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Review of Current Research Advances in Microbial and Phyto-biopesticides

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Abstract—*Biopesticides are the formulated form of active ingredients originating from bacteria, viruses, fungi and plants extracts. We present a detailed review about recent advances in field of biopesticides with particular emphasis on different type of biopesticides and their mechanism of action. In this report we discuss principle features of these biopesticides and the target pest that they counter along with the host plants that can be protected by specific biopesticides. Further we incorporate how recent molecular techniques including recombinant DNA technology, fermentation technology, nanotechnology, and molecular studies are helping to derive new age biopesticides as bioinoculants for biocontrol in integrated pest management. A significant feature of this review article is about an understanding on how metagenomics - the latest field of biotechnological innovation, can be applied to offer a community perspective and an ecosystem based approach to design more effective biopesticides in coming future.*

1. INTRODUCTION

Pesticides are used to protect the crops from harmful pests and to prevent economic losses. Use of chemicals in excessive manner can also show bad impact on nature and natural resources. The notion that '*pesticide as panacea'* had been changed in recent times due to the hazardous effects on human, animals and nature [1]. Synthetic pesticides like DDT methyl bromide, organophosphates and pyrethroids etc., are showing various environmental, health issues including resistance development in target pests. Hence there is increased research on organic agriculture and a shifting focus on alternatives to develop new biopesticides. WHO estimated deaths as high as 20000 worldwide every year apart from other dangerous effects of pesticides such as: carcinogenicity, teratogenicity, neural disorders, high and acute residual toxicity, longer degradation periods and accumulation as food residues [2,3]. In present scenario, the emphasis has shifted from pesticides to biopesticides a possible way of Crop protection. Biopesticides are the pest control agents which are formulated from plant, animal and microbial sources. The United States Environmental Protection Agency (EPA) classified three classes of biopesticides i.e. microbial pesticides, plant-incorporated protectants (PIPs), and biochemical pesticides [1]**.**

1.1 Chemical pesticides vs Biopesticides

Chemical pesticides provide significant benefits by suppressing pests that invade agricultural crops. Consequently, there is an increasing public pressure to discover alternatives for crop protection. Biopesticides offer several advantages including complete biodegradability and water solubility over traditional chemical/synthesized pesticides [4]. Microorganisms and plant based biochemical represent an alternative path because of their safety to humans and non-target organisms, both in individual applications and within integrated pest management (IPM) [5].

1.2 Types of Biopesticides

Semiochemicals

Chemical compounds or their synthetic analogues excreted by animals or plants for defensive purposes or to pass information amongst interacting species are referred to as semiochemicals. Most widely used semiochemicals for crop protection are insect sex hormones used for pest control and mass trapping. Straight chained Lepidopteran pheromones are used as pesticides, which are used in insecticidal traps. Market available semiochemicals are cyromazine, chlorbenzuron and diflubenzuron etc [6]. Sero X is a new semiochemical developed from *Clitoria ternatea* against cotton pest *Helicoverpa spp* [7].

Microbial pesticides

Bacteria, Fungi, Viruses, Protozoa, Oomycetes are used for biological control of plant pathogens and weeds.Most widely used is bacterium *Bacillus thuringinesis* which produces an endotoxin during spore formation and causes lysis of gut cells when consumed by insects. *Agrobacterium radiobacter* is used to control crown gall. Other products based on baculoviruses and fungi are also known. In Europe *Cydia pomonella granulovirus* is used as biopesticide against codling moth in apples majority of fungal biopesticides products are based on ascomycetes that is *Beauveria bassiana* or *Metarhizium anisopliae* used against spittlebugs of sugarcane and grasslands .*Trichoderma harzianum* is another important fungal Biocontrol against used against *Fusarium,Pythium* and other soil borne pathogens [8]. Some examples of market available Biocontrol products are BioTam, Regalia and NoFly etc (Table 1)

Plant extract and vegetable oil based products
Citronella oil, Garlic extract, Neem extract, Datura, orange oil , tea tree extract, Basil, Lemon grass, Apple mint, mustard, Castor, Mahagony, sesame and many more secondary metabolites of plant are used as biopesticide against pests. Most widely used botanical compoundc is neem oil. Pyrethins are also used as insecticides and extracted from *Chrysanthemum cinerariaefolium* plants - mainly found in flower. Pyrethins have low toxicity to mammals and degrade rapidly after application resulting in the development of synthetic pyrethrins [9]. PhomaTech is a market available plant based Biocontrol agent (Table 1)

1.3 Mechanism of action of Biopesticides

Biopesticides can be categorized according to their source (structure) and mechanism by which they mitigate or kill the pests. Naturally occurring or genetically altered bacteria, fungi, algae, viruses, or protozoans are used as pesticides. They control pests by different modes of action i.e. by producing pest specific toxic metabolites that prevent establishment of other microorganisms for causing disease [10].

Though Bacterial biopesticides are generally used as insecticides, they can also be used to suppress the growth of disease causing bacteria and fungi. Bacterial pesticides come into contact with the target pest and may be required to may be ingested for showing their toxicity they disrupt the digestive system by producing endotoxins that are often specific to the particular insect pest [11].For instance *Moraxella osloensis* associated with *Phasmarhabditis hermaphrodita* produces an endotoxin which is heat and protease tolerant and biologically control mollusk pests (slug-parasitic nematode). This bacterium-feeding nematode acts as a vector and transports *M. osloensis* into the shell cavity of the slug, and the bacterium is the killing agent in the nematode-bacterium complex. *M. osloensis* produces an endotoxin(s), which kills the slug after injection into the shell cavity [12].

Fungal biopesticides can be used to control insects, bacteria, nematodes, fungi and weeds [13]. Mechanism of biocontrol is varied and depends on both the pesticidal fungus and the target pest .*Trichoderma* secretes enzymes such as chitinolytic enzymes, glucanases, cellulases, and proteases that help in the biological control of plant diseases. These enzymes might degrade the cell walls of the other fungi, consume/dissolve susceptible cells and multiplies its own spores by growing into the main tissue of the disease-causing pathogenic fungus [10].

Viral Biopesticides are host specific; infecting only one or a few closely related species *viz*. Bacteriophage is a virus that infects bacterial cell walls. These bacteriophages can be used as pesticide if they can attack bacteria that cause plant disease[10], Baculovirus are enveloped viruses and are insect specific with circular, supercoiled double stranded DNA genomes in range of 80-180 kbp.Two phenotypes found that is Occlusion derived virus(ODV) and Budded virus (BV). Different Baculoviruses are characterized by OBs (Occluded Budded Virus) containing either a single virion or multiple virions. Based on morphology 2 major groups of baculoviridae are defined namely Nucleopolyhedrovirus (NPVs) and Granulovirus (GVs) . Some examples of viral biocontrol agents are presented here *Cydia pomonella* GV (CpGV) control the codling moth in apple, pears and on various fruit plants. *Spodoptera frugiperda* Mononucleopolyhedrovirus (SfMNPV) and Granulovirus (GV) for the control of the fall armyworm in the maize crops. *Spodoptera litura* Nucleopolyhedrovirus (SlNPV) act against *Spodoptera litura* which attack important crops such as rice, tomato, maize, groundnut, cotton [14]. Microencapsulation of Colombian *Spodoptera frugiperda* NPV with Eudragit S 100 polymer to minimize activity loss due to solar radiation. [15]

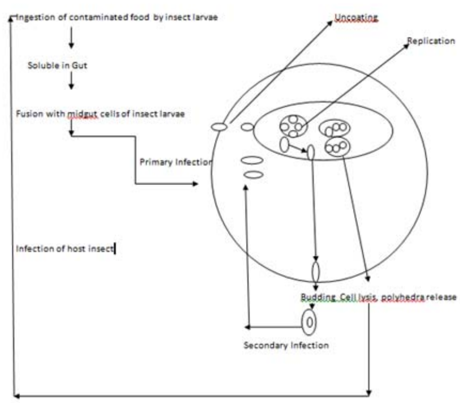

Fig. 1: Insect larvae infection by *Autographa californica* mononucleopolyhedro virus (AcMNPV)

Plant based biopesticides consists active components of plants such as neem, pyrethrins, limonene and rotenone etc. These components can affect by causing neurotoxicity at site-of-action and by sublethal effects observed in some of the essential oil compounds. Neem , pyrethrins, limonene, Rotenone, Sabadilla are the various plant products registered as biopesticides. Neem based biopesticides have multiple biological activities on more than 400 insect species. Neem extract works against green peach aphid *Myzus persicae* which attack vegetables and ornamental crop plants [16] and also toxic to the *Diaphorina citri* which is a vector responsible for citrus greening disease [17]. Incidence of tomato leaf curl viral disease caused by *Bemisia tabaci* vector also inhibited by use of neem base formulations.[18] Also a new biopesticide prepared from oils of *Azadirachta indica* and *Pongamia glabra* human vector control mosquitoes [19]. *Clerodendron infortuntum L*., Indian bhant tree, well known medicinal plant was reported to have antifeedant effects against cotton bollworm, *Helicoverpa armigera* due to the presence of clerodin and other compounds [20]. Water extract of tropical non-economic plants *Polygonum hydropiper L.* ,*Annona squamosa L.* , *Clerodendrum viscosum Vent.* , *Argyreia speciosa L.* and *Leucas aspera* (Wild) L. were observed earlier to control the black inch looper *Hyposidra talaca* which is a major pest of tea [21].

1.4 Market Available Products of Biopesticide

Name	Active Ingrdient	Class	Company
Bio-Tam	*Trichoderma gamsi*(2%) *and T.asperellum* (2%)	Fungicide.soil	AgraQuest
Rootshield	*T.harzianum* 1.15%	Fungicide.soil	BioWorks
Tenet	*T.gamsi* 2% and *T.asperellum* 2%	Fungicide.soil	Isagrow
Actinovate	*Streptomyces lydicus* 0.0371 %	Fungicide.soil	Natural Industries
Mycostop	*Streptomyces griesiovirdis*,35%	Fungicide.soil	Ag Bio
Regalia	*Reynoutria* ,5%	Fungicide.foliage	Marrone
Regalia Maxx	*Reynoutria*,20%	Fungicide.foliage	Marrone
Polyversum	*Pythium oligandrum*	Fungicide.soil	Gowan
Bio-Save	*Pseudomonas syringae* ESC-10,29.8%	Fungicide.Postharvest	Jet Harvest
Blightban A506	*Pseudomonas fluroescenes* ,71%	Fireblight.Fungicide	NuFarm
Phoma Tech	*Phoma macrostoma*,92%	Herbicide	Scotts Company
Econem	*Pasteuria usage*,0.002%	Nematicide	Pasteuria Bioscience
Melocon	*Paecilomyces lilacinus*,6%	Nematicide	Certis
NoFly	*P.fumosoroseus strain* FE9901	Insecticide	Natural Industries
DiTera	*Myrothecium verrucaria*,90%	Nematicide	Valent Biosciences
Madex HP	*Codling moth granulosis virus*	Insecticide	Certis
Grandevo	*Chromobacterium subtsugae*,30%	Insecticide	Marrone
Kodiak	*Bacillus subtilis,GB03*,2.75%	Fungicide.soil	Bayer
Double Nickle	*Bacillus amyloliquefaciens* D747,98.85%	Fungicide.soil.foliage	Certis

2. ADVANCES IN BIOPESTICIDES RESEARCH

2.1 Nanotechnology

Nano level materials facilitate the atomic level specificity and action of pesticides only in the targeted environment including specific pH, temperature and presence of specific compounds. Nanopesticide can reduces the problems which are evaluated in chemical pesticides (uncertainty on the long-term causing cancer, liver damage, neural problem and immunotoxicity) and exhibit more bioavailability than traditional biopesticides. Nanoparticle *Bacillus thuringiensis* have shown increased productivity, good dispersion and wettability, biodegradable in soil and environment, less toxic and more photo-generative, with well understood toxicokinetics and toxicodynamics, and are found to be stable [22]. In case of fungus, nano-chitosan formulation had been prepared by different methods. Radical graft polymerization of acrylic acid onto chitosan showed antifungal and insecticidal activity against some selected soybean seed borne fungi. *Trichoderma* based enzymes (chitin and glucans) are known to show pest resistant activity [23]. On the other hand plant based nano formulations such as eucalyptus based nanoemulsions were found to exhibit antimicrobial activity [24].

2.2 Recombinant DNA Biotechnology

Recombinant DNA technology have been used to improve bacterial insecticide efficacy and applied to improve larvicides by manipulating and recombining gene for vector control. Mosquitocidal *Cyt* and *Cry* proteins of *Bacillus thuringiensis* along with the binary toxin of *Bacillus sphaericus* were combined to exhibit improved efficacy against culex species. These recombinant constructs were not only used as insecticides but also showed effective control of the mosquito vectors for Dengue fever, filariasis and malaria [25]. Transgenic Expression of the *Trichoderma* endochitinase Gene in Tobacco and Potato can be used to control diseases in plants. *ThEn-42* & *chit42* that encode a powerful endochitinase, were cloned from *T.harzianum strain P1* and strain *CECT*. Binary vectors were constructed that contain both gene under the control of the cauliflower mosaic virus 35S subunit (CaMV35S) promoter region and the *Agrobacterium* nopaline synthase terminator. Plasmid p35S-ThEn42 and pBin19:p35S-CHIT42 formed. *Agrobacterium tumefaciens strain LBA4404* having the vectors with the chimeric fungal endochitinase gene and corresponding empty vectors was used to transform leaf disks of *Nicotiana tabacum cv. Samsun NN* and *cv. Xhantii* and stem segments of *Solanum tuberosum cv. Desiree* (only *p35S-ThEn42*). Screening and Molecular analyses of transgenic lines by using different experimental test were performed. *T. harzianum* derived endochitinase genes were not only expressed at higher levels in tobacco and potato but also the secretion peptides from fungus and tomato were correctly cleaved and able to drive the accumulation of the transgenic enzyme outside the plant cell [26]. Resistance to pathogens can also be determined by plant resistance (R) gene and a cognate pathogen avirulence gene. Resistance genes for vascular disease plants have yet to be described molecularly [27]. To investigate interaction of *Trichoderma* strain, crop plants, and soil borne fungal pathogens many tools such as proteomic analysis (MALDI-TOF, CSI and in silico analysis), use of gene expression reporter systems, and high throughput methods to study gene function are used which explore various signalling molecules that influence the life and physiology of many crops. These studies can give data about *Trichoderma spp*. and their interaction with pathogens and plants that could improve our understanding on how these fungi search for the pathogen, talk to the plant, and protect themselves from toxicants [28]. Also with the help of genetic engineering genetic improvement of Baculovirus Biopesticides can be achieved. To improve its insecticidal activity development of recombinant baculovirus can be attempted by deleting the viral ecdysteroid UDP-glycosyltransferase (egt) gene. Product of the viral egt gene prevents larval molting during infection, by inactivating ecdysone, thus increasing feeding activity of infected larvae. Infections with an egt defective recombinant were found to increase the biocontrol efficiency baculovirus by about 20%–30%. Expression of a group of baculovirus genes such as enhancins, cathepsins and chitinases that damage the host peritrophic membrane could be another method to improve speed of kill.however more specific results were reported with insect-specific toxins. Insect predators use venoms to immobilize their prey. Arthropod venoms are also a mixture of toxins that act against various organisms other than insects. It is possible to isolate toxin genes that target insects with high specificity which is been investigated by many researchers [14]. For example to improve the speed of kill and to increase the insecticidal activity, development of *Neutrobactus* a recombinant baculovirus was attempted by inserting foreign genes.[29]

2.3 Encapsulation

Encapsulation is a strategy to maintain the components of formulation in close contact. Encapsulated viral particles have been a preferred delivery system to minimize activity loss due to solar radiation and also maintain the viral insecticidal activity. For example, *Bacillus thuringiensis* and the *Nucleopolyhedrovirus* of *Heliotis* were encapsulated in starch granules. For encapsulation substances like gelatin, pectin, chitin, calcium alginate and maize starch were used that do not affect the viability of the virus. When *Spodoptera frugiperda* Nucleopolyhedrovirus(SfNPV) was encapsulated in Eudragit-S100 microparticles (MPs), it was found that resulting particles were more resistant to UV-inactivation than *Nucleopolyhedrovirus* alone [30,14].

2.4 Novel Formulations

New and innovative formulations were obtained by mixing active ingredients of various components to improve the efficacy , stability and handling of pesticide. Azadirachtin was found to be more effective when formulated in a neem oil medium with other natural products of neem as compared to pure compound alone.

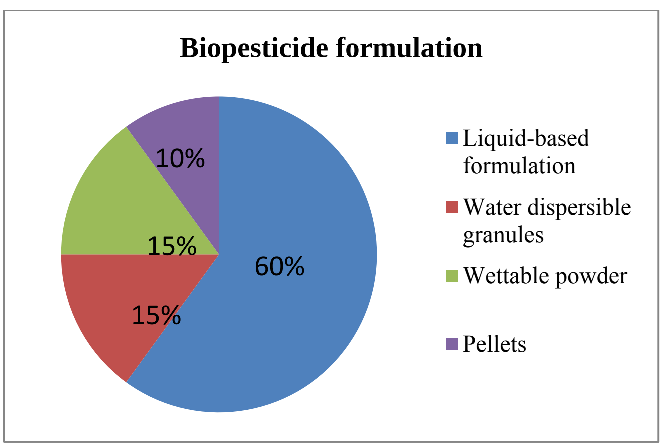

Fig. 2: Global market for biopesticides by formulation (2003-2005)

Worldwide there are 100 commercial neem formulations like Azatin, Bio-Neem, Neemies, Neemguard etc [2]. Other components added to the formulations are surfactants, U.V protectors, thickners, adherents. Liquid based formulations are mostly used when biopesticides are applied to larger areas [14]. As shown in Fig. 2

3. METAGENOMIC APPLICATIONS

Metagenomic study studies comprise isolation, characterization and functional analysis of as yet unreported and non culturable from environmental samples consists isolation of suppressive compounds from microorganisms and extracting fragments of DNA from soil. [31] Application of new genomic techniques, next generation sequencing (NGS) technologies represent new, cost-efficient and fast strategies to depict microbial diversity without the need for culturing the respective organisms and is accomplished by metagenomic studies [32]. By using metagenomics identification of different microbial associations, endophytes and their effects on pest can be studied. This can be very significant because some fungal endophyte exhibits toxic response for plant pathogens affecting growth and secondary metabolite production. such integrated community based studies were made possible studies due to metagenomics [33].

4. CONCLUSIONS

Biopesticides can be a satisfactory alternative to the chemical pesticide when used as part of an overall IPM plan. Advances in biopesticide technology like use of nanopesticide, encapsulation, Recombinant DNA technology make biopesticide more effective, selective or specific and cause less environmental pollution and less toxic to mammals as compare to conventional pesticides.

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