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Review Article

Microbial Bio-Pesticides and Their Use in Integrated Pest Management

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Abstract: Crop pest management in agriculture is important to safeguard crop yield and increase productivity. The need for sustainable and eco-friendly pest management practices is strongly felt with the increasing awareness of the harmful effects of the synthetic insecticides on the non- target organisms, humans and the environment. The demand for bio-pesticides is rising steadily in all parts of the world. Therefore, the current study was carried out with the objectives to review, note and have the know-how all about microbial bio-pesticides and their use in pest management program. When used in integrated pest management systems, bio-pesticides efficacy can be equal to or better than conventional products, especially for crops like fruits, vegetables, nuts and flowers. Microbial bio-pesticides can be smart inputs and components in integrated pest management program. By combining performance and safety, they perform efficaciously while providing the flexibility of minimum application restrictions, superior residue and resistance management potential, and human and environmental safety benefits. It is very likely that in future their role will be more significant in agriculture and forestry. Bio-pesticides clearly have a potential role to play in development of future integrated pest management strategies hopefully, more rational approach will be gradually adopted towards bio-pesticides including microbial bio-pesticides in the near future and short-term profits from chemical pesticides will not determine the fate of bio-pesticides.

Keywords: Bio-pesticides, Entomopathogenic Fungi, Microbial Pesticides, Pest Management

1. Introduction

crop pest management in agriculture is important to safeguard crop yield and increase productivity. The need for sustainable and eco-friendly pest management practices is strongly felt with the increasing awareness of the harmful effects of the synthetic insecticides on the non- target organisms, humans and the environment. Biopesticide is the contraction of bilological pesticide includeing sevral type of pest management intervention: through predatory, parasite or chemical relationship. They can be living organisms (natural enemies) or their products (phytochemicals, microbial products) or byproducts (semiochemicals) which can be used for the management of pests that are injurious to plants. Biopesticides have an important role in crop protection, although most commonly in combination with other tools including chemical pesticides as part of Bio-intensive integrated pest management [1]. Biopesticides or biological pesticides based on pathogenic microorganisms specific to a target pest offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health.

Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or algae) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that control certain weeds, and other fungi that kill specific insects. The most widely known microbial pesticides are varieties of the bacterium Bacillus thuringiensis, or Bt., which can control certain insects in cabbage, potato, and other crops. Bt. produces a protein that is harmful to specific insect pest. Microbial pesticides need to be continuously monitored to ensure that they do not become capable of harming non-target organisms, including humans [2]. Microbial pesticides are considered promising alternatives to chemical pesticides, and have opened up new vistas in insect pest management to aid in the promotion of safe, eco-friendly pest management. Due to biodegradable nature, they do not leave any residues on crops, and do not contaminate the aquatic systems [3].

Objective

To review, note and have the know how all about microbial pesticides and their use in pest management program.

2. Microbial Bio-pesticides

The damage and destruction inflicted on crops by pests have had a serious impact on farming and agricultural practices in the world. These pests include insects, fungi, weeds, viruses, nematodes, animals and birds. In an attempt to avoid such damage, the primary strategy employed has been to eliminate the pests by using chemical pesticides such as chlorinated hydrocarbons, organophosphates and carbamates causing huge costs for chemical purchase.

Pesticides based on microorganisms and their products have proven to be highly effective, species specific and eco-friendly in nature, leading to their adoption in pest management strategies around the world. The microbial bio-pesticide market constitutes about 90% of total bio-pesticides and there is ample scope for further development in agriculture and public health, although there are challenges as well. Out of all the bio-pesticides used today, microbial bio-pesticides constitute the largest group of broad-spectrum bio-pesticides, which are pest specific (i.e., do not target non-pest species and are environmentally benign). There are at least 1500 naturally occurring insect-specific microorganisms, 100 of which are insecticidal. Over 200 microbial bio-pesticides are available in 30 countries affiliated to the Organization for Economic Co-operation and Development (OECD) [4]. There are 53 microbial bio-pesticides registered in the USA, 22 in Canada and 21 in the European Union (EU) although reports of the products registered for use in Asia are variable [5, 1]. Overall, microbial bio-pesticide registrations are increasing globally, the expansion of various technologies has increased the scope for more products and the change in the trend to develop microbial products is definitely on the rise [6, 7].

2.1. Advantages and Disadvantages of Microbial Pesticides

2.1.1. Advantages

The advantages include: Microbial pesticides are non-toxic and non-pathogenic to non-target organisms and the safety offered is their greatest strength; Action of microbial is specific to a single group or species of pests, therefore, do not affect directly beneficial animals such as predators and parasitoids; Microbial pesticides can be used in many habitats where chemical pesticides have been prohibited, such habitats include recreational and urban areas, lake and stream borders of watersheds, and near homes and schools in agricultural settings; Residues of microbial pesticides are non-hazardous and are safe all the time, even close to harvesting periods of the crops; They have a potential to control vectors, some pathogenic microbes can establish in a pest population or its habitat and provide control during subsequent seasons or pest generations [8].

2.1.2. Disadvantages

The disadvantages include: Owing to the specificity of the action, microbes may control only a portion of the pests present in a field and may not control other type of pests present in treated areas, which can cause continuous damage; As heat, UV light and desiccation reduces the efficacy of microbial pesticides, the delivery systems become an important factor; Special formulations and storage procedures are necessary, shelf life is a constraint, given their short shelf lives; Given their pest specificity, markets are limited, the development, registration and production costs cannot be spread over a wide range of pest control sales, for example, insect viruses are not widely available; Some insects develop resistance to several insect pathogens resistance management will have to be practiced, as it is with chemical pesticides [8].

2.2. Registration of Microbial Pesticides

Plant protection against pathogens, pests, and weeds has been progressively re-oriented from a remedial approach to a rational use of pesticides in which consumer health and environmental conservation prevail over any other consideration. Microbial pesticides have been introduced for crop protection, and a new generation of microbial pesticides is being promoted for pest management. The development of microbial pesticides requires several steps to be addressed right from its isolation in pure culture to bio-efficacy assays performed in vitro, ex vivo, in vivo, or in pilot trials under field conditions. For commercial delivery of a microbial pesticide, the bio-control agent must be produced at an industrial scale (fermentation), preserved for storage, and formulated by means of biocompatible additives to improve its survival and application and stability of the final product. Because of the unique nature of bio-control agents, some data requirements are different from those necessary for registration of chemical pesticides, but the general principle that the product should demonstrate effectiveness and should not be hazardous to users, consumers of treated foods, or to the environment including natural enemies and beneficial organisms, still applies [8].

2.3. Impact of Microbial Pesticides on the Environment

The rationale for the development and deployment of microbial insecticides for pest management is their environmental safety, specificity, and biodegradability. Some pathogens selected for commercial development, such as viruses and bacteria, may infect only a single or small number of closely related insect species. Others, such as fungi and nematodes, may affect a fairly wide range of insects and related arthropods. However, the commercially available microbial pathogens are target specific and have not been shown to infect vertebrates or plants. The biodegradable nature of the microbial pesticides does not leave any harmful residues in the environment, and does not enter the food chain.

Spores of entomopathogenic fungi do not withstand high temperatures and cannot persist on the foliage for long. However, infected cadavers that drop to the soil sporulate under congenial microclimatic conditions and overwinter in the soil. A meager percentage of these conidia survive through the summer and express in the subsequent rainy season after the pest population builds up. Baculoviruses, among the insect viruses, are regarded as safe and selective bio-insecticides, and are restricted to invertebrates. They have been used worldwide against many insect pests, mainly Lepidoptera. Their application as microbial pesticides, however, has not met their potential to control pests in crops, forests, and pastures, with the exception of NPV of the soybean caterpillar, Anticarsia gemmatalis (Hub.), which is used on approximately one million ha annually in Brazil. Problems that have limited the use of baculoviruses include narrow host range, slow killing speed, technical and economical difficulties for in vitro commercial production, timing of application based on host population monitoring, and variability in their efficacy in the field under diverse climatic conditions. Epizootics of baculovirus diseases are frequent in Lepidoptera and sawflies with very high larval mortality, resulting in a substantial reduction in insect population. Baculoviruses survive for a long period in the soil [9].

2.4. Impact of Microbial Pesticides on the Natural Enemies

Research pertaining to the development of microbial pesticides in India has focused on the identification of virulent isolates for effective management of the target pests. Information pertaining to their effects on natural enemies, non-target pests, and the environment is quite scanty. Research on the microbial pesticides over the past decade has focused on generation of information pertaining to their safety to the natural enemies, persistence in the environment, phytotoxicity, etc. in addition to generating information on the bio-efficacy [9].

2.5. Use of Microbial Pesticide in Pest Management

Among the different microbial agents developed and tested for pest management, bacteria, fungi, and baculoviruses are quite promising for pest control. Bacteria and fungi are gaining importance due to their amenability for mass multiplication on artificial media. Microbial insecticides currently used in India for controlling economically important pests affecting agricultural and horticultural crops include Bacillus thuringiensis var. kurstaki (Btk), nuclear polyhedrosis virus (es) (NPV) of Helicoverpa armigera (Hubner) and Spodoptera litura (Fab.), the entomopathogenic/nematicidal fungi, Beauveria bassiana (Balsamo) Vuillemin, Verticilliumlecanii (Zimm.) Viegas, Paecilomyces lilacinus Thom [10]. And Metarhizium anisopliae (Met.) Sorokin. Antagonistic fungi and bacteria found promising for plant disease management include Trichoderma viride Pers., T. harzianum Rifai, and Pseudomonas fluorescens Migula. The development of microbial pesticides for effective pest control in the context of sustainable agriculture will be a major challenge. A truly integrated approach to address the present day plant protection issues is to obtain maximum benefit. Because of the low adverse environmental impact and high specificity of the microbial agents microbial pesticides are suitable for pest management program [7].

2.5.1. Fungal Bio-pesticides

The pathogenic fungi are group of microbial pest management organisms that grow in both aquatic as well as terrestrial habitats and when specifically associated with insects are known as entomopathogenic fungi [11]. These are obligate or facultative, commensals or symbionts of insects. The pathogenic action depends on contact and they infect and kill sucking insect pests such as aphids, thrips, mealy bugs, whiteflies, scale insects, mosquitoes and all types of mites. Entomopathogenic fungi are promising microbial bio-pesticides that have a multiplicity of mechanisms for pathogenesis. They belong to 12 classes within six phyla and belong to four major groups; Laboulbeniales, Pyrenomycetes, Hyphomycetes and Zygomycetes. Some of the most widely used species include Beauveria bassiana, Metarhizium anisopilae, Nomuraea rileyi, Paecilomyces farinosus and Verticillium lecanii. Many of them have been commercialized globally. These fungi attack the host via the integument or gut epithelium and establish their conidia in the joints and the integument. Some species such as B. bassiana and M. anisipoliae cause muscardine insect disease and after killing the host, cadavers become mummified or covered by mycelial growth. Some fungi, primarily streptomycetes, also produce toxins that act against insects [12]. About 50 such compounds have been reported as active against various insect species belonging to Lepidoptera, Homoptera, Coleoptera, Orthoptera and mites. The most active toxins are actinomycin A, cycloheximide and novobiocin. Spinosyns are commercially available bio-pesticidal compounds that were originally isolated from the actinomycete Saccharopolyspora spinosa and are active against dipterans, hymenopterans, siphonaterans and thysanopterans but are less active against coleopterans, aphids and nematodes [13].

Potential Benefits of Entomopathogenic Fungi

Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses. Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application. The use of fungal entomopathogens as alternative to insecticide or combined application of insecticide with fungal entomopathogens could be very useful for insecticide resistant management [14]. The commercial mycoinsecticide 'Boverin' based on B. bassiana with reduced doses of trichlorophon have been used to suppress the second-generation outbreaks of Cydiapomonella L. Khashaveh observed 68-92% mortality in the population of

Sitophilus granarius and Tribolium castaneum adults against which Beauvaria bassiana applied [15].

A long term example of a classical biological control project using fungi is the program targeting the cassava green mite (CGM), Mononychellustanajoa (Bondar) in Africa. It was in 1988, that exploration for potential natural enemies in Brazil revealed that the entomophthoralean N. tanajoae was one of the most important natural enemies of CGM in northeastern Brazil. During the last 20 years, a series of studies was undertaken to make the release of this pathogen in Africa possible. The impact of the fungus Neozygitesfloridana on the tomato red spider mite, Tetranychusevansi Baker & Pritchard was demonstrated in the field and under screen houses during four crop cycles of tomato and nightshade in Piracicaba, SP, Brazil [16].

Fungal biocontrol agents, including 10 isolates of Beauveria bassiana, Metarhiziumanisopliae, and Paecilomyces fumosoroseus were bioassayed for their lethal effects on the eggs of the carmine spider mite, Tetranychus cinnabarinus. Results confirmed the ovicidal activity of the three fungal species and suggested the feasibility to search for more ovicidal isolates from fungal species that may serve as biocontrol agents against spider mites such as T. cinnabarinus. Two isolates of entomopathogenic fungi, Beauveria bassiana SG8702 and Paecilomyces fumosoroseus Pfr153, were also bio-assayed against T. cinnabarinus eggs [17].

(i) Trichoderma spp. Characteristics, Mechanism of action, Use, Advantages and Limitation

Characteristics of Trichoderma

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 101-103 cultivable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the teleomorph (genus Hypocrea) has most often been found. However, many strains, including most biocontrol strains, have no known sexual stage. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. Several strains belonging to the genus Trichoderma have been identified so far as biocontrol agents of plant diseases and nematodes and a few of them have been developed and registered as biofungicides. Several species have been found to be very effective as biocontrol agents, namely *T. atroviride, T. asperellum, T. harzianum, T. viride, T. gamsii, T. polysporum*, etc. [18].

Mechanism of action Trichoderma

Trichoderma strains may have one or all mechanisms of action according to species and strain. In some strains prevails the direct, hyper-parasitic activity against pathogens, in others the induction of resistance mechanism prevails or they compete with the pathogens for space and nutrients. Trichoderma strains produce a great variety of lytic enzymes most of which play a great role inbiocontrol (cell wall degrading enzymes, CWDEs). CWDEs from different Trichoderma strains showed antifungal activity towards a broad spectrum of fungal pathogens (i.e. species of Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia and Colletotrichum, as well as funguslike organisms such as the OomycetesPythium and Phytophthora which lack chitin in their cell walls).

Trichoderma produces also many secondary metabolites, some of them specifically involved in the direct antibiosis, like i) volatile antibiotics, i.e. 6-pentyl-a-pyrone (6PP) and most of the isocyanide derivates; ii) water-soluble compounds, i.e. heptelidic acid or koningic acid; iii) peptaibols, which are linear oligopeptides of 12-22 amino acids rich in a-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol (Pheol or Trpol) at the C-terminus. The production of lytic enzymes and antibiotic metabolites may greatly vary among strains. Each strain shows specific characteristics and adaptation to the environment. Some strains are very good colonizers of soil other prefer wood material, others are found on dead plant tissue in the phyllosphere or colonizing the rhizosphere of plants and the root tissues. Several strains can also promote growth [18].

Use of Trichoderma

Being soil inhabitants and good colonizers of organic matter the main use of Trichoderma strains is against soil-borne pathogens, wood disease and grey mould (Botrytis cinerea). In particular some strains represent the only possible solution against wood diseases of grape (no chemical substance have been shown to be effective so far), in fact they can colonize the pruning wounds on the plant for several months thus preventing the entrance of the pathogens. In infested soils they can significantly reduce several soil-borne pathogens and thus the related diseases. On grape, strawberry, tomatoes and other crops they can colonise the dead plant material as leaf residues, dead leaves, etc. preventing the establishment of B. cinerea on those materials and thus reducing the disease. Some strains have been used to induce resistance; however this mechanism seems to be very limited in term of efficacy [18].

Advantages, Limitations and way to Improve Efficacy

Trichoderma can offer several advantages over synthetic chemical fumigants: they are not toxic for mammals and environment, they do not leave toxic residues, they have no adverse effect on soil microflora and microfauna and they do not negatively affect the natural bio-control population. To be effective the Trichoderma strains should be carefully chosen according to their mechanism of action and adaptation to the specific environment. The intrinsic capability of the strain of producing lytic enzymes and secondary antimicrobial metabolites can make the difference. The use of poorly competitive strains or not adapted to soil characteristics may also impede the efficacy. Most of the failures in term of efficacy can be related to the use of product with poor viability of conidia (this often happens when Trichoderma-based fertilizers are applied), insufficient concentration in the soil, short time for activity (Trichodermas need a certain amount of time to kill the pathogen), too high disease pressure. In few weeks after its application the concentration of exogenous Trichoderma tends to decrease to the basal level of that specific soil. There are several possible way to improve its efficacy as: the increase of the applied quantity per unit of soil, which is commonly limited by the cost of the commercial product, the use of carriers or substrates which can guarantee a longer persistency (i.e. cellulose, or chitin rich substrates), to give sufficient time to Trichoderma to reduce inoculum before planting, to apply when the temperature of the soil is sufficiently high, to combine with other practices, for example as bio-inoculum after solarisation or anaerobic soil disinfestation or bio-fumigation [18].

(ii) Coniothyrium minitans Characteristics, Mechanism of Action, Use, Advantages and Limitation

Characteristics of Coniothyrium minitans

Coniothyrium minitans is a coelomycete with a worldwide distribution. In nature it occurs mainly in sclerotia of Sclerotiniasclerotiorum and S. trifoliorum, but can colonise other Sclerotinia species. Spore germination, mycelial extension and sclerotial infection take place at temperatures between 5 and 25 °C. Field control of S. sclerotiorum and S. minor can be achieved following soil incorporation of solid-substrate formulations before planting. C. minitans can survive and spread in soil for at least 2 years and continue to give some control of disease although limited.

Mechanism of action Coniothyrium minitans

Coniothyrium minitans enters through the small pores or a lacerated surface of the target organism, and/or lysing by chitinase and glucanase for entry. Upon entry, Coniothyrium minitans penetrates the inter- and intra-cellular sub-cortex and medulla, producing fruiting bodies, through asexual reproduction. The infection process causes the cell to shrink, due to high osmotic potential, and the membrane is detached and/or degraded. Coniothyrium minitans is highly specialized antifungal agent that targets sclerotia of Ascomycotina and Deuteromycotina (e.g., Sclerotinia sclerotiorum and Sclerotinia minor). Coniothyrium minitans requires a host to be in a vegetative stage.

Use of Coniothyrium Minitans

The use is mainly in high value crops as lettuce, beans, peas, but also in sunflower, oilseed rape. Application is commonly by spraying onto soil and incorporating into upper soil layer either in pre-plant, by applying the product to the soil 3 to 4 months prior to the onset of disease or in postharvest, by applying product to harvest residues and incorporate into the soil.

Advantages, Limitations and Ways to Improve Efficacy

The main advantage is that C. minitans controls specifically Sclerotinia species without any interference with the soil microflora, the treatment is easy to apply and with no risk for the operators. Main limiting factors are the cost per hectare (the concentration should be sufficiently high per unit of soil and sufficiently deeply incorporated) and the time needed to reduce the inoculum (few months) which is incompatible with frequent growing cycles as for lettuce in some areas. If the time between the application of the bio-control agent and the subsequent growing cycle is too short, they bio-control activity and the reduction of inoculum can be insufficient. Another limiting factor is the habit of yearly renting of land by growers, which is common in some countries. In such context farmers do not invest in a soil treatment the year before, which can benefit the next farmer renting that land.

(iii) Gliocladium catenulatum Characteristics, Mechanism of action, Use, Advantages and Limitation

Characteristics of Gliocladium catenulatum

Gliocladiumcatenulatum is anaturally-occurring saprophytic fungus which is widespread in the environment.

Mechanism of action

The mode of action is reported to be by an enzymatic mechanism. There are no reports indicating that Gliocladiumcatenulatum produces any toxins or antibiotics of concern.

Use of Gliocladium catenulatum

It can be used to control damping-off, seed- root- and stem-rots, and wilt diseases caused by Rhizoctonia, Pythium, Phytophthora, Fusarium, Didymella, Botrytis, Verticillium, Alternaria, Cladosporium, Helminthosporium, Penicillium, and Plicaria. It can be applied by soil incorporation, soil drench, foliar spray, and dipping of cuttings, bulbs, and tubers. Timing of application: at sowing, potting or transplanting and 2-6 weeks later for potted plants and 2-8 weeks later for other plants. Cuttings, bulbs, or tubers may be sprayed after planting or treated before planting or storage.

Advantages, Limitations and ways to Improve Efficacy

Advantages, limitations and ways to improve efficacy are the same as reported for the Trichoderma spp [18].

(iv) Purpureocillium lilacinum Characteristics, Mechanism of Action, Use, Advantages and Limitation

Characteristics of Purpureocillium lilacinum

The characteristics Purpureocillium lilacinum is a naturally occurring fungus commonly found in soils. Unlike many other P. lilacinum strains, the registered strain does not produce mycotoxins or paecilotoxins. It grows optimally at 21-27 °C, and does not grow or survive above 36 °C. The registered strain was isolated from infected nematode eggs. Mechanism of action P. lilacinum parasitizes and subsequently kills eggs, juveniles, and adult females of various plant parasitic nematodes.

Use of Purpureocillium lilacinum

As a pesticide active ingredient, *P. lilacinum* can be used to control plant root nematodes on many food and non-food crops. It acts by infecting eggs, juveniles, and adult females of various plant pathogenic nematodes including *Meloidogyne* spp. (root knot nematodes); *Radopholus similis* (burrowing nematode); *Heterodera* spp. and *Globodera* spp. (cyst nematodes); *Pratylenchus* spp. (root lesion nematodes). It can be applied to agricultural soil through drip irrigation, or water in the suspension around base of each plant. If neither of these methods is possible, spray the soil surface around the base of each plant, and drench in afterwards using the irrigation system. The timing varies with crop. In general, 14 days pre-plant; just before planting, 6 weeks after planting, repeat every 6 weeks to 4 months. The main advantages are related to safety and the possibility to treat the soil immediately before planting or after. Main limitations are related to the short shelf-life, temperature and the cost per hectare.

(v) Phlebiopsis gigantea Characteristics, Mechanism of

Action, Use, Advantages and Limitation

Characteristics of Phlebiopsis gigantea

Phlebiopsis gigantea is a common and widely distributed saprophytic wood-decay fungus in the coniferous forests of the Northern Hemisphere. The mechanism of action *Phlebiopsis gigantea* is able to prevent colonization of stumps by *Heterobasidion annosum* through competition for resources. *Phlebiopsis gigantea* can be used as a biological control of annosum root rot, caused by *Heterobasidion* spp., in Western Europe. Annosum root rot is primarily a problem in conifer plantations that have been partially cut (e.g. thinned). The main advantage is that it can support the phase out the use of chemical agents in forestry. The main limitations are the cost of the products and its application.

2.5.2. Viral Bio-pesticides

Over 700 insect-infecting viruses have been isolated, mostly from Lepidoptera (560) followed by Hymenoptera (100), Coleoptera, Diptera and Orthoptera (40) [11]. About a dozen of these viruses have been commercialized or use as bio-pesticides. The viruses used for insect control are the DNA-containing baculoviruses (BVs), Nucleopolyhedrosis viruses (NPVs), granulo viruses (GVs), acoviruses, iridoviruses, parvoviruses, polydnaviruses, and poxviruses and the RNA-containing reo-viruses, cytoplasmic polyhedrosis viruses, noda viruses, picrona-like viruses and tetra viruses. However, the main categories used in pest management have been NPVs and GVs. These viruses are widely used for control of vegetable and field crop pests globally, and are effective against plant-chewing insects. Their use has had a substantial impact in forest habitats against gypsy moths, pine sawflies, Douglas fir tussock moths and pine caterpillars. Codling moth is controlled by Cydia pomonella GVs on fruit trees and potato tuber worm by Phthorimaea operculella GVs in stored tubers [19]. Virus-based products are also available for cabbage moths, corn earworms, cotton leaf worms and bollworms, beet armyworms, celery loopers and tobacco budworms. The mechanism of viral pathogenesis is through replication of the virus in the nuclei or in the cytoplasm of target cells. The expression of viral proteins occurs in three phases. First is the early phase, i.e. 0-6 h post infection second is the late phase, i.e. 6-24 h post infection and the third phase is very late phase, i.e. up to 72 h post infection. It is at the late phase that virions assemble as the 29 kDa occlusion body protein is synthesized. Numerous virions of NPVs are occluded within each occlusion body to develop polyhedra. However, the GV virion is occluded in a single small occlusion body, to generate granules. Infected nuclei can produce hundreds of polyhedra and thousands of granules per cell. These can create enzootics, deplete the pest populations, and ultimately create a significant impact on the economic threshold of the pest. The viral bio-pesticides are usually only active against a narrow host spectrum and after their application to plant surfaces, and baculovirus occlusion bodies (OBs) are rapidly inactivated by solar ultraviolet (UV) radiation, particularly in the UV-B range of 280-320 nm. However, their efficacy can be improved by the use of formulations that include stilbene derived optical brighteners, which increase susceptibility to NPV infection by disrupting the peritrophic membrane or inhibiting sloughing or virus-induced apoptosis of insect mid gut cells.

2.5.3. Bacterial Bio-pesticides

Bacterial bio-pesticides are probably the most widely used and cheaper than the other methods of pest bio-regulation. Insects can be infected with many species of bacteria but those belonging to the genus Bacillus are most widely used as pesticides. One of the Bacillus species, *Bacillus thuringiensis*, has developed many molecular mechanisms to produce pesticidal toxins; most of toxins are coded for by several cry genes. Since its discovery in 1901 as a microbial insecticide, *Bacillus thuringiensis* has been widely used to control insect pests important in agriculture, forestry and medicine. Its principal characteristic is the synthesis, during sporulation, of a crystalline inclusion containing proteins known as dendotoxins or Cry proteins, which have insecticidal properties. To date, over one hundred *B. thuringiensis* based bio-insecticides have been developed, which are mostly used against lepidopteran, dipteran and coleopteran larvae. In addition, the genes that code for the insecticidal crystal proteins have been successfully transferred into different crops plants, which have led to significant economic benefits. Because of their high specificity and their safety in the environment, *B. thuringiensis* and Cry proteins are efficient, safe and sustainable alternatives to chemical pesticides for the control of insect pests [20]. The toxicity of the Cry proteins has traditionally been explained by the formation of transmembrane pores or ion channels that lead to osmotic cell lysis. In addition to this, Cry toxin monomers also seem to promote cell death in insect cells through a mechanism involving an adenylyl cyclase/PKA signalling pathway. However, despite this entomopathogenic potential, controversy has arisen regarding the pathogenic lifestyle of *B. thuringiensis*. Recent reports claim that *B. thuringiensis* requires the co-operation of commensal bacteria within the insect gut to be fully pathogenic [21]. In clear opposition, genomic and proteomic studies have been argued as the most solid data to convincingly demonstrate that *B. thuringiensis* is a primary pathogen rather than a soil-dwelling saprophyte. In any case, what is certainly not doubtful is that *B. thuringiensis* is one of the most successful examples of the use of microorganisms in agricultural biotechnology, with about 70% of the global bio-pesticide market involving products based on *B. thuringiensis*, and will continue to be one of the most important microbial weapons to defend our crops from insect pests. At the end of the twentieth century worldwide sales of bacterial pesticides amounted to about 2% of the total global insecticide market but their share in pesticide market steadily in *Bacillus* spp.

(i) Bacillus spp. Characteristics, Mechanism of Action, Use, Advantages and Limitation

Characteristics of Bacillus spp.

Members of the Bacillus genus are among the beneficial bacteria mostly exploited as bio-pesticides to control plant diseases but also nematodes. The most known species hosting bio-control agents are *B. subtilis and B. amyloliquefasciens, butalso B. firmus and B. pumilus*. They are Gram positive rod-shaped bacteria that can form a protective endospore that can tolerate extreme environmental conditions.

Mechanisms of action Bacillus spp.

Bacillus spp. can control fungal pathogens by competition, direct antibiosis and induced resistance. In the rhizosphere, competition takes place for space at the root surface and for nutrients, noticeably those released as seed or root exudates. Competitive colonization of the rhizosphere and successful establishment in the root zone is a prerequisite for effective bio-control, regardless of the mechanism(s) involved. Direct antagonism involves the production of several microbial metabolites among which lipopeptides play the major role. Surfactin in particular is involved in the mechanism of resistance induction. The bio-nematicide Bacillus firmus also colonizes the rhizosphere of the plant where it parasitizes the eggs and larvae of nematodes especially of the root knot nematodes.

Use of Bacillus spp.

Bacillus strains are effective against a broad spectrum of plant pathogens and they can be used either as foliar application or root application before transplanting. In case of soil or root application the ability of the specific strains to colonize and permanently establishes on the roots of the specific crop is crucial. Sometime the colonization is not simply crop-specific, but cultivar specific. Root application of Bacillus spp. should be preferably targeted to improve resistance of the plant or to protect the early stage of seed germination, rather to directly control soil-borne inoculum of pathogens.

Advantages, Limitations and ways to Improve Efficacy

The advantage of Bacillus spp. based products is their long shelf-life at room temperature, given by the fact that they produce endospores. There is also a lot of knowledge gained in the fermentation process and the ways of production are commonly optimized. It is a relatively easy microorganism to ferment with relatively low cost of production compared for example to fungal bio-control agents. The Main limitation is due to the fact that the Bacillus strains should colonize the roots in order to be active. Since no major direct effect on the pathogen's inoculum is expected, its efficacy may be low in case of high pathogen's inoculum in soil or against very aggressive soil-borne pathogens. The highest efficacy is seen on germinating seeds or young plantlets, because of the combination of direct antibiosis, induced resistance and grow promotion of several Bacillus strains. Possible ways to improve efficacy are: verification of compatibility between strains and cultivars, application in strategies where another bio-control agents are targeted to reducing soil-borne inoculum, application in hydroponic culture or fertigation, selection of Bacillus strains with high production of antimicrobial metabolites.

(ii) Pseudomonas spp. Characteristics, Mechanism of Action, Use, Advantages and Limitation

Pseudomonas spp. are aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. Pseudomonads possess many traits that make them well suited as bio-control and growth-promoting agents. The Mechanism of action the traits related to bio-control include the ability to rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth promoting substances), compete aggressively with other microorganisms; and adapt to environmental stresses. The Use Pseudomonas strains are commonly uses to treat seeds or roots of plants before planting. They can also be used to treat tubers and bulbs. One of the major advantages is that they grow rapidly in vitro and can be mass produced at relatively low cost. Other advantages are the good compatibility with several fungicides. The major weakness of pseudomonads as bio-control agents is their inability to produce resting spores (as do many Bacillus spp.), which complicates formulation of the bacteria for commercial use. The identification of new formulations, which can prolong their shelf-life, can dramatically increase their use in practice. Other point to be implemented is the understanding of the best combination between the specific strain and plant species, soil type and environmental conditions.

2.5.4. Nematode Bio-pesticides

Another group of microorganisms that can control pests is the entomopathogenic nematodes, which control weevils, gnats, white grubs and various species of the Sesiidae family. These fascinating organisms suppress insects in cryptic habitats (such as soil-borne pests and stem borers). Commonly used nematodes in pest management belong to the genera Steinernema and Heterorhabditis, which attack the hosts as infective juveniles (IJs). IJs are free-living organisms, which enter the hosts through mouth, anus, spiracles or cuticle. They are able to release their bacterial symbionts in to the haemocoel of hosts, killing the host within 24-48 h [12]. The nematodes can complete up to three generations within the host, after which the IJs leave the cadaver to find the new hosts [22]. Entomopathogenic nematodes (EPN) can be mass-produced in vivo and in vitro in solid media or liquid fermentation. Nematodes that have been successfully produced in fermenters (7500-80000 litre capacity) include *Steinernema carpocapsae, S. riobrave, Steinernema glaseri, Steinernema scapterisci*, *Heterorhabditis bacteriophora and Heterorhabditis megidis*, with a yield capacity up to 250000 IJs/ml [23]. The use of nematodes is done using a curative rather than prophylactic approach, for instance, as demonstrated in the case of *Synanthedon exitiosa*, using *S. carpocapsae and H. bacteriophora* nematode species to induce field suppression of the pest in a curative manner; 150000-300000 Us/tree were used three times during September and October for three consecutive years in order to obtain as much control as was achieved with chemical pesticides. Some commercial products are available based on Steinernema and Heterorhabditis nematode formulations.

However, extensive studies are required to optimize application parameters and develop efficient strains to achieve significant control of pests through nematodes.

2.5.5. Protozoa as Insect Pathogens

Entomopathogenic protozoans are extremely diverse group of organisms comprising around 1000 species attacking invertebrates including insect species and are commonly referred as microsporidians. They are generally host specific and slow acting, producing chronic infections with general debilitation of the host. The spore formed by the protozoan is the infectious stage and has to be ingested by the insect host for pathogenicity. The spore germinates in the mid gut and sporoplasm is released invading the target cells causing infection of the host. The infection results in reduced feeding, vigour, fecundity and longevity of the insect host.

Although they are undoubtedly important in natural biological regulation of insect populations, they do not possess the required attributes for a successful microbial insecticide. The most notable entomopathogenic protozoa belong to *Nosema* spp. and *Variomorpha necatrix*. *Nosema locustae* is the only commercially available species of the microsporidian and is marketed for control of grass hoppers and crickets in USA, Canada, Argentina, China and Mali. However, the utility of *N. locustae* as a grasshopper bio-control agent remains questionable because of the great difficulty in assessing the efficacy in case of a highly mobile insect [24]. *Nosema pyrausta* is another beneficial microsporidian that reduces fecundity and longevity of the adults and also causes mortality of the larvae of European corn borer [25].

3. Conclusions

The demand for bio-pesticides is rising steadily in all parts of the world. When used in integrated pest management systems, bio-pesticides efficacy can be equal to or better than conventional products, especially for crops like fruits, vegetables, nuts and flowers. Microbial pesticides can be smart inputs and components in IPM program. By combining performance and safety, they perform efficaciously while providing the flexibility of minimum application restrictions, superior residue and resistance management potential, and human and environmental safety benefits. It is very likely that in future their role will be more significant in agriculture and forestry. Bio-pesticides clearly have a potential role to play in development of future integrated pest management strategies hopefully, more rational approach will be gradually adopted towards bio-pesticides including microbial pesticides in the near future and short-term profits from chemical pesticides will not determine the fate of bio-pesticides.

4. Future Directions

Integrated Pest Management (IPM) has been developed as a way to control pests without relying solely on pesticides. IPM is a systematic plan which brings together different pest control tactics into one program. Microbial pesticide can be one of a good input for such IPM program to reach at effective, economically feasible and ecologically sound management of diseases and pests. It is not surprising that most of the information on IPM flows through the agrochemical suppliers and distributors, which may explain why pest control consists mostly of pesticide use. Relevant information on IPM such as the use of microbial pesticides against different pests, however, is mostly published in academic journals where they are not accessible to most growers. These calling for further scientific solution to change these theories into practices by searching different ways of application.

One of the challenges facing in using microbial pesticides in pest management is the shelf life of these microorganisms before and after application is low; this is because of various internal and external factors (environmental factors). Therefore, detailed studies on the properties, mode-of-action and pathogenicity of such organisms are still needed. Ecological studies on the dynamics of diseases in insect populations are necessary because the environmental factors play a significant role in disease outbreaks and ultimate control of the pests. It is expected that with the recent advancements in microbial research coupled with dedicated efforts from extension specialists, farmers, pest management regulators and the general public, microbial bio-pesticides could play a prominent role in future IPM program.

Commercialization is the final and most difficult step in the development of a microbial product. The most critical factors are developmental cost and time to market. Therefore, to examine all these critical factors in the successful commercialization of microbial pest control products is essential in the developmental process of a product and these critical factors should be comprehensively discussed.

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