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BIOFERTILIZERS: A SUBSTITUTE FOR CHEMICAL FERTILIZERS AND GOOD FOR PLANT AND SOIL HEALTH

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Abstract

Fertilizers provide the important plant nutrients, which contain mainly nitrogen (N), potassium (K), and phosphorous (P). They lead to increase in the yield of the crops, at the same time they cause several health hazards. Hence nowadays farmers are getting cautious about it and try to prefer organic farming because of its beneficial uses. Biofertilizers have emerged as an important alternative for synthetic chemicals. Biofertilizers are found to be economically profitable and ecologically healthy in providing required nutrients to the plants. Biofertilizers are also considered as cost effective renewable source of nutrients and also a good substitute for chemical fertilizers. Biofertilizers are becoming popular among farmers as they improve the quality of soil in agriculture fields.

Keywords: - Biofertilizers, N₂ Fixers, plant growth promoting rhizobacteria (PGPR), Organic agriculture, AM fungi.

Introduction

(Biofertilizers boon for 2nd Green revolution.)

Biofertilizers contains living microorganisms and then they colonize the rhizosphere of the plant and increase the availability of primary nutrients for growth

of the plants. More than thousands of soil bacteria strains are known, which colonize in the rhizosphere of the plant. These bacteria together are known as plant growth promoting rhizobacteria (PGPR). These

bacteria help in enhancing the plant growth thus acting as biofertilizers.

Biofertilizers mainly contains microorganisms like nitrogen fixer, bacteria and cyanobacteria, phosphate solubilizer and *mycorrhizae* as phosphate mobilizers.

The microorganisms used for the biofertilizers are bacteria of *Bacillus*, *Pseudomonas*, photosynthetic bacteria.

Fungi (*Trichoderma*, Yeast), nitrogen fixing bacteria. Biofertilizers has proved to be of great importance as it is eco friendly and also renewable. Biofertilizers are ready to use formulations. Biofertilizers when applied to the seeds or the soil, they mobilize the availability and utility of the microorganisms and help in improving the soil texture and make it healthy (Ismail *et al.*, 2014). In other words, biofertilizers are basically living cells of different useful microorganisms having their ability to mobilize plant nutrients in the soil from unusable to usable form through their metabolic activities.

For maintaining these biofertilizers in good condition, they are packed in suitable carrier

Table 1: Carriers

Form	Liquid	Powder	Granular
Appearance	Without strange smell	Brown	Brown
Fast-growing <i>Rhizobium</i>	$>0.5 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$
Slow-growing <i>Rhizobium</i>	$>1.0 \times 10^9/\text{ml}$	$>0.2 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$
N fixing bacteria	$>0.5 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$
Si bacteria	$>1.0 \times 10^9/\text{ml}$	$>0.2 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$
Organic P	$>0.5 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$	$>0.1 \times 10^9/\text{ml}$
In Organic P	$>1.5 \times 10^9/\text{ml}$	$>0.3 \times 10^9/\text{ml}$	$>0.2 \times 10^9/\text{ml}$

such as lignite or peat. Carrier also plays an important role in maintaining sufficient self life (Singh *et al.*, 1999).

Rhizobium is observed to be the most important genera of nitrogen fixing bacteria (Odame, 1997). *Azospirillum* spp., also is considered to increase the yields of cereal and forage grasses by improving root development in well colonized roots, by increasing the rate of water and mineral absorption from the soil and also by biological nitrogen fixation (Okon, 1985). Biofertilizers show lots of promises as a supplementary, environmental friendly and renewable sources of plant nutrients and are an important component of Integrated Nutrient Management (INM) and Integrated Plant Nutrition System (IPNS) (Raghuwanshi, 2012).

Biofertilizers not only help in better plant growth but are also eco-friendly as they don't harm the farmers as chemical fertilizers do and are also low cost so every farmer can afford to use it in his farm (Mishra and Dash, 2014).

Multi-strain bio-fertilizer	>1.0*10 ⁹ /ml	>0.2*10 ⁹ /ml	>0.1*10 ⁹ /ml
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ISOLATION TECHNIQUES

Isolation of phospho bacteria from the rhizosphere

- Soil samples were collected from the different agricultural lands.
- Serial dilution method:

10g of soil sample was dissolved in 100 ml of sterile distilled water and the sample is mixed thoroughly and by dilution making the sample 10¹. Then the soil sample in sterile water is serially diluted upto 90% dilution. Then 10⁵, 10⁶, 10⁷ dilution is taken into spread plate technique.

- Nutrient agar is poured into the plate after solidification of medium 0.1 ml of medium is poured into the agar medium plate, then they are incubated at 25⁰C for 24 hrs.

Isolation Techniques for *Rhizobium* spp.

Intact root nodules from a healthy plant were selected and was transferred to a drop of sterile water in a petri dish. The nodule in the drop of water was crushed in between two glass slides causing the release of nitrogen fixing bacteria *Rhizobium* into the drop of sterile water. The smear of the crushed root nodule was streaked onto yeast extract mannitol agar (YEMA) plate containing 1% Congo red dye. The culture

was then incubated at 20-25⁰C for three days (Boraste, 2009).

Isolation technique for *Azospirillum* spp

Juvenile roots from a healthy sugarcane plant was taken and kept in saline for 5 min. with a forceps, root was immersed to a semisolid Bromothymol blue medium borth containing 0.8% agar in a test tube and incubated at 20 to 25⁰C for atleast a week. A loopful of culture from the broth containing the root was transferred to Bromothymol blue media plates. The culture was incubated at 20 to 25⁰C for a week.

Carriers used in biofertilizers (Table 1):

It helps in preserving the quality maintaining applying consistency of biofertilizers and makes it more effective. It also makes it easy to handle and also increases the shelf life. The ingredients mostly used for making carriers for solid type of biofertilizers are carbon as minerals, clay, diatomaceous soil, rice and wheat bran, peat, lignite, humus, wood charcoal. To give a high coating of inoculants on seed surface, use of adhesive like gum Arabic methylethyl-cellulose and vegetable oil, etc.

MASS PRODCUTION OF BIOFERTILIZERS

Criteria for strain selection

Efficient nitrogen fixing strains were selected and then these were multiplied on the nutritionally rich artificial medium

before inoculating on the seeds and in the soil.

Culturing broth

The isolated stain was inoculated in the flask having a suitable medium for healthy inoculum production. Then the carrier is autoclaved at 15 psi at 121⁰C for 20 min. The culture broth was mixed with carrier (300 ml of broth for 1 kg carrier). The mixture was then dried by spreading it on the plastic sheet in shade room. For large scale production of inoculum culture fermenters are used.

Quality control

For the good and desired results quality control is a must. Some standards are to be followed for maintaining good quality of biofertilizers. These standards are as stated below:

- The inoculants should be carrier based.
- It should contain 10⁸ viable cells per gram of carrier on dry mass basis within 15 days of manufacture.
- The inoculums should have an expiry period of 12 month from the date of manufacture.
- The inoculums should not be contaminated as they are prone to get easily contaminated. This can lead into degrading the quality of biofertilizer.
- The pH of the inoculants should be maintained at 6.0-7.5.
- Each packet containing biofertilizer should display the respective information on the packet.

Table 2. Shows the different types of biofertilizers

Biofertilizers	Microorganisms
Nitrogen fixers	<i>Azolla pinnata</i> , <i>Rhizobium spp</i> , <i>Azotobacter chroococcum</i> , <i>Azospirillum lipoferum</i> , <i>Azotobacter diazotrophicus</i> , <i>Derxia gummosa</i>
Phosphate solubilizers	<i>Bacillus congulans</i> , <i>Bacillus circulans</i> , <i>Torulospora globasa</i> , <i>Pseudomonas fluorescens</i> , <i>Thiobacillus</i> , <i>Aspergillus niger</i> , <i>Trichoderma sp.</i> , <i>Paecilomyces sp.</i>
Potash mobilizers	<i>Bacillus spp</i> , <i>Pseudomonas spp.</i>
Zinc mobilizer	<i>Pseudomonas spp.</i> , <i>Bacillus spp.</i> , <i>Rhizobium spp.</i>

Types of biofertilizers available (Table 2)

Commonly used commercially available biofertilizers are

- Phosphorus solubilising biofertilizers (PSB).
- Nitrogen fixing biofertilizers.
- Phosphorus mobilizing biofertilizers.

- Plant growth promoting biofertilizers.

ROLE OF BIOFERTILIZERS

They help in fixing of nitrogen of soil and also in the root nodules of leguminous crops so as to make it available in the easiest form. Their metabolic activities help in enhancing root growth and plant biomass. They also help in solubilising the insoluble form of phosphate eg. iron, aluminium, phosphate, tricalcium into available form as biofertilizers contain living microbials. It also help in the decomposition of organic matter by their metabolic activities. Once a biofertilizers is applied to the surface of the seeds or mix in the soil, by its metabolic activity it helps in increasing the yield by 10-20% without harming the ecosystem and makes the soil further healthy as the microbes grows in multiples and further decrease the need of addition of any external fertilizer. Therefore, significantly increase the plant biomass viz., shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight (Gharib *et al.*, 2008; Ismail *et al.*, 2014; Leithy *et al.*, 2009; Ateia *et al.*, 2009; Mahmoud, 2009; Ezz EI-Din and Hendawy, 2010).

Phosphorus solubilising biofertilizers

There are many microorganisms which can solubilize phosphate, as these microorganisms can survive in a medium containing tricalcium, iron and aluminium phosphate, hydroxy apatite, bonemeal, rock phosphate and some insoluble phosphate



compound. Some of the most efficient PSM include Bacillus and Pseudomonas bacteria and Aspergillus and Penicillium belonging to fungi group (Gaur, 1990). Many varieties of PSM have been isolated from the rhizospheric soil of the plants. Bacterial organisms are seen to lead the Phosphate solubilizing. Therefore, these bacteria, and fungi are used in making biofertilizers. It is observed that their application in increasing the yield of crops like cereals, legumes, fruit crops, vegetable have been observed (Kundu *et al.*, 2009). A metabolite such as organic acid is observed to be released by Phosphate solubilising microorganisms (PSH), later on which is converted into the soluble form (Nahas, 1996).

Phosphate solubilising microbes dissolves the P present in the soil by producing organic compound with low molecular weight mainly gluconic and ketogluconic acid (Khan *et al.*, 2009).

PSB can be used for all the crops like paddy, millets, oil seeds, pulses and vegetable.

Methods of application of PSB

1. Seed treatment: 10 kg of normal size seeds of lentil, mung, berseem, treated with 200g of PSB.
2. Seedling dip: This method is useful for vegetable and also for transplanted seedling. Inoculants of the seedling are prepared in 1:10 ratio. Roots of seedling are dipped in this suspension for 5 min.

3. Soil application: 3-5 kg of inoculants is mixed with 50kg of farm yard manure.

Phosphorus deficiency is observed mostly in tropical regions. Phosphorus makes 0.1% of the body weight. So it is considered as an important element required for the healthy growth of the crops. As phosphorus is observed to play an important role in cell division, cell development, photosynthesis, nuclear transport with in the plant.

***Mycorrhizal* fungi**

They can utilize phosphorus from extremely low concentration. Therefore, this *Mycorrhizal* fungi is very beneficial as it can use phosphorus from the waste land and supply it to the plants. *Mycorrhizal* also makes the soil fertile by adding phosphorus in it (Mahdi *et al.*, 2010). AM hyphae excrete gluey, sugar-based compounds, called Glomalin, that helps the soil to bind the soil particles together and make stable soil aggregates (Peter, 2002).

***Mycorrhizal* fungi available in the market**

Vesicular Arbuscular Mycorrhiza root inoculant (VAMRI)

It is a chopped dried root of corn having endophytic arbuscular *Mycorrhizal* fungus, Glomus mosseae or Glomus fasciculatum. VAMRI is used in biofertilizers and also helps in controlling soil-borne diseases under various conditions.

Mycorrhizal shows some resistance and tolerance against soil borne pathogens like harmful bacteria, fungi and nematodes. Therefore, it can be said that VAMRI can reduce the use of chemical fertilizers and pesticide percentage to some extent.

Azospirillum: It is a Nitrogen fixer. It belongs to the family Spirillaceae. It has an ability to fix nitrogen of about 20-40 kg/ha, and also observed to produce growth regulating substances.

The different species of *Azospirillum* are *Azospirillum amazonense*, *Azospirillum halopraeferens*, *Azospirillum brasilense*. These *Azospirillum* form associative symbiosis with many plants especially with those with C4-dicarboxylic path way of photosynthesis (Hatch and Slack pathway), as they grow and fix nitrogen on salts of organic acids such as malic, aspartic acid (Arya, 2007). It is used mostly for crops like pearl, millet, sorghum, sugarcane, maize, etc. *Azospirillum* species belong to the carbon compounds and adequately low level of facultative endophytic diazotrophs groups which colonize combined nitrogen (Andrew *et al.*, 2007). *Azospirillum* directly benefits the plant by improving the nitrogen fixing metabolic activities of bacteria present in the rhizosphere of the plants (Nghia and Gyurjan, 1987). It also helps in the development of shoot and root of the plant (Gonzalez *et al.*, 2005).

Azotobacter

Azotobacter is a free living bacterium that can fix atmospheric nitrogen in the soil. It

helps in increasing the yield of the crop by fixing nitrogen for non leguminous plants under field conditions. These bacteria are observed to develop close relation called as symbiosis with the root legumes which is one of the most important relationship with roots of various wild plants (Doroshenko and Rawia, 2007; Rawia *et al.*, 2009).

Algal biofertilizers

Many algae after their death provide nutrients to plants. Blue green algae in free living or symbiotic form fix the atmospheric nitrogen and are useful.

Azolla spp

It is a nitrogen fixer. It is also a diazotrophic symbiont. Symbiotic association is seen between *Azolla* and Cyanobacteria *Anabaena*. The host *Azolla* is observed to provide carbon to the *Anabaena* and Cyanobacteria provides nitrogen to *Azolla* by its nitrogen fixing property. They are the potential source of nitrogen for wet rice land. The percentage of nitrogen from *Azolla* spp., to wet land rice has been found to be maximum when incorporated into the soil as green manure (Galal., 1997). It is beneficial to grow *Azolla* as a biofertilizer for its N and K nutrients and it is also a useful mosquito repellent. An increase in the yield of paddy is seen ranging from 9-39% in field experiment, when *Azolla* was used in the soil (Singh, 1977).

Table 4: Different species of *Azolla* which can be used as biofertilizer

Azolla spp	Reference
Pinnata	Singh and Srivastava, 1984
Mexicana	Thanh and Hang, 1988
Filiculoides	Singh and Srivastava, 1984
Rubra	Stergianou and Fowler, 1990
Niloticaa	Stergianou and Fowler, 1990
Caroliniana	Thanh and Hang, 1988
Microphylla	Stergianou and Fowler, 1990

Cyanobacteria

They are seen more widely used as nitrogen fixer. Cyanobacteria are a diverse group of prokaryotes. The activity of nitrogen fixing organisms provide an important source of nitrogen to the marine ecosystem (Gonzalez *et al.*, 2005). They also grow and fix nitrogen in terrestrial environment from rain

forest to desert (Peter *et al.*, 2002). As Cyanobacteria can tolerate extra climatic conditions and also can fix nitrogen so they are used as a biofertilizer. They also have other benefits like adding of organic matter, secreting growth promoting substances like auxin , vitamins, mobilize insoluble phosphate and also help in improving physical and chemical texture of the soil.



Table 5. Biofertilizers which are used against crops

Biofertilizer	Recommended crop	Fertilizer saving
<i>Azolla pinnata</i> (fresh)	Low land rice	30-50 kg N
<i>Azolla pinnata</i> (dry)	Wheat, potato, tobacco	30-50 kg N
BGA	Low land rice	30-50 kg N
<i>Azotobacter chroococcum</i>	Pearlmillet, , rajma,sugar cane	20-40 kg N
<i>Azospirillum lipoferum</i>	Paddy, fingermillet, pearlmillet	20-40 kg N
<i>Azotobacter diazotrophicus</i>	sugar cane	100 kg N
<i>Rhizobium spp</i>	Pigeonpea, chick pea, green gram	30-50 kg N
<i>Bacillus circulans</i>	Cow pea	20-50 kg P ₂ O ₅
<i>Bacillus brevis</i>	Wheat, pearlmillet	20-50 kg P ₂ O ₅
<i>Bacillus congulans</i>	Pearlmillet, Cow pea,groundnut	20-50 kg P ₂ O ₅

Rhizobium

It belongs to the Rhizobiaceae family and it is symbiotic in nature. It fixes 50-100 kg/ha nitrogen in legumes plants. The known genera are as follows:

Rhizobium, Bradyrhizobium, Sinorhizobium, azorhizobium, Mesorhizobium and *Allorhizobium* (Vance, 2001; Graham and Vance, 2000). It is beneficial for legume pulses like chick pea, red gram, pea , lentil, black gram, etc., oil-seed legumes like soyabean and ground nut and forage legumes like berseem and Lucerne. *Rhizobium* colonizes the roots of legume and form a tumor like structure defined as root nodules, which are said to be a manufacturing unit of ammonia.

It is observed that *Rhizobium* has the ability to fix atmospheric nitrogen in symbiotic association with legumes and some non-legume plants like Parasponia.

Frankia

It is a nitrogen fixing genus of actinomycetes (Benson and Silvester, 1993; Huss-Danell, 1997). They are also known as actinorhizal plants and they are also used in land reclamation for timber and fuel wood production in mixed plantations for wind breaks (Schwencke and Caru, 2001). The percentage of nitrogen fixed by *Frankia* is estimated to be similar to rhizobial symbiosis (Torrey, 1978; Dawson,1986; Dommergues, 1995).

Plant growth promoting rhizobacteria

Various bacteria can promote plant growth (Bashan, 1998). Together, such bacteria are called plant growth promoting rhizobacteria (PGPR). These bacteria are different in their metabolic activities but ultimately help in influencing the plant growth by either fixing of nitrogen or P solubilization , nutrient uptake enhancement or by promoting hormones useful for plant growth (Bashan *et al.*, 1990; Okon and Labandera-Gonzalez, 1994; Goldstein *et al.*, 1999; Richardson ,

2001; Bertrand *et al.*, 2000) showed that a rhizobacterium belonging to genus *Achromobacter* could enhance root hair number and length in oilseed (*Brassica napus*).

ROLE OF BIOFERTILIZER IN AGRICULTURE

The biofertilizers play an important role in improving the fertility of the soil (Kachroo and Razdani., 2006; Son *et al.*, 2007). Application of the biofertilizers improves the texture of the soil and reduces the use of chemical fertilizer. There was tremendous increase in the grain yield and harvest index. *Azotobacter* + *Rhizobium* +VAM gave the highest increase in straw and grass yield of wheat plants with rock phosphate as a P fertilizer.

Azolla is less expensive, economical friendly and also provide carbon and nitrogen to the soil (Kaushik and Prassana., 1989). The microorganisms like *B. Subtilis*, *Thiobacillus thiooxidans* and *Saccharomyces* spp., can be used as biofertilizers for solubilisation of fixed micronutrients like zinc (Raj, 2007). It is observed that soyabean plant can provide 80-90% of nitrogen through symbiosis (Bieranvand *et al.*, 2003).

Bio-control is a modern approach towards disease management which can play an important role in agriculture (Tverdyukev *et al.*, 1994; Hoffmann-Hergarten *et al.*, 1998; Yang-Xiu Juan *et al.*, 2000; Sharon *et al.*, 2001; Senthil Kumar and Rajendra, 2004; Li-Bin *et al.*, 2005; Hossain *et al.*, 2009).



Root knot disease of French beans is observed to be controlled by *Trichoderma* based BAU-biofungicide (Rahman, 2005). Antagonist bacteria like *Rhizobium* and *Bradyrhizobium* have helped in controlling the root knot of mungbean (Khan *et al.*, 2006).

There is significant increase in the biomass of various plants after implementation of biofertilizers containing bacterial nitrogen fixer, phosphate and potassium solubilizing bacteria and microbial strains of some bacteria (Youssef and Eissa, 2014).

Constraints in the use of biofertilizer

- 1. Unavailability of suitable carrier:**
In absence of a suitable carrier, it becomes difficult to maintain the shelf life of the microbes present in the biofertilizers.
- 2. Unavailability of suitable strains:**
Due to lack of a suitable strain in a big population of microbes, it becomes difficult for the production of a particular biofertilizers. As it is known that selective strains have ability to survive both in the broth and the inoculants carrier.
- 3. Lack of awareness among farmers:**
Farmers are not aware of benefits of biofertilizers and also its eco friendly qualities along with the increase in the yield with low cost.
- 4. Inadequate and inexperience staff:**
It is a result of not giving proper

education and required information about the respective product.

The estimated figures of production of biofertilizers in the country is 10,000 mt/annum and the production capacity is 18,000 mt/annum. Average annual consumption of biofertilizer in the country is about 64g/ha.

Limitations of biofertilizers

1. Biofertilizers cannot be mixed with chemical fertilizers.
2. Biofertilizers can't be exposed to direct sunlight as the quality of it may get reduced.
3. Biofertilizers can't be applied with any fungicides, plant ash at the same time.
4. It has to be stored at room temperature so as to maintain its quality.

CONCLUSION

Biofertilizers are nowadays becoming famous in many countries due to its usefulness. As we all know that biofertilizers are made up with useful microorganisms which increases the fertility of the soil by their metabolic activities and they also multiply themselves which again results in increasing the fertility of the soil to a larger extent. In India, the soil is losing its fertility due to various factors like soil erosions, loss of nutrition, accumulation of toxic elements,

water logging and unbalanced nutrient compensation. Organic manure and biofertilizers are some of the options for completing the required nutrients demand of the crops. Some of the biofertilizers which are helping the crops are *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter*. Biofertilizers can also increase the plants resistance to adverse environmental stress. Root knot disease caused by *Meloidogyne javanica* in soyabean can be controlled by the use of BAU-Biofungicide and BINA-Biofertilizer as this is ecofriendly as compared to the chemical pesticides. The proper use and application of biofertilizers will not only help in the increase of the yield but also will keep the environment healthy and ecofriendly.

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