Biofertilizers: types, production and application

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Biofertilizers

- Biofertilizers is a substance which contains living microorganisms, which when applied to seed, plant surface, or soil, colonizes the rhizosphere or interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

OR

- Biofertilizers are carrier-based microbial inoculants containing sufficient cells of efficient strains of specific microoranisms, that help in enhancing the soil fertility by fixing atmospheric nitrogen, solubilization/ mineralization of phosphorus or decomposing organic wastes, by augmenting plant growth promoting substances with their biological activities.

Types of biofertilizers (on the bases of nature and function)

1. **<u>Nitrogen fixing:</u>** Nitrogen is most abundant and ubiquitous in the air, yet becomes a limiting nutrient due to difficulty of its fixation and uptake by the plants. However, certain microorganisms, some of which can form various associations with plants as well, are capable of considerable nitrogen fixation. These microbes can be:
   - **Bacteria**
     - **Free living:** Free-living in the soil eg. *Azotobacter*
     - **Associative:** Living in rhizosphere (associative/associated) without endophytic symbioses. Eg. *Azospirillum*
     - **Symbiotic:** Having symbiotic and other endophytic associations with plants. Eg. Rhizobia, *Frankia*
   - **Blue grean algae (Cyanobacteria):** have been reported to be helpful in enhancing rice-field fertility for the cultivation of rice in many parts of the world. BGA can further provide natural growth hormones, 172 proteins, vitamins, and minerals to the soil. Eg. A*nabaena*, *Nostoc*, *Tolypothrix*, *Cylindrospermum* etc.
   - **Azolla:** is a floating pteridophyte, which contains as endosymbiont the nitrogen-fixing cyanobacterium *Anabaena azollae*. *Azolla* is either incorporated into the soil before rice transplanting or grown as a dual crop along with rice.

2. **Phosphate solubilizing:**

The phosphorus-solubilizing bacteria (PSB) can increase phosphorus availability to plants by dissolution of bound phosphates in soil by secreting organic acids characterized by lower pH in their vicinity. Eg. *Bacillus* spp., *Paenibacillus* spp., *Pseudomonas* spp. etc.

3. **Phosphate mobilizing:**

The mycorrhizal fungi form obligate or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is dependent on host for photosynthates and energy and in return provides a plethora of benefits to its host. The mycelium of the fungus extends from host plant root surfaces into soil, thereby increasing the surface area for more efficient nutrient access and acquisition for the plant, especially from insoluble phosphorus sources and others like calcium, copper, zinc, etc, eg. ectomycorrhiza (*Laccaria* spp., *Pisolithus* spp., *Boletus* spp., *Amanita* spp.), endomycorrhiza (eg. arbuscular mycorrhiza- *Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., and *Sclerocystis* sp.)

4. **Mineral-Solubilizing Biofertilizers**
   - Potassium solubilizing: Certain rhizobacteria can solubilize insoluble potassium forms, which is another essential nutrient necessary for plant growth. Eg. *Bacillus edaphicus*, *B. mucilaginosus*, and *Paenibacillus glucanolyticus*
   - Silicate and zinc solubilizing: Another important mineral is zinc, which is present at a low concentration in the Earth's crust, due to which it is externally applied as the costlier soluble zinc sulfate to overcome its deficiencies in plant. Certain microbes can solubilize insoluble cheaper zinc compounds like zinc oxide, zinc carbonate, and zinc sulfide in soil. Similarly, microorganisms can hydrolyze silicates and aluminum silicates by supplying protons (that causes hydrolysis) and organic acids. Eg. *Bacillus subtilis, Thiobacillus thioxidans*, and *Saccharomyces* sp.

5. **Plant growth promoting rhizobacteria:** Besides nitrogen-fixing, phosphorus and minerals solubilizing microbes, there are microbes that are suitable to be used as biofertilizers as these enhance plant growth by synthesizing growth-promoting chemicals like growth hormones (auxins, gibberellin etc.). These bacteria shows more than one mechanism of plant growth promotion viz. nitrogen fixation, phosphorus solubilization, production of antibiotics, cytokinins, chitinase, and other hydrolytic enzymes and enhancement of soil porosity. Eg. *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas fluorescens*, *Rhizobium*, *Bradyrhizobium* etc.

6. **<u>Compost Biofertilizers</u>**: Compost is a decomposing, brittle, murky material forming a symbiotic food web within the soil, which contains about 2% (w/w) of nitrogen, phosphorus, and potassium, along with microorganisms, earthworms, and dung beetles.

- The microbial organic solid residue oxidation causes the formation of humus-containing material, which can be used as an organic fertilizer that sufficiently aerates, aggregates, buffers, and keeps the soil moist, besides providing beneficial minerals to the crops and increasing soil microbial diversity.
- Compost is produced from a wide variety of materials like straw, leaves, cattle-shed bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, factory waste, etc.
- The compost is formed from these materials by different decomposing microorganisms like *Trichoderma viridae, Aspergillus niger, A. terreus, Bacillus* spp., several Gram-negative bacteria (*Pseudomonas, Serratia, Klebsiella, and Enterobacter*), etc. that have plant cell wall-degrading cellulolytic or lignolytic and other activities, besides having proteolytic activity and antibiosis (by production of antibiotics) that suppresses other parasitic or pathogenic microorganisms .
- Another important type (vermicompost) contains earthworm cocoons, excreta, microorganisms (like bacteria, actinomycetes, fungi), and different organic matters, which provide nitrogen, phosphorus, potassium, and several micronutrients, and efficiently recycles animal wastes, agricultural residues, and industrial wastes cost-effectively and uses low energy.

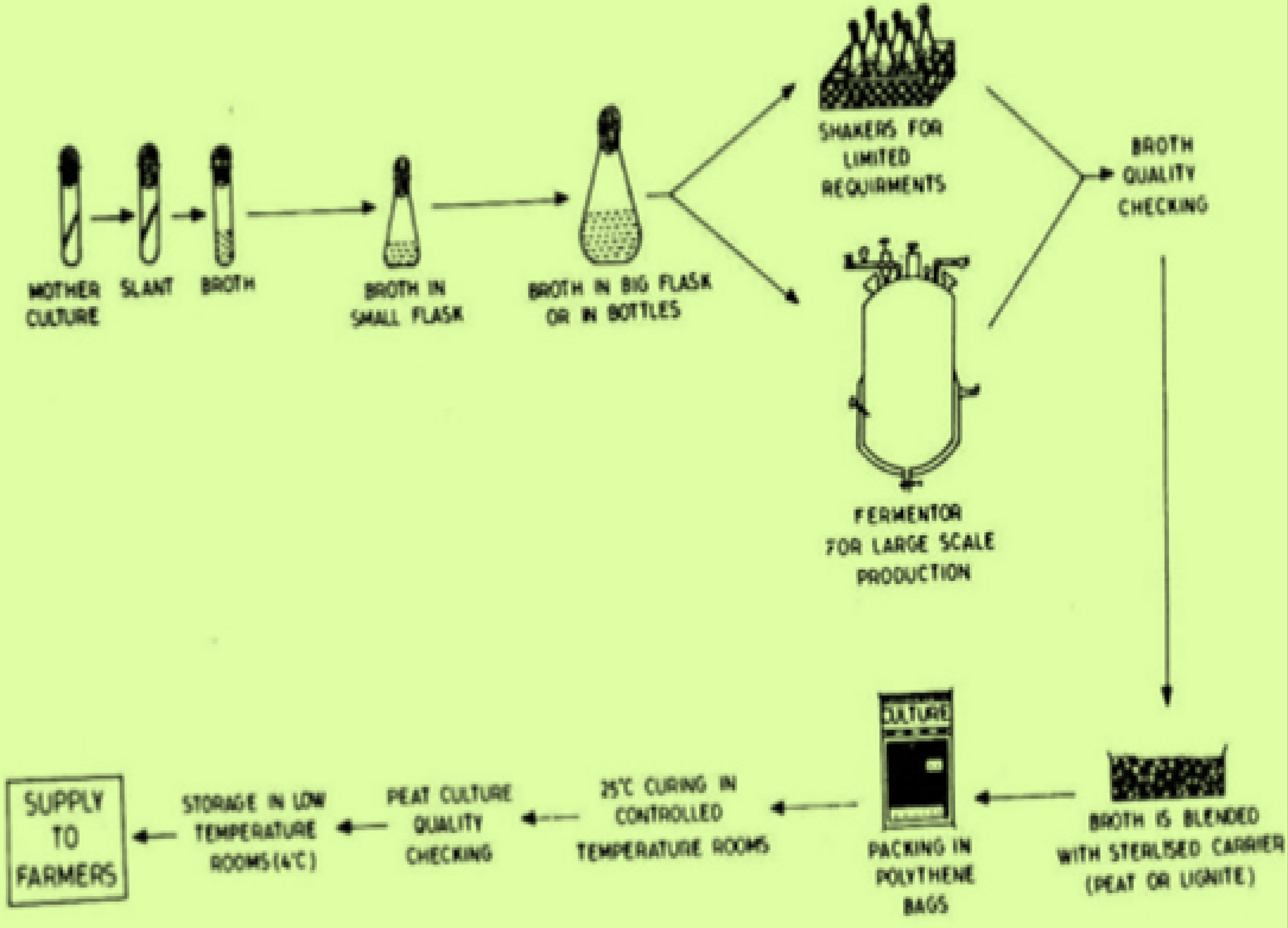
Production of Biofertilizers

Bacterial

Solid carrier based formulation

- The mass production of biofertilizers involves culturing of microorganisms, processing of carrier material, mixing of carrier material with the broth culture, and packing.
- The ideal carrier materials used in the preparation of biofertilizers must be cheaper, locally available, and easier to process; must be non-toxic and organic in structure (so that they remain biodegradable) with high water-holding capacity; and should carry higher bacterial cells and support their survival for longer durations.
- The carriers can be of various origins (organic, inorganic, or synthetic) and can be classified into four main categories:
  - Soils: peat, coal, clays, lignite, inorganic soil
  - Plant waste materials: charcoal, composts, farmyard manure, cellulose, soybean meal, soybean and peanut oil, wheat bran, press mud, corn cobs
  - Inert materials: vermiculite, perlite, ground rock phosphate, bentonite, calcium sulfate, polyacrylamide gels, alginate beads
- In many parts of the world, inoculants are formulated using peat (soil carrier). Peat is made of partially decomposed flora accumulated over the years. It provides a nutritive and defensive growth environment of an extensive variety of microorganisms.

- Once dried, peat is ground, commonly to pass through at least a 250-μm sieve. Generally the peat deposits have a low pH, which must be corrected to pH 6.5–7.0. The peat is then sterilized and an adequate amount of liquid inoculum is added to it. A final moisture content of 40–55 % is generally acceptable.
- Inoculated peat is incubated for a certain period of time to allow bacteria multiplication in the carrier. This step, also called maturing or curing is of major importance since it improves the bacteria survival rate during storage and on seeds.
- Often in peat sticking agents are incorporated thus enhancing its uniformity of coverage on seed. The adhesives used in current agricultural practices are different polymers: polysaccharides (such as gum arabic or carboxymethylcellulose), polyalcohol derivatives, or caseinate salts. Important prerequisites are:
  - nontoxic to seed or microorganisms,
  - easily dispersible in water
  - offering a better adhesion and survival to microorganisms on seed.
- Other materials added to the inoculant formulation include macro- and micronutrients, carbon or mineral sources, hormones, and even fungicides. The aim is to supply microorganisms with protective and/or a nutrient source, to assure better adhesion to seed thus improving the inoculant quality, to make the product more stable, to inactivate the toxins, or to enhance the strain(s) survival during storage and after exposure to environmental stress conditions (high temperature, desiccation).
- Packaging: Polythene bags used for packaging should be of low density grade (50-75 micron).


MOTHER CULTURE
SLANT
BROTH
BROTH IN SMALL FLASK
BROTH IN BIG FLASK OR IN BOTTLES
SHAKERS FOR LIMITED REQUIRMENTS
FERMENTOR FOR LARGE SCALE PRODUCTION
BROTH QUALITY CHECKING
BROTH IS BLENDED WITH STERLISED CARRIER (PEAT OR LIGNITE)
CULTURE
PACKING IN POLYTHENE BAGS
25°C CURING IN CONTROLLED TEMPERATURE ROOMS
PEAT CULTURE QUALITY CHECKING
STORAGE IN LOW TEMPERATURE ROOMS (4°C)
SUPPLY TO FARMERS

Liquid formulation (Bacterial)

- Solid carrier based formulatons can have disadvantages of possessing lower shelf-life, temperature sensitivity, being contamination prone, and becoming less effective by low cell counts. Consequently, liquid formulations have been developed
- Liquid Formulations use liquid materials as carrier, which is usually water, oil or some solvents in form of suspension, concentrates or emulsions. Most popular liquid inoculant formulations contain particular organism's broth 10-40%, suspender ingredient 1-3%, dispersant 1-5%, surfactant 3-8% and carrier liquid (oil and/or water) 35-65% by weight. Viscosity is adjusted at equal to the setting rate of the particles, which is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

Blue green algae production

1. **Trough or tank method**

- Prepare shallow trays (2mx1mx23 cm) of galvanised iron sheet or permanent tank. The size of the tank can be increased if more material is to be produced.
- Spread 4 to 5kg of river soil and mix well with 100g of superphosphate and 2g Sodium molybdate.
- Pour 5 to 15cm of water in the trays.
- In order to avoid the nuisance of mosquitoes and insects add 10 to 15g Furadon granules or malathion, or any other.
- The mixture of soil and water will settle within 8-10hours. At this time, add 200 to 250g mother culture of blue green algae to the surface of water. Then don't disturb water.
- The reaction of the soil should be neutral. If the soil is acidic then add $CaCo_3$ in order to bring the pH of the soil to neutral.
- Within 10-15 days the growth of the blue green algae will look hard flakes on the surface of the water/soil.
- This way water in the tray/pit is allowed to evaporate and the growth of the algae flakes is allowed to dry.
- These pieces of algal growth are collected and stored in plastic bags. In this way from one sq.m. tray or/pit about half tonnes kg blue green algal growth is obtained.
- Again add water to trays and stair the soil well. Then allow the algae to grow in this way. This time it is not necessary to add mother culture of algae or superphosphate. In this manner one can harvest growth of algae 2-3 times. After this effect of superphosphate and soil is reduced.

2. **Pit Method**

- Instead of troughs or tanks pits are dug in the ground and layered with thick polythene sheet to hold the water or one half cement plastered tanks. Other procedure is the same as in the trough method. This method is easy and less expensive to operate by small farmers.

3. Field scale method

- The field scale production of blue green algae is really a scaled up operation of trough method to produce the material on a commercial scale.
- Demarcate the area in the field for algal production: - The suggested area is $40m^2$.
- Prepare a bund with earth so as to store the water.
- Flood the area with water to a depth of 2.5cm.
- Then apply superphosphate $12kg/40m^2$.
- To control the insect-pests attach, apply carbofuran (3% granules) or Furadon 250g $40m^2$.
- After inoculation iIn clayey soils, good growth of algae takes place in about two weeks in clear, sunny weather, while in loamy soils it takes three to four weeks.
- Once the algae have grown and formed floating mats they are allowed to dry in the sun in the field and the dried algal flake, are then collected in sunny bags for further use.
- One can continually harvest algal growth from the same area by reflooding the plot and applying super phosphate and pesticides.

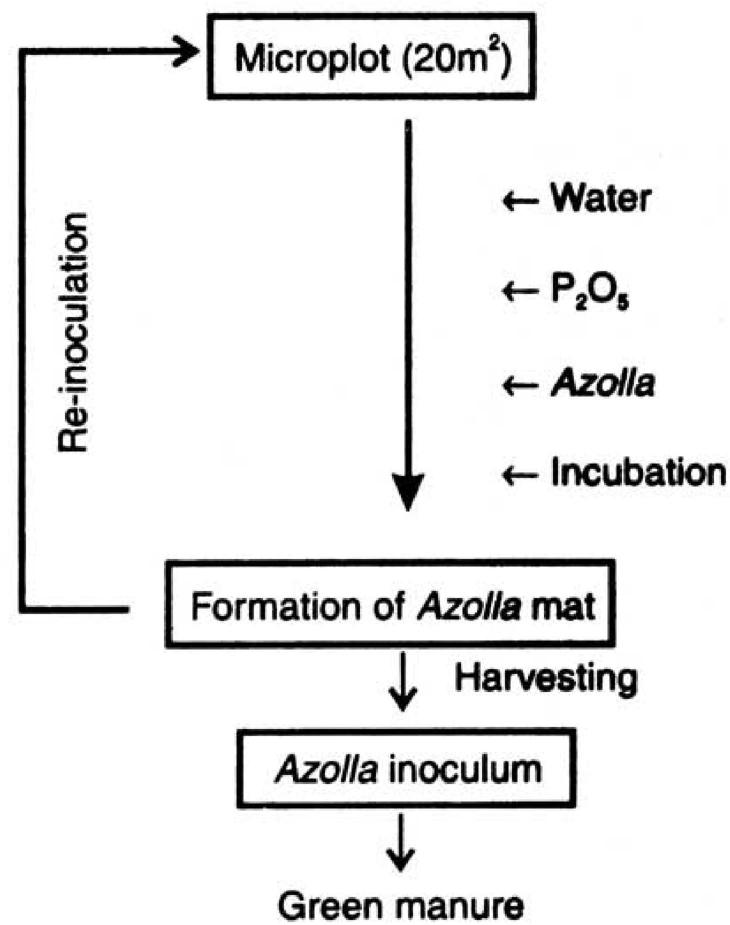
4. Nursery cum algal production

- If $320m^2$ of land are allotted to prepare a nursery, an additional $40m^2$ alongside can be prepared for algal production as described above. By the time rice seedlings are ready for transplantation about 15-20kg of algal material will be available. This much quantity of algal mass will be sufficient to inoculate one and half hectares of area.

- **Recommendation of algal biomass for field application-**10-15kg/ha one week after transplanting the seedlings.

Azolla production

- Microplots ($20m^2$) are prepared in nurseries in which sufficient water (5-10 cm) is added.
- For good growth of Azolla, 4-20 Kg P_2O_5/ha is also amended.
- Optimum pH (8.0) and temperature (14-30°C) should be maintained.
- Finally, microplots are inoculated with fresh Azolla (0.5 to 0.4 Kg/ m^2).
- An insecticide (furadon) is used to check the attack of insects.
- After three, week of growth mat formed by *Azolla* is harvested and the same microplot is inoculated with fresh *Azolla* to repeat the cultivation.



Mycorrhiza production

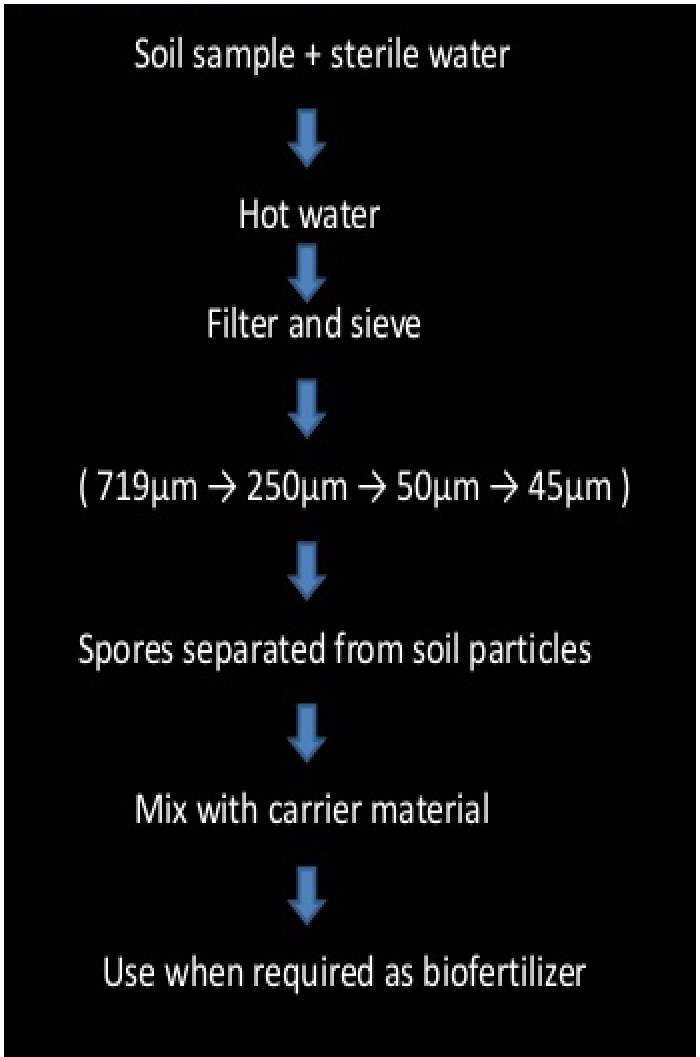
Ectomycorrhiza

- Ectomycorrhiza generally are grown in glucose containing medium and produced spores are used for inoculation.
- Pure mycelia cultures are preferred as they suppress growth of pathogens and contaminants.
- Ectomycorrhizal inoculants may be formulated using a carrier made of vermiculite and 5–10 % peat moisturized with salts and glucose nutrient medium.
- This formulation provides a strong buffering capacity (keeping pH below 6) and enhances the production of fulvic acid that stimulates growth.
- Formulation is then mixed in nursery beds to form association with roots of cultured plants.

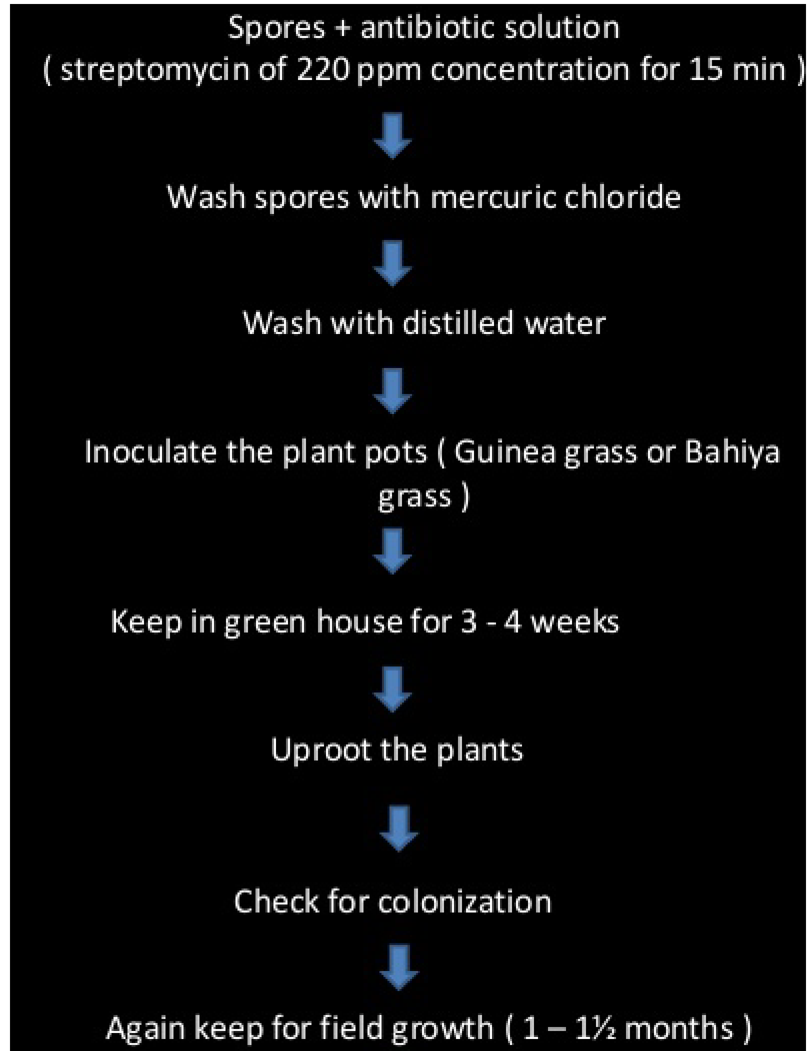
Arbuscular mycorrhiza

- **Constitute a group of root obligate biotrophs that can not grow in media that why a different approach is in use for their production.**

ISOLATION



MASS PRODUCTION



Application of biofertilizer

- **Seed treatment:** Seed treatment is a very effective, economic, and most common method implemented for all types of bacterial inoculants. The seeds are mixed and uniformly coated in a slurry (inoculant mixed with 200 mL of rice kanji or 1% jeggey) and then shade-dried, before being sown within 24 h. Coating of lime after rhizobia inoculant treatment enhances the efficacy.
- **Seedling root dipping:** This application is common for plantation crops such as cereals, vegetables, fruits, trees, sugarcane, cotton, grapes, banana, and tobacco where seedling roots are dipped in a water suspension of biofertilizer (nitrogen-fixing *Azotobacter* or *Azospirillum* and phosphorus-solubilizing microbial biofertilizer) for sufficient period of time.
- **Soil application:** In this practice, biofertilizer is applied directly to the soil either alone or in combination. Some examples of biofertilizers in which soil application is employed are *Rhizobium* (for leguminous plants or trees) and Azotobacter (for tea, coffee, rubber, coconuts, all fruit/agroforestry plants), BGA and azolla in rice field, mycorrhiza in nursery beds.

QUESTIONS

- **Define biofertilizers. Explain the industrial production of biofertilizers.**
  Ans. Give in short about all types.
- **What are the types of biofertilizers. Discuss with example.**
- **Write an essay on industrial production of bacterial biofertilizers and application methods.**
- **Write short note on Organic fertilizer.**
  Ans. Write compost and vermicompost
- **Write short note on phosphate mobilizing biofertilizer production**
  Ans. Mycorrhiza
- **Short note on BGA production.**
- **Short note on azolla production**