BIOFERTILIZERS: A GENERAL STUDY

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
FOR THE B.SC (GENERAL) DEGREE OF

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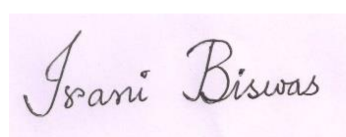
CERTIFICATE

*Certified that the dissertation entitled **“Biofertilizers: A General Study”** has been carried out entirely by **SheuliPramanik**, student of Sem VI, B.Sc (Gen) in the Department of Botany, M.U.C. Women’s College, Burdwan University, PurbaBardhaman under my supervision. It is further certified that the candidate has fulfilled all the conditions necessary for the partial fulfilment of her B.Sc. (Gen) degree achievement under this University and this work has not been submitted anywhere for any other degree to the best of my knowledge.*

Place: PurbaBardhaman

Date: 19. O7.2021



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Place: PurbaBardhaman

Date: 19.07.2021

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Sem-VI (G)

CONTENT	**PAGE NUMBER**

INTRODUCTION

The role of essential macro nutrients such as Nitrogen, Phosphorus, Potassium and other secondary elements is well known for increasing the productivity of land. Population explosion has escalated the pressure on higher productivity per unit of land. Modern agriculture emphasized using hybrid seeds, high yielding varieties that are highly responsive to large doses of chemical fertilizers and irrigation. This has resulted in soil being deprived of essential plant nutrients and nourishing organic matter that had always been available to plants when natural farming was being practiced historically. Chemical fertilizers which are now being used extensively since the Green revolution have depleted soil health by making the soil ecology non - inhabitable for soil micro flora and micro fauna which are largely responsible for maintaining soil fertility and providing some essential and indispensable nutrients to plants. In order to overcome this situation the need for an alternative form of soil reclamation came into focus in the form of biofertilizers.

The term **biofertilizers** includes selective micro-organism like bacteria, fungi and algae which are capable of fixing atmospheric nitrogen or convert solublephosphate and potash in the soil into forms available to the plants.Biofertilizers or more appropriately "Microbial inoculants" can generally be defined as preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solublizing or cellulolytic microorganism used for application to seeds, soils or composting areas with the objective of increasing the number of such microorganisms and accelerating those microbial process which augment the availability of nutrients that can be easily

assimilated by plants. Biofertilizer is a cost effective, eco-friendly & renewable source of land nutrient and they play avital role in maintaining a long term soil fertility & sustainability. It refers to the use of microbes instead of chemicals to enhance the nutrition of the soil. As a result, it is also less harmful and does not cause pollution.The biofertilizer with nitrogen fixer & phosphate solubilizer fixes 20-40 Kg of nitrogen per acre.Continuous use of biofertilizer makes the soil veryfertile for good yield.Thebiofertilizercanbe manufactured in solid form or in liquidform for spraying on the plants.

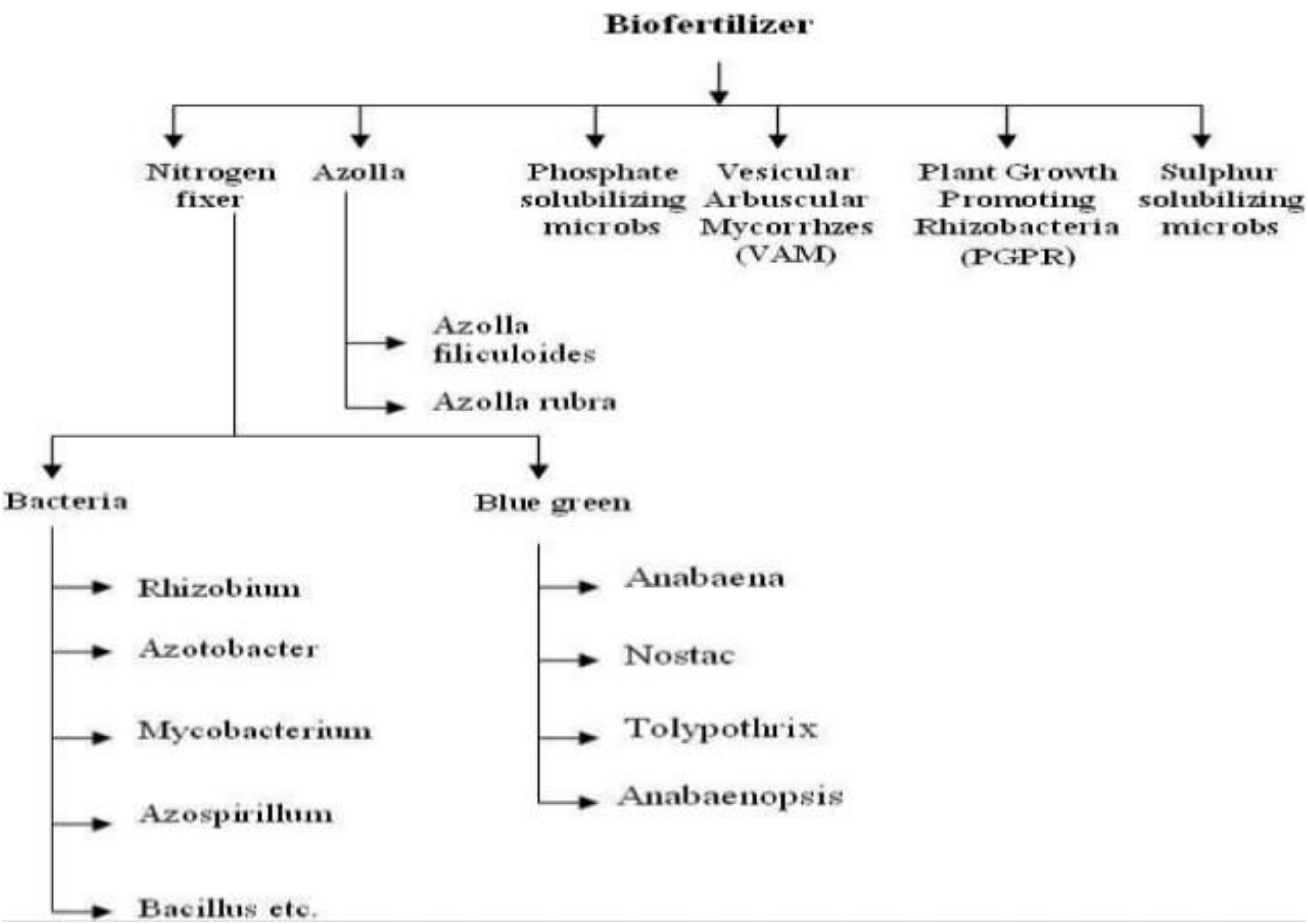
OBJECTIVES

- To get an idea on the basic concept in biofertilizer.
- To learn about the characteristics and method of application of some commonly used biofertilizers.
- To understand the prospects and difficulties of using biofertilizers,
- To learn the method of application of biofertilizers by the farmers directly in the field.

Historical development of biofertilizers

Inoculation of plants with beneficial bacteria can be traced back to centuries. Although bacteria were not proven to exist until Von Leeuwenhoek in 1683 discovered microscopic 'animals', their utilization to stimulate plant growth in agriculture has been exploited since ancient times. Eventually the practice of legume inoculation with non-symbiotic, associative rhizosphere bacteria like *Azotobacter*, was used on a large scale in Russia in 1930s and 1940s. *Bacillus megaterium* for phosphate solubilization was used in the 1930s on large scale in Eastern Europe. In India, from 1920 onwards Joshi Desai, Vyas, Biswas and Acharya worked on phosphate requirements of legumes for better recuperation of soil nitrogen and on anaerobic digestion of organic matter at Imperial Agricultural Research.

CLASSIFICATION OF BIOFERTILIZERS



Biofertilizers may be broadly classified into two main groups:

- **Biological nitrogen fixing biofertilizers----**Biological nitrogen fixing biofertilizers consist of micro-organisms which have the ability to fix biological molecular nitrogen (N_2) either symbiotically or asymbiotically in the plants.
- **Phosphate solubilising (mobilising) biofertilizers----**Phosphate solubilisingbiofertilizers are capable of solubilising or mobilising the fixed insoluble phosphates of the soil

However, specifically biofertilizersare divided into five main categories.These five types are again divided in sub-types as follows:

- **Nitrogen fixers:**

  Symbiotic: *Rhizobium, Frankia*, *Anabaena azollae.*

  Free living: *Azolla*, *Nostoc, Anabaena.*

Associative symbiotic: *Azospirillum.*

- **Phosphate solubilizers :**

  Bacteria: *Bacillus megaterium, Phosphaticum, Bacillus circulans*, *Pseudomonas striata*, *Pseudomonas* sp..

Fungi: *Penicilliumsp, Aspergillusawamori.*

- **Vesicular ArbuscularMycorrhiza (VAM) /Phosphate absorber biofertilizers:**

  Glomus sp., Gigaspora sp., Acaulospora sp., Scutellospora sp. and *Sclerocystis* sp., *Ectomycorrhiza: Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp. Orchid mycorrhiza: Rhizoctoniasolani*.

- **Plant growth promoting rhizobacteria (PGPR):**A group of microbes colonizing root hairs and promoting growth and yield of plant by mobilizing or chelating nutrients from the soil.

  *Bacillus, Rhizobium, Pseudomonas, Mycobacterium, Azospirillum, FRankia, Mesorhizobium*etc.

- **Sulphur supplier:***Thiobacillusnovellus*, *Aspergillus*.

CHARACTERISTICS OF SOME IMPORTANT BIOFERTILIZERS

1. Nitrogen fixing organisms:

A. *Azotobacter (A. chroococcum, A. vinelindii, A. Lipoferum):*

The genus *Azotobacter* is comprised ofbacteriathat require the presenceof oxygen to grow and reproduce, and which are inhabitants of the soil.There are six species of*Azotobacter .*

The representative species is*Azotobactervinelandii.*The bacteria are rod-shaped and stain negative in theGramstainingprocedure. Some species are capable of directed movement, by meansof a flagellum positioned at one end of the bacterium. Furthermore, somespecies produce pigments, which lend a yellow-green, red-violet, or brownish-black hue to the soil where they are located. Relative to other bacteria,*Azotobacter* is very large. A bacterium can be almost the samesize as ayeastcell, which is a eucaryotic single-celled microorganism. *Azotobacter* hasseveral features that allow it to survive in the sometimesharsh environment of the soil.

The bacteria can round up and thicken their cell walls, to produce what is termed a cyst. A cyst is not dormant, like aspore, but does allow the bacterium to with stand conditions that would otherwise be harmful to an actively growing vegetative cell, when in a cyst form *Azotobacter* is not capable of nitrogen fixation.

Benefits of *Azotobacter* :

• It improves seed germination and plant growth

• *Azotobacter* is tolerant to high salts.

• It can benefit crops by Nitrogen fixation, growth promotingsubstances, fungi static substances.

•*Azotobacter* is heaviest breathing organism and requires a largeamount of organic carbon for its growth.

•It is poor competitor for nutrients in soil and hence its growthpromoting substances, fungistatic substances.

•It thrives even in alkaline soils.

•*Azotobacter* is less effective in soils with poor organic matter content.

B. *Rhizobium(R. japonicum):*

Rhizobium is a genus of Gram-negative soil bacteria that fix nitrogen. Rhizobium forms an endo symbiotic nitrogen fixing association with roots of legumes. The bacteria colonize plant cells within root nodules; here the bacteria converts atmospheric nitrogen to ammonia and then provides organic nitrogenous compounds such as glutamine or urea to the plant. The plant provides the bacteria organic compounds made by photosynthesis.

Classification of *Rhizobium*:

The genus Rhizobium will consist of three reorganized species: *R. leguminosarum*, which will contain three biovars (biovartrifolii, biovarphaseoli, and biovarviceae) ;*R. meliloti* ; and *R. loti.*

The reorganization combines into one the former species of *R.leguminosarum,R. trifolii* , and*R. phaseoli* .The fast-growing members ofthe cowpea rhizobiaand the former species*R. lupines*havebeenincluded in the species*R. loti*. The new genus,*Bradyrhizobium*, is made upof one species,*B. japonicum*, which consists of the former species*R. japonicum*, plus the slow-growing members of the cowpea rhizobia. Thenewly proposed classification of*Rhizobium*is as follows.

GENUS I:

*R. leguminosarum*biovartrifoliibiovarphaseolibiovar viceae

R. meliloti; R. loti-fast-growing, sub-polar flagellated strains fromLotus and *Lupinus*with strong affinity for *L. corniculatus,L. densiflorus,* and *Anthyllis vul neraria*(but also nodulates*Ornithopussativum*).

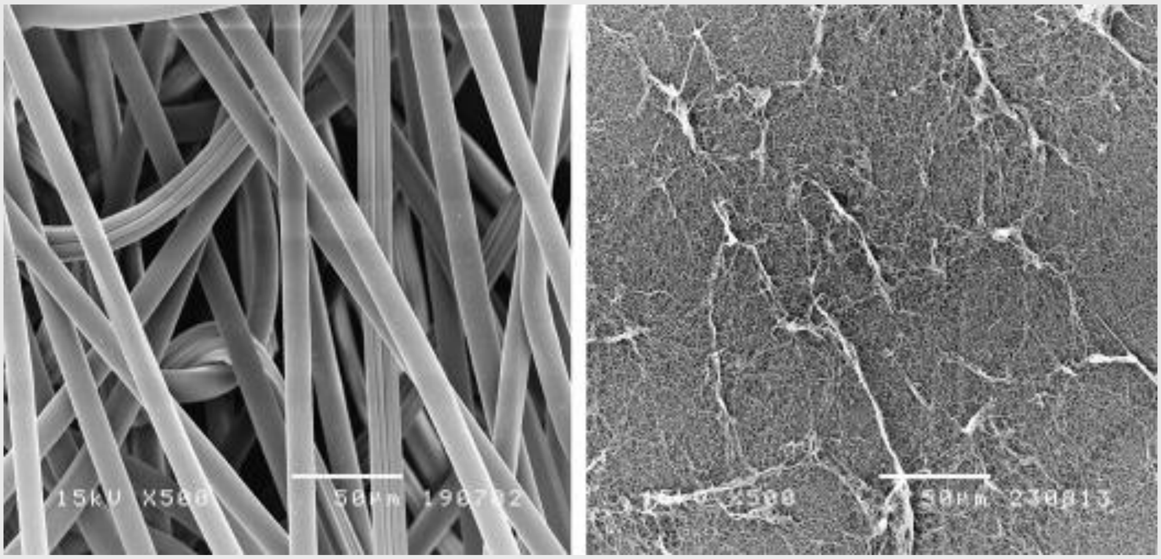
Includes thefast-growing strains nodulating*Cicer, Sesbania, Leucaena, Mimosa,*andLablab.

GENUS II:

*Bradyrhizobium*Slow-growing, polar or sub-polar flagellatedstrainsnodulating soybean, Lotus uliginosus,*L. pendutulatus*, and *Vigna*.Includes those slow-growing strains nodulating*Cicer, Sesbania, Leucaena,Mimosa,* Lablab, and *Acacia*. The possibility exists that other species willeventually be defined withinthis genus, but for the present it is suggestedthat, other than*B. japonicum*(the type species), the various cultures be designated eg.*Bradyrhizobiumsp*. (*Vigna*),*Bradyrhizobium sp*. (*Cicer*),etc.Recently, two more genera have been added to the familyRhizobiaceae. They are *Sinorhizobium* and *Azorhizobium*, nodulatingsoyabean and *Sesbania,* respectively. The combined results of bothsomatic and flagellar reactions have served to distinguish strains within across-inoculation group.

C. *Acetobacterxylinum:*

Acetobacter bacteria, such as *Acetobacter diazotrophicus* that can be isolated from coffee plants or sugarcane, are acid-producing, nitrogen-fixing bacteria. In fact, the *A. diazotrophicus*-sugarcane relationship, firstobserved in Brazil, was the first report of a beneficial symbiotic relationshipbetween grasses and bacteria through nitrogen fixation. Nitrogen-fixingbacteriaareimportant in modern agriculture-exploiting these bacteria would decrease the present dependency on nitrogen fertilizers, whichwould have positive results for the ecosystem and the health of humansand other animals. Other strains can be found in samples from Japaneserice vinegar (komesu) or unpolished rice vinegar (kurosu).



Acetobacter is an obligatory aerobic, nitrogen-fixing bacteria that is known for producing acid as a result of metabolic processes. While allnitrogen-fixing bacteria contain nitrogenase in order to utilize atmospheric nitrogen gas as a source for metabolic biosynthesis, different nitrogen-fixing microorganisms protect the oxygen-sensitive micro organsims from oxygen exposure in different ways.

A. diazotrophicus has been calledinteresting because it carries out nitrogen fixation under aerobic conditions. It needs oxygen for the production of large quantities of ATP required for nitrogen fixation; however, little is known about themechanism or system that protects the nitrogenase under aerobicconditions.

*A. diazotrophicus*is a plant endophyte and has been said tobe capable of excreting about half of its fixed nitrogen in a form thatplants can use.

Acetobacter bacteria can be found in symbiotic relationships with manydifferent plants, such as sugarcane and coffee plants, as well as infermenting vinegar. Endophytes are prokaryotes that associate with plantsby colonizing their internal tissues.

D. Free-Living Nitrogen-Fixing Cyanobacteria:

A number of free-living cyanobacteria, or blue-green algae, have the property of nitrogen fixation, e.g., ***Anabaena, Nostoc, Aulosira, Totypothrix, Cylindrospermum, Stigonema***.

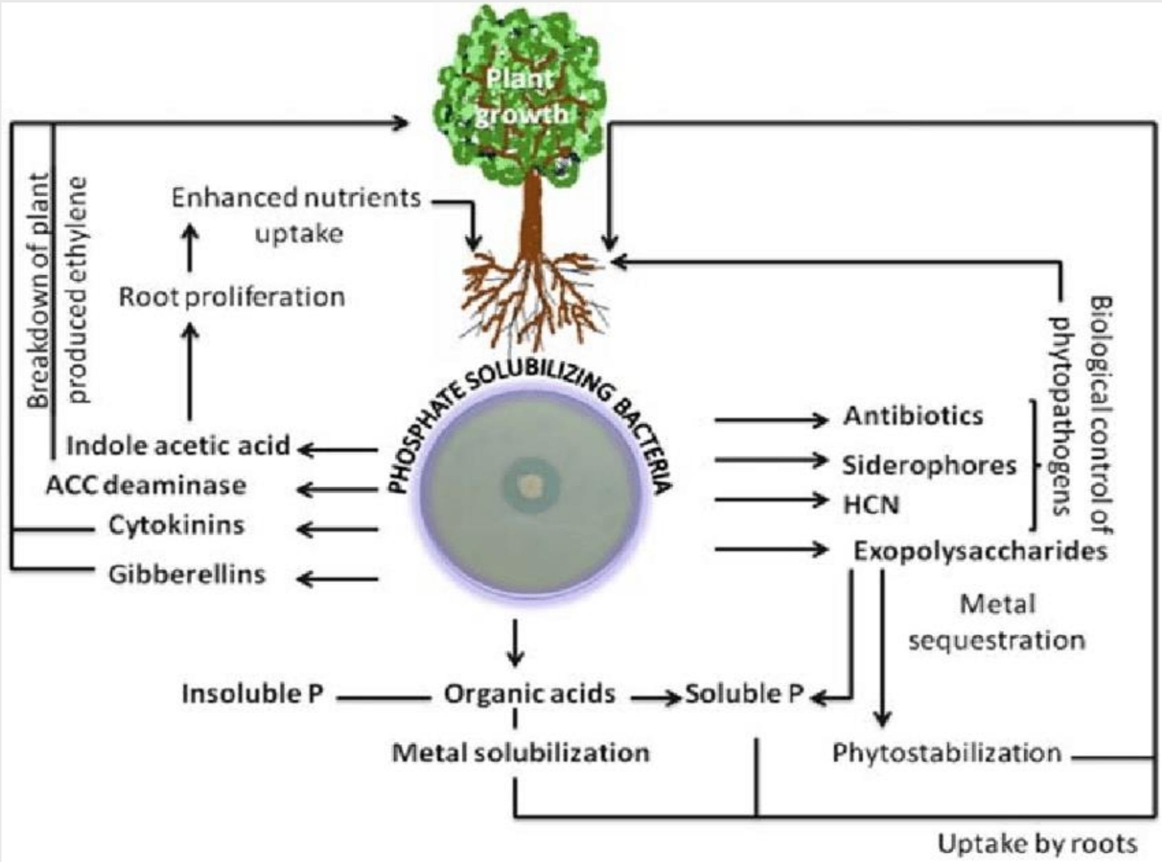
Cyanobacteria are photosynthetic microorganisms. Therefore, they add organic matter as well as extra nitrogen to the soil. These chlorophyll-containing prokaryotic organisms fix atmospheric nitrogen. ***Aulosirafertilissima***is considered to be the most active nitrogen fixer of rice fields. ***Cylindrospermumlicheniforme*** grows in sugarcane and maize fields. Cyanobacteria are extremely low-cost biofertilisers. Phosphate, molybdenum and potassium are supplied additionally.

2.**Phosphate solubilizing micro-organisms**:

Phosphorus is second only to nitrogen in mineral nutrients most commonlylimiting the growth of crops. Phosphorus is an essential element for plantdevelopment and growth making up about 0.2 % of plant dry weight. Plantsacquire P from soil solution as phosphate anions. However, phosphate anionsare extremely reactive and may be immobilized through precipitation withcations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable toplants. As the results, the amount available to plants is usually a smallproportion of this total.Several scientists have reported the ability of different bacterial species tosolubilize insoluble inorganic phosphate compounds, such as tricalciumphosphate, dicalcium phosphate and rock phosphate.

Mechanisms of phosphate solubilization :

The principal mechanism for mineralphosphatesolubilization is the production of organic acids, and acidphosphatases play a major role in the mineralization of organic phosphorus insoil. It is generally accepted that the major mechanism of mineral phosphatesolubilization is the action of organic acids synthesized by soil microorganisms.Production of organic acids results in acidification of the microbialcell and itssurroundings. The production of organic acids by phosphate solubilizingbacteria has been well documented. Gluconic acid seems to be the mostfrequent agent of mineral phosphate solubilization. Also, 2-ketogluconic acid is another organic acid identified in strains with phosphate solubilizing ability.Strains of *Bacillus*were found to produce mixtures of lactic, isovaleric,isobutyric and acetic acids. Other organic acids, such as glycolic, oxalic,malonic, and succinic acid, have also been identified among phosphatesolubilizers. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers .



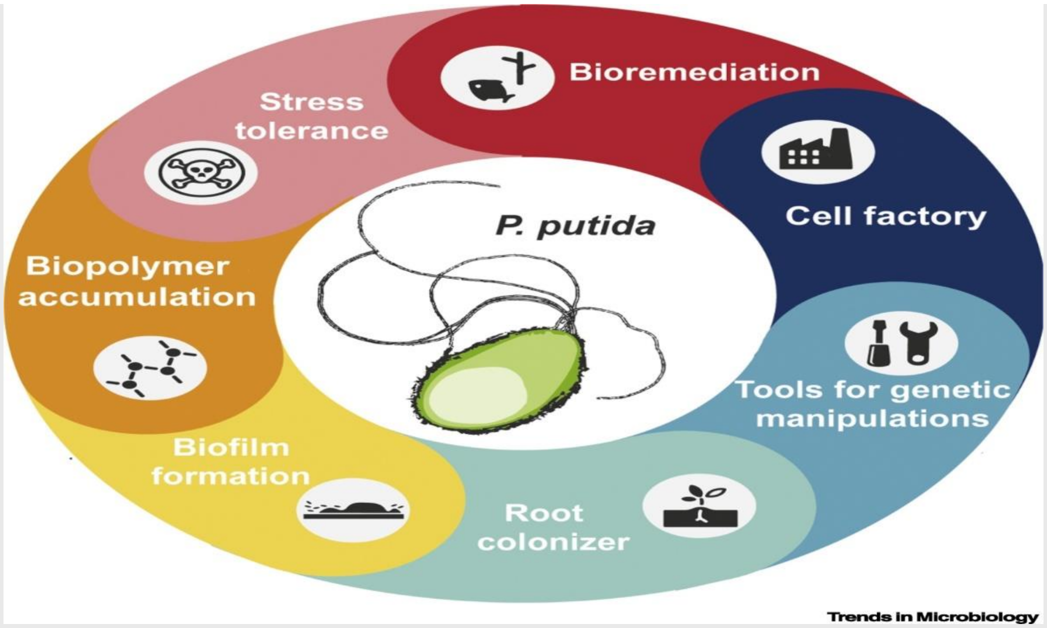
<u>*A.Bacillusmegatherium:Bacillusmegaterium* is phosphate solubilizer.</u> It is a rod-shaped, Gram-positive, endospore forming, species of bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillusform.

*Bacillus megaterium*is a rod shaped bacteria and one of the largesteubacteria found in soil. Groups of thebacteria are often found in chainswhere the cells are joined together by polysaccharides on the cell walls.

*Bacillus megaterium*is able to survive in some extreme conditions such asdesert environments due to the spores it forms. Where there are favourableconditions the spores can survive.

<u>B. *Pseudomonas putida*:</u>

*Pseudomonas putida*is a phosphate solubilizer. It is gram-negative rod-shaped saprotrophic soil bacterium. Based on 16S rRNAanalysis,*P. putida*has been placed in the*P. putida*group, to whichitlends its name.



3.Potash Mobilizers:

This bacterium help to mobilize the insoluble form of potassium for cropgrowth at a faster rate. Seventy percent of insoluble potassium is madeavailable to the crop plants within 25 days of bio-potash application in soil.Reduces cost of potash application by 50-60 %.

•Improves resistance of crop plants

•Resistant to a wide range of soil pH and temperature.

•Suitable to apply for all crops.

•Improves crop growth and yield by 20-30%

•Compatible with other bio-fertilizers

Frateuriaaurentia: The best example of potash mobilizer is*FrateuriaAurentia*. It is a bacteriumand useful in plant nutrition.

4. *Azolla* as biofertilizer:

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, *Anabenaazollae.*

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.

5. Vesicular ArbuscularMycorrhiza (VAM) :

- The term mycorrhiza was taken from Greek language meaning 'fungus root'.
- The 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 aseptatedphycomycetous fungi.
- VAM help in nutrient transfer mainly of phosphorus, zinc and sulphur. Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae.
- VAM plays a great role in including plant growth.
- VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants.
- It increases the resistance to root borne or soil borne pathogens and Nematodes.

METHOD OF PRODUCTION OF BIOFERTILIZERS FOR MARKETING:

Good quality biofertilizers ready for application in the field can be obtained in the following steps:

- Mass production of inoculum in the laboratory.
- Choice of carrier material.
- Preparation of inoculation packets .
- Specification of polythene bags.
- Bioencapsulation
- Checking of quality control of inoculum and certification tagging for marketing.

▪ METHOD OF APPLYING BIOFERTILIZERS DIRECTLY TO THE FIELD:

The important method of biofertilizerapplications are listed below:

- **Seed Treatment:**
  depending on the seed rate, the required quantity of jaggeryis boiled in water and cooled. Rhizobium inoculum is mixed in the jaggery solution and sprinkled over the seeds followed by mixing of seeds with inoculum over the entire surface of seeds. Seeds are dried under shade and sown immediately.
  - **Set Treatment**:sugarcane, cut pieces of potato and base of banana suckers.

  Prepare the culture suspension by 1kg of biofertilize with 40-50lL of water(1:50)

▪ Seedling root dipping:

- The seedlings are uprooted from nursery and cleaned their roots in water dipped in solution of biofertilizer and kept in atleast 20 mins and transplant immediately

▪ Ratio about 1:10

▪For root dipping: Dissolve the 1 pkt of biofertilizer with 20 litres of water (200-300 plants)

▪One packet in 2 litres is sufficient to treat 200-300 sets under cutting method.

- This method is usually applicable for rice crops.

- **Soil application:**

▪The mixture of biofertilizer+ compost + soil applied on land before sowing of seed or transplanting of the main field.

▪The mixture of biofertilizer and cattle manure/soil of sprinkled with water is then

broadcasted into the soil at the timebiofertilizer with of seed soil or and water cattle thenBroadcasted into the soil at the time of sowing or at the time irrigation in standing crop.
Phosphjate solubilizing microorganism are used for this treatment.

▪Soil Treatment:

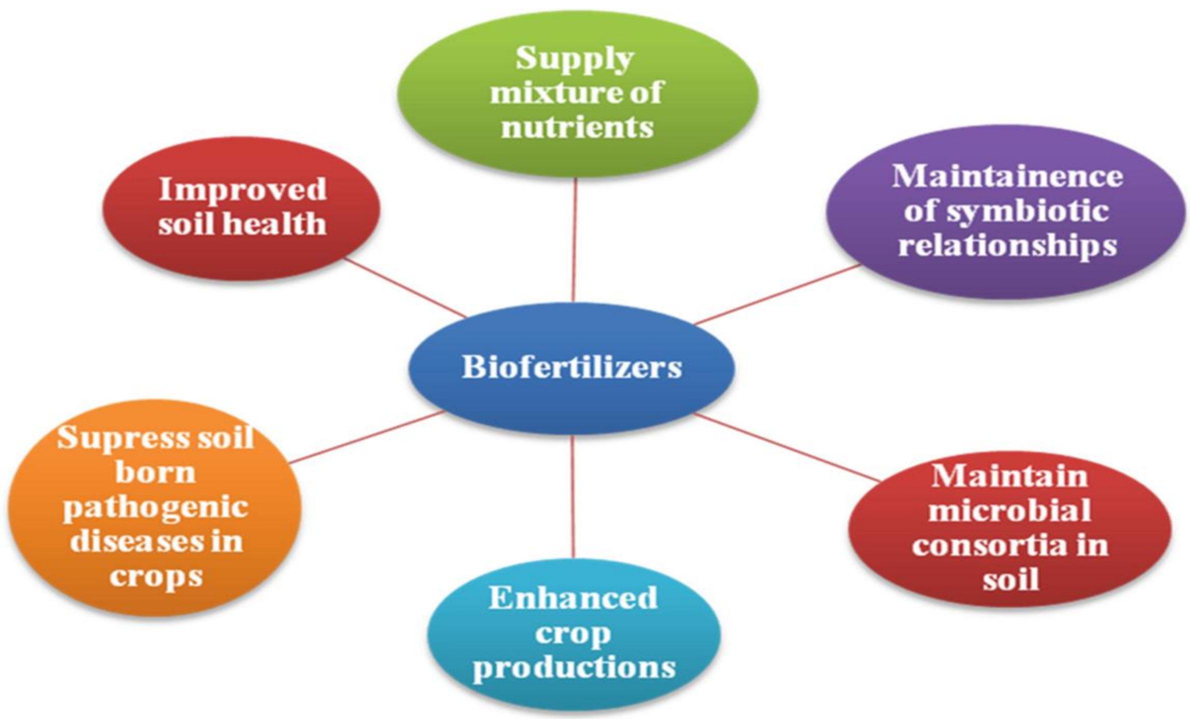
- The biofertilizers along with the compost fertilizers are blended and kept for one night. This mixture is then scattered on the soil where the seeds have to be sown.

IMPOTRTANCE OF BIOFERTILIZERS

1. Advantages of biofertilizers

- Biofertilizers act as supplements to chemical fertilisers.
- Biofertilizers are cost-friendly and can aid to decrease consumption of such fertilisers.
- Microbes in biofertilizers provide atmospheric nitrogen directly to plants.
- They aid in solubilisation and mineralisation of other plant nutrients like phosphates.
- Better synthesis and availability of hormones, vitamins, auxins and other growth-promoting substances improves plant growth.
- On an average crop yield elevates by 10–20 percent by their use.

- They help in the multiplication and survival of beneficial micro-organisms in the root region (rhizospheric bacteria).
- They control and inhibit pathogenic soil bacteria.
- They enhance soil texture by increasing amount of humus and maintain soil fertility.
- Eco-friendly in nature, reduce environmental pollution and cut down the cost of chemical fertilizers
- Release the nutrients gradually and prevent leaching of nutrients from the soil thus improving soil health and providing protection against some soil borne diseases.



2. Disadvantages of biofertilizers

- Biofertilizers are supplement to chemical fertilizers but not substitute to it.
- Biofertilizers only result in 20 to 30 percent increase in crop production. They do not cause marked increase in productivity like chemical fertilizer.
- Specific fertilizers are required for specific crops. This is more applicable to symbiotic micro-organisms. If non-specific *Rhizobium* is used as fertiliser, then it will not lead to root nodulation and increase in crop production.
- During the production of microbial fertiliser, strict aseptic precaution is needed. Contamination is a common issue during microbial mass production.
- If exposed for long time in sunlight, microbes get killed as they are light-sensitive.
- Microbial fertilizer must be used within six months after production when stored at room temperature and within two years if stored at chilling temperature.
- Efficiency of microbial fertilizer depends on soil character, such as, moisture content, pH, temperature, organic matter and types of micro-organisms present. When these factors are unfavourablemicrobial fertilizer may not be effective in enhancing the soil fertility.

3. Precautions taken for its proper usage

● 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.

DISCUSSION AND CONCLUSION:

As proven by many authors, vermicomposting is an effective strategy for converting a variety of organic wastes into biofertilizers. Consequently, it fits well into the circular economy trend, showing many advantages compared to other options of waste management, for example, landfilling or incineration. The low-cost installation and relatively easy course of the process make vermicomposting an interesting solution for treatment of organic wastes in the place that they were produced, e.g., sewage sludge at the watewater treatment plant. Most of the studies also show a positive impact of the application of the obtained vermicompost on soils, if applied as an amendment. The results are encouraging as the substrates in the process are relatively "clean," for example, they come from the food industry or directly from households. Nevertheless, some information on the long-term impact of the application of vermicomposts shall be supplemented, mostly in relation to the presence of some emerging contaminants such as heavy metals and their nanoparticles.

Biofertilizers enhance the nutrient availability to crop plants by processes like fixing atmospheric N or dissolving P present in the soil and also impart better health to plants and soil thereby enhancing crop yields in a moderate way. It is a natural method without any problems like salinity and alkalinity, soil erosion etc. In the vast areas of low input agriculture and oil seeds production, as also in crops like sugarcaneetc, these products will be of much use to give sustainability to production. In view of the priority for the promotion of organic farming and reduction of chemical residues in the environment, special focus has to be given for the production of biofertilizers.

Government intervention

▪National biofertilizer development centre- Ghaziabhad.

▪ Central sector scheme- NPDB-National Project on Development and use of Biofertilizers.

▪ Financial Assistance increased from 13 lakh to 20 lakh per unit.

▪ Government plays a dominant role in marketing by three ways

i.Stategoverment via district level officers and village level workers.

ii. State marketing federation via co-opertive bodies and farmers.
iii. State agro industries co-operation via agro service centres.

Despite the Indian Government's efforts to promote the production and use of biofertilizers, various studies found that biofertilizers have found little acceptance among farmers in India.

The present policy of providing grants and low interest loan to biofertilizers producers should be abolished ; this has resulted in the setting up of a large number of inefficient plants ; which cannot produce good qualitybiofertilizers.

The policy of marketing biofertilizers at very low prices should also be stopped. These prices are too low to adequate investment in modern manufacturing facilities.

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