

ROLE OF BIOFERTILIZERS IN PLANT GROWTH PROMOTING, NUTRIENT SOLUBILIZATION AND SALINITY TOLERANCE: A REVIEW

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Abstract

Biofertilizers (BF) are an eco-friendly additive that decreases reliance on chemical fertilisers, alleviates their adverse impacts, and aids in reducing expenses and agricultural requirements. They are bioactive microbe formulations that enhance plant growth and development by enhancing nutrient uptake. In addition, they enhance soil productivity through the process of nitrogen fixation, wherein they convert atmospheric nitrogen into a form that can be utilized by plants. They also facilitate the dissolution of phosphorus in the soil and enhance the accessibility of potassium, so increasing its availability to plants. This application shows great potential in the realm of sustainable crop production. The purpose of this review is to provide a clear understanding of the key issues concerning biofertilizers, including their many types, uses, and the benefits they offer when applied. Additionally, it will discuss the primary methods of administering biofertilizers to plants and their function in enhancing plant resistance to salinity stress.

Keywords: Biofertilizers, PGPR, Nitrogen Fixation, Nutrient Solubilization, Salinity Stress.

INTRODUCTION

Biofertilizers

The anticipated doubling in food demand due to population growth necessitates increased irrigation to enhance agricultural yields and ensure stability. This approach, however, may exacerbate environmental challenges like soil salinization, particularly in arid areas, thereby hindering agricultural output (1,2). Additionally, reliance on chemical fertilizers and pesticides in traditional farming methods presents risks such as eutrophication(3,4). Consequently, the pursuit of environmentally sustainable practices becomes essential to counter these detrimental effects. There is a growing shift towards the sustainable cultivation of nutrient-dense foods to promote biosafety (5). In this context, biofertilizers serve as organic alternatives that enhance soil biodiversity, bolster nutrient availability, and facilitate sustainable agricultural practices without adverse environmental consequences (6–10). Moreover, the ability of plants to withstand abiotic stresses, such as salt, is improved. Therefore, the utilization of advantageous microorganisms as biofertilizers is essential, considering their substantial contribution to enhancing food safety and fostering sustainable agriculture. An essential eco-friendly approach in agriculture entails utilizing biofertilizers that employ plant growth-promoting rhizobacteria

(PGPR). PGPR refers to a diverse range of microorganisms found in the rhizosphere that promote plant growth through one or more unique pathways (11). In addition, both endo- and ectomycorrhizal fungi, as well as cyanobacteria and other beneficial microbes, play a crucial role. The combined presence of these organisms leads to better absorption of nutrients, improved growth of plants, and higher ability to withstand both living and non-living challenges (5).

Biofertilizers (BF) are comprised of living microbes such as bacteria, fungi, and algae, either alone or in combinations, as highlighted by (12). These formulations enhance nutrient availability to plants, offering a greener and more cost-effective alternative to synthetic fertilizers, thus promoting sustainable farming practices and improving soil health (13). Essentially, BF contain microorganisms that transform nutrients from non-bioavailable to bioavailable forms via natural processes, potentially reducing or eliminating the need for chemical fertilizers (12,14). (15) and (16) elucidate on biofertilizers as microbial concoctions that harbor plant growth-promoting rhizobacteria (PGPR), which bolster plant growth through mechanisms like nitrogen fixation, nutrient solubilization, hormone production, and siderophore production. Additionally, (15) describe these agents as consisting of viable microorganisms, which, upon application to soil, seeds, or plant surfaces, colonize the rhizosphere or the plant interiors, thereby enhancing growth by boosting the availability of primary nutrients.

(17) Characterize biofertilizers as blends or formulations comprised of live or dormant microbes, tailored for prolonged preservation, straightforward management, and effective transportation of beneficial microorganisms from labs to agricultural fields. These biofertilizers are globally acknowledged as potent microbial agents that foster plant development by enhancing nutrient assimilation within the plant's rhizosphere. Commonly, they are identified by various terms such as bioformulations, microbial inoculants, microbial cultures, bioinoculants, and bacterial fertilizers or inoculants. It is essential to recognize that the act of administering biofertilizers to plants is termed inoculation (18,19). Furthermore, the utilization of biofertilizers is projected to alleviate salt stress, thereby reducing its detrimental impact on plant growth (20,21).

Types of biofertilizers depending on the source of the formulation

Biofertilizers are pivotal to the promotion of sustainable agriculture, enhancing soil fertility, boosting plant growth, and reducing dependence on synthetic fertilizers. They can be broadly categorized into three types (Table 1) based on their source of composition: bacterial, fungal, and algal biofertilizers.

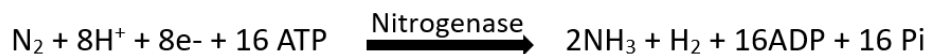
Bacterial biofertilizers comprise nitrogen-fixing bacteria (NFB) like *Rhizobium*, *Azotobacter*, and *Azospirillum*, which facilitate the conversion of atmospheric nitrogen into a usable form for plants. Additionally, phosphate-solubilizing bacteria (PSB) such as *Bacillus* and *Pseudomonas* convert insoluble phosphate into forms accessible to plants. These bacteria play a vital role in increasing nutrient availability to crops and do so without the negative environmental effects often associated with chemical fertilizers (22). **Fungal biofertilizers** include mycorrhizal fungi, which form symbiotic associations with plant

roots to boost water and nutrient absorption. Trichoderma, another significant fungal biofertilizer, is recognized for enhancing plant growth and controlling soil-borne diseases. **Algal biofertilizers** comprise macro algae, like seaweed extracts, which are abundant in growth-stimulating hormones, vitamins, and minerals. Additionally, microalgae such as chlorella and spirulina are utilized for their rich concentrations of essential nutrients, including potassium, phosphorus, and nitrogen (23). A thorough understanding of the various biofertilizers and their sources is crucial for leveraging their potential to sustainably improve crop yields and soil health. This knowledge aids in making informed decisions in agricultural practices, contributing to environmental preservation and increased agricultural productivity.

Types of biofertilizers depending on their function

BF include many microorganisms and vary according to their intended use. BF can be classified according to the nature of their behavior and effect or their functions the plant:

The role of BF in nitrogen fixation: PGPR are integral components of the plant microbiome, performing a variety of beneficial functions within the plant rhizosphere, such as nitrogen (N₂) fixation (24,25). This biological transformation involves the conversion of atmospheric nitrogen into ammonia, a reaction facilitated by the bacterial enzyme nitrogenase. The following equation illustrates the process of biological nitrogen fixation (BNF):



While the primary product of this reaction is ammonia (NH₃), it rapidly converts to ammonium (NH₄⁺), with the predominant forms available to plants being NH₄⁺ and NO₃⁻ (26). This transformation occurs alongside the hydrolysis of 16 ATP molecules and results in the production of hydrogen gas. Biological nitrogen fixers enzymatically convert the ammonia produced by nitrogenase into glutamate using glutamine synthetase. It is important to note that the enzymes activating nitrogenase are highly susceptible to oxygen damage. Consequently, many bacteria cease enzyme production in aerobic environments (27). Numerous nitrogen-fixing organisms thrive solely under anaerobic conditions, employ respiration to decrease oxygen concentrations, or associate with proteins like leghemoglobin, which carries oxygen. This protein bears a resemblance to blood hemoglobin and plays a crucial role in sustaining the low oxygen levels essential for effective nitrogen fixation. Leghemoglobin's primary functions include facilitating oxygen supply to nitrogen-fixing bacteria and shielding nitrogenase from oxygen-induced deactivation (28). Annually, approximately 50-465 kilograms of nitrogen per hectare are fixed through these interactions. Recent data indicate that over 90% of the global soil nitrogen is restored via biological fixation processes. In contrast, a minor portion, between 0.5% and 5%, is fixed using the Haber-Bosch Method (HBM), which synthesizes ammonia by combining atmospheric nitrogen with hydrogen derived from natural gases, under elevated pressure and temperature, in the presence of ferric oxide.

Nitrogen fixation by microbes occurs in two ways: (1) symbiotic fixation and (2) non-symbiotic fixation: **(1) symbiotic fixation:** occurs by bacteria that carry out BNF and increase the N supply to crops in the soil, they are an important component of nitrogen-fixing biofertilizers. In addition to some fungi that contribute to this type of fixation. Mycorrhiza means (fungal roots); in which the mycorrhizal fungi form a symbiotic relationship with the roots of the plant (facilitating the supply of phosphorus to the plant, as the presence of phosphorus is transferred) (29). There are two main types of them: (a) - Ectomycorrhiza: in this type, the fungus does not penetrate the cell wall of the plant roots. Type (b) - Endomycorrhiza: They penetrate the cell walls of the plant root cells. We can therefore define symbiotic biofertilizers as products of microorganisms that live together with the roots of the plant; and provide the plant with some nutrients while obtaining their nutritional needs especially the carbon source from the plant (mutualism relationship).

The genus *Rhizobium*, a prominent example of nitrogen-fixing microbes, establishes symbiotic relationships with the roots of legumes like peas, soybeans, and clover. These bacteria adhere to the roots and form nodules that assimilate atmospheric nitrogen and convert it into ammonia, crucial for the plant's growth. Despite being aerobic, *Rhizobium* effectively engages in nitrogen fixation. Extensive studies demonstrate that legumes inoculated with *Rhizobium* fix significantly more nitrogen compared to uninoculated ones (30–33). Similarly, the genus *Frankia*, belonging to the actinomycetes and further categorized into various groups, also engages in a symbiotic relationship with non-leguminous plants. *Frankia* induces the formation of nitrogen-fixing nodules on the roots of plants such as alders (*Alnus spp.*) and Casuarina trees, exemplifying another type of symbiotic nitrogen-fixing microbe (34).

(2) Non-symbiotic fixation: Independently living microorganisms that fix atmospheric nitrogen are called free-living diazotrophs. These microorganisms can be divided into two categories: First, bacteria and second; cyanobacteria (blue-green algae). **Bacteria** are divided into the following types according to the (carbon, nitrogen, oxygen, and the need for reducing groups): **(a)** Aerobic bacteria: e.g. *Azotobacter*, *Gluconacetobacter* and *Burkholderia* (35,36) **(b)** Facultative anaerobic bacteria: e.g. *Bacillus*, **(c)** Anaerobic bacteria: e.g. *Clostridium*, **(d)** Photosynthetic bacteria: e.g. *Rhodospirillum rubrum*. **Cyanobacteria:** Both heterocystous and non-heterocystous species of cyanobacteria fix atmospheric nitrogen, for example. *Anabaena*, *Anabaenopsis*, *Nostoc* etc. (37). Heterocysts, also known as heterocytes, are very effective nitrogen-fixing cells produced by some filamentous cyanobacteria, including *Nostoc punctiforme*, *Cylindrospermum stagnale*, and *Anabaena sphaerica*, when nitrogen is deficient (14,38).

The process of BNF, both symbiotic and non-symbiotic, is influenced by several factors, namely: **(1) - Factors** influencing **symbiotic nitrogen fixation** include (a) soil factors such as good aeration, sensitivity to high heat, moisture and salinity and pH; the process requires a neutral pH. (b) The soil's mineral nitrogen content (NH_3^+ , NO_3^-). (c) The availability of certain elements such as Ca, K, Mn and PO_4 . (d) biotic factors: e.g., the difference between bacterial strains and a sufficient number of bacteria. In addition, host

plant specificity, nodule formation and genetic compatibility: The genetic compatibility between the host plant and the rhizobia strain (39). **(2)- Factors influencing non-symbiotic nitrogen fixation** include (a.) the mineral nitrogen content of the soil. (b.) the availability of certain elements (Ca, Mo, Fe and Co). (c.) the availability of energy sources: e.g., carbohydrate compounds. (d.) pH of soil: non-symbiotic N₂ fixation does not take place in acidic conditions. (e.) Soil moisture: Non-symbiotic N₂ fixation requires sufficient moisture (40).

Research indicates that the development of biofertilizers incorporating nitrogen-fixing bacteria such as *Rhizobium* and *Azotobacter* has produced favorable outcomes (41). Commonly available nitrogen biofertilizers in the market include *Rhizobium*, along with other bacteria like *Azotobacter* and *Azospirillum*, and are frequently applied to legumes (41,42). These strains of nitrogen-fixing biofertilizers, including *Rhizobium* and *Azotobacter*, are known to enhance plant growth and overall health, and they improve plant resistance to salinity stress.

A. BF Role in solubilization of nutrients: The PGPR also play an important role in solubilization of nutrients (43,44).

1. Phosphorus solubilization (P): is an essential process that is increasingly influenced by global warming, which accelerates soil salinization and calcification, thus reducing phosphorus availability in arid and semi-arid regions globally (45). In soil, phosphate ions (H₂PO₄, HPO₄, and PO₄) exist in two primary forms: they either adhere to clay particles or form complexes with cations such as calcium phosphate and magnesium phosphate in alkaline soils, or iron phosphate and aluminum phosphate in acidic soils, rendering them inaccessible to plants (46,47). Phosphate-solubilizing bacteria (PSB), like *Pseudomonas* and *Bacillus*, offer a more sustainable alternative to mineral phosphorus fertilizers. These bacteria release compounds including phenolic substances, protons, and both organic and mineral acids, which acidify the soil and liberate phosphorus from Ca₃(PO₄)₂ in alkaline environments (48–50). Organic acids further increase phosphorus bioavailability by chelating cations such as Ca²⁺, Al³⁺, and Fe³⁺(51). Additionally, by secreting growth-promoting substances like indole acetic acid (IAA), gibberellins, and cytokinins, phosphate-solubilizing bacteria enhance nitrogen fixation, phosphorus availability, and plant growth(52). Other mechanisms, including the activity of alkaline phosphatases, protonation by H⁺ ions, anion exchange, chelation, and siderophore production, also improve soil and plant phosphorus nutrition(53–56). Furthermore, inoculating crops with PSB has been shown to increase crop yields and enhance phosphorus nutrition in cereals such as rice and maize (57–59). PSB enable plants to access and utilize phosphorus effectively, even in salinized soils, ensuring adequate phosphorus for critical biological functions.

2. Solubilization of sulfur (S): Mineral sulfur added as a fertilizer to alkaline soils to reduce their alkalinity and increase the level of sulfates SO₄ that plants need. Mineral sulfur is a powder that is insoluble in water, and chemolithotrophs oxidize mineral sulfur in well-aerated soils into sulphuric acid (Fig.1). The most important of these microbes

(Sulfur-Solubilizing Bacteria (SSB)) are *Thiobacillus*, *Leptospirillum*, *Sulfobacillus* and *Acidianus* (37).

- 3. Zinc solubilization (Zn):** is crucial for plant productivity, especially given the widespread zinc deficiency in soils globally. This deficiency is primarily due to nutrient depletion from continuous crop harvesting (60). While chemical zinc fertilizers are commonly applied at about 5 kg per hectare to address this issue, they are costly and often not readily available in forms that plants can absorb (61). Recent research highlights the effectiveness of zinc-solubilizing biofertilizers (ZSB) in enhancing plant development and yield by increasing zinc uptake (62,63). For instance, a study by (64) demonstrated that biofertilizers containing *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Rhizobium* significantly boosted zinc absorption in wheat plants. Additionally, a study on soybeans found that 134 bacilli strains from the rhizosphere notably enhanced zinc levels in the plants compared to those that were not inoculated (65). Recent findings also show that various ZSB strains isolated from the rhizospheres of wheat and sugarcane, including *Pantoea dispersa*, *Pseudomonas fragi*, *Pantoea agglomerans*, *Rhizobium* sp., and *Enterobacter cloacae*, have been effective in increasing zinc content and promoting growth in potted wheat plants (62). Moreover, a greenhouse experiment by (66) testing several rhizospheric ZSBs revealed that treated soil and plants exhibited higher zinc concentrations than untreated controls.
 - 4. Solubilization of iron (Fe):** Iron-solubilizing bacteria (ISB) utilize specific mechanisms to capture iron (Fe) in environments where it is scarce. These biofertilizers produce siderophores, metabolites that bind strongly to iron, facilitating its uptake in iron-deficient settings (67). The Fe^{3+} complexes of microbial siderophores are produced in the microbial membrane, where they are then reduced to Fe^{2+} and released into the cell via an input process. Plants have access to the Fe^{2+} of bacterial siderophores and take it up directly the Fe-siderophore complexes or through ligand exchange during this process (68). An example of how rhizobacterial inoculants in BF can improve Fe nutrition is the development of siderophores (69). Siderophilic bacteria have been shown to play an important role in both preventing disease and promoting plant growth.
- B. BF Role in facilitating or extracting potassium (K) from clay minerals:** Plants require potassium in significant amounts as it is a vital element for their nutrition. A significant portion of the potassium is tightly bound to the mineral component of the soil, making it unavailable for exchange. Recent research has revealed the presence of certain microorganisms, including *Pseudomonas*, *Bacillus*, *Penicillium*, and *Aspergillus*. It has the ability to analyse the presence of aluminium silicate in clay minerals, with potassium being a distinguishing characteristic of these minerals. *Bacillus circulans* has the ability to extract silicon and potassium from silicate clay minerals like biotite and orthoclase. These microbes thrive in specific culture media, where they are cultivated, harvested, and then introduced into the soil. Research has indicated that certain bacteria have the ability to break down minerals containing potassium and transform it into a soluble form that plants can absorb. *Acidothiobacillus ferrooxidans*, *Paenibacillus* spp., *Bacillus licheniformis*, *Burkholderia cenocepacia*,

Klebsiella variicola, *Enterobacter cloacae*, and *Bacillus cereus* have the ability to dissolve K minerals like biotite, feldspar, illite, muscovite, and orthoclase through the release of organic acids (70).

C. BF Role in improving phytochemical composition and disease resistance: PGPR are essential for producing key substances that promote plant development(71). These bacteria also bolster plant defenses against various pathogens (72–74). Notable among these are *Azotobacter*, *Azospirillum*, *Mycorrhiza*, and other Phosphate-Solubilizing Bacteria (PSB). A symbiotic relationship exists between legumes and *Rhizobium* bacteria, which leads to the synthesis and emission of several phytohormones such as IAA, cytokinins, lumichrome, rhizobitoxine, gibberellins, jasmonate, ethylene, brassinosteroids, and growth-promoting enzymes (75,76). Indoleacetic acid, for instance, encourages the growth of longer roots and a greater number of root hairs (77). Research has shown that bacteria including *Rhizobium*, *Glomus* spp., *Azotobacter* spp., *Azospirillum* spp., and *Pseudomonas* are instrumental in reducing plant diseases (78,79). The use of biofertilizers has been linked to improved plant growth in environments with certain agricultural pests and pathogens, highlighting their capacity to foster stress resistance by triggering induced systemic resistance (ISR) (80). ISR involves a plant's enhanced defensive capabilities when exposed to pests and pathogens, activating multiple physical and chemical defenses. Studies confirm that the presence of beneficial microbes in the rhizosphere can activate and enhance the plant's innate defense systems (81–83). Additionally, biofertilizers are pivotal in alleviating salinity stress by producing compounds that support root growth, improve nutrient uptake, and overall bolster plant resilience to saline environments.

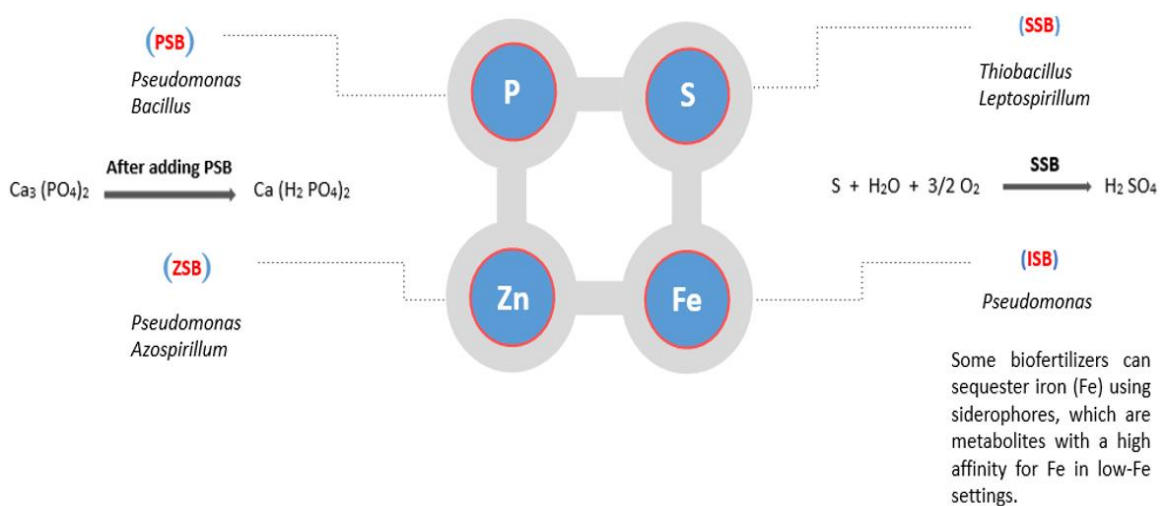


Figure 1: Microorganisms used as biofertilizers aid in the solubilization of certain macro and micronutrients. Bacteria that solubilize phosphorus, sulphur, zinc, and iron are referred to as PSB, SSB, ZSB, and ISB, respectively.

Table 1: Some types of BF according to their source (produced from bacteria, fungi or algae) and their role in promoting plant growth

Types of BF	Inoculants used	Treated Plant	BF Roles	Ref.
Bacteria	<i>Pseudomonas Azotobacter Azospirillum</i>	<i>Helianthus annuus</i>	To improve growth	(84)
Bacteria	<i>Bacillus sp Pseudomonas sp Azospirillum spp</i>	<i>Triticum aestivum</i>	N-fixing & P-solubilizing	(85)
Algae	<i>Laurencia obtusa, Corallina elongata and Jania rubens</i>	<i>Zea mays L.</i>	To increase in P and N content, and enhancement of plant growth	(86)
Bacteria	<i>Pseudomonas, Azospirillum Azotobacter & Bacillus</i>	<i>Capsicum annum</i>	To improve growth & yield	(87)
Bacteria	<i>Azospirillum</i>	<i>Lactuca sativa</i>	Tolerance salinity stress	(88)
Algae	Seaweed <i>Ascophyllum nodosum</i>	<i>Solanum melongena</i>	Salinity tolerance and Increased K content	(89)
Bacteria	<i>Pseudomonas spp. LYT-1</i>	<i>Triticum aestivum</i>	To improve the productivity	(90)
Bacteria	<i>Azospirillum</i>	<i>Triticum aestivum</i>	Biocontrol and Tolerance salinity stress	(91)
Bacteria	<i>Rhizobium, Azospirillum & Pseudomonas</i>	<i>Triticum aestivum</i>	Zn-solubilizing	(64)
Fungi	<i>Trichoderma sp & consortia of BF</i>	<i>Oryza sativa</i>	To increase productivity , diseases resistance & soil fertility	(15)
Algae	(green algae) <i>Ulva compressa</i> and Enteromorpha intestinalis (brown algae) <i>Sargassum muticum and Cystoseira amentacea</i>	<i>Vigna unguiculata L.</i>	To improve growth and Tolerance salinity stress	(92)
Bacteria	<i>Sinorhizobium meliloti, Bacillus flexus & Bacillus megaterium</i>	<i>Zea mays L.</i>	P-solubilizing	(43)
Bacteria	<i>Pseudomonas fragi, Pantoea dispersa, Pantoea agglomerans, Enterobacter cloacae & Rhizobium sp.</i>	<i>Triticum aestivum</i>	Zn-solubilizing	(62)
Bacteria	<i>Mesorhizobium sp., Paenibacillus sp. & Arthrobacter sp.</i>	<i>Lolium perenne</i>	K- solubilizing (extracting)	(56)
Bacteria	<i>Azospirillum lipoferum & Azotobacter chroococcum</i>	<i>Dodonaea viscosa L.</i>	Tolerance salinity stress	(19)
Bacteria	PGPR	<i>Triticum aestivum</i>	Tolerance salinity stress	(93)
Bacteria	<i>Bacillus cereus</i>	<i>Oryza sativa</i>	Zn-solubilizing	(63)
Bacteria	<i>Serratia plymuthica</i>	<i>Vicia faba L.</i>	P-solubilizing	(44)
Bacteria	Bioform “ <i>Azotobacter, Azospirillum and Pseudomonas</i> ” and	<i>Triticum aestivum</i>	To improve growth and biotic stress resistance	(80)

	Probio96 (<i>Bacillus subtilis</i> UTB96)			
Bacteria	<i>Bacillus cereus</i>	<i>Solanum tuberosum</i>	K- solubilizing (extracting)	(70)
Fungi	<i>Aspergillus niger</i>	<i>Lolium multiflorum</i>	S-solubilizing	(94)
Fungi	Arbuscular Mycorrhizal <i>Glomus intraradices</i>	<i>Colocasia esculenta</i> L.	Increase productivity and mitigation salinity stress	(95)
Bacteria	<i>Enterobacter AS19</i>	Pepper, Maize & <i>Gynura divaricata</i>	Fe-solubilizing Promote the germination and growth	(67)
Bacteria	<i>Bacillus mojavensis</i> I4	<i>Triticum aestivum</i>	Tolerance salinity stress	(96)

Importance and possible applications

BF are of great importance and are therefore: **Firstly**, one way to lessen the financial and ecological burden of farming is to use less chemical fertilisers, as most of the nutrients plants need are already present. Utilizing BF can enhance crop yields by around 25% while decreasing the need for inorganic nitrogen and phosphorus fertilisers by approximately 25-50% and 25%, respectively (97). In the same context and more generally using these BF to inoculate crops and farms, can significantly reduce the amount of commercial fertilizer used while still providing crops with the required amounts of N and nutrients (16). **Secondly**, the positive effect on the developing plant, which leads to the following: (a)-acceleration of seed germination, (b)-improvement of the growth and performance of the root system, contributing to increased absorption, increased aeration rate, and increased stress resistance. Also (c) - improving the growth of the vegetative system, (d) - improving plant productivity: biofertilizers help the plant to come into production earlier, improve quality in quantity and quality, and help to increase crop yield by 10-25% (12), (e) - protecting the plant from soil-borne pathogens: by increasing its immunity to injury or by increasing its ability to tolerate injury when it occurs (98). **Thirdly**, they maintain the fertility of the soil in the long term by adding them to the soil in large quantities, which leads to a change in the microbial balance in the soil in favor of beneficial microbes, activates the biological processes in the soil and improves the natural properties of the soil. Thus, BF maintain a soil ecosystem rich in all kinds of micro and macronutrients, by fixing nitrogen, solubilizing or mineralizing phosphate and potassium, biodegrading organic material in the soil, releasing compounds that control plant growth and producing antibiotics (99).

Techniques for applying biofertilizers to plants:

BF are applied using different methods (on seeds, plant surfaces or soil). Each method has advantages and disadvantages (Fig.2), but several factors must be considered during application (12,100), including: Environmental conditions, Type of crop, Prosperities of the inoculant, Technical background and Farmers' constraints.

Seeds inoculation: is the most commonly used method for all types of inoculants. In seed treatment, beneficial microorganisms are transferred directly into the root system of the plant and BF can be sprayed on the seed before sowing. This technique promotes

the development of a symbiotic relationship in young plants. It can be applied by seed treatment using BF with the help of a sticky material such as acacia gum (19) jiggery solution, or other sugary liquid (101). For example, 200 g of biofertilizer can be suspended in 300–400 ml of water and carefully mixed with 10 kg of seeds.

Plant inoculation (seedling root watering): with this method, BF can be mixed into the irrigation systems and supply the roots with nutrients; this guarantees effective nutrient up take. This method is used for transplanted plants. A planting substrate is prepared and filled with water. The roots of the seedlings are immersed in this water for eight to ten hours in the presence of the recommended biofertilizers and then transplanted (12).

Inoculating the Soil: The use of BF in the soil can be accomplished in two ways: either by using it as a top dressing during the growing season or by incorporating it into the soil prior to planting. According to (67), this method enhances the quality of the soil and increases the amount of nutrients that are accessible to the plants. As an illustration, 200 kilogrammes of compost is combined with four kilogrammes of each of the best biofertilizers, and the mixture is then permitted to sit overnight. It is necessary to incorporate this mixture into the soil before planting or sowing an item.

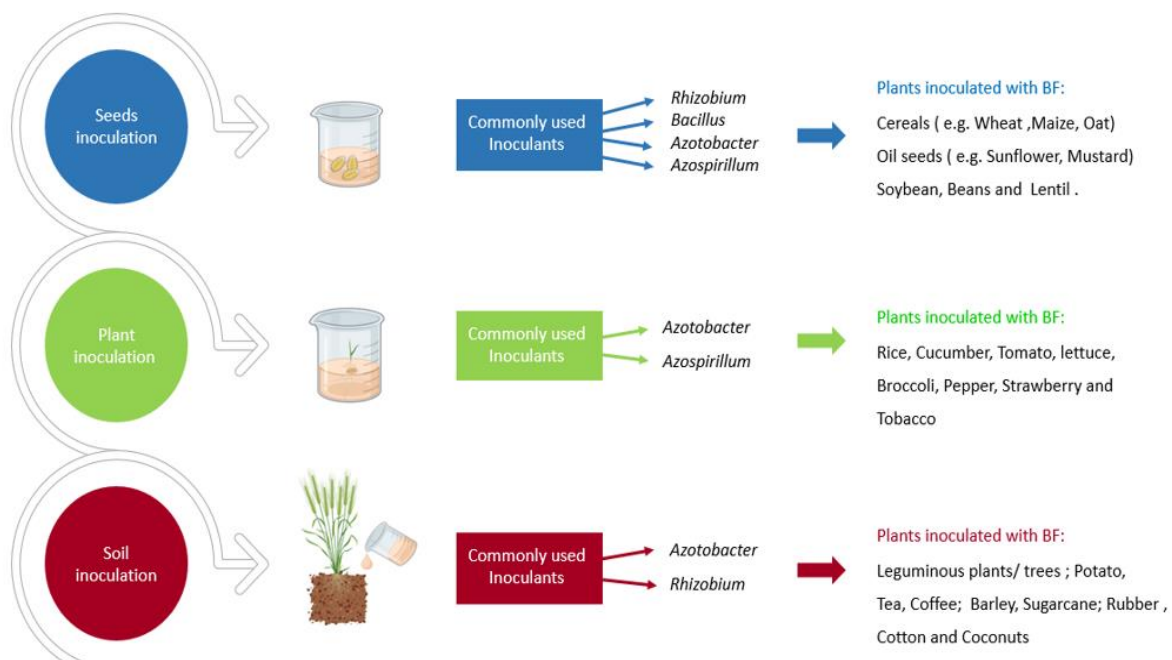


Figure 2: Methods of treating plants with biofertilizers (BF) and the main types of inoculants used in each case.

Role of biofertilizers in the tolerance of salinity stress

Salinity stress

Salinity, stemming from natural origins (primary salinity) and anthropogenic activities (secondary salinity), is increasingly deteriorating. Forecasts suggest that by 2050, salinity

will affect half of the world's agricultural lands (102). This situation results from an overabundance of soluble salts, mainly sodium chloride (NaCl), in soil and water environments, severely restricting the growth and viability of many crops. Plant growth is curtailed by salinity through three principal mechanisms: (a) osmotic stress, involving the buildup of phytotoxic ions, (b) ionic stress, occurring within the cytosol, and (c) oxidative stress, triggered by reactive oxygen species (ROS).

These salinity-induced stressors impede water uptake, cause ion and hormonal disruptions, enhance ROS production, and reduce photosynthetic efficiency, collectively diminishing plant growth and overall productivity (2,103). Moreover, the detrimental impacts of salinity also include soil erosion, ion toxicity, nutrient shortages, and oxidative damage, all contributing to a decline in agricultural output and economic benefits (93).

Some of the roles of biofertilizers in salinity tolerance

1. Improvement of morphological properties

Research has demonstrated that plant tolerance to abiotic stress factors, such as salinity, improves with inoculation (104,105). Specific rhizobacterial species, notably *Azospirillum*, *Pseudomonas*, and *Azotobacter*, significantly enhance seedling germination and growth, as reported by (84). Furthermore, (19) observed that the application of biofertilizers, particularly with *Azotobacter chroococcum* and a combination of *Azotobacter chroococcum* and *Azospirillum lipoferum*, effectively bolstered the resistance of *Dodonaea viscosa* to salt stress. Biofertilizer treatments produced several beneficial effects on plant morphology, including increases in stem dry weight, regenerative development, seed output, germination rates, and overall vegetative growth. These positive outcomes are likely attributable to the productive symbiotic relationships formed between rhizobacteria and plant roots.

These associations not only shield plants from the adverse effects of salt stress but also mitigate the broader impacts of salinity on plant development. Additionally, the benefits are linked to the enhanced absorption of growth-promoting elements and nutrients facilitated by bacterial inoculation. This interaction aids plant growth and development by maintaining a robust root connection, which allows for better nutrient uptake and environmental stress tolerance (106).

Research indicates that rhizobacteria, including *Azotobacter*, contribute to increased plant height and regeneration by synthesizing phytohormones and supporting plant nutrition, producing antibiotics, and protecting against root infections (88,107,108). Studies on various cereal varieties have also shown that inoculation with *Azospirillum* bacteria positively affects plant characteristics such as bush height, root length, leaf size, and the volume and dry matter content of plants (11,91). The maximum vigour index and percentage germination of 99% of pepper seeds were recorded by (87) in a combined inoculation of four different bacteria, namely *Pseudomonas*, *Azospirillum*, *Azotobacter* and *Bacillus*.

2. Improvement of biochemical properties

According to (19), the use of BF treatment improves plant tolerance to salinity stress by minimizing the impacts of osmotic stress and lowering the levels of ionic toxicity. In addition to this, it has a beneficial effect on the plant's capacity to absorb water as well as its efficiency in water utilization. It is possible that the promotion of root growth and elongation that occurs as a result of PGPR treatment is responsible for the improvement in water use efficiency.

This is because the treatment enables roots to extend deeper into the soil. This more extensive root system allows the plant to more efficiently take in water and nutrients, which is beneficial to the plant's overall health. Root-associated bacteria mitigate the negative effects of salinity by producing the enzyme ACC deaminase, which reduces plant ethylene levels, a response highlighted in studies by (109).

This reduction is a key mechanism that enhances plant salinity tolerance and mitigates its detrimental impacts. (105) also identified additional factors contributing to salinity tolerance, including the enhancement of hormonal balance and root system development, which further elevate the resistance levels of inoculated plants.(110) found that applying biofertilizer at a rate of 10 liters per hectare increased chlorophyll content in *Amaranthus tricolor* L. under salinity stress, suggesting that biofertilizers are more effective when they contain a diverse microbial consortium.

These microbes possess capabilities such as nitrogen fixation, phosphate solubilization, production of plant growth regulators, and decomposition of organic matter. Nitrogen, being a fundamental component of proteins and chlorophyll molecules, significantly influences chlorophyll synthesis and chloroplast development, playing a critical role in forming green pigments in leaves.

Moreover, enhanced nitrogen levels contribute to increased chlorophyll production and photochemical efficiency of leaves. This enhancement also boosts the activity of antioxidant enzymes and improves the permeability of cell membranes, thereby preserving cell structure and function (111,112).

Challenges in the use of biofertilizers in plant production

Every technology and application, for all its advantages can also have some disadvantages and BF technology is rather positive in the current period. However, some negative aspects are related to the difficulty of producing inoculants, preserving them and ensuring their longevity (113).

There is also a lack of talented workers researchers to manufacture and handle the subsequent requirements. Moreover, the continuous support for industrialization and research in this field is still lower than expected.

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