COURSE TITLE: ENTREPRENEURSHIP IN MICROBIOLOGY

Course Code: 24MICEPSC3

Courser Teacher: Dr. R. Vijayakumar

BIOPESTICIDES

Includes naturally occurring substances that control pest (biochemical pesticides), microorganisms that control pests (microbial pesticides) and pesticidal substances produced by plants containing added genetic material (plant incorporated protectants or PIPs)

CLASSES OF BIOPESTICIDES

1. BIOCHEMICAL PESTICIDES

- Naturally occurring substances that control pests by non-toxic mechanisms
- Includes substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps

CLASSES OF BIOPESTICIDES

2. MICROBIAL BIOPESTICIDES

- Consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient.
- Active ingredient is relatively specific for its target pest
- Eg: some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes.

CLASSES OF BIOPESTICIDES

3. PLANT – INCORPORATED PROTECTANTS (PIPs)

- Pesticidal substances that plants produce from genetic material that has been added to the plant.
- Eg: scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest.

NEEDS FOR BIOPESTICIDES

- Proper pest management is important factor for healthy and high yielding crop to fulfill the food demand for increasing population.
- Chemical pesticides have accelerated land, air and water contamination.
- They have been the main cause of insect resistance as well as adverse impacts on natural enemies and humans.

ADVANTAGES OF BIOPESTICIDES

- Less toxic than conventional pesticides.
- Effect only the target pest and closely related organisms whereas conventional pesticides are broad spectrum pesticides.
- Effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management programs, biopesticides can greatly reduce the use of conventional pesticides while crop yields remain high.

MICROBIAL BIOPESTICIDES

- Constitute the largest group of broad-spectrum biopesticides which are pest specific.
- There are atleast 3000 naturally occurring insectspecific microorganisms, 100 of which are insecticidal.

1. Bacterial biopesticides
2. Viral biopesticides
3. Fungal biopesticides
4. Protozoan biopesticides

BACTERIAL BIOPESTICIDES

Mainly 4 categories:

1. Crystalliferous spore formers (*Bacillus thuringiensis)*
2. Obligate pathogens (*Bacillus papiliae)*
3. Potential pathogens (*Serratia marcesens)*
4. Facultative pathogens (*Pseudomonas aeruginosa)*

Out of these four, 1 and 2 are important biopesticides.

Bacillus thuringiensis

- GP, spore forming, facultative bacterium with nearly 100 subspecies and varieties divided into 70 serotypes.
- Specific, safe and effective tool for insect control.
- Insecticidal property resides in Cry family of crystalline proteins that are produced in the parasporal crystals and are encoded by the cry genes

Bacillus thuringiensis – CRY proteins

- Cry proteins are globular molecules with 3 structural domain connected by single linkers.
- This 3 domain family is characterised by protoxins of two different lengths, one being longer with C

  – terminal extension necessary for toxicity.
- This extension also has a characteristic role in

crystal formation within the bacterium.

- ry proteins are responsible for feeding cessation and death of the insect.

Bacillus thuringiensis- mechanism

- Cry protoxins are ingested and then solubilised, releasing a protease resistant biologically active endotoxin, before it is being digested by protease of the gut to remove amino acids from its C and N terminal ends.
- The C terminal domain of the active toxin binds to the specific receptors on brush border membranes of the midgut.
- It is followed by the insertion of the hydrophobic region of the toxin into the cell membrane.
- This creates a disruption in the osmotic balance because of the formation of transmembrane pores.
- Ultimately cell lysis occurs in the gut wall leading to leakage of gut contents.
- This induces starvation and lethal septicaemia of the target pest.

Bt subspecies and targets
B. thuringiensis tenebrionis Colorado potato beetle and elm leaf beetle larvae
B. thuringiensis kurstaki Variety of caterpillars
B. thuringiensis isralensis Mosquito, blackfly and fungus gnat larvae
B. thuringiensis aizawai Wax moth larvae and various caterpillars especially diamond back moth caterpillar

Bacillus sphaericus

- GP, strict aerobic bacterium, which produces round spores in a swollen club like terminal or subterminal sporangium.
- Produces an intracellular protein toxin and a parasporal crystalline toxin at the time of sporulation.
- The mosquito – larvicidal binary toxin produced by *B. sphaericus is composed of Bin* A (51.4 kDa) and Bin B (41.9 kDa).
- Bin proteins forms a crystal and in solution it can exist as an oligomer containing 2 copies each of Bin A and Bin B.
- Some toxin strains also produces 100 kDa toxins encoded by *mtx genes.*
- Mainly used for mosquito control.

VIRAL BIOPESTICIDES

- The viruses used for pest control are:

DNA containing baculoviruses (BVs)	**Nucleopolyhedrosis viruses (NPVs)**
Granuloviruses (GVs)	Acoviruses
Parvoviruses	Polydnaviruses
Pox viruses	RNA containing recoviruses
Cytoplasmic polyhedrosis viruses	Nodaviruses
Picorna like viruses	Tetraviruses

VIRAL BIOPESTICIDES

They are narrow spectrum.

- After application to plant surface, baculovirus occlusion bodies (OBs) are rapidly inactivated by solar UV radiation (280 – 320nm).
- Efficacy can be improved by the use of formulations that include stilbene derived optical brighteners, which increase susceptibility to NPV infection.
- UV inactivation can be controlled by creating systems which filter UV radiation such as plastic greenhouse structures

MECHANISM

- Replication of virus occurs in the nuclei or cytoplasm of the target cell
- The expression of viral proteins occurs in 3 phases:
- Early phase ie, 0-6 h post infection; Late phase ie, 6-24 h post infection
- Very late phase ie, upto 72 hr post infection
- It is at the late phase that the virions assemble as the 29kDa occlusion body protein is synthesised.
- Virions of NPVs are occluded within each occlusion body to develop polyhedra whereas the GV virion is occluded in a small occlusion body to generate granules
- Infected nuclei can produce 100s of polyhedra and 1000s of granules per cell.
- These can create enzootics, deplete the pest populations and ultimately create significant impact on the economic threshold of the pest

VIRUSES AND THEIR TARGETS

VIRUSES	TARGETS
Cydia pomonella GV	Codling moth on fruit trees
Phthorimaea operculella GV	Potato tuberworms
Virus based products	Cabbage moth, corn earworms, cotton leafworms, bollworms, celery loopers, tobacco budworms

FUNGAL BIOPESTICIDES

- Fungi specifically associated with insects (aphids, thrips, mealy bugs, whiteflies, scale insects, mosquitoes and mites) are known as
- entomopathogenic fungi.
- Obligate or facultative, commensals or symbionts of insects.
- Belong to 4 major groups:
- Laboulbeniales
- Pyrenomycetes
- Hyphomycetes
- Zygomycetes

- Most widely used species include:
- *Beauveria bassiana*
- *Metarhizium anisopliae*
- *Nomurea rileyi*
- *Paecilomyces farinosus*
- *Verticillium lecanii*
- The fungi attack the host via integument or gut epithelium and establish their conidia in the joints and the integument.

- *B. bassania and M. anisiphliae causes muscardine*
- insect disease and after killing the host, cadavers
- become mumified or covered by mycelial growth.
- *Streptomycetes produce toxins.*
- Most active toxins are actinomycin A, cyclohexamide and novobiocin.
- Spinosyns are isolated from *Saccharopolyspora spinosa and are active against dipterans,* hymenopterans, etc. and less active against
- coleopterans, aphids and nematodes.

PROTOZOAN BIOPESTICIDES

- Although they infect pests, induce chronic and debilitating effects on targets, the use of protozoa as biopesticide has not been very successful.
- *Microsporan* protozoans are used as possible component of IPM.
- Microsporidia are ubiquitous, obligate intracellular parasites.

Eg: *Nosema and Vairimorpha have some potential* to attack lepidopteran and orthopteran insects.

Nosema pyrausta

- A microsporidian which infect European corn borer, *Ostrinia nubilalis.*
- Spores eaten by corn borer larvae germinates in the midgut and injects sporoplasm into midgut cell.
- The sporoplasm reproduces and then forms more spores, which can infect other tissues
- Spores in infected midgut cells are sloughed into the gut lumen and are eliminated along with the faeces to the plant.

Nosema pyrausta

- These spores remain viable and are consumed during larval feeding so that infection is repeated in midgut cells of new host. This is horizontal transmission.
- *Nosema can be passed by vertical transmission.*
- As the infected female larva develops to an adult, the ovarian tissue and developing oocytes become infected.
- The embryo and hatched larvae is infected.
- It suppresses by reducing oviposition, % hatch and survival of infected larvae.

Nosema locustae

- Infects grasshoppers
- Most effective when ingested by nymphal stages of grasshoppers and kills them within 3

– 6 weeks post infection.

- Not all infected grasshoppers are killed by this protozoan infection.

MICROBIAL PRODUCTS IN BIOPESTICIDES

- Microorganisms are known to produce anti – pest chemical compounds.
- Antinsectan compounds derived from:
- Non – filamentous bacteria (eg: aminolevulinic acid, thiolutin, thuringiensin, xenorhabdins)
- Actinomycetes
- Fungi (eg: actinomycin A, avermectins, citromycin, nikkomycin, spinosyns, various cyclic peptides) are well known as toxins, growth inhibitors, antifeedants and physiological disrupters.
- Some transgenic crops can be considered among microbial based products

  Eg: *Bacillus thuringiensis based genetically* engineered crops like Bt cotton and maize.
- Genetically modified (GM) sugar beet, papaya, sweet pepper, tomato etc are successfully grown.

MAIN CHALLENGES TO MICROBIAL PESTICIDES

- The utilization of microbial pesticides in IPM model requires high scientific study such as systematic surveys on properties, mode of action, pathogenicity, etc.
- Ecological studies are necessary on the dynamics of diseases in insect populations because the environmental factors play a vital role in disease outbreaks to control the pests.
- In order to improve mass production technologies; contamination should be reduced with the improvement of formulation potency and increase in shelf-life of microbial biopesticides.
- Dry formulations should be commercially focused than the liquid formulations with the improvement of slow speed with which microbial pathogens kill their host. Genetic and biotechnological tools would lead to the production of strains with improved pathogenesis and virulence.
- Due to narrow specificity mostly forces biopesticide application with common conventional insecticides. However, this practice can also lead to incompatibility problems such as inhibition or death of the living organism.
- All aspects study should be done especially; persistence, resistance, dispersal potential, the range of non-target organisms affected directly and/or indirectly in order to solve the problem of regarding the regulatory and registration.

POSITIVE ASPECTS OF MICROBIAL PESTICIDES

The bioactive agents are basically non-toxic and nonpathogenic to non-target organisms, communities and humans.

• They have narrow area of toxic action, mostly specific to a single group or species of insect pests and do not directly affect beneficial insects (predators, parasites, parasitoids, pollinators) in treated areas.

• They can be used in combination with synthetic chemical insecticides because in most cases the microbial product is not deactivated.

Their residues have no adverse effects on humans or other animals, therefore, microbial insecticides can be used in near harvesting time.

• Sometime, the pathogenic microorganisms can become established in a pest population or its habitat and provide control pest generation to generations or season after seasons.

• They improve the root and plant growth by encouraging the beneficial soil microflora and also increase yield.

DEMERITS OF MICROBIAL PESTICIDES

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.

REFERENCES

✓Nawaz M, Juma I, *et.al.Current status and advancement of biopesticides* Microbial and botanical pesticides; Journal of Entomology and Zoology studies 2016 (*www.entomo/journal.com/archives/2016/vol 4 issue 2/Part D/ 4-2-45.pdf*)

✓B.ramanujam*, et al.management of insect pests by microorganisms, Poc* Indian Natn Sci Acad 80 no.2 June 2014 Spl. Sec pp.455-471 *(www.insa.nic.in/writeraddata/ UpLoadedFiles/ PINSA/ Vol80-2014-2-Art27.pdf)*

✓Koul Opender, Microbial biopesticides: opportunities and challenges; CAB reviews Perspectives in agriculture, veterinary studies, nutrition and natural resources, 2011No. 056;pdf (http://www.cabi.org/cabreviews)

✓http://www.epa.gov/ingredients-used-pesticide-products/what -arebiopesticides.