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Review

Microbial Biopesticides in Agroecosystems

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Abstract: Microbial biopesticides include several microorganisms like bacteria, fungi, baculoviruses, and nematode-associated bacteria acting against invertebrate pests in agro-ecosystems. The biopesticide sector is experiencing a significant growth and many discoveries are being developed into new biopesticidal products that are fueling a growing global market offer. Following a few decades of successful use of the entomopathogenic bacterium *Bacillus thuringiensis* and a few other microbial species, recent academic and industrial efforts have led to the discovery of new microbial species and strains, and of their specific toxins and virulence factors. Many of these have, therefore, been developed into commercial products. Bacterial entomopathogens include several Bacillaceae, *Serratia*, *Pseudomonas*, *Yersinia*, *Burkholderia*, *Chromobacterium*, *Streptomyces*, and *Saccharopolyspora* species, while fungi comprise different strains of *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Verticillium*, *Lecanicillium*, *Hirsutella*, *Paecilomyces*, and *Isaria* species. Baculoviruses are species-specific and refer to niche products active against chewing insects, especially Lepidopteran caterpillars. Entomopathogenic nematodes (EPNs) mainly include species in the genera *Heterorhabditis* and *Steinernema* associated with mutualistic symbiotic bacteria belonging to the genera *Photorhabdus* and *Xenorhabdus*. An updated representation of the current knowledge on microbial biopesticides and of the availability of active substances that can be used in integrated pest management programs in agro-ecosystems is reported here.

Keywords: entomopathogens; bacteria; fungi; entomopathogenic nematodes (EPNs); biological control; integrated pest management (IPM)

1. Introduction

Biological pesticides, or biopesticides, represents a range of bio-based substances acting against invertebrate pests with different mechanisms of action. Based on a technical definition provided by the United States Environmental Protection Agency (EPA), they can be classified into three main classes: (i) naturally-occurring biochemicals that act through non-toxic mechanisms; (ii) microbial entomopathogens; and (iii) plant-incorporated protectants deriving from genetically engineered plants [1]. The basic concept of employing living organisms and natural products leverages the properties of natural ecosystem components to counteract the biotic and reproductive potential of pests. In agricultural ecosystems, the growth of harmful insect and other invertebrate populations is, in fact, favored by an oversimplification of living communities [2], so that biological control methods based on the use of natural enemies (i.e., predators and parasitoids) [3], and pest disease agents [4] may restore a lost ecological balance. Invertebrate pathogens are represented by several microbials, and in particular by bacteria, fungi, baculoviruses, and nematodes. In the last decades, numerous research projects conducted in the academic and industrial context have led to the discovery, development, and market launch of several microbial biopesticides [5]. The interest in this specific field of study is internationally fostered by recently revised legislative frameworks, like the European Pesticide Regulation (EC) No. 1107/2009, encouraging the use of safer pesticides with less environmental impact [6].

The present review is intended to give an updated overview of the current knowledge and of the availability of active substances, mostly bioinsecticides, that can be used for integrated pest management programs in agro-ecosystems.

2. Entomopathogenic Microorganisms

2.1. Bacteria

Different bacterial species in the family Bacillaceae have, for a long time, been the object of studies investigating their pathogenic relationship with invertebrates, especially insects [7,8]. This group of entomopathogens is well represented by *Bacillus thuringiensis* (*Bt*), the most studied and commercially used bacterial species. The insecticidal activity of this bacterium relies on the biosynthesis of crystal toxins (Cry and Cyt) associated with parasporal bodies produced during the sporulation phase and other toxins and virulence factors, some of which produced and released by the cell during the vegetative phase of growth (VIP) [9]. Varying toxin gene sequences result in different affinity with insect midgut receptors, so that different strains are characterized by diverse insecticidal protein toxins and strain-specific insecticidal properties [10]. Consequently, different *Bt* strains are effective only against a narrow target range. For these reasons the search for new strains and insecticidal toxins is fostered at the scientific and industrial level. After being ingested, these toxins specifically bind to insect midgut receptors thus triggering a pore-forming process that determines the alteration of the epithelial membrane permeability with consequent disruption of the intestinal barrier functions and eventual bacterial septicaemia leading to insect death [11]. A similar mechanism is associated with the *Lysinibacillus sphaericus* (formerly *Bacillus sphaericus*) species group that act against mosquitoes and blackflies through the production of complementary crystal proteins BinA and BinB, and mosquitocidal toxins Mtx [12]. Insecticidal toxins showing high homology with *Bt* Cry toxins have been found in entomopathogenic species belonging to the *Paenibacillus* genus. Another bacterium in the same bacterial family, but showing a wider spectrum of pesticidal activity is *Brevibacillus laterosporus*, a species characterized by a swollen sporangium containing a spore with a canoe-shaped parasporal body attached to one side [13,14]. This bacterium holds several virulence factors [15] and its use in integrated management of different pests has been proposed [16,17].

The worldwide market is represented by several bacterial products based on different species and strains (Table 1). Among these, Gammaproteobacteria represents an heterogeneous group of species including several entompathogens like the endosymbionts of insecticidal nematodes *Photorhabdus*, *Xenorhabdus*, and *Serratia* species, whose insecticidal action is a toxin mediated process [18,19]. The same group includes the non-spore forming species *Yersinia entomophaga* producing the toxin complex Yen-Tc, containing toxins and chitinases [20], and *Pseudomonas entomophila* holding a toxin secretion system [21], both acting by ingestion.

Table 1. Selection of commercial products based on entomopathogenic bacteria.

Active Substances	Commercial Names [1]	Main Targets
Bacillus thuringiensis aizawai	Able-WG, Agree-WP, Florbac, XenTari	Armyworms, diamondback moth
Bacillus thuringiensis kurstaki	Biobit, Cordalene, Costar-WG, Crymax-WDG, Deliver, Dipel, Foray, Javelin-WG, Lepinox Plus, Lipel, Rapax	Lepidoptera
Bacillus thuringiensis israelensis	Teknar, VectoBac, Vectobar	Mosquitoes and Black flies
Bacillus thuringiensis tenebrionis	Novodor, Trident	Colorado potato beetle
Bacillus thuringiensis sphaericus	VectoLex, VectoMax	Mosquitoes
Burkholderia spp.	Majestene, Venerate	Chewing and sucking insects and mites; nematodes
Saccharopolyspora spinosa	Tracer™ 120, Conserve	Insects
Chromobacterium subtsugae	Grandevo	Chewing and sucking insects and mites
Bacillus firmus	Bionemagon	Nematodes

[1] Different products may refer to different microbial strains. The representative trade names are those shown on the relevant company websites to which reference should be made for details.

Betaproteobacteria represents another class including species with a significant potential as biocontrol agents. An insecticidal strain of *Burkholderia rinojensis* has recently been discovered and developed into a product acting by ingestion and contact against diverse chewing and sucking insects and mites [22]. The insecticidal action relies on different metabolites and the commercial product is based on heat-killed cells and spent fermentation media. Another commercially successful betapropteobacterium is a strain of *Chromobacterium subtsugae* whose metabolites show a broad spectrum insecticidal activity against different species of Lepidoptera, Hemiptera, Colepotera, and Diptera [23].

Actinobacteria include different *Streptomyces* species producing a variety of insecticidal toxins, such as the macrocyclic lactone derivatives acting on the insect peripheral nervous system [24]. Within the same phylum, *Saccharopolyspora spinosa* produces potent and broad spectrum insecticidal toxins known as spinosins, whose natural and semisynthetic derivatives have a good commercial success [25].

2.2. Fungi

Invertebrate pathogenic fungi include a variety of genera and species acting against diverse targets and showing varying degrees of specificity. The infection process normally starts with the germination of conidia or spores that have come into contact with the host cuticle. Due to a combined enzymatic and mechanical action, the fungus penetrates the host body and the mycelium develop internally, often producing different types of conidia or spores colonizing the host. During the vegetative growth the fungus may produce and release a variety of metabolites, favoring its growth or acting as virulence factors or toxins. To ensure spread in the environment, new conidia or spores will be produced outside the infected host. Before this stage, the host affected by both the biochemical and mechanical action of the fungus, normally dies. The infection is triggered by the first conidium or spore germination, which normally requires specific environmental conditions (i.e., temperature, relative humidity). Main fungal entomopathogens include species in the following phyla: Chytridiomycota, Zygomycota, Oomycota, Ascomycota, and Deuteromycota [26]. Most commercial products are based on suspensions of conidia (Table 2).

Table 2. Selection of commercial products based on entomopathogenic fungi.

Active Substances	Commercial Names [1]	Main Targets
Beauveria bassiana	Bio-Power, Biorin/Kargar, Botanigard, Daman, Naturalis, Nagestra, Beauvitech-WP, Bb-Protec, Racer, Mycotrol	Wide range of insects and mites
Beauveria brongniartii	Bas-Eco	*Helicoverpa armigera*, Berry borer, Root grubs
Hirsutella thompsonii	No-Mite	Spider mites
Isaria fumosorosea	Nofly	Whitefly
Metarhizium anisopliae	Biomet/Ankush, Bio-Magic, Devastra, Kalichakra, Novacrid, Met52/BIO1020 granular, Pacer	beetles and caterpillar pests; grasshoppers, termites
Metarhizium brunneum	Attracap	*Agriotes* spp.
Paecilomyces lilacinus	Bio-Nematon, MeloCon, Mytech-WP, Paecilo	Plant pathogenic nematodes
Paecilomyces fumosoroseus	Bioact WG, No-Fly-WP, Paecilomite	Insects, Mites, Nematodes, Thrips
Verticillium lecanii	Bio-Catch, Mealikil, Bioline/Verti-Star	Mealy bugs and sucking insects
Lecanicillium lecanii	Lecatech-WP, Varunastra	Aphids, leafminers, mealybugs, scale insects, thrips, whiteflies
Myrothecium verrucaria	DiTera	Nematodes

[1] Different products may refer to different microbial strains. The representative trade names are those shown on the relevant company websites to which reference should be made for details.

Beauveria bassiana represents one of the most used fungal bioinsecticide, and it was the first example of insect microbial control at the end of the 19th century. Within the same genus, *B. bassiana* and *B. brongniartii* strains showing varying level of virulence against diverse targets are now used as active substances in diverse formulations [27]. Recent studies have highlighted the potential of some *B. bassiana* and *B. brongniartii* strains as endophytes in biological control applications [28].

Metarhizium anisopliae represents another well exploited fungal species with diverse strains acting against a wide range of targets [29]. A variety of insecticidal toxins and virulence factors produced by *M. anisopliae* strains have been identified [30]. Other fungal species commercially exploited worldwide for pest management include *Verticillium lecanii* [31], *Lecanicillium* spp. [32], *Hirsutella* spp. [33], *Paecilomyces*, and *Isaria* spp. [34], whose action is associated with the production of insecticidal metabolites. Several other entomopathogenic fungal species are associated with insects and play an important role in their natural control through the spread of their spores [35]. A limitation of fungal based biopesticides is their action by contact and a strict range of conditions for conidia and spore germination [26]. Improved products are expected to be developed employing endophitic strains targeting insects after their penetration inside the plant [36]. Another aspects to be considered before applying fungal entompathogens on the crop is the lack of fungicide residues used against phytopathogenic fungi.

2.3. Baculoviruses

Species in the family *Baculoviridae* represents DNA viruses establishing pathogenic relationships with invertebrates and showing potential in biological control [37,38]. The virus infectivity is associated with the production of crystalline occlusion bodies, containing infectious particles, within the host cell. Based on the morphology of these occlusion bodies Baculoviruses are divided into two main groups: the nucleopolyhedroviruses (NPVs), in which these bodies are polyhedron-shaped and develop in cell nuclei, and the granuloviruses (GVs), in which these bodies are granular-shaped. A different, double-stranded RNA virus family (Reoviridae), presents polyhedron-shaped occlusion bodies in the cell cytoplasm (CPVs) [39].

Baculoviruses act orally against insects, and the first infection normally takes place after ingestion of contaminated food. Ingested occlusion bodies within the midgut environment release specific types of virions, called occlusion-derived viruses (ODVs), that interact directly with the membrane of microvillar epithelial cells through the action of their envelop proteins (i.e., PIFs). Within the nucleus of infected midgut cells, a second type of virions, called budded viruses (BVs), are produced ensuring the successive spread of the virus throughout the host. As the infection spread, the dead insect body progressively liquefies, favoring the dispersal of virus particles in the environment [40]. Viral infections are also able to induce behavioral changes in the hosts, affecting their gene expression mechanisms [41].

Given the close relationship and specificity with the host, the name of the entomopathogenic virus includes the initial of the host name. For instance, *Ld*MNPV refers to the *Lymantria dispar* multicapsid nucleopolyhedrovirus. Due to their mode of action, commercially available baculovirus-based products are active only against chewing insects, especially Lepidopteran caterpillars (Table 3). Because of the low stability of baculovirus formulations in the environment and their high production costs related to the need to reproduce them within their host, their use in biological pest management is limited to specific niche market segments [42,43].

Table 3. Selection of commercial products based on entomopathogenic viruses.

Active Substances	Commercial Names [1]	Main Targets
Helicoverpa zea nucleopolyhedrovirus	Heligen	*Helicoverpa* spp. and *Heliothis virescens*
Spodoptera litura nucleopolyhedrovirus	Biovirus–S, Somstar-SL	*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*

[1] Different products may refer to different microbial strains. The representative trade names are those shown on the relevant company websites to which reference should be made for details.

2.4. Nematodes

Entomopathogenic nematode (EPN) species in the genera *Heterorhabditis* and *Steinernema* act as obligate parasites and because of their mutualistic symbiosis with insect pathogenic bacteria in the genera *Photorhabdus* and *Xenorhabdus*, respectively, possess a significant insecticidal potential [44].

Insect pathogenic nematodes normally enter actively the host through its natural openings (oral cavity, anus, and spiracles) and release their symbiotic bacteria in the hemocoel. The following bacterial proliferation is accompanied by the release of toxins and virulence factors that weaken the host, and by the production of metabolites that favor the creation of a suitable environment for nematode reproduction [45]. Among other virulence factors, *Photorhabdus* and *Xenorhabdus* species produce an insecticidal toxins complex (Tc), including different subunits that show toxicity against insects by ingestion [46]. A variety of improved in vivo and in vitro methods, for nematode production at small- and large-scale, have been developed. The quality of the final formulation plays a major role in the efficacy of nematode-based biological control applications against pests [47]. A variety of products based on different nematode species are commercialized worldwide targeting specific pest species and market segments (Table 4).

Table 4. Selection of commercial products based on entomopathogenic nematodes (EPNs).

Active Substances	Commercial Names [1]	Main Targets
Steinernema carpocapsae	Capsanem, Carpocapsae-System, Exhibitline SC, Optinem-C, NemaGard, Nemastar, NemaTrident-T, NemaRed, Nemasys C, Palma-Life	Borer beetles, caterpillars, cranefly, moth larvae, *Rhynchophorus ferrugineus, Tipulidae.*
Steinernema feltiae	Entonem, NemaShield, NemaTrident-F, Nemapom, Nemaplus, Nemaflor, NemaFly, Nemafrut, Nemasys F, Nematrip, Nematech-S SP, NemaTrident-S, Nemax-F, Nemycel, Steinernema-System, Optinem-F	*Bradysia* spp., *Chromatomyia syngenesiae, Phytomyza vitalbae*, soil dwelling pests, codling moth larvae, sciarids, thrips
Steinernema kraussei	Kraussei-System	Vine Weevil larvae
Heterorhabditis bacteriophora	Larvanem, Nemaplant, NemaShield-HB, Nematop, Nematech-H NemaTrident-H, NemaTrident-C, Nema-green, Optinem-H	*Otiorhynchus* spp., chestnut moths, black vine weevil and soil-dwelling beetle larvae, *Melolontha melolontha*, caterpillars, cutworms, leafminers
Heterorhabditis downesi	NemaTrident-CT	Black Vine Weevil *Otiorhynchus sulcatus*
Phasmarhabditis hermaphrodita	Slugtech-SP	Molluscs

[1] Different products may refer to different microbial strains. The representative trade names are those shown on the relevant company websites to which reference should be made for details.

3. Benefits of Microbial Biopesticides and Market Scenario

Invertebrate pathogenic microorganisms employed as active substances in pest management are recognized as generally safe for the environment and non-target species, in comparison with synthetic chemicals. This is in relation to the specificity of their mode of action, limiting their efficacy against one or a narrow range of pest species [4]. On the other hand, biological pesticides exhibit a multi-site action, which hinders the development of resistant pests, fostering their use in resistance management programs. Accordingly, the employment of microbials in combination or rotation with conventional pesticides is encouraged [48]. A good efficacy against their targets can, however, be achieved employing these biopesticides as stand-alone products in organic farming. Additional advantages of biopesticides, include a reduced pre-harvest interval and the lack of significant residues on crops.

The method of application in the field and the formulation features play a key role in the performance of any biopesticide. With the aim of enhancing efficacy, proprietary technologies have been developed by industry maximizing the effects on the target and improving product application features (e.g., adhesion, dispersion, spry drift distribution) [49].

The whole market of biopesticides has significantly grown during the last years, as a result of an increased awareness of their potential and a growing attention to the environmental and health risks associated with conventional chemicals [50,51]. The global biopesticides market is predicted to reach nearly $7.7 billion in 2021, growing at a five-year Compound Annual Growth Rate (CAGR) of 14.1% [52].

This trend is in line with the implementation of legislative frameworks fostering the registration and use of environmentally friendly products in different world regions. In this context, the pre-market authorization remains an important factor that slows down this innovation process, even if it is a necessary and indispensable tool to guarantee health safety.

4. Conclusions

The availability of biopesticides acting against diverse crop pests is essential to ensure the management of agro-ecosystems respecting the environment and human health. The growing demand from farmers is accompanied by an increasing market offer of newly introduced and improved products that can be used alone and in rotation or combination with conventional chemicals.

Academic and industrial investments in the biopesticide sector is experiencing a significant growth and many discoveries are being developed into new biopesticidal products that are enlarging the global market offer. This includes the development of novel solutions against new targets or the introduction of new technologies that enhance the efficacy of already available active substances. Advanced molecular studies on insect microbial community diversity are also opening new frontiers for the development of innovative pest management strategies [53,54]. On the other hand, recent findings are contributing to foster a deeper understanding of the insect-microbial interactions within the plant ecosystem [55].

The modern legislative frameworks requiring to follow criteria and principles of integrated pest management (IPM) in agro-ecosystems, are further fueling a significantly expanding market. Added to this are the efforts made by scientists working in the field of invertebrate pathology, whose studies aim to give light to new and increasingly effective microbial derived active substances.

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