Entomopathogens, Pathological Symptoms and their Role in Present Scenario of Agriculture: A Review

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Abstract

Agriculture plays an important role in the economy of India. Insect infestation causes drastic level of loss to the agricultural crop production. This is a challenging task to meet the requirements of the increasing human population in India and other developing nations. Indian agricultural system needs a sustainable agricultural produce for future needs of future generation. This article highlights the present scenario of Entomopathogens in Agriculture sector. Synthetic Chemical Pesticides are commonly used to control insect pest which causes hazardous impact on our environment and non-targeted beneficial organism’s system including human being. Entomopathogens as a biopesticides are natural, sustainable, cost effective and good alternative source to replace those dangerous chemical pesticides. At present time few entomopathogenic formulations are available at market, which are insufficient to meet the demand of farmers due to lack of innovative advancements in research and policies in India. Production and consumption of entomopathogens are limited as compared to synthetic chemical pesticides. This article suggests enhancing technology oriented research and boosting entomopathogenic biopesticides formulations for increasing agricultural production in an eco-friendly manner.

Keywords: Biopesticides, Microbial pesticide, Crop protection, Bacillus thuringiensis, Bacterial, Fungal, Viral, Nematode pesticides

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Introduction

Entomopathogens are microorganism that is pathogenic to arthropods such as insect, mites and ticks. Several species of naturally occurring bacteria, fungi, nematode and viruses infects a variety of arthropods pests and play an important role in their management. Some Entomopathogens are mass produced in-vitro (bacteria, fungi and
nematode) and in-vivo (nematodes and viruses) and sold commercially. In some cases, they are produced on small scale for non-commercial local use. Using Entomopathogens as biopesticides in pest management is called microbial control, which can be critical part of integrated pest management (IPM) against several pests.

Farmers using Chemical pesticides for the control of insect pest from so many decades; however, the effect on non-targeted organisms, residues on food crops, groundwater pollution, and development of insect resistance to chemical pesticide increase the requirement of alternative eco-friendly methods. The use of fungi biocontrol is the most effective alternative pest control method among all biological control process to decrease the insect pest population or density with the aim to lower down the disease developing activities subsequently crop damage. In sustainable pest management program plant protection with the help of entomopathogenic fungi plays a key role. A compared to conventional insecticides biocontrol with entomopathogenic fungi have various advantages like high efficiency, low cost, safety of beneficial organisms, lowering the chemical residues in the environment, and increased biodiversity in human-managed ecosystems (Gul et al., 2014).

Entomopathogens, which can be bacterial, viral or fungal, are pathogens that kill or seriously disable insects. They play a vital role in natural regulation of insect pest population (Sandhu et al., 2012). A wide range of entomopathogens have been studies and used as bioinsecticides, including the soil bacterium Bacillus thuringiensis (Bt.), baculoviruses and entomopathogenic fungi such as Metarhizium and Beauveria species.

Generally, biopesticides are classified into numerous classes: microbial pesticides containing of entomopathogenic bacteria (e.g., Bacillus thuringiensis), viruses (e.g., Baculoviruses) or fungi (e.g., B. bassiana), as well as their metabolites, entomopathogenic, protozoa and nematodes. The associate of Bacillaceae family, Bacillus thuringiensis, is extensively exhausted as biopesticides, meanwhile it produces a toxin that is active against several classes of insects (Fisher and Garchynski, 2012) (Table 1–5).

**Entomopathogenic fungi**

Entomopathogenic fungi with broad based and abundant diversity is a boon sustainable solution concerning Integrated pest management. These entomopathogenic fungi because of environment friendly and bio persistence prefer to kill insect at distinct stages of their life cycle. A very diverse range of myco species are discovered from various classes that contaminate insects. These insect pathogens are found in an extensive kind of modifications and contaminating abilities using facultative and obligate pathogens. These Entomopathogenic fungi are originated from the divisions, Deuteromycota, Ascomycota, and Zygomycota.

Myco-biocontrol is the use of fungi in biological processes to lower the insect density with the aim to reduce disease-producing activity and consequently crop damage. Biological plant protection with entomopathogenic fungi has a key role in sustainable pest management program.

Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low costs, high efficiency, safety for beneficial organisms, reduction of residues in the environment, and increased biodiversity in human-managed ecosystems (Gul et al., 2014).
Some important entomopathogenic fungi

**Genus – Beauveria**

These enter the host insect body through food or in contact with the host cuticle and reproduce inside the insect body. It produces toxins namely beauvericin, bassianocide etc. inside the host body causes paralysis of the host insects and ultimately kills the insects within four or five days. They are used particularly to control sucking pests and caterpillars infesting crop plants. These entomopathogenic fungi are utilize to ecologically manage the caterpillars of yellow stem borer and leaf folder of rice, white grub of groundnut, coconut rhinoceros beetle, sugarcane pyrilla, caterpillars of pulses, tomato and cotton, diamond back moth, leaf eating caterpillars of tobacco and sunflower etc.

**Genus-Verticillium**

*Verticillium chlamydosporium* and *Verticillium lecanii* are the imperative species of this genus. Fungus *V. lecanii* is broadly distributed, which can cause huge epizootic in tropical and subtropical regions, as well as in warm and humid environments (Nunez et al., 2008). It is an effectively work against *Trialeurodes vaporariorum* in greenhouses (Kim et al., 2002). This biocontrol agent attacks nymphs and adults and sticks to the leaf underside by means of filamentous mycelium (Nunez et al., 2008). *Verticillium lecanii* is most effective to manage the whitefly and several aphid species.

**Genus-Metarhizium**

The genus *Metarhizium* has three important species, *Metarhizium anisopliae*, *Metarhizium album*, and *Metarhizium flavoviride*. *Metarhizium anisopliae* is a potential entomopathogenic fungus. This pathogenic fungus is used to control mainly coconut rhinoceros beetle, groundnut cut worm, rice brown plant hopper, diamond back moth and early shoot borer, top shoot borer and internode borer of sugarcane (Sandhu et al., 2000).

**Genus-Nomuraea**

*Nomuraea rileyi* is a potential insect-infecting fungus, is a dimorphic hyphomycete that can cause epizootic death in various insects. The host specificity of *N. rileyi* and its eco-friendly nature encourage its use in insect pest management. This bio control is an effective against several insect’s hosts such as *Trichoplusia* sp., *Heliothis zea*, *Bombyx mori*, *Plathypena scabra*, etc.

**Genus-Paecilomyces**

*Paecilomyces* is one of the most important bio control agent against whiteflies worldwide and causes the sickness called “yellow muscardine.” It is effective against *Bemisia* and *Trialeurodes* spp. both in field and greenhouse conditions.

**Genus-Hirsutella**

Genus *Hirsutella* includes three important species, *H. thompsonii*, *H. gigantea*, and *H. citriformis*. *H. thompsonii* is utilized for the control of citrus rust mite. This biocontrol is also effective to Acarida, Lepidoptera, and Hemipteran groups of insects.

**Toxin production**

These toxins have been shown to have diverse effects on various insect tissues. The cytotoxin of entomopathogenic fungi caused cellular disruption prior to hyphae penetration. Behavioral symptoms such as partial or general paralysis, sluggishness, and decreased irritability in fungal-infected insects are consistent with the action of
neuromuscular toxins (Charnley, 1984). Toxins such as beauvericin, bassianolide, beauverolides and isarolides have been isolated from *B. bassiana* (Eyal *et al.*, 1994).

**Mode of action of entomopathogenic fungi**

The infective unit in most fungi is a spore, usually a conidium. In most of the cases, the conidia are adhesive to the cuticle, or secrete adhesive mucus as the conidium swells during pre-germination. In favourable conditions, the conidium, germinates into a short germ tube which gives out small swellings called appresoria. The appresorium 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. After that the hyphae penetrate to the insect cuticle by enzymatic dissolution chitin and protein, it first dissolves the cuticle then enter in to the haemocoel and internal organ of the insect. The invasion by the contagious fungal mycelium proceeds until the insect is filled with the fungus and gets to be very firm to touch. After that conidiophores are produced which explode through the cuticle and produce spores on the outside of the insect, which infecting nearby healthy insects also. Death of the host is caused by mechanical blocking of the tissues and also by the toxins produced by the fungus.

**Symptoms of entomopathogenic fungi**

The point from where fungus has gained entry into the cuticle turns black in colour. Some behavioural symptoms are also appeared such as loss of appetite, weakness, and restlessness. As the fungal hyphae grows, host body becomes hard and no change occurs in the shape and structure of insects. However, some Lepidopterous larvae with certain entomophthorales appears flaccid with fragile integument. The colour of insect also changes in later stages. Insect host filled with zygomycota conidia are turn into yellow and host insect having resting spores turn into black in colour. The conidia of Duteromycetes fungi also produce several colours in cadaver. The cadavers which is covered with white colour may infected with *Beauveria* whereas *Paecilomyces* conidia are usually gray, yellow, or in some cases, pink or pink-gray (*P.fumosoroseus*). Conidia of *Nomuraea rileyi* and *Metarhizium anisopliae* are green, and those of *Aspergillus flavus* are characteristically yellow-green. The infection by *Sorosporella* (Deuteromycota) is called red muscardine. The infected larvae shortly after death turns from creamy white to pink and increase red as fungal complete its development and cadaver filled with reddish spores (Speare, 1920). *Verticillium* also known as white halo fungus because of the presence of white mycelium on the edges of scale. The infection by *Aspergillus*, makes the cadaver very hard so resulted in stone like mummies. In honeybees *Aspergillus* causes stone brood (Gilliam, 1978).

**Entomopathogenic bacteria**

Entomopathogenic bacteria (EPB) used for insect control are spore forming and rod-shaped bacteria, and the genus *Bacillus* in general and *Bacillus thuringiensis* (Berliner) in particular is one of the most important, widely researched and commercially deployed species of this group. Insecticidal activity of the bacterium is due to three proteins, crystal (Cry), cytolytic (Cyt) and vegetative insecticidal protein (Vip) produced during the bacterial infection on the larvae. These proteins are accumulated in the crystalline inclusion bodies produced by the bacterium on sporulation (Cry proteins, Cyt proteins) or expressed during bacterial growth (Vip proteins). Considering the environmental safety and the effectiveness in pest management, several other bacteria such as *Streptomyces avermitilis* species nova and
Saccharopolyspora spinosa species nova, have also been commercially deployed in pest management.

The insecticidal activity of Bt was first discovered in 1911 and commercially available after 1950s. In the past 50 years’ numerous bacteria have been isolated, classified and demonstrated in the laboratory to be pathogenic for various insects. Insect pathogenic bacteria occur in the families, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, Micrococcaceae and Bacilliaceae (Tanada and Kaya, 1993).

**Mode of action of entomopathogenic bacteria**

*Bacillus thuringiensis* produces a parasporal inclusion, a protein crystal body during sporulation. A huge number of related crystal proteins are known and more than one protein type can co-assemble in one crystal. Many distinct crystal protein (*Cry*) genes have been described. The gut epithelium is the primary target tissue for *Bt* delta-endotoxin action. The crystal proteins exert their effect on the host by causing lysis of midgut epithelial cells, which leads to gut paralysis.

The insect stops feeding and, if it does not recover, eventually dies. Upon ingestion, the crystals dissolve in the alkaline environment of the midgut and then the protoxin is proteolytically processed to produce the actual toxin. Activation of actual toxin usually involves the removal of a small number of N-terminal amino acid residues along with the cleavage of the C-terminal half (Gill et al., 1992). After that the activated toxin binds to specific receptors present on the membranes of the host insect epithelial midgut cells and induces the formation of pores in the membrane of midgut epithelial cells. This is followed by permeability of cell membrane increase which finally leads to cell lysis, disruption of gut integrity and eventually to the death of the insect from starvation or septicemia (Adang 1985; Gill et al., 1992; Bauer, 1995).

**Symptoms**

Bacterial infections in insects can be broadly classified as bacteremia, septicemia, and toxemia. Bacteremia occurs when the bacteria multiply in the insect's haemolymph without the production of toxins. This situation occurs in the case of bacterial symbionts and rarely occurs with bacterial pathogens. Septicemia occurs most frequently with pathogenic bacteria, which invade the haemocoel, multiply, produce toxins, and kill the insect. Toxemia occurs when the bacteria produce toxins and the bacteria are usually confined to the gut lumen, as in the case of brachytosis of the tent caterpillar.

Pathogenic bacteria, upon ingestion by a susceptible insect, multiply and produce toxins in the midgut lumen. The insect loses its appetite, becomes diarrheic, discharges watery feces, and may vomit. The invasion of the bacteria into the haemocoel results in septicemia and death of the insect. The bacteria, in general, are extracellular pathogens except for the pathogenic rickettsia and mollicutes.

**Entomopathogenic viruses**

These viruses are specific and highly virulent to their hosts. They are restricted in their virulence to the class insect, they are often genus or species specific. About 60 percent of the total known insect viruses (1200) belong to the family Baculoviridae and it is estimated that such viruses can be used against nearly 30 percent of all the major pests of food and fiber crops. Nucleopolyhedrosis virus (NPV) is known for high epizootic levels and is naturally occurring, self-perpetuating, safe to
natural enemies due to host specificity and eco-friendly. Since, NPV is an obligate parasite, it multiplies only in living insect larvae. So, mass production of NPV is a difficult and requires skilled workmen (McIntosh et al., 1987).

**Baculoviruses**

Baculoviruses are one of the largest and most diverse groups of double-stranded DNA viruses pathogenic only for insects mostly of the orders Lepidoptera (butterflies, moths), Hymenoptera (sawflies) Coleoptera (beetles) and Diptera. Individual baculovirus usually have a narrow host range restricted to a few closely related species. *Autographa californica* nucleopolyhedrovirus (AcNPV) is the most widely studied baculovirus.

Baculoviruses are arthropod viruses and they are well known due to their potential as agents of biological control of pests in agriculture, forestry and horticulture. The family Baculoviridae contains the occlusion bodies (polyhedra) of nucleopolyhedrovirus contain many occlusions derived virions (ODV) surrounded by a matrix of polyhedrin protein, a major structural protein (Braunagel et al., 2003). Polyhedra are relatively stable and the protected virions can survive in the environment for more than twenty years under favourable conditions.

**Mode of action**

As the susceptible larvae feed the leaves infected with baculoviruses, the occluded bodies get dissolved in the alkaline pH of insect midgut and the virus start multiplying within the host cell nuclei. NPV can be multiplied in any type of host tissue but the GV multiplied only in the fat bodies. Infected host may die within 3-4 days when environmental conditions are favourable.

**Symptoms of entomopathogenic viruses**

The host insect infected with Baculoviruses shows shiny oily appearance of body. Larvae become sluggish and reduce its apatite or do not feed at all. The colours of the infected larva become dark or brown. Infected larvae appear to be swollen as the body filled with fluid containing virus and in the advance of infection, the larvae ruptures and release infective virus particle. Larvae have the characteristic symptom of climbing up to the top of the plant part and hanging upside down. This is also known as tree top disease.

These viruses may infect healthy larvae if they eat leaves contaminated with the infective larvae (Ramanujam.et al., 2014). Diseased larvae behave differently, becoming easily agitated when disturbed, and are frequently found lying at the edge of the rearing beds before death. In addition, the integument becomes very fragile, and the haemolymph turns milky and turbid.

**Entomopathogenic nematode**

Nematodes are non-segmented, elongated roundworms that are colorless, without appendages, and usually microscopic. There are non-beneficial and beneficial nematodes. Non-beneficial nematodes cause damage to crops and other types of plants are also called “plant parasitic nematodes”. Beneficial nematodes attack soil-borne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms (Dennoet et al., 2008).

Beneficial nematodes cause disease in insect are referred to as “entomopathogenic” and have the ability to kill insects. Indiscriminate use of chemical pesticides for the
management of insect in different agro ecosystems has been raised many environmental concerns viz. ground water contamination, residue in food, resistance development, soil pollution, air pollution, secondary pest outbreak, pest resurgence, etc. (Zimmerman and Cranshaw, 1990). As a substitute to pesticides, biological control agents like entomopathogenic nematodes have gained more importance due to its eco-friendly properties. Entomopathogenic nematodes are belongs to the genera Heterorhabditis and Steinernema which are obligate parasites of insects (Lewis and Clarke, 2012). Nematodes have a symbiotic relationship with a bacterium (Photorhabdus spp. are associated with Heterorhabditis and Xenorhabdus spp. are associated with steinernematids) (Poinar, 1990).

**Entomopathogenic nematodes biology**

The parasitic cycle of nematodes is started by the third infective juveniles stage. These non-feeding juveniles find and invade appropriate host insects through natural body openings likes anus, mouth, and spiracles or even through the cuticle when the genus Heterorhabditis is concerned. Once interior the host, infective third juveniles stage invade the haemocoel and release a symbiotic bacterium, which is held in the nematode’s digestive system (Poinar, 1990).

In insect the bacteria cause a septicemia and killing the host within 24 to 48 hours. The infective juveniles feed on the fast multiplying bacteria and disintegrated host tissues. About 2-3 generations of the nematode are completed within the insect cadaver. When food reserves are exhausted, nematode reproduction ceases and the offspring develop into resistant infective juveniles which disperse from the dead host, and are able to survive in the environment and to seek out new hosts.

**Host range**

The nematode-bacterium complex kills insects so rapidly that the nematodes do not form the close, highly adapted, host-parasite relationship characteristic of other insect nematode associations, e.g., mermithids. This fast mortality permits the nematodes to exploit a range of hosts that spans nearly all insect orders, a spectrum of activity well beyond that of any other microbial control agent. In laboratory tests alone S. carpocapsae infected more than 250 species of insects from over 75 families in 11 orders (Poinar, 1975).

The nematodes attack a wide spectrum of insects in the laboratory where host contact is secure, environmental conditions are best, and no ecological or behavioral barriers to infection exist (Gaugler et al., 1997). For example, the lepidopteran larvae which feed on foliage are highly susceptible to infection in Petri dishes, but are infrequently impacted in the field, where nematodes tend to be quickly inactivated by the environmental extremes (i.e., desiccation, UV radiation, temperature) characteristic of exposed foliage. Behavioral obstacles also restrict nematode efficacy to a few selected hosts or host groups (Gaugler et al., 1997).

**Host finding mechanism of entomopathogenic**

Nematodes Host-searching strategies of entomopathogenic nematode will help in to find the appropriate nematode species to pest insects to ensure infection and control (Gaugler, 1999). Only infective juvenile stage of entomopathogenic nematodes will survive in the soil and find and penetrate insect host. Infective juvenile finds their hosts in soil by means of ambushing and cruising strategies (Gaugler et al., 1989). Ambusher species include Steinernema carpocapsae and S.
scapterisici; cruisers include *Heterorhabditis bacteriophora* and *S. glaseri*. *S. riobrave* and *S. feltiae* do a bit of both ambushing and cruising (Campbell and Gaugler, 1997).

**Ambushing:**

Entomopathogenic nematodes that use the ambushing strategy, the IJs mostly remain in the same spot for a long period of time waiting for the prey to cross the boundary of their strike area. Chemical cues are not important for them. Nematodes belonging to the category of ambushers are also capable to nictate, i.e. to stand on their tails with more than 75% of the body held straight. Nictation is a relatively stationary tactic which is applied by infective juveniles (Campbell and Gaugler, 1993). These are ambusher entomopathogenic nematodes most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets.

**Cruising**

The cruisers move continuously in the environment in search of hosts hence they may become preys themselves. The cruising strategy are highly mobile and able to move throughout the soil profile. They largely use long-range chemical cues (carbon dioxide, vibration and other volatiles released by the host) to discover the location of resources (Kaya and Gaugler, 1993). Cruiser entomopathogenic nematodes are most effective against sedentary and slow-moving insect pests at various soil depths, such as white grubs and root weevils.

**Mode of action EPN**

They only stage that survives outside of a host is the non-feeding third stage infective juvenile. The first juvenile stage of *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* carry bacterial symbiont of genus *Photorhabdus* and *Xenorhabdus* respectively in their intestinal tract. After locating the suitable host, the first juvenile stage invades it through natural openings such as mouth, spiracles, anus & penetrate into the host haemocoel. Start feeding on multiplying bacteria and disintegrated host tissues. Toxins produced by nematodes and multiplying bacteria in the body cavity kill the insect host usually within 48 hours.

**Pathological symptoms**

Steinernematids nematodes, infected larvae turn creamy/dark brown colour and *Heterorhabditid* nematodes infected larvae will turn reddish/purplish colour.

The sticky nature of infected cadavers is the characteristic of *H. bacteriophora* is also used in the identification (Aliyu *et al*., 2019).

The important character of the symbiotic bacteria (*Xenorhabditis luminescens*) of *Heterorhabditis* spp. was its ability to fluorescens. The entire infected insect cadaver glowed in the dark and the infected larvae can be easily detected (Poinar *et al*., 1980).

**Entomopathogenic protozoa**

Entomopathogenic protozoans occur in all the major groups of protozoa, but microsporidia is the major class of such pathogens. Protozoa as a diverse group of unicellular Eukaryotic organisms, whereas bacteria are Prokaryotic. Protozoa has membrane enclosed organelles like mitochondria and digestive vacuoles, whereas it’s absent in bacteria. Protozoa causes diseases like Malaria, dysentery and Bacteria causes Cholera, tuberculosis, lyme disease. Protozoa is unicellular Eukaryotic organisms and possess at least one motile stage powered by cilia, flagella, or pseudopodia. The vegetative cells of most
protozoa are membrane-bound and lack the rigid cell wall structure common to both fungi and plants.

**Mode of action:**

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 pathogenicity. The spore germinates in the midgut and sporoplasm is released invading the target cells causing infection of the host.

**Symptoms**

The infected insect become whitish, is reduced in size, and remain in immature stages much longer than a healthy insect. The protozoal spores are present in fat, midgut and haemolymph may cause these structures to turn milky color. The integument of infected insects remains firm and does not easily disintegrate (Fernando E. Vega, 2012). No toxins have been detected in protozoal infection in insects, but Weiser (1961) that toxin may be produced by microsporidia that cause tumor like growth and inflammatory response in insects.

**Use in pest management**

They are successfully not control the insect but some are used for pest control like *N. locustae* as a bio-control agent of grasshopper. *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.

**Role of Entomopathogens in Present Scenario of Agriculture**

Agriculture and its allied sectors are the largest contributors to India’s Gross Domestic Product and the principal livelihood for an estimated 58% of its population (Kamat and Rajendra, 2016). With limited land resources and an increasing human population, minimizing pest-associated crop losses is becoming increasingly critical (Pandey and Seto, 2015). Yield losses from pests on farm have been estimated at 10–30% (Thind, 2015), while supplementary post-harvest losses can be significant. A recent country-wide study (Jha et al., 2015) estimated that up to 18% fruits, 13% vegetables, 10% oil seed and 6% cereals were lost during harvesting, handling and storage. In the intervening time, many traditional chemical pesticides have been withdrawn from use as a result of environmental and health concerns (Damalas and Eleftherohorinos, 2011). As of October 2015, 32 pesticide active ingredients are banned for manufacture, importation and use, with a further 8 inhibited from the Indian market and 13 more restricted for use (CIBRC, 2017).

In India commercial level production of microbial pesticides has gained momentum in the recent years. As of 2017, a total of 188 mycoinsecticides, 51 bacterial insecticides, 39 myconematicides and 27 nucleopolyhedrovirus (NPV) products were registered through the CIBRC. In addition, 9 products based on 4 species of entomopathogenic nematodes (EPN) are sold in the India which are exempted from CIBRC registration. A large number of bio fungicides as wettable powders are registered and sold in India. These pathogens colonize the rhizosphere or phylloplane and products are often marketed as soil inoculants, due to their ability to outcompete or exclude infection of fungal pathogens (Woo et al., 2014). However, microbial pesticides comprise a small portion of the pesticide market and minute portions of indigenous entomopathogens isolates have been registered. As of 2014, ICAR-National Bureau of Agriculturally Important Microorganisms
(NBAIM), maintain 5375 microbial cultures in the National Agriculturally Important Microbial Culture Collection (NBAIM, 2017). Presently, the NBAIR maintains a repository of potential microbial biopesticides, which recently included 284 strains of Bt, 210 strains of entomopathogenic fungi, 119 strains of EPN and six NPVs (NBAIR, 2017a).

Table 1 Some of the important toxins produced by the entomopathogenic fungi are as follows

| Toxin     | Fungi                          | Function                                                                                   |
|-----------|-------------------------------|-------------------------------------------------------------------------------------------|
| Efrapeptins | *Tolyposcladium niveum*       | Inhibitors of mitochondrial oxidative phosphorylation and ATPase activity.              |
| DestruXins | *Metarhizium anisopliae*      | Immunodepressant activity in insect and cytotoxic effect.                               |
| Beauvericin | *Beauveria bassiana*          | Cytotoxic effect and insecticidal properties.                                           |
| Bassianolide | *B. bassiana Verticillium lecanii* | Acts as ionophore, toxic effect on insects.                                              |
| LeucinostatinS | *Paecilomyces lilacinus*         | Insecticidal activity by interfering with oxidative phosphorylation.                       |

| Table 2 Commercial Formulations of Entomopathogenic Fungal Pesticides in Different Countries |
|---------------------------------|---------------------------------|----------------------------------|---------------------------------|
| Fungi                           | Target pest                     | Crop                            | Product and company             | Formulation                    |
| *Beauveria bassiana*            | Sucking insects                 | Cotton,                         | Naturalis™, Tray Bioscience, USA | Liquid formulation             |
| *B. bassiana*                  | Whiteflies/ Aphids/Thrips       | Field crops                     | Mycontrol-WP/ Mycotech Corp, USA | Wettable powder                |
| *B. bassiana*                  | Corn borer                      | Maize                           | Ostrinil/Natural plant protection/France | Microgranules of mycelium   |
| *Metarhizium anisopliae*        | Termites                        | Houses                          | Bio-path™/ EcoScience/USA       | mycelium placed in trap/chamber|
| *M. anisopliae*                | Locusts, Grasshoppers, Red-headed, cockchafer | Pasture/Turf, Field crops | Biogreen/Biocare Technology Pvt. Ltd./ Australia | Conidia produced on grains.   |
| *Verticillium lecanii*          | Aphids, Whiteflies and Thrips   | Glasshouse crops                | Mycotal/Koppert/ Netherlands     | Wettable powder                |
Table.3 List of Bacterial Entomopathogens developed to control pest attack on various crop plants

| Name of the bacteria                        | Target pest                                      |
|---------------------------------------------|--------------------------------------------------|
| *Bacillus popilliae*                        | Members of Coleoptera                           |
| *Paenibacillus popilliae*                   | Coleoptera: Scarabaeidae: *Popillia japonica*    |
| *Bacillus thuringensis var. kurstaki*       | Members of Lepidoptera and Coleoptera           |
| *B. thuringiensis var. aizawai*             | Lepidoptera                                     |
| *B. thuringensis var. galleriae*            | Helicoverpa armigera and *Plutella xylostella*  |
| *B. thuringensis var. israelensis*          | Diptera: Culicidae, Simuliidae                  |
| *B. thuringiensis subspecies japonensis*    | Coleoptera: Scarabaeida                         |
| *B. thuringiensis subspecies*               |                                                  |
| *B. thuringiensis subspecies*               |                                                  |
| *B. thuringiensis subspecies*               |                                                  |
| *Lysinibacillus sphaericus*                 | Diptera: Culicidae                              |
| *Serratia entomophila*                      | *Costelytra zealandica*                         |
| *Chromobacterium subsugae,*                 | *Leptinotarsa decemlineata*                     |

Table.4 Commercial Baculovirus formulations Available for use in field Crops

| Product Name  | Manufacturer                        | Baculovirus | Pests                                    |
|---------------|-------------------------------------|-------------|------------------------------------------|
| Spod-X        | Thermo Trilogy                      | SeNPV       | Beet armyworm                            |
| GemStar       | Thermo Trilogy                      | HzNPV       | *Heliothis/Helicoverpa*                   |
| Madmex        | Andermatt Biocontrol, Switzerland    | CpNPV       | Codling moth                             |
| Elcar         | Novartis                            | HaNPV       | *Helicoverpa armigera*                    |
| VPN           | Agricola El Sol, Brazil             | AgNPV       | Velvetbean caterpillar                    |
| Gusano        | Thermo Trilogy                      | AcNPV       | *Autographa californica*                  |
| Spodopterin   | NPP, France                         | SINPV       | *Spodoptera litura*                      |

Table.5 Commercially used nematode formulation

| Name of Nematode Formulation | Targeted Insects          | Trade Name of Formulation                     |
|------------------------------|---------------------------|-----------------------------------------------|
| *Steinernema carpocapsae*    | Lepidopterous larvae      | X-Gnat, Nemastar, Bio-safe                    |
| *Heterorhabditis bacteriophora* | Soil-dwelling insects | Nema Green, nema Top, Hetromask               |
| *Steinernema feltiae*        | Dipterous insect          | Magnet, Entonem, Nemasys                      |

Entomopathogenic fungi

In general, entomopathogenic fungi are compatible with most parasitoids and predators (Goettel and Hajek, 2001; Sterk *et al.*, 2003). Among the microbial biological control agents, fungal pathogens have received particular interest because of their effective management of pests with piercing and sucking mouthparts (Wraight *et al.*, 2001). Besides the members of Tetranichyidae which are not able to be
controlled by Bt, are managed successfully using EPF such as *Hirsutella* sp. EPF have comparative advantage of having a multiple site of action (Khachatourians, 1991) which further minimizes the chances of resistance development in insect pest.

**Entomopathogenic bacteria**

*Bt.* formulations are species specific against several insect order including Lepidoptera, Coleoptera and Diptera. *Bacillus papillae* cause s milky disease in Japanese beetle larvae. Non target organism is not directly affected by exposure to commercial products Cry toxins used for control of lepidopteran pests (Sims, 1997). By avoiding more toxic insecticides that would disrupt natural enemy populations, the use of *B. bassiana* has been shown to preserve the parasitoids of *C. indicus* (Ramasubramanian et al., 2014; Srikanth et al., 2016). *B. bassiana* plays an important role in the management of several human disease vectors in many regions of India (Amalraj Mittal, 2003; Poopathi, 2012).

**Entomopathogenic viruses**

The most extensively studied baculovirus that is used for controlling lepidopteran pests is the alfalfa looper virus, *Autographa californica* (Speyer) NPV (AcMNPV) Koul and Dhalwal, 2002). These baculoviruses may kill the host within 4-7 days under favourable environment and may take 3 weeks in the absence of favourable conditions.

**Entomopathogenic nematodes**

Various formulations of EPN have been prepared commercially till now and used against various soil and foliar insect pest. The wax moth larva *Galleria mellonella* (L.) is most commonly used to rear nematodes and liquid fermentation technique for large-scale production of nematodes (Friedman 1990).The product (Pusa Nemagel) has an extended shelf life and activity against white grubs, termites, and many lepidopteran pests (Ganguly et al., 2008; Ganguly and Rathour, 2014). Interestingly, *S. thermophilum* was the first reported EPN to have pathogenicity to lepidopteran eggs (Kaliya et al., 2014).

Conclusion of the study:

Since prehistoric time, agriculture has been facing devastating harm caused by weeds, viruses, nematodes, fungi, insect pests, animals, and birds which has directed to the diminish in crop production. It has been estimated that there has been an excessive harm of crop yield due to insects, diseases and weeds. To overcome this problem, many strategies were working. One of the greatest common practice to get rid of the insect pests is to use chemicals/synthetic pesticides (e.g. carbamates, chlorinated hydrocarbons, organophosphates, etc.). In spite of the achievement increased by the use of chemical pesticides, there are potential health and environmental threats/risks connected with them. These chemical pesticides have long perseverance period. Likewise, insensitive and nonstop application of these chemical products lead to in intensified residual problems, resistance among the insect pests and loss of some beneficial species. To overcome the hazards associated to chemical pesticides, there is an essential requirement to adopt a coherent and eco-friendly practice. One such enhancement in pest control tactic is to develop biopesticides which are derivative of naturally occurring material such as plants, animals, microorganisms or their products. These are effective and decomposable and pose less effect on the environment. The term ‘biopesticides’ is misleading in the sense it is not essential that microbial agent for pest control will totally eliminate the pest, rather it suppresses and allow the crop to sufficiently
develop some deleterious effect on the pest so that crop yield is not affected.

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