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Microbial control: As a component of natural farming

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Abstract
Pest problem has resulted in major limitation on the agricultural output as well as the quality of agricultural produce. To mitigate this increasing pest problems, there has been a continuous search for an alternative method which is environment friendly, safe to non-target organisms and sustainable. Microbial control which involves the disease causing mechanisms of microorganisms like bacteria, fungi, virus and nematodes called entomopathogens to bring about death of target insect pest has been recently explored extensively as an eco-friendly alternative to the use of synthetic pesticides. In the natural world, entomopathogens naturally control the populations of many arthropods. In addition, entomopathogens have been used as traditional biological pest control agents for non-native insect pests and habitat modification has improved their capacity for natural pest management. All of these are widely distributed in the environment and naturally infect various pest species. Many of these entomopathogens can be manufactured in large quantities and prepared for use in the field to control pest populations in a way that is similar to the use of chemical pesticides. The potential for pest control is expanded by the use of entomopathogens.

Keywords: Entomopathogens, microbial control, insect pest management, entomopathogenic bacteria, entomopathogenic fungi, entomopathogenic virus

Introduction
India is the world's second most populous country. The livelihood of majority of the population depend solely on agriculture. Due to the ever-increasing population of India, there is a constant pressure on the farming community to meet the rising food demand of the population. As human civilization and population growth took hold, agricultural activity gradually grew. Since the beginning of cultivation of agricultural crops, insect pests and pathogens have been a major limiting factor causing an estimated 40 percent of agricultural losses globally (FAO, 2021) [44]. A recent estimate of an 18-26% decline in global agricultural output, valued at $470 billion annually, was also made in the year 2020 (Mantzoukas and Eliopoulos 2020) [93]. During the Green Revolution Era, crop output was increased tremendously to fulfil the food needs of low-income countries by heavily utilising inputs like inorganic fertilisers, synthetic pesticides, and genetically modified organisms. The farming community has increased their dependence on agrochemicals as these pesticides have quick effect on the pests and diseases. But with time, intensive farming using new varieties and synthetic agrochemicals, along with pressured anthropogenic activities in the name of modernization, have recently not only restructured the ecosystem but also negatively impacted the natural balance and completely exhausted the abundant native micro fauna in the soil, rendering it almost lifeless. Excessive use of agrochemicals has led to the problem of environmental pollution, loss of ecological diversity, pesticide residue in food, resistance development, etc. In addition to these, agrochemicals effect the soil ecosystem by altering the soil biology, affecting the beneficial soil microbes which in turn results in crop failure and reduced crop yields. These problem faced by using agrochemicals can be overcome by shifting our management practices to biological control. According to Elena and Lenski, 2003 [40], microbes have existed on this planet since time immemorial and are considered fundamental to life on Earth (Nisbet and Sleepju, 2001) [103]. Earlier investigations described only certain specific functions of microbes. But, nowadays, their applications as biological control of pest insect species (Janzen 1977; McCarthy and Williams 1992) [77, 94] has gained huge attention and researchers as well as scientists are continuously exploiting these organisms for management of diverse group of insect pests. In recent years, commercial-scale development of microbial insecticides has accelerated in India.

The Central Insecticides Board and Registration Committee (CIBRC) has registered 970 microbial formulations for their commercial use in India (NBAIR, 2017) [102]. Additionally, there are roughly 200 commercially accessible products made from entomopathogenic fungi. Among them, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, *Beauveria bassiana*, *B. brongniartii* etc. are commonly utilised against arthropods. There are 45 different *Bt* products now on the market commercially used for the control of loopers, bollworms, and other lepidopterans (NBAIR, 2017) [102].

Importance

Synthetic chemical insecticides have very quick effect on insect pests, but they also severe risks to human health and our environment. The high demand for pesticide free food products, vegetables and fruits among the healthy population has made the farmers to shift from chemical farming to natural farming rapidly. As the name suggests, natural farming is a type of farming where there are no costs associated with cultivating and harvesting the plants. This indicates that farmers do not need to buy pesticides and fertilizers to ensure that the crop growth is healthy. The method involves combining cutting-edge technology with conventional farming methods based on biological processes that occur naturally with easily accessible, locally manufactured, naturally biodegradable materials. This method raises the yield, quality, and fertility of the soil. The soil health depends on earthworms which act on the plants and animals debris, breaking them down which adds humus to the soil. Apart from these, this method helps to increase the water retention capacity as well as soil aeration.

According to FAO - "Zero Budget Natural Farming (ZBNF) is a holistic agro-ecological alternative based on modern and traditional science that mitigates the consequences of climate change, reduces input costs and creates sustainable agricultural livelihoods" (Sowmyalatha and Sahana, 2022) [142].

Natural Farming works on four basic Principles:

1. No plant protection
2. No weeding
3. No chemical fertilizers
4. No ploughing

Microbial insecticides provides the appropriate levels of pest control and possess very less risk to the environment emerging as a successful insect management practice. Microbial pesticides are substances which are naturally occurring derived from microbes or their products and their by-products that can effectively bring the pest population below their economic injury levels by employing non- toxic mechanisms. The main active ingredient in these pesticides are the disease causing fungi, bacteria, virus and nematodes. Microbial pesticides are crucial, especially in natural farming, because of their extraordinarily minimal toxicity to non-target organisms.

Entomopathogenic fungi

Entomopathogenic fungi are those disease causing fungi that target and infect their hosts and kills them (Singkaravanit *et al.*, 2010) [138]. There are a wide variety of fungal species from several types that infect insects in a variety of ways, including obligate and facultative infections. Of late, all of the major groups of fungus, roughly 700 species and 90 genera are thought to be insect-infecting fungi (Hajek and St. Leger, 1994: Moorhouse *et al.*, 1992) [64, 99]. While some additional unusual species are found in other classes, majority of the disease causing fungi are found in as comycetes and Zygomycetes. The specificity of entomopathogenic fungi varies greatly among species and within genera. The concept of controlling insect pest using fungi evolved in the 1980s when the early insect pathogenic studies were carried out to develop methods for controlling the silkworm disease (Steinhaus, 1975) [141]. Bassi became the pioneer by proposing his germ theory in 1835 by infecting silkworms with the white muscardine fungus *B. bassiana*. This gave rise to the concept of controlling insect pests by using fungi that can infect insects. Among the commonly researched fungi are *B. bassiana* and *M. anisopliae*, which have a diverse spectrum of hosts that includes hundreds of insect species. Several other hosts, in Coleoptera, Lepidoptera, Diptera, Homoptera, and Hymenoptera orders have also been noted in recent years. Coleopteran species, including more than 70 species of scarab, serve as the main hosts of *M. anisopliae* (Vestergaard *et al.*, 2003) [148]. Several entomopathogens like *Beauveria bassiana*, *Aspergillus* spp., *Fusarium* spp., *Metarhizium anisopliae*, *Cordyceps unilateralis*, *Verticillium lecanii*, *Arthrinium urticae*, *Ashersonia aleyroides*, *Mucor* spp. etc. have been isolated and characterized from the North East of India which is considered as a hub of microflora and fauna, *Nomuraea rileyi* occurring naturally is one of the potent entomopathogenic fungi and acts against majority of the noctuids. This pathogen has been recorded to occur naturally on *Spodoptera litura* in Assam (Dutta *et al.*, 2014) [37]. Bioassays conducted with *N. rileyi* against *Helicoverpa armigera* showed very high virulence and efficacy of this pathogen against the target pest. (Hazarika *et al.*, 2016). Rice leaf hoppers and plant hoppers are infected by several fungal pathogens like *Fusarium* sp., *Hirsutella citriformis*, *B. bassiana*, *Erynia delphacid*, *Entomophthora coronata* and *Metarhizium flavoviride*. Rice hispa, *Dicladispa armigera* which emerged as a major pest in the rice fields of Assam was managed by the application of *B. bassiana* which works as an excellent mycoinsecticide in moist and wet environmental conditions (Hazarika *et al.*, 2005; Puzari *et al.*, 2006) [69, 117]. Baruah *et al.*, 2003 [6] documented several entomopathogenic fungi mainly infecting rice pests. He reported *Aspergillus flavus* attacking rice grasshopoper, *Hieroglyphus banian*, *Conidiobolus thrombosis* infecting rice bug, *Leptocorisa acuta*, *Nomuraea rileyi* attacking all the larval stages of the rice cut worm. Few important pathogenic fungi were characterised and identified infecting cowpea aphid, *Aphis craccivora*. These fungi were *M. anisopliae, B. bassiana, N. rileyi* and *Fusarium* sp. (Pegu *et al.,* 2012; Pegu *et al.,* 2013) [111-112]. Compatability tests of entomopathogenic fungi particularly *B. bassiana* and *M. anisopliae* with commonly used insecticides has shown positive effects on the successful management of various insect pests which holds great promise for their use in integrated pest management. (Puzari *et al.*, 2006; Hazarika and Puzari, 1990; Hazarika and Puzari 1991, 1992; Kakati *et al*, 2018, Dutta *et al.*, 2015) [117, 118, 119, 66, 79, 39]

Mode of action

In insects, the cuticle acts as both the main infection barrier and the main entry point for fungi. Therefore, some kind of physical or enzymatic technique must be used to break through the tough cuticle. In addition to entering through the integument, entomopathogenic fungi can also infect insects through ingestion, wounds, or the trachea (Holder *et al*., 2005) [72]. Depending on how they function, insect pathogenic fungi can kill insects in a variety of ways, such as by starving them or by creating toxins. These insect pathogenic fungi produce several toxins and extracellular enzymes, such as proteases and chitinases. The infection process is started when the spores come into contact with the host's cuticle. During the early phases of infection, fungal conidia adhere to the surface via common hydrophobic and electrostatic principles. Additionally, certain strains secrete mucous substances that help conidia adhere to the cuticle and start dissolving it (Boucias and Pedland, 1991) [16]. Some infectious sessile spores (Capilliconidia), have an adhesive drop at the spore end to make it easier for insects to adhere and ingest them (Glare *et al*., 2010) [58]. There are several common structures and processes involved in the penetration of the host cuticle, while each fungus may employ different methods. In order to promote adherence and germ tube penetration, *M. anisopliae* builds certain structures from conidia (Hajek, 1994) [64]. Under optimum cuticular environment, spore germination may take place. Specialised structures called appressoria are developed by these fungi in order to pierce the cuticle. According to Ferron 1985; Boucias and Pedland 1998 [17], these enlarged cells develop into penetration hyphae (also known as penetration pegs) that uses both mechanical (Pressing) and enzymatic methods to penetrate thevarious cuticular layers and reach the hemolymph. The appressoria infection pegs facilitate the penetration of the germ tube. After piercing the cuticle and insect epidermis via the germ tube, the fungus multiplies inside the insect's body cavity. When the fungus gets within the insect cavity, it starts to generate hyphal bodies. Depending on the exact fungal isolate, these cells can finally infiltrate the entire insect through a tissue-specific sequential process, leading to insect mummification. Additionally, they have the capacity to generate toxic compounds known as secondary metabolites, which can either aid in the invasion of a host by fungus or serve as immunosuppressive agents that weaken the host's defences. (Boucias and Pedland, 1998; Ferron, 1985, Roberts *et al.*, 1992; Trienens and Rohlfs, 2012) [17, 46, 144, 125]. The first multiplication or proliferation may be carried out by protoplasts (Some Entomophthorales), or by blastospore (*M. anisopliae*). Some insect pathogenic fungi produces toxins while some produces antimicrobial metabolites having insecticidal property. Insect pathogenic fungal metabolites are secreted by several fungi such as destruxins from *Metarhizium* species, Species of *Beauveria* produce bevericins. *B. bassiana* also produces isarolides, bassianolides, and beauverolides, all of which are toxic. *Isaria fumosoroseus* and a few plant pathogenic *Fusarium* species have also been shown to contain certain beauvericins (Vestergaard *et al.,* 2003) [148]. Early infection stages do not show any noticeable behavioural symptoms. However, indications such as decreased coordination, eating (such as *M. anisopliae* infected grasshopper and locust), and activity start to show up a few days before death. Behavioural fever, increased eating (Such as in *B. bassiana* infected Colorado potato beetles), positive or negative photo- or geotropism, and changed mating are a few other behavioural reactions (Noma and Strickler, 2000) [105]. Another fungal infection reaction is called "behavioural fever," in which the body temperature of the insect raises by taking a sunbath or resting on warm surfaces, as in the case of locusts and grasshoppers infected with *M. anisopliae* and *B. bassiana* (Blanford and Thomas, 2001) [14].

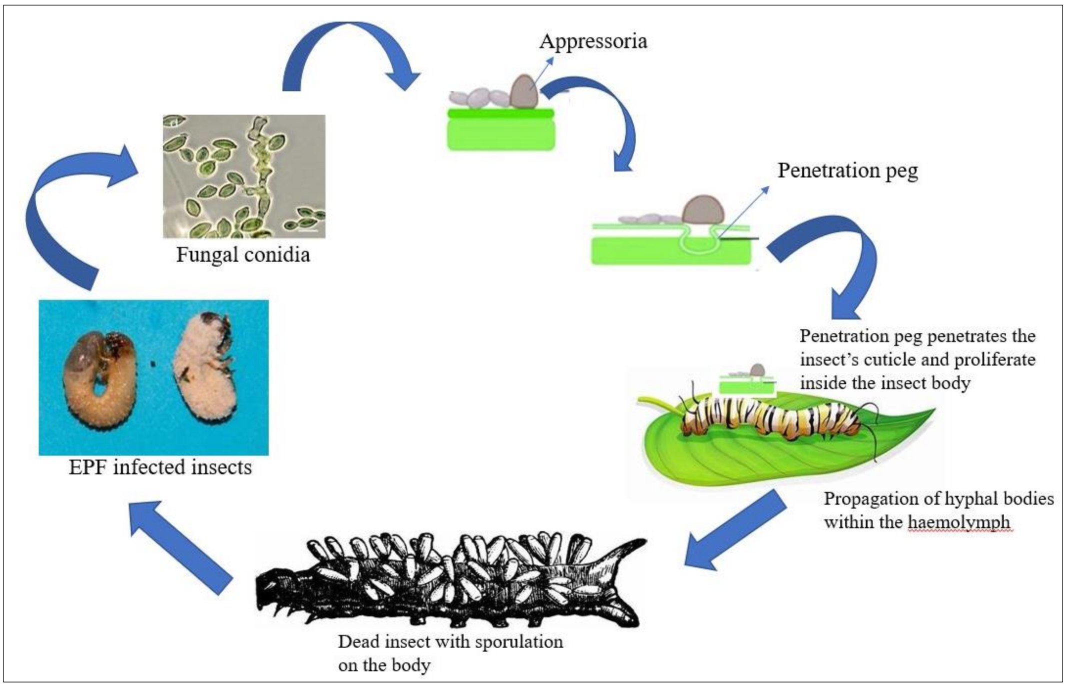

Fig 1: Mode of action of entomopathogenic fungi

Entomopathogenic bacteria

The biological control model took a massive turn with the discovery of the entomopathogenic bacteria. Entomopathogenic bacteria have been employed the most frequently so far. The first event of using entomopathogenic bacteria was the introduction of *Paenibacillus* (Former *Bacillus popilliae*) Dutky against Japanese Beetle *Popillia japonica* Newman (Steinhaus, 1975) [141]. In fact, *Bacillus thuringiensis* (*Bt*) (Firmicutes: Bacillaceae), first identified by Ishiwata in 1901 and later rediscovered by Berliner (Berliner,

1915) [10], has been a very promising microbial agent used widely against various insect pests. The creation of bacterial spray formulations (Wilcox, 1986) [155] and transgenic plants producing bacterial toxins (Fischhoff, 1987 and Hilder, 1987) [48, 70] was specifically spurred by the revelation that *Bt*spore-associated toxins are very pathogenic and may remain in the environment with huge potency. In terms of effectiveness and cost of manufacturing, the *Bacillus thuringiensis* (Bt) strains are competitive with traditional insecticides due to their high toxicity against certain insects.Several commercially accessible strains of *Bt*. belonging to the Cry1 and Cry2 families such as SA-11, SA-12, PB 54, ABTS- 351 and EG2348 expressing a variety of toxins. Have been isolated from insects and soil. Other widely used strains from various subspecies include kurstaki (HD-1) (De Barjac and Lemille, 1970), israelensis (Bti), and tenebrionis, which are employed to control mosquitoes, simulids, and lepidopteran pests in forestry and agriculture (Goldberg and Margalit, 1977) [59]. The capacity of this bacteria to create parasporal structures (crystals) that contain distinct insecticidal endotoxins (Cry proteins) is its main trait. These endotoxins cause injury to the insect stomach epithelium through ingestion through a pore-forming method of action. This species' strains produce parasporal crystals inside the exosporium that are closely related to the endospore and are toxic to mosquitoes as a result. Few other entomopathogenic bacteria showing management against diverse insect pests are *P. popilliae, P. lentimorbus*, *Serratia entomophila*. The endosymbionts of insecticidal nematodes, particularly those belonging to the genera *Xenorhabdus* and *Photorhabdus*, are another type of entomopathogenic bacteria that is of great interest. The first belongs to the genus *Steinernema* of nematodes, whilst the second inhabits the intestines of *Heterorhabditis* species. When nematodes infest susceptible insect hosts, symbiotic bacteria are typically secreted in the hemocoel, where they produce a range of pathogenecity factors that aid in weakening the host's immune system and killing the insect.

Mode of action

Bt acts as a stomach poison. When an insect consumes *Bt*, the gut pH of the insect which is generally alakaline causes the parasporal inclusions (Delta endo toxins) to dissolve and produce protoxins, which are smaller poisonous polypeptides in the size range of 27 to 140 kDa. These polypeptides attach to glycoprotein receptors on the cell membrane of the midgut, which are particular receptors. Pores produced in the cell membrane causes disturbance in the osmotic equilibrium which results in expansion and lysation of the cells, a process known as "Colloid-osmotic lysis"—and causes swelling and lysis. Ion imbalance in the hemolymph caused by the leakage of ions from the midgut causes septicemia. Following these procedures, many toxins will bond together and cause the insect to become paralysed. The crystals act upon the insect gut wall breaking it down which further allows spores and normal gut bacteria to enter the body. The spores and gut bacteria multiplies within the insect body and finally the insect stops feeding and dies. Typical symptoms seen in insects infested by entomopathogenic bacteria are

- Larvae becomes inactive and stops feeding.
- Regurgitation/vomiting or have watery excrement.
- Larger head capsule, larger than the body size.
- Larva becomes soft, droopy and dies within a the body contents on decomposing turn into brownish-black colour.

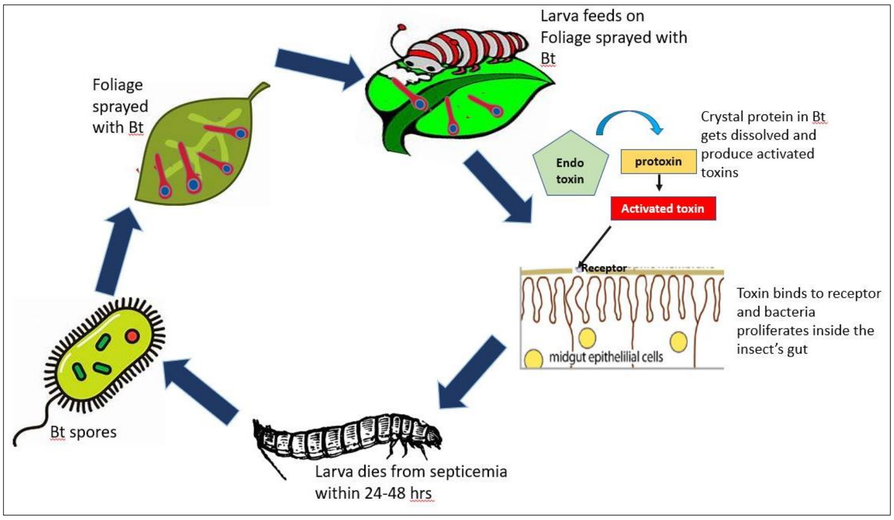

Fig 2: Mode of action of Entomopathogenic bacteria

Entomopathogenic Virus

Insects have been found to be infected by members of many virus families, having either DNA or RNA genomes. Among all the known disease causing virus families, the family *Baculoviridae* has been subjected to the most ecological, biological, molecular, and applied research. Entomopathogenic viruses have been classified into 13 families and a total of 73 known virus families belong to *Baculoviridae* (Murphy *et al.* 1995) [98]. *Baculoviridae* generally consist of a double-stranded circular DNA, and they consist of a protein matrix mostly build up by protein polyhedrin which protects them from any environmental effects (Rohrmann 2019) [127]. The family *Baculoviridae* of insect viruses are confined to Lepidoptera, Hymenoptera, and Diptera larvae and these can be isolated from hundreds of host species. Normally baculo viruses may be of two types depending on inclusion bodies- occluded and non- occluded virus particles. Occluded viruses, consist of mature virion particles (virions) which remain embedded within a protein matrix. The other category of viruses known as the non-occluded viruses typically contain virions that occur freely or sporadically and lack occlusion body protein found interspersed among the virions. (Federici, 1999) [45]. These two different types of baculoviruses can be divided into two groups of major viruses: The nucleopolyhedrosis viruses (NPVs) and the *granulovirus* (GVs) (Murphy *et al*., 1995) [98]. NPVs typically only have one host species or genus as their range, with the exception of the NPVs of *Autographa californica* (Speyer*), Anagrapha falcifera* (Kirby), and *Mamestra brassicae* (Linnaeus), which have limited host ranges. The genus Granuloses virus (GV) has granule-shaped viral occlusions (capsules) that occasionally contain two or more virions and one nucleocapsid per envelope. The virus dose is measured in terms of larval equivalents (LE), and one LE equals 2 X10^9 POB. Three fully grown virus-infected larvae can produce one LE. Since the 16th century, entomopathogenic viruses have been linked to many diseases in insects. A condition known as jaundice grasserie now known to be caused by the nucleopolyhedrosis virus was discovered in silkworm (*Bombyx mori*) rearing establishments. Maestri and Cornalia, two Italian researchers, provided the initial description of the occlusion bodies (OBs) of silkworms in 1856. Steinhaus and his colleagues (1950-1970) used a nucleopolyhedrovirus (NPV) to control the lucerne caterpillar (*Colias eurytheme*) in order to test baculo viruses as biological control agents in the field.

Mode of action

Typically for a viral infection to occur, it has to enter through the mouth of the insect host. Initially, the susceptible host insect feeds on virus infected plants. The protein matrix of the OBs breaks down when the insect consumes them, thereby the infectious particles (virions or ODV/PDV) are released into the alkaline midgut (pH 8.0) of the insect. The alkaline pH of the midgut region is a major factor determining the ease of dissolving ability of the OBs. The ODVs may also be released by breaking down polyhedrine, digestive proteinases in the stomach of the host insect. According to Sajjadian and Hosseininaveh (2015) [131] and Saxena *et al*. (2018) [132], the midgut contains a layer of extracellular matrix that serves as a barrier for the gut epithelial cells against abrasion and harmful microbes. The released ODVs must transcend this layer, known as the peritrophic membrane (PM), which mainly comprises of chitin, mucins, glycoproteins, and proteoglycans in addition to other minor components. Baculoviruses have developed strategies to get beyond the PM barrier as a result of their evolution and can degrade and increase permeability of the PM. The ODV/PDVs are then uncoated in the following phase before entering the nuclear pores. With cellular activity, these uncoated ODV/PDVs go into the nucleus. Due to the phosphorylation of the DNA-binding protein, the nucleocapsid's DNA becomes uncoated and unwound in the nucleus. The viral DNA-polymerase enzyme then causes expression and replication of the viral DNA. The newly developed nucleocapsids sprout through the nucleus and acquire a nuclear membrane encasing. The virus-coded glycoprotein spikes are shed in the cytoplasm, and a second envelope made up of cytoplasmic membrane is obtained by budding through the midgut basal membrane. These virus variants are referred to as budded viruses (BVs). After their release into the haemolymph, BVs multiply again in the cells of tissues that are vulnerable. PDVs are produced when the infection is already advanced. The OBs are eventually formed when the occlusion body protein (polyhedrin) crystallizes and is discharged into the environment. Death of the target insect occurs as a result of the disruption of the chitinous exoskeleton caused by viral proteases and chitinases. During the secondary infection of haemoglobin and the tracheal matrix, BVs infect majority of other tissues of the host. The virus enters several additional tissues, including the epidermis and fat body, as the infection proceeds along the tracheal epithelium from the primary point of infection.During the second cycle of virus replication, the infected cells not only produce BV but also encapsulate virus particles into polyhedra in the nucleus. The whole body of the insect becomes full of viral particles. (Cunningham, 1995; Flexner and Belnavis, 2000) [26, 49]. Several enzymes like ecdysteroid (UDP)-glucosyltransferase (EGT), a baculovirus-encoded enzyme activate at this time and act upon the hormones that larvae use to moult, extends their feeding time and inhibits ecdysis (Wang & Hu 2019) [151]. The infected insect produces the typical tree-top disease symptom, which is characterized by the hyperactivity and larval death at elevated positions on plant foliage. As the disease progresses, the insect liquefies and releases polyhedra, which when eaten by other insects causes the virus to spread quickly. At the time of its death, a single caterpillar can have over 109 OBs left over from a dose of 1000. The infected larvae exhibit negative geotropism before succumbing to the virus infection, assisting in the widespread transmission of the virus. (Cunningham, 1995; Flexner and Belnavis, 2000) [26, 49].

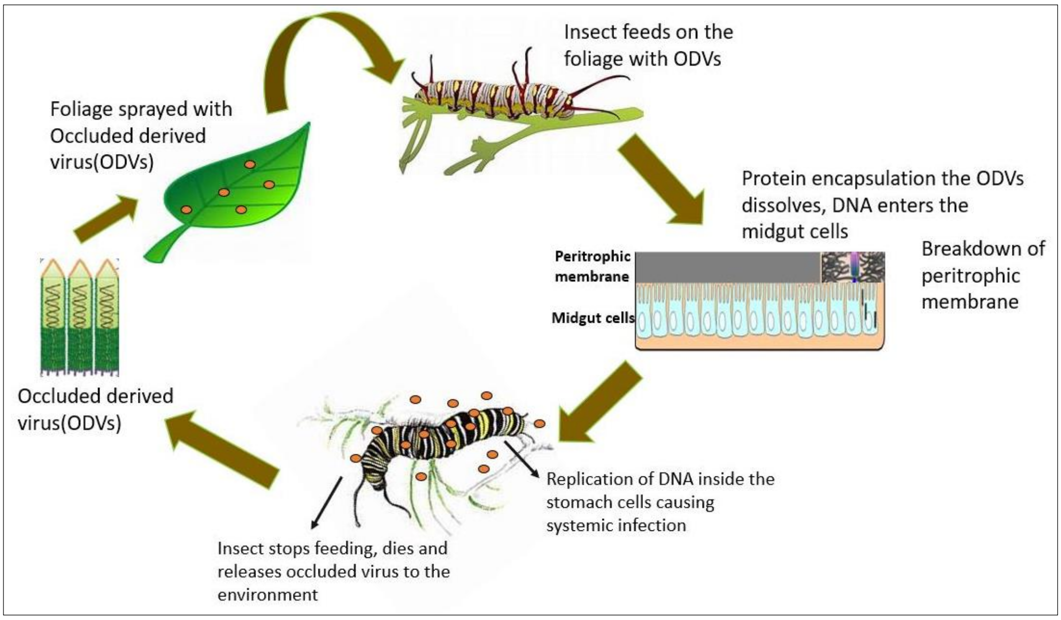

Fig 3: Mode of action of virus on insect

Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) are obligatory parasites of insects that are softbodied, non-segmented, and measure roughly 0.5 mm in length (Koppenhöfer, 2007) [86]. Steinemernatidae and Heterorhabditidae are two prominent families of entomopathogenic nematodes that are employed to biologically manage insects. (Klein, 1990; Koppenhöfer 2007) [85, 86]. These infectious juveniles of free-living, non-feeding nematodes have traits in common with both microbial pathogens and insect parasitoids or predators. Similar to parasitoids and predators, they have chemoreceptors, are mobile, and are extremely virulent, killing their hosts quickly. They are simple to cultivate *in vitro* and have a high capacity for reproduction. The Heterorhabditidae have 3 species (Poinar, 1990) [112], the *Steinernematidae* have 10 species (de Doucet, 1990 and Poinar, 1990) [112], and both entomopathogenic nematode families are monogeneric. In laboratory where ideal environmental conditions prevails and no barrier in the host contact, *Steinemematid* and *Heterorhabditidae* nematodes attack a much wider variety of insects than other biological control agents do (Gaugler, 1981, 1988) [56]. However, they are unable to swim in pools or streams, thus they quickly leave the host feeding area. Infectious juveniles are equally very harmful to mosquito and black fly larvae in the lab (Finney, 1981; Gaugler, 1983 and Gaugler, 1981) [47, 53, 55]. Nematode survival is improved in soil because it protects them from harsh environmental conditions. A tropical *Heterorhabditidae* isolate cannot survive at 10 degrees Celsius (Molyneux, 1985) [96], but they can endure slow desiccation at high relative humidity levels and low temperatures (Molyneux, 1985; Schmidt, 1979) [96, 133]. EPNs are soil inhabitants that develop a symbioticand cooperative relationship with bacteria to biologically control insect pests. All *Heterorhabditis* nematode species form symbiotic relationship with *Photorhabdus* bacteria and all *Steinernema* species form symbiotic association with *Xenorhabdus* bacteria (Boemare *et al.*, 1993) [15]. This association enhances nematode pathogenicity and reproduction. Nematodes operate as carriers of bacteria when they are placed into a host, where they can grow, and the bacteria then create the perfect environment for nematode survival and reproduction inside the insect carcass. EPNs can easily be made in large quantities and applied with common spray equipment. They are environment-friendly and adaptable to a variety of situations.

Mode of action

EPNs are obligate pathogens. The only stage that can endure outside of a host is the non-feeding third stage infective juvenile (IJ), also referred to as a dauer juvenile (IJ). Bacterial symbionts from the genera *Photorhabdus* and *Xenorhabdus* live in the digestive tracts of IJs from the families *Steinernematidae* and *Heterorhabditidae*, respectively. IJs immediately after locating their host enter the host hemocoel invading through natural holes like the mouth, spiracles, anus, or thin portions of the host cuticle. In order to suppress the host immune response, the IJs work with bacteria and nematodes after emerging from their developmental arrest. They then release the symbionts. The bacteria grow and produce substances that quickly kill the host while preventing other microorganisms from colonising the corpse. After starting to develop, the nematode feeds on host tissues and microorganisms while going through one to three generations. The host dies within 24 to 48 hours due to septicemia. Due to continuous multiplication of the EPNs, new generation of IJs develops in search of a new host. The nematode relies on the bacterium (symbiont) to infect its host and for the production of antibiotics that stifle secondary microorganism competition, disintegrate the host tissues into usable nutrients, and serve as a food source. The nematode is required for the bacterium to enter into the hemocoel, defend itself from the environment and inhibition of the antibacterial proteins of the host. (Prasad, 2019) [115].

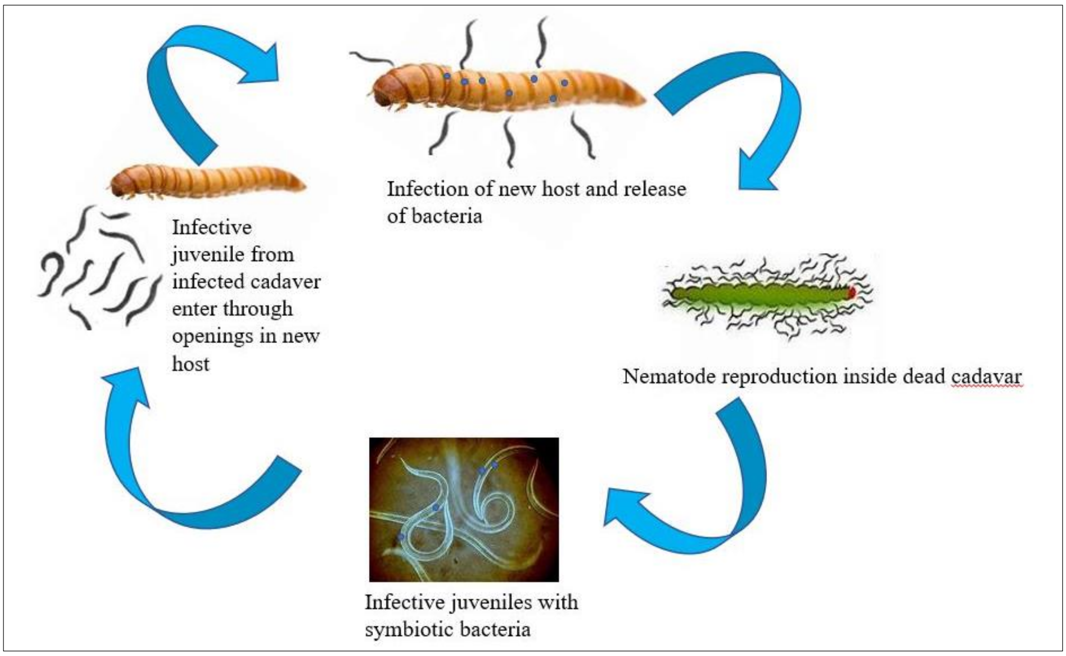

Fig 4: Mode of action of EPN

Mode of action of different entomopathogenic agents and their target pests
(Grewal and Peters 2005; Okano *et al.*, 2006, Kalha *et al.*, 2014; CIBRC, 2017 and Dara, 2017) [61, 106, 81, 20, 28].

Entomopathogenic agent	**Mode of Action**	**Target pests**
Bacteria	Acts as stomach poison Ingestion of entomopathogenic bacteria, toxins are produced which disrupts midgut epithelium, insect stops feeding, dies due to septicemia	*Manduca sexta*, *Tuta absoluta*, *Helicoverpa armigera*, *Leptinotarsa decemlineata*, *Pectinophora gossypiella*
Fungi	Acts as contact poison Conidia produces penetration peg to penetrate the insect' cuticle, multiply inside the insect haemocoel, loose appetite, coordination and less active, cadavars hard with fungal sporulation on the body.	*Holotrichia* sp., thrips, mites, mosquito larvae and aphids, whiteflies, *Callosobruchus* sp., *Microtermes obesus*
Nematodes	Enters through natural body openings or directly breaking through the insect cuticle. Bacteria released inside the host body inside which they continue to multiply	*H. armigera*, *Conogethes punctiferalis*, *Athalia proxima*, *Agrotis ipsilon*, *Microtermes obesi*
Viruses	Acts as stomach poison EPV disrupt the peritrophic membrane and reproduce in the insect haemocoel killing the insect.	*Autographa californica*, *Bombyx mori*, *Spodoptera exigua*, *Trichoplusia ni*, *Spodoptera frugiperda*, *Helicoverpa armigera*

Commonly available formulations and their effectiveness against target pest (Prasad, 2019) [115]

Agent	**Microorganism**	**Trade name of microbial insecticides**	**Effective against**
Bacteria	*Bacillus thuringiensis* var. *kurstaki*	ABTECBtk, Agni, Bioasp, Bio-Dart (BTK), Biolep, Biobit, B.T. Killer, Caterpilin, Cezar, CID, Deflin WG, Dipel, Dipole, Gold Btk, Halt, Jas BT, Kavach Bt, Krishi Bio Prasar, Lipel SP, Mahastra, Minchu, Neelstaki, R.B. Bt, VBT	Lepidopteran pests
	Bacillus thuringiensis var *galleriae*	Spicturin	Lepidoptera
	Bacillus thuringiensis var. *tenebrionis*	Di Terra, M - one, M-Trak, Novovdor, Trident	Coeloptera
	Bacillus thuringiensis var. *israelensis*	Acrobe, Skeetal, Teknar, Vectobac	Diptera
	Bacillus sphaericus	Larvicide, Spherimos, Vectobac and Spherifix	Mosquitoes and black flies
	Bacillus popilliae	Doom	Japanese beetle
	Bacillus thuringiensis var. *aizawai*	Certan, Agree and Xentari	Caterpillars
	Bacillus thuringiensis var. *sandiego*	M - Trak, Foil and Novodor	Colorado potato beetle
Virus	Nuclear polyhedrosis virus (Ha- NPV)	Biokill-H, BioVirus-H, Heli-Cide®, Heliokill®, Helimar-NPV, Helivax, Jas Viro-H, Helicop, Heligard, Somstar™-Ha, VBL-Utkranti	*Helicoverpa armigera*
	Nuclear polyhedrosis virus (Sl-NPV)	BioVirus-S, Jas Viro-S, Spodo-Cide, Spodopterin, Somsta-SL	*Spodoptera litura*
	Nuclear polyhedrosis virus	*Aa* - NPV	*Amsacta albistriga*
	Nuclear polyhedrosis virus	VPN 80	*Autographa californica*
	Nuclear polyhedrosis virus	Spodopterin	*Spodoptera littoralis*
	Nuclear polyhedrosis virus	SPOD - X	*Spodoptera exigua*
	Nuclear polyhedrosis virus	Elcar, Helicide, Helimar, Biovirus-H, Biokill-H, Helicop, Helistop	*Helicoverpa armigera*
	Nuclear polyhedrosis virus	Virin, Gypchek	*Lymantria dispar*
	Nuclear polyhedrosis for Gypsy moth	Gypchek virus	Gypsy moth caterpillars
	Tussock moth NPV TM	Biocontrol-1	Tussock moth caterpillars
	Pine sawfly NPV	Neochek-S	Larvae of pine sawfly
	Granulosis virus for Codling moth (GV)	Madex, Carpovirusine, CYD-X	Codling moth caterpillars
	Granulosis virus	Carpo virusine, CYD-X, Granusal, Madex	*Cydia pomonella*
	Granulosis virus	Agrovir	*Agrotis segetum*
	Granulosis virus	PTM baculovirus	*Phthorimaea operculella*
Fungi	*Beauveria bassiana*	Mycotrol, Naturalis, Conidia, Ostrinol, Boverin, Myco-Jaal, Biosoft, ATEC Beauveria Larvo-Guard, Biorin, Biolarvex, Biogrubex Biowonder,	Effective against a variety of insects such as rickets, white grubs, fire ants, flea beetles, plant bugs, grasshoppers, thrips, mites, mosquito larvae and aphids, fungus gnats, whiteflies and to stored pest
	Beauveria brongniartii	Betel, Engerlingspilz, Melocont	Scarab beetle larvae
	Trichoderma viride	EcosomTV, Tricon, Trieco	Effective against Rot disease
	Trichoderma harzianum	Rootshield, BioTrek, Supresivit	Effective against a variety of soil inhabiting micro organisms
	Metarrhizium anisopliae	BIO 1020, Bio-Blast, ABTEC, Verticillium Meta-Guard, Biomet, Biomagic, Meta Biomet, Sun Agro Meta, Bio-Magic	Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, grubs
	Metarrhizium flavoviride	Green muscle BioGreen	Locusts, Grasshoppers Red headed cock chafer

	Verticillum lecanii	Vertalec, Mycotal, Verisoft, ABTEC, Verticillium, Vert-Guard, Bioline, Biosappex, Versitile, Ecocil, Phalada 107 V, Biovert Rich, ROM Verlac, ROM Gurbkill, Sun Agro Verti Bio-Catch	Whitefly, coffee green bug, homopteran pests
	L. lecanii and *Hirsutella thompsonii*	ROM-Biomite	Mites especially eriophids in coconut
	Paecilomyces fumosoroseus	PFRE - 97, PreFeral Paecilomite, Mycomite, Priority	Whiteflies and thrips
	Purpureocillium lilacinum	ABTEC Paecilomyces, Bionemat, Bioniconema, Bio-Nematon, Ecogreen, Krishi Bio Vikalp, Paceilo®, Mysis, ROM-Pelicide, Nematofree, Nemato Guard, Nematolin-LF, Niyanthran, Nemator, Panther-PL, Paecilocon, Platinum, Yorker	Plant pathogenic nematodes
Nematode	*Steinernema carpocapsae*	BioVectorEcomask Exhibit, Vector TL, X-Gnat, NemaStar, Green commandos, Guardian Helix, Ortho, BioSafe	Lepidopterous larvae
	Steinernema thermophilum	Pusa Nemagel	Lepidopterans and termites
	Steinernema feltiae	Entonem, Magnet, Nemasys, Scanmask, Stealth, Gnat Not, NemaPlus	Dipterous insects
	Steinernema capterisci	Proactant	Adult mole crickets
	Heterorhabditis bacteriophora	GrubStake HB, Heteromask, J-3 Max Hb, NemaGreen, Nema Top, Larvanem, Lawn Patrol, Otinem, Soil commandos	Soil-dwelling insects
	Heterorhabditis indica	Soldier, Nema Power, BCS-Grub Terminator, Grubcure, Calterm, Aarmour	lepidopterans and termites
	H. downesi	Nema Trident-CT	Black vine weevil

Microbial semiochemicals

The Microbial volatile organic compounds (MVOCs) are substances produced by fungi, molds and bacteria during primary and secondary metabolism as side-products. These compounds have been used since a long time back for detection of contaminants in food and as pollution indicators (Pasanen *et al.*, 1998; Wessen and Schoeps 1996) [107, 154]. In addition to this, some MVOCs can imitate plant hormones, attract or repel insects, restrict the growth of microbes that compete with the insects they are linked with, stimulate oviposition, or even cause plants to become resistant (Davis *et al.*, 2011; Ryu *et al.*, 2003, 2004) [29, 129, 130]. Although few researchers have shown MVOCs functioning as pheromonal signals, Wertheim *et al.* (2005) [153] studied that aggregation behaviour shown by anumber of are significantly related with specific microbial ecosystems. Many insects also use MVOCs specifically to find food sources including nectar, fruit, rotting tree or animal tissues, weak creatures, or material resources (DeVries 1987) [133]. Guaiacol, a part of the locust pheromone made from the faeces of the insect, encourages the gathering of locusts for mating. Bacteria in the gut of locusts, including *Pantoea agglomerans*, are the principal producers of guaiacol. By creating antimicrobial phenolic chemicals, gut bacteria assist the locust in its defence against microbial infections (Dillon *et al.*, 2002) [34]. Millipedes and hemipterans may also synthesise guaiacol (Duffey *et al.*, 1977) [36]. Hoyt *et al.* (1971) [73] discovered an unidentified bacteria that produces phenol and attracts male beetles in the collatorial glands of the female grass grub beetle, *Costelytraz ealandica* White (Coleoptera: Scarabaeidae). A number of gut symbionts in bark beetles (Coleoptera: Curculionidae) can produce substances that their host uses as pheromones. The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytinae), is connected with two yeasts, *Hansenula capsulata* and *Pichia pini*, which are capable of producing verbenone, an anti-aggregation pheromone that prevents mass attacks on particular host trees (Hunt and Borden, 1990) [76]. The mutualistic fungus *Grossmania clavigera* produces aggregation pheromones, which *D. ponderosae* females need in order to synthesise aggregation pheromones (Bentz and Six, 2006) [9]. Dipterans can change how they lay their eggs in reaction to MVOCs. For instance, when exposed to MVOCs produced by the bacterium *Enterobacter agglomerans*, *Rhagoletis pomonella* (Diptera: Tephritidae), an apple maggot fly, oviposits more frequently on fruit (Lauzon *et al*., 1998) [91]. In order to direct egg laying in favourable habitats, gravid *Aedes aegypti* (Diptera: Culicidae) mosquitos utilise volatiles in the form of carboxylic acids and methyl esters released by alpha and gamma proteobacteria (Ponnusamy *et al.*, 2008, 2010) [113-114]. MVOCs are also known to prevent mosquito oviposition. MVOC complexes found in a natural larval habitat which are released by a mixture of various bacterial species (*Enterobacter*, *Klebsiella*, *Pseudomonas*, *Stenotrophomonas*, *Pantoea*, *Acinetobacter*, *Aeromonas*, and *Bacillus*) were crucial to evoke oviposition in gravid *A. gambiae* (Huang *et al.*, 2004) [74]. Vespid wasps (Hymenoptera: Vespidae) are semiochemically active in response to volatile emissions from an epiphytic bacterium (Davis *et al*. 2012) [30]. Among the commonly utilised entomopathogenic fungi in microbial control, *Metarhizium anisopliae* and *Beauveria bassiana* produce a variety of volatile secondary metabolites. Volatiles released by entomopathogenic *B. bassiana* repell termites. Other insects have also been observed to recognise and avoid *B. bassiana* volatiles, including the seven-spot ladybirds *Coccinella septempunctata* L.A range of insect repellents made by microbes may help with pest management. Styrene and 3-methylanisole, two volatiles generated by the fungus *Penicillium expansum*, were discovered to have a negative impact on the attraction of the pine weevil, *Hylobius abieties* to Scots pine twigs (Azeem *et al.*, 2013) [5]. The same is true for endophytic fungi, which can create volatile insecticides and antibacterial substances. Naphthalene, which is powerfully insecticidal, is produced by the endophyte *Muscodor vitigenus* (Daisy *et al.* 2002) [27]. The leaf beetles, *Oreina* spp. (Coleoptera: Chrysomelidae), have significantly reduced weight, increased development rate, and reduced growth rate when growing in the presence of the phytopathogenic rust *Uromyces cacaliae* (Roder *et al.* 2007) [126], which may signify decreased plant nutritional quality or the production of toxic fungal metabolites. According to Hatcher *et al.* (1994) [65], Uromyces on *Rumex* spp. similarly delays development and reduces pupal weight in *Gastrophysa viridula*. Ants, wasps, and other foragers that would normally feed on cadavers were deterred by MVOCs generated by a bacteria-nematode complex that colonised insect cadavers (Gulcu *et al*., 2012) [62].

Some VOCs released by entomopathogens and their target pest

Volatile organic compounds	Source	Target pest	References
6-pentyl- α-pyrone	*Trichoderma asperellum*	*Tetranychus urticae*	Kottb, 2017 [87]
Methyl salicyclate	*T. harzianum*	*Macrosiphum euphorbiae*	Coppola *et al.* 2019 [22]
(*Z*)-3-hexen-1-ol	*T. harzianum*	*Aphididae* species	Contreras-Cornejo *et al.* 2020 [23]
Not determined	*T. harzianum*	*Periplaneta Americana, Amrasca bigutulla bigutulla, Aphis gossypii*	Abdul-Wahid and Elbanna, 2012 [1], Nawaz *et al.* 2020 [100]
Not determined	*T. harzianum*	*Aedes aegypti* *Tenebrio molitor* *Tribolium castaneum* *Pectinophora gossypiella*	Sundaravadivelan and Padmanabhan, 2014 [143] Shakeri and Foster, 2007 [136] Rahim and Iqbal, 2019 [121] El-Massry *et al.* 2016 [41]
1-octen-3-ol and 6-pentyl- α-pyrone	*T. atroviride*	*Spodoptera frugiperda*	Contreras-Cornejo *et al*., 2018a [24]
6-pentyl- α-pyrone	*T. atroviride*	*Spodoptera frugiperda*	Contreras-Cornejo *et al*., 2018b [25]
methyl salicylate, (Z)-3-hexen-1-ol, and β-caryophyllene	*T. longibrachiatum*	*Macrosiphum euphorbiae*	Battaglia *et al.* 2013 [18]
Verbenone	*Hansenula capsulata*	*Dendroctonus ponderosae*	Hunt and Borden 1990 [76]
Dimethyl trisulfide; 2-phenylethanol	*Phoma* spp.; *Fusarium* spp. *Rhizophus* spp.	*Musca domestica* (Diptera: Muscidae)	Lam *et al.* 2010 [90]
1-octen-2-one; (R, S)-3-octanol; 3-octanone;	*Penicillium corymbiferum*	*Oryzaephilus surinamensis*; *Oryzae Philus mercator*	Dolinski and Loschiavo 1973 [35]
Dialkyl sulphides	*Klebsiella oxytoca, Bacillus* spp.	*Diadromus pulchellus*	Thibout *et al.* 1993 [145]; Thibout *et al.* 1995 [146]
cis- and trans-octa-1,5-dien-3-ol	*Trichothecium roseum*	*Tyrophagus putrescentiae*	VanHaelen *et al.* 1980 [147]
Not determined	*Pythium aphanidermatum*	*Bradysia impatiens*	Braun *et al.* 2012 [18]
Methyl linoleate and linoleic acid	*Trichoderma* sp.	*Myzus persicae*	Kaushik *et al.* 2020 [83]
Fumonisin B1 and beauvericin	*Fusarium proliferatum, Paecilomyces fumosoroseus, Trichoderma harzianum*	*Schizaphis graminum*	Ganassi *et al.* 2001 [50]
Citrantifidiene and citrantifidiol, bisorbicillinoids	*T. citrinoviride*	*Schizaphis graminum*	Evidente *et al.* 2008 [42], Evidente *et al.* 2009 [43]

Successful examples of implementation of microbial pesticides in IPM

In Indian agriculture, a number of success stories involving the use of microbial insecticides have been documented (Kalra and Khanuja 2007) [80]. Beauveria infects mango mealy bugscoffee pod borer and mango hoppers. NPV infects gram pod borer *Helicoverpa armigera*. *Bacillus thuringiensis* infects diamond back moths and *Helicoverpa* on cotton, pigeon pea, and tomato. In Ludhiana, Punjab, ICAR-IIOR, Hyderabad, India have synthesized a suspension concentrate formulation of *B. bassiana* and a commercial formulation (Daman 1% WP) which were found to enhance sunflower seed yield significantly a along with early season *Helicoverpa armigera* inhibition. Numerous studies conducted in India also indicate that *B. bassiana* products can be effectively used against hemipteran pests. Foliar application of HaBa (Hyderabad strain) brought about 85% mortality of Cowpea aphid, *Aphis craccivora*, one week after treatment on fenugreek in greenhouse conditions (Selvaraj and Kaushik, 2014) [135]. A number of field research utilizing *B. bassiana* to combat aphids and other pests were also reported by the NBAIR. A *B. bassiana* isolate Bb-5a oil-based experimental formulation reduced the cabbage aphid, *Brevicoryne brassicae*, by 76% and enhanced yield by over 30% when compared to control plots (NBAIR, 2016) [101]. In Andhra Pradesh, sugarcane damage by white grub, *Holotrichia consanguinea*, was decreased by 88% over two years by the application of talc-based *B. bassiana* formulations to the soil (51013 spores/ha) that were enhanced with farmyard manure (FYM) (Visalakshi *et al*., 2015) [149]. Another species showing promising and effective results against *Holotrichia serrata*is the *B. brongniartii* which survives for a long time in field conditions (Srikanth *et al*., 2010; Bhattacharyya and Pujari, 2014) [140, 13]. Field testing in Andhra Pradesh utilising talc-based formulation of *M. anisopliae* (5 1013 spores/ha) supplemented with FYM reduced grub populations and damage to sugarcane by 93% over a two-year period.

Liquid formulations of *Btk* have not only successfully controlled *H. armigera* but also used to manage other noctuid pests in pigeon pea and sunflower (Kumar and Kaur, 2017) [89]. Microbial formulations available commercially like Delfin, Biobit, Dipel and Halt have brought the populations of shoot borer, *Chilo infuscatellus* in sugarcane in Tamil Nadu below economic limits (Kesavan *et al*., 2003) [84]. A study done in Andhra Pradesh, *Btk* also caused cent percent control of the citrus leafminer, *Phyllocnistis citrella*, for up to 10 days after spraying. (Rao *et al*., 2015) [124]. The rice hispa, *Dicladispa armigera* which was a major pest of rice in Assam was found to be successfully controlled by the use of entomopathogenic *fungus B. bassiana*, *A. flavus*, *F. heterosporum*, *Penicillium cyclopium*, *Geotrichum* sp. and *Mucor* sp. A study conducted in the vegetable growing areas of Majuli recorded five entomopathogenic fungi *M. anisopliae*, *B. bassiana*, *N. rileyi* and *Fusarium* sp. were found to be effective against seven different insect species from members of the orders Lepidoptera, Isoptera, Orthoptera, Homoptera and Hemiptera (Pegu *et al*., 2012 and Pegu *et al*., 2013) [111-112]. The workers of the termite *Microtermes obesus*, which is an important agricultural insect pest were successfully controlled by the use of *Metarhizium anisopliae* and *B. bassiana* (Singha *et al*., 2010) [139]. During a study in Assam Agricultural University, Jorhat, infected cadavars of potato pest, *Agrotis ipsilon* were found. The isolated fungus recovered from this cadavars were that of *Metarhizium anisopliae* showing that *M. anisopliae* can successfully control *Agrotis ipsilon* (Bhattacharrya *et al*., 2008) [13]. Four disease causing fungi associated successful pathogenic relationship with six species of insect belonging to orders Hemiptera, Orthoptera and Lepidoptera in the rice growing places of Assam (Baruah *et al.*, 2003) [6]. Several institutions like IARI in New Delhi, NBAIR in Bengaluru, the Sugarcane Breeding Institute in Coimbatore etc.conducted research on EPN and developed a new EPN formulation having a shelf life of 12 months. The commercial manufacturing of this product was granted a licence in 2012 and works brilliantly against numerous states to control white grubs and other soil pests in areca nut, banana, brinjal, cardamom, groundnut, and sugarcane (NBAIR, 2017c) [102]. Another such research was conducted by IARI alone and created a *S. thermophilum*gel formulation (Pusa Nemagel) having a long shelf life and acts against white grubs, termites, and many lepidopteran pests (Ganguly *et al.*, 2008; Ganguly and Rathour, 2014) [51, 52]. This EPN is first of its kind to work against lepidopteran eggs. (Kaliya *et al*., 2014) [82].

Advantages and Disadvantages

Merits

1. In general, microbial pesticides are not pathogenic and are not hazardous to non-target organisms, natural enemies and human.
2. They are generally specific, have a narrow toxicological spectrum, do not directly affect non target organisms (parasites, pests, pollinators, and predators) in the treated fields.
3. Microbial pesticide residues are safe for use during harvest and do not possess any harmful effect on people or other animals.
4. Microbial pesticides enhance the plant growth by promoting beneficial soil organisms and earthworms and boosting production.
5. No secondary pest outbreak, no pesticide residue are encountered with application of microbial pesticide.

Demerits

1. The number of applications required to bring effective management are more than chemical pesticide.
2. The effectiveness of microbial insecticides are affected by heat, desiccation (drying out), or exposure to ultraviolet radiation. For some products, proper timing and implementation methods are particularly essential.
3. Certain microbial pesticides are need to be synthesized into special formulation and their storage is also different from the other products.
4. The pest specificity nature of these products make their market restricted to only specific pests. Apart from these, few products are not available easily and are costly compared to other products.
5. To effectively control the target pests, a protracted deadly infection phase is necessary in case of microbial pesticides.
6. Some pathogens are difficult to mass produce.

Registration of a microbial pesticides

A microbial pesticide must go through numerous stages of development. First, the microorganism must be isolated in pure culture or enhanced. Next, it must be identified, characterized, and subjected to efficacy bioassays, which can be conducted *in vitro*, ex vivo, or *in vivo* depending on the target pathogen or pest organism. Finally, pilot tests under actual application conditions must be conducted. e. Each nation has its own set of regulations that affect registration. The Office of Pesticide Programmes in the EPA in the US is in charge of approving the registration of biopesticides. The register is managed by the Directorate of Consumer Health Protection of the European Union and is governed by Directive 91/414/CEE, which was explicitly revised for biopesticides by Directive 2001/36/EC. According to the Insecticide Act (1968) in India, any microbiological organism produced or sold for the control of pests and diseases must be registered with the Central Insecticides Board (CIB) of the Ministry of Agriculture. This includes biopesticides products gain from expedited registration processing, streamlined registration procedures, and the acceptance of generic registration information for new products containing strains already registered in order to promote registration. Manufacturers can register their products under either 9(3) B (temporary registration) or 9(3) (regular registration).By allowing commercial producers of microbial pesticides that have been determined to be generally safe to get provisional registration and continue market development while the product is pending full registration, this strategy decreases the financial obstacles to product development. Comparatively speaking, less information is needed for registration under 9(3) B than under 9(3). As an illustration, 9(3) B calls for efficacy data on certain crops from two locations over two seasons, whereas 9(3) calls for the same data from three locations. Information on the product's characteristics, effectiveness, safety, toxicity, and labelling must be included in the registration application. The product must adhere to the CIB's established quality standards with regard to content, virulence of the organism as determined by LC50, moisture content, shelf life, and secondary non-pathogenic microbial load. For the collection of these datas, there are established protocols for evaluating these quality indicators (Rabindra, 2005). For registration of a microbial pest control agent, few important information regarding the identity (MCPA, Applicant, manufacturer, scientific information of microbes, taxonomy, composition and concentration of A.I. quality control and storage stability data), physical and chemical properties (density, viscosity, surface tension, explosivity, corrosiveness & oxidizing properties), *biological properties* (origin, isolation method, type of strain, physiolt, life cycle, mode of action etc.), *analytical methods* (Production process, quality control and post registration monitoring, determination of shelf-life, storage stability, quantifying residues of viable and non-viable compounds (toxins), human health - toxicology (Acute oral infectivity, pathogenicity, cell culture study for viruses, specific bacteria with intracellular replication) Metabolism and residues data (Rationale, claiming that residues are non-hazardous to mammals, environmental fate (behaviour in the environment, persistency and mobility in soil) and ecotoxicology (toxic effects on non-target organisms like fish, bees, soil microbes, beneficial, earthworms etc.) (Dutta, 2022) [39].

Conclusion and future prospects

The use and demand of biological originated pesticides is increasing rapidly all over the globe. In natural farming systems, microbe-based biopesticides will not only increase food production but will also increase the soil fertility and enrich the soil. Microbe-based biopesticides are effective and provide advantages for both individual as well as ecosystem safety, flexible and low application constraints, and greater prospects for residue and strength leadership. In order to control insect pests and diseases that limit agricultural production, microbes like bacteria, fungi, viruses and nematodes are often employed to generate microbe-based biopesticides since they attack certain targets and provide no or very little threat to people, animals, and the environment. The usage of plant- and microbe-based biopesticide has led to safer and healthier farming practices as opposed to other chemical-based pesticides. By the adoption and use of microbial pesticides, the environmental as well as human health will improve greatly. (Mishra *et al*., 2015) [95]. Currently, challenges with the status quo are frequently what motivates the adoption of microbial insecticides. However, if a commitment is made to the development of high quality products, a more responsive registration process, government support for research and extension, and successful collaboration between researchers, industry, and farming community, microbial pesticides will be more widely accepted in sustainable crop production practises. The commercial development and implementation of microbial pesticides in India has not yet reached a broad level, despite the fact that they have a number of advantages over conventional pest control solutions. The Indian market of microbial pesticides is constrained by several issues. Some of the major constraints are the market's unregistered product sales, low microbial counts that result in subpar performance in the field, and a lack of large-scale production facilities (Alam, 2000; Arora *et al*., 2010; Gupta and Dikshit, 2010 and Mishra *et al*., 2015) [2, 4, 63, 95]. In India, 50-70% of microbial biopesticide-based products had issues, such as less colony propagules than claimed on the label, too much moisture in solid formulations, or contaminants, and as a result, did not adhere to the published CIBRC standards (Ramanujam *et al*., 2016) [123]. Farmers should be made aware as well as encouraged to use microbial pesticides as well as the benefits associated with it.

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