Vivekanand College, Kolhapur
(Empowered Autonomous)

Department of Botany

B.Sc. I: Open Elective

Topic: Biofertilizers

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M.Sc., Ph.D.

BIOFERTILIZERS

INTRODUTION

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

- Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.

What is Bio fertilizer?

- Biofertilizers are natural fertilizers that are microbial inoculants of bacteria, algae and fungi (separately or in combination).
- which may help biological nitrogen fixation for the benefit of plants.
- They help build up the soil micro-flora and there by the soil health.
- Biofertilizer also include organic fertilizers(manure, etc.)
- Use of bio-fertilizer is recommended for improving the soil fertility in organic farming

TYPES OF BIOFERTILIZERS

- Bacterial
- Fungal
- Algal
- Aquatic fern
- Earthworms

- **Bacteria:**
  - Symbiotic nitrogen fixers.
    - *Rhizobium, Azospirillum* spp
  - Free living nitrogen fixers.
    - *Azotobacter, Klebsiella* etc.,
- **Algal biofertilizers:**
    - BGA in association with Azolla
    - *Anabena, Nostoc, Ocillatoria*
- **Phosphate solubilising bacteria:**
    - *Pseudomonas, Bacillus megaterium*
- **Fungal biofertilizer**
    - VAM
- **Earthworms**

Bacterial biofertilizers

- The live cells of bacteria used as a biofertilizers
- These microbes contains unique gene called as Nif-Gene which make them capable of fixing nitrogen.
- The nitrogen fixing bacteria work under two conditions,
  - Symbiotically
  - Free living bacteria (non-symbiotic).
- The symbiotic bacteria make an association with crop plants through forming nodules in their roots.
- The free living bacteria do not form any association but live freely and fix atmospheric nitrogen.

Symbiotic nitrogen fixers.

Most important symbiotic Nitrogen fixing bacteria is *Rhizobium* and *Azospirillum.*

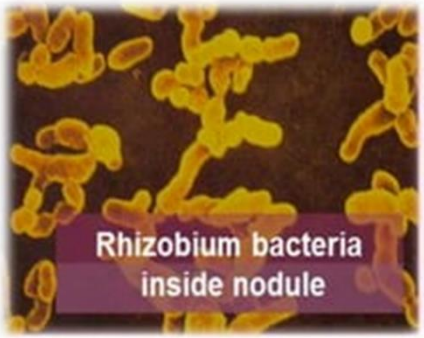
➧ ***Rhizobium:***

- ✓ ***Rhizobium*** lives in the root hairs of the legumes by forming nodules
- ✓ Plant root supply essential minerals and newly synthesized substance to the bacteria
- ✓ The name ***Rhizobium*** was established by Frank in 1889.
- ✓ This genus has seven distinct species based on "Cross Inoculation Group Concept".

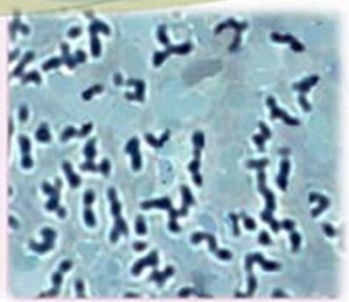

Legume root nodules

- ✓ More than twenty cross-inoculations groups have been established.
- ✓ A new classification has been established for ***Rhizobium.***
- ✓ That is 'slow growing rhizobia' known as ***Bradyrhizobium*** and the other group is 'fast growing rhizobia' called ***Rhizobium.***
- ✓ ***Rhizobium*** can fix **50-300** kg/ha

Rhizobium



Azospirillum:



- ✓It mainly present in cereal plants.
- ✓inhabits both root cells as well as surrounding of roots
- ✓forming symbiotic relation and increasing nitrogen fixing potential of the cereal plant.
- ✓Azospirillum is recognized as a dominant soil microbe
- ✓nitrogen in the range of 20- 40 kg/ha in the rhizosphere in non-leguminous plants such as cereals, millets, Oilseeds, cotton etc.
- ✓Considerable quantity of nitrogen fertilizer up to 25-30 % can be saved by the use of *Azospirillum* inoculant.
- ✓These species have been commercially exploited for the use as nitrogen supplying Bio-Fertilizers.

Free living bacteria

- Large number of free living or non -symbiotic bacteria (does not form nodules but makes association by living in the rhizosphere) present in soil.
- Commonly used free living bacteria are
  - *Azotobacter*
  - *Klebsiella*

it will not associated with plant.

Azotobacter is a biofertilizer which provides the required amount of nitrogen to the plant from the soil.

Azotobactor

- ***Azotobactor*** is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils.
- ***Azotobactor*** is the most commonly occurring species in arable soils of India.
- Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances such as auxins and gibberellins and also to some extent the vitamins.

- Many strains of *Azotobactor* also exhibit fungicidal properties against certain species of fungus.
- Response of *Azotobactor* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops.
- It improves seed germination and plant growth.
- *Azotobacter* is heaviest breathing organism and requires a large amount of organic carbon for its growth.

Mass production

- isolated bacterial cultures were subculture in to nutrient broth
- The cultures were grown under shaking condition at 30±2°C
- The culture incubated until it reaches maximum cell population of 10^{10} to 10^{11}
- Under optimum condition this population level could be attained within 4-5 days for *Rhizobium* 5-7 days for *Azospirillum* and 6-7 days for *Azotobacter*.
- The culture obtained in the flask is called **Starter culture**
- For large scale production , inoculum from starter culture is transferred in to large flasks / fermentor and grown until required level of cell count is reached

prepare appropriate media for specific to bacterial inoculant in required quantity

⇩

Inoculated with specific bacterial strain for aseptic condition

⇩

Incubated at 30±2ºC for 5-7 days in rotary shaker

⇩

Observe growth of the culture and estimate the population

⇩ (starter culture)

The above the media is prepared in large quantities in fermentor

⇩

Sterilized and cooled well

Media in a fermentor is inoculated with the log phase of culture grown in large flask (usually 1-2 % of inoculum is sufficient)

cells are grown in fermentor by providing aeration & continuous stirring

Broth is checked for the population of inoculated organisms

Cells are harvested with the population load of 109 cells/ml

Carrier material

- the use of ideal carrier material is necessary for the production of god quality of biofertilizer
- Peat soil, lignite, vermiculture, charcoal, press mud, farmyard manure and soil mixture are used as a carrier materials
- Neutralized peat soil/lignite are found to be better carrier materials
- Ideal carrier material should be
  - **Cheaper in cost**
  - **Locally available**
  - **High organic matter content**
  - **No toxic chemical**
  - **Water holding capacity of more than 50%**
  - **Easy to process**

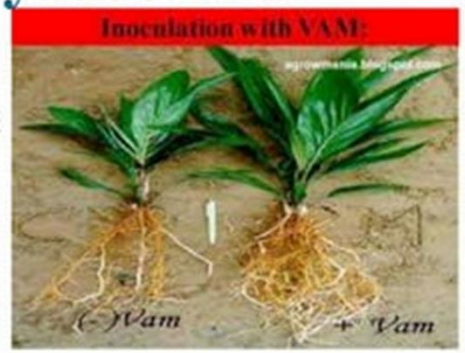
Preparation of inoculants packet

- ✓ Neutralized and sterilized carrier material is spread in a clean, dry, sterile metallic or plastic
- ✓ Bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual or mechanical mixer
- ✓ Inoculants are packed in a polythene bags sealed with electric sealer

Specification of the polythene bags

- Polythene bags should be of low density grade
- Thickness of bag should be around 50-75 micron
- Packet should be marked with the
  - **Name of the manufacture**
  - **Name of the product**
  - **Strain number**
  - **The crops to which recommended**
  - **Method of inoculation**
  - **Date of manufacture**
  - **Batch number**
  - **Date of expiry**
  - **Price**
  - **Full address**
  - **storage instruction**

Vesicular Arbuscular Mycorrhiza (VAM)



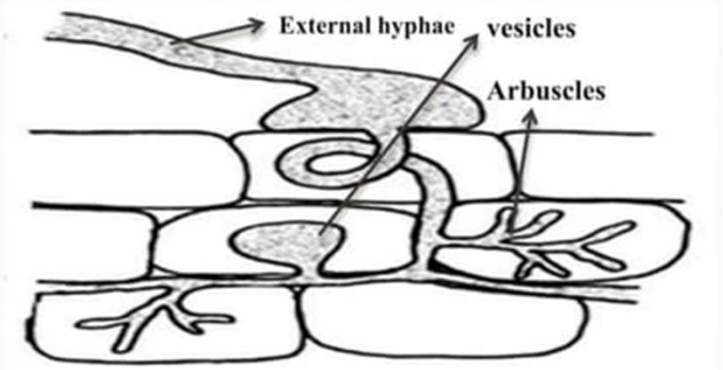
- The term mycorrhiza was taken from Greek language meaning **'fungus root'**. term was coined by Frank in 1885
- The mycorrhiza is a mutualistic association between fungal mycelia and plant roots.
- VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi.
- VAM help in **nutrient transfer** mainly of phosphorus, zinc and sulfur.

- Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae,
- VAM plays a great role in inducing plant growth.
- VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants.
- The VAM hyphae also help is retaining moisture around the root zone of plants
- It increases the resistance to root borne or soil borne pathogens and Nematodes.

- They also **mobilize different nutrients** like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron)and Mg (magnesium) from the soil to the plant roots.
- They posses vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system.

Morphology

- ✓ **External hyphae**
- ✓ **Arbuscles**
- ✓ **Vesicles**



Mechanism of Action

- The VAM forms an association with plant roots.
- It **penetrates in the root cortex** and spreads around the roots of the plant.
- As the name indicates, they posses sac like structure called **vesicules** which stores phosphorus as phospholipids.
- The other structure called arbuscule helps bringing the distant nutrients to the vesicules and root.

Mass production

VAM spores isolated

↓

Spores mixed with sterilized soil

↓

Soil filled in pots

↓

Host plant transplanted in pots

↓

Kept 3-4 months in green house

↓

Soil in the pot along with roots of host plant is macerated

Dried till it attains 5% moisture

Dried soil inoculants used for field application

Uses of VAM

- Enhances the feeding areas of the plant root is as the hyphae spreads around the roots.
- Mobilizes the nutrients from distantance to root.
- Stores the nutrients (sp. phosphorus).
- Removes the toxic chemicals (example : phenolics) which otherwise hinder nutrient availability.
- Provide protection against other fungi and nematodes
- It increase growth rate in plants (*citrus, maize, wheat, etc.*)
- It reduces sensitivity of crop towards high level of salts and heavy metals

Algae as a biofertilizer

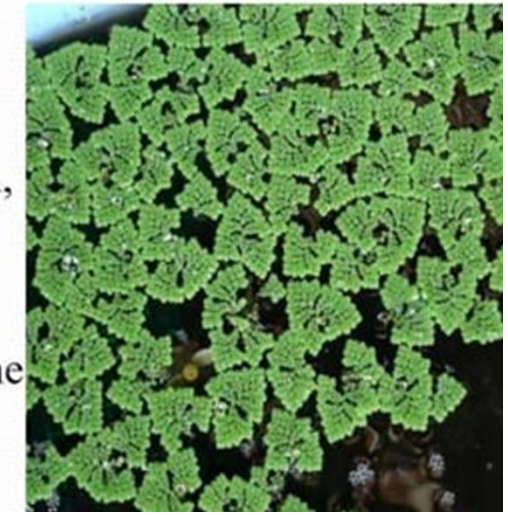
- Another group of free living nitrogen fixers are cyanobacteria.
- Commonly called as **Blue green algae**.
- More than 100 species of BGA can fix nitrogen.
- Nitrogen fixation takes place in specialized cells called **'Heterocyst'**
- BGA very common in rice field.
- Unlike *Azotobacter* BGA are not inhibited by the presence of chemical fertilizers.
- No chemical fertilizers added, inoculation of the algae can result in 10-14% increase in crop yields.

- They are easy to produce
- Usually they are mass produced in cement tanks filled with fresh water.
- Not require any processing
- Quite and cheap
- Cost of 10kg may be Rs.30-40 only
- Beneficial in certain crops like vegetables, cotton, sugarcane.
- Eg. of some algal biofertilizers are
  - ***Anabena***
  - ***Nostoc***
  - ***Oscillatoria***

Azolla as a bio fertilizer

- Azolla is a tiny fresh water fern common in ponds, ditches and rice fields.
- It has been used as a biofertilizer for a rice in all major rice growing countries including India, Thailand, Korea, Philippines, Brazil and West Africa.
- The nitrogen fixing work is accomplished by the symbiotic relationship between the fern and BGA, ***Anabena azollae.***

In addition to nitrogen the decomposed Azolla also provides K, P, Zn and Fe to the crop



- *Azolla* biomass gets doubled within 5-7 days by vegetative methods.
- fix 40-80 kg nitrogen / ha / year.
- good manure for flooded rice.
- Increase of crop yield up to 15-20% has been observed while fertilizing the rice with *Azolla*
- Hybrids are growing faster
- Tolerant to heat and cold
- Fix 4-5% more nitrogen

Bio - fertilizers application methods

There are three ways of using these N-fixing/P.S.M. bacteria.

- **Seed treatment**
- **Root dipping**
- **Soil applications**

Seed Treatment

- Seed treatment is a most common method adopted for all types of inoculant. The seed treatment is effective and economic.
- Seed treatment with ***Rhizobium, Azotobacter, Azospirillum*** along with ***P.S.M.***
- seed treatment can be done with any of two or more bacteria.
- no side effect.
- important things has the seeds must be coated first with *Rhizobium* or *Azotobacter* or *Azospirillum* when each seeds get a layer of above bacteria then the P.S.M. inoculant has to be treated on outer layer of the seeds.

- This method will provide maximum number of population of each bacteria required for better results.
- Mixing the any of two bacteria and the treatment of seed will not provide maximum number of bacteria of individuals.

Root dipping

- Application of *Azospirillum* with the paddy/vegetable plants this method is needed.
- The required quantity of *Azospirillum* has to be mixed with 5-10 ltr of water at one corner of the field and all the plants have to kept for minimum ½ an hour before sowing .

Soil application

- P.S.M. has to be used as a soil application use 2 kgs of P.S.M. per acre. Mix P.S.M. with 400 to 600 kgs of Cowdung along with ½ bag of rock phosphate if available. The mixture of P.S.M., Cowdung and rock phosphate have to be kept under any tree shade or celling for over night and maintain 50% moisture.
- Use the mixture as a soil application in rows or during leveling of soil.

Precautions

- Store biofertilizer packets in cool and dry place away from direct sunlight and heat.
- Use right combination of biofertilizers
- *Rhizobium* is crop specific, so use in specified crop
- Do not mix with chemicals
- Use the packet before expiry, only on the specified crop, by the recommended method.

Advantage of biofertilizers

- Renewable source of nutrients
- Sustain soil health
- Supplement chemical fertilizers.
- Replace 25-30% chemical fertilizers
- Increase the grain yields by 10-40%.
- Decompose plant residues, and stabilize C:N ratio of soil
- Improve texture, structure and water holding capacity of soil
- No adverse effect on plant growth and soil fertility.

- Stimulates plant growth by secreting growth hormones.
- Secrete fungistatic and antibiotic like substances
- Solubilize and mobilize nutrients
- Eco-friendly, non-pollutants and cost effective method

Disadvantages

- Biofertilizers require special care for long-term storage because they are alive.
- must be used before their expiry date.
- If other microorganisms contaminate the carrier medium or if growers use the wrong strain, they are not as effective.
- Biofertilizers lose their effectiveness if the soil is too hot or dry.

THANK YOU