Soyabean: white grub, *Holotrichia longipennis*	Soil application formulation applied @ $5x10^{13}$ conidia/ha, 61.50per cent reduction in grub population	Pandey (2010)
Verticilium lecanii	Coffee green scale, *Coccus viridis*	Spraying spores@ 16 X 10^6 spores/ml along with Tween-80 twice at 2 weeks interval caused 97.6 per cent mortality of the pest	Jayaraj (1989)
	Citrus green scale, *Coccus viridis*	Spraying of spore ($2x10^6$spores/ml) along with 0.005 per cent quinalphos and 0.05per cent Teepol was found highly effective killing 95.58per cent and 97.55per cent scales in coffee and citrus respectively	Singh (1995)
	Indian mustard and Rapeseed: Mustard aphid, *Lipaphis erysimi*	Spray @ (10^6 spores/ml). There was a significant reduction in aphid infestation at 10 DAS	Rana *et al*. (2002)
Nomuraea rileyi	Castor: *Spodoptera litura* in AP	Spraying of spore ($10x10^{10}$spores/ml) along with 0.02per cent Tween-80.	Vimala Devi and Prasad (1997)
	Soybean: *Spodoptera litura* *Helicoverpa armigera* *Thysonoplusia orichalcea*	*N. rileyi* spores spraying @ $2X10^8$/ml twice at 10 days interval during Kharif in North Karnataka was cheaper than insecticidal treatment and cost effective	Lingappa *et al*. (2002)

Table 5: Status of production of microbial biocontrol agents used against insect pests in India

Agent	2005-06 (T/L)	2006-07 (T/L)	2009-2010	
			Solid formulations (T)	Liquid formulations (L)
*Ha*NPV	15639 (L)	7958 (L)	-	12522
*Sl*NPV	3438 (L)	2387 (L)	-	1673
Bacillus thuringenesis	52	0.07	5.0	-
Beauveria bassiana	141	82	244	19478
Metarhizium anisopliae	29	35	20	4730
Verticillium lecanii	45	30	190	10242
Paecilomyces lilacinus	50	3.0	14	26436
P. fumosoroseus	-	-	2.0	4000

Table 6: Quality analysis of products of entomofungal pathogens in India

Year	No. of samples analyzed	% of samples	
		As per CIB standard	Not as per CIB standard
2010-11	42	50	50
2009-10	48	40	60
2008-09	44	40	60
2007-08	47	35	65
2006-07	35	30	70
2005-06	32	30	70

quality products to the farmers, Government regulatory agencies (Central Insecticides Board, India) have made registration of microbial pesticides mandatory before commercial production/import/ sale. Data on non-target organisms, toxicological reports on laboratory animals and eco-toxicity have to be provided along with other efficacy data at the time of registration. The biological product should not show allergenicity, pathogenecity and toxicity to the non-target organisms. Entomogenous fungi intended for biological control should be safe to the beneficial insects like, silkworms, honey bees, lac insects and natural enemies of crop pests (parasites and predators).

So far, most of the germplasm collections of entomofungal pathogens are made from the agricultural ecosystems. A large wealth of microbial diversity from undisturbed natural ecosystems like forests, mangroves etc. are yet to be exploited for biological control of pests. For this, systematic surveys are to be carried out especially in North-East, Sub- Himalayan, and Western Ghats regions for augmenting the germplasm collection. Strains with desirable characteristics like better efficacy, wider host range, tolerance to pesticides, survival ability in the environment, tolerance to adverse environmental conditions, vigorous growth and longer shelf life have to be identified for effective utilization in the field. The biodiversity of Entomophthorales group (obligate pathogens) has not been exploited for pest management in India and some of them like, *Erynia neoaphidis***,** *Neozygites parvispora Entomophthora thripidum*, and *Zoopthora radicas* have potential for successful management of crop pests like, aphids, thrips and lepidopteran pests. Research on this line has to be intensified in India.

Protozoa as Insect Pathogens

Entomopathogenic protozoans are extremely diverse group of organisms comprising around 1000 species attacking invertebrates including insect species and are commonly referred as microsporidians. They are generally host specific and slow acting, producing

chronic infections with general debilitation of the host. The spore formed by the protozoan is the infectious stage and has to be ingested by the insect host for pathogenecity. The spore germinates in the midgut and sporoplasm is released invading the target cells causing infection of the host. The infection results in reduced feeding, vigour, fecundity and longevity of the insect host.

Although they are undoubtedly important in natural biological regulation of insect populations, they do not possess the required attributes for a successful microbial insecticide. The most notable entomopathogenic protozoa belong to *Nosema* spp. and *Variomorpha necatrix*. *Nosema locustae* is the only commercially available species of the microsporidian and is marketed for control of grass hoppers and crickets in USA, Canada, Argentina, China and Mali. However, the utility of *N. locustae* as a grasshopper biocontrol agent remains questionable because of the great difficulty in assessing the efficacy in case of a highly mobile insect [87]. *Nosema pyrausta* is another beneficial microsporidian that reduces fecundity and longevity of the adults and also causes mortality of the larvae of European corn borer [88].

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