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Soil Microbes as Bio pesticides: Agricultural Applications and Environments

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Abstract

Bio pesticides, including entomopathogenic viruses, bacteria, fungi, nematodes , and plant secondary metabolites, are gaining increasing importance as they are alternatives to chemical pesticides and are a major component of many pest control programs. The virulence of various bio pesticides such as nuclear polyhedrosis virus (NPV), bacteria, and plant product were tested under laboratory conditions very successfully and the selected ones were also evaluated under fi eld conditions with major success. Bio pesticide products (including benefi cial insects) are now available commercially for the control of pest and diseases. The overall aim of bio pesticide research is to make these bio pesticide products available at farm level at an affordable price, and this would become a possible tool in the integrated pest management strategy. Moreover, bio pesticide research is still going on and further research is needed in many aspects including bio formulation and areas such as commercialization. There has been a substantial renewal of commercial interest in bio pesticides as demonstrated by the considerable number of agreements between pesticide companies and bio product companies which allow the development of effective bio pesticides in the market.

Crops damage due to pests and diseases are always encountered by farmers. It can be significant constraints to production influencing human life and the environment. Mostly plant protection methods recently use synthetic pesticides, that are toxic chemicals noxious the environment. Because of the hazardous effect, one of environmentally friendly methods is developed to protect plant from plant pathogens, that is, the use of biological control or referred to as biopesticides. Biopesticides encompass a broad array of microbial pesticides, biochemicals derived from microorganisms and other natural sources, and processes involving the genetic incorporation of DNA into agricultural commodities. Bio pesticides have benefits and limitations effect for the environment, human life, or agricultural products. The media is biodegradable material. Beside the microbial content, carrier media for formulating bio pesticide were consisted of several organic materials, such as animal broth, organic materials, or organic waste product. Biopesticides supported stability and sustainability of agro ecosystem because they did not affect negatively on environment It also can increase the farmers' income, through the high price of their yield organic products.

Keywords: Soil Microbes; Bio pesticides; **Integrated Pest Management**

Introduction

Bio pesticides, a contraction of 'biological pesticides', include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with [biological control] – and by implication – the manipulation of living organisms. Regulatory positions can be influenced by public perceptions, thus:

In the EU, bio pesticides have been defined as "a form of pesticide based on micro-organisms or natural products".

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> The US EPA states that they "include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protect ants) or PIPs".

They are obtained from organisms including plants, bacteria and other microbes, fungi, nematodes, etc [1, 2]. (They are often important components of integrated pest management (IPM) programmes, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs).

Modern agriculture largely relies on the extensive application of agrochemicals, including inorganic fertilizers and pesticides. Indiscriminate, long-term and over-application of pesticides have severe effects on soil ecology that may lead to alterations in or the erosion of beneficial or plant probiotic soil micro flora. Weathered soils lose their ability to sustain enhanced production of crops/grains on the same land. However, burgeoning concern about environmental pollution and the sustainable use of cropping land have emphasized inculcation of awareness and the wider application of tools, techniques and products that do not pollute the environment at all or have only meagre ecological concerns. This section covers the types of, concerns about and current issues regarding the extensive application of agrochemicals, in particular pesticides, on a variety of microorganisms integrated in successive food chains in the soil food web.

Microbial bio pesticides include several microorganisms like bacteria, fungi, *baculoviruses*, and nematode-associated bacteria acting against invertebrate pests in agro-ecosystems. The bio pesticide 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.

Some entomopathogens have been or are being used in a classical microbial control approach where exotic microorganisms are imported and released for managing invasive pests for long-term control. The release of exotic microorganisms is highly regulated and is done by government agencies only after extensive and rigorous tests. In contrast, commercially available entomopathogens are released through inundative application methods as bio pesticides and are commonly used by farmers, government agencies, and homeowners. Understanding the mode of action, ecological adaptations, host range, and dynamics of pathogen-arthropod-plant interactions is essential for successfully utilizing entomopathogen-based bio pesticides for pest management in agriculture, horticulture, orchard, landscape, turf grass, and urban environments.

Food security and safety are the major concern in ever expanding human population on the planet earth. Each and every year insect pests cause a serious damage in agricultural field that cost billions of dollars annually to farmers. The loss in term of productivity and high cost of chemical pesticides enhance the production cost. Irrespective use of chemical pesticides (such as Benzene hexachloride, Endosulfan, Aldicarb, and Fenobucarb) in agricultural field raised several types of environmental issues. Furthermore, continuous use of chemical pesticides creates a selective pressure which helps in emerging of resistance pest. These excess chemical pesticide residues also contaminate the environment including the soil and water. Therefore, the biological control of insect pest in the agricultural field gains more importance due to food safety and environment friendly nature. In this regard, bacterial insecticides offer better alternative to chemical pesticides. It not only helps to establish food security through fighting against insect pests but also ensure the food safety.

Types of Bio pesticides

Bio pesticides can be classified into these classes-

- Microbial pesticides which consist of bacteria, entomopathogenic fungi or viruses (and sometimes includes the metabolites that bacteria or fungi produce). Entomopathogenic nematodes are also often classed as microbial pesticides, even though they are multi-cellular [3, 4]
- Bio-derived chemicals. Four groups are in commercial use: pyrethrum, rotenone, neem oil, and various essential oils [5, 6] are naturally occurring substances that control (or monitor in the case of pheromones) pests and microbial diseases.
- Plant-incorporated protect ants (PIPs) have genetic material from other species incorporated into their genetic material (*i.e.* GM crops). Their use is controversial, especially in many European countries [7].
- RNAi pesticides, some of which are topical and some of which are absorbed by the crop.

Biopesticides have usually no known function in photosynthesis, growth or other basic aspects of plant physiology. Instead, they are active against biological pests. Many chemical compounds have been identified that are produced by plants to protect them from pests so they are called antifeedants. These materials are biodegradable and renewable alternatives, which can be economical for practical use. Organic farming systems embraces this approach to pest control *[6].*

RNA:

RNA interference is under study for possible use as a spray-on insecticide by multiple companies, including Monsanto, Syngenta, and Bayer. Such sprays do not modify the genome of the target plant. The RNA could be modified to maintain its effectiveness as target species evolve tolerance to the original. RNA is a relatively fragile molecule that generally degrades within days or weeks of application. Monsanto estimated costs to be on the order of $5/acre [8].

RNAi has been used to target weeds that tolerate Monsanto's Roundup herbicide. RNAi mixed with a silicone surfactant that let the RNA molecules enter air-exchange holes in the plant's surface that disrupted the gene for tolerance, affecting it long enough to let the herbicide work. This strategy would allow the continued use of glyphosate-based herbicides, but would not per se assist a herbicide rotation strategy that relied on alternating Roundup with others [8].

They can be made with enough precision to kill some insect species, while not harming others. Monsanto is also developing an RNA spray to kill potato beetles one challenge is to make it linger on the plant for a week, even if it's raining. The Potato beetle has become resistant to more than 60 conventional insecticides [8].

Monsanto lobbied the U.S. EPA to exempt RNAi pesticide products from any specific regulations (beyond those that apply to all pesticides) and be exempted from rodent toxicity, allergenicity and residual environmental testing. In 2014 an EPA advisory group found little evidence of a risk to people from eating RNA [8].

However, in 2012, the Australian Safe Food Foundation posited that the RNA trigger designed to change the starch content of wheat might interfere with the gene for a human liver enzyme. Supporters countered that RNA does not appear to make it past human saliva or stomach acids. The US National Honey Bee Advisory Board told EPA that using RNAi would put natural systems at "the epitome of risk". The beekeepers cautioned that pollinators could be hurt by unintended effects and that the genomes of many insects are still unknown. Other unassessed risks include ecological (given the need for sustained presence for herbicide and other applications) and the possible for RNA drift across species boundaries [8].

Monsanto has invested in multiple companies for their RNA expertise, including Beeologics (for RNA that kills a parasitic mite that infests hives and for manufacturing technology) and Preceres (nanoparticle lipidoid coatings) and licensed technology from Alnylam and Tekmira. In 2012 Syngenta acquired Devgen, a European RNA partner. Startup Forrest Innovations is investigating RNAi as a solution to citrus greening disease that in 2014 caused 22 percent of oranges in Florida to fall off the trees [8] .

Examples:

Bacillus thuringiensis, a bacteria capable of causing disease of Lepidoptera, Coleoptera and Diptera, is a well-known insecticide example. The toxin from *B. thuringiensis* (Bt toxin) has been incorporated directly into plants through the use of genetic engineering. The use of Bt Toxin is particularly controversial. Its manufacturers claim it has little effect on other organisms, and is more environmentally friendly than synthetic pesticides.

Other microbial control agents include products based on:

- Entomopathogenic fungi (*e.g.* Beauveria bassiana*,* Isaria fumosorosea*,* Lecanicillium and Metarhizium spp.),
- Plant disease control agents: include Trichoderma spp. and Ampelomyces quisqualis (a hyper-parasite of grape powdery mildew); Bacillus subtilis is also used to control plant pathogens *[3]*.
- Beneficial nematodes attacking insect (*e.g. Steinernema feltiae*) or slug (*e.g. Phasmarhabditis hermaphrodita*) pests
- Entomopathogenic viruses (*e.g.*. Cydia pomonella granulovirus).
- Weeds and rodents have also been controlled with microbial agents.

Various naturally occurring materials, including fungal and plant extracts, have been described as biopesticides. Products in this category include:

- Insect pheromones and other semiochemicals
- Fermentation products such as Spinosad (a macro-cyclic lactone)
- Chitosan: a plant in the presence of this product will naturally induce systemic resistance (ISR) to allow the plant to defend itself against disease, pathogens and pests [9].
- Biopesticides may include natural plant-derived products, which include alkaloids, terpenoids, phenolics and other secondary chemicals. Certain vegetable oils such as canola oil are known to have pesticidal properties. Products based on extracts of plants such as garlic have now been registered in the EU and elsewhere.

Applications:

Bio pesticides are biological or biologically-derived agents, that are usually applied in a manner similar to chemical pesticides, but achieve pest management in an environmentally friendly way. With all pest management products, but especially microbial agents, effective control requires appropriate formulation [10] and application [11, 12] .

Bio pesticides for use against crop diseases have already established themselves on a variety of crops. For example, bio pesticides already play an important role in controlling downy mildew diseases. Their benefits include: a 0-Day Pre-Harvest Interval (see: maximum residue limit), the ability to use under moderate to severe disease pressure, and the ability to use as a tank mix or in a rotational program with other registered fungicides. Because some market studies estimate that as much as 20% of global fungicide sales are directed at downy mildew diseases, the integration of bio fungicides into grape production has substantial benefits in terms of extending the useful life of other fungicides, especially those in the reduced-risk category.

A major growth area for bio pesticides is in the area of seed treatments and soil amendments. Fungicidal and bio fungicidal seed treatments are used to control soil borne fungal pathogens that cause seed rots, damping-off, root rot and seedling blights. They can also be used to control internal seed–borne fungal pathogens as well as fungal pathogens that are on the surface of the seed. Many bio fungicidal products also show capacities to stimulate plant host defence and other physiological processes that can make treated crops more resistant to a variety of biotic and a biotic stresses.

Disadvantages:

- High specificity: which may require an exact identification of the pest/pathogen and the use of multiple products to be used; although this can also be an advantage in that the bio pesticide is less likely to harm species other than the target
- Often slow speed of action (thus making them unsuitable if a pest outbreak is an immediate threat to a crop)
- Often variable efficacy due to the influences of various biotic and abiotic factors (since some bio pesticides are living organisms, which bring about pest/pathogen control by multiplying within or nearby the target pest/pathogen)
- Living organisms evolve and increase their resistance to biological, chemical, physical or any other form of control. If the target population is not exterminated or rendered incapable of reproduction, the surviving population can acquire a tolerance of whatever pressures are brought to bear, resulting in an evolutionary arms race.
- Unintended consequences: Studies have found broad spectrum biopesticides have lethal and nonlethal risks for non-target native pollinators such as Melipona quadrifasciata in Brazil [13].

Generally, bio pesticides are made of living things, come from living things, or they are found in nature. They tend to pose fewer risks than conventional chemicals. Very small quantities can be effective and they tend to break down more quickly, which means less pollution.

Some bio pesticides are targeted in their activity, often working on a small number of species. However, users need more knowledge to use biopesticides effectively. This is because they are often most effectively used as part of an Integrated Pest Management approach.

Types of Bio pesticides:

- **Microbes -** These are tiny organisms like bacteria and fungi. They tend to be more targeted in their activity than conventional chemicals. For example, a certain fungus might control certain weeds, and another fungus might control certain insects. The most common microbial bio pesticide is Bacillus thuringiensis.
- **Substances Found in Nature –** These include plant materials like corn gluten, garlic oil, and black pepper. These also include insect hormones that regulate mating, malting, and food-finding behaviours. They tend to control pests without killing them. For example, they might repel pests, disrupt their mating, or stunt their growth. Some synthetic substances are allowed. However, they must be similar in shape and makeup to their natural counterparts. They must also work in the exact same way against pests.
- Plant-Incorporated Protect ants (PIPs) – These are the genes and proteins, which are introduced into plants by genetic engineering. They allow the genetically modified plant to protect itself from pests, like certain insects or viruses. For example, some plants produce insect-killing proteins within their tissues. They can do this because genes from Bacillus thuringiensis were inserted into the plant's DNA. Different types of proteins target different types of insects.

Microbial control agent	Tradenames of biopesticides	Target pests
Bacteria		
Bacillus thuringiensis subsp. *aizawai*	Agree WG and XenTari DF	Lepidoptera
B. thuringiensis subsp. *israelensis*	Mosquito Beater WSP	Diptera
B. thuringiensis subsp. *kurstaki*	CoStar, DiPel ES, Monterey B.t., and Thuricide	Lepidoptera
B. thuringiensis subsp. *tenebrionis*	Novodor FC	Coleoptera
Paenibacillus popilliae	Milky Spore Powder	Japanese beetle, *Popillia japonica*
Fungi		
Beauveria bassiana	BotaniGard ES, Mycotrol-ESO, Myco-Jaal, and Naturalis-L	One or more pests of Acarina,
Hirsutella thompsonii	ABTEC Hirsutella	Coleoptera, Diptera, Hemiptera,
Isaria fumosorosea	NoFly WP and Pfr-97 WDG	Hymenoptera, Lepidoptera,
Lecanicillium lecanii	Phule Bugicide	Orthoptera, Thysanoptera, and
L. longisporum	Vertalec	others
Metarhizium anisopliae	BioCane, Metarril and Ory-X	
M. brunneum	Met52 EC	
Paecilomyces lilacinus	MeloCon WG	Plant-parasitic nematodes
Nematodes		
Heterorhabditis bacteriophora	Nemasys and Terranem	Several orders of soilborne pests
Steinernema carpocapsae	Ecomask and NemAttack	
S. feltiae	Entonem, Fungus Gnat & Rootknot Exterminator, and Scanmask	
H. heliothidis and *S. carpocapsae*	Double-Death	
Viruses		
Granulovirus (GV)		Lepidoptera
Cydia pomonella GV	CYD-X and MADEX HP	
Nucelopolyhedrovirus (NPV)		
Helicoverpa zea NPV	Gemstar LC	
Spodoptera exigua NPV	Spod-X LC	

Microbial control and Integrated Pest Management:

There are several examples of entomopathogen-based bio pesticides that have played a critical role in pest management. Significant reduction in tomato leaf miner, *Tuta absoluta*, numbers and associated yield loss was achieved by *Bt* formulations in Spain [14]. *Bt* formulations are also recommended for managing a variety of lepidopteran pests on blueberry, grape, and strawberry [15-18].

Lecanicellium muscarium-based formulation reducedgreenhouse whitefly (*Trialeurodes vaporariorum*) populations by 76-96% in Mediterranean greenhouse tomato [19]. In other studies, *B. bassiana* applications resulted in a 93% control of twospotted spider mite (*Tetranychus urticae*) populations in greenhouse tomato [20] and 60-86% control on different vegetables [21]. The combination of *B. bassiana* and azadirachtin reduced rice root aphid (*Rhopalosiphum rufiabdominale*) and honeysuckle aphid (*Hyadaphis foeniculi*) populations by 62% in organic celery in California [22]. *Chromobacterium subtsugae* and *B. rinojensis* caused a 29 and 24% reduction, respectively, in the same study. IPM studies in California strawberries also demonstrated the potential of entomopathogenic fungi for managing the western tarnished plant bug (*Lygus hesperus*) and other insect pests [23, 24]. Entomopathogenic fungi also have a positive effect on promoting drought tolerance or plant growth as seen in cabbage [25] and strawberry [26] and antagonizing plant pathogens [27].

Application of SeMNPV was as efficacious as methomyl and permithrin in reducing beet armyworms (*S. exigua*) in head lettuce in California [28]. Several studies demonstrated PhopGV as an important tool for managing the potato tubermoth (*Phthorimaea operculella*) [29].

The entomopathogenic nematode, *S. feltiae*,reduced raspberry crown borer (*Pennisetia marginata*) populations by 33-67% [30]. For managing the branch and twig borer (*Melagus confertus*) in California grapes, *S. carpocapsae* is one of the recommended options [18].

Entomopathogens can be important tools in IPM strategies in both organic and conventional production systems. Depending on the crop, pest, and environmental conditions, entomopathogens can be used alone or in combination with chemical, botanical pesticides or other entomopathogens.

Conclusion

Current problems with the use of chemical insecticides and emphasis on low inputs sustainable agriculture have pushed the microbial agents to the fore front for use in IPM systems. The microorganism provides certain distinct advantages over many other control agents and methods. The major advantage of exploiting microorganism for pest control is their environmental safety primarily due to the host specificity of these pathogens. Microorganisms have natural capability of causing disease at epizootic levels due to their persistence in soil and efficient transmission. Many insect pathogens are compatible with other control methods including chemical insecticides and parasitoids. The cost of development and registration of microbial insecticides is much less than that of chemical insecticides. There is a minimum effect on nontarget organism. There is a long term regulation of a pest population in most the cases whereas in others fairly a good check of pest population has been established. The large scale culture and application is relatively easy and inexpensive. The self-perpetuating nature of most of the pathogens in both space and time would certainly prove to be an asset in sustainable agriculture.

The availability of bio pesticides 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 bio pesticide 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 [31, 32]. On the other hand, recent findings are contributing to foster a deeper understanding of the insect-microbial interactions within the plant ecosystem [33]. The modern legislative frameworks requiring following criteria and principles of integrated pest management (IPM) in agro-ecosystems, are further fuelling 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.

In addition to the continuous search for new bio molecules and improving the efficiency of the known bio pesticides, recombinant DNA technology is also being used for enhancing the efficacy of bio pesticides. Better understanding of genes from microorganisms and crop plants has enabled the isolation of genes effective against particular pest. Fusion proteins are also being designed to develop next-generation bio pesticides. This technology allows selected toxins to be combined with a carrier protein which makes them toxic to insect pests when consumed orally. The fusion protein may be produced as a recombinant protein in substitutes. The human and environmental safety of the bio pesticides and compatibility with integrated pest management systems will drive continued expansion of this industry. The industry has recognized the need to work together and has formed the Bio pesticide Industry Alliance (BPIA), with a mission to improve the global market perception of bio pesticides as effective products. BPIA plans to develop industry standards for product quality and efficacy.

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