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Role of biocontrol agents in agriculture: A review

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Abstract

Use of agrochemicals is not sustainable in long run, and we can see consequences of their use, however during 1960s and 1970 when agrochemicals were in use in India and their use was accelerated, we didn't recognize their side effects at that time and their side effects were not given any importance, however at this point of time there is enormous information, reports, books on their use and harmful effects on environment, human, animal and plant life and soil quality.

During 1960's when HYV seeds were introducing in India, particularly Rice and Wheat, they demanded extensive input care, they demanded use of heavy dose of fertilizers, water and also plant protection chemicals. Because of plant breeding, development of varieties using common ancestor, genetic erosion (as land races and wild forms had genes for biotic and abiotic stress), we are narrowing the genetic base and going towards uniformity and this let to susceptibility of developed varieties to races of pathogens, insects and made plant more prone to biotic stress, hence there was extensive use of plant protection chemicals.

We use wide range of plant protection chemicals to kill weeds, insects, fungi, bacteria, nematode and etc which we term Herbicides, Insecticides, Fungicides, Nematicides, Acaricides and etc. And use of these chemicals not just harm environment on a whole but also human (farmer) in particular and increase cost of production. Hence to achieve sustainability and to decrease cost of production we must go through Holistic approach of pest management and use different tools to keep pest population below ETL and this Holistic approach is what we call Integrated pest management, Biocontrol agents are one attractive method of pest management because it is target specific, effective in control, environmentally friendly.

Biocontrol involves use of live microorganisms i.e., bacteria, fungi, viruses, protozoans and nematodes to kill the insects. Hence these microbes either parasitize the insect to kill it, or release toxins to disturb physiology of insect. Moreover, these microbes must be use with care and their specifications must be known and understood orelese use of these will back fire us, for instance, use of *Bacillus thuringiensis* near silkworm farm or its use near Moriculture will lead to death of silkworm catterpillar and destroy silk industry.

Keywords: Tree top disease, grasseries, endotoxin, npv, muscadine, biocontrol, strains, mass multiplication

Introduction

Live microbial strains such as bacteria, fungi, virus, protozoans, and nematodes are used to kill Insects which harm crop production. Living organisms show different kinds of interactions such as symbiosis/Mutualism, Commensalism, Antagonistic, Amensalism, competition of which symbiosis/Mutualism, Commensalism, synergism show positive interaction and others being negative interaction which we are going to utilize to control insects of crop production importance.

Genus, *Bacillus* is most important for biocontrol perspective as there are many species from this genus which are used as biocontrol, species such as *thuringiensis, papillae* and etc, of which *Bacillus thuringiensis* is most important. It was 1st discovered in Japan by Ishiwata during a silkworm disease, and Ishiwata names it as Bacillus soto, however later it was rediscovered in Thurigin province of Germany in flour moth, where it was explained in more detail hence it was named *Bacillus thuringiensis.* The bacteria when enter into catterpillar body through mouth, the protein from Bacillus thuringiensis, modifies, activates in alkaline environment of Insect GUT and then cause pores in Digestive canal leading to leakage of fluids from GUT into body and putrefies the insect body. *Bacillus papillae* causes milky disease in Japanese beetle. Viruses are yet another important biocontrol agents, Nuclear polyhedrosis virus (NPV), (Nuclear because its propagation happen in nucleus, Polyhedrosis for its polyhedral shape), is taken again through mouth, virus parasitice insect body, leading to fading of insect body, loss of hunger and insect enters hypoxemia stage where it feels lack of oxygen, hence it crawls to top of tree in suffocation and get paralyses (hence called tree top disease).

Apart from bacteria and viruses, fungi are one of important biocontrol agents, causing Muscadine (mummification of body i.e., body inside and outside is filled with fungal conidia) *Beauveria bassiana* and *Metarhizium anisopliae* are two common fungi which cause white and green Muscadine.

Nematodes such as *Xenorhabdus* and *Photorhabdus* are biocontrol in nature

Use of these biocontrol agents in Integrated pest Management is definitely a path towards sustainability along with use of Biorationals (Rational means intelligence, I mean able to differentiate) such as Semio-chemicals (chemicals used for communication) which include Pheromones (Intra such as sex pheromone, alarm pheromone and inter such as Allomones which are repellents, Kairomones i.e., attractants, Apneumones i.e., synthetic pheromones and Synomone), botanicals such as neem, Pongamia spp, Custard apple an etc.

Bacterial Biocontrol Agents

Bacillus spp i.e, rod shaped, spore producing, gram positive bacteria, there are very few bacteria which produce spore, *Bacillus spp*, are one among them, which produce spore, more specific, endotoxin which are produced during extreme situations such as high heat, lack of nutrients, as at that point of time bacteria want to sustain its life hence produce endotoxin which is resistant to extreme environment because of presence of calcium DPA (Di picolinic acid – approx 15% dry weight), and along with endospore there is a toxin released during this event which we call Delta Endotoxin (delta in order of discovery other being alpha, beta and gamma) in *Bacillus thuringiensis.* And this delta Endotoxin when taken through insect during feeding, the toxin enter GUT, and in alkaline PH of insect gut, modifies protoxin to toxin (proteolysis) which is the active form.

This endotoxin is like a receptor with a particular shape, this particular shaped endotoxin binds with GUT wall, and then chain of events takes places leading to puncturing of GUT wall, because of which all the contents from digestive track get into body. In initially 2 days the catterpillar doesn't feed any food, slowly it becomes lethargic and then putrefied.

Endotoxin is a protein produced during sporulation and it's coded by genes which are called cry genes, cry standard for Crystal which are harmful for insects hence insecticidal, however they cause little or no harm to humans, most beneficial insects and other non-target organism. There are different cry genes in different strains and each of this gene lead to a particular crystal protein which is of particular shape and molecular weight and each of this is insect specific. For instance:

Table 1: Strains of *Bacillus thuringiensis*

Strain/ subsp	Protein size	Target Insects	Cry	Shape
berliner	130 – 140 kDa	Lepidoptera	Cry 1	Bipyramidal
kurstaki	130 – 140 kDa	Lepidoptera	Cry 1	Bipyramidal
entomocidus	130 – 140 kDa	Lepidoptera	Cry 1	Bipyramidal
aizawai 7.29	130 – 140 kDa	Lepidoptera	Cry 1	Bipyramidal
aizawi IC 1	135 kDa	Lepidoptera, Diptera	Cry 1	Cuboidal
kurstaki HD 1	71 kDa	Lepidoptera, Diptera	Cry 2	Cuboidal
tenebrionis (sd)	66 – 73 kDa	Coleoptera	Cry 2	Flat/ irregular
morrisoni PG14	125 – 145 kDa	Diptera	Cry 4	Bipyramidal
israelensis	68 kDa	Diptera	Cry 4	Bipyramidal

Bacillus thuringiensis, was discovered in Japan in 1901 by Ishiwatari by a Japanese Botanist during a silkworm disease and then in 1911 (10 years later), Berliner isolated it from Mediterranean flour moth in Thuringia province of Germany and described it in 1915. Ishiwatari initially named it as *Bacillus soto*. Bt is closely related to yet another species i.e., *Bacillus cereus* which causes food poisoning and both are transformable and discovery of this also led to understanding that the cry gene is located on plasmids.

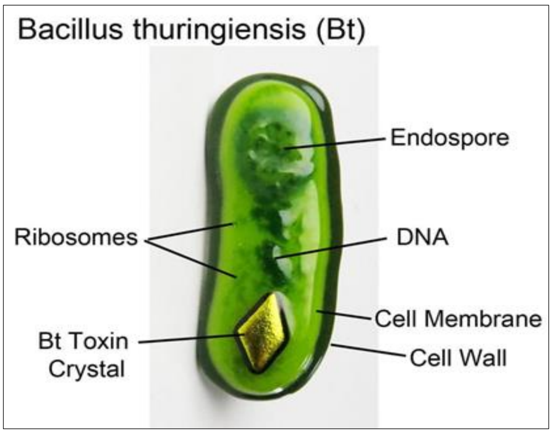
And there must be a realization at this point of time that, this delta endotoxin is released during sporulation (endospore release) and this can be realized as dead flour moth caterpillars were found to be loaded with spores and crystals. And these spores when came in contact with insect integument let to catterpillar slowly showing symptoms, however these spores as such are not virulent, the catterpillar show symptoms only when spores. Crystals are coated on leaves.

After recognizing the potential of Bt as an insecticide, Mattes (1927) isolated the Bt strain discovered by Ernst and subsequent field trails against the European corn borer gave promising results. This work eventually led to the development of Sporeine, a commercial Bt insecticide, which was used for the first time in 1938.

Apart from Bt which is used in Agriculture to control Lepidopteran catterpillar, beetles and flies. There are strains in Bt which is used to control a common domestic pest i.e., mosquito which transmit many diseases. Bt var *israeliensis*, was isolated in Israel in 1976 and it is seen to be effective against flies i.e., mosquito. And crystal genes lethal to flies are located on a megaplasmid pBtoxis which contains 4 Cry proteins and 2 Cyt protein encoding genes (Cry 4Aa, Cry 4Ba, Cry 11 Aa, Cry 11 Aa, Cyt 1Aa and Cyt 2Ba) and Cry and Cyt act in Synergy. Cyt act as receptor by interacting with lipid membrane of larval midgut and Cry bind to it.

Cry and Cyt belong to a class of toxins called pore forming toxins (PFT).

Sporeine was 1st commercial developed Bt based insecticide which was developed in 1930s in France, however till 1961 no Bt pesticide was registered in United States. But Thuricide was already in market which was based on *kurstaki strain HD 1* and this was used against Lepidopteran. However, in 1960s new strains were developed and this led to replacement of many early products with new Bt strains that were more effective that previous products.



Source: Ohio State University (Caroline Mifsud PHR 7588 Blog, May 18, 2019)

Picture 1: Typical structure of Bacillus thuringiensis

Viral Biocontrol agents

Nuclear Polyhedrosis Virus

It's an obligate pathogen. The virus consists of a proteinaceous polyhedral occlusion body inside which the virions or virus rods are embedded. And similar to *Bacillus thuringiensis*, the alkaline PH of GUT favor virus for its virulence, in alkaline GUT, the virus is liberated, and attack nuclei of cells of GUT tissues, tracheal matrix, haemocytes, ganglia and brain.

Infected insects become dull in color, less active and larvae become pinkish white on ventral side. In advanced stages larvae become flaccid, the skin become fragile and eventually ruptures. Diseases larvae hang upside from the plants. This is called "tree top disease" or "wipfel krankeit". Disease caused by NPV in silk worm is called as Grasserie.

NPV is genus/ species specific. NPV formulations are commercially available for *H. armigera* (HaNPV), *Spodoptera litura* (SlNPV) and *Amsacta albistriga* (AaNPV). The recommended dose for field application is 250-500 LE/ha. LE stands for larval equivalent.

Granulosis Virus

Granulosis virus (GV): Virions are occluded singly in small inclusion bodies called capsules. It attacks cytoplasm or nucleus of epidermis, trachea and fat bodies. Infested larvae lose appetite, become sluggish and show milky white colour on ventral surface. GV is also specific and available for sugarcane early shoot borer. The recommended dose for field application is 750 diseased larvae/ha twice at 35 and 50 DAP.

Picture 2: Showing tree top disease (also called Wipfel krankeit)

Fungal Biocontrol Agents

Conidia is the infective part of fungi, it's an asexual/ mitotic, aplanospore (absence of flagella), and Conidia disperse from conidiophore through various means with air, water, animals and etc. And conidia get attached to integument of insect catterpillar and then produce germ tube and penetrates the cuticle and drains the nutrients from the insect body. And all of these processes are interestingly very much similar to the process through which fungal is infected. And here the conidia are green in colour hence we call Green Muscadine, there is yet another most common fungal biocontrol agent which causes White Muscadine.

Biological Interactions which contribute to biological control

Table 2: Different types of interspecies that lead to biological control of plant viruses.

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/predation	Lytic/some nonlytic mycoviruses, *Ampelomyces quisqualis, Lysobacter enzymogenes, Pasteuria penetrans, Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol, Phenazines, Cyclic lipopeptides
	Lytic enzymes	Chitinases, Glucanases, Proteases
	Unregulated waste products	Ammonia, Carbon dioxide, Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores, Germination signals consumption, Molecular cross-talk confused
Indirect antagonism	Competition	Exudates/leachates consumption, Siderophore scavenging, Physical niche occupation
	Induction of host resistance	Contact with fungal cell walls, Detection of pathogen-associated, molecular patterns, Phytohormone-mediated induction

Antibiotics

These are chemicals which are low concentrations kill other germs. I mean one organism being antagonistic to other because of chemical that it secretes. Antibiotics can be anti – fungal or anti – bacterial.

Many methods have been developed to determine, when and where antibiotics are produced by a biocontrol agent.

Table 3 is intended to explain about different antibiotics produced by microbes which are having potential to be used in agriculture.

Table 3: Some of antibiotics by BCA

Antibiotic	Source	Target pathogen	Disease	Reference
2, 4-diacetyl-phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium* spp.	Damping off	Shanahan *et al*. (1992)
Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall	Kerr (1980)
Bacillomycin D	*Bacillus subtilis* AU195	*Aspergillus flavus*	Aflatoxin contamination	Moyne *et al*. (2001)
Bacillomycin, fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium, oxysporum*	Wilt	Koumoutsi *et al*. (2004) [6]
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*	Damping off	Islam *et al*. (2005)
Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rots	Wilhite *et al*. (2001)
Herbicolin	*Pantoea agglomerans* C9-1	*Erwinia amylovora*	Fire blight	Sandra *et al*. (2001)
Iturin A	*B. subtilis* QST713	*Botrytis cinerea* and *R. solani*	Damping off	Paulitz and Belanger (2001), Kloepper *et al*. (2004) [5]
Mycosubtilin	*B. subtilis* BBG100	*Pythium, aphanidermatum*	Damping off	Leclere *et al*. (2005) [8]
Phenazines	*P. fluorescens* 2-79 and 30-84	*Gaeumannomyces, graminis* var. *tritici*	Take-all	Thomashow *et al*. (1990)
Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	*Pythium ultimum* and *R. solani*	Damping off	Howell and Stipanovic (1980)
Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*R. solani* and *Pyricularia oryzae*	Damping off and rice blast	Homma *et al*. (1989)
Zwittermicin A	*Bacillus cereus* UW85	*Phytophthora medicaginis* and *P. aphanidermatum*	Damping off	Smith *et al*. (1993)

Conclusion

Farmers, plantation and orchard owners, floriculturists all must be educated with basic practices of IPM (Integrated Pest Management). Farmer depending entirely on chemicals is not sustainable hence must be avoided and there are many well established facts, regarding how detrimental it's to use insecticides such as incidents such as Kasargod Endosulfan.

During 1990's till early 21st century, a strong fact established in Kerala, that use of Endosulfan (Broad-spectrum Organochloride, insecticide and acaricide used on food and nonfood crops) in mango, cashew and many other orchards continuously for many decades had led to pollution of air, water, animal and human. And here Endosulfan was not just used as such, in fact it was sprayed through helicopter through great heights to large hectares of land in cashew and mango orchards (to control Tea mosquito bug in cashew – which is a Hemipteran, a sucking pest), and as sprayed from great heights through helicopter, it contaminated water, soil and also crop and people in Kasargod who were drinking the water and living in that land suffered a lot in long run.

And farmers exposed to these chemicals are facing long term problems, use of agrochemicals leading to high cost of production and farmers going into debt trap and distress sale.

Hence to overcome all these side effects, its important to use ecofriendly pest control solutions, one among them is Biocontrol agents which are live microbes such as Bacillus thuringiensis, Bacillus papillae, Metarhizium anisopliae, Beauveria bassiana, NPV, and Photorhabdus and Xenorhabdus.

Evolution of Insecticides

Chlorinated Hydrocarbons: DDT, Chlordane, Dieldrin – 1940's – 1950's.

Organophosphates and Carbamates: Dimethoate, Acephate, Chlorpyrifos – 1960s – 1970s.

Pyrethroids – Deltamethrin, Bifenthrin: 1980s – 1990s.

Reduced Risk Insecticides: 1990 – 2000s.

Naturalytes – Spinosad, Neem and etc.

Insect Growth Regulators:

Neonicotinoids: Imidacloprid, Thiamethoxam.

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