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Entomopathogens: Role of Insect Pest Management in Crops

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ABSTRACT

Entomopathogens are microorganisms that pathogenic to insect pest. Several species of naturally occurring viz; fungi, bacteria, viruses and nematodes, infect a variety of insect pests and play an important role in agricultural crops controlling insect pest management. This kind of biopesticide has many advantages and alternative to chemical insecticides, highly specific, safe, and environmentally sustainable. Pest problems are an almost inevitable part of agriculture. They occur largely because agricultural systems are simplified and modifications of natural ecosystems. Viruses, bacteria are host specific and fungi generally have broader host range and can infect both underground and aboveground pests, soil-dwelling nature nematodes are more suitable for managing soil pests. Growing crops in monoculture provides concentrated food resource that allows pest populations to achieve higher densities in natural environments. Some of the most important problems occur when pests develop resistance to chemical pesticides. These cause highly significant damage to crops, there are also threats from emerging new strains of pests. Crops cultivation can make the physico-chemical environment more favourable for pest activity. Agricultural pests are reducing the yield and quality of produce by feeding on crops, transmitting diseases. Agricultural production significantly loss crop yields, suggest that improvements in pest management are significant forward for improving yields. Crop growers are under immense pressure to reduce the use of chemical pesticides without sacrificing yields, but at the same time manage of pests is becoming difficult due to pesticide resistance and the decreasing availability of products. Alternative methods are needed urgently. These need to be used as part of Integrated Pest Management safety and environmental impact.

***Keywords*:** Entomopathogenic Fungi; Bacteria; Viruses; Nematodes; Management

1. Introduction

Agricultural pests include plant pathogens (fungi, oomycetes, bacteria, viruses and nematodes), weeds, arthropods (primarily insects and mites), molluscs (slugs and snails) and a small number of vertebrates. They reduce the yield and quality of produce by feeding on crops. There are estimated different pest species worldwide. They are a significant constraint on agricultural production, responsible for around 40% loss of potential global crop yields. These losses occur despite the considerable efforts made at pest management in significant way and improving yields.

Insect pest problems are an almost foreseeable part of agriculture. These losses occur despite the significant efforts made at pest management for improving yields. They occur largely because agricultural systems are simplified and modifications of natural ecosystems. The management of agricultural disrupts the ecological and forces potential to pest species in natural ecosystems food availability, predation, and competition. Thus, growing crops in monoculture provides a concentrated food resource that allows pest populations to achieve higher densities than they in natural environments. Cultivation can make the physico-chemical environment more favourable for pest activity. Using broad spectrum pesticides is destroying natural beneficial microorganisms (entomopathogens). Bacteria, fungi, virus, Protozoa and nematodes are frequently used as bio-insecticides. Entomopathogens are microorganisms that are pathogenic to insect pests and mites. Several species of naturally occurring bacteria, fungi, nematodes, and viruses infect a variety of insect pests and play an important role in their management. Some entomopathogens are mass-produced in vitro (bacteria, fungi, and nematodes) or in vivo (nematodes and viruses) and sold commercially. Using entomopathogens as biopesticides in pest management is called microbial control, which can be a part of integrated pest management (IPM) against insect pests.

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There are two main taxonomic orders of entomopathogenic fungi. The Entomophthorales occur in the phylum Zygomycota and include genera such as Pandora, Entomophthora and Conidiobolus. These fungi often cause natural epizootics in insect and mite populations. Many of the species are obligate pathogens that show specific ecomorphological adaptations to the life cycles of their hosts, they are very difficult to mass produce in culture. Despite this, they can be used for pest control applied in the field. An isolates of Neozygites floridana from S. America investigated for classical biological control of the cassava green mite (Mononychellus tanajoa), a major pest of cassava in Africa. Natural epizootics of entomophthoralean fungi are being used as a natural form of pest control as developed by Don Steinkraus at the University of Arkansas is used to inform cotton growers in the southeast USA about outbreaks of Neozygites fresenii in cotton aphids (Aphis gossypii). These outbreaks can reduce aphid populations by about 80% in 4 days.

The second major order of entomopathogenic fungi is Hypocreales, occurs in the phylum Ascomycotina. There are many species in the Ascomycotina in which the sexual phase (teleomorph) is not known, these fungi are commonly known as anamorphic fungi. Sexually reproducing hypocrealean fungi occur in the genera Cordyceps and Torrubiella. Cordyceps species are important natural parasites of many insect and mite species in tropical forests. Cordyceps sinensis is a pathogen of ghost moth (Thitarodes) caterpillars that occur at high altitude in the Himalayas (Tibet and China). Important anamorphic genera of entomopathogenic fungi include Beauveria, Isaria, Metarhizium and Lecanicillium. Species from all these genera are used as biopesticides of insect pests. The spores of many species of the anamorphic entomopathogenic fungi can be mass produced on variety of culture media and suitable for development as biopesticides which are applied inundatively to pest populations. They have range of desirable characteristics including safety to people, compatibility with other natural enemies, lack of toxic residues and possibility of providing persistent control by multiplying in the pest population. Because they have contact action, they are good for the control of sap feeding pests, like aphids and whiteflies, which cannot be infected by other types of biopesticide (bacteria and viruses) which are active only when ingested. Commercial products are available to growers, such as Vertalec for control of glasshouse aphids is based on fungus Lecanicillium longisporum and Mycotal for glasshouse whitefly is based on fungus Lecanicillium muscarium. Beauveria bassiana and Metarhizium anisopliae are for the control of vine weevils. Insect pest are the largest group of animals and causes the most damage in cultivated crops. It is important to understanding the biology of their natural enemies. Entomopathogenic fungi are very heterogeneous group, pathogenic to insect effectiveness in infecting their hosts. Entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems, some of them can be found practically throughout the world. Such species may be Beauveria bassiana, Metarhizium anisopliae and Isaria farinosa which is reported. There are different groups of entomopathogenic fungi in different habitats commonly found in soil. B. bassiana seems to be very sensitive to the disturbance effects of cultivation and thus restricted to natural habitats. The ability of M. anisopliae to persist in cultivated soils is well established. Therefore the first is more frequent in forest, and second in arable soils (Rath *et al*., 1992; Vänninen, 1995; Quesada-Moraga *et al*., 2007; Sánchez-Pena *et al*., 2011). The great majority of species belong in the genus Cordyceps (Ascomycota: Hypocreales) (Evans, 1982; Aung *et al*., 2008). While other species of Hypocreales such as Beauveria, Metarhizium and Isaria were the dominant fungi found on soil insects (Keller & Zimmerman, 1989).

Biological control agents (BCAs) of parasites and microbial pathogens, such as nematodes, fungi, bacteria, viruses and protozoa, which cause lethal infections. Some agents are being used as application of parasitic nematodes against slugs, use of viruses to control codling moth in apple orchards. The natural enemies inhabiting an agro-ecosystem play a key role in preventing pests reaching damaging levels. Biological control agents are used vary according to the type of pest the biological characteristics of the control agent. BCAs have a range of attractive properties that include host specificity, lack of toxic residue, no phytotoxic effects, human safety and the potential for pest management to be self-sustaining and successful use requires fundamental knowledge of the ecology of both the natural enemy and the pest. The roles played by many entomopathogenic fungi and propose new research strategies focused on alternate uses for these fungi. It seems likely that these agents can be used in multiple roles in protecting plants from pests and at the same time promoting plant growth.Their mode of action against insects involves attachment of the spore to the insect cuticle followed by germination, cuticle penetration, and internal dissemination throughout the insect. During this process, which may involve the production of secondary metabolites, the internal organs of the insect are eventually degraded. The development of Entomopathogens (fungi, bacteria, viruses and nematodes) as effective biological control agents requires knowledge of bioassay methods as well as production, formulation and application methodologies.

2. Entomopathogenic fungi

Entomopathogenic fungi are important biological control agents worldwide Entomopathogenic fungi are a group of phylogenetically diverse, heterotrophic, eukaryotic, unicellular or multicellular (filaments) microorganisms that reproduce via sexual and asexual or both and produce a variety of infective propagules. Environmental factors such as ultraviolet light, temperature, and humidity can influence the effectiveness of fungal entomopathogens in the field. Entomopathogenic fungi are concentrated in the orders: Hypocreales, Onygenales (Ascosphaera genus), Entomophthorales, and Neozygitales (Entomophthoromycota). The earliest known fossil of an insect pathogenic fungus, a scale insect (Hemiptera) infected by an Ophiocordyceps-like fungus (Sung *et al*., 2008), and later fossils include a termite (Isoptera) infected by an Entomophthora-like fungus and an ant (Hymenoptera) infected by Beauveria, both from (Poinar and Thomas, 1984) . Most of the taxonomic groups contain entomopathogenic genera, such as Metarhizium, Beauveria, Verticillium, Nomuraea, Entomophthora and Neozygites (Desphande, 1999). Entomopathogenic fungi are found in the divisions Zygomycota, Ascomycota and Deuteromycota (Samson *et al*., 1988), as well as Chytridiomycota and Oomycota, which were previously classified within Fungi. Entomopathogenic fungi most of them belong to the order Entomophthorales of the phylum Glomeromycota and Hypocreales of the phylum Ascomycota (Hibbett *et al*., 2007) Recent phylogenetic studies within entomopathogenic fungi resulted in significant revision of many species of entomopathogenic fungi, such species as Paecilomyces farinosus and P. fumosoroseus currently belong to the genus Isaria (Luangsa-ard *et al*., 2004) and species Verticillium lecanii to the genus Lecanicillium (Zare & Gams, 2001). Genus Beauveria (anamorphic fungus) is monophyletic within the Cordycipitaceae (Hypocreales), and has been linked developmentally and phylogenetically to Cordyceps species. Genus Cordyceps has many anamorphs, of which Beauveria, Lecanicillium and Isaria are the best known and described (Blackwell, 2010).

Entomopathogenic fungi can attack insects from different orders: Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, Hymenoptera. Some species of fungi (belongs to the Hypocreales) have a very wide spectrum of potential victims and Entomophthorales are pathogens only one particular species of insect. They are reported to infect a very wide range of insect pest and mite species including lepidopterous larvae, aphids and thrips which are of great concern in agriculture worldwide (Roberts and Humber, 1981). Entomopathogenic fungi cause lethal infections and manage insect and mite population in nature. They are host specific with a very low risk of attacking non-target organisms.

The fungi produce spores (conidia and blastospore) which infect their host through the external cuticle by germinating on its surface and then growing into its body. The process of infection involves: adhesion of the spore on the insect cuticle, penetration of the cuticle by the germ tube, development of the fungus inside the insect body and colonization of the hemocoel by fungal hyphae. The spores of the entomopathogenic fungi are usually covered with a layer of mucus composed of proteins and glucans, which facilitates their attachment to the insect cuticle and produce specialized structures called appressoria (attachment of germinating spore). The process of penetration of the insect cuticle is a result of mechanical pressure and hydrolytic enzymatic activity (lipases, proteases and chitinases) of the germ tube (Xiao *et al*., 2012. Zheng *et al*., 2011) Vegetative growth in the insect hemocoel is common to most entomopathogenic fungi (Roberts and Humber, 1981). Death of an insect is usually a result of mechanical damage caused by growing mycelia inside the insect (mummification), or toxins produced and released by the pathogen. Beauveria, Metarhizium, and Tolypocladium are known that secrete toxins such as destruxin, bavericin, and efrapeptins are fully described chemically, and is known their action and contribution in the process of pathogenesis (Roberts, 1981;

Hajek & St. Leger, 1994). After death, the fungus produces thousands of new spores on the dead body, which disperse and continue their life cycle on new hosts.

3. Entomopathogenic bacteria

The majority of bacterial pathogens of insects occur in the family Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae. Most of these bacteria are weak pathogens that infect insects subject to environmental stress, but a minority are highly virulent. Most attention has been given to the Bacillaceae. Bacillus popillae causes milky disease in scarbaeids, while Bacillus sphaericus is a lethal pathogen of mosquitoes. Bacillus thuringiensis (Bt) is widespread in soil and most widely used entomopathogenic agent and control of caterpillars, beetles. Bt is a spore forming bacterium. Sporulation is usually associated with the synthesis of a proteinaceous protoxin crystal that has insecticidal activities. Ingested crystals dissolve within the gut and cleaved by host proteases to form an active toxin, termed the δ-endotoxin. This bacterium is the production of parasporal bodies (crystals) containing specific insecticidal endotoxins (Cry proteins) acting by ingestions through a pore-forming mechanism of action detrimental for the insect gut epithelium (Pigott and Ellar, 2007). Different studies to evaluate the effects of Bt toxins on both insect pests (Pérez-Guerrero *et al*., 2012) and non-target species (Marchetti *et al*., 2012). The mode of action of this new entomopathogenic bacterium is complex and different are the metabolites that have recently been associated to the insecticidal effects (Asolkar *et al*., 2014).

Entomopathogenic spore forming bacteria, especially Bacillus thuringiensis (Bt), have been used extensively for control of insect pests in crops, Bt, the insecticidal activity of which is based on Cry toxins (δ-endotoxins), are now commercially available for use against a wide variety of insect pests including species of Lepidoptera, Coleoptera and Diptera. The specificity of toxins is determined by the molecular configuration of the toxin and the physiology of the host midgut and presence of toxin receptors on the midgut epithelium (Gill et, al., 1992., Schnepf *et al*., 1998). Non-target organism are not directly affected by exposure to commercial products Cry toxins used for control of lepidopteran pests (Krieg and Langenbruch, 1981, Melin and Cozzi,1990., Sims, 1997). A study of five weekly applications of low and high label rates of a genetically engineered isolate of Bt for control of L. decemlineata, resulted in fair to good control of the beetle with no detectable effects on non- target organism including predatory Hemiptera (Lacey *et al*., 1999).

Varieties of B. thuringiensis (Bt) have been referred to as entomopathogens, soil organisms, or saprophytic inhabitants of the phylloplane. Applications of Bt bioinsecticides to agroecosystems and other habitats usually do not result in a build-up of spores in the environment and decline in the viability of spores is observed, especially those exposed to sunlight (Ignoffo, 1992). As a microbial control agent, Bt is applied to infestations of insects and results in rapid kill of target insects usually without recycling. Large scale control programs that utilize Bt for suppression or eradication of pests. The safety and environmental impact of entomopathogenic bacteria should be evaluated in light of the risk for non-target organisms in comparison with other interventions an ecosystem. The genes that encode the Bt δ-endotoxin can be expressed in plants, which allows them to be protected against some species of insect pest.

Surveys of farmers field that growing Bt crops can result in significantly reduced applications of conventional insecticides. Secondary pest problems caused by mirid bugs have occurred on Bt cotton grown in China. These bugs were controlled previously by broad spectrum pesticides but are not controlled by Bt cotton. Problems with mirids in China did not occur until a few years after the widespread uptake of Bt cotton. Genetically modified (GM) maize and cotton crops that express lepidopteran active endotoxins have been available for a number of years and have revolutionised farming in the countries in which they are grown. Eight countries now grow of GM crops (India, USA, Canada, China, S Africa, Paraguay, Argentina, Brazil.) and negative effects do occur from GM crops.

4. Entomopathogenic viruses

Entomopathogenic viruses that are harmful to insect and have emerging recent years. A great variety of viruses attack and kill many insects. These viruses are called entomopathogenic viruses and have been found in many insect orders. Some insect pests are also susceptible to viral infections and hence, these viruses can be used as biological control agents. Insect viruses may be double or single-stranded DNA (dsDNA and ssDNA) or RNA (dsRNA and ssRNA).

Diseases caused by entomopathogenic viruses are known since the 16th Century. Several viruses attacked many pests of plants in agro-ecosystems of the world. A disease grasserie (Jaundice) now identified as nucleopolyhedrosis (GV) observed in silkworm (Bombix mori L. Lepidoptera) and another viral disease was describe in the honeybee (Apis mellifera L Hymenoptera). Virus partials contain a nucleic acid (RAN or DNA) is encapsulated with a protein coat known as capsid, which is play an important role in host cell infection process. Once viral partial gains entry in to the cell, its nucleic acid take charge host metabolic system and creating grate number of copies until cell die. Virus is considered as obligate parasite and cannot replicate ïn vitro". Entomopathogenic viruses are classified into 12 viral families by the International Committee on Taxonomy of Viruses (ICTV) (van Regenmortel *et al*, 2000). Viruses are very host specific and can cause significant reduction of host populations. Three families (Baculoviridae, Polydnaviridae, Ascoviridae) are specific for insects. The baculoviridae, has long been recognized as an environmentally safe potential alternative to chemical pesticides as the viruses are highly host specific, non-pathogenic to beneficial insects and other non-target organisms, including mammals. The baculovirus (ds DNA) is frequently studied and to identify the virus in this family that will most potential to control lepidopteran pests on crops and grouped into two main groups. Nucleopolyhedrovirus (NPV) and Granulovirus (GV). Both groups contain circular double-stranded DNA genome (Okano *et al*, 2006; van Oers and Flak, 2007). The baculovirus has been widely used for the production of numerous recombinant proteins in insect cells and mammals (Condreay and Kost, 2007). The development of external symptoms of entomopathogenic virus infection is very different in each group. The first detectable signs appear when the insect becomes slow in its movements, stops feeding, and growth practically ceases.

The mode of pathogenesis and replication of entomopathogenic viruses varies according to the family, but infection nearly always occurs by ingestion. Virions bind to receptors in the gut and penetrate epithelial cells. In the Baculoviruses, the infection often spreads to the haemocoel and then to essential organs and tissues, particularly fat bodies. Acute infections lead to host death in 5 – 14 days. In baculoviruses, infected insects become whitish in color because of the massive infection of the fat body, visible through a more translucent integument (exoskeleton), which turns thinner as the infection advances until it ruptures. A greysh to creamy liquid is released, were billions of occlusion bodies (OBs) are suspended, after the larva crawled up and hang head down from its crochets in an inverted "V" position, which facilitates the spread of inocula in the field (Mazzone, 1985; Granados and Williams, 1986). Lepidopteran pests of cotton, such as Spodoptera exigua and Pectinophora gossypiella as well as the Heliothis/Helicoverpa complex significant levels of control. The host range of baculoviruses is restricted to the order, and usually the family of the host of origin and commercial baculovirus biopesticides are considered to present a minimum risk to people and wildlife. Mass production of Baculoviruses can only be done in vivo, but is economically viable for larger hosts such as Lepidoptera. The majority of these products are targeted against Lepidoptera. Such as codling moth granulovirus (Cydia pomonella Granulovirus- CpGV) is an effective biopesticide of codling moth caterpillar pests of apples.

5. Entomopathogenic nematodes (EPNs)

Entomopathogenic nematode worms are about 0.5 mm in length, soft bodied, non-segmented roundworms that are obligate parasites of insects. Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families (Koppenhöfer, 2007). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs (Koppenhöfer, 2007, Kaya and Gaugler, 1993). Entomopathogenic nematodes occur naturally in soil environments and locate their host in response to carbon dioxide and other chemical (Kaya and Gaugler 1993). Entomopathogenic nematodes are being produced commercially and used as biological control agents against many soil insect pests and insects (Kaya and Gaugler, 1993; Kaya and Stock, 1997; Boemare, 2002).

Entomopathogenic nematodes are soil organisms, which live with bacteria in symbiotic-mutualistic relationship. Entomopathogenic nematodes have potential for biological control of insect pests. Entomopathogenic nematodes can be easily mass-produced and applied by using conventional spray equipment. They have a broad host range and are safe to the environment. Once inside the host, infective juveniles invade the hemocoel and release a symbiotic bacterium, which is held in the nematode's intestine (Poinar, 1990). The bacteria cause a septicemia, killing the host within 24-48h. The infective juveniles feed on the rapidly multiplying bacteria and disintegrated host tissues. About 2-3 generations of the nematode are completed within the host cadaver. The symbiotic association of entomopathogenic nematodes with specific bacteria facilitates reproduction (bacteria serve as food) and pathogenicity of the nematodes. Nematodes act as vectors to transport the bacteria into a host within which they can multiply, and the bacteria create conditions necessary for nematode survival and reproduction within the insect cadaver. It is well known that entomopathogenic nematodes, which are classified into Steinernematidae and Heterorhabditidae families, have great potential as biological control agents in plant protection (Klein, 1990). All species of Steinernema are associated with bacteria of the genus Xenorhabdus and all Heterorhabditis nematode species are associated with Photorhabdus bacteria (Boemare *et al*. 1993). Steinernematids and heterorhabditids are ubiquitous in distribution and have been recovered from soils throughout the world (Hominick *et al*. 1996). Their activity against different pest insects is already well studied (Kaya and Gaugler, 1993; Ebssa, 2005). Entomopathogenic nematodes can be mass-produced by in-vivo or in-vitro methods. The wax moth larva Galleria mellonella (L.) is most commonly used to rear nematodes and liquid fermentation technique for large-scale production of nematodes (Friedman 1990). Entomopathogenic nematodes are currently produced by different methods either in vivo or in vitro (Shapiro-Ilan and Gaugler 2012). In vivo use of a surrogate host larvae of wax moth (Galleria mellonella) trays, shelves and White trap (White 1927). In vitro culturing of entomopathogenic nematodes is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium, utilizing large fermenters are used to produce large quantities of entomopathogenic nematodes for commercial use. The quality of the nematode product can be determined by nematode virulence and viability assays, age and the ratio of viable to non-viable nematodes (Grewal *et al*. 2005).

EPNs juvenile parasitize their host insect via the spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle, and then enters into the hemocoel (Bedding and Molyneux 1982). They then introduce symbiotic bacteria, which multiply rapidly and cause death by septicaemia, often within 48 hours. The insect cadaver becomes red if the insects are killed by heterorhabditids and brown or tan if killed by steinernematids (Kaya and Gaugler 1993). The bacteria break down the insect body, which provides food for the nematodes. After the insect has died, the juvenile nematodes develop to adults and reproduce. A new generation of infective juveniles emerges 8 – 14 days after infection. The infective juvenile stage is the only free living stage of entomopathogenic nematodes.

Both Heterorhabditis and Steinernema are mutualistically associated with bacteria of the genera Photorhabdus and Xenorhabdus, respectively (Ferreira and Malan, 2014). The juvenile stage release cells of their symbiotic bacteria from their intestines into the hemocoel. The bacteria multiply in the insect hemolymph and the infected host usually dies within 24 to 48 hours. After the death of the host, nematodes continue to feed on the host tissue, mature and reproduce. The progeny nematodes develop through four juvenile stages to the adult. Reproduction differs in heterorhabditid and steinernematid nematodes. Infective juveniles of heterorhabditid nematodes become hermaphroditic adults but individuals of the next generation produce both male and females whereas in steinernematid nematodes all generations are produced by males and females (Grewal *et al*. 2005). Entomopathogenic nematodes work best in sandy soil, pH between 4 and 8. Entomopathogenic nematodes are susceptible to freezing, hot temperatures, desiccation, and UV light.

6. Conclusions

Chemical insecticides are commonly used in plant protection. The effect of this is to increase the resistance of insects to various chemical substances contained in plant protection product. In recent years more attention paid to the possibility of using natural enemies, including entomopathogens in control of insect pests. Entomopathogenic microorganisms (fungi, bacteria, viruses, and nematode) which cause illnesses of insect pests. This can create new opportunities for the management of certain insect pests. Entomopathogens are being developed as environmentally friendly alternatives in agriculture crops. They can be exploited for insect pest management as biological control agents and improve the sustainability of Agro-ecosystem. Biological control is defined as the use of living organisms to suppress the population density of a specific pest organism, making it less damaging and reducing its population below the economic threshold of harmfulness. One of environmentally accepted that biological suppression of harmful pests on cultivated crops. The area of microbial pesticides offers a unique possibility to do prospective and prognostic research in the pesticide world.

References

1. Aung OM, Soytong K, Hyde KD. Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand. Fungal Diversity. 2008; (30): 15-22.
2. Asolkar R, Huang H, Koivunen M, *et al*. Chromobacterium bioactive compositions and metabolites.- US Patent Application (US 14/293,728) Pubblication, 2014.
3. Boemare NE, Akhurst RJ, Mourant RG. DNA relatedness between Xenorhabdus spp. (Enterobacteriacae) symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer Xenorhabdus luminescens to a new genus, Photorhabdus gen. novo. Int. Syst. Bacteriol. 1993; 43: 249-255.
4. Blackwell M. Fungal evolution and taxonomy. Bio Control 2010; 55(1): 7-16.
5. Bedding R, Molyneux A. Penetration of insect cuticle by infective juveniles of Heterorhabditis spp. (Heterorhabditidae: Nematoda). Nematologica 1982; 28: 354-359.
6. Boemare N. Biology, taxonomy and systematics of Photorhabdus and Xenorhabdus. In: Gaugler R. ed. Entomopathogenic Nematology. CABI Publishing. Wallingford, UK 2002; 35-56,
7. Condreay JP, Kost TA. Baculovirus Expression vectors for insect and mammalian cells. Current Drug Targets 2007; 8: 1126-1131
8. Deshpande MV. Mycopesticide production by fermentation: Potential and challenges. Crit. Rev. Microbiology 1999; 25: 229-243
9. Evans HC. Entomogenous fungi in tropical forest ecosystems: an appraisal. Ecological Entomology 1982; 7 (1): 47–60.
10. Ebssa, L. Efficacy of entomopathogenic nematodes for the control of the western flower thrips Frankliniella occidentalis. Ph. D. Thesis, Hannover University: 2005; 141
11. Ferreira T, Malan AP. Xenorhabdus and Photorhabdus, bacterial symbionts of the entomopathogenic nematodes Steinernema and Heterorhabditis and their in vitro liquid mass culture: a review. African Entomology 2014; 22: 1-14.
12. Friedman MJ. Commercial production and development. Biocontr. Sci. Technol. 1990; 153-172.
13. Grewal PS, Ehlers R-U, Shapiro-Ilan DI. Nematodes as biological control agents. Wallingford: CABI Publishing 2005.
14. Granados RR, Williams KA. In Vivo infection and Replication of Baculoviruses. In: Granados, R.R. and Federici, B.A. (Eds.). The biology of baculoviruses. Vol.I. CRC Press. Boca Ratón, Florida 1986; 89-108.
15. Gill SS, Cowles EA, Pietrantonio PV. The mode of action of Bacillus thuringiensis endotoxins. Annu. Rev. Entomol. 1992; 37: 615-636.
16. Hominick WM, Reid AP, Bohan DA, *et al*. Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Sci. Technol. 1996; 6: 317-331.
17. Hibbett DS, Binder M, Bischoff JF, *et al*. A higher-level phylogenetic classification of the Fungi. Mycological Research 2007; 111(5): 509-547.
18. Hajek AE, St. Leger RJ. Interactions between fungal pathogens and insect host. Annual Review of Entomology 1994’39: 293-322.
19. Ignoffo CM. Environmental factors affecting persistence of entomopathogens. Florida Entomol. 1992; 75: 516-525.
20. Krieg A, Langenbruch GA. Susceptibility of arthropod species to Bacillus thuringiensis. In: Burges HD, Ed., Microbial control of pests and plant diseases. Academic Press, London 1981; 837-896.
21. Keller S, Zimmerman G. Mycopathogens of soil insects. In: Insect-Fungus Interactions. Wilding, N.; Collins, N.M.; Hammond, P.M. & Webber, J.F. (Eds.) Academic Press, London 1989; 240–270.
22. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annual Review of Entomology. 1993; 38: 181–206.
23. Koppenhöfer AM. Nematodes. In L. A. Lacey and H. K. Kaya, eds. Field manual of techniques in invertebrate pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests, second ed.

Dordrecht: Springer 2007; 249–264.
24. Kaya HK and Gaugler R. Entomopathogenic nematodes. Ann. Rev. Entomol1993; 38: 181-206.
25. Kaya HK, Stock SP. Techniques in insect nematology. In: Lacey L. ed. Manual of Techniques in Insect Pathology. Academic Press. San Diego, CA 1997; 281-324.
26. Klein MG. Efficacy against soil-inhabiting insect pests. Entomopathogenic Nematodes in Biological Control (Gaugler, R., Kaya, H. K., eds). CRC Press, Boca Raton, FL 1990; 195-214.
27. Luangsa-ard JJ, Hywel-Jones NL, Manoch L, *et al*. On the relationships of Paecilomyces sect. Isarioidea species. Mycologia 2004; 96 (4): 773-780.
28. Lacey LA, Horton DR, Chauvin R, *et al*. Comparative efficacy of Beauveria bassiana, Bacillus thuringiensis, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. Ent. Exp. Appl. 1999.
29. Melin BE, Cozzi EM. Safety to nontarget invertebrates of lepidopteran strains of Bacillus thuringiensis and their β−exotoxins. In: Laird M, Lacey LA & Davidson EWD, Eds., Safety of Microbial Insecticides. CRC Press, Boca Raton 1990; 149-167.
30. Mazzone HM. Pathology associated with baculovirus infection. In: Maramorosh, K., and Sherman, K.E. (Eds.). Viral insecticides for biological control. Academic Press. Orlando, Florida 1985; 81-120.
31. Marchetti E, Alberghini S, Battisti A, *et al*. Susceptibility of adult Exorista larvarum to conventional and transgenic Bacillus thuringiensis galleriae toxin-Bulletin of Insectology 2012; 65: 133-137.
32. Okano K, Vanarsdall AL, Mikhailov VS, *et al*. Conserved molecular systems of the Baculoviridae. Virology 2006; 344: 77–87.
33. Pigott CR, Ellar DJ. Role of receptors in Bacillus thuringiensis crystal toxin activity- Microbiology and Molecular Biology Reviews 2007; 71: 255-281.
34. Pérez-Guerrero S, Aldebis HK, Vargas-Osuna E, Toxicity of six Bacillus thuringiensis Cry proteins against the olive moth Prays oleae.- Bulletin of Insectology 2012; 65: 119-122.
35. Poinar GO, Thomas GM. Fossil entomogenous fungus from dominican amber. Experientia 1984; 40: 578-579.
36. Poinar Jr. GO. Taxonomy and biology of Steinernematidae and Heterorhabditidae, p.23-61. In R. Gaugler & H.K. Kaya (eds.), Entomopathogenic nematodes in biological control. Boca Raton, CRC Press, 1990; 365
37. Quesada-Moraga, E, Navas-Cortés JA, Maranhao EAA, *et al*. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research 2007; 111(8): 947– 966.
38. Rath AC, Koen TB, Yip HY. The influence of abiotic factors on the distribution and abundance of Metarhizium anisopliae in Tasmanian pasture soils. Mycological Research 1992; 96 (5): 378–384.
39. Roberts DW. Toxins of entomopathogenic fungi. In: Microbial control of pests and plant diseases. Academic press, London and New York. H.D. Burges (Ed.) 1981; 441-464.
40. Roberts DW, Humber RA. Entomogenous fungi. In: Cole GT, Kendrick B, (Eds.). Biology of Conidial Fungi. Academic Press, New York 1981; 201-236
41. Samson R A, Evans HC, Latg JP. Atlas of entomopathogenic fungi. Springer, Berlin Heidelberg New York 1988.
42. Sánchez-Peña SR, San-Juan Lara J, Medina RF. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Santillo, Mexico, and their virulence towards thrips and whiteflies. Journal of Insect Science 2011; 11(1): 1-10.
43. Sung GH, Poinar GO, Spatafora JW. The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses. Molecular Phylogenetics and Evolution 2008; 49:.495-502.
44. Shapiro-Ilan DI, Han R, Dolinksi C. Entomopathogenic nematode production and application technology. Journal of Nematology 2012; 44: 206-217.
45. Schnepf E, Crickmore N, Van Rie J, *et al*. Bacillus thuringiensis and its pesticidal proteins. Microbiol. Mol. Biol. Rev. 1998; 62: 775-806.
46. Sims SR. Host activity spectrum of the cryIIa Bacillus thuringiensis subsp. kurstaki protein: effects on Lepidoptera, Diptera, and non-target arthropods. Southwest. Entomol. 1997; 22: 395-404.
47. Vänninen I. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycological Research 1996; 100 (1): 93–101.
48. van Regenmortel, MHV, Fauquet CM, Bishop DHL. *et al*. Virus taxonomy. Seventh report of the international committee of taxonomy of viruses. Academic Press. San Diego. 2000; 1162.
49. van Oers MM, Flak JM. Baculovirus Genomics. Current Drug Targets. 2007; 8: 1051-1068
50. White GF. A method for obtaining infective nematode larvae from cultures. Science1927; 66: 302-303.
51. Xiao G, Ying SH, Zheng P, *et al*. Genomic perspectives on the evolution of fungal entomopathogenicity in Beauveria bassiana. Sci. Rep. 2012; 2: 483.
52. Zheng P, Xia YL, Xiao GH, *et al*. Genome sequence of the insect pathogenic fungus Cordyceps militaris, a valued traditional chinese medicine. Genome Biol. 2011; 12 (11) R116.
53. Zare R, Gams W. A revision of Verticillium section Prostrata. IV. The genera Lecanicillium and Simplicillium.

Nova Hedwigia 2001; 73: 1-50.