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# BIO-FERTILIZER



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# OBJECTIVES

- After going through this Unit, you will be able to learn biofertilizers and
  - their characteristics
  - understand the prospects and
  - difficulties of biofertilizers
  - select a low cost, suitable and efficient bio-fertilizer for your organic farming.



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# INTRODUCTION

- ▶ Generally, agricultural land gets impoverished after long term cultivation, if not supplemented properly with inputs.
- ▶ To supplement the soil nutrient content under conventional farming system, we need to apply high doses of agrochemicals which in turn pollute the ecosystem.
- ▶ Therefore, in order to make agriculture sustainable, it is necessary to implement a balanced and responsible use of organic agriculture.
- ▶ The principles of organic farming also outline the similar concepts where the soil health and biodiversity is built up to sustain the plant growth in longer term.



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# What is Biofertilizer?

- The name itself is self explanatory.
- Biofertilizer is a ready-to-use live formulation of such **beneficial microorganisms** which on application to seed, root or soil, mobilize the availability of nutrients by their biological activity.
- They help build up the soil micro-flora and there by the soil health.
- As we know, organic farming excludes the use of any chemical.
- Use of bio-fertilizer is recommended for improving the soil fertility in organic farming



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A simple form of classification of biofertilizers is given below:

### **1) For Nitrogen**

- ▶ Rhizobium for legume crops.
- ▶ Azotobacter / Azospirillum for non legume crops.
- ▶ Acetobacter for sugarcane only.
- ▶ Blue -Green Algae (BGA) and Azolla for low land paddy.

### **2) For Phosphorous**

- ▶ Phosphatika for all crops to be applied with Rhizobium, Azotobacter,
- ▶ VAM(Vesicular-arbuscular mycorrhiza).

### **3) For Enriched Compost**

- ▶ Cellulolytic fungal culture.
- ▶ Phosphatika and Azotobacter culture



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# *Types of biofertilizer*

## **Nitrogen Fixing Biofertilizers**

- The nitrogen fixing bacteria work under two conditions, symbiotically and as
- 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.
- Now let us examine the features of these microbes in details.



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- ***Rhizobium***
- ***Rhizobium*** lives in the root hairs of the legumes by forming nodules
- The name ***Rhizobium*** was established by Frank in 1889.
- This genus has seven distinct species based on "Cross Inoculation Group Concept".
- More than twenty cross-inoculations groups have been established so far.
- 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***. Still this classification is discretely not distinguishable because the bacteria of one group may infect to another group.
- This is called "the principle of cross inoculation" which relies on the assumption that legumes within a particular infection group may be nodulated by another species of nodule forming bacteria



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## Methods of Application of *Rhizobium* Inoculants

- ▶ The seed treatment has been found to be the suitable method of *Rhizobium* inoculation.
- ▶ Some adhesive is used to make proper contact between seeds and
- ▶ inoculants (bacteria).
- ▶ About 900 g soil base culture is sufficient to inoculate the seeds for one hectare area in case of legumes.
- ▶ A 10 % jaggery (*gur*) solution is used as sticker for *Rhizobium* cells to seed.
- ▶ First the solution is spread over the seeds and mixed to build up a thin coat over the seeds.
- ▶ After ascertaining the proper coating of slurry over the seeds, the inoculant is sprinkled over the seeds and the content is again mixed thoroughly.
- ▶ Then content is dried in the shade by spreading thinly on a polythene sheet at least for overnight



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- This is a free living or **non -symbiotic bacteria** (does not form nodules but makes association by living in the rhizosphere). Azospirillum species establish an association with many plants particularly with C, plants such as maize, sorghum, sugarcane, etc.
- It is the most common organism and can form associative symbiosis on a large variety of plants.
- Azospirillum is recognized as a dominant soil microbe



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- **Azotobactor**
- *Azotobactor* is a heterotrophic **free living nitrogen fixing bacteria** present in alkaline and neutral soils.
- *Azotobactor chroococcum* 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 *viz.*, **auxins**, and **gibberellins** and also to some extent the vitamins.
- Many strains of *Azotobactor* also exhibit **fungicidal properties** against certain species of fungus.



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- Response of *Azotobactor* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops.
- Field experiments carried out on *Azotobac*



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- **Features of Azotobacter**
- Azotobacter contributes to the moderate benefits.
- Azotobacter is heaviest breathing organism and requires a large amount of organic carbon for its growth.
- It is **poor competitor** for nutrients in soil.
- *It can benefit crops by Nitrogen fixation, release of growth promoting substances, and fungicidal substances.*
- Azotobacter is less effective in soils with poor organic matter content.
- *It improves seed germination and plant growth.*



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- **Acetobactor:-**
- *Acetobactor diazotrophicus* is a newly discovered nitrogen fixing bacteria associated with sugarcane crop.
- This bacterium belongs to the alpha group of *proteobacteria*.
- 
- It was isolated from leaf, root, bud and stem samples of sugarcane.
- Acetobator is located in apoplastic fluid of sugarcane stem and to some extent in xylem vessels.
- It is an acid and high salt tolerant and sucrose loving bacteria which can *fix up to 200 kg nitrogen per hectare*.



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- **Vesicular Arbuscular Mycorrhiza (VAM)**
- The term mycorrhiza was taken from Greek language meaning '**fungus root**'. This term was coined by Frank in 1885
- As indicated above, 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.
- 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.



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## 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 possess sac-like structures called *vesicles* which store phosphorus as phospholipids.
- The other structure called arbuscule helps bring distant nutrients to the vesicles and root.

### *Actions of Mycorrhiza*

- Enhances the feeding areas of the plant root as the hyphae spread around the roots.
- Mobilizes nutrients from a distance to the root.
- Stores nutrients (sp. phosphorus).
- Removes toxic chemicals (example: phenolics) which otherwise hinder nutrient availability.
- Provide protection against other fungi and nematodes.



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- ***Azospirillum***
- Bacteria of the genus *Azospirillum* ( $\alpha$ -subclass of proteobacteria) are known for many years as plant growth promoting rhizobacteria (PGPR) .
- They were isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates .
- Both in greenhouse and in field trials, *Azospirillum* was shown to exert beneficial effects on plant growth and crop yields .
- At present, five species have been described: *Azospirillum lipoferum*, *Azospirillum brasilense*, *Azospirillum amazonense*, *Azospirillum halopraeferens* and *Azospirillum irakense*.



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- Azospirilla are Gram-negative free-living nitrogen-fixing rhizosphere bacteria.
- They display a versatile C- and N-metabolism, which makes them well adapted to establish in the competitive environment of the rhizosphere.
- Ammonium, nitrate, nitrite, amino acids and molecular nitrogen can serve as N-sources .
- In unfavorable conditions, such as desiccation and nutrient limitation, azospirilla can convert into enlarged cyst-like forms . This morphological change is accompanied by the development of an outer coat of polysaccharides and by the accumulation of abundant poly- $\beta$ -hydroxybutyrate granules, which can serve as C- and energy source under conditions of stress and starvation



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- ***NOSTOC***
- *Nostoc* is a genus of cyanobacteria found in various environments that forms colonies composed of filaments of moniliform cells in a gelatinous sheath.
- The name *Nostoc* was coined by Paracelsus.
- *Nostoc* can be found in soil, on moist rocks, at the bottom of lakes and springs (both fresh- and saltwater), and rarely in marine habitats.
- It may also grow symbiotically within the tissues of plants, such as the evolutionarily ancient angiosperm *Gunnera* and the hornworts (a group of bryophytes), providing nitrogen to its host through the action of terminally differentiated cells known as heterocysts. These bacteria contain photosynthetic pigments in their cytoplasm to perform photosynthesis.



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- *Anabaena*
- *Anabaena* is a genus of filamentous cyanobacteria that exist as plankton. They are known for nitrogen-fixing abilities, and they form symbiotic relationships with certain plants, such as the mosquito fern.
- They are one of four genera of cyanobacteria that produce neurotoxins, which are harmful to local wildlife, as well as farm animals and pets.
- Production of these neurotoxins is assumed to be an input into its symbiotic relationships, protecting the plant from grazing pressure.



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- *Frankia*
- It is a genus of nitrogen-fixing, filamentous bacteria that live in symbiosis with actinorhizal plants, similar to the *Rhizobia* bacteria found in the root nodules of legumes in the Fabaceae family. Bacteria of this genus also form root nodules.
- *Frankia alni* is the only named species in this genus, but a great many strains are specific to different plant species. The bacteria are filamentous and convert atmospheric nitrogen into ammonia via the enzyme nitrogenase, a process known as nitrogen fixation. They do this while living in root nodules on actinorhizal plants. The bacteria can supply most or all of the nitrogen requirements of the host plant. As a result, actinorhizal plants colonise and often thrive in soils that are low in plant nutrients.



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- ***Bacillus***
- It is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic). They will test positive for the enzyme catalase when there has been oxygen used or present.
- Ubiquitous in nature, *Bacillus* includes both free-living (nonparasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods.
- These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of the Firmicutes



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- ***Pseudomonas***
- It is a genus of Gram-negative, aerobic Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species.
- The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches.
- Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*.



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## Method of biofertilizer inoculation

### • **Seed Inoculation**

- This is the most common practice of applying biofertilizers.
- In this method, the biofertilizers are mixed with 10 per cent solution of jaggary.
- The slurry is then poured over the seeds spread on a cemented floor and mixed properly in a way that a thin layer is formed around the seeds.
- The treated seeds should be dried in the shade overnight and then they should be used.
- Generally, 750 gram of biofertilizer is required to treat the legume seeds for one hectare area.



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## Soil Application

- This method is mostly *used* for fruit crops, sugarcane, and other crops where localized application is needed.
- At the time of planting of fruit trees, 20 g of biofertilizer mixed with compost is to be added in the ring of one sapling.
- Sometime, the biofertilizers are also *broadcasted* in the soil but we may require four to ten times more bio fertilizers.
- Before broadcasting, the inoculants should be incubated with the desired amount of well decomposed granulated FYM for 24 hours.



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# Self Inoculation or Tubez Inoculation

- This method is suitable for application of Azotobactor. In this method, 50 litres of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly.
- Planting materials required for one acre of land are dipped in this mixture.
- Similarly, if we are treating the potato, then the tubers are dipped in the mixture and planting is done after drying the materials in the shade



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# ADVANTAGES

- There are many advantages of using the biofertilizers. They form an important *association with other soil microbes and help in nutrient supply.*
- Fixes atmospheric nitrogen.
- Increase availability or uptake of nutrients through solubilization or increased absorption.
- *Stimulate plant growth* through hormonal or antibiotics action or by decomposing organic waste.
- They are cheap, hence, reduced cost of cultivation.
- *Improves soil properties* and sustaining soil fertilityLead to soil enrichment.
- Are compatible with long term sustainability.
- *Build up soil fertility in the long term.*
- They are eco-friendly and pose no damage to the environment



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# Disadvantages

- As such there *is no harmful impact of biofertilizers* if it is used properly some constraints:
- Specific to the plants. *Rhizobiurn* spp. culture doesn't work well in high nitrate tolerant strains of soybean.
- The acceptability of biofertilizers has been rather low chiefly because they do not produce quick and spectacular responses.
- Require skill in production and application. Difficult to store.



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