## M.Sc. 2<sup>nd</sup> semester Generic Elective course (IDC course)-Advanced Microbiology

#### Biofertilizer: Mass cultivation of microbial inoculants; Green manuring; Algalization

Bio-fertilizers are microbial innoculants which bring about nutrient enrichment of soil by enhancing the availability of nutrients to crops. The micro-organisms which act as bio-fertilizers are bacteria, cyanobacteria (blue green algae) and mycorrhizal fungi. Bacteria and cynobacteria have the property of nitrogen fixation while mycorrhizal fungi preferentially withdraw minerals from organic matter for the plant with which they are associated.

#### Types of biofertilizers

- symbiotic nitrogen fixers, Rhizobium spp.;
- non-symbiotic, free-living nitrogen fixers (Azotobacter, Azospirillum, etc.);
- algal biofertilizers (Cyanobacteria);
- phosphate-solubilising bacteria (Acinetobacter sp., Bacillus sp.);
- mycorrhizae

#### Importance of Bio-fertilizers:

- (i) They increase the yield of plants by 15-35%.
- (ii) Bio-fertilizers are effective even under semi-arid conditions,
- (iii) Farmers can prepare the inoculum themselves,
- (iv) They improve soil texture,
- (v) Bio-fertilizers do not allow pathogens to flourish,
- (vi) They produce vitamins and growth promoting bio-chemical,
- (vii) They are non-polluting.

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#### Mass cultivation of Microbial inoculants

Bacteria to be inoculated in soil as biofertilizer need to be multiplied on artificial media to harvest on a large scale so that it can be supplied to farmers. Mass cultivation of rhizobia is discussed below:

Species of *Rhizobium* are kept in different specialization groups. Inoculum of different strains are prepared separately and cultivated on large scale, as required. Strains of *Rhizobium* sp. are grown in Yeast Extract Mannitol (YEM) broth in a small or large container as needed. Following are the steps of mass cultivation of *Rhizobium*.

(a) sterilize the growth medium and inoculate with broth of mother culture prepared in advance, (b) incubate for 3-4 days at 30 - 32°C, (c) test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration), (d) allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand, (e) check the quality of broth, (f) blend the broth with sterile carrier e.g. peat, lignite, farmyard manure and charcoal powder, (g) pack the culture in polyethylene bags and keep at 25°C, (h) check the quality of carrier culture, (i) store at 4°C in a controlled-temperature room, and (j) supply to farmers.

#### **Green Manuring**

Green manuring is defined as the growing of green manure crops & then turning off these crops directly in the field by ploughing the field so as to make the field richer in nitrogen which is the most deficient nutrient of the soil. Green manuring crops help in improving the structure of soil & also increase its physical properties. One of the main objectives of the green manuring is to increase the content of nitrogen in the soil to increase the crop production. The green manuring can be practised as in-situ or green leaf manuring. Green in-situ manuring refers to the growing of green manuring crops in the border rows or as intercrops along with the main crops for example: Sunn hemp, Cowpea, Dhaincha, Berseem, Green gram etc. whereas green leaf manuring is the collection of green leaves from outside places such as waste or forestlands; for example collection of wild dhaincha leaves & then incorporating them into the crop field for improving the soil properties. Most of the green manuring crops are incorporated in the fields after 6 to 8 weeks of sowing with the application of water so that they should be easily incorporated into the soil. The flowering stage of the green manuring crops is the best time for incorporation of these crops.

There are many advantages of green manuring such as:

- 1. It will lead in building the soil structure & improving the soil physical properties like soil aeration, water holding capacity of the soil, soil bulk density etc.
- 2. This will also help in bringing the nutrients into the upper layers so that they should become available for the plant absorption from the lower layers.
- 3. Green manuring also fulfils the crops need of nitrogen which is the most deficient nutrient in the soil these days.
- 4. It will also increase the soil availability of different micro and macronutrient like those of calcium, phosphorus, potash, magnesium etc.

The different green manuring crops are listed in detail below:

- 1. Crotolaria juncea (or Sunnhemp): it is a green manuring crop with vigorous cropping habits & it does not withstand the waterlogging. Its seed rate is 25 -30 kg. per hectare. It is incorporated into the soil after a period of 9 or 10 weeks of sowing. The quantity of nitrogen fixed by this crop is very good i.e.80 to 130 kg/ha. It contains about 2.80 to 3.15 % nitrogen content percent.
- 2. Sesbania aculeate (Daincha): It is a quick growing succulent crop which can be grown in any conditions of soil & climate. It can be incorporated into the soil after 8 to 10 weeks after sowing & its recommended seed rate is 20 to 25 kg/ha. The quantity of nitrogen fixed by this crop is 130 to 18 5 kg/ha & N content in it is 2.55 to 3.21 %.
- 3. Sesbania rostrata: It is different from S.acumeate in the sense that it has nodules on both stem & root. It grows well under waterlogged conditions. It's recommended seed rate is 35 to 40 kg/ha & the seed should be sown after treating it with sulphuric acid for 15 minutes for effective germination. It can fix a nitrogen dose of 170 to 220 kg/ha within a period of 8 to 10 weeks in soil & contains 3.20 to 3.37 % N content.

#### **Algalization:**

Algalization is a technique for mass cultivation of blue-green algae to be used as biofertilizer in paddy fields. Venkataraman (1961) coined the term 'algalization' to denote the process of application of blue-green algal culture in field as biofertilizer. He initiated algalization technology in India and demonstrated the way how this technology could be transferred to farmer level who hold small lands (Vekataraman, 1972).

#### Mass cultivation of cyanobacterial biofertilizers

For outdoor mass cultivation of cyanobacterial biofertilizers, the regional specific strains should be used. However, many germplasm collection laboratories have been established by the D.B.T. in different parts of the country for the development of starter inoculum. Mixture of 5 or 6 regional acclimatized strains of cyanobacteria, e.g. species of *Anabaena, Aulosira, Cylindrospermum, Gloeotrichia, Nostoc, Plectonema, Tolypothrix* are generally used for starter inoculum.

The following four methods are used for mass cultivation: (i) cemented tank method., (ii) shallow metal troughs method, (iii) polythene lined pit method, and (iv) field method. The polythene lined pit method is most suitable for small and marginal farmers to prepared algal biofertilizer. In this method, small pits are prepared in field and lined with thick polythene sheets. Mass cultivation of cyanobacteria is done by using any of the four methods under the following steps:

- (i) Prepare the cemented tanks, shallow trays of iron sheets or polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m. This will facilitate the proper handling of culture.
- (ii) Transfer 2 -3 Kg soil (collected from open place for Im<sup>2</sup> area of the tank) and add 100 g of superphosphate. Water the pit to about 10 cm height. Mix lime to adjust the pH 7. Add 2 ml of insecticide *e.g.* malathion to protect the culture from mosquitoes. Mix well and allow to settle down soil particles.
- (iii) When water becomes clear, sprinkle 100 g of starter inoculum on the surface of water.
- (iv) When temperature remains between 35-40° during summer, optimum growth of cyanobacteria is achieved. Always maintain the water level to about 10 cm during this period,
- (v) After drying, the algal mat will get separated from the soil and forms flakes. During summer about 1 kg pure algal mat per m<sup>2</sup> area is produced. These are collected, powdered, kept in sealed polythene bags and supplied to the farmers.

(vi) The algal flakes can be used as starter inoculum if the same process is repeated.

#### **Biopesticides:**

Biopesticides are ecofriendly compounds used to manage agricultural pests by means of specific biological effects unlike harmful chemical pesticides. It contains biocontrol agents – i.e., natural organisms or substances derived from natural materials (such as animals, plants, bacteria, or certain minerals), including their genes or metabolites, for controlling pests.

Biopesticides cover a wide spectrum of potential products that can be classified as follows:

- Microbial pesticides and other entomopathogens: pesticides that contain microorganisms, like bacteria, fungi, or virus, which attack specific pest species, or entomopathogenic nematodes as active ingredients. Although most of these agents attack insect species (called entomopathogens; products referred to as bioinsecticides), there are also microorganisms (i.e., fungi) that control weeds (bioherbicides).
- Plant-Incorporated Protectants (PIPs): these include pesticidal substances that are produced in genetically modified plants/organisms (GMO) (i.e., through the genetic material that has been incorporated into the plant).
- Biochemical pesticides: pesticides based on naturally occurring substances that control pests by non-toxic mechanisms, in contrast to chemical pesticides that contain synthetic molecules that directly kill the pest. Biochemical pesticides fall into different biologically functional classes, including pheromones and other semiochemicals, plant extracts, and natural insect growth regulators.

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SI. No	Types	Active Ingredients/ Substances it includes	Example
1.	Microbial pesticides	Bacterium, fungus, virus or protozoan.	Strains of Bacillus thuringiensis (Bt).
2.	Biochemical pesticides/ herbal pesticides	Naturally occurring substances	Scented plant extracts.
3.	Plant- Incorporated- Protectants (PIPs)	Genetic material/ toxin	Plant producing Bt toxin.

For any doubt or clarification, feel free to contact me through

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