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Review Article

Entomopathogens and their role in insect pest management

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ABSTRACT: Agricultural production faces significant challenges due to the loss of crop yields, highlighting the need for improvements in pest management methods to enhance productivity. Crop growers are increasingly pressured to minimize the use of chemical pesticides without compromising yields. However, managing pests has become more challenging due to pesticide resistance and limited product availability. Consequently, there is an urgent requirement for alternative approaches. Entomopathogens such as fungi, bacteria, viruses, and nematodes emerge as promising alternatives to broad-spectrum chemical insecticides. They have been widely employed to control insect pests in cultivated crops, employing successful strategies such as augmentation and classical biological control. These methods involve applying or introducing bacteria, baculoviruses, fungi, and nematodes. Utilizing entomopathogens offers numerous benefits beyond their effectiveness. These advantages encompass the capacity to treat expansive areas with a sole application, ensuring safety for both humans and non-target organisms. Additionally, their use leads to decreased pesticide residues in food, the conservation of natural enemies, and a boost in biodiversity within managed ecosystems. Entomopathogens present a viable solution by offering effective pest control while addressing environmental, human health, and ecosystem sustainability concerns. The primary emphasis of this review is on the present condition of bio-formulations, the pathogenicity associated with entomopathogens, their mode of action, and the possible implementation of diverse microbial formulations aimed at achieving sustainable pest management.

KEYWORDS: Bacteria, entomopathogens, fungi, insect pest management, nematodes, viruses

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INTRODUCTION

Agricultural pests are comprised of a diverse range of organisms, including but not limited to weeds, arthropods (such as insects and mites), molluscs (like slugs and snails), and a limited number of vertebrates. Furthermore, plant pathogens such as fungi, oomycetes, bacteria, viruses, and nematodes pose significant threats to crop health and productivity. Their impact on agricultural output and quality is substantial, primarily through crop consumption. It is estimated that there are millions of pest species globally. The obstacles faced by agriculture significantly impede its production, leading to an estimated substantial reduction of around 40% in potential global crop yields (Mantzoukas & Eliopoulos, 2020). Chemical pesticides exert detrimental effects on non-target organisms, resulting in adverse consequences such as biodiversity depletion, compromised food safety, insect resistance emergence, and pests' resurgence in previously unaffected areas. These ramifications have prompted scientists to explore and develop more environmentally friendly alternatives. For instance, one promising approach involves utilizing entomopathogens to combat insect pests (Venkatesh *et al.*, 2022). Moreover, bacteria trace their ancestry back to unicellular microbes—the earliest known life forms on Earth that emerged approximately 4 billion years ago (Hall, 2008). Mites and diverse insect pests harbour a multitude of naturally existing bacteria, fungi, nematodes, and viruses that affect a broad spectrum of organisms. According to Dhaliwal *et al.,* 2015), the activities of insect pests have been identified as contributing to around 10.80% of global agricultural losses following the Green Revolution, signifying their substantial impact. Mantzoukas and Eliopoulos 2020) further estimated a substantial decline in global agricultural output, ranging from 18% to 26%, equating to an annual loss of $470 billion. To combat these losses, insecticides have become a pivotal method due to their ease of application, effectiveness, and prompt action, establishing themselves as a crucial tool in managing insect pest infestations (Sharma, 2019). However, in as many as 80% of cases, the progress of resistance to one or more types of insecticides has been caused by concentrated chemical application (Sharma, 2019). Entomopathogenic Microorganisms (EM) are effective against insect pests and environmentally safe for both people and non-target animals (reduced pesticide residues) when used as pest control agents. Natural enemies within agroecosystems are pivotal in averting the escalation of insect populations to damaging levels. Biological Control Agents (BCAs) are utilized variably based on the specific pest and the biological attributes of the control agent. These agents boast several beneficial traits, including host specificity, absence of harmful residues, non-phytotoxic effects, human safety, and the potential to sustainably manage pests. Nevertheless, their effective application necessitates a thorough comprehension of both the insect's ecology and its natural antagonist. Entomopathogens, encompassing fungi, bacteria, viruses, and nematodes, serve as effective biological control agents, but their development is pivotal. Scientific communities recognize their significance in integrated pest management for creating eco-friendly systems for crop protection and improvement (Gangwar *et al.*, 2021). Achieving this goal demands a comprehensive understanding of bio-assay processes, along with expertise in manufacturing, formulation, and application strategies.

ENTOMOPATHOGENIC FUNGI

Entomopathogenic Fungi (EPF) refer to fungal species that exhibit pathogenicity towards insects, playing a crucial role in managing insect populations, thus serving as some of the earliest agents for controlling insect pests. Before the introduction of insecticides, early agricultural methods depended on the interaction between predators, pathogens, and the resistance of host plants to control insect pests. The origins of significant field trials related to EPF can be traced back to 1888 when Russian microbiologist Elie Metchnikoff conducted pioneering experiments. Metchnikoff's groundbreaking work in this field eventually led to the identification of *Metarhizium anisopliae*, a discovery that earned him a Nobel Prize (Vega *et al.*, 2009). Entomopathogenic fungi, a group of fungi that eliminate insects by infecting their hosts, have been extensively studied (Singkaravanit *et al.*, 2010). Many bio-insecticides based on insect pathogenic fungi have been developed and are commercially available (Hafiza *et al.*, 2014). The growing utilization of EPF in biological control arises from a heightened awareness of the environment, worries about food safety, and the decreasing effectiveness of traditional chemicals caused by the emergence of insecticide-resistant species (Shahid *et al.*, 2012). Beyond their impact on herbivores, endophytic fungi and mycorrhizae often maintain symbiotic relationships with plants, exerting adverse effects on generalist herbivores (Hartley *et al.*, 2009). Likewise, EPF present in different plant settings such as the phyllosphere, rhizosphere, or endophytes, can combat plant pathogens using methods like parasitism, competition, antibiosis, or by triggering systemic resistance within the plant (Ownley *et al.*, 2010). The classification of fungi encompasses divisions such as Ascomycota, Zygomycota, and Deuteromycota, along with previous inclusions of Oomycota and Chytridiomycota (Samson *et al.*, 1988) (Table 1). At present, around 90 genera encompassing over 700 species are acknowledged as insect-infecting fungi, covering a wide array of major fungal classes (Moorhouse *et al.*, 1992). These entomopathogenic fungi play a substantial role in naturally controlling diverse insect pests and their populations. Many of these fungal species are commercially grown and globally employed as biological control agents against a variety of insect pests (Sevim *et al.*, 2015).

Action mechanism of entomopathogenic fungi

Entomopathogenic fungi have gained recognition as effective biological agents for controlling insects, notably replacing chemical-based biopesticides in agricultural applications. The advancement of fungal biopesticides for controlling pests in forestry and agriculture is significantly reliant on our comprehension of the action mechanisms of entomopathogenic fungi. This area of research is particularly intriguing and holds promise for effective pest management strategies. They have developed several strategies to attack, settle, and eventually destroy their insect hosts as shown in Figure 1. This is a description of how entomopathogenic fungi work.

The virulence of entomopathogenic fungi relies on their enzymatic machinery (Table 2), The enzymes consist of lipases, proteases, and chitinases, which effectively degrade the integument of insects. Moreover, according to research conducted by Mondal and colleagues in 2016, the researchers investigated the presence of β-galactosidase, β-glutaminase, and catalase within these entomopathogenic fungi. To adhere to the insect's cuticle, which serves as its exterior layer of protection, entomopathogenic fungi generate specialised structures called sticky appressoria or conidia, which are asexual spores. Hydrophobic interactions commonly occur between fungal spores and the waxy cuticle of insects, facilitating adhesion (Butt *et al.*, 2001). Following adhesion, entomopathogenic fungi secrete aqueous substances and apply pressure to the insect's cuticle. Enzymes present in these substances break down chitin and cuticular proteins, enabling the fungus to penetrate the insect's body (Robert *et al.*, 2004). The fungus begins to develop and spread as soon as it enters the body of the insect. It creates filamentous structures called hyphal bodies, which take in nutrients from the insect's hemolymph (blood). The insect is weakened by the fungus's competition with the host for resources (Vega & Blackwell, 2005). Entomopathogenic fungi generate various toxins and metabolites that exhibit toxicity towards the insect host. These substances have the potential to interfere with the insect's physiological functions, which could ultimately result in the mortality of the host (Pell *et al.,* 2003). Certain

Table 1. Taxonomic distribution of EPF used against different insect pests

Fungal Division	Entomopathogenic Fungal Genera	Targeted Insect Pest	Reference
Zygomycota: Entomophthorales			
Family: Ancylistaceae	*Conidiobolus*	Aphids (or other Homoptera)	Keller, 2006
Family: Entomophthoraceae	*Batkoa*	Aphids, other hemipterans, small flies, many other insects, diverse flies, Lepidopterans, Coleopterans	Gryganskyi *et al.*, 2022
	Entomophaga	Lymantriidae (Lepidopteran), Orthopteran-like melanoplinae spur-throated grasshoppers and non-melanopline grasshoppers	Lomer *et al.*, 2001
	Entomophthora	Mosquitoes, midges or blackflies, Dipteran-like muscoid flies, Homopteran-like aphids	Cossentine *et al.*, 2011
	Pandora	Dipteran, Homopteran like aphids, Lepidopterans	Keller, 2006
	Zoophthora	Coleopterans, Homopteran like aphids	Eilenberg *et al.*, 2006
Family: Neozygitaceae	*Neozygites*	Acarina, Homopteran like aphids, Thysanoptera	Montalva *et al.*, 2014
Ascomycota			
Class: Sordariomycetes Order: Hypocreales Family: Clavicipitaceae	*Cordyceps*	Dipterans, Hymenoptera like wasps and ants, Lepidopterans	Sung *et al.*, 2007
	Aschersonia	Scale insects, white flies	Meekes *et al.*, 2002
	Metarhizium	Coleopterans, Hemipterans, Lepidopterans, Orthopterans	Milner 2000)
	Nomuraea	Lepidopterans, Spiders	Lacey *et al.*, 2001
Family: Cordycipitaceae	*Beauveria*	Aphids, whiteflies, thrips, grasshoppers and certain types of beetles	Sun *et al.*, 2023
	Evlachovaea	Hemipteran bugs like *Triatoma infestans*, *Rhodnius* spp., *Panstrongylus herreri, Dipetalogaster maximus*	Luz *et al.*, 2004
Family: Ophiocordycipitaceae	*Hirsutella*	Acarida, Hemipterans, Hymenopterans, Lepidopterans, mites and nematodes	Simmons *et al.*, 2015
Order: Glomerellales Family: Plectosphaerellaceae	*Lecanicillium [Verticillium]*	Aphids, mealy bugs, scales, thrips and whiteflies	Prince & Chandler, 2020
Class: Eurotiomycetes Order: Eurotiales Family: Thermoascaceae	*Paecilomyces /Isaria*	Aleurodids, aphids, Coleopterans, Dipterans, Hymenopterans, Lepidopterans and Thysanopterans	Weng *et al.*, 2019

entomopathogenic fungi could inhibit an insect's immune system, preventing the immune system from recognizing and attacking the fungus. This facilitates the fungus's ability to successfully infect (Cerenius *et al.*, 2008). As the fungal infection worsens, the insect host becomes weaker and experiences disruptions to its essential organs. The bug eventually perishes. After that, the fungus proceeds through sporulation, which results in the production of fresh spores that can be dispersed into the environment and infect more hosts (Roy *et al.*, 2006).

Entomopathogenic bacteria

Microbial pesticides, derived from living organisms or their products, present eco-friendly alternative management tactics characterized by their specific targeting of pests. Their effectiveness lies in employing non-toxic mechanisms that are harmful to the targeted pests. Within this group, the *Bacillus* genus of entomopathogenic bacteria is widely employed as agents for controlling insects at a microbial level (Table 3). Although Gram-negative bacteria, which can cause diseases in insects, show potential as control agents, their connection to opportunistic pathogens of vertebrates initially impeded their progress in development. For instance, *Bacillus thuringiensis* (Bt), a spore-producing bacterium within the Bacillaceae family, has been widely employed to manage caterpillars and beetles. Its efficacy stems from the production of insecticidal proteinaceous protoxin crystals during sporulation (Deka *et al.*, 2021). In the 1960s, the utilization of entomopathogenic

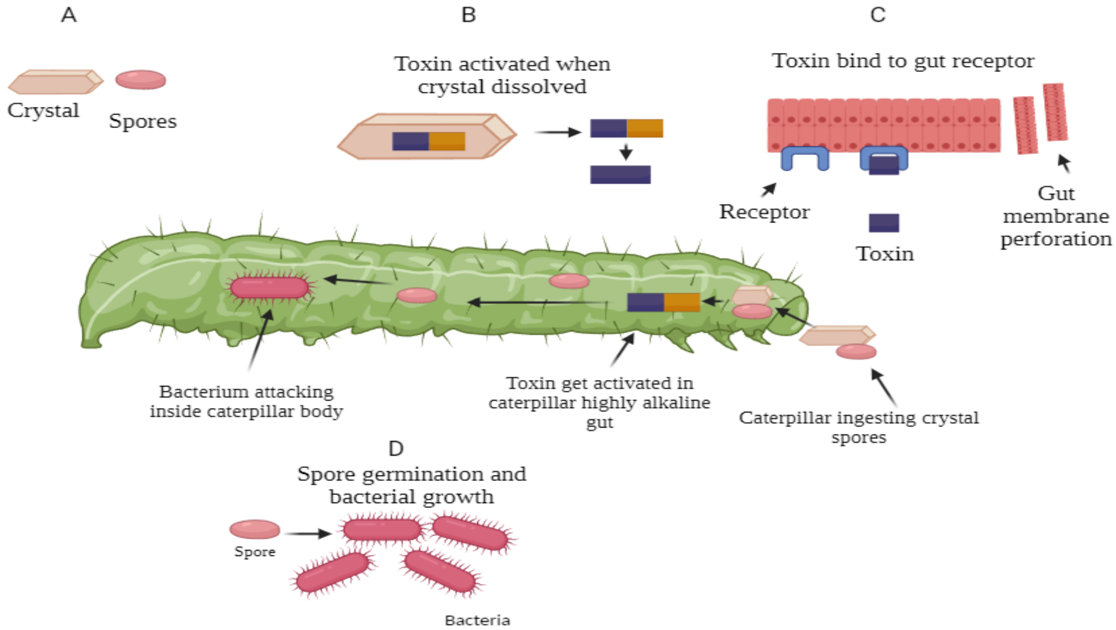

Figure 1. Entomopathogenic fungi mode of action with modification (Krishgupta *et al.*, 2019).

Table 2. Entomopathogenic fungi (EPF) utilized as commercial bio-insecticides in India

EPF Species	Released Enzymes	Commercial Product	Formulation	Application Method	Reference
Beauveria bassiana	Lipase, protease, chitinase, β-galactosidase, catalase and L-glutaminase	Baba, Beavera, BBC, Bb-Protec, Beauvitech-WP, Bio-Be-Ba, BioGaurd Rich, Bio-power, Biorin/Kargar, Botanigard, Brigade, Daman, Jas Beesi, Mycojaal, Nagestra, Naturalis, Metabeave, Racer, Toxin ,	Powder Dispenser tray	Place filter paper between the frames or sprinkle it along the path of the hive entrance using a dispenser	Reddy *et al.*, 2013); Mondal *et al.*, 2016); Ruiu, 2018); Kumar *et al.,* 2020)
			Powder/Flour/ Carnauba wax powder	Blown between frames	
			Fungal culture	Walk for a few minutes	
			Spore solution	Immersion for a few seconds	
			Commercial preparation suspended in water	Sprayed frames	
			Wettable Powder	Sprinkled amidst the frames / interspersed among the frames	

Table 2. Continued...

Metarhizium anisopliae	Lipase, protease, chitinase and β-galactosidase	Bioblast, Bio-Magic, Biomet Rich, Biostorm, Cropmet, Devastra, Green Muscle, Kalichakra, Metrocid, Metaz, Jasmeta, Metarhizium, Met52/ BIO1020 granular, Novacrid, Pacer	Powder/Liquid	Placing filter paper within frames or dispersing it along the hive entrance path	Reddy *et al.*, 2013; Mondal *et al.*, 2016; Ruiu, 2018
			Powder Dispenser tray	Placing filter paper between frames or sprinkling it along the frames or within the path of the hive entrance is a recommended method for beekeepers	
			Powder	Dust particles scattered amidst the frames / tiny particles lodged between frames	
			Liquid	Sprayed frames	
			Fungal culture	Walk for a few minutes	
			Spore solution	A brief period of submersion	
			Commercial preparation suspended in water	Sprayed frames	
Hirsutella spp.	Chitobiase and chitinase	Almite, Green Hirsutella, Lancer, No-mite	Fungal culture	Walk for a few minutes	Mondal *et al.*, 2016; Ruiu, 2018
			Spore solution	Immersion for a few seconds	
Lecanicillium lecanii (formerly *Verticillium lecanii*)	Catalase, chitinase and protease	Bio-Catch, Biogade-V, Biovert Rich, Cropfit, Green Heal, Mealikil, Varunastra, Verticill, Verticide, Vertimust, Vertifire-L, Mycotal/ Koppert/Netherlands	Fungal culture	Take a few minutes to stroll	Reddy *et al.*, 2013; Mondal *et al.*, 2016; Ruiu, 2018; Kumar *et al.*, 2020
			Liquid	Sprayed frames	
			Spore solution	Immersion for a few seconds	
			Wettable Powder	Particles dispersed among the frames/ particles scattered amidst the frames	
Paecilomyces spp.	Protease	Bioact WG, Bio-Nematon, MeloCon, Mytech-WP, Mysis, Nematonashak, Nematox, Nematofree, Niyantran, No-Fly-WP, Paecilo, Paecilomite, Paci Hit Rich	Spore solution	Immersion for a few seconds	Vega & Blackwell, 2005; Reddy *et al.*, 2013); Mondal *et al.*, 2016); Ruiu, 2018)
			Wettable Powder	Dusted between frames/strips between frames	
Myrothecium verrucaria	Bilirrubin oxidases endochitinase, pectinases and xylanases	DiTera	Fungal culture	Walk for a few minutes	Millhollon *et al.*, 2003); Moreira *et al.*, 2005); Khalil *et al.*, 2010)
			Spore solution	Immersion for a few seconds	

Table 3. Important commercial products of entomopathogenic bacteria

Entomopathogenic Bacteria	Commercial Product	Used Against	References
Bacillus thuringiensis var. *aizawai*	Certan	Caterpillars of the wax moth	Kumar *et al.,* 2020
Bacillus thuringiensis var. *galleriae*	Spicturin	*Earias vitella, Helicoverpa armigera* and *Plutella xylostella*	Srinivasan & Ganandhi, 2020
Bacillus thuringiensis var. *israelensis*	Aquabee, Bactimos, Gnatrol, LarvX, Mosquito Attack, Skeetal, Teknar, Vectobac	Immature stages of Aedes and Psorophora mosquitoes, black flies, and fungus gnats	Filha *et al.,* 2014
Bacillus thuringiensis var. *kurstaki*	Bactur, Bactospeine, Bioworm, Caterpillar Killer, Dipel, Futura, Javelin, SOKBt, Thuricide, Topside, Tribactur, Worthy Attack	Caterpillars (larvae of moths and butterflies)	Kalha *et al.,* 2014
Bacillus thuringiensis var. tenebrionis	Foil, M-One, M-Track, Novardo, Trident	larvae of the Colorado potato beetle and the adult elm leaf beetles	
Paenibacillus (formerly *Bacillus*) *popilliae* and *Bacillus lentimorbus*	Doom, Japidemic, Milky Spore Disease, Grub Attack	Larvae (grubs) of Japanese beetle	Glare *et al.,* 2017
Bacillus sphaericus	Vectolex CG, Vectolex WDG	The immature stages of Culex, Psorophora, and Culiseta mosquitoes, along with the early developmental phases of specific Aedes species' mosquitoes	
Burkholderia spp.	Majestene, Venerate	Chewing and sucking insects and mites	Ruiu, 2018
Chromobacterium subtsugae	Chromo, Grandevo	Colorado potato beetle (*Leptinotarsa decemlineata*), *Nezara viridula,*	Martin *et al.,* 2007; Kumar *et al.,* 2020
Saccharopolyspora spinosa	Tracer 120, Conserve	Spinosad is used against leafminers (*Liriomyza* spp.)	Schoonejans & van der Staaij, 2001; Ruiu, 2018
Serratia entomophila, Serratia proteamaculans	Bioshield, Invade	Triggering amber disease in the New Zealand grass grub (Costelytra zealandica)	Townsend *et al.,* 2004

bacteria for insect control emerged with the unveiling and progress of *Bacillus thuringiensis* (Bt) strains, which produced insecticidal proteins proven effective against agricultural pests. A significant milestone occurred in 1978 with the discovery of *B. thuringiensis* serovar *israelensis* (Bti) by Goldberg and Margalit, revolutionizing the fight against Diptera larvae (de Barjac, 1978). This particular entomopathogenic bacterium swiftly evolved from its initial characterization to practical field application (Margalit & Dean, 1985; Guillet *et al.*, 1990; Becker, 1997). Its rapid development was primarily triggered by the formidable resistance issues that synthetic insecticides faced in vector management programs during that period.

ACTION MECHANISM OF ENTOMOPATHOGENIC BACTERIA

Entomopathogenic bacteria refer to bacteria that pose harm to insect pests. Researchers have extensively investigated the potential of these bacteria as agents for biological pest control. How entomopathogenic bacteria infiltrate insect hosts varies based on the specific bacterium and type of insect. Insects often ingest various entomopathogenic bacteria through consumption. They can be found in the soil or on plant surfaces, and insects are exposed to them while eating. *It* is a well-known entomopathogenic bacteria that generates insecticidal toxins. Insect larvae commonly enter by the ingestion of *Bt* spores or crystals (Bravo *et al.*, 2007). Some entomopathogenic bacteria can pass through the cuticle (the insect's exterior protective covering) and infect the insect straight via the integument. The nematodes transport the bacteria into the insect host, and the bacteria subsequently enter the insect by cuticle penetration (Eleftherianos *et al.*, 2007). Some entomopathogenic bacteria can penetrate the respiratory system of the insect host. This method of entrance is less prevalent, but it has been detected in a few cases (Akhurst *et al.*, 1990). The mechanism through which entomopathogenic bacteria operate encompasses multiple strategies to invade, proliferate within, and ultimately eliminate their insect hosts. These bacteria produce toxins that pose a threat to insects. These toxins are often specific to particular insect types and significantly contribute to the bacteria's ability to cause disease. For instance, *Bacillus thuringiensis* (*Bt*) generates crystal proteins (known as Cry toxins) which, upon ingestion by susceptible insects, harm the lining of the insect gut and induce septicemia (Bravo *et al.*, 2007). Certain bacterial entomopathogens generate insecticidal toxins, including proteins such as Cry, Cyt, Vip, and Bin toxins (Adang *et al.*, 2014). Spore-forming bacterial entomopathogens encompass *Bacillus* spp., *Paenibacillus* spp., and *Clostridium* spp., while non-spore-forming types include *Pseudomonas, Serratia, Yersinia, Photorhabdus,* and *Xenorhabdus*. In susceptible insect hosts, ingestion of these bacteria leads to infection (Dara, 2017). Entomopathogenic bacteria frequently contain mechanisms to inhibit the immune system of the insect, allowing the bacteria to avoid host defences and infect the insect. Some bacteria, for example, interfere with the insect's cellular immune response, making it simpler for the bacteria to multiply (Eleftherianos *et al.*, 2007). Extracellular enzymes produced by certain entomopathogenic bacteria destroy the tissues of the insect, allowing invasion and colonization of the host. *Serratia* spp., for example, produces chitinases and proteases that aid in the breakdown of the insect cuticle and other tissues (Blackburn *et al.*, 2003). Through the collaborative action of enzymes and the formation of appressoria, these biological components facilitate the penetration of the insect's cuticle. Upon penetrating the host, they commence an infection procedure, nourishing themselves with the insect's tissues, ultimately resulting in the insect's demise (Venkatesh *et al.*, 2022). Some entomopathogenic bacteria create biofilms on the cuticle of insects, offering protection and assisting in infection establishment. *Xenorhabdus* spp., for example, is known to generate biofilms that contribute to their pathogenicity (Yang *et al.*, 2015).

Biological control methods, such as utilizing bacterial entomopathogens, are widely acknowledged as safer alternatives to chemical pesticides and present several advantages. These methods offer a more intricate mode of action compared to conventional pesticides, as they target multiple locations where resistant pests are prone to develop (Ruiu, 2015). While entomopathogenic bacteria can be used as a standalone pest management technique, their optimal effectiveness and environmental sustainability are achieved when used in rotation or conjunction with insecticides. Numerous studies have highlighted the synergistic potential of chemical substances and entomopathogenic microorganisms (Musser *et al.*, 2006). Further advantages include the incorporation of biopesticides into pest control strategies, improved safety for workers, decreased crop residues, and greater flexibility in harvesting, often requiring minimal to no pre-harvest intervals. Many bacterial infections in insects are attributed to specific groups, including Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae. While some of these bacteria pose significant threats, the majority are moderate pathogens that target insects under stress. Research has primarily concentrated on the Bacillaceae family. For instance, *Bacillus sphaericus* (Bacillales: Bacillaceae) is transmitted by mosquitoes, while Bacillus popillae (Bacillales: Bacillaceae) induces milky disease in scarabaeids. One of the well-known entomopathogenic agents, *Bacillus thuringiensis* (Bacillales: Bacillaceae), commonly referred to as *Bt* is frequently utilized to manage caterpillars and beetles. *Bt* bacteria produce spores and a proteinaceous protoxin crystal possessing insecticidal properties, often associated with sporulation. When these crystals are ingested, they degrade in the insect's stomach and, upon interaction with host proteases, release an active toxin known as endotoxin.

Entomopathogenic viruses

Insect viruses have a historical record dating back over 2000 years. Palaeontologists studying insects preserved in amber have made a significant discovery — adult sand fly fossil. This fossilized insect contained a range of pathogens, including fungi, trypanosomes, nematodes, cytoplasmic polyhedrosis viruses, and nucleopolyhedrovirus. This finding suggests a historical association between viruses and insects, dating back approximately 15 to 200 million years ago (Poinar & Poinar, 2005). The field of insect virology, which resides within the realms of invertebrate pathology and insect pathology, has historical roots in the identification of infections in honeybees. Aristotle, around 330 BC, was among the earliest to document the recognition of infections in honeybees through the observation of symptoms. Girolamo Fracastoro, an Italian physician from the sixteenth century, was among the first to propose the idea that infectious agents could cause the spread of diseases (Taylor, 2014). Entomopathogenic Viruses (EPVs), commonly referred to as insect-killing viruses, have emerged relatively recently. The introduction of a virus-based insecticide in the United States took place in 1970 to control the cotton bollworm. This step marked a significant milestone, considering the extensive global testing of various viruses for managing insect pests, a practice that has been ongoing since the early 1900s (Ignoffo, 1973). Several viruses have been approved for controlling insect pests (Table 4), while ongoing research aims to identify and evaluate additional viral candidates (López-Ferber, 2020). Viruses have demonstrated the capacity to infect and eliminate various insects across different orders. EPVs, identified as RNA (comprising both double-stranded and single-stranded) and DNA (comprising both double-stranded and single-stranded), have been acknowledged historically since the seventeenth century. Numerous viruses have been intentionally engineered to target different pests within worldwide agricultural ecosystems.

Nucleopolyhedrosis, previously known as brasserie or jaundice, was detected in both honeybees (*Apis mellifera* L., Hymenoptera: Apidae) and silkworms (*Bombyx mori* L., Lepidoptera: Bombycidae). Viral particles encapsulate nucleic acids within a protein coat called a capsid, crucial for infecting host cells. Once infected, the virus seizes control of the host's metabolic system, prompting rapid cell division until the host cell's demise. Viruses, as obligatory parasites, are unable to multiply outside a host cell. The EPVs (Entomopathogenic Viruses) have been classified into 12 viral families by the International Committee on Taxonomy of Viruses (ICTV) as documented by van Regenmortel 2000). These viruses are known for their significant host specificity, often leading to a reduction in host populations. Notably, viruses belonging to three insect-specific families—Baculoviridae, Polydnaviridae, and Ascoviridae—demonstrate high host specificity and pose no threat to beneficial insects and non-target animals, including mammals. Among these families, Baculoviridae has garnered attention as an environmentally friendly alternative to chemical pesticides due to its perceived ecological benignity. Among baculoviruses (dsDNA), Granulovirus (GV) and Nucleopolyhedrovirus (NPV) are extensively studied for their potential to control lepidopteran pests on crops. Virus particles can enter an insect's digestive system, attaching themselves to specific receptors and passing through epithelial cells to infect the haemocoel, critical organs, and tissues, particularly targeting the fat bodies. Insects affected by baculoviruses often display a whitish appearance due to a substantial infection in their fat bodies, which leads to a gradual thinning of their exoskeleton. As the illness progresses, the integument may rupture. In the advanced stages of the disease, infected larvae may position themselves in a distinct inverted 'V' shape and discharge a greyish to creamy liquid along with numerous Occlusion Bodies (OBs). These OBs play a crucial role in transmitting the infection within the environment (Granados & Williams, 1986).

Baculoviruses

The family Baculoviridae comprises some of the most extensively researched groups of entomopathogenic viruses. Their application in agricultural systems is extensive, and there has been notable progress in the development of bio-pesticides based on baculovirus. They lessen the need for chemical insecticides because they are target-specific and environmentally friendly. They have been employed in the management of numerous pests, including the coddling moth and gipsy moth (Cory & Myers, 2003). NPVs are a subclass of baculoviruses that have been successfully employed in the management of several significant insect pests. Because of their strong host specificity, they have less of an effect on non-target species (Harrison & Hoover, 2019). To manage harmful insect pests like the western tent caterpillar and the spruce budworm, baculoviruses have been employed in forest management. This method aids in preventing damage and defoliation of forests (Skinner & Hunter, 2001).

Cytoplasmic Polyhedrosis Viruses (CPVs)

One such class of entomopathogenic viruses are called CPVs. They mostly infect insects that belong to the Lepidoptera order. Entomopathogenic viruses have undergone extensive examination for potential applications in agriculture and are recognized for their effectiveness in combating forest pests. Utilizing these viruses alongside other biological control agents, such as parasitoids and predators, has been explored as a strategy to enhance the overall effectiveness of pest management techniques (Hajek & Eilenberg, 2018). The potential use of CPVs in managing aquatic insect larvae—which can be pests in aquaculture—has been studied. In aquaculture ponds, the viruses can infect and kill insect larvae (Tang & Lightner, 2007). Potential applications of CPVs in biotechnology have been investigated, such as serving as viral vectors in insect cell culture systems to produce proteins and express genes (Huger & Käufer, 2008).

Cypovirus

Studying entomopathogenic cypoviruses has advanced our understanding of their interaction with the immune system of insects and shed light on the pathophysiology of viral infections in these creatures. The primary threat to apple and pear orchards is the codling moth, known to be susceptible to the *Cydia pomonella* granulovirus (CpGV). It is employed in this pest's biological control (Jehle *et al.*, 2009). Potential uses for CPVs in biotechnology include the use of these viruses as viral vectors in insect cell culture systems to produce proteins and express genes (Huger & Käufer, 2008).

Iridovirus (EIVs)

EIVs are a more environmentally friendly option to chemical insecticides. Wang *et al.* 2015). To comprehend the mechanisms underlying viral replication, pathogenicity in insects, and interactions with hosts, EIVs are researched. The field of insect virology benefits from these investigations Wang *et al.* 2009). EIVs can be used as model systems for studying antiviral strategies, such as developing antiviral medications and analysing how the host immune system responds to viral infections in insects (Wang *et al.*, 2008).

To increase the effectiveness and shelf life of entomopathogenic viruses, future research may concentrate

Table 4. Entomopathogenic viruses for the management of some important insect pests

Entomopathogenic Virus	Used against	Order	Cultivated host crops	References
Cydia pomonella Granulovirus (CPGV)	Codling moth *(Cydia pomonella)*	Lepidopteran	Apple, pear, walnut, etc.	Zimmermann, 2008
Helicoverpa armegera Nucleopolyhedrovirus (HaNPV)	Cotton bollworm (*Helicovarpa armegera)*	Lepidopteran	Cotton, okra, Chilly, etc.	Fuxa, 2004
Aphidius ervi picorna- like virus (AePV)	Aphids	Hemipteran	Potato, cabbage, mustard, apple, etc.	Liu *et al.,* 2016
Autographa californica Nucleopolyhedrovirus (AcMNPV)	Green peach aphids *(Myzus persicae)*	Hemipteran	Peach, tomato, pepper, potato, Etc.	Yao *et al.,* 2018
Drosophila C Virus (DCV)	*Drosophila* sp.	Dipteran	Berries, citrus, melons, grapes, etc.	Fleuriet, 2018
Colorado Potato Beetle Virus (CPBV)	Colardo potato beetle *(Leptinotarsa decemlineata)*	Copleoptera	Tomato, eggplant, etc.	Huger, 2005
Western Corn Rootworm Virus (WCRV)	Corn Rootworm *(Diabrotica barberi)*	Coleopteran	Corn, sorghum, grasses, etc.	Paul & Storer, 2013
Amsacta moorei entomopoxvirus (AMEV)	Spotted cutworm *(Amsacta moorei)*	Lepidopteran	*Chrysanthenum*, dahlia, gladiolus, etc.	Harrison *et al.*, 2014
Agrotis segetum granulovirus (AgseGV)	turnip moth *(Agrotis segetum)*	Lepidopteran	Lettuce, melon, Asparagus, etc.	Zwart & Dicke, 2007
Spodoptera exigua nuclear polyhedrosis virus (SeNPV)	armyworm *(Spodoptera exigua)*	Lepidopteran	Maize, sorghum, cotton, etc.	Moscardi, 1999
Mamestra configurata entomopoxvirus (McEPV)	bertha armyworm *(Spodoptera frugiperta*)	Lepidopteran	Canola, alfalfa, rapeseed, etc.	Arif *et al.*, 2007
Junonia coenia densovirus (JcDNV)	Common buckeye *(Junonia coenia)*	Lipidoptera	Plantain, snapdragon, Toadflax, Monkeydragon, etc.	Li *et al.*, 2002

on refining their formulation and delivery techniques. Scientists are attempting to create formulations that increase the commercial viability of entomopathogenic viruses by extending their shelf life. This entails keeping the virus safe from environmental stresses and optimizing storage conditions (Lacey *et al.*, 2015). It is possible to create formulations that increase the viral particles' adherence to plant surfaces or pests. The chance of infection rises with increased persistence on the target insects (Gindin *et al.*, 2006).

Wang and Jehle 2009) suggest that genetic engineering of entomopathogenic viruses could be a promising avenue for improving host specificity and reducing non-target impacts. To ensure that entomopathogenic viruses only infect particular pest species and spare non-target organisms, genetic engineering can be employed to limit the host range of these viruses (Huber *et al.*, 2004). By expressing viral proteins in response to certain cues from target insects, pest-selective promoters in genetic engineering enable the virus to become active only when it is near the target (Cory & Franklin, 2012). Further investigation and the identification of novel entomopathogenic viruses could expand the range of pests that can be targeted (Stasiak *et al.*, 2021).

The entomopathogenic virus is typically named after its specific target host, as indicated by the host's initial. For instance, LdMNPV denotes Lymantria dispar multicapsid nucleopolyhedrovirus. Commercially available products based on baculovirus exhibit primary effectiveness against chewing insects, particularly Lepidopteran caterpillars (Table 5). Nevertheless, due to the inherent instability of baculovirus formulations in the environment and the high production costs associated with their replication within their host, their utilization in biological pest management remains confined to niche market segments (Harrison & Hoover, 2012; Sun, 2015).

Entomopathogenic nematode

Entomopathogenic nematodes are diminutive round worms. Over 35 species of these nematodes have been identified, found widely across all continents except Antarctica, primarily isolated from soil or natural hosts. In their natural habitat, these nematodes act as obligate pathogens of insects, offering potential as bioinsecticides to combat arthropod pests. They utilize symbiotic bacteria to assist in the termination of their hosts; Heterorhabditid nematodes form partnerships with the bacteria *Photorhabdus* spp., whereas steinernematids form associations with *Xenorhabdus* spp.

Table 5. Selection of commercial products based on entomopathogenic viruses

Active Substances	Commercial Names	Main Targets
Helicoverpa zea nucleopolyhedrovirus	Heligen	*Helicoverpa* spp. and *Heliothis virescens*
Spodoptera litura nucleopolyhedrovirus	Biovirus-S, Somstar-XL	*Spodoptera litura*
Adoxophyes orana granulovirus (AoGV)	Capex	Summer fruit tortrix moth (*Adoxophyes orana*)
Cryptophlebia leucotreta granulovirus	Cryptex	False codling moth (*Thaumatotibia leucotreta*)
Helicoverpa armigera nucleopolyhedrovirus (HearNPV)	Biovirus-H, Helicovex, Helitec, Somstar-Ha	African cotton bollworm (*Helicoverpa armigera*), Corn earworm (*H. zea*) and other *Helicoverpa* species (*H. virescens*, *H. punctigera*)
Helicoverpa zea Nuclear Polyhedrosis virus	Gemstar	*Heliothis* and *Helicoverpa* species
Plutella xylostella granulovirus	Plutellavex	*Plutella xylostella*
Spodoptera littoralis nucleopolyhedrovirus (SpliNPV)	Littovir	African cotton leaf worm (*Spodoptera littoralis*)
Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV)	Gypchek	*Lymantria dispar*
Cydia pomonella granulovirus (CpGV)	CYD-X, Madex, Carpovirusine	*Cydia pomonella*
Neodiprion abietis nucleopolyhedrovirus (NeabNPV)	Neodiprion abietis NPV	*Neodiprion abietis*
Spodoptera exigua nucleopolyhedrovirus (SeNPV)	Spexit, Spod-X	*Spodoptera exigua*

Table 6. The utilization of Entomopathogenic Nematodes (EPN) for commercial purposes as bio-insecticides

EPN Species	Major pest(s) targeted	Host/Habitat	Commercial product	Formulation	Reference
Steinernema abbasi (=*S. thermophilum*)	Asian tiger mosquito (*Aedes albopictus*), cotton bollworm (*Helicoverpa armigera*)	Cotton, corn, pigeon pea, chickpea, sunflower, chilli, tomato, and okra	CS-39	Hydrogel, talc	Srivastava *et al.*, 2022
Steinernema carpocapsae	Common pests found in gardens or agricultural settings include billbugs, cutworms, armyworms, sod webworms, chinch bugs, crane flies, banana moths, codling moths, cranberry girdlers, various clearwing borer species, black vine weevils, peachtree borers, and shore flies like those from the Scatella species.	Orchards, ornamental crops, vegetables and turfgrass	Boden-Niitzlinge, Biosafe, Biovector, Bouncer, CAPSANEM, Carpocapsae-System, DD136, Green Commandos, Helix, Mioplant, Nemabakt, Sanoplant, X-GNAT	Alginate gel, bulk, clay, dispersable granule, granular, flowable gel, liquid concentrate, polymer, sponge, vermiculite, water-dispersible granule, wettable powder	Ruiu, 2018; Askary & Abd-Elgawad, 2021; Sarwar & Mukhtar, 2021
Steinernema feltiae	Fungus gnats (*Bradysia* spp.), shore flies, western flower thrips, leaf miners	Oak, deciduous forests, new plantation and fruit orchards	Entonem, Exhibit, Magnet, Nema Globe, Nemasys, Owinema SC, Stealth	Alginate gel, bulk, clay, dispersable granule, granular, polymer, sponge, vermiculite, water-dispersible granule, wettable powder	Ruiu, 2018; Askary & Abd-Elgawad, 2021; Sarwar & Mukhtar, 2021

Table 6. Continued...

Steinernema glaseri	White grubs (scarabs, especially scarab beetles, *Adoretus tenuimaculatus*, Japanese beetle, *Popillia* sp.), *Anomala* sp., banana root borers, *Cosmopolites sordidus*	small fruits, tree fruits, truck, garden crops, ornamental shrubs, vines, vegetable fields and turfgrass	Fifty thousand IJs of *Steinernema glaseri* strain SBILN1 per gram of powder	Flowable gel, wettable powder	Ruiu, 2018; Li *et al.*, 2023
Steinernema kraussei	Black vine weevil (*Otiorhynchus sulcatus*)	Fruit orchards/soil	Nemasys L	Clay, polymer	Ansari *et al.*, 2010; Ruiu, 2018
Steinernema kushidai	scarabaeid beetle larvae (*Anomala cuprea*)	Vegetable fields	SDS Biotech	Clay, polymer	Deans & Krischik, 2023
Steinernema riobrave	Citrus root weevils (*Diaprepes* spp.), mole crickets	Fruit orchards	Bio vector	Liquid concentrate, water-dispersible granule	Lacey & Georgis, 2012; Koppenhöfer *et al.*, 2020
Steinernema scapterisci	Mole crickets (*Scapteriscus s*pp.)	Fruit orchards	Proactant Ss	Flowable gel, wettable powder	Frank, 2009
Heterorhabditis bacteriophora	Agricultural pests include white grubs, known as scarabs, cutworms, black vine weevils, flea beetles, corn rootworms, and citrus root weevils	Oak, deciduous forests, new plantation and fruit orchards	E-Nema Gmbh, Otinem, Soil Commandos	Bulk, clay, dispersable granule, granular, polymer, sponge, wettable powder	Ruiu, 2018; Sarwar & Mukhtar, 2021; Li *et al.*, 2023
Heterorhabditis downesi	Black vine weevil (*Otiorhynchus sulcatus*)	Ornamental crops as well as strawberries and other fruit crops	Nemamax, NemaTrident-CT	Wettable powder	Ruiu, 2018;
Heterorhabditis indica	Fungus gnats, root mealybugs, grubs	Fruit orchards	Soldier	Talc, wettable powder	Li *et al.*, 2023
Heterorhabditis marelatus	White grubs (scarabs), cutworms, black vine weevils	Berries/fruit orchards and ornamentals	-	Sponge	Kajuga *et al.*, 2018; Shakeel *et al.*, 2022
Heterorhabditis megidis	Weevils	Vegetable fields	Nemasys, Larvanem, NovoNem	wettable powder	Lacey & Georgis, 2012
Heterorhabditis zealandica	Scarab grubs	Ornamentals, vegetable fields and turf	-	wettable powder	Lacey & Georgis, 2012
Phasmarhabditis hermaphrodita	Grey field slug (*Deroceras reticulatum*), snails	Oilseed rape crop	Nemaslug	polymer, clay	Ruiu, 2018; Schurkman *et al.*, 2022

The intrinsic characteristics of entomopathogenic nematodes make them promising agents for pest control. Notably, they actively seek out their hosts (Tumialis *et al.*, 2019). Entomopathogenic nematodes, specifically those within the *Steinernema abbasi* and *Heterorhabditis* genera, exhibit effectiveness as insect biological control agents when produced through *in vitro* or *in vivo* methods (Table 6). *In vivo,* production generates high-quality nematodes, but the associated costs, stemming from obtaining insects and labour, resulting in a low economy of scale. There's a possibility of cost reduction if growers can produce their nematodes. However, current grower-based production systems are not sustainable as they rely on external sources to acquire or calibrate inoculum (Oliveira-Hofman *et al.*, 2023).

CONCLUSIONS WITH FUTURE PERSPECTIVE

Ensuring the sustainability of agroecosystems while safeguarding the environment and human health necessitates a wide range of biopesticides targeting various crop pests. Farmers' escalating needs are being met with a rising array of newly developed and enhanced products in the market. These can be applied individually, in rotation, or tandem with traditional chemicals, catering to diverse pest management strategies. In integrated pest management (IPM), insect pests are managed with the use of entomopathogens. They offer a sustainable substitute for chemical insecticides. Entomopathogens support sustainable and ecologically friendly farming methods by reducing the need for chemical pesticides, thereby lessening the negative effects of agriculture on the environment. Entomopathogens support biodiversity and are a component of natural ecosystems. They contribute to the control of insect populations and the preservation of ecological equilibrium. Studying entomopathogens and their application in pest control presents valuable educational prospects in entomology, microbiology, and environmental science. This field offers significant implications for understanding disease dynamics and sheds light on the co-evolution of pathogens alongside their hosts, thereby providing crucial insights into host-pathogen interactions.

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