Chapter

# Applications and Constraints of Plant Beneficial Microorganisms in Agriculture

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## Abstract

At present time, chemical fertilizers are more in practice for crop production, which failed to upkeep soil and environment quality and affected the sustainability of the agricultural production system. Conversely, biofertilizers are ecosystem friendly, one of the best modern tools for agriculture, and are used to improve soil fertility and quality. Biofertilizers have now emerged as a highly potent alternative to inorganic fertilizers and offer an ecologically sound and economically attractive route for augmenting nutrient supply and increasing crop production. These include live cells of diverse genera of microorganisms and have the potential to fix atmospheric nitrogen and solubilize and mobilize plant nutrients from insoluble form through microbiological process. It has also the potential to diminish the gap between nutrient supply through fertilizers and nutrient removal by crops. Hence, biofertilizers can be a feasible option to the farmers to increase crop productivity and should find greater acceptance from the extension workers and commercial biofertilizer manufacturers.

**Keywords:** N fixers, P-K mobilizers, biofertilizer formulation, current advances

## 1. Introduction

Biofertilizers, more appropriately microbial inoculants, are the preparations containing one or more species of microorganisms which have the ability to capture or mobilize nutritionally important plant nutrients from non-usable to usable form through the biological processes such as N fixation, P solubilization, excretion of plant growth enhancers, or cellulose degradation in soil, compost, and other environments [1–3]. Biofertilizers are low-cost and environment-friendly supplement to chemical fertilizers and manures. Recently, biofertilizers are gaining momentum due to its ability to maintain soil health, minimize environmental degradation, and cut down the use of inorganic fertilizers in agriculture. These inputs gained added importance in rainfed agriculture in view of their low cost, as small to marginal farmers across the globe cannot afford expensive chemical fertilizers [4]. Biofertilizers could be an ideal input for cutting the cost of production and for practicing organic and conservation farming [5]. These organisms can be engaged in maintaining long-term soil fertility and sustainability [6, 7]. For the generations to come, biofertilizers are indispensable to ensure healthy soils and food.

The emphasis on chemical fertilizers, which sometimes led to unscientific and non-judicious application, has meant that the soil be regarded as an inert substrate for plant roots, instead of a living biosphere, the rhizosphere, containing a myriad of organisms [3]. The blanket use of inorganic fertilizers has also led to pollution of the soils and surface water bodies in many regions of the world [5]. Nevertheless, the importance of fertilizers, essential for achieving increased crop production, will further increase because there is little scope for bringing more areas under cultivation and majority of soils are deficient in many macro- and micronutrients. It is now realized that in agricultural lands under intensive monoculture system, including rice, which receives heavy application of chemical fertilizers alone, productivity slowly is declining, and environmental quality is deteriorating [8]. Intensification of agriculture has also widened the gap between nutrient removal and supplies and, thus, soil fertility depletion [9]. The role of biofertilizers in agriculture, therefore, assumes special significance, particularly in the present context of increased cost of inorganic fertilizers and their hazardous effects on soil health. The success with biofertilizers is reported for more than 100 years in many parts of the world, and statistically significant increase in yields has been observed [2]. However, their response varies with crops, host cultivars, locations, seasons, agronomic practices, bacterial strains, soil fertility, and interaction with native soil microflora.

## 2. Types of biofertilizers

Biofertilizers may be broadly classified into nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, and organic matter decomposers (**Figure 1**). Nevertheless, it also includes organic fertilizers (manure, etc.), which are rendered in an available form due to the interaction of microorganisms or due to their association with plants.

### 2.1 Nitrogen-fixing biofertilizer (NFB)

Nitrogen-fixing organisms are used in biofertilizer as a living fertilizer composed of microbial inoculants or groups of microorganisms which are able to fix

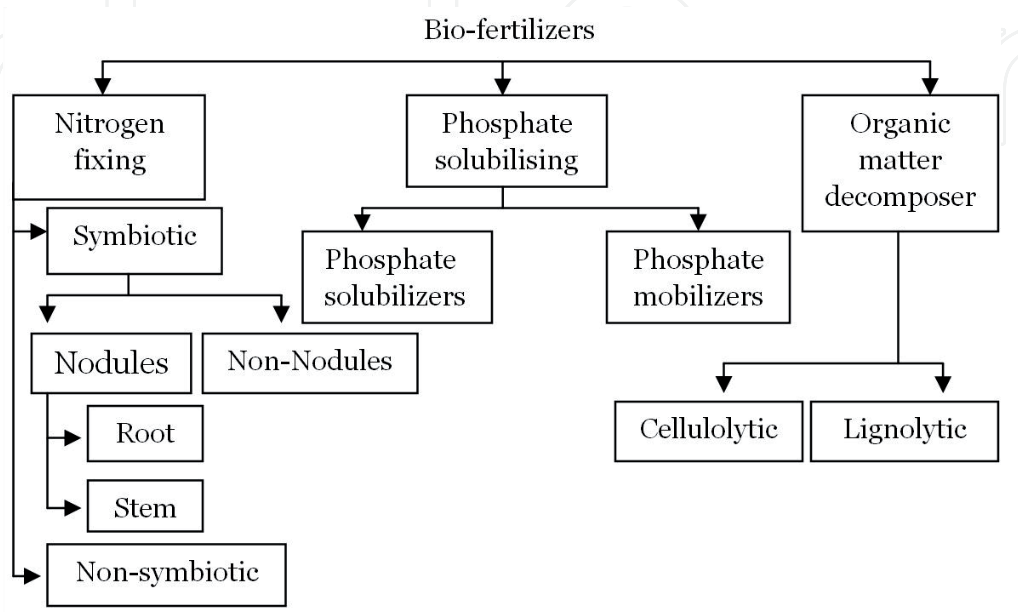


**Figure 1.**
*A broad classification of biofertilizers.*

atmospheric nitrogen, which is transformed into organic nitrogenous compound. The nitrogen-fixing bacteria work under two conditions, symbiotically (*Rhizobium*, *Frankia*) and as free-living bacteria (nonsymbiotic) such as *Azotobacter* and *Azospirillum*. The $N_2$-fixing bacteria associated with nonlegumes include species of *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*, *Desulfovibrio*, *Coryne bacterium*, *Campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirillum*, *Rhodopseudomonas*, *Xanthobacter*, *Mycobacterium*, and *Methylosinus* [10].

#### *2.1.1 Symbiotic*

The most exploited symbiotic $N_2$-fixing bacteria are those belonging to the family *Rhizobiaceae*. *Rhizobium* inoculants are of greatest importance because of their ability to fix atmospheric $N_2$ in association with certain legumes [11]. It is estimated that $N_2$ fixation by *Rhizobium* in root nodules of legumes is of the order of 14 million tons on a global scale and is almost 15% of the industrial N fixation. Yield of many legumes can be increased substantially by the use of appropriate *Rhizobium* cultures. For successful nodulation each legume requires a specific species of *Rhizobium* to form effective nodules. Many legumes may be modulated by diverse strains of rhizobia, but growth is enhanced only when nodules are produced by effective strains of rhizobia [12]. *Rhizobium* can be used for legumes crop and trees (e.g., lucerne) and is a crop-specific inoculant, for example, *Rhizobium trifolii* for berseem, *Rhizobium meliloti* for lucerne, *Rhizobium phaseoli* for green gram and black gram, *Rhizobium japonicum* for soya bean, *Rhizobium leguminosarum* for pea and lentil, *Rhizobium lupini* for chickpea, and *Rhizobium* spp. for cowpea. *Rhizobium* is however limited by cross-inoculation group, and only certain legumes are benefited by this symbiosis.

Similar to the *Rhizobium*, other filamentous bacteria of genus *Frankia* belonging to the family *Frankiaceae* are found in the root nodules of nonlegumes such as trees and shrubs. These bacteria live in symbiosis with actinorhizal plants. These actinorhizal plants are used for timber and fuel wood production, for wind breaks, and for shelterbelts in coastlines and desert, as well as for land reclamation [13]. In arid areas where actinorhizal plants are not present, inoculation of *Frankia* (*Frankia alni*) can be advantageous [13]. Despite their potential importance, very limited information is available for inoculation practice and their use for *Frankia* symbiosis. However, their potential could be harnessed in agroforestry system.

#### *2.1.2 Nonsymbiotic*

In nonsymbiotic or free-living nitrogen, fixation does not require host plant, and bacteria do not form nodules. An example of such free-living bacteria is *Azotobacter*. They fix atmospheric $N_2$ nonsymbiotically, and the extent of fixation is directly depends upon the amount of carbohydrates utilized by them [14, 15]. Azotobacter comprises seven species: *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. paspali*, *A. armeniacus*, *A. nigricans*, and *A. salinestri* [16]. Soils containing poor organic matter and antagonistic relationship with other soil microorganism adversely affect the population of *Azotobacter*. Besides nitrogen fixation, it can also synthesize growth-promoting substances, viz., auxins, gibberellins, and to some extent the vitamins. It also helps to improve seed germination and crop growth due to positive response of B vitamins, naphthalene acetic acid (NAA), gibberellic acid (GA), and chemical produced during the biochemical process showing antagonistic relationship with root pathogen [17].

### 2.1.3 Associative

Apart from symbiotic and nonsymbiotic nitrogen fixers, some bacteria form a close associative symbiosis with the higher plants. These bacteria live on the root surface and sometimes also penetrate into the root tissues but do not produce any visible nodule or outgrowth on the root tissue. *Acetobacter diazotrophicus* and *Herbaspirillum* spp. associated with sorghum, maize, and sugarcane [18–20] and *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, and *Rhizobium* associated with rice and maize [21] are examples of associative nitrogen-fixing microorganism.

*Azospirillum* produces growth-regulating substances, which help to protect from soilborne diseases. It improves leaf area index and ultimately crop yield. Apart from many species across the globe, the major species under this genus are *A. lipoferum* and *A. brasilense*. *Azospirillum* species mainly identified as rhizosphere bacteria and its colonization of the rhizosphere have been studied extensively [22–24]. *Azospirillum* with the plant having $C_4$-dicarboxylic pathway (Hatch and Slack pathway) of photosynthesis formed associative symbiosis because they fix nitrogen in salts of organic acids such as malic and aspartic acid [25]. So, it is mainly beneficial for $C_4$ plants like maize, sorghum, sugarcane, etc. Despite all these benefits that bear great promise as a growth-promoting $N_2$-fixing biofertilizer, the main problem that limits the use of *Azospirillum* is great uncertainty and unpredictability of the results [26].

### 2.1.4 Cyanobacteria

Blue green algae (BGA) are known as cyanobacteria. Cyano means blue, so that means it is blue bacteria. These belong to eight different families, phototrophic in nature, and produce auxins, indole acetic acid (IAA), and GA. N-fixing blue green algae have been shown to be the most important in maintaining and improving the productivity of rice fields [27]. Favorable condition for biological nitrogen fixation by BGA is considered to be one of the reasons for relatively stable yield of rice under flooded condition. BGA forms symbiotic association capable of fixing nitrogen with fungi, fern, and flowering plants, but the most common symbiotic association has been found between a free floating aquatic fern, the *Azolla* and the *Anabaena azollae* (BGA) [28]. This association produces 40–60 tons of organic matter per hectare per year. Despite the importance of $N_2$-fixing cyanobacteria in rice cultivation, the production and application are poorly developed. Biofertilizers should be seriously considered for supporting sustainable agriculture practice [29].

### 2.1.5 Azolla

*Azolla* is known as free floating water fern that fixes atmospheric $N_2$ in symbiotic association with BGA (*Anabaena azollae*) in rice field. They are free-living organism and use energy derived from photosynthesis to fix nitrogen. It is a fast-growing water fern and can double its weight within a week [30]. The most common species occurring in India is *A. pinnata*. *Azolla* is rich organic manure and mineralizes soil nitrogen rapidly which can be available to the crop in a very short period. *Azolla* can help rice or other crops through dual cropping or green manuring of soil [31].

## 2.2 Phosphate-solubilizing biofertilizer (PSB)

Several experiments have showed the ability of different bacterial species to solubilize insoluble inorganic phosphate minerals, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. Phosphate-solubilizing bacteria are common in the rhizosphere, and secretion of organic acids like citric, oxalic, tartaric, acetic, lactic, gluconic, glyoxylic, maleic, and fumaric helps to convert insoluble form of phosphorus to plant available form [32]. Some of the bacterial genera are *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Enterobacter*, and *Erwinia*. Among the soil bacterial communities, ectorhizospheric *Pseudomonas* and *Bacillus* and endosymbiotic rhizobia are found most effective phosphate solubilizers [33]. A higher amount of organic substances is present in the rhizosphere attracting the phosphate-solubilizing bacteria, and population is more in rhizospheric soil compared to the non-rhizospheric soil [34, 35]. Application of rock phosphate with PSB (*Bacillus megaterium* var. *phosphaticum*) showed that without phosphorus application PSB amendment could increase sugarcane yield up to 12.6% and it also improved sugar yield and juice quality [36]. Results of a greenhouse pot experiments with onion (*Allium cepa* L.) showed that application of *G. fasciculatum* along with *A. chroococcum* and 50% recommended P rate resulted in greater root length, plant height, bulb fresh weight, root colonization, and P uptake. Also the rate of chemical phosphatic fertilizer can be brought down [37]. Phosphate-solubilizing bacteria may be of greatest value in allowing the use of cheaper P sources.

### 2.3 Phosphate-mobilizing biofertilizer (PMB)

The symbiotic association between plant roots and fungi is termed as "mycorrhizal association." Arbuscular mycorrhizal fungi (AMF) form symbiotic relationship with about 90% of land plant species [38]. These are of two types, ectomycorrhiza found in trees and found beneficial for forest trees, and endomycorrhiza for crop plants [39]. The functional symbiosis in mycorrhizal fungus is obligatory and depends on host photosynthates and energy. The plant acquires carbon for various mycorrhizal benefits to the host plant. The fungi capture nutrients from soil solution with the help of mycelium that extends from the root surfaces into the soil matrix. So, it results more efficient nutrient uptake and improved plant growth when mycorrhizal fungi colonized the root systems [40].

In higher plants, phosphorus and other nutrients are often mediated with mycorrhizal association, in which symbiotic association is performed by higher plants and associative fungi (*Glomus*) [41]. Hyphae of AMF do not solubilize the insoluble unavailable phosphorus but assimilate them from soil for their own requirement. Mycorrhizal roots can take up several times more phosphorus per unit root length than non-mycorrhizal roots. Mycorrhizal symbiosis also increased the tolerance of heavy metal contamination or drought, as well as lesser susceptibility of root pathogens. AMF also helps to improve soil quality by having a direct influence on soil aggregation [42]. This association is generally found very effective in agroforestry. The other crops benefited from AMF are sorghum, barley, wheat, tobacco, cotton, soybean, apple, citrus, grape, etc.

### 2.4 Organic matter decomposer

Composting is a key technology to use different types of organic wastes (crop residues, rural and urban wastes), and it takes about 4–6 months for its maturity for use as a source of plan nutrients. To decompose these organic waste, some cellulolytic and lignolytic microorganisms are introduced which help to decompose that organic wastes at a faster rate and make it ready for use within 2–3 months. Many soilborne fungal species like *Aspergillus niger*, *Penicillium*, *Trichoderma viride*, *Trichurus spiralis*, *Phanerochaete chrysosporium*, etc. act as an activator in the decomposition process of plant bodies containing cellulose or lignin [43].

### 2.5 Potassium-solubilizing biofertilizer (KSB)

Some soil microorganisms are capable of solubilising potassium from K-bearing minerals such as muscovite, mica, orthoclase and illite. These minerals are the potential source of available K in soil. Microorganism produces organic substances which react with these K bearing minerals to solubilize K and enhances available K in the soil solution [44]. These organisms also produce various types of amino acids, growth-promoting compounds (IAA, GA, etc.), and vitamins, promoting the crop growth and yield [45]. *Frateuria aurantia*, a K-solubilizing bacteria, is capable of mobilizing mixture of potassium from mica into a usable form for the plants, which has fairly been applied to crops in association with other biofertilizers without any antagonistic effects [46, 47]. Application of high-K-bearing clay mineral with K-solubilizing bacteria can help to mitigate the K requirement in agricultural soils [48].

### 2.6 Sulfur-solubilizing biofertilizer (SSB)

Sulfur is one of the major elements in oil seed crops and some vegetables (onion, oat, cauliflower, etc.) and some species (ginger, garlic, etc.). It is essential for biochemical synthesis of some important glycosides, pungent compound, and disease resistance properties. Khandkar et al. [49] observed that the nodule in black gram was increased due to sulfur application. Deficiency of sulfur in agricultural soils could be corrected by application *Azotobacter pasturianam* as biofertilizer [50].

### 2.7 Zinc-solubilizing biofertilizer (ZSB)

Zinc is one of the micronutrients whose deficiency affects the crop growth and crop yield [5, 8]. Zinc fertilizers are very costly and its availability is also limited. So, zinc solubilizers can play a vital role for providing adequate supply of zinc to the crop and enhancing the crop growth and yield. The microorganisms which are well known for solubilization of zinc are *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* sp. [51]. These strains are used as zinc biofertilizers and get positive response to the crop. Sometime application of zinc fertilizers combination with zinc biofertilizers (*Bacillus* sp.) gave better response and increased zinc concentration in the soil [46].

### 2.8 Plant growth-promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR), when grown in association with host plant, result in stimulation of growth of their host. It represents a wide variety of soil bacteria. These bacteria vary in their mechanism of plant growth promotion but generally influence growth via P solubilization, nutrient uptake enhancement, and plant growth hormone production [33, 52, 53]. Bertrand et al. [54] showed that a rhizobacterium belonging to the genus *Achromobacter* could enhance root hair number and length in rapeseed. The PGPR inoculants promote growth by any of the following mechanism: (i) suppression of plant disease (bioprotectants), (ii) improved nutrient acquisition (biofertilizers), and (iii) phytohormone production (biostimulants).

## 3. Potential of biofertilizers

The competent strains of nitrogen-fixing, phosphate-solubilizing, or cellulolytic microorganisms are used for application in seed, soil, and roots of saplings or composting areas with the intention to amplify the number of such microorganisms and speed up those microbial processes which supplement the availability of nutrients that can be easily assimilated by plants (**Table 1**).

### 3.1 *Rhizobium*

They can fix nitrogen 50–100 kg/ha with legumes only. The symbiotic relationship between leguminous crops and *Rhizobium* is very important for crop production system. It has been proven to be useful for pulse legumes like chickpea, red gram, pea, lentil, black gram, oil seed legumes like soybean and groundnut, and forage legumes like berseem and lucerne [77]. The suitable strain is capable to increase the crop yield up to 10–35% since N is fixed at 40–200 kg/ha which is able to meet up to 80–90% of N need of the crop [46].

### 3.2 *Azotobacter*

The presence of this organism has been reported from the rhizosphere of various crop plants such as rice (*Oryza sativa* L.), maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), bajra (*Pennisetum glaucum* L.), vegetables, and plantation crops [78]. It can fix N up to 25 kg/ha under optimal conditions and increase yield up to 40–50% [5]. It has been observed that *Azotobacter* improved the seed germination and crop growth owing to the affirmative response of B vitamins, NAA, GA, and other chemicals produced during the biochemical process that exhibited antagonistic relationship with root pathogens [17].

### 3.3 *Azospirillum*

Apart from their nitrogen-fixing ability of about 20–40 kg/ha, they are also known to produce various growth-regulating substances. The *Azospirillum* form associative symbiosis with plants having the $C_4$-dicarboxylic pathway of photosynthesis (Hatch and Slack pathway), as they grow and fix nitrogen on salts of organic acids such as malic and aspartic acid [25]. Thus, *Azospirillum* is mostly recommended for $C_4$ plants like maize, sugarcane, sorghum, pearl millet, etc. [5].

### 3.4 *Azolla*

*Azolla* can fix 100–150 kg N/ha/year in rice fields along with *Anabaena* [79]. It can also be incorporated as green manure by adding in the fields prior to rice planting. The most widespread species in India is *A. pinnata* and can be reproduced on commercial scale by vegetative means. India has recently introduced some species of *Azolla* (*A. caroliniana*, *A. microphylla*, *A. filiculoides*, and *A. mexicana*) for their large biomass production [80].

### 3.5 Blue green algae (BGA)

In India, rice is one of the main staple food crops grown by farmers by using of BGA and *Azolla* as a plant nutrient provider. Generally, BGA has been reported to be able to supply 50–100 kg/ha nitrogen through biological N fixation, and in addition, it is also known to supply plant growth-promoting substances to crop under puddled condition [81].

Keeping in view the importance of biofertilizer for sustainability in agriculture sector, the government of India has also ensured the quality and production of biofertilizers under Section 3 of essential commodities, Act 1955. The government

| Biofertilizer | Recommended crop | Effect | Reference |
|---|---|---|---|
| Nitrogen-fixing biofertilizers | | | |
| *Rhizobium* | Bean | Increased straw and grain yield, harvest index, and agronomic fertilizer use efficiency | Yanni et al. [55] |
| | | Increased nodule dry weight and seed yield | Koskey et al. [56] |
| | Cowpea, common bean, peas, fenugreek | Increased vegetative growth parameters, shoot minerals, and yield | Arafa et al. [57] |
| | Pea | Increased mean seed yield | Abera and Abeba [58] |
| | Faba bean | Improved enzymatic activity in inoculated soil | Beshir et al. [59] |
| *Bradyrhizobium* | Pigeon pea | Induced improvement in nodule dry weight, plant biomass, and shoot N uptake | Youseif et al. [60] |
| *Azotobacter* | Mulberry | Increased trends in silk filament length, cocoon weight, shell weight, and shell ratio | Moorthi et al. [61] |
| | Pearl millet | Improved plant height, dry matter accumulation, no. of effective tillers, grain per ear, and grain and stover yield | Yadav et al. [62] |
| | Cauliflower | Increased morphological character and yield | Subedi et al. [63] |
| | Wheat | Enhanced grain yield | Mahato and Kafle [64] |
| *Azospirillum brasilense* | Maize | Increased plant growth and improved biochemical traits | Zeffa et al. [65] |
| | Wheat | Enhanced plant growth and increased root depth, fresh weight of roots and shoots, and nutrient use efficiency | Sayed et al. [66] |
| *Azospirillum lipoferum* | Foxtail millet | Improved seed weight, panicle, dry weight of shoot and root, total N content of shoot, and root and grain yield | Rao and Charyulu [67] |
| *Cyanobacteria* | Rice | Improved yield | Bhoosan et al. [68] |
| *Azolla* | Rice | Increased grain and straw yield | Mishra et al. [69] |
| | Rice | Reduction in weed emergence | Biswas et al. [70] |
| Phosphate-solubilizing biofertilizers | | | |
| *Pseudomonas spp.* | Chickpea | High nodulation and stimulation of plant growth | Malik and Sandhu [71] |
| *Bacillus* spp. | Amaranth | Improved nutrient use efficiency | Pandey et al. [72] |
| *Aspergillus niger* | Wheat | Improved growth and P uptake | Xiao et al. [73] |

| Biofertilizer | Recommended crop | Effect | Reference |
|---|---|---|---|
| *Bacillus thuringiensis* | Rice | Increased shoot length | David et al. [74] |
| Phosphate-mobilizing biofertilizers | | | |
| VAM | Jatropha | Reduced salt stress | Kumar et al. [75] |
| | Maize | Enhanced concentration of P in plant | Sudova and Vosatka [76] |

**Table 1.**
*Effect of biofertilizers on crop improvement.*

has issued a fertilizer (control) amendment order (FCO), 2006, with the gazette notification, S.O. 391 (E), dated on March 24, 2006, for biofertilizer production. After coming into enforcement of this order, four biofertilizers came under the FCO, i.e., *Rhizobium*, *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria [82]. Though the effect of biofertilizers on the crop production is slow, they possess vast potential for meeting plant nutrient requirements and sustaining soil quality while curtailing the use of chemical fertilizers. The development of biofertilizers has paced up in the last 20 years, and phosphate-solubilizing bacteria (PSB) have been reported to be used most widely among the farming community [83, 84].

## 4. Role of biofertilizers in alleviating abiotic stress in plants

### 4.1 Salinity

The condition of soil salinity generally inhibits the crop growth. High concentration of salts imparts pessimistic effects on plant metabolism and growth owing to the osmotic stress and accumulation of $Na^+$ and $Cl^-$ ions [85]. Salt stress is responsible for obliteration of the microbial communities and carbon cycling in the soil [86]. Several researchers have recommended various chemical, physical, and biological methods for improving crop growth and performance under salt-affected soils [87–89]. Apart from this, various other advancements, counting traditional breeding and genetic engineering, have also been tried to improve the salinity tolerance in plants. However, such intercessions have little success rate, owing to the intricacy of salinity tolerance and slight genetic variability among germplasm accessions [90]. Among these methods, the biological means of improving crop growth has identified some promising outcomes so far.

Several researches of recent past have suggested the efficiency of cyanobacteria for remediation of salt-affected soil in laboratory studies and field trials [91–95]. There have been a variety of suggested mechanisms involved in reclaiming the salt-affected soils and promotion of plant growth by cyanobacteria. Li et al. [96] suggested the nitrogen fixation, extracellular polymeric substance production, the accumulation of compatible solutes, plant growth hormone production, active export of ions through $K^+/Na^+$ channels and $Na^+/H^+$ antiporters, and defense enzyme productions as possible mechanisms for salt-affected soil remediation using cyanobacteria. Khalilzadeh et al. [97] suggested that enhanced grain filling speed, photosynthesis, plant water accumulation, and flag leaf salt accumulation were some plausible mechanisms for cycocel and PGR-induced salt tolerance shown in wheat plants under pot experiment. After investigating the salt stress and inoculation effect on nodulation and growth of forage cowpea (*Vigna unguiculata* cv. Baladi), Omara and Tamer [98] reported the alleviation of detrimental effects of salt stress by applying dual inoculation with tolerant *Bradyrhizobium* SARSRh3 + *Bradyrhizobium* SARS-Rh5 due to improvement in nodulation, growth dynamics, increase in K uptake, and reduced Na uptake in forage cowpea plants.

The use of bacterial inoculation, specifically, plant growth-promoting rhizobacteria (PGPR), has proved to be effective in improving plant stress tolerance. Several reports claimed that PGPR successfully improved growth of a wide range of agricultural crops under environmental stress conditions [99–104]. The PGPR are also known to use several mechanisms to sustain the plant growth under salt stress. Rhizobacteria trigger the plant antioxidant defense mechanism by modifying the key enzymes activity, viz., superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) that forage the overproducing reactive oxygen species (ROS) and ultimately defend the plants from salt toxicity [100, 105]. PGPR-inoculated plants have also been reported to have changes in their root architecture owing to the increased indole-3-acetic acid (IAA) level that facilitates the plants to take up more nutrients under salinity stress condition in soil [106, 107]. In a field trial, Kamaraj and Padmavathi [108] reported that the seeds treated with triple inoculation of biofertilizer such as *Rhizobium*, phosphate-solubilizing bacteria, and VAM at 600 gm/ha gave higher crop growth and seed yield parameters under saline stress condition.

The use of microorganisms as biofertilizers has also been reported to alleviate the effect of salinity on vegetables. The inoculation of seeds of various vegetables, such as tomato, pepper, bean, and lettuce, with PGPR has resulted in augmented root and shoot growth, dry weight, fruit, and seed yield and improved the resistance of plants to salt stress [109]. Mahmood et al. [110] revealed that PGPR and Si synergistically improved the salinity tolerance in mung bean. The use of arbuscular mycorrhiza (AM) has also been recorded to improve salt stress in tomato, onion, and lettuce [111–113].

### 4.2 Drought

Drought stress influences a range of growth parameters and stress-responsive genes in plants under the situation of stress. Inadequate quantity of water generally reduces the cell size and membrane integrity; create reactive oxygen species; and lowers down the crop productivity by promoting leaf senescence [114]. The plant-associated microbes possess a variety of mechanisms to deal with harmful impact of drought on plants and soil. Apart from the water content, these microbes also supply nutrients and provide favorable environmental conditions for the sustainable growth of plants. These microbes are known to encourage plant growth and development by various potential mechanisms which include:

a. Synthesis of various phytohormones such as IAA, cytokinins, and abscisic acid

b. Production of bacterial exopolysaccharides

c. Production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase

d. Promoting systemic tolerance

The PGPR have the ability to produce plant hormones like IAA that encourage plant growth under stress condition. IAA is the most vigorous auxin that regulates the vascular tissue differentiation, adventitious and lateral root differentiation, cell division, and shoot development under drought stress [115]. The exopolysaccharides synthesized by microbes also enable certain plants to tolerate drought. Three drought-tolerant bacterial strains, viz., *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3), inoculated in maize crop resulted in increased relative water content, protein, and sugar [116]. Sandhya et al. [117] have also reported the improved plant resistance against drought stress by the use of exopolysaccharide-producing bacteria. Under the stress environment, ACC is an immediate precursor of ethylene. The ACC deaminase produced by bacteria hydrolyzes ACC into ammonia and alpha-ketobutyrate [118]. Vardharajula et al. [119] have reported the decrease in antioxidant activity and enhanced production of proline, free amino acid, and sugar in plants with microbial inoculants under drought stress. The mycorrhizal inoculation in consortium with specific bacteria has also been recorded to improve plant growth, nutrient uptake, and relative water content to decrease the effect of drought. Ortiz et al. [120] revealed that the association of *Pseudomonas putida* and *Bacillus thuringiensis* reduced the stomal conductance and electrolyte leakage owing to the accumulation of proline in shoot and root.

Tomato (*Lycopersicon esculentum* Mill) cv. Anakha treated with phosphate-solubilizing bacteria (*Bacillus polymyxa*) was reported to secrete excess proline to resist the drought condition [121]. Giri et al. [122] studied the physiological response of peas (*Pisum sativum* L.) when inoculated with ACC deaminase bacteria *Variovorax paradoxus* 5C-2 under moisture stress and watering conditions. It was reported that the bacterial effects were more apparent and consistent in moisture stress condition. The AM fungal inoculation reduced the concentration of malondialdehyde and soluble protein in plant leaf and enhanced the activities of SOD, POD, and CAT, which ultimately led to the improved osmotic adjustment and drought tolerance of mycorrhizae citrus-grafting seedlings [123]. Inoculation of *Glomus versiforme* in citrus plants has also been reported to improve the osmotic status of the plant in drought condition owing to the enhanced levels of nonstructural carbohydrates, $K^+$, $Ca^{+2}$, and $Mg^{+2}$, which helped the plants to resist the drought condition [124]. Ruiz-Sanchez et al. [125] revealed the increase in photosynthetic efficiency and the antioxidative response of rice plant in drought stress after inoculation of arbuscular mycorrhiza.

Phosphate-solubilizing microorganisms have positively increased the plant growth and phosphorus absorption in maize resulting in increasing the efficiency of plant tolerance to drought stress conditions [126]. Inoculation of *Pseudomonas* spp. to basal plants under water stress improved their antioxidant and photosynthetic pigment content. *Pseudomonas* spp. were also found to have affirmative influence on the seedling growth and seed germination under water stress [127]. Chavoshi et al. [128] reported that phosphorus- and potassium-solubilizing bacterial consortium was able to increase biomass and important physiological traits in red bean under limited irrigation conditions. Li et al. [129] investigated the response of synergistic application of superabsorbent polymer (SAP) and biofertilizers (*Paenibacillus beijingensis* BJ-18 and *Bacillus* sp. L-56) on plant growth, including wheat and cucumber in drought stress. Both the biofertilizers amended with SAP were recorded to promote germination rate of seeds, plant growth, and soil fertility (urease, sucrose, and dehydrogenase activities). Moreover, the quantitative real-time PCR analysis revealed that biofertilizer + SAP significantly regulated the expression levels of genes involved in ethylene biosynthesis, stress response, salicylic acid, and transcription activation in plants in the drought stress condition.

## 5. Application and doses of biofertilizers

Biofertilizers are usually applied along with carrier material in order to enhance their efficacy. Khosro and Yousef [130] elucidated that the use of these microorganisms along with carrier material makes it possible for the users to handle them easily, facilitate their long-term storage, and augment their effectiveness. The biofertilizers are usually used as seed treatment in which the inoculant is mixed with water to make form of slurry and then mixed with seeds (**Table 2**). In this case, the carrier material is generally used as fine powder to get the tight coating of inoculant on the seed surface. For this purpose the use of adhesive, such as gum arabic, methyl ethyl cellulose, sucrose solutions, and vegetable oils, is recommended.

### 5.1 Seed treatment

The seed treatment of biofertilizer is done by suspending 200 g of biofertilizer in 300–400 mL of water and mixed tenderly with 10 kg of seeds using an adhesive-like acacia gum, jiggery solution, etc. Thereafter, the seeds are spread on a clean sheet/cloth under the shade to dry. The shade dried seeds should be sown within 24 hours.

| Name of organism | Mode of action | Host crops for which used | Method of application | Rate of inoculant | Remarks |
|---|---|---|---|---|---|
| *Rhizobium* | Symbiotic $N_2$ fixation | Legumes like pulses, soybean, groundnut | Seed treatment | 200 g per 10 kg seed | Leaves residual N in soil for the next crop |
| *Azotobacter* | Nonsymbiotic $N_2$ fixation | Cereals, millets, cotton, vegetable | Seed treatment | 200 g per 10 kg seed | Also controls certain diseases |
| *Azospirillum* | Associative $N_2$ fixation | Nonlegumes like maize, barley, oat, sorghum, millet, sugarcane, rice, etc. | Seed treatment | 200 g per 10 kg seed | Produces growth-promoting substances, can be applied to legumes as co-inoculant |
| Phosphate solubilizers | Phosphorus solubilization | Soil application for all crops | Seed treatment | 200 g per 10 kg seed | Can be mixed with rock phosphate |
| Blue green algae (BGA) | Nonsymbiotic $N_2$ fixation | Rice | Soil application | 10 kg/ha | Reduces soil alkalinity, has growth-promoting effects |
| *Azolla* | Symbiotic $N_2$ fixation | Rice | Soil application | 1 ton dried material/ha | — |
| Mycorrhiza (VAM) | Symbiotic association | Many tree species, wheat, sorghum, ornamentals | Soil application | — | Usually seedlings are inoculated |

**Table 2.**
*Application and doses of biofertilizers for various crops [43].*

### 5.2 Seedling root dip

This method is generally applied for transplanted crops. For rice crop, a bed filled with water is prepared in the field, and recommended biofertilizers are mixed in this water. The roots of seedlings are dipped for 5–10 min and then transplanted.

### 5.3 Soil treatment

Four kilograms of the recommended biofertilizer is mixed in 200 kg of compost and kept overnight. This mixture is then incorporated in the soil at the time of sowing or planting.

### 5.4 Liquid biofertilizers

Bhattacharyya and Kumar [131] stated that biofertilizers manufactured in India are mostly carrier based and the microorganisms have the shelf life of only 6 months. The advantage of liquid biofertilizer over powder based is that microorganisms have longer shelf life up to 2 years and they are tolerant to UV radiations and high temperature (55°C). The count is as high as 109 c.f.u/ml, which is maintained constant up to 2 years. Since they are liquid formulation, the application in the field is very easy and simple. They are applied using hand sprayer, power sprayer, and fertigation tanks and as basal manure mixed along with farm yard manure (FYM) [132, 133].

For all leguminous crops, *Rhizobium* is generally applied as seed inoculant. *Azospirillum*/*Azotobacter* is inoculated through seed, seedling root dip, and soil application methods in transplanted crops. For direct sown crops, *Azospirillum* is usually incorporated through seed treatment or soil application.

## 6. Constraints in biofertilizer use

Despite little investment, eco-friendly character, and advantages of biofertilizers, adoption of this organic input by farmers has remained far from satisfactory. There are several constraints at production, marketing, and field level which limit the adoption of biofertilizers among the wide community of farmers.

### 6.1 Production constraints

- **Raw material:** Biofertilizers are generally prepared as carrier-based inoculants with effective microorganisms. Granular form of carrier material like peat, perlite, charcoal, etc. is commonly recommended for soil inoculation of the biofertilizer [46]. These carrier materials for seed and soil treatment are not easily available and accessible to the small and marginal farmers. In India, these carriers are neither available in adequate quantities nor in desirable quality, which is one of the reasons for the lack of popularity of biofertilizers among the Indian farmers [134].

- **Specificity of strains for different agroclimatic regions:** The majority of the strains of biofertilizers is not only crop specific but is also soil and agroclimate specific. The lack of region-specific strains is one of the major constraints associated with biofertilizer use. This confines their extensive and optimum use with expected performance [46, 135].

- **Biological constraints:** There is likelihood of presence of ineffective or antagonistic strains in the bio-inoculants, and removal of these strains from the bio-inoculant is generally a complicated task. The selected strains should also have the ability to compete with other strains, N-fixing or nutrient-solubilizing/nutrient-mobilizing ability over a range of environmental conditions, and ability to survive in broth and in inoculants carrier [134, 136]. This largely affects the efficiency of desired microorganism as biofertilizer.

- **Technical constraints:** Biofertilizers possess the tendency to mutate during fermentation which increases the cost of production and quality control. A broad range of research is needed to reduce such undesired changes [5].

- **Economic constraints:** For the production of quality product, the use of high-tech instruments and equipment is required. In the absence of these facilities, production of contamination free product is uncertain. Moreover, the lack of trained human resources in the production units and lack of suitable training on the production techniques also serve as a limitation of the widespread use of biofertilizers [137].

### 6.2 Marketing constraints

- **Limited transportation and storage facilities:** The serviceable life of biofertilizers prepared with common carriers like peat or lignite is usually less than 6 months. It has been recommended that best results of biofertilizers are possible only if the material is used within 3–4 months of production. But often the biofertilizers are subjected to very high temperature during transportation and storage which reduces their efficiency and leads to lack of interest among the dealers due to nominal profit margin [138, 139].

- **Low demand:** Owing to the lack of adequate promotion and awareness about the advantages of biofertilizers, farmers refrain themselves from adopting this sustainable practice due to different methods of inoculation and no visual variation in the crop growth immediately as in the case of inorganic fertilizers [46].

### 6.3 Field-level constraints

- Soil conditions like acidity, presence of salts and toxic elements, application of pesticides, water logging and drought [140]

- Poor organic matter content of many soils around the world

- Extreme annual and diurnal variation in soil temperature

- Poor competition and adaptability as compared to native soil microflora [46]

## 7. Conclusion

Enhancing agricultural crop production needs to be ushered through new horizons without causing any harm to the natural resources and environmental quality. So, low-cost and eco-friendly biofertilizers could play a critical role in increasing crop yield by cutting the use of chemical fertilizers and increased nutrient use efficiency vis-à-vis maintaining long-term soil fertility and quality. However, lack of consistent responses in different soils and environmental conditions, difficulties in application, limited shelf life, and slow action are reasons restraining the widespread commercialization of biofertilizers. We need to apprehend that biofertilizers are extremely specific to crops, soils, and edaphic factors and their sustainability in soils largely depends on pH, soil organic matter, native microbiota, and soil moisture and temperature regime. Our understanding on particular strain effectiveness with specific to crop, soil, and climate needs to be strengthened through extensive research and development. Research should also focus on standardizing biofertilizer dose in a particular soil and crop. Efforts from the government should be emphasized on frequent monitoring of the biofertilizer manufacturing units to assure proper method of production and top quality of the produce and storage. Wide publicity and large-scale utilization of this new era technology through research institutions, nongovernment organizations (NGOs), scientific training, farmer fairs or exhibitions, extension workers, and media are urged.

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