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Review

Biofertilizer, a way towards organic agriculture: A review

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Fertilizers supply essential plant nutrients, mainly nitrogen (N), potassium (K) and phosphorous (P). These fertilizers increase the yield of the crop but they cause several health hazard. Due to the several health hazard, consumer preferences shift towards the use of the organic food grown without use of any chemical. In recent years, biofertilizers have emerged as an important component for biological nitrogen fixation. They offer an economically attractive and ecologically sound route for providing nutrient to the plant. Biofertilizers are low-cost renewable source of nutrient that supplements the chemical fertilizer. Biofertilizers gained importance due to its low cost amongst small and marginal farmer.

Key words: Biofertilizer, isolation, mass multiplication, N_2 fixers, plant growth promoting rhizobacteria (PGPR), organic agriculture.

INTRODUCTION

The term biofertilizer, represent everything from manures to plant extracts. “Biofertilizers” are those substances that contain living microorganisms and they colonize the rhizosphere of the plant and increase the supply or availability of primary nutrient and/or growth stimulus to the target crop. There are numerous species of soil bacteria that colonize mainly in the rhizosphere of plants. These bacteria are collectively known as plant growth promoting rhizobacteria (PGPR). Some PGPR promote the growth by acting as biofertilizer. Microorganisms mainly nitrogen fixer, phosphate solubilizer and mycorrhizae are the main sources of biofertilizer. The microorganisms used for the biofertilizer are bacteria of *Bacillus, Pseudomonas, Lactobacillus*, photosynthetic bacteria, nitrogen fixing bacteria, fungi of *Trichoderma* and yeast. Biofertilizers have shown great potential as a, renewable and environmental friendly source of plant nutrient. Biofertilizers are ready to use and used as a live formulation of beneficial microorganisms, when it amended to seed, root or soil, it mobilizes the availability and utility of the microorganisms and thus improves the soil health. In general, bio-fertilizers are microbial preparations containing living cells of different microorganisms which have the ability to mobilize plant nutrients in soil from unusable to usable form through biological process. Bio-fertilizers are used in live formulation of beneficial microorganism which on application to seed, root or soil, mobilize the availability of

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Table 1. Carriers.

Form	Liquid	Powder	Granular
Appearance	Without strange smell	Brown	Brown
Fast-growing *Rhizobium*	>0.5 x 10^9/ml	>0.1 x 10^9/g	>0.1 x 10^9/g
Slow-growing *Rhizobium*	>1.0 x 10^9/ml	>0.2 x 10^9/g	>0.1 x 10^9/g
N fixing bacteria	>0.5 x 10^9/ml	>0.1 x 10^9/g	>0.1 x 10^9/g
Si bacteria	>1.0 x 10^9/ml	>0.2 x 10^9/g	>0.1 x 10^9/g
Organic P	>0.5 x 10^9/ml	>0.1 x 10^9/g	>0.1 x 10^9/g
In organic P	>1.5 x 10^9/ml	>0.3 x 10^9/g	>0.2 x 10^9/g
Multi-strain bio-fertilizer	>1.0 x 10^9/ml	>0.2 x 10^9/g	>0.1 x 10^9/g

nutrients particularly by their biological activity and help to build up the lost microflora and in turn improve the soil health in general (Ismail et al., 2014). Their mode of action differs and can be used alone or in combination. For easy application, biofertilizers are packed in suitable carrier such as lignite or peat. Carrier also plays an important role in maintaining sufficient shelf life (Singh et al., 1999).

Rhizobium is the most studied and important genera of nitrogen fixing bacteria (Odame, 1997). *Azospirillum* spp. contribute to increased yields of cereal and forage grasses by improving root development in properly colonized roots, increasing the rate of water and mineral uptake from the soil, and by biological nitrogen fixation (Okon, 1985). Biofertilizers have shown great potential as supplementary, renewable and environmental friendly sources of plant nutrients and are an important component of Integrated Nutrient Management (INM) and Integrated Plant Nutrition System (IPNS) (Raghuwanshi, 2012). Naturally grown biofertilizers not only give a better yield, but are also harmless to humans and lead to better sustainable economic development for the farmers and their country (Mishra and Dash, 2014).

ISOLATION TECHNIQUES

Isolation technique for *Rhizobium* spp.

Intact root nodules from a healthy *Sysbania exaltata* plant were selected. One of the pink juvenile root nodule was selected and 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 *Rhizobium* bacteria into the drop of sterile water. The smear of the crushed root nodule was streaked onto yeast extract mannitol agar (YEMA) plate with 1% Congo red dye. The culture was then incubated at 20 to 25°C for three days (Boraste, 2009).

Isolation technique for *Azospirillium* spp.

Juvenile root from a healthy sugar cane plant was taken and kept in saline for 5 min. With a forceps, root was immersed to a semisolid Bromothymol blue medium broth containing 0.8% agar in a test tube and incubated at 20 to 25°C for at least a week. A loopful of culture adjacent to the root in the broth was transferred to bromothymol blue media plates. The culture was incubated at 20 to 25°C for at least a week.

Isolation of phospho bacteria from the rhizoids

1. Soil samples: They are collected from the different agricultural land.
2. Serial dilution method: 10 g of soil sample is dissolved in the 100 ml of distilled water and the sample is mixed well, and by dilution making the sample10^1. Then the soil sample in sterilized water is serially diluted up to 10^7 dilution. Then 10^5, 10^6, 10^7 dilution is taken into spread plate technique.
3. Spread plate technique: Nutrient agar are poured in to the plate, after solidification of medium 0.1 ml of medium are poured into the agar medium plate, then they are incubated at 37°C for 24 h.

Carriers

Carriers (Table 1) increase the effectiveness of the biofertilizer. It enables easy handling and increases the storage or shelf life. Carriers which are used for making solid type of biofertilizer products are clay mineral, diatomaceous soil, and white carbon as mineral; rice, wheat bran, peat, lignite, peat soil, humus, wood charcoal and discarded feed as organic matter. However, clay mineral and rice bran are most often used as carriers. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum arabic, methylethylcellulose and vegetable oil is also available.

MASS PRODUCTION OF BIOFERTILIZERS (FIGURE 1)

Criteria for strain selection

Efficient nitrogen fixing strains is selected and then

Isolation of microbes from the soil

↑↓

Laboratory screening of microbes for plant growth

↑↓

Greenhouse screening of microbes to promote growth in potted soil

↑↓

Field screening of most effective microbes in cropped soil

(Crop variety and different soil types examined)

↑↓

Refinement of inoculum

↑↓

Production of biofertilizer

Figure 1. Production of biofertilizers.

multiplied on the nutritutionally rich artificial medium before inoculating in the seed and soil.

Culturing in the flask containing broth

The isolated strain is inoculated in the small flasks containing suitable medium for inoculums production. Now, the carrier was autoclaved at 15 psi at 121°C for 20 min. The culture broth was mixed with the carrier at 30%, that is, for 1 kg carrier; 300 ml of culture broth was used. The mixture was spread on a plastic sheet in a closed room for air drying. The biofertilizer was packed in sterile plastic air tight bags and stored. For large scale production of inoculums, culture fermenters are used.

Quality control

Like every product, the biofertilizers should also follow some standards. The inoculants should be carrier based, and it should contain 10^8 viable cells per gram of carrier on dry mass basis within 15 days of manufacture. The inoculums should have a maximum expiry period of 12 month from the date of manufacture. The inoculants should not have any contaminant. The contaminant is one of the biggest problems faced by the biofertilizers industry. The pH of the inoculant should be 6.0-7.5. Each packet containing the biofertilizer should be marked with the information eg. name of the product, leguminous crop for which intended, name and address of the manufacturer, type of carrier, batch or manufacture no, expiry date. Each packet should also be marked with the ISI mark. The biofertilizer should be stored in the cool place and keep away from direct heat.

Types of biofertilizer (Table 2) available

1. Nitrogen fixing biofertilizer: *Rhizobium*, *Azotobacter*, *Azospirillum*, *Bradyrhizobium*.
2. Phosphorus solubilising biofertilizer (PSB): Bacillus, *Pseudomonas*, *Aspergillus*.
3. Phosphorus mobilizing biofertilizer: *Mycorrhiza* (Plate 1).
4. Plant growth promoting biofertilizer: *Pseudomonas.*

Mode of action of biofertilizer

They fix nitrogen in the soil and the root nodules of the legumes crop and make it available to the plant. They solubilise the insoluble form of the phosphate like tricalcium, iron and aluminium phosphate into the available form. They produce hormones and anti metabolites which promote root growth. They also decompose the organic matter. When biofertilizers are applied to the seed and the soil they increases the

Table 2. Different types of biofertilizers.

Biofertilizer	Microorganism
Nitrogen fixers	*Azolla pinnata, Rhizobium* spp., *Azotobacter chroococcum,* *Azospirillum lipoferum, Acetobacter diazotrophicus, Derxia gummosa*
Phosphate solubilizers	*Bacillus circulans, Bacillus coagulans, Torulospora globasa,* *Pseudomonas fluorescens (siderophore), Thiobacillus (SOM),* *Aspergillus niger (avirulent), Trichoderma* sp., *Paecilomyces sp*
Potash mobilizers	*Bacillus* spp., *Pseudomonas* spp.
Zinc mobilizer	*Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp.

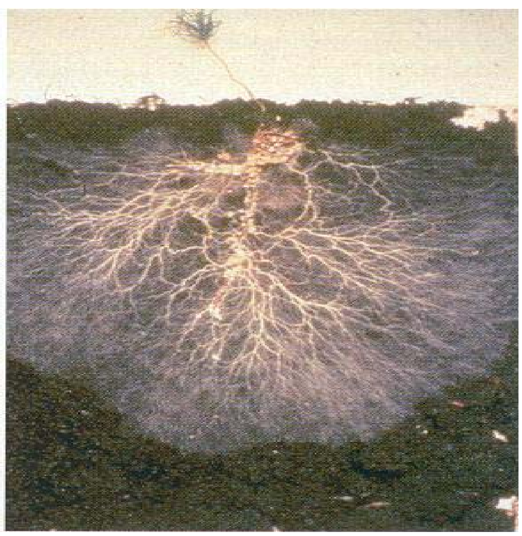

Plate 1. Mycorrhizae (Peters, 2002).

availability of the nutrient to the plant and increases the yield up to 10-20% without producing any adverse effect to the environment. Therefore, significantly increase the plant growth parameters *viz*., plant height, number of branches, number of roots, root length, shoot length, dry matter accumulation in plant organs and vigour index etc. (Ezz El-Din and Hendawy, 2010; Ateia et al., 2009; Mahmoud, 2009; Leithy et al., 2009; Gharib et al., 2008; Ismail et al., 2014).

Phosphorus producing biofertilizer

Phosphate solubilising microorganisms include several bacteria and fungi which can grow in the medium containing tricalcium, iron and aluminium phosphate, hydroxy apatite, bonemeal, rock phosphate and some insoluble phosphate compound. The most efficient PSM belong to the genera *Bacillus* and *Pseudomonas* among bacteria and *Aspergillus* and *Penicillum* amongst fungi (Gaur, 1990). Several varieties of PSM have been isolated from the rhizospheric soil of the crop. Majority of bacterial organisms are known to solubilize phosphate. These bacteria and fungi are used as a biofertilizer. Their application in several crop tend to increase their yield in crop such as cereals, legume, vegetable, fruit crops (Kundu et al., 2009). Phosphate solubilising microorganisms release metabolite such as organic acid latter being converted into the soluble form (Nahas, 1996). Phosphate solubilising microorganisms dissolve soil P through production of low molecular weight organic compound mainly gluconic and ketogluconic acid (Khan et al., 2009).

Low cost medium preparation for PSB

Alternative for King's B broth (*Pseudomonas* spp.); 1. Fish extract, 10 ml; 2, Algal water, 25 ml; 3, *Aloe vera* extract, 5 ml; 4, Tap water, 100 ml; 5, pH, 7.2.

The broth was prepared, inoculated with *P. flourescens* and incubated at 27°C for 24 h on rotary shaker.

Uses of PSB

PSB can be used for all the crops including paddy, millets, oilseeds, pulses and vegetable.

Method of application of PSB

1. Seed treatment: 10 kg of normal size seeds of lentil,

Plate 2. VAMRI.

Plate 3. Brown magic.

mung, berseem treated with 200 g of PSB. Large size seeds like groundnut, chickpea, soyabean require 400-600 g inoculants for 10-12 kg seeds.

2. Seedling dip: This method is useful for the transplanted seedling and also useful for the vegetable crop. Inoculant suspensions are prepared in 1:10 ratio. Dipping the root of the seedlings in this suspension for 5 min.

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

Phosphorus deficiency is one of the major limiting factors in crop growth and nitrogen fixation in the tropical regions. Phosphorus requirement is next to the nitrogen. It makes up 0.2% of the body weight. It plays an important role in cell division, cell development, photosynthesis, breakage of sugar, nuclear transport within the plant. Nowadays, phosphorus is a non-renewable and costly input, and these phosphate fertilizers also have pollution problems associated with them. Mycorrhizal fungi (Plate 1) can utilize phosphorus from extremely low concentration. Mycorrhiza based biofertilizer technology is one of such successful technology capable of wasteland reclamation and beneficial in agriculture because it provides phosphorus nutrition to the plant. Mycorrhizae also benefit plants indirectly by enhancing the structure of the soil (Mahdi et al., 2010). AM hyphae excrete gluey, sugar-based compounds called Glomalin, which helps to bind soil particles, and make stable soil aggregates (Peters, 2002).

Marketable product of mycorrhizal fungi

Vesicular Arbuscular Mycorrhiza Root Inoculant (VAMRI) is a chopped dried corn roots infected with arbuscular mycorrhizal fungus, either *Glomus mosseae* or *Glomus fasciculatum*. VAMRI serves as bio-fertilizers and bio-control agents of soil-borne diseases of different crops under various conditions. They show a degree of resistance or tolerance against soil-borne pathogens like nematodes, bacteria and fungi. VAMRI can substantially reduce or substitute the chemical fertilizer and pesticide requirements of crops. This inoculant can be used for pepper, eggplant, tomato, papaya, banana, pineapple, watermelon, onion, corn, sugarcane, peanut, fruit crops/trees and ornamental plants.

Application

VAMRI (Plate 2) can be applied by seed pelleting or coating for direct seeding crops, by mixing with the sowing medium .VAMRI can replace 50-100% of chemical fertilizers.

Brown magic is a mycorrhizal fungal inoculant that can be utilized as biological fertilizer and bio-control agent of root diseases of orchids. This fungus was selected from 200 isolates composed of sclerotium or fruiting bodies of fungi and mycelia collected and isolated from orchid roots.

Application of brown magic (Plate 3)

It increases the growth and survival of *in vitro* cultured orchid and it also increases the tolerance and resistance of plants to pathogens and diseases; induces early flowering and enhances the production of more suckers and longer spikes. This inoculant is environment-friendly, economical and easy to use. This inoculant is available at BIOTECH Sales office at UPLB.

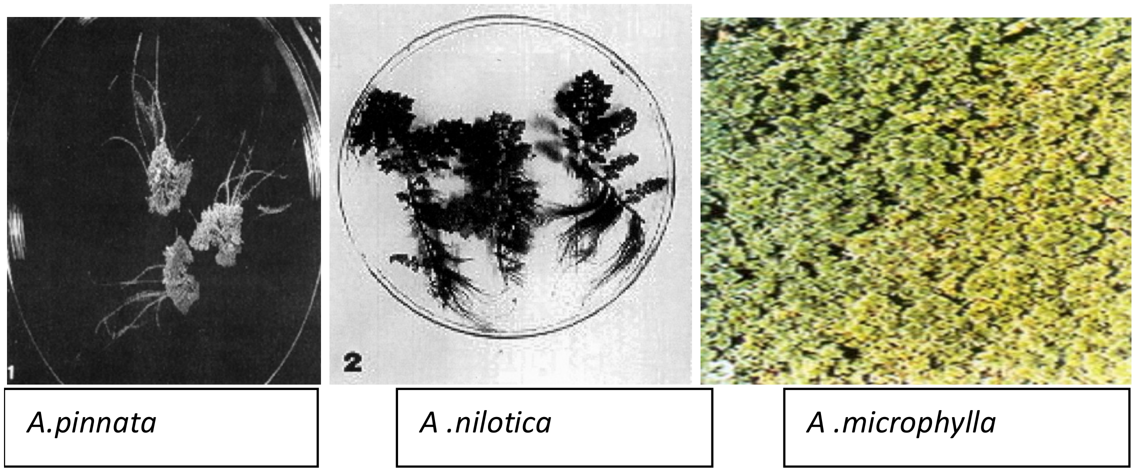

Plate 4. Different types of *Azolla* spp. (Pabby et al., 2004).

Application of mycorrhizae

Endomycorrhizae should be applied at a rate of 3,600,000 propagules per Mycorrhizae via hand seeding, seed drilling, hydroseeding, broadcast and till, planting, or as a nursery medium.

Facultative endophytic diazotroph (*Azospirillum*)

Azospirillum belongs to family Spirilaceae. It is heterotrophic and associative in nature. It has also an ability to fix nitrogen of about 20-40 kg/ha, they also produce growth regulating substances. The different species of *Azospirillum* are *Azospirillum amazonense, Azospirillum halopraeferens, Azospirillum brasilense*. The *Azospirillum* form associative symbiosis with many plants particularly with those having the C4-dicarboxyliac path way of photosynthesis (Hatch and Slack pathway), because they grow and fix nitrogen on salts of organic acids such as malic, aspartic acid (Arun, 2007). It is mainly used for some recommended crops like maize, sugarcane, sorghum, pearl millet 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 plants by improving the fixing activity of bacteria in rhizosphere of plants (Nghia and Gyurjan, 1987). It helps in shoot and root development (Gonzalez et al., 2005).

Obligate endophytic diazotrophs (*Azotobacter*)

Azotobacter is an obligate aerobe, bacteria of the genus *Azospirillum* are a well-known or endocytobionts live in special cells of their hosts which are known as *Rhizobia* found in the root nodules of legumes or *Frankia* widespread in the soils of tropical, subtropical. Azotobacter as nitrogen-biofertilizer increase the growth and yield of various crops under field conditions (Table 3). These bacteria develop in close-legume symbiosis which is one of the most efficient fixing relationships with the roots of various wild plants (Doroshenko and Rawia, 2007; Rawia et al., 2009).

Algal biofertilizers (N fixers)

Azolla spp.

It is a diazotrophic symbiont, it is well known for its utilization as a nitrogen fertilizer. *Azolla* spp. (Plate 4) float in water in large number and in soils where there is appropriate number of fern species. These are mostly found in the tropical and temperate ecosystems. There is a symbiotic association between *Azolla* and a *cyanobacteria Anabaena*. The host *Azolla* provide carbon source to the anabaena while its nitrogen requirement is fulfil by atmospheric nitrogen fixation by *Cyanobacteria*. It has the ability to fix atmospheric nitrogen through bacteria and the infection always occurs during the life of symbiosis with blue green algae (*Nostoc anabaena*).They are the potential source of nitrogen especially for wet land rice (Table 4). The contribution 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). The benefit of growing azolla as a biofertilizer for both N and K is its usefulness as human feed and it is also used as a mosquito repellent. An increase in the yield of paddy ranging from 9-39% has been obtained in the field experiment when *Azolla* was incorporated in the soil (Singh, 1977).

Cyanobacteria

N_2 fixing *Cyanobacteria* are most wide spread N_2 fixers on earth. *Cyanobacteria* or blue green algae are the

Table 3. Effect of *Azotobacter* on crop yield.

Crop	Increase in yield over yields obtained with chemical fertilizers (%)	Crop	Increase in yield over yield obtained with chemical fertilizers (%)
Food grains		Other	
Wheat	8-10	Potato	13
Rice	5	Carrot	16
Maize	15-20	Cauliflower	40
Sorghum	15-20	Tomato	2-24
		Cotton	7-27
		Sugarcane	9-24

Table 4. *Azolla* spp. which can be used as biofertilizer.

Azolla spp.	Reference
A. pinnata	Singh and Srivastava, 1984
A. mexicana	Thanh and Hang, 1988
A. filiculoides	Singh and Srivastava, 1984
A. rubra	Stergianou and Fowler, 1990
A. niloticaa	Stergianou and Fowler, 1990
A. caroliniana	Thanh and Hand, 1988
A .microphylla	Stergianou and Fowler, 1990

diverse group of prokaryotes. The activities of nitrogen-fixing organisms provide an important source of nitrogen to the marine eco system (Gonzalez et al., 2005). They also grow and fix nitrogen in terrestrial environment, from rain forest to desert (Peter et al., 2002). *Cyanobacteria* are able to survive in the extreme environment and have ability to fix nitrogen because of the capacity to fix nitrogen; they are used as a bio fertilizer. In addition to contributing N, the *Cyanobacteria* add organic matter, secrete growth promoting substance like auxin, vitamins, mobilise insoluble phosphate and improve physical and chemical nature of the soil. *Cyanobacteria* act as a supplement to the N fertilizers contributing up to 30 kg N/ ha. It increases the crop yield between 5-25%.

Mass multiplication of *Azolla*

For mass multiplication of *Azolla*, microplots (20 m^2) are prepared in the nurseries in which sufficient water (5-10 cm) is added. For profuse growth of *Azolla* 4-20 kg P_2O_5 is amended. Optimum pH 8.0 and temperature of 14-30°C should be maintained. Finally, microplots are inoculated with fresh *Azolla*. An insecticide (Furadon) is used to check the insect’s attack. After 3 weeks, the mat of *Azolla* is ready for harvest and the same microplots are inoculated with fresh azolla to repeat the cultivation. Azolla mat is harvested and dried to use as green manure.

Mass multiplication of *Cyanobacteria*

The following methods are used for mass cultivation:

a) Cemented tank method
b) Shallow metal trough method
c) Polythene lined pit method
d) Field method

i) Prepare the cemented tank, shallow trays of iron sheets are polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m.This will facilitate the proper handling of culture.
ii) Transfer 2-3 kg soil and add 100 g superphosphate. Water the pit to about 10 cm height, Mix lime to adjust the pH. Add 2 ml of insecticides to protect the culture from mosquitoes. Mix well and allow to settle down soil particles.
iii) When water become clear, sprinkle 100 g starter culture on the surface of water.
iv) When temperature remains around 35-40°C during summer, optimum growth of *Cyanobacteria* is achieved. The water level is always maintained at about 10 cm during the period.
v) After drying, the algal mass is separated from the soil that form flaskes. Then it is collected, powdered and packed in the polythene bag and supplied to the farmers after sealing the packets.
vi) The algal flakes can be used as starter inoculums again.

Gluconacetobacter diazotrophicus

G. diazotrophicus is a nitrogen-fixing, acetic acid bacterium first isolated from sugarcane plants. It belongs to phylum *Proteobacteria* (comprising Gram negative bacteria) section a-Proteobacteria, order *Rhodospirillales* and family *Acetobacteraceae*. Currently, this family contains three nitrogen-fixing genera, comprising of seven species, namely *Acetobacter nitrogenifigens, Gluconacetobacter kombuchae, Gluconacetobacter johannae Gluconacetobacter azotocaptans, G.*

Table 5. Biofertilizers which are used against crops.

Biofertilizer	Recommended crop	Fertilizer saving
Azolla pinnata (fresh)	Low land rice	30-50 kg N
Azolla pinnata (dry)	Wheat, potato, tobacco	30-50 kg N
BGA	Low land rice	30-50 kg N
Azotobacter chroococcum	Pearlmillet,sorghum, rajma, sugarcane, maize, potato, pigeonpea, onion, cotton	20-40 kg N
Azospirillum lipoferum	Pearlmillet, fingermillet, paddy, sorghum, maize, tobacco, onion	20-40 kg N
Acetobacter diazotrophicus	Sugarcane	100 kg N
Rhizobium spp	Pigeonpea, chickpea, greengram	30-50 kg N
Bacillus circulans	Cow pea	20-50 kg P_20_5
Bacillus brevis	Sorghum, wheat, pearlmillet	20-50 kg P_20_5
Bacillus congulans	Sorghum, cowpea, pearlmillet, groundnut	20-50 kg P_20_5

diazotrophicus, Swaminathania salitolerans and *Acetobacter peroxydans*.

Rhizobium

Rhizobium belongs to family *Rhizobiaceae,* it is symbiotic in nature, it fixes 50-100 kg/ha nitrogen with legumes only. It includes the following genera: *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium* and *Allorhizobium* (Vance, 2001; Graham and Vance, 2000). It is useful for the pulse legumes like chickpea, red-gram, pea, lentil, black gram, etc., oil-seed legumes like soybean and groundnut and forage legumes like berseem and lucerne (Table 5). It colonizes the roots of specific legumes to form tumour like growths called root nodules, which acts as factories of ammonia production.

Rhizobium has ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like *Parasponia*. Population of the *Rhizobium* population in the soil depends on the presence of legume crops in the field. In the absence of legumes, the population decreases.

Frankia (N fixers)

Frankia is the genus of N2-fixing actinomycetes (Benson and Silvester, 1993; Huss-Danell, 1997). These are also called actinorhizal plants and they are also used in land reclamation, for timber and fuel wood production, in mixed plantations, for windbreaks, (Schwencke and Carù, 2001). *Frankia* N_2 fixation has been estimated to be similar to rhizobial symbioses (Torrey, 1978; Dawson, 1986; Dommergues, 1995).

Plant growth promoting rhizobacteria

Various bacteria can promote plant growth (Bashan, 1998). Collectively, such bacteria are called plant-growth-promoting rhizobacteria (PGPR). These bacteria vary in their mechanism of plant growth promotion but generally influence growth via P solubilization, nutrient uptake enhancement, or plant growth hormone production (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 the genus *Achromobacter* could enhance root hair number and length in oilseed rape *(Brassica napus*).

POTENTIAL ROLE OF BIOFERTILIZER IN AGRICULTURE

The biofertilizers play an important role in improving the fertility of the soil (Kachroo and Razdan, 2006; Son et al., 2007). In addition, their application in soil improves the structure of the soil minimizes the sole use of chemical fertilizers. Under low land conditions, the application of BGA + *Azospirillum* proved significantly beneficial in improving LAI. Grain yield and harvest index also increase with use of biofertilizers. Inoculation with *Azotobacter* + *Rhizobium* + VAM gave the highest increase in straw and grain yield of wheat plants with rock phosphate as a P fertilizer. *Azolla* is inexpensive, economical, friendly, which provide benefit in terms of carbon and nitrogen enrichment of soil (Kaushik and Prassana, 1989). Some commercially available biofertilizers are also used for the crop (Table 6). Raj (2007) recorded that microorganisms (*B. subtilis, Thiobacillus thioxidans* and *Saccharomyces* sp.) can be used as bio-fertilizers for solubilization of fixed micronutrients like zinc. Soybean plants, like many other legumes can fix atmospheric nitrogen symbiotically and about 80 to 90% nitrogen demand could be supplied by soybean through symbiosis (Bieranvand et al., 2003). Bio-control, a modern approach of disease management can play a significant role in agriculture (Tverdyukev et al., 1994; Hoffmann-Hergarten et al., 1998; Yang-Xiu Juan et al*.,* 2000; Sharon et al., 2001; Senthilkumar and

Table 6. Commercially available biofertilizer and their manufacture, beneficial crop and associated microorganisms.

Product	Manufacture's name	Microbe used	Benefical crop
Nitragin TM	Nitragin Sales Corpn.Wisconsin, 53209	Rhizobium	Soyabean
Rhizocote	Coated Seed Ltd, Nelson, New Zealand	Rhizobium	Legumes
Nodosit	Uniob Chemiques S.A. Belgium	Rhizobium	Legumes
Rhizonit	Phlylaxia Allami Budapest, Hungary	Rhizobium	Legumes
Nitrazina	Wytwornia Walcz Poland	Azotobacter	Cereals and vegetables
N-germ	Laboratoire de Microbiologie, France	BGA	Rice
Tropical inoculants	Tropical inoculants	Azotobacter	Rice and wheat
Nodulaid	Brisbane, Queensland Agricultural Lab. New South Wales, UK	Rhizobium	Legume
Azotobacterin	Tashkent laboratories Moscow	Azotobacter	Vegetable and cereals.
Nodion	Indian Organic Chems. Ltd. Mahew Mahal, Bombay	Rhizobium	Legumes
Azoteeka	Bacifil, 25 Nawal Kishore Rd.Lucknow	Azotobacter	Cereals
Agro-teeka	National Fertilizers and Chemicals 11, Ind Area-II, Ramdarbar, Chandigarh	Azotobacter	Wheat, rice, maize, tea, sugarcane, potato.
Rhizoteeka	Microbes India,87.Lenin Savabe, Calcutta	Rhizobium	Legumes
Nitrogeron	Root Nodne Pvt. Ltd. Australia.	Rhizobium	Legumes

Rajendran, 2004; Li-Bin et al., 2005; Hossain et al., 2009). *Trichoderma* based BAU-biofungicide has been found promising to control root knot diseases of French bean (Rahman, 2005). Use of antagonist bacteria like *Rhizobium* and *Bradyrhizobium* also has significant effect in controlling root knot of mungbean (Khan et al., 2006). Growth, yield and quality parameters of certain plants significantly increased with 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 the biofertilizer

1. Unavailability of suitable strain: Due to the lack of the availability specific strain it is one of the major constraint in the production of the biofertilizer. Based on the fact that selective strain have ability to survive both in the broth and the inoculants carrier.
2. Unavailability of suitable carrier: If suitable carrier is not available it is difficult to maintain the shelf life of the biofertilizer. As per the suitability, the order is peat, lignite, charcoal, FYM, soil, rice husk. Peat of good quality is rarely found in India. Good quality carriers have a good moisture holding capacity, free from toxic substances.
3. Lack of awareness among farmers: Farmers of India are not aware of the biofertilizers, their usefulness in increasing crop yields.
4. Inadequate and inexperienced staff: This is because the unskilled and the inadequate staff farmers are not given proper instruction about the application. The production of biofertilizers in the country is 10,000 mt/annum and the production capacity is 18,000 mt/annum. Average annual consumption of biofertilizers in the country is about 64 g/ha.

Limitation of biofertilizer

1. Biofertilizers never mix with the chemical fertilizers.
2. Biofertilizers are never applied with the fungicides, plant ash at a same time.
3. Biofertilizers are never exposed to direct sunlight.
4. Stored at room temperature not below 0 and 35°C.

CONCLUSION

Biofertilizers are becoming increasingly popular in many countries and for many crops. Biofertilizers are fertilizers containing living microorganisms, which increase microbial activity in the soil. Often, organic food is included to help the microbes get established. In India soil fertility is diminishing gradually due to soil erosions, loss of nutrition, accumulation of toxic elements, water logging and unbalanced nutrient compensation. Organic manure and bio fertilizers are the alternate sources to meet the nutrient requirement of crops. biofertilizers, benefiting the crops are *Azotobacter, Azosprillium, Phosphobacter* and *Rhizobacter* which are very important. The role of biofertilizer in agricultural production is of great importance. Inoculation of nitrogen fixing bacteria with biofertilizer increases the phosphorus level that influences the sunflower seed oil content and the proportion of fatty acids (unsaturated/saturated fatty acids ratio). Biofertilizers can also make plant resistant to adverse environmental stresses. Control of root-knot disease of soybean caused by *Meloidogyne javanica* may be explored through use of BAU-Biofungicide and BINA-Biofertilizer for eco-friendly management of this nemic disease avoiding chemical nematicides. The proper application and use of biofertilizers will not only have an impact on sustainable agriculture's economic development but it will also contribute to a sustainable ecosystem and the holistic well-being.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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