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Biofertilizer and their role in sustainable agriculture-A review

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Abstract
Biofertilizers consist mainly of beneficial microorganisms that can be released nutrients from raw materials and plant residues in the soil and make them available commercially where specific strains are used as biological fertilizers. Bio-fertilizer contains micro-organisms which promotes the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of biofertilizers. Only those microorganisms are used which have specific functions to enhance plant growth and reproduction. There are different types of microorganisms which are used in the bio-fertilizers. Bio-fertilizers offer great potential for not only improving soil fertility but also provide for efficient use of various resources for increasing crop production on sustainable basis. They helps in maintaining long term soil fertility and sustainability by fixing atmospheric N2, mobilizing fixed macro and micro nutrients or convert insoluble P in the soil into forms available to plants, there by increases their efficiency and availability. Nowadays, the liquid biofertilizers are showing a very good response in various crops which overcomes the previous restrictions of the application of biofertilizer. While dealing with biofertilizers there are some species of micro-organism which are using for enhancing the use efficiency of the micronutrient and along with that the decomposer of the organic residue. After all, we are in the need of cost effective option and responsive one which can reduce the degradation of soil by chemical application and we can say them as an eco-friendly input material for the sustainable agriculture.

Keywords: Biofertilizers, micro-organisms, soil fertility, plant growth, sustainable agriculture

Introduction
Soil is a dynamic living body and contains enormous numbers of diverse living organisms. Organic agricultural practices aim to enhance biodiversity, biological cycles and soil biological activity so as to achieve optimal natural systems that are socially, ecologically and economically sustainable. Biofertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. The production technology for biofertilizers is relatively simple and installation cost is very low compared to chemical fertilizer plants. Bio-fertiliser are the low cost source of plant nutrients, environment-friendly and have auxiliary role with chemical fertilizers. Historically, the bio-fertilizers were initially identified by a Dutch scientist in 1888 there after bio-fertilizer use started with the launch of Nitragin by Nobe and Hiltner with a laboratory culture of Rhizobia in 1895 (Ghosh, 2004) [4]. Then subsequently *Azotobacter*, *Blue green algae* etc. were discovered. Among the recent discoveries of the most popular bio-fertilizers are *Azospirillum*, *Vesicular Arbuscular Mycorrizae*, etc. They plays potential role in evolving judicious combinations with chemical fertilizers to further supplement the nutrient requirements of crop (Nyekha *et al.* 2015) [8]. First time in India, the legume-*Rhizobium* symbiosis study was conducted by N.V. Joshi in 1920 with commercial production of Biofertilizers in 1956 (Rana *et al.* 2013) [10]. The use of inoculants has considerably increased over the years and a number of private producers have come into operation. Accordingly, the Bureau of Indian Standards has sprung into action and specific standards for *Rhizobium, Azotobacter*, *Azospirillum* and PSB inoculants have been brought out. Some of the nitrogen fixers colonize the root zones and fix nitrogen in loose association with plants. In recent years, various other rhizobacteria such as *Aeromonas veronii*, *Azotobacter sp*., *Azoarus sp*, *Cyanobacteria* (predominantly of the genera *Anabaena* and *Nostoc*) *Alcaligenas, Burkholderia, Comamonas acidororans, Enterobacter, Erwinia, Flavobacterium, rhizobia* (including the *Allorhizobium, Azorhizobium, Bradyrhizoblum, Mesorhizobium, Rhizobium and Sinorhizobium*) *Gluconacetobacter diazotrophicus, Herbaspirillum seroepdicae, Serratia,*

Variovorax paradoxus and *Xanthomonas maltophilia* have been identified for their use either as biofertilizers or biological control agents (Bioinoculants).

Need of bio-fertilizers: Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility. Demand is much higher than the availability. Depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers. Depleting soil fertility is due to widening gap between nutrient removal and supplies. Besides above facts, the long term use of bio-fertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers (Venkataraman and Shanmugasundaram, 1992) [14].

Some of the important biofertilizers are mentioned below-

Rhizobium: Is the symbiotic bacteria that fix atmospheric N2 gas in plant through root nodules and have a mutually helpful relationship with their host plants. The plant roots supply essential minerals and newly synthesized substances to the bacteria. It is reported that rhizobium can fix 50-300 kg N/ha. A strain of Rhizobia that nodulates and fixes a large amount of nitrogen in association with one legume species may also do the same in association with certain other legume species. That's need to be verified by testing. Leguminous plants that demonstrate this tendency to respond similarly to particular strains of Rhizobia are considered "effectiveness" group (Wani and Lee 2002) [15].

Azotobacter: It is free living and non-symbiotic nitrogen fixing organism that also produces certain substances which would like to be claimed as a good for the growth of plants and antibodies that suppress many root pathogens belongs to family *Azotobacteriaceae*, aerobic and heterotrophic in nature. *Azotobacter* are present in neutral or alkaline soils and *A. chroococcum* is the most commonly occurring species in arable soils. *A. vinelandii, A. beijerinckii, A. insignis* and *A. macrocytogenes* are other reported species. The number of *Azotobacter* rarely exceeds of 104 to 105 g-1 of soil due to lack of organic matter and presence of antagonistic microorganisms in soil. The bacterium produces anti-fungal antibiotics which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent. The population of *Azotobacter* is generally low in the rhizosphere of the crop plants and in uncultivated soils. The occurrence of this organism has been reported from the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops. They can fix 15-20 kg/ha N per year. *Azotobacter* can also produce antifungal compounds to fight against many plant pathogens. They also increase germination and vigour in young plants leading to improved crop stands. The *Azotobacter* colonizing the roots not only remains on the root surface but also a sizable proportion of them penetrates into the root tissues and lives in harmony with the plants.

Azospirillum: *Azospirillium* is a nitrogen-fixing micro-organism beneficial for non-leguminous plants. *Azospirillum* transcend nitrogen enrichment through production of growth promoting substances. It belongs to family *Spirilaceae*, heterotrophic and associative in nature. In addition to their nitrogen fixing ability of about 20-40 kg ha-1, they also produce growth regulating substances. Although, there are many species under this genus like, *A. amazonense, A. halopraeferens, A. brasilense* but worldwide distribution and benefits of inoculation have been proved mainly with the *A. lipoferum* and *A. brasilense*.

Phosphate-solubilizing microbes (PSM): Phosphate solubilising microbes are differs in their habits and functionalities on the basis of content of phosphorus present in the soil and accordingly response of these communities towards the phosphorus availability varies. That's why the management strategies toward these communities are depends on their response. Mode of action of these PSB's include increasing the surface area of the plant roots increasing the availability of the nutrients in the soil to the plants assisting the N2 fixation and enhancing the other beneficial effects of symbiotically. PSB solubilise phosphate by production of organic acids. These acids can either dissolve the phosphorus directly by lowering the pH of soil which can help in ion exchange of PO42- by acid ions or they can chelate heavy metal ions such as calcium, aluminium, iron and release associated phosphorus with them (Moghimi *et al*. 1978) [7].

Phosphate absorbers (mycorrhiza)/*Vesicular arbuscular mycorrhiza* (VAM): Mycorrhizae are mutually beneficial (symbiotic) relationships between fungi and plant roots. VAM fungi infect and spread inside the root. They possess special structures known as vesicles and arbuscules. The plant roots transmit substances (root exudates) to the fungi, and the fungi aid in
transmitting nutrients and water to the plant roots. The fungal hyphae may extend the root lengths 100-fold. The hyphae reach into additional and wetter soil areas and help plants absorb many nutrients, particularly the less available mineral nutrients such as phosphorus, zinc, molybdenum and copper. The solubilization effect of phosphobacterins is generally due to the production of organic acids that lower the soil pH and bring about the dissolution of bound forms of phosphate. It is reported that PSB culture increased yield up to 200-500 kg/ha and thus 30 to 50 kg of superphosphate can be saved.

The term Mycorrhiza denotes "fungus roots". It is a symbiotic association between host plants and certain group of fungi at the root system, in which the fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. These fungi are associated with majority of agricultural crops, except with those crops/plants belonging to families of *Chenopodiaceae, Amaranthaceae, Caryophyllaceae, Polygonaceae, Brassicaceae, Commelinaceae* and *Cyperaceae.* VAM fungi infect and spread inside the root. They possess special structures known as vesicles and arbuscules. The plant roots transmit substances to the fungi, and the fungi aid in transmitting nutrients and water to the plant roots. The fungal hyphae may extend the root lengths 100-fold.

Phosphate solubilising bacteria (PSB): PSB include *Pseudomonas, Bacillus, Enterobacter, Azospirillum* and *Rhizobium* etc, also called as Rhizobacteria because they colonize the plant roots and promote plant growth (Anthony and Kloepper, 2009) [2]. These are free living bacteria which are beneficial to crop, they are capable of enhancing crop growth by colonizing the roots of the plants. Plant growth promoting rhizobacteria or nodule promoting bacteria are associated with rhizosphere of the soil.

Plant growth promoting rhizobacteria (PGPR): It represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of host growth. PGPR modes include fixing N2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology and promoting other beneficial plant–microbe symbioses. Some researchers have indicated that PGPR will often have multiple modes of action. Ratti *et al*. (2001) [11] found that a combination of the arbuscular mycorrhizal fungi *Glomus aggregatum*, the PGPR *Bacillus polymyxa* and *Azospirillum brasilense* maximized biomass and P content of the aromatic grass palmarosa (*Cymbopogon martinii*) when grown with an insoluble inorganic phosphate. PGPR are divided into iPGPR and ePGPR.

Zinc solubilizers: The nitrogen fixers like *Rhizobium, Azospirillum, Azotobacter*, *BGA* and Phosphate solubilizing bacteria like *B. magaterium, P. striata,* and phosphate mobilizing Mycorrhiza have been widely accepted as bio-fertilizers (Subba Roa, 2001) [13]. However these supply only major nutrients but a host of microorganism that can transform micronutrients are there in soil that can be used as bio-fertilizers to supply micronutrients like zinc, iron, copper etc., zinc being utmost important is found in the earth's crust to the tune of 0.008 per cent but more than 50 per cent of Indian soils exhibit deficiency of zinc with content must below the critical level of 1.5 ppm of available zinc (Katyal and Rattan, 1993) [6]. The plant constraints in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate ($ZnSO_4$). But the fate of applied zinc in the submerged soil conditions is pathetic and only 1-4% of total available zinc is utilized by the crop and 75% of applied zinc is transformed into different mineral fractions (Zn-fixation) which are not available for plant absorption (crystalline iron oxide bound and residual zinc). There appears to be two main mechanisms of zinc fixation, one operates in acidic soils and is closely related with cation exchange and other operates in alkaline conditions where fixation takes by means of chemisorptions, (Chemisorptions of zinc on calcium carbonate formed a solid-solution of $ZnCaCO_3$), and by complexation by organic ligands (Alloway, 2008) [1]. The zinc can be solubilized by microorganisms viz., *B. subtilis, T. thioxidans* and *Saccharomyces sp.* (Raj, 2007) [9].

Cyanobacteria: These are free-living as well as symbiotic cyanobacteria (blue green algae) and described by a group of one-celled to many-celled aquatic organisms. These can be brown, purple or red in colour, found in wet and marshy conditions, only used for rice cultivation and do not survive in acidic conditions. The activities of nitrogen fixing organisms provide an important source to the marine ecosystem (Gonzalez *et al*. 2005) [5]. *Cynobacteria* are able to survive in the extreme environment and have ability to fix nitrogen. They also add organic matter, secrete growth promoting substances like auxin, vitamins, mobilize insoluble phosphate and improve physical and chemical properties of soil.

Blue green algae (BGA): It is photosynthetically nitrogen fixers and is free living microorganism. They are found in abundance in India. They too add growth-promoting substances including vitamin B12, improve the soil's aeration and water holding capacity and add to bio mass when decomposed after life cycle.

Azolla: Is a diazotropic symbiont is capable of fulfilling the N requirements of the plant by the process of nitrogen-fixation. They are the potential source for wet land rice. 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) [3]. An increase in the yield of paddy ranging from 9-39% recorded when azolla was incorporated (Singh, 1977) [12]

Liquid biofertilizer: Liquid biofertilizers which contains living or dormant microbes (bacteria, fungi, algae, actinomycetes etc.) alone or in combination which help in fixing atmospheric nitrogen or solubilizers of different soil nutrients as well as the secretion of growth promoting substances for enhancing crop growth and yield.

Application of biofertilizers

Seed treatment: The seeds are uniformly mixed in the slurry of the inoculants and then dried for 30 minutes in shade. The dried seeds are to be sown within 24 hours. One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip: This method is used for transplanted crops. Two packets of the inoculant are mixed in 40 litres of water. The root portion of the seedlings is dipped into the mixture for 5 to 10 minutes and then transplanted.

Foliar application: Liquid biofertilizer can be applied through fertigation as well as foliar application to the suitable crop. It can also applied through seed treatment and root dipping.

Main field application: Four packets of the inoculant are mixed with 20 kg of dried and powdered farm yard manure and then broadcasted in the main field just before transplanting.

Set treatment: This method is recommended generally for treating the sets of sugarcane, cut pieces of potato and the base of banana suckers. Culture suspension is prepared by mixing 1 kg (5 packets) of bio-fertilizer in 40-50 litres of water and cut pieces of planting material are kept immersed in the suspension for 30 minutes. The cut pieces are dried in shade for some time before planting. For set treatment, the ratio of bio-fertilizer to water is approximately 1:50.

Improved nutrient uptake (macro and micronutrients): The improvement of P nutrition of plants has been the most recognized beneficial effect of mycorrhizas. It is also reported that the AM- fungi also increases the uptake of K and efficiency of micronutrients like Zn, Cu, Fe etc. By secreting the enzymes, organic acids can makes fixed macro and micronutrients mobile and as such are available for the plant.

Better water relation and drought tolerance: AM fungi play an important role in the water economy in the plants. Their association improves the hydraulic conductivity of the root at lower soil water potentials and this improvement is one of the factors contributing towards better uptake of water by plants.

Improved Soil structure (A physical quality): Mycorrhizal fungi contributes to soil structure by growth of external hyphae into the soil to create a skeletal structure that holds soil particles together, creation of conditions by external hyphae that are conducive for the formation of micro-aggregates, enmeshment of micro aggregates to form macro aggregates and directly tapping carbon resources of the plant to the soils.

Enhanced phytohormone activity: The activity of phytohormones like cytokinin and indole acetic acid (IAA) is significantly higher in plants inoculated with AM. Higher hormone production results in better growth and development of the plant.

Crop protection (interaction with soil pathogens): AM-inoculation considerably increases production and activity of phenolic and phytoalexin compounds due to which the defense mechanism of plant becomes stronger there by imparts the resistance to plants.

Future perspective of bio-fertilizers

Excess nutrients are accumulated in soils, particularly P as a result of over application of chemical fertilizers by farmers during intensive agricultural practices. Hence, major research focus should be on the production of efficient and sustainable bio-fertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems. In view of overcoming this bottleneck, it will be necessary to undertake short-term, medium, and long-term research, in which soil microbiologists, agronomists, plant breeders, plant pathologists, and even nutritionists and economists must work together. The most important and specific research needs should highlight on following points:

1. Selection of effective and competitive multi-functional bio-fertilizers for a variety of crops.
2. Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant- microorganism symbiosis.
3. Study of microbial persistence of biofertilizers in soil environments under stressful conditions
4. Agronomic, soil, and economic evaluation of bio-fertilizers for diverse agricultural production systems.
5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation.
6. Establishment of "Bio-fertilizer Act" and strict regulation for quality control in markets and application.

Constraints in biofertilizer use

(i) Production level constraints: Unavailability of appropriate and efficient strains, unavailability of suitable carrier, mutation during fermentation.
(ii) Market level constraints: Lack of awareness of farmers, inadequate and inexperienced staff, lack of quality assurance, seasonal and unassured demand.
(iii) Resource constraint: Limited resource generation for Biofertilizer production. Field level constraints: Soil and climatic factors, native microbial population, etc.

Conclusion

To improve and maintain the productivity of agricultural lands, the integrated approach to determine the most favorable plant-microorganism interaction is vital. Biofertilizer have an important role to play in improving nutrient supplies and their crop availability in the years to come. They are of environment friendly non-bulky and low cost agricultural inputs. A biofertilizer is an organic product containing a specific micro-organism in concentrated form which is derived either from the plant roots or from the soil of root zone (Rhizosphere). Among the biofertilizers *Azotobacter*, *Azospirillum*, *Acetobacter* are the important for nitrogen fixation, *Bacillus sp.* and *Aspergillus sp.* are important for phosphate solubilisation and other soil mineral nutrients. The current trend of low input chemicals in sustainable agricultural systems will contribute to the goal. The application of inorganic chemical fertilizers was thus significantly reduced to 30-50%. This helps in the realization of environmental friendly and sustainable agriculture.

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