A REVIEW ON INTERGRATED PEST MANAGEMENT SYSTEM WITH A FOCUS ON ENTOMOPTHOGENIC BIO-CONTROL AGENTS

A Dissertation report submitted in Partial Fulfilment of

The Degree of M. Sc. In Biochemistry

By:

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To the

SCHOOL OF CHEMICAL SCIENCES

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May 2022

DECLARATION

I hereby declare that the matter presented in the dissertation entitled, "**Review on Integrated Pest Management System with a Focus on Entomopathogenic Biocontrol Agents**" is based on the results of investigations carried out by me in the School of Chemical Sciences, Goa University under the supervision of Ms. Snigdha Mayenkar and the same has not been submitted elsewhere for the award of a degree.

WINOINA FERNANDES

20P046004

CERTIFICATE

This is to certify that the dissertation entitled, "**Review on Integrated Pest Management System with a Focus on Entomopathogenic Biocontrol Agents**" is a bonafide work carried out by 'Miss Winoina Fernandes' under my supervision in partial fulfilment of the requirements for the award of the degree of Master of Science in Biochemistry at the School of Chemical Sciences, Goa University.

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CERTIFICATE

This is to certify that the dissertation entitled, “**Review on Integrated Pest Management System with a Focus on Entomopathogenic Biocontrol Agents**” is a bonafide work carried out by ‘Miss Winoina Fernandes’ in partial fulfilment of the requirements for the award of the degree of Master of Science in Biochemistry at the School of Chemical Sciences, Goa University.

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ACKNOWLEDMENT

Throughout the writing of this dissertation I have received a great deal of support and assistance. This thesis becomes a reality with the kind support and help of many individuals and I would like to extend my sincere gratitude towards all of them.

On the very outset of this report, I would like to extend my sincere thanks to Ms. Snigdha Mayenkar, as the completion of this undertaking would not have been possible without their assistance, cooperation and encouragement. Their contributions are sincerely appreciated and gratefully acknowledged. Their insightful feedback pushed me to sharpen my thinking and brought my work to a higher level.

I would also like to acknowledge with much appreciation the crucial role of all faculty of the Biochemistry department under the School of Chemical Sciences and the University itself in providing me with an opportunity to undertake this project.

Last but not the least, I would like to thank my parents and friends for their continuous support and encouragement.

ABSTRACT

Agriculture is vital for the survival of any economy. It not only provides food and raw materials to a large section of the population but it also generates various employment opportunities. However, one of their primary problems is pest control, which if not managed well leads a huge loss of economy. The human population has turned to pesticides as a solution to the ever-increasing need for food diversity and the decline of arable land. Pesticides serve an important role in enhancing crop yields and production. Since their discovery, the use of agrochemicals has dominated the world of insect pest management. Insect resistance, loss of biodiversity and the presence of hazardous residues in food and feed have all been linked to the indiscriminate use of these agrochemicals, which has had negative consequences on animal health, soil heath and water quality. Biopesticides are an environmentally beneficial alternative to its chemical counterparts. Biochemicals obtained from microorganisms and other natural sources, as well as the genetic incorporation of DNA into agricultural plants to provide pest resistance, all fall under the umbrella of biopesticides. Microbial pesticides are made up of bacteria, fungi, viruses and other microbes that work in a nontoxic and ecologically sustainable way to manage pests. This review is intended to highlight the commercially available entomopathogenic biocontrol agents -bacteria, fungi, nematodes and viruses, and the possibility of employing them as an alternative to synthetic pesticides. As a result, biopesticides have the potential to play a vital role in an integrated pest management (IPM) programme for insect pest management that is both effective and relatively safe. Biopesticides have been found to be effective in controlling insect pests in economically important crop cultivation; however, successful marketing and utilisation of these products has been slow, owing to high costs, low production efficiency, poor performance under difficult environmental conditions and a lack of awareness. Biopesticides are an important part of IPM systems for pest control, as they provide more natural, eco-friendly, and safer alternatives to chemical pesticides. To achieve this, intensive research is needed to improve pathogen virulence and range of action, as well as their performance under difficult environmental circumstances, formulations that will boost persistence and have a longer shelf life. The existing entomopathogens as biocontrol agents, their mode of infection, future prospects and the challenges of using biopesticides in pest management programmes are discussed in this article.

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1. Introduction

The Indian economy is predominantly agro-based with about 70% of the population of the country being linked to agriculture in some way or the other. A number of plants are grown for their economic benefits rather than for the sustenance of livelihood. The vendibles thus produced can be consumed as fruits, flowers, leaves, stems, roots, latex and other parts can be processed for products such as fibre, rubber, sugar, beverages and biofuel. Crops such as wheat, maize, oats, potatoes, cherries, apples, strawberries, soybeans, bananas, cotton, jute, oranges, jojoba, jatropha, nuts, and brassicas fall under the umbrella of economically attractive crops. These cash crops are often prone to climate change. Any losses in the production or storage of such crops showcase a very deflating pattern in the country's economy. Therefore, protecting the existing diversity and producing new varieties is very important. (Heckel, 2021; Hussain, 2014; Keswani, 2019; Kidanu, 2020)

The human population has turned to pesticides as a solution to the ever-increasing need for food diversity and the decline of arable land. Pesticides serve an important role in enhancing crop yields and production. This has led to the exploitation of agrochemicals in our farmlands, causing abiotic and biotic components of the soil and water ecosystem to be perturbed. Pesticides are the only harmful substances that are intentionally introduced in significant quantities into the environment. (Samada et al., 2020; Syromyatnikov et al., 2020). Therefore, the objective of this article is to overview the growing dependence on pesticides, it's consequences and the introduction of more modern day, eco-friendly methods as a solution to it.

2. Reliance on Pesticides

Increased crop productivity is dependent on the use of appropriate fertilisers, the development of new crop varieties and improved disease and pest control strategies. Pest control is a critical component of a healthy, high-yielding crop that can meet the demands of ever-increasing populations (Birch et al., 2011). A previous study found that 50,000 fungi, 10,000 insects, 15,000 nematodes and 1800 types of weeds harm fibre and food plants around the world (Thakur et al., 2020). It also leads to the annihilation and extinction of their natural predators, with disastrous consequences for human health and the environment (Pathak et al., 2017; Yadav et al., 2020).

Due to their structure, xenobiotic pesticides have a slower disintegration rate, which promotes bioaccumulation and biomagnification across the food web, resulting in biodiversity loss and groundwater contamination. Increasing plant output globally is a difficult undertaking due to regional variations in the environmental conditions (abiotic variables). It is vital to handle the challenges created by numerous pests in order to achieve long-term agricultural production. Insects, weeds, fungi, bacteria, nematodes, birds and animals are among some of these pests. (Syromyatnikov et al., 2020; Ruiu, 2018)

We are currently dealing with a slew of issues as a result of decades of indiscriminate usage of agrochemicals. Many pesticides are difficult to decompose, so they remain in the soil, contaminate groundwater and pollute the environment (Samada et al., 2020). Pesticides do not always stay in the area where they are administered and frequently migrate through air, water and soil, coming into contact with other beneficial organisms and showcasing an adverse effect on them (Abhiram et al., 2018). When synthetic pesticides enter an organism's body, they are not broken down, and the compounds accumulate in the body, resulting in bioaccumulation and biomagnification, in which the chemical compound's concentration rises with each level of the food chain. (Pathak et al., 2017)

Excessive use of chemical pesticides impairs the soil texture (Kumari et al., 2014) not to mention the observed effects on plant life, causing their general physiology and biochemistry to be harmed (Lengai et al., 2020). Sooner or later, with the continuous use of synthetic pesticides, it is observed that the target pests are being rendered resistant. This situation calls for an increase in the dosage of the existing chemical, or the synthesis of a newer, stronger one. Furthermore, non-target creatures such as microorganisms, bacteria, fungi and algae, which are an important element of the soil ecosystem, may be affected, which could have an indirect impact on food supply and its security. (Meena et al., 2021)

3. Integrated Pest Management (IPM)

Due to the above-mentioned shortcomings of chemical pesticides, a newer approach to pest management has been formulated which has the potential to be more effective than the sole use of synthetic pesticides i.e. Integrated Pest Management system. The European Commission Directive 2009/128/EC (2009) developed a framework to limit pesticide exposure hazards and consequences towards the environment and human health. Its main purpose is to promote integrated pest management systems (IPM) and other pest control strategies in order to achieve sustainable pesticide use (i.e alternative to synthetic pesticides). To combat pest resistance issues, integrated pest management system employs biological control, cultural control, autocidal treatments, crop rotation, chemical control, semiochemicals, host plant resistance and genetically modified (GMO) plants. (Srivastava et al., 2020; Thakur et al., 2020). IPM is an effective approach devised to protect plants/crops against pests while avoiding the excessive use of pesticides and is currently gaining popularity among farmers. The following features of a good IPM programme should be prioritised:

I. Pest identification and problem monitoring
II. Choosing the most effective management plan
III. Keeping records for the evaluation program.
IV. IPM approaches should be expanded because they are helpful to both farmers and the environment.

(Abraha et al., 2021; Thakur et al., 2020)

Following are some of the advantages of IPM over pesticides:

- IPM is critical for the ecosystem's equilibrium, as pesticides can disrupt the ecosystem's balance by harming beneficial species.
- Pesticides may become inefficient when pests get resistant and they may also survive and outspread if proper treatment is not provided, while IPM will be useful most of the time.
- Pesticides contaminate groundwater, soil, and air and they persist in the environment, causing harm to living things.
- In general, IPM can save farmers' costs by eliminating the use of pesticides they don't need. It's important to remember that the methods chosen are effective throughout implementation.
  (Abraha et al., 2021; Thakur et al., 2020; Srivastava et al., 2020)

During implementation of IPM, it is worth noting that the methods chosen are both effective and non-harmful to the people and the environment. The greatest strategy to ensure the agricultural sector's long-term viability is to use natural pest control methods. The agricultural sector can use a multitude of pest management techniques in conjunction with other methods outlined below.

I. Cultural Control

Cultural control is the process of altering the host or pest's environment in order to prevent or decrease infection. "Cultural practices" and "sanitation" are used to maintain cultural control. Pest-resistant cultivars, crop rotation, sowing and harvesting at the right times, and proper irrigation management are all examples of cultural practises. Weeds, insects, mites, germs, and other pests can all be prevented or reduced by using trap crops. Another efficient weed management approach is mulching. Sanitation, on the other hand, is accomplished by removing pests' basic necessities for survival, such as shelter, food and water. Removal of pest-hosting weeds, as well as their eradication prior to seed development, is also a desirable technique. Infected plant material should be discarded and pest breeding places in agricultural fields and nearby regions should be investigated. (Barzman et al., 2015; Kumar et al., 2021; Srivastava et al., 2020; Buragohain et al., 2021)

II. Mechanical Control

This involves the use of traps, obstacles, fences and nets, as well as the use of equipment, various gadgets and other physical methods for monitoring pests. The use of tillage devices such as ploughs, disc blades, rollers, cultivators and other similar devices during cultivation destroys weeds and changes the soil environment, making it undesirable for harmful microorganisms and insects. Exclusion tools such as fences and ditches for vertebrate pests and wire or cloth meshes to keep birds away from fruit trees, are also quite effective. Traps are also mechanical devices that are commonly used to relocate or eliminate pests. Sticky surfaces are sometimes maintained in order to catch crawling insects. (Barzman et al., 2015; Kumar et al., 2021; Ahissou et al., 2021)

III. Biological Control

Natural enemies are utilised in this strategy to effectively destroy or regulate the pest population in a variety of situations. Pathogens or insects that control weeds, fungi, mites, other insects and pests are examples of natural enemies. Predatory mites, for example, can help manage spider mites that feed on plants. They must be released on a regular basis because they have no long-term effects. Commercial rearing and cultivation of several natural enemies is also practised. (Barzman et al., 2015; Copping et al., 2000; Idris et al., 2020)

IV. Genetic Control

This encompasses both traditional and molecular breeding techniques for plants and animals with the goal of avoiding or resisting specific pests. Transfer of genetic information from some pest-destroying organisms to hybrid seeds could help plants fight pests on their own. These gene-editing techniques are frequently utilized and could be a useful tool in future pest-control initiatives. (Barzman et al., 2015; Kiran et al., 2018; Srivastava et al., 2020)

V. Chemical Control

Pesticides, either naturally occurring or synthesised, are used in chemical control methods. Pesticides are widely and regularly used by farmers and they play a vital role in pest management. Synthetic pesticides are frequently used because of their efficacy, quickness, ease of application and pest management. (Barzman et al., 2015; Idris et al., 2020; Ahissou et al., 2021).

VI. Transgenic approaches in developing pest resistant plants

Pest resistance can be found in a variety of microorganisms as well as plants, and this resistance material (the gene encoding the protein that confers resistance to that organism) can be obtained through transgene introduction, molecular breeding and the production of recombinant species employing host resistance. (Barzman et al., 2015; Koul et al., 2003; Kumar et al., 2021)

As a result, the transgenic method appears to be a revolutionary technique for introducing new pest resistance genes into desired plants (Arthurs et al., 2018). To battle the pests, one or more genes that impair the metabolic and biological processes of the targeted pests are utilised. Several genetically engineered plants are created by combining genetic variants from various species. Transgenic techniques allow farmers to manage agricultural yield by allowing them to generate herbicide and insect resistant plants and this approach can fulfil the world's growing food demands, which are predicted to exceed 6 billion by 2050. (James, 2003). Furthermore, by using traditional breeding processes, the integrated genes can be transferred to desired species (Srivastava et al., 2020). The production of *Bt* plants is an excellent example of genetically engineered self-protecting plants. It contains transgenic expression of the *Bt* gene, which allows plants to synthesize a bacterial protein that kills pests such as bollworms, budworms and armyworms without the application of any physical pesticides (Heckel, 2021). Another remarkable example of induced pest resistance in plants is by utilising RNA interference/silencing technique which showed promising results in pest control of the TMV (Tobacco Mosaic Virus) against tobacco plants. RNA interference (RNAi) is a biological mechanism in which double-stranded RNA molecules restrict gene expression in a sequence-specific manner via translational or transcriptional repression. (Duan et al., 2012)

Cultural practices, physiochemical control measures and the regulation of mediated biological / phytochemical ways are all examples of traditional pest control methods. Chemical, biological and phytochemical pesticides look to be promising in terms of reducing productivity losses, but one of the major downsides of these pesticides is the development of resistance to them, as well as the targeting of beneficial insects/pests. As a result, the optimum method would be to use pesticides and insecticides in the cultivation of crops without affecting the output or yield of the plants. In such a scenario, the use of biopesticides be could the ultimate solution.

4. Biopesticides

The non-biodegradability, persistence and toxicity of chemically synthetized pesticides lead to the adoption of long-term pest control measures that are cost effective. Biopesticides, also known as natural pesticides, are organic products made from living organisms such as plants, nematodes and microorganisms including bacteria, fungi and viruses that inhibit or reduce pest populations (Pathma et al., 2021). Bio-pesticides control pests using non-toxic mechanisms in an environmentally safe manner. Biopesticides is an expression that encompasses many aspects of pest control, including: Microbial (viral, bacterial and fungal) organisms, entomopathogens, insect predators and parasites, genes used to transform crops to express resistance to insects, fungal and viral attacks or to render them tolerant to herbicide application. (Ruiu, 2018; Thakur et al., 2020)

These pesticides are based on nature's biological pest control mechanisms. Bio pesticides usually target a specific pest, unlike broad-spectrum chemical pesticides, which destroy not only the pests but also other beneficial organisms in that environment. Hence, microbial pesticides pose a lower risk to the environment and human health. Additionally, there are a variety of microbial biopesticide products on the market that can be used to eliminate a variety of pests. (Wraight et al., 1999; Ruiu, 2018)

Pests and viruses are now controlled using a range of environment friendly approaches in India and around the world. Biopesticides are an environmentally acceptable pest control alternatives that should be widely promoted because they are effective and sustainable compared to their chemical counterparts (Thakur et al., 2020). Biopesticides protect plants during the growing seasons, do not develop toxins in the plants and decrease disease transmission to a variety of insects (Ignacimuthu and Sen, 2001; Koul et al., 2003; Rabindra, 2001). Advances in the field of biopesticide research and development have improved the sustainability and reduced the pollution caused by synthetic pesticides (Suman et al., 2016).

Many microbial biopesticides have been produced from fungi, viruses, bacteria, nematodes and protozoa, and are currently employed in pest management systems throughout the world (Islam and Omar, 2012; Mazhabi et al., 2011). *Bacillus thuringiensis* Berliner (Abtew et al., 2015) is one of over 100 kinds of bacteria that have been identified as pathogens against insect pests. The entomopathogenic bacteria *B. thuringiensis* has proven to be beneficial, but new species, strains, specific toxins and risk factors must be identified through educational and industrial research. Many of them are available on the market and are for commercial use. Few examples of entomopathogenic bacteria are *Bacillaceae* sp., *Burkholderia* sp., *Chromobacterium* sp., *Pseudomonas* sp., *Saccharopolyspora* sp., *Serratia sp.*, *Streptomyces sp.* and *Yersinia* species and fungi include a variety of *Metarhizium anisoplia, Beauveria bassiana, Hisrsutella* species, *Passlomycium* sp., *Lesarcasutella* sp. *and Hisarcasutella* species. Baculoviruses (insect pathogenic viruses) are species-specific and active against chewing and biting insects, particularly lepidopteran caterpillars. As of 2017, the Central Insecticides Board and Registration Committee have approved 188 mycoinsecticides, 39 myconematicicides, 51 bacterial insecticides and 27 nucleopolyhedrovirus products. (Kumar et al., 2018).

5. Entomopathogenic Biocontrol Agents

Entomopathogenic bio-control agents are those which live in close association with insect pests. These associations may be in the form of commensalism, symbiosis or parasitism, mainly facultative or obligatory in nature (Rana et al., 2019, 2020; Suman et al., 2016). Given below is a list of the widely studied and commercialised entomopathogens as biocontrol agents.

Bio-control Agent	Organism name	Target Pest	Source
Bacterium	*Psuedomonas fluorescens*	Larvae of the ladybird beetles, *D. melanogaster*	Thakur et al., 2018
Bacterium	*Bacillus thuringiensis* (*Bt*) subsp. kurstaki/aizawai	Beet armyworm, European corn borer, *Ostrinia nubilalis*, budworms, ballworms, *Heliothus* spp. and tortricid leafrollers.	Arthurs et al., 2018
Fungus	*Beauveria bassiana*	Rice leaf folder/roller (*Cnaphalocrosis medinalis*), diamond back moth (*Plutella xylostella*), pod borer (*Helicoverpa armigera*) fruit borer and spotted bollworm	Thakur at al., 2020
Fungus	*Metarhizium anisopliae*	Coleoptera, Diptera, Hemiptera, Isoptera, brown plant hopper (BPH), shoot and fruit borer (*Leucinodes orbonalis*)	Thakur et al., 2020
Nematode	*Heterorhabdits indica*	Lepidopteran pests and termites	Mansour et al., 2020
Nematode	*Steinernema carpocapse*	Root grubs, cutworms, root weevils	Mansour et al., 2020
Virus	*Helicoverpa zea* nucleopolyhedrovirus	Cabbage looper, *Trichoplusia ni*, *Heliothis zea* and beet armyworm(*Spodoptera exigua)*	Abhiram et al., 2018
Virus	*Plutella xylostella* granulovirus	Cotton leaf worm, corn earworm	Abhiram et al., 2018

5.1 Entomopathogenic Bacteria as biocontrol agents

Bacillus thuringiensis, widely known as *Bt*, is one of the most frequently used microbial pesticide. The bacterium makes crystalline proteins and kills only one or a few insect species that are closely related. The target insect species is determined by the *Bt* crystalline protein's binding to the insect gut receptor (Kumar et al., 2018). During sporulation, *B. thuringiensis* develops parasporal, proteinaceous, crystal inclusion-bodies. The presence of extrachromosomal plasmids in the cell is primarily responsible for *Bt*'s insecticidal mechanisms. These contain cry genes, which code for a wide range of protein crystalline inclusion bodies that are poisonous to insects. The crystal proteins are solubilized by the insect gut proteases, which then transform the original pro-toxin into a mixture of up to four smaller toxins. These hydrolysed toxins bind with high-affinity to particular receptor binding sites in the insect's midgut cells, interfering with the potassium ion-dependent active amino acid symport mechanism. This rupture results in the creation of large cation selective holes which increases the cell membrane's water permeability. A considerable amount of water intake induces cell swelling and eventual rupture, causing the midgut lining to disintegrate which eventually leads to death of the pest (Copping et al., 2000; Srivastava et al., 2020). *Pseudomonas fluorescens* is a bacterium that lives in soil and water and has numerous flagella. Infection with *P. fuorescens* SBW25 causes discoloration and a halt in feeding in the infected larvae, resulting in stunted growth and a delay in larval development. Adult flies emerging from infected larvae have deformities in the head capsule, legs, eyes and wings, among other things (Miao-Ching et al., 2021).

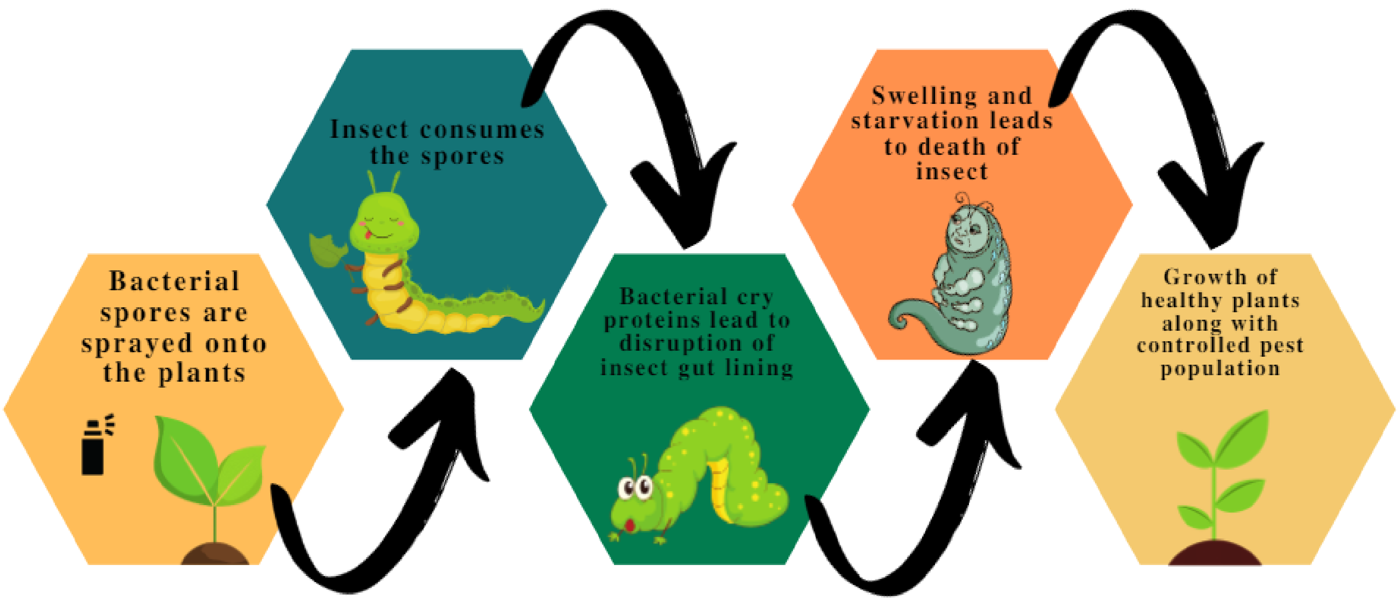

Fig. 1: Mode of infection in case of entomopathogenic *B. thuringiensis*

5.2 Entomopathogenic Fungi as biocontrol agents

"The use of mycoinsecticides is a popular method of managing the insect population in agricultural fields. Due to low productivity, higher cost and lack of knowledge, the rate of utilization and marketing is comparatively slower for mycoinsecticides. These mycoinsecticides also plays an important role in IPM(Integrated Pest Management)" (Maina et al., 2018).

Among the several biocontrol agents, entomopathogenic fungi(EPF) are a crucial component of an integrated approach that can provide significant and selective pest management, among the many biocontrol agents. Entomopathogenic fungi are a type of fungi that destroys insects by infecting and attacking their hosts by living in association with it (Singkaravanit et al., 2010) . EPF can be found in soil and leaf litter all over the world, however temperate forests have a smaller variety of EPF than tropical habitats (Aung et al., 2008). They're potentially the most versatile biological control agents because of their vast host range. The destruction of an insect is dependent on the interaction of the fungi with it's insect pests. More than 800 fungal species have been identified to be causing pathogenicity in the insect species, according to the findings. EPF are a crucial component of integrated pest management strategies in horticulture, forestry and agriculture as biological control agents against insect pests and other arthropods. (Inglis et al., 2000). *M. anisopliae, B. bassiana*, and *Isaria fumosoroseus* include the majority of the commercially available products as biopesticides. (Thakur et al., 2020).

The majority of EPF are soil-borne fungi species with a common mechanism of infection. EPF attack insects directly through spiracular apertures in the cuticle, unlike other biopesticides that act by ingestion by the host insect. The infective stage of fungi is either asexual or sexual (conidia) spores. EPF efficiently eliminate pests when enough infective propagules (primarily conidia) touch a vulnerable host and the conditions are perfect for a lethal mycosis to develop. The conidia or spores grow in the bloodstream of the bug and the germinating mycelia kill the afflicted host over time. (Alfina et al., 2022; Bava et al., 2022; Maina et al., 2018)

Entomopathogenic fungi are opportunistic pathogens that kill insects through nutritional deprivation, tissue damage and the production of toxins. Entomofungal pathogens' cuticle degrading enzymes, such as chitinase, protease and lipase, are vital in the pathogenicity of these organisms on insects because they break down the insect cuticle to allow the fungal germ tube to penetrate into the insect body. Mechanical pressure and enzymatic degradation work together to allow the EPF to enter the insect cuticle (Brunner-Mendoza et al., 2022; Kidanu et al., 2020)

When fungal conidia come into contact with the host, they use hydrophobic methods to adhere to the cuticle and germinate to create germ tubes under favorable conditions. The fungi creates a number of specialized infection structures during this phase, including penetration pegs and/or appressoria, which allows the developing hyphae to pierce the host integument. Other enzymes, such as metalloid proteases and aminopeptidases, assist the germ tube in penetrating the cuticle. Once within the insect, the fungus forms hyphal bodies that spread through the haemocoel and infect various muscle tissues, fatty bodies, malpighian tubes, mitochondria and haemocytes, causing the insect to die 3 to 14 days later (Kidanu et al., 2020; Selvaraj et al., 2014)

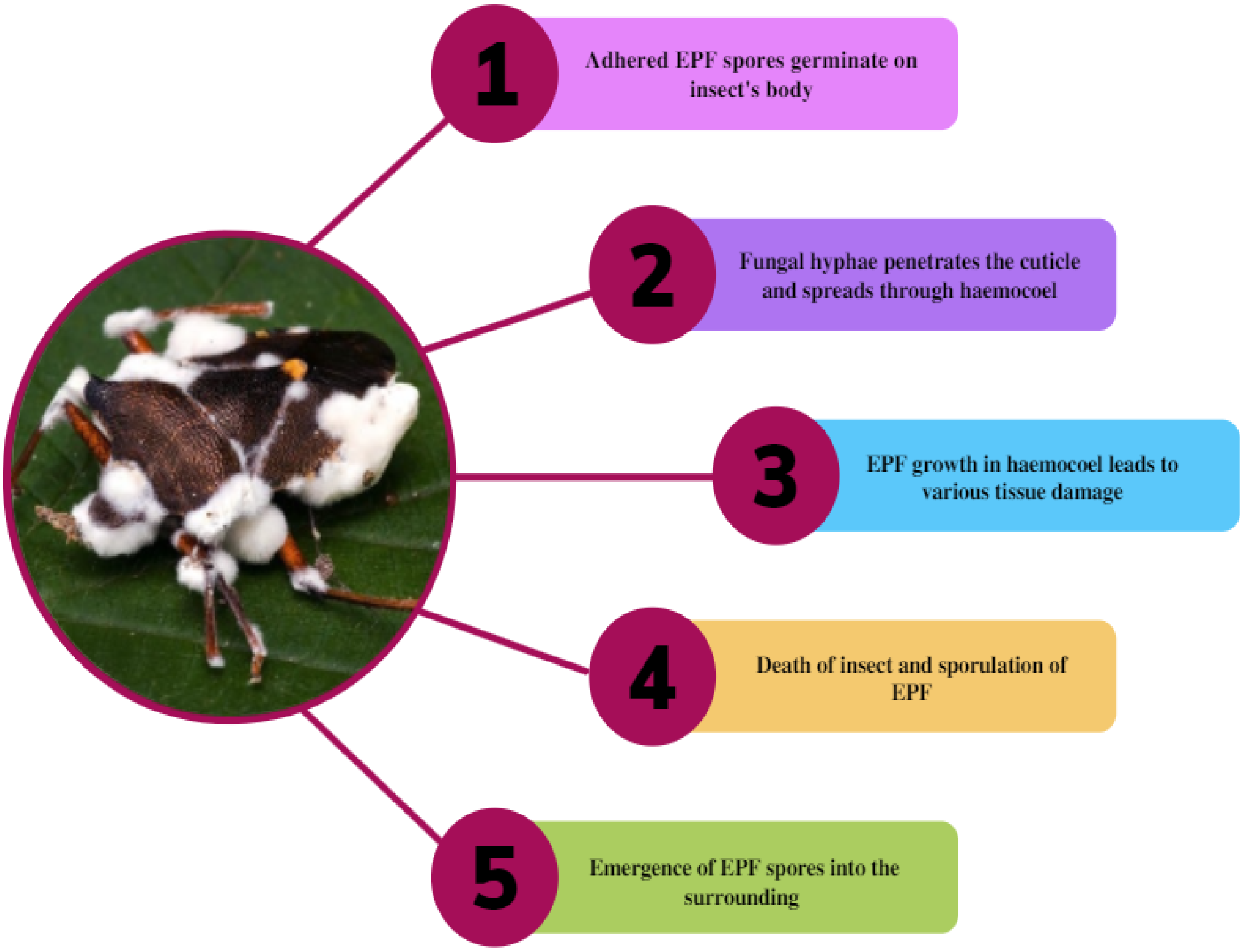

Fig. 2: Mode of infection in case of entomopathogenic *B. bassiana*

Several studies in India have found that *B. bassiana* products are effective against hemipteran pests. Under greenhouse conditions, Selvaraj and Kaushik (2014) found that foliar sprays of the HaBa (Hyderabad strain) on fenugreek caused 85 percent mortality of the cowpea aphid, *Aphis craccivora,* one week after treatment. In India, around 30 products based on *M. anisopliae* s.l. have been used to manage foliar and soil pests, particularly in the areca nut, coconut, coffee, corn, potato, pigeon pea, soybean and sugarcane industries (Vidhate et al., 2022). In several circumstances, these products have provided an astounding level of pest control. When compared to untreated plots in Andhra Pradesh, field tests with a talc-based formulation of *M. anisopliae* ($5x10^{13}$ spores/ha) enhanced with farmyard manure(FYM) reduced white grub damage to sugarcane by 93 percent and grub populations by 77 percent over two years. When compared to treatments applied one month after planting, treatments applied at the time of planting were generally more effective (Visalakshi et al., 2015).

5.3 Entomopathogenic Nematodes as biocontrol agents

Entomopathogenic nematodes (EPNs) are parasitic nematodes that live in a mutualistic symbiotic relationship with the bacteria *Xenorhabdus* sp and *Photorhabdus* sp (Ruiu, 2018). The *Steinernema* and *Heterorhabditis* nematode genera , exert an insecticidal effect on the larval stages of the insects. There exists 55 species of genera *Steinernema* and 12 species of genera *Heterorhabditis* living symbiotically with the two bacterial genera- *Xenorhabdus* and *Photorhabdus,* respectively (Koul, 2011). EPN are microscopic (400-800mm), delicate organisms that are susceptible to desiccation, temperature fluctuations and sun radiation, all of which limit their use.

EPNs begin their parasitic life cycle with third-stage infective juveniles that infect target insects through body holes. EPNs invade the host through natural openings (the oral cavity, anus, spiracles, and wounds) and deliver the symbiotic bacteria into the hemocoel. They disseminate symbiotic bacteria (stored in the nematode intestines) throughout the body cavities, inflicting extensive septicemia and killing the host within 24 to 48 hours. The bacteria and host tissues are consumed by the juvenile nematodes. The insect carcass contains two to three generations of nematodes. Toxins and virulence factors that are released weaken the host and cause the generation of metabolites that aid in the formation of a hospitable environment for nematode proliferation. In pursuit of a new host, the nonfeeding generation migrates out of the deceased insect. (Miao-Ching et al., 2021; Ruiu, 2018; Thakur et al., 2020)

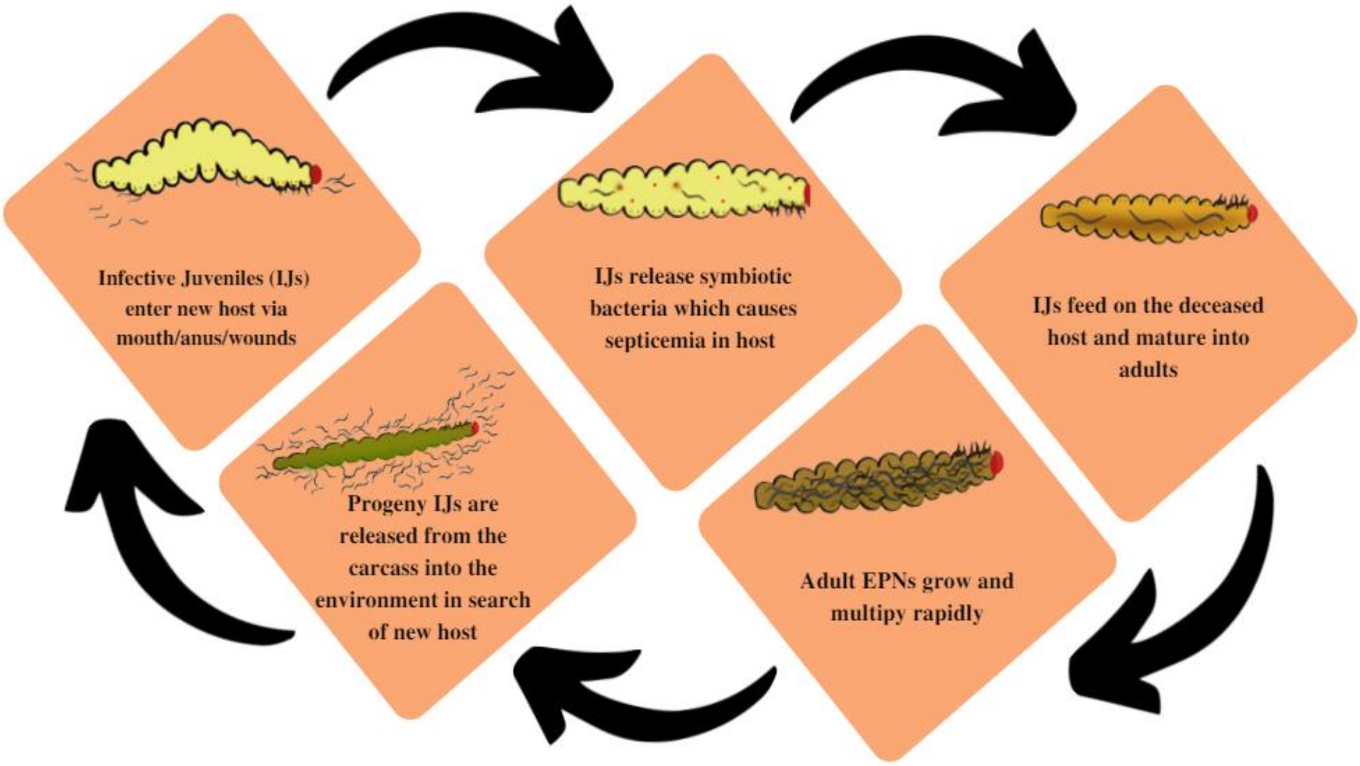

Fig. 3: Mode of infection in case of entomopathogenic *Steinernema* sp.

5.4 Viruses as biocontrol agents

Baculoviruses are used to combat insect pests of vegetables, rice and cotton. The mode of infectivity is via epizootics i.e during mating or egg laying, an insect swallows a virus and transmits it to another insect (Thakur et al., 2020). Inclusion viruses (IV) and non-inclusion viruses (NIV) are the two types of entomopathogenic viruses (Narasimha et al., 2015). IV create inclusion bodies inside the host cell and is categorized as polyhedron viruses (PV) and granulosis viruses. The existence of nucleus and cytoplasm in polyhedral viruses causes them to be classified as nuclear polyhedrosis viruses (NPV) and cytoplasmic polyhedrosis viruses (CPV). Baculoviruses attack insects orally, and the first infection usually occurs after consumption of contaminated matter. Occlusion-derived viruses (ODVs) are virions that are released into the midgut environment by ingested occlusion bodies and interact directly with the membrane of microvillar epithelial cells due to their envelop proteins. Budded viruses (BVs), a second type of virions, are produced within the nucleus of infected midgut cells, ensuring the virus's continuous transmission throughout the host. The dead insect body liquefies as the infection spreads, allowing virus particles to diffuse more easily in the environment. Viral infections can also impact the hosts' gene expression processes, causing behavioural abnormalities. (Thakur et al., 2020; Ruiu, 2018; Arthurs et al., 2018)

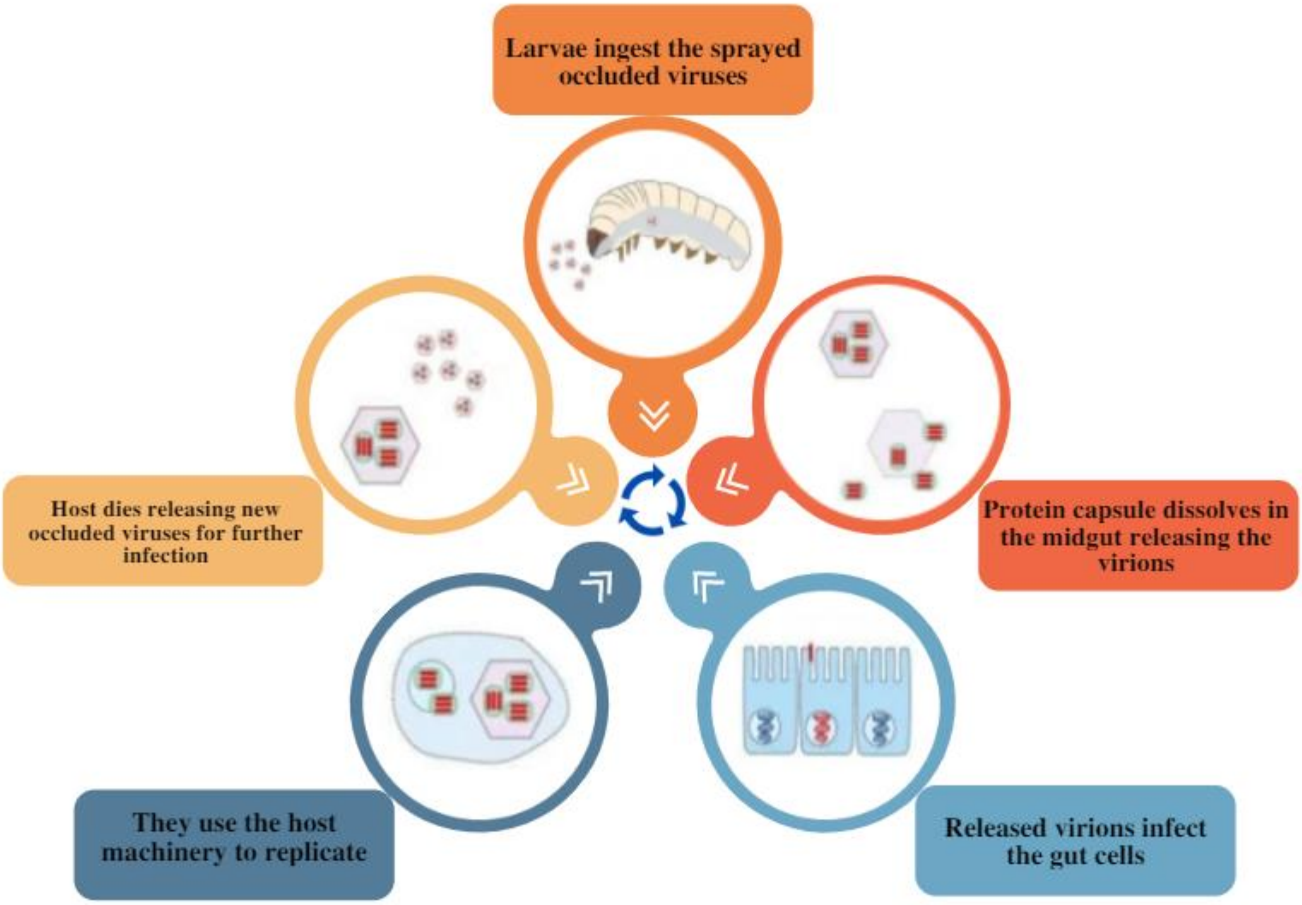

Fig. 4: Mode of infection in case of entomopathogenic *Helicoverpa zea* nucleopolyhedrovirus

6. Delivery systems for Entomopathogenic biopesticides

Biopesticides come in a variety of forms, depending on shelf life, stability and microorganisms. These formulations might be dry or liquid in terms of their physical state. Stabilizers, additives, spreads, synergists, stickers, surfactants, colouring agents, nutrients, dispersants, melting and anti-freezing agents can all be used to maintain the active components (Brar et al., 2006; Knowles, 2008).

Fig. 5: Delivery Systems for Entomopathogenic Biocontrol agents

Source of information: (Brar et al., 2006; Knowles, 2008).

7. Benefits of Biopesticides

Biopesticides are beneficial in pest management in several ways. For instance, their remnants have no known negative environmental consequences. Since they have a limited range of toxicity, usually limited to a single group or a few species, non-target organisms are relatively unaffected by biopesticides, this in turn, leads to protection of the biodiversity. They can also be employed in conjunction with synthetic chemical insecticides and other IPM techniques and are self-perpetuating in the right environment. Biopesticides have the potential to minimize the use of agrochemicals. Because of their particular mode of action, pest resistance to microbial insecticides is less prevalent or may develop more slowly. (Ignacimuthu and Sen, 2001; Koul et al., 2003; Rabindra, 2001)

8. Limitations of biopesticides

Although biopesticides appear to be a novel solution in pest control, it has its fair share of drawbacks. Firstly, to germinate and spread infection, the spores/conidia require ideal environmental conditions. Secondly, biopesticides can be altered or rendered ineffective if exposed to UV radiation, harsh temperatures or a climatic change. Commercial production and distribution can be highly expensive as they have a rather short shelf life. Moreover, because the pathogen can only be used if the pest is present, preventative treatment is difficult. Unlike agrochemicals which are fast-acting, biopesticides often produce delayed responses. Another restriction is the requirement for biosafety testing of microorganisms prior to biopesticide registration and propagation. Additionally, due to the lack of persistence and low infection rate, they require frequent applications which increases the costs. (Ignacimuthu and Sen, 2001; Koul et al., 2003; Rabindra, 2001; Thakur et al., 2020)

9. Conclusion

To recapitulate, bioinsecticides have the potential to play an important role in an IPM program for successful and reasonably safe insect pest management in field crops. To achieve this, intensive research efforts must be made to improve pathogen virulence and range of action, as well as their performance under difficult environmental conditions, simplicity of application, formulations that will boost persistence and have longer shelf life.

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