Biological alternates to synthetic fertilizers: efficiency and future scopes

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ABSTRACT
The nutrient availability to plants is major limiting factor determining the crop production. Chemical fertilizers are, no doubt, a milestone to fulfill the nutrient deficiency but presently mankind is facing a huge threat of environment damage as well as resource depletion. At the same time population explosion is also a major concern. To feed such a large population (8.5 × 10^9 in 2025) unexploited resources should be used to enhance the crop production and to improve quality of soil. The various plant specific nitrogen fixing, phosphate solubilizing, potassium solubilizing and zinc mobilizing microorganisms can be used to enhance the bioavailability of nutrients to plants. This biological method is not only sustainable for long run but also economical and thus can be used as biofertilizers. These microorganisms can be commercially made available to farmers in the form of carrier based, liquid or encapsulated formulations containing latent or active forms. Apart from nutrient mobilization, they can also act as bioenhancers and biopesticides. However, efficiency and acceptance of biofertilizer among farmers is still a big concern. This review article focuses on efficiency of biofertilizers to replace or supplement the synthetic fertilizers for soil fertilization.

Key words: Biofertilizers, Nutrient mobilization, Synthetic fertilizers.

INTRODUCTION
The use of synthetic fertilizers, high yielding varieties of seed, assured irrigation has helped booming productivity in Indian agriculture. No doubt, productivity boomed for several decades, but long term environmental repercussions of green revolution were not taken into account. Soil infertility has increased due to the imbalanced use of chemical fertilizers (Kumar et al. 2017) due to which agricultural yields have begun to drop. Every year 20–25% of land worldwide is being degraded (Edrisi et al. 2016). Expected world population will be approximately 8.5 × 10^9 in 2025 and with shrinking fertile land resources, dealing with food insecurity will be a great challenge in future. It indicates that to deal with global food insecurity that is likely to aggravate with climate change and scarcity of arable land, food yield substantially need to be increased to approximately 2.4 × 10^9/yr. For increasing productivity, soil fertility has to be increased with regular addition of balanced nutrients. The ill effects of chemical fertilizers are not just confined to environment but also with farmer’s condition. Chemical fertilizers have led to deterioration of soil and environmental quality (Nath et al. 2017), water eutrophication, groundwater pollution, ecosystems change, air pollution (Tilman et al. 2002; Khan et al. 2008) and loss of beneficial soil microorganisms (Seneviratne and Kulasooryia, 2013). Affordability of expensive chemicals and their efficiency in light of climate change is progressively becoming a major concern in view of increasing farmer’s debts. Heavy cost of fertilizers and their inefficient utilization has made income of farmers stagnated. These concerns regarding chemical fertilizers have triggered to rethink about sustainable methods of nutrition and to change pattern of fertilizer use. A large number of human diseases are known to be caused due to chemical fertilizers; therefore, organic food is gaining popularity among buyers.

General overview of biofertilizers: The nutrient-rich part of the soil under the direct effect of plant roots, habituating millions of microbes including bacteria, fungi, actinomycetes, protozoa, algae thriving on root exudates (Garcia-Gutiérrez et al. 2013) is referred to as rhizosphere. Plant health is strongly determined by microbial diversity sustaining in plant roots. Exudates secreted by plants contain sugars, organic acids, amino acids chemotactically attract microorganisms (Huang et al. 2014) to multiply in the plant roots (Shrivastava et al. 2014). The microbes that establish a positive interaction with plant and benefit it through a number of mechanisms are termed as plant growth-promoting rhizobacteria (PGPR) (Zhou et al. 2015). These bacteria produce microbe-associated molecular pattern (MAMPs), various volatile organic compounds (VoCs) and exopolysaccharides, that enable the plants to induce defense related genes. In response to various physical and chemical changes, plants exhibit Induced systemic tolerance (IST) which increases the survival of plants under abiotic and biotic stress (Mishra et al. 2013; Iha and Saraf, 2015). This positively affect the metabolism of plants by enhancing their

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nutrient absorption capacity through nitrogen fixation (Gupta et al. 2015), phosphate solubilization (Xie et al. 2003), zinc solubilization, siderophore production (Beneduzi et al. 2008), suppression of harmful rhizobacteria by producing antibiotics (Jahanian et al. 2012), stress and ethylene concentration reduction (Shanmugam and Kanoujia, 2011) and phytohormone production (Rashid et al. 2012, Kukreja et al. 2004), hence stimulate plant yield.

However, it is important to note that all PGPRs cannot be considered biofertilizers. Bacterial species that promote the plant growth by controlling harmful organisms are regarded as biopesticides, not biofertilizers. Similarly, microorganisms producing phytohormones are regarded as bioenhancers (Bergottini et al. 2015). This interaction between soil microbes and plants can act as a sustainable alternative way of assured nutrient supply to plants and can decrease the dependency of agriculture on chemical fertilizers.

Biofertilizers contain in vitro cultured living or latent microorganisms capable of nitrogen fixation, zinc and phosphate solubilization, and potassium mobilization. Use of biofertilizers is future mandate for agriculture. It forms a part of plant nutrient supply system (IPNS) and organic farming that can effectively increase macro, micro and secondary nutrients in soil in eco friendly, sustainable and cost effective manner. They colonize the rhizospheric soil and help in the mobilization of nutrients for plants. Biofertilizers help to restore depleting soil nutrients and maintains a balance between soil sustainability and plant growth (Hazarka and Ansari, 2007). Chemical fertilizers can be supplemented with biofertilizers to partially reduce their dosage. Soil microorganisms belonging to different taxa of the bacteria, fungi, and possibly, protozoa kingdoms, colonizing the rhizosphere, can be utilized for biofertilizers production (Kamkar, 2016). The current biofertilizer market represents about 5% of the total chemical fertilizer market. The global biofertilizer market is currently dominated by nitrogen-fixing organisms followed by phosphate solubilized and others (Timmusk et al. 2017).

### Table 1: Crop specific nitrogen fixing bacteria.

| Bacterial Species | Crop | Nature of association | Effect on the crop | Reference |
|-------------------|------|-----------------------|--------------------|-----------|
| R. japonicum      | Soybean | Symbiotic | Enhanced root nodule formation | Ponnmurugan and Gopi (2006) |
| R. leguminosarum  | Pea | Symbiotic | Increase in dry weight of root and shoot length | Ponnmurugan and Gopi (2006) |
| R. lupini         | Alfalfa | Symbiotic | Higher Nitrogen shoot content, enhanced nutrient uptake, increased root and shoot weight | Ponnmurugan and Gopi (2006) |
| R. meliloti       | Bean | Symbiotic | Increase in root nodule weight | Yanni et al. (2001) |
| R. phaseoli       | Clover | Symbiotic | Increase in root nodule formation | Hayat et al. (2010) |
| R. trifolii       | Cowpea | Symbiotic | Increased pod size, decreased pest attack, increase in yield | Baldani et al. (2000) |
| A. lipoforum      | Pearl millets | Heterotrophic and associative | Increased grain yield more than 10% | Malik et al. (2002) |
| A. brasilense     | Sugarcane Maize | Heterotrophic and associative | Increase in plant height, root length and number of roots, stem diameter and length | Dudeja et al. (1981) |
| A. chroococcum    | Rice Maize | Aerobic free living and heterotrophic | Increased growth and germination of seedlings, enhanced root length and plant height, yield increase from 2-45% in vegetables, 9-24% in sugarcane, 31% in maize | Lynch (1990) |
| A. beijerinckii   | Sugarcane Bajra Vegetables | | | |
| A. insignis       | Paddy | Phototrophic | Temperature tolerance, herbicide resistance, increase yield | Mutch and Young (2004) |
| Azolla and Anabaena azollae (BGA) | | | | |
| Gluconacetobacter and Herbaspirillum | Sugarcane | Endophytic | Elicit defense response | Lery et al. (2008) |
nodule formation in plants. In the nodules, molecular nitrogen is fixed into ammonia that can be utilized by plants as nitrogen source. Legume-Rhizobium symbiosis is known as the most efficient system for biological nitrogen fixation (BNF) through nodulation in legume roots. Approximately 60 million tons of nitrogen is fixed by leguminous plants annually. Members of genus *Rhizobium* specifically forms root nodule in leguminous plants, however some rhizobia can also form stem nodules. *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* are other symbions that can form root nodules in leguminous plants, while *Frankia* by is associated with non-leguminous plants, shrubs and higher trees. Table 1 illustrates the results of various research published in literature depicting some crop specific nitrogen fixing bacteria. Application of *Rhizobium* in pea fields has shown beneficial effects on nodulation, yield and biochemical parameters of peas chickpea (Singh and Singh, 2018). Various *Rhizobium* species including *R. trifolii*, *R. meliloti*, *R. phaseoli*, *R. japonicum*, *R. leguminosarum* and *R. lupini* are known to accelerate yield and productivity in berseem, lucerne, green and black gram, soybean, pea and lentil, chickpea, respectively (Lucy et al. 2004). Bacteria belonging to genera including *Azoarcus*, *Glucanacetobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, cyanobacteria and diazotrophs, is capable of fixing nitrogen non-symbiotically in radish, wheat, coconut, rice, maize, tea, beetroot, tobacco, coffee, sugarcane and other non-legume plants (Meeks et al. 2002). *Azotobacter* treatment in soil has shown 10-15% increase in yield of mustard, sunflower, banana, sugarcane, grapes, papaya, watermelon, tomato, ladyfinger, coconut, spices, fruits, flowers, plantation crops and French beans (Saikia et al. 2017). *Beijerinckia*, is associated with sugarcane plantations whereas *Azoarcus*, *Azotobacter* and *Azolla* successfully supplements nitrogen in paddy, wheat, barley, oat, sunflowers, maize, beetroot, tobacco, tea, coffee and coconuts. Strains of *Glucanacetobacter*, *Azospirillum* and *Herbaspirillum* are helpful in supplementation of nitrogen to sugarcanes (Illmer and Schinner, 1992). *Herbaspirillum* has also been isolated from bean and rice.

**Phosphorus solubilizing bacteria:** Phosphorus is another important element for plant nutrition as it is vital for photosynthesis, signal transduction, biomolecule synthesis and metabolic processes. About 0.05% (w/w) phosphorous is present in soil as both inorganic and organic form but it is obsolete for plants as this is present in immobilized, precipitated and insoluble form (Sridave et al. 2007).

Different bacterial strains of *Pseudomonas*, *Bacillus*, *Burkholderia*, *Agrobacterium*, *Micrococcus*, *Achromobacter*, *Aerobacter*, *Flavobacterium*, *Rhizobium* and *Erwinia* have the ability to solubilize inorganic and organic phosphate compounds present in soil through the production of organic acids, protons, hydroxyl ions and extracellular enzymes. Table 2 shows potential phosphorus solubilizing bacteria associated with different crops. These compounds convert inorganic phosphate compounds (tricalcium phosphate, fluorapatite, dicalcium phosphate, rock phosphate and hydroxyapatite into monobasic (HPO$_3^{2-}$) and the dibasic (HPO$_4^{3-}$) ions which can be absorbed by the plants. Fig.1 summarizes the various sources of phosphorous present in soil, and the mechanism of phosphorous solubilization by bacteria. Another group of microorganisms called as root fungus, *Myccorrhiza*, establishes a symbiotic relationship with roots of higher plants and directly absorbs phosphorus through hyphae from soil making it available to the plants, which is otherwise inaccessible to them. *Myccorrhiza* benefits large number of crops, except those belonging to families of Caryophyllaceae, Brassicaceae, Commelinaeaceae, Polygonaceae, Cyperaceae, Juncaceae, Amaranthaceae, and Chenopodiaceae. Of the total bacterial and fungal species, 1-50% and 0.1-0.5% in soil exhibit P solubilization properties respectively. *R. trifolii* (Alloway, 2008), *R. leguminosarum*, and *Rhizobium* species nodulating Crotalaria (Sridave et al. 2007) are reported to solubilize inorganic and organic P and thus improving plant yield.

**Table 2: Phosphorus solubilizing bacteria associated with different crops.**

| Bacterial Species                  | Crop          | Effect on the crop                      | Reference                      |
|-----------------------------------|---------------|----------------------------------------|--------------------------------|
| *Bacillus firmus* Enterobacter spp. | Paddy         | 12% enhancement in yield, increase in number of tillers and yield | Patil et al. (2002)           |
| *Paenibacillus kribbensis*        | *P. melinus*  | 30% increase in yield                  | Patil et al. (2002)           |
| *Burkholderia* spp.* Pseudomonas sp. | *B. megaterium* | Increase in yield up to 70%            | Patil et al. (2002)           |
| *Glomus fasciculatum* *Bacillus megaterium* | Banana       | Increased root and shoot length        | Gulden and Vessey (2000)      |
| *Phosphobacterium*                | *S. SBS 1*    | Increased plant height and yield       | Afzalband Asghari (2008)      |
| *Penicillium* *bicalii*           | Pea           | Enhanced root length, pod number, 100-kernel mass, nodule number | Costa et al. (2015)           |
| *Pseudomonas cepacia* *R85*       | Wheat         | Increased yield upto 74%               | Dey et al. (2004)             |
| *Pseudomonas* *fluorescens* R22   | Peanut        | Stimulate plant growth, increase dry matter yield from 36 to 286% | Kumar et al. (2001)           |
| *Pseudomonas* *fluorescens*       | Wheat         |                                         |                                |
| *Azotobacter* *chroococcum*       | Maize         |                                         |                                |
Potassium solubilizing bacteria: Potassium (K) is the third essential nutrient necessary for plant growth. Potassium is found bound to the surfaces of clay minerals, organic matter or weathered micaceous minerals. Although present abundantly in soil and supplied exogenously as natural or synthetic fertilizers, only 1.0% - 2.0% of this is available to plants. Some bacterial and fungal species are capable of releasing oxalic acid, citric acid and specific enzymes (Sangeeth et al. 2012) which can dissolve insoluble potassium in the soil into a form that plant can access. *Bacillus edaphicus* stimulates potassium uptake in cotton, rape and wheat (Basak and Biswas, 2009), *Paenibacillus glucanolyticus* enhanced the dry weight of black pepper by increasing potassium uptake (Han and Lee, 2005), *Bacillus mucilaginosus* enhance potassium mobilization in Sudan grass (Goss et al. 2003). *Bacillus mucilaginosus* co-inoculation with the phosphate-solubilizing *Bacillus megaterium* promoted the growth of eggplant, pepper and cucumber (Rose et al. 2011).

Apart from nutrient mobilization, rhizobacteria can stimulate the plant growth by various direct and indirect mechanisms. Rhizobacteria secretes various phytohormones like auxins, cytokinins, gibberellins and ethylene that can directly stimulate plant growth by slow release into the soil (Rashid et al. 2012). Indirect mechanism of plant growth stimulation includes the production of antibiotics, hydrocyanic acid and hydrolytic enzymes. Various antibiotics secreted by these microorganisms includes amphisin, 2,4 diacetylphloroglucinol (DAPG), cyclic lipopeptides oomycin A, phenazine, kanosamine, pyoluteorin, pyrrolnitrin, tensin, tropolone, oligomycin A, zwitermicin A, xanthobaccin. These antibiotics stimulate plant growth by preventing the proliferation of plant pathogens. Volatile compounds such as HCN inhibit the growth of certain bacterial diseases like black root rot of tobacco. These microorganisms also secretes a number of hydrolytic enzymes such as chitinases, dehydrogenase, α-glucanase, lipases, phosphatases, proteases and hydroxylases (Neeraja et al. 2010) that degrades the cell wall of pathogenic fungi and parasites. Defensive capacity of plants against environmental stress is also believed to be enhanced by priming the seedlings with certain plant growth promoting bacteria. Thus, the microorganism can be used by plants as an effective tool against biotic and abiotic stress.

**Zinc solubilizing bacteria:** Zinc is important micronutrients which are important for carbohydrate metabolism and functioning of various enzymes and proteins. Zinc present in soil is unavailable to plants as it is found in bound form with minerals (Ahmad et al. 2012). Zinc deficiency is common in plants due to their inability to take up sufficient quantities of divalent zinc cations from soil. Zinc deficiency critically affect crop yield (Joy et al. 2017). The use of zinc solubilizing bacteria can act as a biological alternative for reducing zinc deficiency. These bacteria are capable of decreasing the pH of the nearby soil by releasing organic acids. The anions sequester the zinc cations and enhance their solubility (Wakatsuki, 1995). Also, production of siderophores, protons, oxidoreductive systems, chelating ligands (Hussain et al. 2015) are various other ways of zinc solubilization by these bacteria. Various bacterial species including *Bacillus* spp. (Deepak et al. 2013), *Bacillus aryabhattai* (Naz et al. 2016), *Rhizobium* strains (Narayanan et al. 2007), *Pseudomonas aeruginosa*, *P. striata*, *P. fluorescens* (Pawar et al. 2015), *Gluconacetobacter diazotrophicus* (Abaid-Ullah et al. 2015), *Burkholderia cenocepacia* (Khande et al. 2017), *Serratia liquefaciens*, *S. marcescens*, and *Bacillus*...
thuringiensis (Kamran et al. 2017) and Azospirillum have shown to increase crop yield by zinc augmentation. Pea plants have shown enhanced growth rate and nutrient uptake, when inoculated with zinc solubilizing bacteria. Likewise, zinc solubilizing Bacillus spp. augment the yield, growth and zinc uptake of soybean, wheat (Vaid et al. 2014) and rice (Prajapati and Modi, 2012).

**Types of bioformulations available:** In last two decades, biofertilizer market exhibited phenomenal growth in most of the Asian countries. Still the farmers are reluctant to use biofertilizers because of certain disadvantages associated with them. Biofertilizers have found to have low shelf life (3-4 months), temperature sensitive, bulky to transport, more chances of contamination, problem of proper packing, poor cell protection, poor moisture retention capacity and restriction on use of charcoal as a measure of conservation. Market demands biofertilizers that should be high in quality, reliable, efficient and promising. Viable cell count and the ability of organisms to fix nitrogen, solubilize phosphorus, potassium, zinc, etc. is important efficiency character being taken into consideration while formulating the biofertilizers. Fig 2 describe various types of bioformulations available in market and their general characteristics.

**Carrier based biofertilizers:** Biofertilizers are generally prepared as carrier based inoculants of effective microorganisms. Various carrier materials such as clay (Kloepper and Schratho, 1981), compost, coconut shell powder, peat (Temprano et al. 2002), talc (Dommergues et al. 1979), perlite (Bashan, 1986), zeolite, vermiculite, perlite, rice bran, wheat bran, polyacrylamide (Cassidy et al. 1996), charcoal, sawdust, organic manure are used for production of biofertilizers. These carriers provide support to the microbial culture and help in the survival capacity of microorganism. The choice of suitable carrier material ensures the longer viability and efficiency of the bioinoculant. Apart from it, carrier material is also important in deciding the type of application mode (liquid, powder, granulated or as a seed coating). Generally carrier material should be a biodegradable substance having high water-holding capacity and a good pH buffering capability (Daza et al. 2000). The formulation of carrier based formulations is difficult because a natural product exhibiting all these properties is difficult to find. All the commonly used carriers possess certain advantages and disadvantages of their own. For instance, clay and peat is most dependent carrier with high organic content and water holding capacity but they present a high chemical variability and therefore, have low cell survival rate on storage (Narendranath, 1995). Perlite can be used as the carrier for materials for the survival of Azospirillum and it could maintain a higher population of organisms than peat (Bashan et al. 2000). Soymeal (1%) and molasses (1%) are suitable carriers for Rhizobium inoculants (Vassilev et al. 2001).

**Liquid formulations:** Liquid biofertilizers are broth cultures containing dormant form of desired microorganisms along with required nutrients, minerals and organic oils, allowing them to tolerate adverse conditions. Firstly, Rhizobium based liquid bioformulations were made in Holland that resulted in 2.5 times increase in number of Rhizobia per seed. Liquid bioformulations are easy to produce and can be applied directly on seeds, increasing the adherence of bacteria to plant roots. Liquid bioformulations provides certain advantages like longer shelf life (12-24 months), tolerance to high temperature (up to 45°C), less contamination, high cell number of more than 10^9 cells/ml (with shelf life of 12 to 24 months), high export potential, less dosages requirements. But it is important to note that survival of microorganisms in liquid biofertilizers decreases with time because it does not provide protection to microorganisms against environmental conditions and thus bioformulation is prone to contamination during transportation or storage (Trevors et al. 1992). Also, the bacterial distribution per seed is quite heterogeneous. For maintaining viability an efficiency of liquid bioformulations, storage conditions need to be optimized (Sehrawat et al. 2017).
Encapsulated bioformulations: Biofertilizer technology is projected towards the development of novel carriers that can be environment friendly and can provide suitable environment for microbial survival. This involves the use of synthetic or natural polymer based carrier for encapsulation of bacteria into a biodegradable matrix (Cassidy et al. 1996). Cellulose, chitosan, sodium alginate, starch, lignin, agarose, cellulose sulfate, chondroitin sulfate, hyaluronan, agricultural residues, biochar, agar, agarose and carrageenan are used as natural polymers, while polystyrene, polyacrylamides, and polyurethane, poly (alkylene oxides), poly(vinyl acetate), polyvinylpyrrolidone (PVP), polyethylene glycol and polyethersulfone (Cassidy et al. 1996) are synthetic polymers.

Encapsulation involves protecting the microbe in a suitable matrix and to ensure a gradual and prolonged release. These encapsulated bacteria when applied to plants, slowly release the bacteria into the soil during their degradation process. This leads to increased establishment of microorganism in soil and persistence after application. Encapsulated system has the potential to extend shelf life of biofertilizers and reduce dosage requirements with regard to improved efficiency, easy handling and decreased contamination (Muyone et al. 2009). Encapsulation of microbes improves their metabolic activities by enhancing the production of several hydrolytic enzymes (El-Katatny et al. 2003). Encapsulation protects the microorganism from biotic and abiotic stress by providing a beneficial microenvironment. Matrix act as a “mini-fermenters” in which low biomass of bacteria can maintain their metabolic activity for quite a long time.

Encapsulation studies on enzymes have been extensively reviewed by various researchers (Sheldon, 2007). Various encapsulations techniques are currently available for the therapeutics (Dulieu et al. 1999). However, only some encapsulation methods are considered suitable for living cells. Most of them are based upon ionic gelation using alginate (McLoughlin, 1994).

CONCLUSION

The effects associated with chemical fertilizers have shown increased inclination of consumers towards organic crops grown by using biofertilizers. The biofertilizers provide an easy, effective and low input integrated nutrient management system that can be effectively used for nutrient mobilization, enhance crop production and maintain soil health. These are economically viable and at the same time environment friendly. However, it is important to note that their acceptance among farmers is low because of their efficiency and viability related issues. The biofertilizer technology is growing rapidly and will soon be a vital part of agricultural practices.

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