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2. Classification and Importance of Biofertilizers in Agriculture

Madhumonti Naskar, Sidhu Murmu, Kanu Murmu

Department of Agricultural Chemistry and Soil Science,
Faculty of Agriculture,
BCKV, Mohanpur, Nadia, West Bengal.

2.1 Introduction:

With the ever-increasing population to feed, the sustainability in the food production and security can surely be achieved through the maintenance of soil health. Soil is the holder and carrier of every terrestrial organism. Intensive cultivation with the goal to achieve maximum productivity inorganic fertigation, application of insecticides and heavy tillage procedures have been taken into account since the past century.

This slowly degraded soil physical, chemical and biological properties, collectively known as "Soil Health". Many fertile lands become barren due to intensive cultivation processes.

To restore such lands and decrease the pace of destruction, scientists from every corner of the world contributed their wholesome effort. Introduction of organic farming, conservation agriculture, integrated pest management are some of the important visionary steps inculcated by the scientist community.

The biological health of the soil is the worst affected sector by this type of intensive cultivation. Introduction of biofertilizers is one of the boons to boost the soil health organically. Commercial journey of biofertilizers began with the launch of "Nitragin" by Nobbe and Hiltner in 1895. Inoculation of beneficial fungal and bacterial species with the inert base material are introduced to the field to create a symbiosis between the microorganism and the plant.

Biofertilizers are the natural booster for the nutrient supply, the organisms act as an agent that provides essential nutrients like nitrogen, phosphorus, carbon for the host. In return they rely on the host for energy and growth substances. The species may be facultative or obligatory in nature.

They are responsible for symbiotic or freely fixing atmospheric nitrogen in the soil, solubilization of phosphorus and decomposition of organic substances. They are capable of infecting the roots called rhizobium where the N-N triple bond is broken, and nitrogen is fixed.

Phosphorus solubilizing bacteria or fungi develop structures that are able to store phospholipids and break the phosphate organic compounds or dissolve the inorganic compounds as rock phosphate, slag etc.

According to 2014-16 report of Soil Biodiversity-Biofertilizer research progress more than 2000 isolations of rhizobia of 20 major legumes made all over India including 700 from hyper-arid and arid regions of Rajasthan and Haryana, acidic soil of jharkhand, “taal” lands of bihar and soils of Uttarakhand.

The urge to develop more effective biofertilizer species for bacteria and fungi through biotechnological advancement has clearly paved the way for sustainable crop production. More awareness regarding the effectiveness of biofertilizers as well as the dark side of chemical fertilizer application needs to be focused. In this arena of hunger, the complete reliability of alternative nutrient management is very hard. Identification of regions with gradually degrading soil health and the applicability of suitable biofertilizers are the prime objectives to be focused on.

2.1.1 What are Biofertilizers?

Biofertilizers are the products containing one or more viable microorganisms which have ability to increase the soil fertility by increasing availability of plant nutrients through several biological processes such as nitrogen fixation, phosphorus solubilization, excretion of plant growth promoting substances or cellulose and lignin degradation etc. Use of biofertilizers is one of the important constituents of integrated nutrient management as they are the cost effective and adequate renewable sources of plant nutrients in supplement of chemical fertilizers for sustainable development in agriculture.

2.1.2 Classification of Biofertilizers:

a. Nitrogen Fixing Biofertilizers:

Nitrogen is the most limiting nutrient to plant growth although the atmosphere contains about 80% nitrogen because plants cannot take the nitrogen from air. Some microorganisms are capable of fixing atmospheric nitrogen for plant uptake. Nitrogen fixing biofertilizers are bacteria and blue green algae. Bacteria are both symbiotic and non-symbiotic. Bacteria become associated with different plant parts and fix the nitrogen and make the nitrogen able to plant uptake.

i. Symbiotic Nitrogen Fixing Bacteria:

The best known and effective symbiotic nitrogen fixing bacteria are belonged to the family Rhizobiacieae (Rhizobia) and the following families are included into this family: Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium, and Allorhizobium (Vance, 1998; Graham and Vance, 2000).

The N2-fixing capability of rhizobia varies greatly up to 450 Kg N·ha–1 depending on host plant species and bacterial strains (Stamford et al., 1997; Unkovich et al., 1997; Unkovich and Pate, 2000).

Inoculation is very important because local and resident soil rhizobia population are absent or very low in some particular soil conditions like acidic soils generally contain no or low population densities of the alfalfa rhizobial symbiont *Sinorhizobium meliloti*, whereas basic soils contain a low inoculum potential of *Bradyrhizobium sp.*, a rhizobial symbiont of *Lupinus spp.* (Amager, 1980, Catroux et al., 2001).

Rhizobia inoculant is very effective and cost-effective in terms of proper use, as if the rhizobia are present in high quantities in the soil, then the application of inoculation becomes unnecessary and loss of investment. Catroux et al. (2001) suggested that when rhizobial population density is lower than 100 rhizobia per gram of soil, inoculation is likely to be beneficial for crop productivity. Rhizobia inoculants are prepared and applied in several forms like powder, liquid, and granular formulations. The granular formulations are very favourable and convenient because the application rate and placement can be easily controlled (Stephens and Rask, 2000).

Table 2.a: A list of Common legumes and the Rhizobium strains by which they are inoculated

Rhizobium spp.	Legumes inoculated
1. Rhizobium meliloti	a. a) Melilotus (Sweet clover) b. b) Medicago (Alfalfa) c. c)Trigonella (Fenugreek)
2. R. trifoli	Trifolium (Clover)
3. R. leguminosarum	a. Pisum (Pea) b. Vicia (Vetch) c. Lathyrus (Sweet peas) d. Lens (Lentil)
4. R. phaseoli	Phaseolus (Beans)
5. R. lupini	a. Lupinus (Lupine)
6. R. japonicum	a. Glycine (Soybean) b. Vigba (Cowpea) c. Arachis (Groundnut) d. Crotalaria

ii. Free living Nitrogen Fixing Bacteria:

Many free-living bacteria also fix atmospheric nitrogen such as free-living bacteria such as Azotobacter, Beijerinckia, and Clostridium. The estimation of the N2 fixation by free-living bacteria is quite difficult. In an alfalfa (*Medicago sativa*) stand, the contribution of free-living N2-fixing bacteria was estimated to range from 3 to 10 kg N·ha–1 (Roper et al., 1995). In a greenhouse experiment using different types of bacterial inoculation methods (leaf spray, seed soaking, side dressing), *Beijerinckia mobilis* and *Clostridium spp.* stimulated growth in cucumber and barley plants (Polyanskaya et al., 2002).

But the inoculation of these free-living bacteria help in the plant growth especially in non-legumes fixing atmospheric nitrogen without forming nodules. Azotobacter act in temperate zone with the pH range 6.5-8.0 whereas in tropical zone Beijerinckia act with wide pH range 5.0-9.0. Clostridia are tolerant in anaerobic conditions with pH range 5.0-9.0.

iii. Nitrogen fixing associated Bacteria:

Besides symbiotic and free-living nitrogen fixation, some bacteria are capable of nitrogen fixing with association living within the roots of several crop plants like sorghum, pearl millet, rice, maize, wheat and sugarcane.

Examples of such bacteria include *Acetobacter diazotrophicus* and *Herbaspirillum spp.* associated with sugarcane, sorghum, and maize (Triplett, 1996; James et al., 1997; Boddey et al., 2000), *Azoarcus spp*. associated with kallar grass (*Leptochloa fusca*) (Malik et al., 1997), and *Alcaligenes, Azospirillum, Bacillus, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas*, and *Rhizobium* associated with rice and maize (James, 2000).

The genus Azospirillum makes an association by colonizing plant roots more than other bacteria mentioned earlier. They possess not only as a great root colonizer but also can increase the growth of plants. These include sunflower, carrot, oak, sugarbeet, tomato, eggplant, pepper, and cotton in addition to wheat and rice (Bashan et al., 1989; Bashan and Holguin, 1997). The yield increases can be substantial, up to 30 percent, but generally range from 5 to 30 percent.

These yield increases by Azospirillum are possibly a result of the production of growth-promoting substances rather than N2 fixation (Okon, 1985). They can fix atmospheric nitrogen upto 15-30 kg/ha.

iv. Blue Green Algae or Cyanobacteria:

Soil algae are microscopic, chlorophyll containing organisms which can fix atmospheric nitrogen by obtaining energy from sunlight. Blue green algae are also known as Cyanobacteria. The first agronomic potential of blue green algae in rice was recognised by P. K. Dey (1939). The rice field ecosystem provides a favourable environment for development of blue green algae with respect to their requirements like light, water, temperature, air, nutrient requirements etc.

They contribute about 20-30 kg N/ha, and they can also add organic matter, excrete several growth promoting substances and also amend the physical and chemical properties of the soil. Nostoc, Anabaena, Calothrix, Tolypothrix, Aulosira etc.are considered as dominant nitrogen fixers and can be used as soil based mixed algal cultures for growth of rice plants.

v. Azolla:

Azollae are the free floating water which fix the atmospheric nitrogen in symbiotic association with blue green algae in rice fields. They fix nitrogen by using energy through sunlight through photosynthesis contributing about 40-60 kg N/ha/year.

There are several species of Azolla present in the environment, among them Azolla pinnata is most widely distributed in India. Azolla can be used both as green manure before transplanting and as a dual crop after transplanting of rice.

b. Phosphorus Solubilizing Biofertilizers:

The total phosphorus determines the amount of phosphorus availability to the plants. The solubility of phosphate compounds is of great concern. Most of the soils are deficient to soluble forms of phosphorus thus causing deficiency syndromes. It is the second most limiting nutrient after N2 that consists of 0.2% of the total plant dry weight (Schachtman et al., 1998). Phosphorus is mainly available in inorganic form as rock phosphate (RP) and slag.

Bone meal, fish meal and other plant residue consists of organic phosphate compounds.

Microorganisms such as bacteria and fungi play a vital role converting them into plant available form. They are able to solubilize and mineralize the inorganic and organic forms respectively.

Pseudomonas, Bacillus, Penicillium, Aspergillus etc are some of the important fungi and bacterial species that act as PSM. Stalstron (1903) and Sacket et al. (1908) first showed the capability of bacterial species to solubilize the rock phosphate into plant uptake form.

They are even capable of breaking the organic phosphate bonds and converting them to orthophosphates. PSM consists of 0.5 - 1% of the total soil microbial population mainly from the bacterial species. Though the aerobic bacterial species are more effective they are highly disturebed through the tillage process. While the fungi population and efficiency regarding phosphate solubilizing in the tilled soil is undisturbed.

The symbiosis between the plant and the root colonizing mycorrhizal fungi may be a facultative or an obligatory relationship.

Fungus is mostly dependent on the host for the carbon, photosynthates and energy. In return they provide several benefits to the host plant. The extended surface area of the hyphal structure of the mycorrhiza is capable of extracting nutrients from a larger volume of soil matrix.

Thus, this type of symbiosis increases the crop yield demonstrated by many scientists. They are also heavy metal accumulators and moisture absorbers and help them avoid toxicity and drought. Mycorrhizal growth on plant roots also increases the soil aggregation and provides better soil health. The mycorrhizal growth formed due to fungal association may be classified into two major categories, as follows

i. Vesicular Arbuscular Mycorrhizal Fungi:

This type of inoculation can be made through the application of spores, fragments or roots along with some carrier substances like pumice or clay, sand, vermiculite. The hyphal system creates vascular (vesicles and arbuscules) structure inside the host body, this is also known as “endomycorrhiza”. This obligate symbiont plays a key role in efficient use of Phosphate fertilizers and enhancing nitrogen fixation. They also enhance the uptake of K, S, Cu and Zn. The arbuscules help in nutrient transfer and vesicles store phospholipids. Application of excess P-fertilizers ceases their further growth and mostly proliferates in agroforestry. Sorghum, barley, wheat, upland rice, tobacco, citrus, cotton, guava, apple showed beneficial effects of VAM inoculation.

ii. Ectomycorrhizal Fungi:

Ectomycorrhizal symbiosis of the fungi are the externally infected fungi species that connect with the hosts through infectious peg. The fungi suck root sap through the infectious peg and also provides nutritional elements to the host plant. Frank (1885) hypothesized the presence of ectomycorrhizal fungi and their benefits. *Pisolithus tinctorius* is one of the widespread ectomycorrhizal species in the plantation orchards. Inoculum for the ectomycorrhizal fungi can be developed through vegetative mycelium in a peat of vermiculite carrier. Alternative techniques as application of liquid and spore based mycelial inoculum were lately introduced. But their proliferation time may vary due to delayed establishment and fragmentation.

c. Organic Matter Decomposer:

Composting is a fruitful technology to use a wide variety of organic wastes including rural and urban wastes. This process takes a long time about 4-6 months for maturity, but it is very rich in plant nutrients. In order to increase the decomposition of the organic wastes, there are some microorganisms present in the composting mass. They are mainly two types: cellulolytic and ligninolytic microorganisms. These microorganisms decompose the organic matter at a much faster rate and make the compost ready within 2-3 months for application. The efficient microorganisms for rapid decomposition used as biofertilizers are *Trichoderma viride, Trichurus spiralis, Aspergillus niger, Phaenerocheate crysosporium, Paecilomyces fusisporus* etc.

d. Plant Growth Promoting Rhizobacteria (PGPR):

Bacteria which are capable in promoting the plant growth present in the rhizosphere, collectively they are called plant growth promoting rhizobacteria (PGPR). These group of bacteria help in stimulate the plant growth by colonizing in the root rhizosphere. They promote the growth through the suppression of several diseases, by improving nutrient acquisition or by producing phytohormones to stimulate the growth. These PGPR generally produce different kind of phytohormones like indole-acetic acid, cytokinins, gibberellin and inhibitors of ethylene production. Species of *Pseudomonas* and *Bacillus* can produce phytohormones or growth regulators that helps crops to have greater amounts of fine roots and ultimately increasing the absorptive surface of plant roots for uptake of water and nutrients. Bertrand et al. (2000) showed that a rhizobacteria belonging to the genus *Achromobacter* could enhance root hair number and length in oilseed rape (*Brassica napus*). *Achromobacter* increased NO3 and K uptake and, consequently, shoot and root dry weights by 22 to 33 percent and 6 to 21 percent respectively (Bertrand et al., 2000).

Table 2.b: Biofetilizers and their mode of action, host crops, methods of application and rate of inoculants used

Name of the organisms	Mode of action	Host crops which used	Method of application	Rate of application	Remarks
Rhizobium strain	Symbiotic nitrogen fixation	Legumes (pulses, soybean, groundnut)	Seed treatment	200 g/ 10 kg seed	Leaves residual nitrogen in soil for next crop
Azotobacter	Non-symbiotic nitrogen fixation	Cereals, millets, cotton, vegetables	Seed treatment	200 g/10 kg seed	Also control certain diseases
Azospirillum	Associated nitrogen fixation	Non-legumes (maize, barley, oats, sorghum, millets, sugarcane etc.)	Seed treatment	200 g/ 10 kg seed	Produces growth promoting substances
Phosphate solubilizes	Phosphate solubilization	Soil application for all crops	Seed treatment	200 g/ 10 kg seed	Can be mixed with rock phosphate
Blue green algae (BGA)	Non-symbiotic nitrogen fixation	Rice	Soil application	10 kg/ha	Reduces soil alkalinity; has growth promoting effects
Azolla	Symbiotic nitrogen fixation	Rice	Soil application	1 t/ha (dried material)	Increases organic carbon, soil physico-chemical properties
Mycorrhiza (VAM/AM)	Symbiotic association	Many trees and crops, wheat, sorghum	Soil application	varied	Usually, seedlings are inoculated

2.1.3 Methods of Application of Biofertilizers:

There are three types of application of microbial inoculum for application as biofertilizers: a) seed treatment, b) seedling root tip, and c) soil treatment.

a. Seed Treatment: In the case of seed treatment, 200g of biofertilizers is suspended in 300-400ml of water and mixed gently with about 10kg seeds by using an adhesive like gum acacia, jaggery solution etc. Due to these processes, the bioinoculants may get energy for their prolonged survival. But care must be taken for the seed coat that the seed coat should keep intact. The seeds are then spread over a clean sheet under shade for drying and used for sowing immediately.

b. Seedling Root Dip: Seedling root dipping is generally used for transplanted crops. In the case of rice, a seedbed is prepared in the field and filled with water. Recommended biofertilizers are mixed with the water and then the roots of rice seedlings are dipped into the water for 8-10 hr. And then transplanted into the field.

c. Soil Treatment: Biofertilizers are applied as basal application; About 4 kg each of recommended biofertilizers is mixed with 200 kg of compost and kept overnight. Then the mixture is applied in the soil at the time of sowing the crops.

2.1.4 Advantages of Biofertilizers:

There are some advantages in the application of biofertilizers:

- They are eco- friendly as well as cost effective
- Their use leads to soil enrichment and the quality of the soil improves with time.
- Though they do not show immediate results, but the results shown over time are spectacular.
- These fertilizers harness atmospheric nitrogen and make it directly available to the plants.
- They increase the phosphorous content of the soil by solubilising and releasing unavailable phosphorous.
- Biofertilizers improve root proliferation due to the release of growth promoting hormones.
- Microorganism converts complex nutrients into simple nutrients for the availability of the plants.
- Biofertilizers contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology.
- They help in increasing the crop yield by 10-25%.
- Biofertilizers can also protect plants from soil borne diseases to a certain degree.

2.2 Conclusion:

To meet the demand of food security the sustainable development in agriculture is very necessary now-a-days. To achieve the goal the better agronomic practices in sustainable way will be our main moto. But high application of chemical fertilizers and pesticides cause the harmful impact on the soil and plant growth and ultimately in our food security. To mitigate the problems, application of biofertilizers combined with the chemical fertilizers can help the development of plant growth and increase the yield. Biofertilizers are the very good option for the farmers to increase the productivity per unit area. More future research should increase the biofertilizers application in the field and help in the crop production in sustainable manner to mitigate the problems and achieve our goals.

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