

2. Bio-Pesticides and Their Role in Organic Agriculture

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Abstract:

Chemical pesticides have been widely used by farmers to control plant diseases and insect pests because not only they act on a broad host range, but production technology is also less expensive. However, the distressing part is their unfavorable influence on the environment including the beneficial biota like microorganisms, birds, predators, plants, animals and humans. Despite this, the use of synthetic pesticides has dominated throughout the world in the non-existence of appropriate substitute. Considering this problem, organic farming is receiving prime concern worldwide with the intent of achieving long-term sustainability along with safe, healthy and residue free food. Bio-pesticides have emerged as a prospective eco-friendly input that augment plant protection in organic farming.

An extended range of bio-pesticide products (including as active agent's fungi, bacteria, viruses, nematodes, and beneficial insects) are commercially available for the control of insect pests and plant pathogens. With the onset of greener approach of developing and utilizing bio-pesticides, the condition is slowly improving but actually can proceed far more rapidly in this direction which will be eco-friendly and sustainable. Even though bio-pesticides are gradually replacing the chemical pesticides, an absolute view of the global scenario implies that the former and specifically the industries based on them are still in doubtful state compared to the chemicals which lead the agriculture. This chapter reviews about bio-pesticides, their potential role in organic farming and prospects and challenges of using bio-pesticides.

Keywords:

Bacillus thuringiensis, EPNs, RNA interference, Organic farming, Pesticides

2.1 Introduction:

The global population is continuously increasing which needs to be fed by an agro-ecological system under stress. Consequently, there is growing interest in a productive and ecologically stable agriculture that produces healthy food while preserving environmental integrity for future generations. Not all innovations that increase productivity are free of adverse effects on long-term sustainability. Therefore, there is a need of strategies that are stable, sustainable resilient as well as productive. Organic agriculture is one of the safest strategies to produce non-toxic food for consumption without causing detrimental effects to environment.

Organic agriculture is part of a sustainable approach of food production comprising soil, animals, plants and humans. In organic farming, the control of insect pests, diseases, and weeds is all important since synthetic chemicals used in conventional agriculture are not permitted. The control of insect pests and plant pathogens mainly depends on the preventive and permitted biological measures recommended by the law and regulations on organic production. Bio-pesticides or biological pesticides are potential eco-friendly inputs that supplement plant protection in organic farming. Bio-pesticides 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 protectants) (US EPA, 2015). They have minimum or no risk to the environment, non-target organisms, and natural enemies due to their rapid degradation, mode of action, and the small amounts applied to control pests. Bio-pesticides act slowly, have relatively definite application times, and repress rather than eliminating a pest population (Olson, 2015). They have low field persistence, shorter shelf life and offer no residue problems. Hence, bio-pesticides are accepted for pest management in organic crops.

2.2 Types of Bio-Pesticides:

2.2.1 Biochemical Pesticides:

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms (US EPA, 2015). Plants synthesize a diverse array of essential oils and extracts that prevent their colonization by plant pathogens, insects and other herbivores.

Conventional pesticides, by contrast, are mainly synthetic substances that directly kill or inactivate the pest. Biochemical pesticides include substances that repel approaching insects, prevent feeding and oviposition on the plants, alter behaviour and physiology of insects in several ways and even prove toxic to different developmental stages of many insects (Hikal et al., 2017). Plant families that contain bioactive compounds with activity against important crop pests include Myrtaceae, Rutaceae, Asteraceae, Apocyanaceae, Cupressaceae, Poaceae, Zingiberaceae, Piperaceae, Liliaceae, Solanaceae, Caesalpinaceae, Ranunculaceae and Leguminosae. The use of essential oils extracted from aromatic plants has increased greatly as insecticides due to their popularity with organic growers and environmentally conscious consumers. *Mentha piperita* oil repels flies, lice, ants, and is effective against *Tribolium castanum* and *Callosobruchus maculatus*. Eucalyptus oils rich in cineole is effective against varroa mite, (*Varroa jacobsoni* - an important parasite of honeybee), *Tetranychus urticae*, and *Dermatophagoides pteronyssinus*. The bioactive components of neem (*Azadirachta indica*) are beneficial in controlling a variety of crop pests and diseases. Around 400 species of insect pests of major food crops and 16 plant parasitic nematodes have been found to be controlled by the bioactive compounds of neem. The aerosols from the seed kernel extract and oil of neem suppress *Cercospora* leaf spot, anthracnose, and *Alternaria* leaf blight diseases in black gram. Further, the neem oil is also used to cure plant diseases like rust, powdery and downy mildew, scab and blossom, twig and tip blight, and Botrytis blight. The active compound azadirachtin present in Neem has powerful antifeedant properties against a various insect species. Neem has also been shown to disrupt the metamorphic changes in insects. Pyrethrum, derived from the dried flowers

of *Chrysanthemum cinerariaefolium* is highly efficacious against common household insects. Neem and other plant products have also been found to increase the effectiveness of microbial agents like *B.thuringiensis*, fungi and baculoviruses.

2.2.2 Microbial Pesticides:

Microbial Pesticides include substances derived from microorganisms like fungi, bacteria, protozoa, viruses, and algae to control insect pests and plant diseases. They inhibit pests by producing a pest specific toxin; causing a disease; impeding establishment of other microbes through competition; or other modes of action (Ayilara et al., 2023).

A. Fungi:

Fungal antagonists play an important role in controlling plant pathogens and diseases. These antagonistic fungi have a relatively high reproduction potential (sexually as well as asexually) and a short generation time (Thambugala et al., 2020). Moreover, they are target specific and can survive even in the absence of the host by shifting their mode of parasitism to saprotrophism. These antagonistic fungi species possess several mechanisms, such as mycoparasitism, antibiosis, or production of other inhibitory substances, competition for nutrients or space or induced resistance, that allow them to protect plants from diseases. *Trichoderma* spp. are known efficacious mycoparasites because of their ability to attack and feed on fungal plant pathogens directly.

These biocontrol agents produce cell wall degrading enzymes like chitinases and glucanases that inhibit pathogen growth. Additionally, *Trichoderma* also produce antifungal metabolites that activate the natural defence mechanisms of plants, thereby promoting their disease resistance (Zin et al., 2020). Likewise, *Gliocladium virens*, *G. catenulatum*, non-pathogenic *Fusarium oxysporum*, *Coniothyrium minitans* have been reported as effective biocontrol agents of root-borne fungal pathogens (Postma et al., 2008).

Entomogenous fungi are associated with insects and other arthropods typically as pathogens or parasites. The most common portal entry of fungi is by penetrating the cuticle in a series of competitive interactions with the host (Joglekar et al., 2023). The invasion by fungal mycelium fills the insect with fungus resulting in death of the host by obliteration (choking) of the tissues and also by the toxins produced. The major fungi that can be used for insect control include *Beauveria*, *Isaria* (*Paecilomyces*), *Hirsutella*, *Metarhizium*, *Nomuraea*, *Cordyceps* etc. Entomopathogenic fungi such as *Beauveria bassiana* (White muscardine) and *Metarhizium anisopliae* (Green muscardine) successfully infect susceptible host populations by forming mat on the outer surface of the body (Arthurs et al., 2019).

B. Bacteria:

A number of bacteria from various genera, including *Bacillus*, *Paenibacillus*, *Agrobacterium*, *Acinetobacter*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bradyrhizobium*, *Rhizobium* and *Streptomyces*, have been reported as biocontrol agents to control various diseases in major crops (Massawe et al., 2018; Ayaz et al., 2023). These beneficial bacteria form biofilms and secondary metabolites, which lower the plant pathogen population by

creating plant-microbial interactions in the rhizosphere region (Farzand et al., 2019). *Bacillus* species are one of the most exploited beneficial bacteria as bio-pesticides. They are known to trigger induced systemic resistance in plants against many pathogens by increased defense-related enzyme activity, and modifications such as amino acids and polysaccharides (Bonaterra et al., 2022). Many *Pseudomonas* spp. are effective colonizers of the plant surface. They use plant exudates as nutrients and have a high growth rate, which are required to compete with other microorganisms in the plant environment (Lugtenberg et al., 2001; Ayaz et al., 2023).

Entomogenous bacteria are categorized as spore formers and non-spore formers. The spore forming bacteria (*Bacillus* sp. and *Clostridium* sp.) are more promising in insect control than non-spore forming ones (*Pseudomonas* sp., *Aerobacter* sp., *Serratia* sp.) owing to their high resistant to environmental changes and ability to remain in dormant conditions outside the intended host (Gangwar et al., 2021). Among the spore formers, crystalliferous bacteria are more potent than non-crystalliferous bacteria due to the toxic crystals produced by them. *Bacillus thuringiensis* (*Bt*) is one of the most common crystalliferous bacteria. It produces protein crystals at the time of sporulation. The crystal proteins are regarded as endotoxins to distinguish them from the exotoxins that are produced additionally by bacteria. In the most studied *Bt kurstaki*, large number of disulphide bridges between individual protein molecules stabilize each crystal of δ -endotoxin. Once ingested by susceptible host, *Bt kurstaki* crystals are dissolved by both alkaline (pH >9.5) and reductive environment of the midgut, liberating the full length protoxins of 130 KDa. Then, the midgut proteolytic enzymes (serine proteases) begin their action. These enzymes cleave amino acids leaving behind final toxin core as only half the protoxin size (65-67 KDa). The activated *Bt kurstaki* toxins easily pass across the peritrophic membrane (acts like a molecular sieve that allows molecule up to 100 KDa to easily diffuse through) to interact with the gut epithelium. Thereafter, the toxin molecules inserted into the plasma membrane form a pore that is permeable to small ions and molecules. These pores later enlarge from osmotic swelling, and finally cell lysis occurs. The infected host stop feeding due to gut paralysis (Bravo et al., 2007; Schunemann et al., 2014).

C. Viruses:

Inclusion viruses are effective in controlling insect pests. Inclusion viruses produce polyhedral (Polyhedrosis viruses, PV) or granular bodies (Granular viruses, GV). Polyhedroses inhabiting nucleus are called nuclear polyhedrosis viruses (NPV) while cytoplasm inhabiting is called as cytoplasmic polyhedrosis viruses (CPV). Baculoviruses are the best-known entomogenous viruses (Gelaye and Negash, 2023). When ingested, alkaline gut enzymes dissolve the polyhedral membranes, releasing the virus particles free. These free virus particles penetrate the gut epithelium and infect the haemocytes and tracheal matrix. Inside these cells, they bind themselves to the chromatin in nuclei, multiply and form membranes to reform polyhedral bodies. As proliferation continues, chromatin is consumed. Ultimately, the nuclear and cell membranes rupture to release the polyhedral into the body cavity to infect other cells. The infected insect stops feeding, become pale in color and sluggish. The fragile integument ruptures to discharge blood with fragments of tissues and polyhedra. Before death, the insect ascends to higher positions and dead larvae hang as brown or black cadavers by their prolegs.

D. Nematodes:

Parasitism by entomopathogenic nematodes can have numerous detrimental effects on the insect like reduced fitness, delayed development, sterility and in some cases, mortality. Nematodes in the families Steinernematidae and Heterorhabditidae are obligate parasites of many soil-inhabiting insects and used as potential biological control agents of many insect pests. These nematodes are symbiotically associated with different bacterial species – *Xenorhabdus* (in *Steinernema*) and *Photorhabdus* (in *Heterorhabditis*). The infective juveniles (IJs) present in the soil are attracted towards the gas emitted from insects. IJs usually enter the hosts via natural openings (mouth, spiracles anus), but some nematodes may also pierce soft inter-segmental membranes to enter the hosts. On entering an insect, the associated mutualistic bacteria are released by IJs in the haemolymph. These bacteria proliferate in the haemolymph and secrete toxic metabolites that cause insect mortality due to septicemia. The nematodes then feed on the host tissue, mature and reproduce Shapiro-Ilan et al., 2012).

2.2.3 Plant-Incorporated Protectants (PIPs):

Plant-incorporated protectants (PIPs) are another class of bio-pesticides that are produced by the crop plants from genetic material inserted into the plant genome (Basnet, 2022). The nuclear material of crop plants is stably integrated with genetic material from a naturally occurring microorganism; hence these plants are also called as transgenic plants.

Consequently, transgenic plants have typical characteristics of added genetic material, which are expressed to kill associated pests. Many PIPs have been developed so far. Of them, transgenic plants carrying cry gene (producing toxin) from *Bt* bacterium, *B. thuringiensis*, are most common. This alteration, where a simple plant becomes a transgenic plant, is achieved out via biological or physical manipulation. For example, for transgenic *Bt*, crop plants are inserted with corresponding gene from the *Bt* bacterium by the use of recombinant DNA technology with the help of *Agrobacterium tumefaciens* (Razaq and Shah, 2022).

Proteases from various organisms including plants are also useful to target critical nutrients, metabolic enzymes, or protective barriers. For example, inbred maize lines produce Mir1-CP as part of a defense response to insect feeding. Mir1-CP degrades the insect peritrophic membrane that protects the epithelial lining of the gut; hence, render it vulnerable to ingested pathogens (Pechan et al., 2000).

RNA interference (RNAi) is another promising emerging technology for the development of genetically engineered crops. RNAi (also called as posttranscriptional gene silencing) is often found in eukaryotic organisms, including insects, for sequence-specific gene silencing set off by the presence of double-stranded RNA (dsRNA) (Whangbo and Hunter, 2008). In brief, cleavage of dsRNAs by an enzyme called dicer into sequence-specific effector molecules, called small- or short-interfering RNAs (siRNAs), which target homologous RNAs for destruction is the first step in the RNAi pathway. These siRNA are then put together into a large protein complex called RISC (for RNA-inducing silencing complex). Later, the duplex siRNA is unwound, leaving the antisense strand which guide RISC

towards its target mRNA for endonucleolytic cleavage to ensure perfect siRNA-mRNA sequence complementarity. Gene silencing occurs when the target mRNA is cleaved at a single site in the center of the duplex region between the guide siRNA and the target mRNA (Nelson and Alves, 2014). Many studies have shown that meaningful levels of insect and disease resistance can be achieved by producing dsRNA in plants (Kumar et al., 2012; Zhu et al., 2012; Hariharan et al., 2021).

2.3 Production, Formulation and Commercialization of Bio-pesticides:

Botanical pesticides are developed from plants and plant parts acquired from the environment, natural or man-made. The procured materials are cleaned to remove foreign materials followed by extraction either using solvents or distillation to obtain extracts or essential oils, respectively. The resultant extracts are screened for efficacy in laboratory using different methods such as disc diffusion, agar well diffusion, agar dilution and poisoned food technique against different pests (Jahangiriana et al., 2013). The field trials are then conducted for evaluating the potency of active botanicals. The most active constituents of the specific extracts are then confirmed for optimum formulation. The most productive combination of the active compounds, surfactants, emulsifiers, carrier materials and other components used in pesticide development are optimized by carrying out intensive laboratory and field trials conditions (Nashwa and Abo-Elyousr, 2012). The report of most potent formulation from the laboratory and field trials is sent to the pest control products body for product registration (Lengai et al., 2018).

Microbial pesticides are also prepared following the same procedure as botanicals, except that the antagonistic microbes are collected from different sources like the rhizosphere, compost and manure. The pure cultures of antagonistic microbes are isolated and maintained in agar slants. The active microorganisms are further propagated on a suitable substrate in the laboratory and mixed with carriers, enhancers and stabilizers for field application (Naing et al., 2013). The laboratory and field efficacy trials are conducted for the final registration process (Lengai et al., 2018).

2.4 Prospects and Limitation of Bio-Pesticides:

Bio-pesticides are gaining attraction due to the advantages linked with the environmental safety, biodegradability, efficacy, target-specificity, and suitability in the integrated pest management (IPM) programs (Kumar and Singh, 2015). Although application of agrochemicals is all-important to meet the ever-growing demands of food, feed and fodder, there are many opportunities in selected crops and niche areas where bio-pesticides can be used as a constituent of IPM. In the nearby future, bio-pesticides could replace conventional chemical pesticides without significantly influencing productivity and yield, if their potentials are fully utilized (Mishra et al., 2020). The constraint confronting the full acceptance of bio-pesticides are differing standard method of preparations and guidelines, dose determination of active ingredients, the susceptibility of bio-pesticides to several environmental factors, ephemeral stability, slow action among others and inability to meet global market demand. Many countries have modified their policies to cut down the use of chemical pesticides and encourage the use of bio-pesticides; yet bio-pesticides are still regulated to a large extent by the system originally designed for chemical pesticides.

This has developed market entry barriers on the bio-pesticide industry by inflicting burdensome costs (Singh and Kumar, 2014). One of the major hurdles in promoting bio-pesticides as replacement to chemical pesticides is the lack of profile of bio-pesticide, which shows shortcoming of the supporting policy network. Relative immaturity of the policy network, lack of trust between regulators and producers, and limited resources and capabilities, are some of the serious problems.

Research in production, formulation and delivery can considerably help in commercialization of bio-pesticides. While predicting the limitations to be confronted through research breakthroughs in the coming years, farmers, especially in rural areas, can take advantage of a crude plant extract in avoiding losses due to pests. The efficacy of the application of bio-pesticides is maximized when incorporated in IPM. An IPM is a pest-control program, comparable to a successful multi-faceted approach including the blend of cultural practices and other suitable control measures into one management program for long-lasting reduction of pest population and related problems (Sadof et al., 2021). Further, more research is required for combining biological agents into production system, enhancing capability of developing countries to manufacture and use bio-pesticides. Simultaneously, it is also requisite to encourage pesticide companies, public funded programme and commercial investors to take up bio-pesticide enterprises. Equally essential is the establishment of strict regulatory mechanisms in the developing countries for sustaining the quality and availability of the bio-pesticides at affordable cost (Leahy et al., 2014).

2.5 Conclusion:

The global production and utilization of bio-pesticides is rising at a fast rate. The increasing concern of growing pesticide residue free agricultural produce (organic farming) would definitely assure increased utilization of bio-pesticides by the farmers. To popularize bio-pesticides, production and quality control training to manufacturers, and organizational training to extension workers and farmers is essential. Since environment protection is a global concern, we need to educate the agriculturists, manufacturers, policy makers and the common men to switch-over to bio-pesticides for pest management. If employed properly, bio-pesticides have potentiality to create sustainability to global agriculture for ensuring food security.

2.6 References:

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