Microbes as Biofertilizers

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Biofertilizers are a promising alternative to chemical fertilizers and gaining importance for attaining sustainable agriculture.

biofertilizers microorganisms soil fertility crop productivity sustainable agriculture chemical fertilizers

1. Introduction

To help meet the escalating demand for food arising from the continuous expansion of the world's population, different crop nourishment strategies are being explored by farmers. According to FAO's estimates, the demand for agricultural products will increase to 60% by 2030 [1]. Enhancing the production while keeping the environment safe is one of the major challenges in the 21st century [2]. Fertilizers have been used extensively to increase crop production from arable land. Increasing use of chemical fertilizers in agriculture may make a country self-sufficient in food production, but chemicals have an adverse impact both on the environment and living organisms. In addition, the chemical fertilizers are expensive, affect the soil, reduce its water-holding capacity and fertility, cause imbalance in the soil nutrients, and result in unacceptable levels of water pollution [3]. On the other hand, biofertilizers are eco-friendly, cost-effective, non-toxic, and easy to apply; they help maintain soil structure and biodiversity of the agricultural land. Thus, they serve as a good substitute for chemical fertilizers [4][5].

Biofertilizers, also called microbial inoculants, are organic products containing specific microorganisms, which are derived from plant roots and root zones. They have been shown to improve the growth and yield of the plant by 10–40% [6]. These bioinoculants colonize the rhizosphere and the interior of the plant, promoting plant growth when applied to the seed, plant surface, or the soil [7]. They not only improve soil fertility and crop productivity by adding nutrients to the soil, but also protect the plant from pests and diseases. They have been shown to enhance the growth of the root system, extend its life, degrade the harmful materials, increase the survival of seedlings, and reduce the time to flowering [8]. Another beneficial aspect is that after continuous use of biofertilizers for 3–4 years, there is no need for their application, as parental inocula are sufficient for growth and multiplication [9].

There are 17 essential elements required for proper growth and development of the plant. Among them, nitrogen (N), phosphorous (P), and potassium (K) are needed in relatively large quantities [10]. Several microorganisms are commonly used as biofertilizers including nitrogen-fixing soil bacteria and cyanobacteria, phosphate-solubilizing bacteria, used along with the combination of molds and fungi [11]. Similarly, phytohormone producing bacteria are also used in biofertilizer formulation. They provide the growth-promoting substances like indole acetic acid (IAA), amino acids, and vitamins to the plant and improve the productivity and fertility of the soil while maintaining the crop yield [12].

2. Types of Biofertilizers

Biofertilizers are grouped into different types on the basis of their functions and mode of action. The commonly used biofertilizers are nitrogen fixer (N-fixer), potassium solubilizer (K-solubilizer), phosphorus solubilizer (P-solubilizer), and plant growth promoting rhizobacteria (PGPR) [13]. In one gram of fertile soil, up to 10^{10} bacteria can be present, with a live weight of 2000 kg/ha [14]. Soil bacteria could be cocci (sphere, 0.5 µm), bacilli (rod, 0.5–0.3 µm), or spiral shaped (1–100 µm). The presence of bacteria in the soil depends upon the physical and chemical properties of the soil, organic matter, and phosphorus contents, as well as cultural activities. However, nutrient fixation and plant growth enhancement by bacteria are key components for achieving sustainable agriculture goals in the future. Microbes also facilitate various nutrient cycles in the ecosystem. Table 1 contains a summary of the classification of the biofertilizers based on the type of microbe used and mode of action, with suitable examples.

Table 1. Classification of biofertilizers, mechanism of action, and their examples.

Biofertilizers	Mechanism	Groups	Examples	References
Nitrogen fixing	Increase soil nitrogen content by fixing atmospheric N and make it available to the plants	Free-living	*Azotobacter, Anabaena, Clostridium, Aulosira Bejerinkia, Nostoc, Klebsiella, Stigonema, Desulfovibrio, Rhodospirillum, and Rhodopseudomonas*	[15]
		Symbiotic	*Rhizobium, Frankia, Anabaena azollae, and Trichodesmium*	
		Associative symbiotic	*Azospirillum* spp., *Herbaspirillum* spp., *Alcaligenes, Enterobacter, Azoarcus* spp. *Acetobacter diazotrophicus*	
Phosphorus solubilizing	Solubilize the insoluble forms of P in the soil into soluble forms by secreting organic acids and lowering soil pH to dissolve bound phosphates	Bacteria	*Bacillus circulans, B subtilis, Pseudomonas striata, Penicillium* spp. *B. polymyxa Microccocus Agrobacterium*, *Aereobacter* and *Flavobacterium*	[16]
		Fungi	*Penicillum* spp., *Aspergillus awamori and Trichoderma*	

Biofertilizers	Mechanism	Groups	Examples	References
Phosphorus mobilizing	Transfer phosphorus from the soil to the root cortex. These are broad spectrum bio-fertilizers.	Mycorrhiza	*Arbuscular mycorrhiza*, *Glomus* spp., *Gigaspora* spp., *Acaulospora* spp., *Scutellospora* spp., and *Sclerocystis* spp.	[17]
Potassium solubilizing	Solubilize potassium (silicates) by producing organic acids that decompose silicates and help in the removal of metal ions and make it available to plants.	Bacteria	*Bacillus. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, and *Arthrobacter* spp.	[18]
		Fungi	*Aspergillus niger*.	
Potassium mobilizing	They mobilize the inaccessible forms of potassium in the soil.	Bacteria	*Bacillus* spp.	[19]
		Fungi	*Aspergillus niger*.	
Micronutrient	Oxidizing sulfur to sulfates which are usable by plants.	Sulfur oxidizing	*Thiobacillus* spp.	[20]
	Solubilize the zinc by proton, chelated ligands, acidification, and by oxidoreductive systems.	Zinc solubilizing	*Mycorhiza Pseudomonas* spp., and *Bacillus* spp.	[21]
Plant growth Promoting	Produce hormones that promote root growth, improve nutrient availability, and improve crop yield	Plant growth promoting rhizobacteria	*Pseudomonas* spp. *Agrobacterium, Pseudomonas fluorescens, Arthrobacter, Erwinia, Bacillus, Rhizobium, Enterobacter, Streptomyces*, and *Xanthomonas*	[22]

[23] % of the microbes are required to fix this nitrogen and make it available to the plant. These microorganisms are known as biological nitrogen fixers (BNFs). They transform the inert N_2 into plant-usable organic form [24]. Nitrogen fixation can provide 300–400 kg N/ha/yr and increase the crop yield by 10–50%. In plants, up to 25% of total nitrogen comes from N-fixation. The roots of plants release substances into the soil, which support colonization and nitrogen fixation by bacteria in the rhizosphere of plants. They can efficiently substitute for chemical fertilizers to a varied extent, thus reducing the chemical load from the environment. A rough approximation of such substitution is shown in Table 2. They are grouped into free-living bacteria (*Azotobacter* and *Azospirillium*), blue-green algae, and symbionts, such as *Rhizobium*, *Frankia*, and *Azolla*. The N_2-fixing bacteria associated with legumes include *Rhizobium, Mesorhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium*, and *Allorhizobium* and those with non-legumes include *Achromobacter, Alcaligenes, Arthrobacter, Acetobacter, Azomonas, Beijerinckia, Clostridium, Bacillus, Enterobacter, Erwinia, Desulfovibrio, Derxia, Corynebacterium, Campylobacter, Herbaspirillum, Klebsiella, Lignobacter, Mycobacterium, Rhodospirillum, Rhodo-pseudomonas, Xanthobacter, Mycobacterium*, and *Methylosinus* [25]. Although many genera are isolated from the rhizosphere, mainly members of *Azospirillum* and *Azotobacter* genera have been widely tested to increase yield of legumes and cereals under field condition [26]. The main N_2-fixing bacteria are described below.

Table 2. Substitution of nitrogen by biofertilizers. Modified: [27].

Biofertilizers	Substitutes/Ha/Year
Rhizobium	50–100 kg N
Azolla	9.2–18.4 kg N
Azospirillum	27.6 kg N (maize)
Blue Green Algae	24.8–29.9 kg N
Frankia	89.7 kg N

2.1.1. The Rhizobium

Rhizobium belongs to the bacterial family *Rhizobiaceae* and is the best example of symbiotic nitrogen fixation. It can fix N_2 in legumes as well as in non-legume crops. Rhizobium has been shown to fix up to 300 kg N/ha/year in different legume crops [28]. The bacteria infect the legume root and form nodules, within which they reduce molecular nitrogen to ammonia, which is utilized by the plant to produce proteins, vitamins, and other nitrogen-containing compounds. Thus, these root nodules act as factories of ammonia production [29]. *Rhizobium* species improve the growth of non-legumes by inducing changes in root morphology and growth physiology. The *Rhizobium* application increased crop growth by improving plant height, seed germination, leaf chlorophyll, and N content [30]. Seed inoculation of rice with different strains of *Rhizobium* at graded levels of N increased straw yield by 4% to 19% and rice grain yield by 8% to 22% [31]. *Rhizobium, Bradyshzodium, Sinorhizobium, Azorhizobium*, and *Mesorhizobium* are collectively called rhizobia. They can act directly by fixing nitrogen or influencing plant hormones or indirectly by decreasing the inhibitory effects of pathogens [32]. *Rhizobium* is commonly used in agronomic practices to ensure adequate nitrogen (approximately 80% of biologically fixed N comes from symbiosis) and have potential to replace chemical N fertilizers [33]. *Rhizobium* maintains the soil fertility along with higher crop yields [34]. Datta et al. isolated *Rhizobium* strains and concluded that growth and yield parameters were significantly enhanced by *Rhizobium* application in comparison to the control [35].

2.1.2. Azotobacter

Azotobacter is a free-living, nitrogen-fixing diazotrophic bacterium; it plays an important role in the nitrogen cycle because of its various metabolic functions [36]. *Azotobacter* has the ability to produce vitamins like thiamine and riboflavin [37]. It belongs to the family *Azotobacteriaceae* and is used as a biofertilizer for all non-leguminous plants, especially rice, cotton, vegetables, sugarcane, sweet potato, and sweet sorghum. Dutta and Singh (2002) reported a significant increase in seed yield in rapeseed and mustard following *Azotobacter* inoculation. It fixes almost 30 kg/N/year; it is mainly commercialized for sugarcane crop, as it increases the cane yield by 25–50 tons/hectares and sugar content by 10–15%. *Azotobacter* is present in both alkaline and acidic soils. *A. chroococcum* is the most prevalent species found in the soil, but other species like *A. vinelandii, A. insignis, A. beijerinckii*, and *A.*

macrocytogenes are also found [38]. Eklund et al. (2013) demonstrated that the presence of *A. chroococcum* in the rhizosphere of cucumber and tomato was correlated with increased growth and germination of seedlings [39][40]. Another study showed inoculation with *A. chroococcum* caused a significantly increase in plant growth compared to control [41]. *Azotobacter* also produces antifungal compounds and antibiotics that inhibit the growth of several pathogenic fungi in the root zone and help prevent seedling mortality [42][43]. The major limiting factor for the proliferation of *Azotobacter* is the presence of reduced amount of organic matter in the soils; consequently, the rhizoplane lacks *Azotobacter* cells [31][44].

2.1.3. Azospirillum

This bacterium is also essential, as it fixes a considerable amount of nitrogen in the soil. It is associated with the rhizospheric region and fixes up to 20–40 kg N/ha in non-leguminous plants, such as cereals, millets, oilseeds, cotton, and sorghum [45]. It mostly forms a symbiotic association with plants. Several studies have shown the potential of *Azospirillum* for crop improvement [46][47][48][49]. *Azospirillum*-inoculated wheat seedlings developed good water status; fresh weight was higher from inoculated seeds than from non-inoculated seeds. Somers et al. (2005) showed that *A. brasilense* could synthesize phenyl acetic acid (PAA), an auxin-like molecule with anti-microbial activity. It is demonstrated that the co-inoculation of *A. lipoferum* and *B. megaterium* provided balanced nitrogen and phosphorus nutrition to the plant and produced a higher yield than inoculation with only *Azospirillum* [50].

2.1.4. Anabaena Azollae

It is a symbiotic bacterium and used to fix the atmospheric nitrogen, mostly in rice [51][52]. It is always associated with the free-floating fern known as *Azolla*. *Azolla* leaves contain 4–5% nitrogen (on dry weight basis) and 0.2–0.4% (on wet weight basis), quickly decompose, and provide the nitrogen to the plant. The *Azolla-Anabaena* system contributes 1.1 kg N/ha/day; one crop of Azolla provided 20–40 kg N/ha to the rice crop in about 20–25 days [53]. *Azolla* is used as a biofertilizer in many countries, such as Vietnam, China, Thailand, and the Philippines [54][55][56]. Another benefit of using this biofertilizer is its metal tolerance ability; thus, can be applied to the heavy metal-polluted areas [57].

2.1.5. Blue-Green Algae (Cyanobacteria)

Nitrogen-fixing *cyanobacteria* are the most widespread N_2 fixers on the earth. *Cyanobacteria* or *blue green algae* are a diverse group of prokaryotes consisting of *Nostoc, Anabaena, Oscillatoria, Aulosira*, and *Lyngbya* [58]. They play an important role in nourishing the soil with nitrogen and supply vitamin B complex and growth-promoting substances like auxins, indole acetic acid, and gibberellic acid, which accelerate plant growth. They fix 20–30 Kg/N/ha in submerged rice fields and increase crop yield by 10–15% when applied at 10 Kg/ha. Reportedly, N availability to plants was increased by the application of cyanobacteria in agriculture, particularly in the rice fields [59][60]. Cyanobacteria have been shown to enhance seed germination, shoot and root growth, and yield of wheat and rice. In rice fields, blue-green algae can fix 25–30 kg N/ha/season [61]. In a study, the effect of the exudates of the cyanobacterial strains were tested on the growth parameters of *Sorghum durra* and *Helianthus annuus*. Shoot length was increased to about 120–242% as compared to control with various other positive effects [62]. Strains showed potential for releasing bioactive compounds and enhanced the plant growth and yield. In another study, rice inoculation with cyanobacteria isolated from rice field showed the positive effects simultaneously on rice plant and soil properties [63]. Application of cyanobacteria as biofertilizers is useful for economically weak farmers who are unable to invest in costly chemical fertilizers. Cyanobacterial biofertilizers can be used for a variety of biomes and environments (terrestrial, rain, or desert) [64][65].

2.2. Phosphate Solubilizing and Mobilizing Biofertilizers

Plant contains about 0.2% of phosphorus on a dry weight basis, and it is an essential nutrient for plant growth and development. Compared to other macro nutrients, phosphorus is so far the least mobile nutrient available to plants under most soil conditions. Microorganisms are needed to convert the insoluble forms of phosphate to the soluble forms [66][67]. Several bacteria and a few fungi species are involved in the phosphate solubilizing process [68]. The phosphate-solubilizing bacteria (PSB) convert the insoluble phosphate, such as HPO_4 and H_2PO_4, into the soluble form by using different mechanisms, including the production of organic acids, chelation, and ion exchange reactions. Among the microbial populations, phosphate-solubilizing bacteria account for 1–50%, whereas fungi account for only 0.1–0.5% of phosphate-solubilizing activities [69]. The PSB can release metabolites, such as organic acids, having hydroxyl (gluconic) and carboxyl (ketogluconic) groups that chelate the cation bound to the phosphate and convert it to the soluble form, which is utilized by the plants. The secreted acids also reduce the pH of the soil and dissolve the bound phosphate to make it available to the plants [20]. Along with the organic method, microorganisms also use the proton-extrusion mechanism to solubilize the phosphate [70][71]. The PSB provide the phosphate as well as other trace elements, such as Fe and Zn, ultimately enhancing the plant growth. They also synthesize the enzyme that kills the pathogens, thus protecting the plant from diseases. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Enterobacter*, along with *Penicillium* and *Aspergillus* fungi, are well-known phosphate solubilizers [72]. The application of *Pseudomonas fuorescens* strain in acidic soils of Cameroon significantly improved the shoot length, grain yield, plant dry weight, and seed phosphorus content in maize [73]. In a recent study, phosphorus solubilizer *Aspergillus niger* was evaluated for its efficiency as biofertilizer; it significantly increased the plant height, fruit size, leaf length/width, and number of fruits per plant when compared with untreated plants. However, plants co-inoculated with both phosphorus solubilizing (*A. niger*) and the N fixing *Azotobacter* showed more improved performance than those treated with each biofertilizer alone [74].

Phosphate-mobilizing microbes can mobilize the immobile forms of phosphorous [75]. They transfer and mobilize the insoluble phosphate from soil layers to the root cortex. Arbuscular mycorrhiza is an example of phosphate-mobilizing fungi, in which fungi penetrate the roots and increase the surface area of roots, stimulate metabolic processes, and absorb the nutrients into the roots. Reportedly, phosphorus-solubilizing bacteria (PSB) sometimes act as phosphate mobilizers [17]. Under optimum condition, they have potential to solubilize/mobilize about 30–50 kg P_2O_5/ha, due to which crop yield could increase by 10–20% [76].

2.2.1. Mycorrhiza

It is a symbiotic association between the host plant and a certain group of fungi. It is arguably the most important symbiosis on earth [77][78]. This association provides the essential nutrients to the plant, mostly phosphorous and growth hormones, which promote plant growth. They also increase the surface area of roots to increase the absorption of nutrients from the soil and provide resistance to plants against plant pathogens [79]. The hyphae of fungi absorb the insoluble phosphorus and convert it into the solubilized form, which is taken up by the plant and, in return, the plant provides shelter and other nutrients to the fungi. These fungi are ubiquitous in geographical distribution and are associated with all crops, except *Brasicacea* [13].

2.2.2. Endomycorrhiza or VAM Fungi

Vesicular arbuscular mycorrhiza (VAM) is the symbiotic association between certain phycomycetous fungi and angiosperm roots. These symbiotic soil fungi colonize the roots of approximately 80% of plant families [80][81]. They enhance the transfer of nutrients from the soil into the root system via specialized structures known as vesicles and arbuscules. This association provides many benefits to the plant. The fungal hyphae enhance the uptake of phosphorous and other nutrients as well as increase the root and shoot length. They also help the plant to uptake a large amount of water from the roots. VAM can potentially increase plant tolerance to various biotic and abiotic stresses and could replace the fertilizer requirements of trees and reduce the needs of current levels of chemical fertilizers [82][83][84]. VAM fungi could contribute to more than twofold increased acquisition of the less mobile nutrients like P, S, Ca, Mg, Zn, and Cu from the rhizosphere [85]. Six genera of fungi have been shown to form mycorrhizal associations: *Glomus, Acaulospora, Gigaspora, Sclerocystis, Entrophospora*, and *Scutellospora* [86]. Co-inoculation treatment of VAM fungi, *Glomus fasciculatum* with *Bradyrhizobium* sp. + *Pseudomonas striata* or *Penicillium variable*, significantly increased the nutrient uptake and plant yield [87]. In addition, VAM fungi enhance the uptake of nutrients by secreting the enzymes and organic acids. An increased concentration of K was found in mycorrhizal plants in comparison to non-mycorrhizal plants [27].

2.3. Potassium Solubilizing and Mobilizing Biofertilizers

Potassium (K) is the second most abundant and important plant nutrient after nitrogen and phosphorus. Although K is an abundant element in the soil, only 1–2% is available to plants, whereas the rest is present as mineral K that cannot be taken up by plants. Therefore, a continuous K replenishment of soil solution is required [70][88]. It plays a vital role in the growth and development of plants. If not supplied in adequate quantity, the plants will grow slowly, have poorly developed roots, and produce small seeds and low yields [89][90]. It has been reported that a wide range of bacterial and fungal strains use various mechanisms, including the production of acids, chelation, acidolysis, complexolysis, and exchange reactions to solubilize the insoluble K into soluble forms [18][91][92]. Examples of potassium-solubilizing biofertilizer include *Bacillus* spp. and *Aspergillus niger. Arthrobacter* spp., *Cladosporium*, and *Sphingomonas aminobacter* with varying potential for K solubilization. *B. edaphicus* and *B. mucilaginosus* are known to improve solubilization as well as mobilization. *B. mucilaginosus*, when inoculated in soil, improved the oil content and groundnut biomass by 35.4% and 25%, respectively, along with enhanced K and P availability [93]. Recently, a study has shown that a potassium-solubilizing strain *Bacillus pseudomycoides* enhanced K uptake in tea plants in the mica waste-treated soil by increasing potassium availability [94]. Another strain *Bacillus cereus* significantly increased the plant height, shoot dry weight, and branches number by about

15%, 26%, and 27%, respectively, compared to the control [95]. Some fungi like *Aspergillus* spp., and *Penicillium* spp. also have potential to solubilize and mobilize K from organic and inorganic sources [96]. Thus, role of K solubilizers is significant for ensuring the regular supply of K to crop plants. These also exert positive impact on the availability of other essential nutrients to the soil, and thus play an important role in maintaining soil sustainability [97].

2.4. Sulfur Oxidizing Biofertilizers

Sulfur as a micronutrient is also required by the plants. It has been reported that sulfur plays a key role in improving certain biological and physical properties of the soil. Sulfur is famous for soil buffering from high pH values. Previous studies have shown that sulfur also promotes the efficiency of nitrogenous and phosphorus fertilizers and increases the efficiency of crops to uptake micronutrients [98]. An example of sulfur-oxidizing microbe is *Thiobacillus* spp.; *Thiobacillus thioparous* and *T. thioxidans* can oxidize sulfur to plant usable sulfates that help in nourishment of plants [99][100]. A recent study has shown that inoculation of *Thiobacillus* along with elemental sulfur increases the oxidation of elemental sulfur, resulting in increased nutrients availability in soil and consequently increased plant growth [101]. Sulfur compounds, especially in reduced form, significantly pollute the environment. Sulfur oxidizing bacteria also play significant roles in environmental protection by biological elimination of sulfur pollution [102].

2.5. Zinc Solubilizing Biofertilizers

Zinc is one of the essential micronutrients required at relatively small concentrations (5–100 mg/kg) in tissues for the growth and reproduction of plants. Zinc deficiency is very common in soil that results from the increased application of fertilizers in an imbalanced manner, intensive agriculture, and poor soil health. It is estimated that by 2025, zinc deficiency may increase from 42% to 63% if the contributing reasons are neglected. Zinc is involved in the synthesis of growth hormones. Zinc deficiency in plants leads to retarded shoot growth, reduced membrane integrity and reduced leaf size, chlorosis, and increased susceptibility to light, heat, and fungal infections and affects grain yield, root development, pollen formation, and water uptake and transport [21][103]. Zinc deficiency can lead to yellowing of leaves and stunted growth in wheat. Consuming zinc-deficient wheat can lead to zinc deficiency in humans as well [21]. Zinc deficiency is considered the fifth most important human-related death in less developed countries. Therefore, addressing Zn deficiency in agriculture is getting top priority among other minor nutrients [104][105][106]. Microbial inoculants have been identified to solubilize the complex form of zinc in soil [107]. *Mycorrhiza*, *Saccharomyces* spp., and several genera of *rhizobacteria* such as *Pseudomonas* spp. and *Bacillus* spp. are reported to increase Zn availability in soil. These microbes solubilize the zinc by chelated ligands and oxidoreductive systems [21][108]. These bacteria also produce phytochromes, antibiotics, vitamins, and antifungal substances, and help the plant in many aspects [109]. In a study, rice plants inoculated with a suitable combination of Zn solubilizing bacterial strains (*Burkholderia* spp. and *Acinetobacter* spp.) increased the growth attributes and rice yield and were found more efficient in acquiring Zn from the soil as compared to non-inoculated plants [110]. Biofertilizers containing Zn solubilizing bacteria have been reported to boost up the maize production [111].

2.6. Plant Growth Promoting Rhizobacteria (PGPR)

A group of free-living rhizosphere bacteria that colonize plant roots and exert a beneficial effect on plant growth are referred to as PGPR [112]. They act as biofertilizers by promoting growth and development of plants, facilitating biotic and abiotic stress tolerance, and helping in the mineralization of the soil by decomposing organic matter. Inoculation of PGPR imparts various beneficial effects to the plant. They increase the tolerance of plant to drought [113][114][115][116], salinity [117][118], and biotic stress [119][120]. They enhance the seed germination [121][122] and soil fertility [123][124] and promote growth by producing phytohormones including Auxins, IAA, ethylene, gibberellin, etc. [125][126][127]. They can modulate plant secondary metabolites and bioremediation of heavy metals and pollutants [128][129][130][131][132]. PGPR includes member of several genera, e.g., *Agrobacterium, Arthrobacter, Alcaligenes, Azotobacter, Acinetobacter, Actinoplanes. Bacillus, Frankia, Pseudomonas, Rhizobium, Micrococcus Streptomyces, Xanthomonas, Enterobacter, Cellulomonas, Serratia, Flavobacterium, Thiobacillus*, etc. [133]. The detailed contribution of PGPR to plant growth promotion and their modes of action has been included in several reviews [134][135][136][137][138][139][140][141].

3. The Potential and Suitability of Biofertilizers for Crops

Biofertilizers play an important role in improving soil fertility and enhancing crop yield [142]. When applied to the soil, they participate in nutrient cycling and improve the soil structure and crop productivity [143][144]. The uniqueness of microbes and their capabilities in environmental and cultural conditions have made them potential candidates in the agriculture field for resolving food related issues [145]. The use of potential biofertilizers will not merely play a key role in efficiency and sustainability of soil, but also conserve the environment by reducing the adverse impact of agricultural practices and improving the food quality as well [146]. Biofertilizers solubilize the key nutrients and make them available to the plants. They contribute to the development of root hairs and consequently improve water uptake by plants [147][148]. They produce phytohormones and improve the soil fertility, consequently improving the growth and development of plants without detrimental side-effects [149]. A list of beneficial traits that these microbes impart to the plants can be seen in Figure 1. Researchers are continuously working on the development of biofertilizers to increase their application in agricultural industry for sustainable ecosystem and holistic well-being.

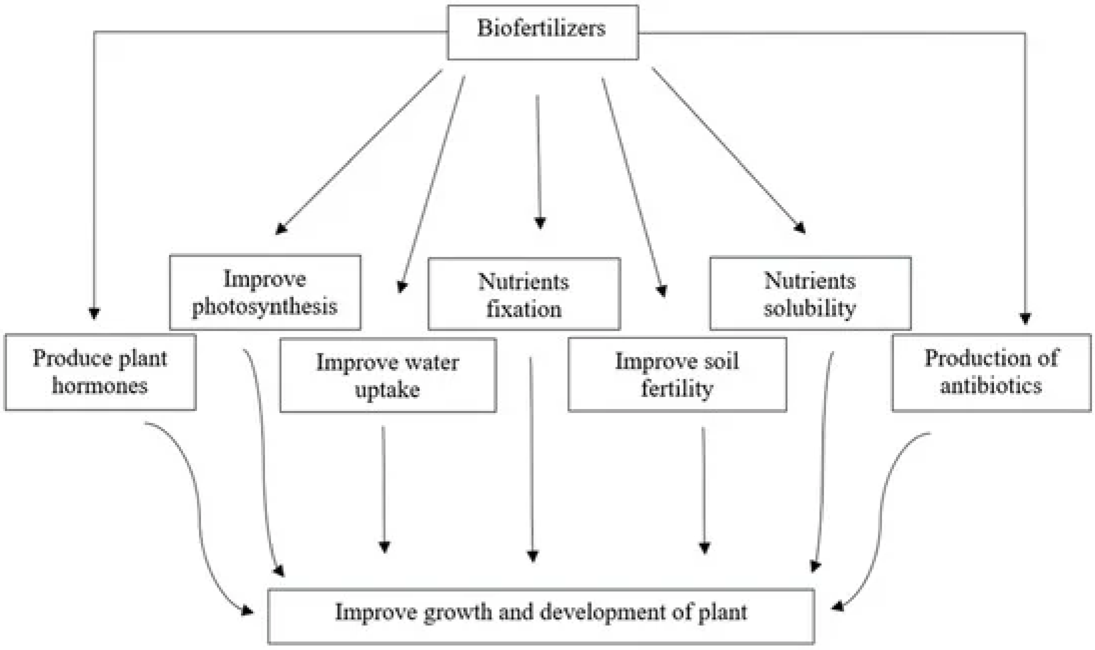

Figure 1. Potential role of microbes as biofertilizers in the growth and development of plant.

Different crops need different biofertilizers for better results. Table 3 is explaining the best biofertilizers for the different crops. Later scientists observed that specific combination of biofertilizers gives better results as compared to inoculation of single/individual fertilizer. For field-grown maize, inoculation with *Azotobacter* and *Azospirillum* significantly increased the grain yield, and total dry weight was increased up to 115% [150]. Similarly, it is reported that inoculation of rice seedlings with *Azospirillum* spp. and *Azotobacter* spp. successfully substituted inorganic nitrogen fertilizer and increased rice yield from 2–3 t·ha^{-1} to 3.9 to 6.4 t·ha^{-1} [151]. Another research has tested the impact of rice root inoculation on the yield under different nitrogen fertility levels. Surprisingly, the significant yield was observed at the lowest level of inorganic N fertilization [152]. Using phosphorus solubilizing bacteria as biofertilizers could increase the sugarcane yield up to 50%, thus saving 50% of costly phosphate fertilizer [153].

Table 3. Biofertilizers for the specific crops.

Biofertilizer	Function	Crops	References
Rhizobium (symbiotic)	Fixes 200–300 kg N/ha/year	Pea, pulses legumes, cow pea, green gram, black gram, groundnut, soyabean, berseem, wheat, jowar, bajra, maize	[27][154]
	Increases yield up to 10–30%		
	Maintain soil fertility		
Azotobacter	Supplies 20–40 kg N/ha/year	Mustard, sunflower, banana, sugarcane, grapes, papaya, watermelon, tomato, chilly, lady finger, coconut	[5][155][156]
	Promote growth substances such as vitamins, IAA, gibberellic acids.		

Biofertilizer	Function	Crops	References
	Increase yield up to 10–15%		
	Maintain soil fertility		
	Fixes 20–160 kg N/ha /year		
	Increase water and mineral uptake		
Azospirillum	Production of plant hormones	Rice, sugarcane, millet, wheat, sorghum, bajra	[85][157]
	Enhance root growth		
	Increase crop yield		
	Fixes 20–40 kg N/ha/year		
Blue-green algae	Promote growth substances such as vitamins, IAA, Gibberelic acids	Rice	[59]
	Fixes 30–60 kg N/ha/year		
Azolla	Used as green manure	Rice	[158]
	Increase root absorption area for nutrient access		
Arbuscular mycorrhizal fungi (symbiotic)	Fixes phosphate	Soybean, wheat, and corn	[159]
	Increase crop yield		
	Production of siderophores and plant hormones		
Pseudomonas	Fixes phosphate	Potato, reddish, sugar beat	[160]
	Increase crop yield		
Bacillus spp.	Solubilize the phosphate and fix the nitrogen in soil	Many vegetables and fruits	[161][162]
	Synthesis of growth hormones		
	Production of antibiotics		

Chickpea is one of the major pulse crops grown in many countries worldwide. Single and combined inoculation of bacterial and fungal strains (*Bacillus* sp. RM−2 and *Aspergillus niger* S−36) significantly enhanced many growth parameters of chickpea over the control. However, dual inoculation of bacterial and fungal species was found more

Biofertilizer	Function	Crops	References
	Increase crop yield		

[163] [164] showed that biofertilizers signif t uptake of chickpea. The effects of soil fertility on chlorophyll, nutrients, grain yield, and yield components of chickpea seed are shown briefly in Table 4. Combined application of PSB and *Trichoderma* produced the highest leaf P content (0.33%) and grain P content (279 mg 100 g^{-1}). The production of acids by *Bacillus* spp. under P-limited conditions may increase the solubility of phosphorus. The same study also revealed that chickpeas inoculated with biofertilizer (PSB+ *Trichoderma*) possess significantly increased grain protein content (15.06%). A meta-analysis study has confirmed that the combined application of N fixers and P solubilizers significantly increases the yield as compared to single inoculation. This indicates the synergies between both fertilizers instead of competition [165]. Another study tested the inoculation of soil with two cyanobacterial species (*Nostoc entophytum* and *Oscillatoria angustissima*) as biofertilizers for pea plants that significantly enhanced the growth, germination percentage, and photosynthetic pigment fraction. However, the most effective results were noted with inoculation of one species and a half dose of chemical fertilizer, which allowed saving of 50% of chemical fertilizers [166]. Another group also tested the efficiency of biofertilizers, and they concluded that inoculation of the wheat plant with biofertilizers (*Azotobacter*, Yeast, and *Azotobacter* + Yeast) resulted in significantly higher values of most of the growth and yield parameters. They noted that mixed inoculums were found better than single inoculums. [167].

Table 4. The effect of soil fertility on chlorophyll, nutrients, grain yield, and yield components of chickpea seed. Modified: Mohammadi et al. [164].

Biofertilizers	Chlorophyll	N	P	K
		mg/100 gm		
PSB	43.41 ^{b}	2269 ^{b}	273.5 ^{b}	1201.1 ^{b}
Trichoderma	43.35 ^{b}	2295 ^{b}	266.2 ^{c}	1176.3 ^{c}
PSB + fungi	44.12 ^{a}	2315 ^{a}	299.8 ^{a}	1232.1 ^{a}
Control	43.22 ^{b}	2167 ^{c}	264.9 ^{c}	1199.8 ^{b}
Biofertilizers	**Grain Yield (kg/ha)**	**Pod Number Per Plant**		**Fertile Pod Per Plant**
PSB	1756.1 ^{c}	39.72 ^{b}		25.84 ^{c}
Trichoderma	1866.2 ^{b}	40.79 ^{b}		27.41 ^{b}
PSB + fungi	2560.3 ^{b}	57.66 ^{a}		35.07 ^{a}
Control	1310.7 ^{d}	30.83 ^{c}		20.73 ^{d}

Mean values in each column with the same superscript(s) do not differ significantly ($p = 0.05$).

Habibi et al. (2011) strongly suggested that using combined strains in biofertilizers plus a half dose of chemical or organic fertilizers could significantly increase oil and grain yield in medicinal pumpkin [168]. They showed that biofertilizers improved the efficiency of traditional chemical fertilizers, ultimately reducing the use of expensive chemical fertilizers and reducing environmental pollution. Another study reported that the integration of biofertilizers with chemical fertilizers produced maximum rice yield [169]. They combined nitrogen, phosphorus, and potassium chemical fertilizers with biofertilizers (*Azospirillum, Azotobacter*, and *Azolla*) and obtained the highest grain yield and straw yield (3.57 and 4.32 t/ha, respectively) of rice. Then they grew peanut crop on the residual soil and found the highest pod yield. This revealed that biofertilizers applied to one crop can have beneficial effects on the next crop too.

The use of biofilm is also getting popular in biofertilizer technology. This technique was tested on wheat crops in which a biofilm prepared from *Anabaena torulosa* was used as a matrix for *Azotobacter, Pseudomonas, Serratia*, and *Mesorhizobium*, and it resulted in 40–50% increased nitrogen fixation [170]. Biofertilizers have been shown to enhance plant tolerance to environmental stresses. The study has shown that inoculation of plants with *arbuscular mycorrhiza* fungi enhances the plant growth under salt stress [171]. A study demonstrated that *Psuedomonas* spp. exert a positive effect on the growth and germination of seeds under water stress. [172]. Another study has shown that the inoculation of mycorrhiza increased the photosynthetic efficiency of rice plant under saline and drought conditions [173]. Biofertilizers are also useful to protect the crops from the hazardous effects of heavy metals. A study revealed that the use of biofertilizers was found effective in moderating cadmium toxicity in the soil for sunflower and maize cultivation [174].

4. Biofertilizers: A Hero or a Villain in the Field

Biofertilizers are very helpful in getting a high yield of crops. They can convert the insoluble form of nutrients to the soluble form, making the soil rich and suitable for the proper growth of plants. The biofertilizers come as an alternative to chemical fertilizers. Chemical fertilizers release the chemicals that are damaging to our soil and environment. Biofertilizers contain the natural component, which does not harm the plant. In fact, they protect the plant from other diseases, fungal attack, and free pollutants [8]. Biofertilizers also protect the plants from strict conditions like drought, alkalinity, etc. Biofertilizers are of lower cost than the chemical fertilizers, but these biofertilizers sometimes deceive the farmer. Regarding the specific type of biofertilizers used for specific crops, the choice should be correct. The knowledge of biofertilizer composition is crucial to exploit the synergistic action of various microbes. The biological and chemical interaction of biofertilizers with crop and soil is very complex. These interactions are highly affected by moisture, pH, temperature, and other environmental variables, which ultimately affect the efficacy of biofertilizers. That is why a good understanding of plant and microbe interrelationships is mandatory [144]. If the conditions are not right for the microbes to multiply and do their work, their populations are likely to peter out. In other words, the user would have wasted time and money on a product that was not suitable according to the soil conditions. Thus, great care should be taken in choosing the biofertilizers to achieve the maximum results. There is a great need to overcome the biofertilizer production, market level, and resources constraints that include improper handling of strains, lack of farmers awareness, limited investment for production units, etc. Biofertilizers being living organisms required proper facilities for handling, transportation, and storage [13].

5. Potential of Biofertilizers in Agriculture Market

The market of biofertilizers is continuously expanding due to rising awareness of biofertilizers towards the growing economy. The market of biofertilizers was valued about $440 million in 2012 globally and is expected to grow at the rate of 10% per annum [175]. Rhizobia is famous for being used as a biofertilizer, constituting 79% of world demand, while phosphate mobilizing biofertilizers constitute about 15% [176]. Manufacturing companies and regulatory government authorities are the main stakeholders of the biofertilizers market. There are many companies in the market ensuring safe production and distribution of biofertilizers. However, there are still some countries like in Africa and Asia that are suffering from hunger and malnutrition but cannot access the latest agricultural technologies. The strategy of using biofertilizers can play a significant role in this direction, as these fertilizers can be easily produced by small companies and can be used in small agricultural lands. *Azospirillum* is an excellent example of this in America; it can dramatically increase plant growth. They selected promising *Azospirillum* strains by rigorous testing in the field and, after suitable formulations, were directed for production and commercialization. Nowadays, more than 100 products of *Azospirillum* strains are commercially available, which aimed to enhance crop yield mainly in wheat, maize, and soybean in South America [177]. Similarly, 167 million hectares area and one lakh hectares area are cultivated as organic farming using biofertilizers in China and India, respectively [178].

6. Limitations in the production of biofertilizers

Though biofertilizers have proven their worth in agriculture with promising results over the past 50 years, desired success is still to be achieved. Several constraints limit the application of this technology at large scale. Some of the possible reasons including competition of bioinoculant and natural flora of soil for niche, poor soil characteristics, presence of environmental and soil pollutants and extreme climatic conditions, unavailability of appropriate strain and suitable carrier material, unavailability of skilled and experienced staff in production unit, unavailability of sufficient funds and equipment from government and private bodies, lack of storage and transport facilities, lack of awareness among farmers, marketing constraints like unavailability of a suitable strain at a suitable place at the right time, lack of regulations and standards for production, and lack of promotion network. A list of possible constraints with recommendations that need to be taken care can be seen in Table 5. Practically, all of these factors determine the potential success of biofertilizers.

Table 5. Constraints/limitations in the production and commercialization of biofertilizers.

Constraints/Limitations		Recommendations
Technical constraints	Unavailability of suitable carrier material	Identification and selection of appropriate economic carrier to maintain shelf life and effectiveness of biofertilizers.

		Skilled staff should be hire and manpower should be trained via proper training.
	Lack of skilled staff in production units	Frequent monitoring of the biofertilizer production units for quality assurance
Marketing constraints	Lack of regulation and standards for biofertilizer	Necessary legislation for monitoring biofertilizers should be done by government.
	Limited transportation and storage facilities	
	Poor and incomplete labeling of biofertilizer products	Proper labeling of biofertilizers should be done (giving genus name, viable count, and expiry date, etc.)
	Lack of promotion network and publicity among the end users.	Wide publicity of biofertilizers should be done through scientific training, fairs, exhibitions, or media.
Biological constraints	Unavailability of appropriate strain Tendency of strain to mutate during fermentation.	Continuous efforts for identification of strains and screening for their efficiency across the type of soil, agro-climatic conditions, and farming situations is recommended.
	Nonavailability of right inoculant at the right place at the right time	Standardization of biofertilizer dose in a particular crop and soil.
		Understanding on strain effectiveness should be strengthened through extensive

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		research in this field
Field-level constraints	Soil conditions like acidity, presence of salts and toxic elements in the soil, and extreme climatic conditions may make the results of biofertilizers inconsistent.	It is needed to strengthen the research and technologies to reduce effects and to counteract stated soil and environmental conditions.
	Inadequate awareness among the farming community about bio-inoculants	A strong training and awareness program may be initiated to aware and motivate farmers.
Financial constraints	Nonavailability of sufficient funds and equipment from government and private bodies.	Use of high-tech equipment is required for quality products.
		Government should provide funding and loans for development of production units.

7. Conclusion and Prospects

Biofertilizers are a good approach to increasing crop productivity. In recent years, the biofertilizers are used to provide the essential nutrients to the plant and significantly increase its yield. These are eco-friendly, cost effective, provides the natural environment to the plant, boost the defense system of the plant, and protect the plant from drought, acidity, and other strict conditions. The advantages of biofertilizers exceed its usage from the other harmful chemical fertilizers. In this review, the most important microorganisms used as biofertilizers are explained along with their mechanism of action. It is also observed that the integration of two different types of biofertilizers increases the yield of crops more significantly than the single biofertilizer or sole chemical fertilizer inoculation. The increasing demand for biofertilizers reflects an eco-friendly and sustainable agriculture system in the future. However, knowledge about soil properties, field environment, and host specificity of strains is mandatory for the successful production and application of biofertilizers. Recent advances in the field of molecular biology, biotechnology, genetic engineering, microbial taxonomy, and nanotechnology have played a significant role in the production of biofertilizers with improved efficiency, higher competitive ability, and multiple functionalities. Biofertilizers can maintain crop productivity with low environmental impact and can be an effective substitute for

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chemical fertilizers. Research efforts are still required in this field to explore and identify soil-specific strains, to gain further insights into potential composition, and to improve the existing strains using biotechnological methods.

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