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Unit -VIII (Applied Microbiology: biopesticides Trichoderma sp. and Pseudomonas)

NEP-2020 M.Sc. Ist sem Botany Microbiology (Paper Code: BOY-60094)

Unit -V (Applied Microbiology: biopesticides)

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BIOPESTICIDES

A Biopesticide is broad term and it may define as

"**A form of formulated product based on beneficial micro-organisms or natural products from plant and their parts used to manage the pathogens and insect pests is called biopesticide"**

It may be based on

(A) Antagonistic fungal biocontrol agents (*Trichoderma* spp.- mycoparasite, *Arthrobotrys ologospora*- nematophagous *Paecilomyces* spp.-nematophagous, *Verticillium* spp.-nematophagous, *Beauveria bassiana-entomopathogenic*, *Metarhizium anisopliae-entomopathogenic*).

(B) Bacterial biocontrol agents (*Bacillus* spp., *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Xanthomonas*),

(C) Entomopathogenic nematodes *Steinernema carpocapsae* (ambush forager) and *Steinernema glaseri* and *Heterorhabditis bacteriophora*a (cruising forager)

(D) Entomopathogenic viruses nucleopolyhedroviruses (NPV) and granuloviruses (GV)

(E) Predators, parasitoides, parasites as natural enemies

(F) Insect pheromones

(G) Botanical antagonists as biopesticide

Mycoparasites as bio-pesticides

Those beneficial fungal biocontrol agents (e. g. Trichoderma spp.) are known to suppress the population of pathogenic fungi (e. g. *Fusarium oxysporum*, *Phytophthora* spp, *Sclerotium rolfsii*, *R. solani*, *S. sclerotiorum*, *Alternaria* spp. etc.) are considered as mycoparasites. Formulated product of myco-parasitic fungi is known as fungal biopesticides to manage disease causing pathogens. The mode of fungal biocontrol agents are as follows

- Through parasitism, mycelium of biocontrol agent coiling around the mycelium of pathogen take food
- Production of antibiotics, by production of secondary metabolites, biocontrol agent used to disrupt metabolism and function of pathogens
- Production of volatile and non-volatiles substances, during the developmental stages, biocontrol agents used to produce volatile and non-volatiles substances to suppress the pathogens by diffusion at their vicinity.
- Production of enzymes (e. g. chitinase, 1,4 β-glucanase etc.) to massacrate the cell membrane of mycelium for lysis.
- Competition for nutrients and niches, microbes which have ability to grow faster required more food as compared to others.
- Induced resistance
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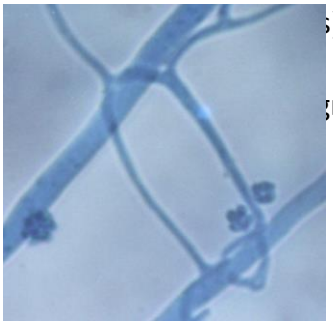

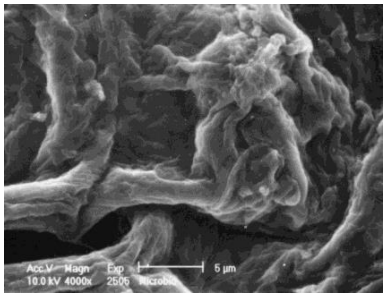

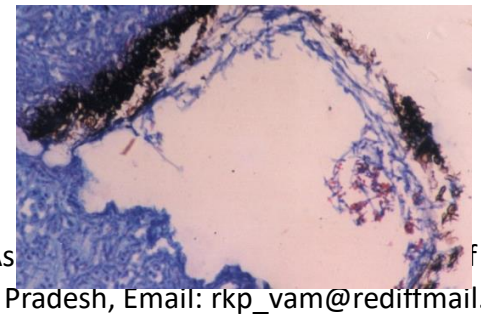

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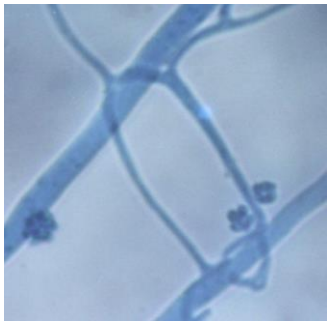

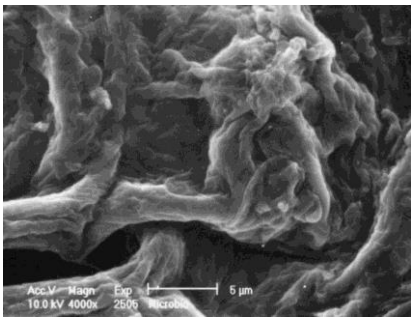

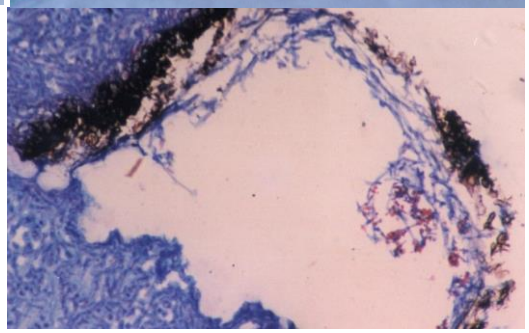

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Inoculation of *Trichoderma* stock solution in bajra grains for mass production

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Mass Production of *Trichoderma* spp.

- Effective strains of *Trichoderma* spp. were mass multiplied by using conventional means (grains like sorghum, maize, wheat et.) and using agricultural wastes like maize cob, tea, and distilled wastes of aromatic plants etc.
- The highest cfu of *Trichoderma* spp. was observed in carbon rich agricultural waste.

Formulations on different wastes

Nematophagous fungi as bio-pesticides

Those beneficial fungal biocontrol agents (e. g. *Arthrobotrys ologospora*, *Paecilomyces* spp., *Verticillium* spp.,) are known to parasitize on plant parasitic nematodes particularly on eggs, egg masses are considered as nematophagous fungi. Formulated product of entomopathogenic fungi is known as nematophagous fungi based biopesticides to manage plant parasitic nematodes and insect pests. The mode of nematophagous fungi are as follows showing the egg-parasitization on egg of *Meloidogyne incognita* by *Paecilomyces lilacinus*

- Through parasitism on eggs, egg masses and nematode body

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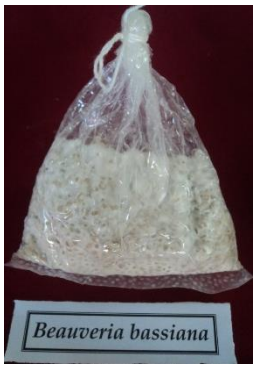
- Production of antibiotics to inhibit the metabolic function of eggs, egg masses and nematode body
- Production of volatile and non-volatiles substances to inhibit the egg hatching and larvicidal
- Production of enzymes (e. g. chitinase, 1,4 β-glucanase etc.) to massacrate the cell membrane for lysis.

Entomopathogenic fungi as bio-pesticides

Those beneficial fungal biocontrol agents (e. g. *Beauveria bassiana*, *Metarhizium anisopliae* etc.) are known to parasitize on eggs, egg masses of insect and their body (e. g. European corn borer, *Ostrinia nubilalis*; codling moth, *Laspeyresia pomonella*; Japanese beetle, *Popillia japonica*; Colorado potato beetle, *Leptinotarsa decemlineata;* Chinch bug, *Blissus leucopterus*; European cabbageworm, *Pieries brassicae* aphids, scale insects, whiteflies and fruit flies etc.) are considered as entomopathogenic fungi. Formulated product of entomopathogenic fungi is known as entomopathogenic fungi based biopesticides to manage insect pests, virus vectors and plant parasitic nematodes. The mode of fungal biocontrol agents are as follows



- Through parasitism on eggs, egg masses of insect and colonization on their body to lead death
- Production of enzymes (e. g. chitinase, 1,4 β-glucanase etc.) to massacrate the cell membrane for lysis of egg and insect body.

Bacteria biocontrol agents as biopesticides

Those beneficial bacteria (e. g. etc.) are known to suppress the population of microbes, insect and their eggs, egg masses by different mode of action (e. g. etc.) are considered as bacteria biocontrol agents. Beneficial bacteria based formulated product are known as bacterial biopesticide to manage disease causing pathogen, insect pests, plant parasitic

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nematodes. The mode of action of bacteria biocontrol agents (*Bacillus* spp., *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Xanthomonas*), biocontrol agents are as follows

Entomopathogenic nematodes as biopesticides

Those nematodes (e. g. *Steinernema carpocapsae*-ambush forager, *Steinernema glaseri*-cruising forager and *Heterorhabditis bacteriophora*a-cruising forager etc.) are known to parasite and multiply in the body of insect pests by entering through anus and among segmented part of insect body (e. g. etc.) are considered as entomopathogenic nematodes. Beneficial nematodes based formulated product are known as entomopathogenic nematodes based biopesticide to manage insect pests. The mode of action of entomopathogenic nematodes e. g. *Steinernema carpocapsae* (ambush forager) and *Steinernema glaseri* (cruising forager) and *Heterorhabditis bacteriophora*a (cruising forager) are as follows: When the infective juveniles of entomopathogenic nematodes are applied as biopesticides to the field to manage the insect pests, they start searching/waiting for their insect pests (hosts). Once the larva of insect pests has been located, the nematode infective juveniles penetrate into the larval body of insect pest cavity via natural opening present on the body of larvae of insect pests such as mouth, anus and spiracles. Once larvae of insect pest reached in the body cavity, infective juveniles release symbiotic bacteria (*Xenorhabdus* spp for *Steinernematidae* and *Photorhabdus* spp for *Heterorhabditidae*) from their gut in insect hemocoel. In the blood, multiplying nematode bacterium complex causes septicemia and kill their insect pest host usually within 48h after infection. Nematodes feed on multiplying nematode, bacteria mature into adult reproduce and then emerge as infective juveniles from the host dead body to seek new larvae in the soil.

Development of commercial formulations through mass production of biocontrol agent:

A. Preparation of Medium:

For mass production of antagonistic fungal/bacterial biocontrol agents, initially prepare the potato dextrose agar (PDA) by using 2% agar agar, 2% dextrose, 250g potato, 1000 ml double distilled water while potato dextrose broth (PDB) medium can be prepared by using, 2% dextrose, 250g potato 1000 ml double distilled water.

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Peeled the skin of potato and sliced it in medium size pieces followed by washing the pieces and keep sliced pieces in tea pan along with double distilled water for starch extraction by boiling to make the soften to tissues of pieces potato.

Filter the starch containing water in clean conical flask from tea pan after boiling sliced pieces of potato by passing through strainer and make up the volume of starch containing water 1000 ml by adding double distilled water.

Make the tea pan clean and pour the starch containing water (1000ml) in tea pan and keep it on burner for boiling on seam flame temperature. When the starch containing water start to boil, add 2% (20g) dextrose followed by 2% agar agar in starch containing water along with constant stirring with stirrer to avoid the coagulation of agar agar. once dextrose and agar agar thoroughly mixed in the starch containing water, off the burner, and wait for cooling down the temperature of PDA medium upto about 50^0C then transfer the PDA medium in 250 ml conical flasks and make cotton plug to close the mouth of conical flaks prior to keep in autoclave for sterilization at pressure 15 psi, temperature 121^0C for 30 minutes. After auto release of pressure in autoclave, take out the conical flask from autoclave and pour the warm PDA medium @20-25ml in per petri plates as desired number under laminar air flow to maintained aseptic conditions. Couples hours later, take nucleus culture test tube of desired microbe (e. g. *Trichoderma harzianum*) and inoculate mycelium mat from culture test tube with the help of inoculation needle on PDA medium containing petri plates under aseptic conditions followed by sealing of the petri plate with paraffins film and keep It in BOD for incubation. After 8 days of inoculation of antagonistic biocontrol agents, entire petri plates will cover with sporulated mycelium and keep it at 4^0C for further use. Similarly, culture of antagonistic fungal biocontrol agent (*Arthrobotrys ologospora* or *Paecilomyces lilacinus*, or *Beauveria bassiana* or *Bacillus subtilis*) can be grow separately in petri plates for further use.

B. Mass culture of biocontrol agent:

For mass culture of antagonistic fungal biocontrol agent, potato dextrose broth medium can be prepared in 5 lite capacity flat bottom conical flak by preparing potato dextrose broth medium. Mouth of flat bottom conical flak is plugged with cotton followed by sterilization in

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autoclave. At ambient temperature and under aseptic condition, small 6mm disc of biocontrol agent (e.g. *Trichoderma harzianum*) from 8 days old culture grown on PDA in petri dish. Likewise *Trichoderma harzianum*, petri dish of *Arthrobotrys ologospora* or *Paecilomyces lilacinus*, or *Beauveria bassiana* or *Bacillus subtilis*, can also use a small 6mm through cork borer and inoculate the same mycelial fragment in 5 liter capacity flat bottom flask containing molasses yeast medium and incubate the inoculated flask at ambient temperature 28±2^{0}C

After 8-10 days of incubation, a thick layer of sporulated mycelial mat grown on potato broth medium. Mycelium mat will separate through muslin cloth from broth and mycelium will keep on sterilized blotting paper for shade drying. Shade dried mycelium will make in fine powder.

C. Development of formulation

For preparing formulation combination for antagonistic microbe (e.g. *Trichoderma harzianum*) along with pre-sterilized carrier (Talc based powder or Kaolin based powder or Clay soil-based powder or Bentonite based powder or Boric acid-based powder) and sticker @5% (carboxy methyl cellulose or *Acacia arabic* gum), amendment of powder of antagonistic microbe in requisite amount in mixture of carrier and sticker will be done after determining the spore load per gram @1x10^0 cfu by haemocytometer. To homogenise the distribution of powder of antagonistic microbe in mixture of carrier and sticker, exhaustive mixing will require to make final formulated product of antagonistic microbe (e. g. *Trichoderma harzianum*) in mixture of carrier (e. g. Talc Fine Powder) and sticker (e. g. CMC). The Prepared product will be store at ambient temperature for further use and also for determining the shelf life of CFU at constant interval.

Application of Biopesticide:

Use of biopesticide for seed treatment:

To avoid the pre and post germination mortality of seeds and seedlings, seed priming with biopesticide is essential before the sowing of seeds. Take 1kg seeds in polybag and add 10g biopesticide formulation for seed coating or seed priming. Seeds containing polybag should

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shake meticulously for proper priming of biopesticide on seed surface. Since, carboxy methyl cellulose (CMC) present in biopesticide formulation act as sticker between colony forming unit and root system as a result a strong network of mycelium will establish in and around of rhizosphere along with growth of root of seedling. Coating/priming of seeds with biopesticide will protect to the spermosphere through suppressing to pathogens and pests present in soil as accordance with their mode of action. After sowing of coated seeds with antagonistic biocontrol agent present in biopesticide will gradually the propagating them self specially in organic rich soil.

Use of biopesticide for bare root dip treatment:

Prior to bare root dip treatment before transplantation of seedlings, 10gram biopesticide will be dissolved in 1000ml for making solution for bare root dip treatment. At transplantation of seedlings (e. g. tomato, chilli, brinjal paddy etc) from uprooted from nursery bed, the roots of seedlings will be dip in solution of 10gram biopesticide dissolved in 1000 ml for 1 hour. Sometimes, jaggary is also used as, additional sticker, by dissolving in 10g biopesticide containing solution for root dip treatment.

Use of biopesticide for nursery bed treatment/furrow treatment/spot treatment:

Prior to sowing of seeds (e. g. tomato, chilli, brinjal paddy etc) to raise seedlings in nursery bed, 1kg biopesticide should be mixed with 25kg farm yard manure (FYM) together and apply mixture in furrow at sowing of seeds in nursery bed.

Mixture of biopesticide and farm yard manure (FYM) can also be used for furrow treatment in directly seeded sown crops (e. g. okra, chickpea, wheat etc) at the time of sowing.

Mixture of biopesticide and farm yard manure (FYM) can also be used for spot treatment prior to the seedling plantation. When a deep spot is made-up with digger for seedling plantation in field, mixture of biopesticide and farm yard manure (FYM) can also be put in spot prior to seedling plantation.

Use of biopesticide for foliar spray:

For foliar spray, 10g biopesticide should use for 1000ml water, it means that 5 kg biopesticide would be required to dissolved in 500 litre water for 1 acre area through tractor

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supported spraying machine. Foliar spray of biopesticide solution should be apply 4-5 time at 15 days intervals.

Use of biopesticide for dusting:

For foliar dusting of biopesticide, 5 kg biopesticide should be mixed in 25kg sterilized soil and should be use for foliar dusting on green foliage to avoid or suppress the infection or infestation of disease-causing pathogen and insect pest. Duster machine should use for dusting the mixture of biopesticide and sterilized soil. Dusting of biopesticide and sterilized soil should be apply 4-5 time at 15 days intervals.

Entomopathogenic viruses as biopesticides

Those virus (e. g. etc.) are known to infect and multiply in the body of several lepidopteran insect pests by entering through chewing of baculoviruses nucleopolyhedroviruses (NPV) and granuloviruses (GV) infected leaves of host plants are considered as entomopathogenic viruses. Beneficial virus based formulated product are known as entomopathogenic virus based biopesticide to manage insect pests. The mode of action of entomopathogenic virus e. g. nucleopolyhedroviruses (NPV) and granuloviruses (GV) are as follows Virus particles invade the nucleus of the midgut, fat body or other tissue cells, compromising the integrity of the tissues and liquefying the cadavers. Before death, infected larvae climb higher in the plant canopy, which aids in the dissemination of virus particles from the cadavers to the lower parts of the canopy. This behaviour aids in the spread of the virus to cause infection in healthy larvae. Viruses are very host specific and can cause significant reduction of host populations. Examples of some commercially available viruses include *Helicoverpa zea* single-enveloped nucleopolyhedrovirus (HzSNVP), *Spodoptera exigua* multi-enveloped nucleopolyhedrovirus (SeMNPV), and *Cydia pomonella* granulovirus (CpGV). Most entomopathogens typically take 2-3 days to infect or kill their host except for viruses and *P. locustae* which take longer. Dead insect pest due to (NPV) and granuloviruses (GV) laid on soil surface, may collect in bulk to make the paste by crushing dead infected insect pests. Paste of dead infected insect pests may dissolve in fresh water and pour in sprayer after filtering by strainer for spray on infested crops e. g. chickpea etc with insect pests.

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Natural enemies (predators, parasitoides, parasites) as biopesticides:

Those insect (e. g. tiphia larval as parasitoids and cotesia larvae as parasitoids) are known to kill other insect pest are considered as natural enemies (predators, parasitoides, parasites). Beneficial insect commercially rears in cage by providing formulated food to survive and used as biopesticide to manage insect pests. The mode of action of natural enemies (predators, parasitoides, parasites) e. g. predator (ladybeetle) is an insect that capture to eat small size prey (100 Aphids in day as insect pest) as food. Parasitoides, (ladybeetle) is a young stage of insect, develop in the body of host (insect pest) and eventually kill to the host (insect pest). Unlike to the predators, the size of pararitoides is smaller than prey. Parasitoides have the character of both predator and parasites. A japanese beetle grub with an external tiphia larval parasitoids and tobacco hornworm that has been killed by cotesia larvae which have pupated outside the host.

Insect pheromones as biopesticides:

The chemical which are emitted from outside of the body of insect (female) to attract the male insect for mating called pheromones. Pheromone is synthetic substance available in lures in rubber septa in market. It may deploy at 1m above crops canopy @2-3/acre. Distance between two traps should be 40m.

Botanical antagonists as biopesticides:

Those plants (e. g. etc.) are known to have an active ingredient to suppress/repel the population of pathogen and insect pest are considered as botanical antagonists. Botanical antagonists based formulated product are known as botanical pesticide. Botanical pesticides have a very diverse mode of action to deter the fungal pathogen, bacterial pathogen, viruses and virus vector, plant parasitic nematode, insect pests. Neem based botanical pesticides are very readily available in market. Non-edible oil seed are also considered as botanical

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pesticides. Extract, oil and other active ingredient of botanical antagonist are known as insect pest repellent. Some Important bioactive compounds reported in different plants are as follows which are being commercialized as botanical pesticides in market.

Bioactive compound pyrethrins is found in *Chrysanthemum* genus

Bioactive compound limonene, indole, pyridine, and phytosterols Nicotine are reported to found in tobacco

Bioactive compound Artemisinin is derived from leaves of *Artemisia annua*

Bioactive compound Atropine, and Scopolamine are found in Datura,

Bioactive compound Calatropin and Calotoxin are found as ingredients in calotropis,

Bioactive compound Vasicine, Vasicinone, and Adhatodin are reported as active ingredients available in the Adhatoda,

Bioactive compound Karanjin is the key ingredient reported in *Pongamia pinnata*.

Bioactive compound Azadiractin, Melantriol, Nimbinin, Nimbidin, Salanin, Nimbin, Nimbolin A, and Nimbolin B are potent active ingredients present in the neem leaf extract.

Bioactive compound Allicin and diallyl sulfide are the chief active ingredient present in garlic extract,

Bioactive compound Capsaicin is known for pungency in Chili

Bioactive compound Lantanolic acid and Lantic acid are potential active ingredient found in Lantana,

Use of botanical pesticide:

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