BIOTECHINTELECT, 2026, 3 (1) e13 (1-19)

https://jbiotechintel.com/index.php/biotechintel

Review Article

eISSN: 3115-7920

OPEN ACCESS

The Role of Organic Biofertilisers Containing Nitrogen-Fixing Bacteria in the Efficiency of Agricultural Products

Maryam Taheri[1], Zarrindokht Emami-Karvani[1*], Seyed Hossein Mirdamadian[1], Alaleh Zoghi[2]

[1] Department of Microbiology, Fal.C., Islamic Azad University, Isfahan, Iran.

[2] Research Department of Food Technology, National Nutrition and Food Technology Research Institute, Faculty of Food and Nutrition Sciences, Shahid Beheshti University of Medical Sciences, P.O. Box: 19395-4741, Tehran, Iran.

Article history:

Received	01 March 2026
Revised	29 March 2026
Accepted	26 April 2026
Published online	29 April 2026

Keywords:

Azotobacter; *Azospirillum*; *Rhizobium*; Crops; Soil fertility; Diazotrophic bacteria

How to cite this article

Taheri M, Emami-Karevai Z, Mirdamadian SH, Zoghi A. The role of organic biofertilisers containing nitrogen-fixing bacteria in the efficiency of agricultural products, *BiotechIntellect,* 2026; 3(1): e13, 1-19. https://doi.org/10.66224/BiotechIntellect.3.1.48

*corresponding author's email: Zarrin Emami Karevani: za.emami@iau.ac.ir

ABSTRACT

Purpose and aim: This review systematically discuss the role of nitrogen-fixing bacteria as biofertilizers in enhancing agricultural productivity, with particular focus on four major genera, *Azotobacter*, *Azospirillum*, *Rhizobium*, and *Gluconacetobacter*, and evaluates their potential as sustainable alter-natives to chemical nitrogen fertilizers.

Methods: A structured literature search was conducted in PubMed, Web of Science and Scopus for English-language papers from 2010 onward, using combinations of the keywords including "nitrogen-fixing bacteria, biofertilizer, *Azotobacter*, *Azosprillium*, *Rhizobium*, *Gluconoacetobacter*, crop yield and sustainable agriculture". We included peer-reviewed original research articles, field trials, meta-analyses, and relevant reviews, excluding editorials, conference abstracts, case reports, duplicate publications. From 1247 initial records, we critically evaluated 82 primary studies and 5 meta-analyses.

Results and Conclusion: Nitrogen-fixing bacterial inoculation significantly enhances crop productivity, with crop-specific responses varying: *Azospir-illum* shows particular efficacy in wheat and maize, *Rhizobium* improves wheat yields, and *Azotobacter* benefits chickpea. Co-inoculation outperforms single-strain applications, increasing common bean yield (34%), chickpea (19%), and soybean (2%). Nitrogen fixation rates range from 20–40 kg N/ha/year for free-living and associative fixers to 50–100 kg N/ha for symbiotic *Rhizobium*. Field evidence indicates biofertilizer application can reduce chemical nitrogen requirements by 25–50% while maintaining yields. Although biofertilizers represent a viable strategy for reducing chemical ferti-lizer dependence, adoption faces barriers including formulation challenges, inconsistent field performance, and regulatory gaps. Future research should prioritise strain selection, formulation technology, and understanding plant-microbe-environment interactions to optimise field efficacy.

What is "already known":	• Chemical fertilizers have low use efficiency (<30%), with the majority contributing to environmental pollution, soil degradation, and human health risks. • Biological N fixation by diazotrophic bacteria (*Azotobacter*, *Azospirillum*, *Rhizobium*, and *Gluconacetobacter*) can reduce dependence on synthetic fertilizers
What this article adds:	• Recent meta-analytical evidence quantifying crop-specific efficacy of nitrogen-fixing bacteria, with effect sizes across wheat, maize, and chickpea. • Co-inoculation strategies, identifies commercialisation barriers including formulation challenges and regulatory gaps

1. INTRODUCTION

Conventional agriculture relies heavily on synthetic fertilizers and pesticides, creating unstable agro-ecosystems and generating environmental pollution that affects agricultural, environmental, industrial, and health sectors [1]. The proper and balanced use of fertilizers, whether chemical, animal, plant compost, or green manure, remains fundamental to maintaining soil fertility and increasing crop yields [2].

Global population growth and rising food demand have driven a substantial increase in chemical fertilizer use over recent decades. This intensification aims to maximize production per unit area but carries high costs: elevated production expenses, water and soil resource contamination, nutrient imbalance, reduced product quality, and risks to human and animal health [3]. Given these consequences, biofertilizers derived from bacteria, fungi, algae, or other soil organisms have gained attention for their ability to enhance plant nutrient uptake through biological mechanisms [4].

Biofertilizers offer economic and environmental advantages beyond simple nutrient provision. They contribute to resource sustainability, increase long-term production capacity, and reduce environmental pollution [4]. They can boost plant growth and yield by 10-40% while improving nutrient use efficiency [5]. Field evidence shows biofertilizer application can reduce nitrogen requirements by 50% and phosphorus requirements by 25% [6].

Composting, the biological decomposition of organic materials, depends critically on microbial activity. More capable microorganisms have shorter processing time and produce more mature biofertilizer. Under controlled moisture and aerobic conditions at temperatures of 60-75°C, organic materials decompose to yield stable compost suitable as organic fertilizer [7]. The microbial population- particularly extremophiles or mesophiles capable of surviving elevated temperatures- determines product quality [8].

Nitrogen stands as the most limiting nutrient for crop production, constituting the primary component of proteins and nucleic acids. Despite its abundance in the atmosphere (78%), plants cannot directly assimilate molecular nitrogen (N_2) [1]. Nitrogen deficiency reduces yields more severely than deficiencies of other nutrients. In meeting food production demands, nitrogen management becomes pivotal. The challenge lies in optimizing nitrogen fertilization to enhance yields while minimizing inefficiency and pollution [4].

Biofertilizers offer a viable pathway to reduce or eliminate chemical fertilizer use. Environmental concerns, rising production costs, and recognition of chemical fertilizers' adverse effects on biological cycles and agricultural sustainability have made this approach imperative [9]. Biofertilizers contribute to nutrient cycling, nitrogen fixation, nutrient provision, pest resistance, improved soil physicochemical properties, and seedling establishment. Their growth-promoting effects characterize them as Plant Growth Promoting Rhizobacteria (PGPR). Genera including *Clostridium*, *Bacillus*, *Azotobacter*, *Azospirillum*, and *Pseudomonas fluorescens* establish mutualistic associations with plant roots, converting unavailable nutrients to available forms through biological processes, thereby enhancing root development and seed germination [10, 11].

Crop quality improvement depends directly on microbial inoculant type and application conditions. Current research largely emphasizes strain development and understanding rhizosphere modes of action, with limited investigation into inoculant nitrogen-fixing bacteria [12]. A recent meta-analysis of 80 studies examining factors influencing nitrogen-fixing bacteria efficacy found significant overall positive effects on crop yield (Hedges' g = 1.263, 95% CI: 1.159–1.367); however, substantial heterogeneity underscores the need for targeted application strategies [13]. This review provides a comprehensive synthesis of nitrogen-fixing bacteria's role in agricultural productivity, with practical recommendations for their integration into sustainable farming systems.

2. FERTILIZERS: TYPES, EFFECTS, AND SUSTAINABLE ALTERNATIVES

Fertilizers fall into four categories: mineral, chemical, biological, and organic. Mineral fertilizers- such as phosphate rock- contain plant nutrients in their raw natural state. Chemical fertilizers result from industrial processing: purification, conversion, or combining abundant elements (like atmospheric nitrogen) with others to create plant-usable compounds

[14]. Simple fertilizers provide a single nutrient (urea supplies only nitrogen); compound fertilizers supply multiple nutrients (nitrofoska contains nitrogen, phosphorus, and potassium). Fertilizers may supply macroelements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur) or microelements (iron, zinc, manganese, copper, molybdenum, chlorine, boron). Complete fertilizers contain all these elements in appropriate proportions, with application rates determined by plant requirements and soil test results [14].

Research across multiple continents indicates that crops absorb only slightly over 30% of applied chemical fertilizers; approximately 70% enters natural cycles in various forms [15]. This inefficiency generates adverse environmental and health consequences affecting soil, living organisms, ecosystems, and surface and groundwater quality (Figure 1) [3, 15].

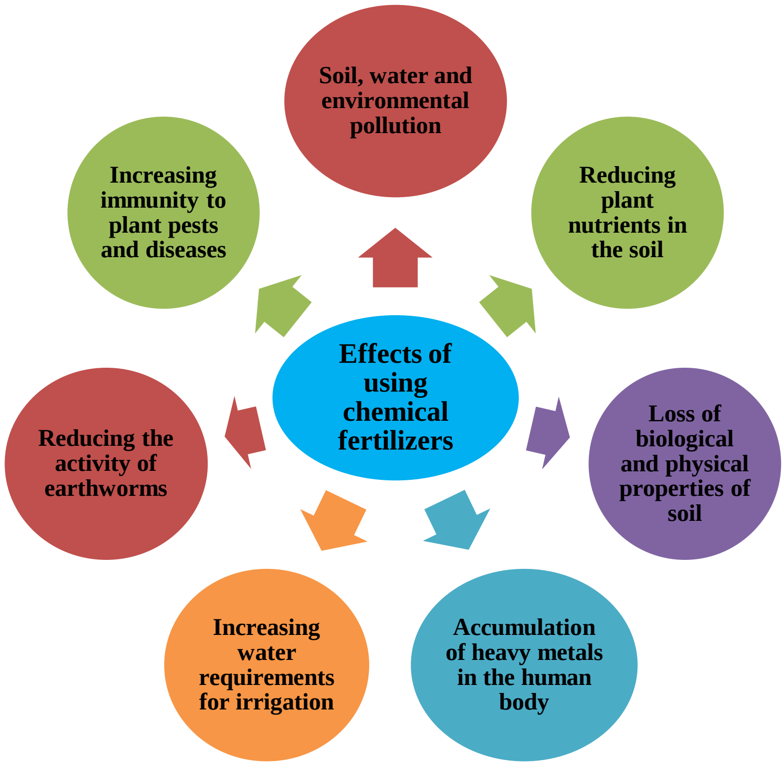

Figure 1. The most important effects of using chemical fertilizers

2.1. Organic Fertilizers

Organic fertilizers derive from natural sources and undergo microbial decomposition, releasing minerals gradually through chemical reactions or microbial activity. Any decomposable substance releasing plant nutrients qualifies as organic fertilizer. Beyond improving soil fertility, organic fertilizers affect soil physical properties. Major organic matter sources include animal waste, plant residues, sewage sludge, and municipal waste compost, materials gaining attention with the rise of organic agriculture. Their key features (affordability, non-polluting nature, and environmental compatibility) support biological activity and enhance various plant capabilities. Organic fertilizers encompass livestock manure, green manure, agricultural waste, and municipal waste compost [16].

2.2 Biofertilizers

Sustainable agriculture requires meeting plant nutritional needs through soil-dwelling living organisms. Biofertilizers provide an effective solution [17]. These products contain sufficient numbers of one or more beneficial soil organisms in a carrier material. Following seed application, they survive in soil, improve fertility, and promote plant growth by increasing beneficial microorganism populations in the root zone [18]. Biofertilizers extend beyond animal manures or plant additives to include products resulting from microbial activity in nitrogen fixation or nutrient availability [12]. Thus, microorganisms are essential to the process. However, constraints such as prolonged environmental stresses (drought, temperature extremes, frost, flooding, excessive pesticide use) and absence of suitable host plants reduce desired microorganism populations in soils [16].

Biofertilizer comprises a high concentration of beneficial soil organisms or their metabolic products combined with a preservative, produced specifically to supply plant nutrients. These microbial inoculants contain effective, highly efficient strains providing one or more plant nutrients [18]. Microorganisms convert nutrients from unavailable to available forms through biological processes. Growth-promoting bacteria act directly through biological nitrogen fixation, phytohormone production, and enhanced nutrient uptake and availability. Optimizing biofertilizer application requires selecting suitable strains with tolerance to environmental stresses [12].

Biofertilizer use reduces chemical fertilizer consumption, consequently lowering transportation, storage, and distribution costs [17]. It maintains and develops soil fertility, prevents pollution from residual chemical fertilizer compounds, and reduces diseases caused by contaminated water and nitrogen-compound-contaminated products [12]. Additional advantages include immediate usability after purchase, small size, light weight, and easy transportability [17]. Classification of biofertilizers has been illustrated in Figure 2.

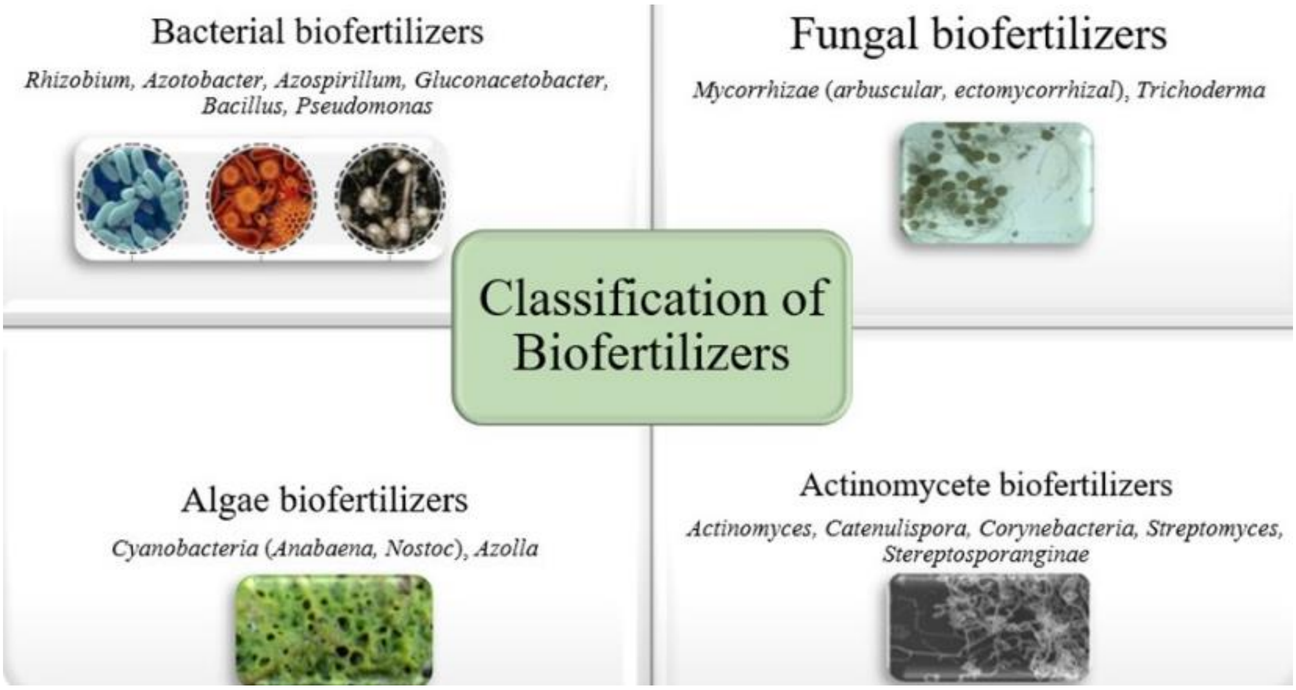

Figure 2. Classification of biofertilizers

3. NITROGEN-FIXING BACTERIA: DIVERSITY, MECHANISMS, AND AGRICULTURAL SIGNIFICANCE

Nitrogen, the most abundant atmospheric element, is essential for living organisms and affects plant growth. Plants cannot directly use atmospheric N_2; diazotrophic microorganisms must transform it through a complex process (Figure 3) into forms absorbable by plant roots [19]. This conversion of N_2 to ammonia by diazotrophic microorganisms constitutes biological nitrogen fixation (BNF). Following photosynthesis, biological fixation of atmospheric nitrogen into ammonia ranks as the second most important biological process on Earth. In the absence of chemical fertilizers or manures, natural ecosystems depend on this biological conversion by diverse prokaryotic microbes [19].

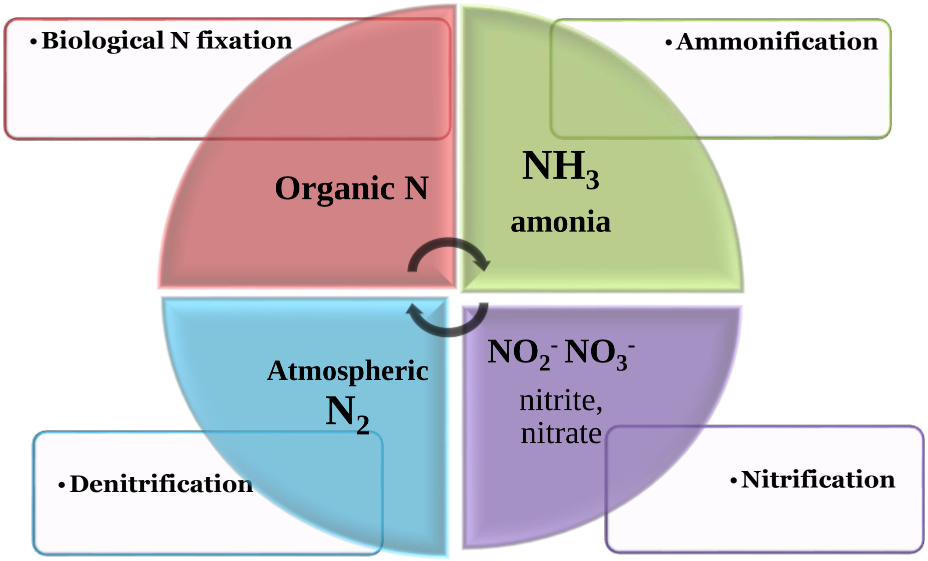

Figure 3. Diagram of the nitrogen cycle.

Bacteria and fungi remain the only organisms capable of converting atmospheric N_2 into plant-usable forms [20]. Symbiotic interactions with plant roots involve various bacterial species: *Azotobacter*, *Enterobacter*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Bradyrhizobium japonicum*, *Serratia*, and *Rhizobium* species [10]. Compost containing nitrogen-fixing microbial strains improves nitrogen metabolism, and adding nitrogen-fixing PGPR to compost enhances plant growth through improved nitrogen availability [16].

In BNF, nitrogenase in diazotrophic microorganisms converts atmospheric N_2 to NH_3. BNF contributes more than 21,013 g of nitrogen globally annually [12]. Nitrogenase comprises two components: a dimeric Fe protein (dinitrogenase reductase) and a heterodimeric MoFe protein (dinitrogenase). The Fe protein functions as an ATP-dependent electron donor; the MoFe protein serves as the catalytic site. Diazotrophs possess molybdenum-iron nitrogenase. Where molybdenum is scarce, some microbes exhibit alternative nitrogenases with vanadium-iron or iron-iron cofactors, documented in *Azotobacter vinelandii* and *Rhodobacter capsulatus*. Nitrogenase is oxygen-sensitive; in the Fe protein, the [4Fe-4S] cluster exposed on the surface bridges the dimer subunits. Nitrogenase requires at least 20 nitrogen fixation (*nif*) genes in aerobic diazotrophic bacteria [12]. Diazotrophic bacteria occur on leaf surfaces and in surrounding areas (phyllosphere) or around roots and rhizosphere of many plant species. Factors affecting diazotroph growth and survival, and consequently nitrogen fixation, include adequate phosphorus supply, inorganic nutrients (especially trace elements), and environmental pH [21]. Nitrogen-fixing bacteria divide into three groups [22]:

Aerobic bacteria: Found in diverse habitats including water, soil, and animal bodies; fix nitrogen in oxygen presence while producing hydrogen during metabolic reactions. Examples include *Azomonas*, *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Derexia*, and *Mycobacterium*.

Anaerobic bacteria: Nitrogen fixation proceeds through enzymatic cycles with nitrogenase transferring atmospheric nitrogen to ammonia. These enzymes are oxygen-sensitive, requiring anaerobic conditions. Important genera include *Clostridium*, *Chlorobium*, *Chromatium*, and *Desulfovibrio*.

Facultative anaerobic bacteria: Include *Bacillus*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Pseudomonas*, and *Rhodospirillum*.

3.1 Azotobacter

Azotobacter biofertilizer meets the nitrogen needs of crops, vegetables, summer crops, and fruit trees [23]. By fixing atmospheric nitrogen and transferring it to plant growth systems, this fertilizer balances absorption of essential substances. Secreting the growth hormone auxin increases root and shoot development, consequently increasing per-unit-area production. Additionally, *Azotobacter* prevents invasion by many soil-borne pathogens through secretion of various antibiotics and hydrogen cyanide [23]. Classification criteria include flagella presence, pigment production, cyst formation, and genetic chara-cteristics (Table 1) [24]. The most common species appear in Figure 4. *Azotobacter chroococcum*, the most important species, produces water-insoluble brown to black pigment, synthesizes and excretes thiamine, riboflavin, pyridoxine, cyanocobalamin, niacin, pantothenic acid, indol-3-acetic acid, and gibberellin, and produces an antifungal antibiotic active against various soil fungi [25].

Table 1. Classification of *Azotobacter*

Domain	Bacteria
Kingdom	Bacteria
Phylum	*Pseudomonadota* (Proteobacteria)
Class	Gammaproteobacteria
Order	*Pseudomonadales*
Family	*Pseudomonadaceae*
Genus	*Azotobacter*
Species	7 species: *A. chroococcum, A. vinelandii, A. beijerinckii, A. nigricans, A. armeniacus, A. salinestris, A. paspali*

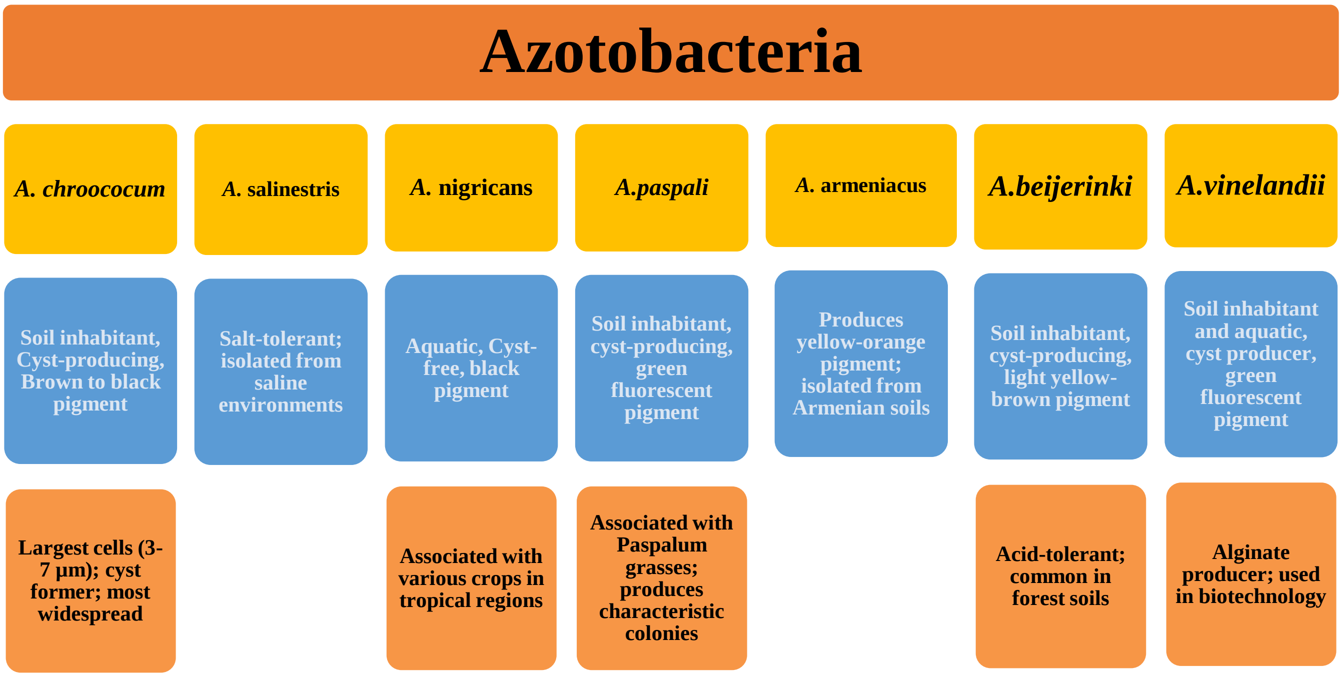

Figure 4. The most common species of *Azotobacter*

Azotobacter is Gram-negative with polymorphic forms (rod, ellipsoid, spherical) 3-7 µm long and 1.5-2 µm diameter, occurring singly, in pairs with regular bundles, sometimes in chains [24]. *Azotobacter* species do not sporulate but typically form cysts. They are motile or non-motile via peripheral flagella. In old cultures, cells become dense and spherical with thickened walls, forming cysts. Cysts resist adverse environmental conditions such as heat and dryness more effectively than vegetative forms. Cyst formation associates with polybeta-hydroxybutyrate (PHB) accumulation, visible as transparent, colorless granules under conventional microscopy. When transferred to a suitable culture medium, cysts germinate: the central part enlarges, cracks appear in the cyst coat, and the cell is released. Under dry conditions, cysts may survive for decades [24]. Optimal activity temperature ranges from 20-30°C. Soil calcium is essential for cyst formation and affects *Azotobacter* distribution [26]. Nutrient levels, especially nitrogen and phosphorus, affect *Azotobacter* growth: phosphate fertilizers increase bacterial growth while excessive nitrogen fertilizers limit it. Typically, *Azotobacter* counts are less than 10^4 per gram of soil. *Azotobacter* grows in habitats including soil, leaf surfaces, freshwater, and various regions from tropical to polar. Abundance varies across soils, primarily in alkaline to neutral soils, and is rare in poor and acidic soils [26]. *Azotobacter* is aerobic and heterotrophic, characterized by non-symbiotic (free-living) nitrogen fixation, distributed in soil, water, and sediments [25].

Its agricultural importance stems from synthesizing plant growth stimulants (auxin, gibberellin, cytokinin), exopolysaccharides, pigments, phosphate solubilization, and siderophore production, which increase growth hormones, root density, and hairy roots, enhancing nutrient absorption, especially macronutrients such as phosphorus and potassium [23, 25]. Additional advantages include antagonistic effects against pathogens, antibiotic and antifungal substance synthesis to combat fungal pathogens (*Fusarium, Sclerotium*, *Rhizoctonia solani*), and production of various vitamins, especially B vitamins, pantothenic acid, and nicotinic acid, vitamin production increases under diazotrophic conditions and in the presence of Lack sufficient carbon nutrition [23]. Azotobacteria also synthesize amino acids including arginine, lysine, tryptophan, histidine, cysteine, and palmitic acid [25].

Azotobacter, alongside *Azospirillum, Klebsiella, Enterobacter*, and *Pseudomonas*, occurs primarily near the roots of gramineous species. Among these, *Azotobacter* receives more attention due to its abundance, wide distribution, and prevalence in temperate soils. In well-drained agricultural soils, free-living *Azotobacter* performs the greatest amount of nitrogen fixation [25].

Molybdenum is essential for nitrogen fixation, though *Azotobacter* can substitute vanadium. Most *Azotobacter* species use ammonium salts, nitrates, and certain amino acids as nitrogen sources [24]. *Azotobacter* possesses active catalase and superoxide dismutase enzymes, essential for protecting nitrogenase from oxygen. Nitrogen fixation rates are influenced by multiple biotic and abiotic factors: carbon, nitrogen, phosphorus, molybdenum, temperature, moisture, nitrification, and soil microbial community [24].

As a heterotrophic bacterium, *Azotobacter* requires soil with high organic matter content for carbon supply. *Azotobacter* exhibits antagonistic relationships with many soil microorganisms, including controlling pathogenic fungal growth [23]. Positive effects of organic matter on *Azotobacter* growth and nitrogen fixation are well established [25]. Increasing carbon supply and creating high C/N ratios enhance *Azotobacter* nitrogen fixation contributions. Plant residues increase soil organic carbon proportions, and growing plant roots also contribute organic carbon. A significant proportion of photosynthetically fixed carbon is released into the rhizosphere as root exudates or decaying root cells. Consequently, soil microorganism populations, including *Azotobacter*, are typically higher in the rhizosphere than in non-rhizosphere soil [25].

3.1.1. Biological control of plant pathogens

Rhizospheric bacteria including *Azotobacter* control diseases such as wheat blight, tobacco black rot, *Fusarium* wilt in tomatoes and beans, and sugarcane root rot [27]. Biological control reduces host plant damage through competitive mechanisms: parasitic relationships, predation, occupation of suitable root sites, or production of growth-inhibiting substances such as bacteriocins, chitinase enzymes, and fungal cell wall dissolution. Beneficial bacteria, including *Azotobacter*, strengthen plants by producing growth-stimulating hormones, amino acids, vitamins, siderophores, and increasing solubility and absorption of nutrients such as iron, phosphorus, and zinc, thus preventing disease development as a secondary complication [27].

3.1.2. Heavy metal bioremediation

Azotobacter effectively removes heavy metals from agricultural and industrial wastewater. These bacteria produce various capsule-like and exopolysaccharide polysaccharides that remove significant amounts of heavy and harmful elements such as lead, nickel, and cadmium. Mercury, harmful to human and animal health, can be decomposed by some *Azotobacter* species synthesizing two enzymes: mercuric reductase and organomercurial lyase. Mercuric reductase converts Hg^{2+} (inorganic mercury compounds) to Hg^0, which volatilizes from the system. Organomercurial lyase first converts organic mercury compounds' carbon bonds to Hg^{2+}, then mercuric reductase converts Hg^{2+} to Hg^0 [28].

3.2 Azospirillum

The genus *Azospirillum* belongs to Alphaproteobacteria. Species are characterized as associative, facultative, endophytic, microaerophilic, and diazotrophic [29]. These microbes are isolated from roots and above-ground parts of various crop plants including forage grasses, cereals, legumes, millets, and soils. They colonise root surfaces and interiors. *Azospirillum* belongs to the family *Rhodospirillaceae* with five major species: *A. brasilense, A. halopraeferense, A. lipoferum, A. amazonense*, and *A. irakense*. *Azospirillum* is Gram-negative, non-nodulating, and an aerobic-associative nitrogen-fixing bacterium. It occurs in plants with C4 dicarboxylic photosynthesis, including sugarcane, maize, sorghum, bajra, and cereals such as wheat, rice, and barley. It produces gibberellins and indole acetic acid, facilitating nutrient uptake and promoting root growth. Carbon sources include organic acids, L-arabinose, D-fructose, D-glucose, sucrose, pectin, and D-gluconate; nitrogen sources include amino acids, ammonium, and ammonia [29]. *Azospirillum* respires aerobically, has nitrate-reducing properties, produces growth-promoting hormones such as auxin, increases water and mineral uptake, root development, and vegetative growth, and fixes 20-40 kg nitrogen per hectare while increasing yields by 15-30% [14].

3.2.1. Azospirillum-based biofertilizers

Azospirillum is applied as a biofertilizer in wetlands across many countries including Italy, Mexico, Belgium, Africa, the USA, Pakistan, France, Germany, Uruguay, Australia, Argentina, and Brazil [12]. Numerous studies investigate *Azospirillum*'s positive impacts on plant life, particularly nitrogen fixation capacity, though recent studies emphasize alternative plant growth-promoting characteristics [30, 31]. A meta-analysis found *Azospirillum* boosted wheat yields (Hedges' g = 1.312) and showed strong efficacy in maize [13]. The beneficial effects of *Azospirillum*-based biofertilizers are shown in **Figure 5.**

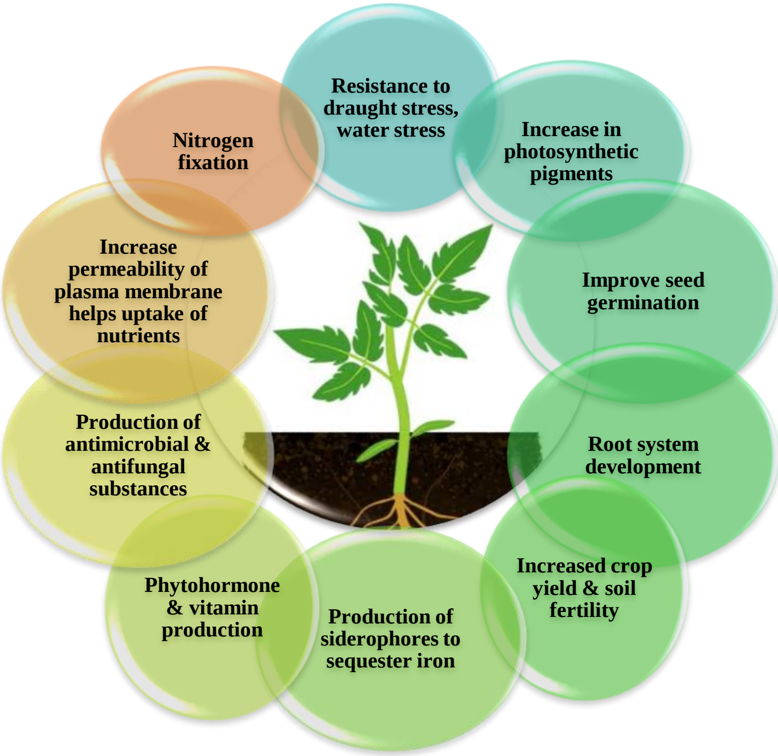

Figure 5. Beneficial effects of *Azospirillum*-based biofertilizers

Siderophores also contribute to growth promotion [29]. Phytohormones enhance nutrient and water absorption by stimulating root system development, and the use of phytohormone-producing *Azospirillum* strains in commercial inoculant formulations is recommended for integration with conventional N-fertilizer doses. Certain *Azospirillum* strains solubilize phosphate, making it bioavailable to plants and contributing to enhanced crop yields [30]. Research on *Azospirillum* inoculation effects across various crops shows notable yield and growth parameter improvements, particularly in wheat and maize, followed by rice and sorghum, with barley and oats receiving less attention. Enhancing efficacy through co-inoculation with other microorganisms improves economic viability [29].

3.3 Rhizobium

Rhizobium occurs naturally in soil and the root environment of leguminous and non-leguminous plants [32]. Nitrogen fixation capacity depends on legume species and environmental conditions [33]. *Rhizobium* fixes 50-100 kg N/hectare per growing season, and biofertilizer application increases legume yields by 10-35% [32]. Meta-analytical evidence shows *Rhizobium* improves wheat yields (Hedges' g =

0.883) [13]. Bacteria grow well on root exudates, though no single exudate component specifically stimulates growth. *Rhizobium* excretes abundant polysaccharides effective in binding soil particles. It survives low temperatures but is sensitive to storage pesticides, antibiotics, and other agricultural chemicals. Acid-producing Rhizobia (*R. phaseoli*, *R. trifolii, R. leguminosarum, R. meliloti*) exhibit fast growth; non-acid-producing Rhizobia (*Azorhizobium, Bradyrhizobium*) grow slowly [33]. *Bradyrhizobium* is unicellular, spherical, rod-shaped, X-shaped, or Y-shaped (0.3-0.5 µm), belonging to *Rhizobiaceae*. They are flagellated, motile, non-nucleated, Gram-negative, chemoheterotrophic, symbiotic with legumes, and aerobically respiring. *Bradyrhizobium* is sensitive to antibiotics produced by other microorganisms and to other bacterial reactions [34]. Various *Rhizobium* strains form nodules on specific leguminous plants, enhancing growth, increasing nutritional value, and improving soil fertility. *Rhizobium* nitrogen fixation reduces legume dependence on chemical fertilizers compared to non-legumes. Inoculating effective rhizobia strains into various legumes significantly increases plant biomass and grain yield [35]. A global meta-analysis of 158 articles from 1980 to 2022 examined synergistic interactions in legume-AMF-rhizobium systems, finding that rhizobial inoculation significantly increased legume plant nitrogen content, phosphorus content, shoot biomass, and yield [36]. Co-inoculation of legume crops with rhizobia and plant growth-promoting rhizobacteria increased yields of common bean (+34%), chickpea (+19%), and soybean (+2%) [37]. Nevertheless, failures and inconsistencies in achieving augmented yields following rhizobia inoculation under field conditions have been documented [35].

3.3.1. Rhizobium-based biofertilizers

The use of rhizobium-based biofertilizers began in India in the early 1970s [32]. The trial's success led to analogous treatments for other legumes and cereal crops [38]. Beneficial effects of *Rhizobium*-based biofertilizers appear in Figure 6.

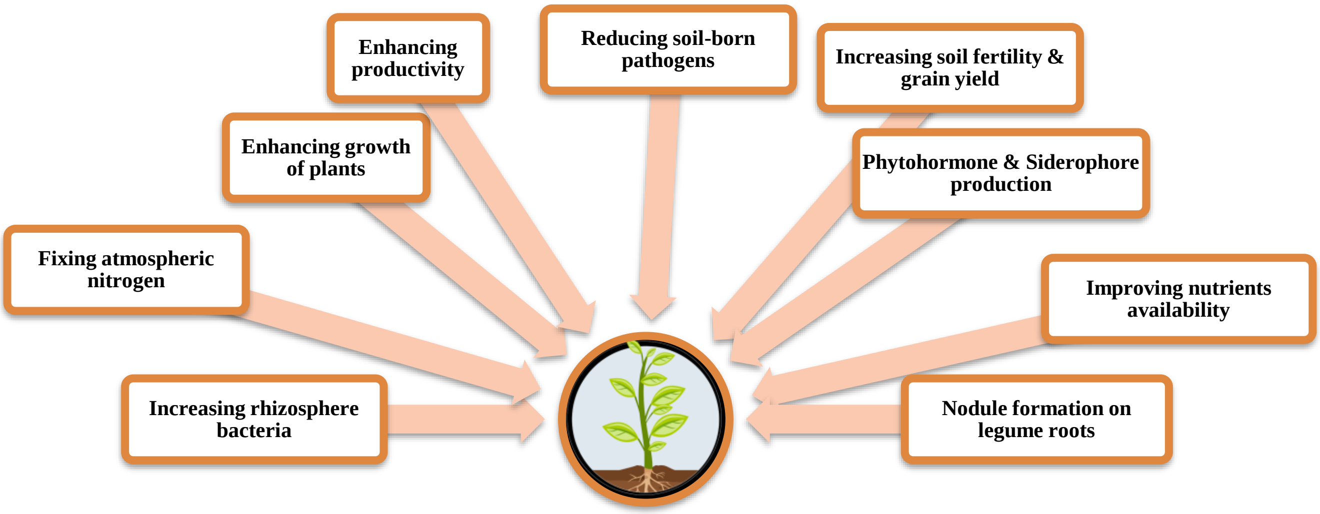

Figure 6. Beneficial effects of Rhizobium-based biofertilizers

Rhizobium biofertilizer production can use molasses as the primary carbon source in growth media, with potential for industrial-scale application [38]. Storage at 4°C is optimal for carrier-based inoculants; sodium alginate is the most effective carrier material [39]. Appropriate media selection positively affects food quality and quantity [38].

Rhizobium specificity and efficacy for leguminous crops depend on host specificity, nodulation capability, nitrogen fixation efficiency, and environmental stress tolerance. Key quality assessment parameters include instructions and labelling, carrier materials, pH, moisture content, cell viability, and shelf life. Regulatory agencies must implement these parameters to guarantee reliability [38]. In the United States, the biofertilizer market is dominated by *Rhizobium* products alongside mycorrhizal fungi and *Azospirillum*. However, the term "biofertilizer" lacks a universally accepted definition, and the US market, projected to exceed $1 billion by 2029, lacks clear production and sale guidelines [6]. *Rhizobium*-derived biofertilizer plays a pivotal role in promoting plant growth. *Rhizobium* enhances growth through phytohormone and siderophore pro-duction [41]. Inoculating cereal grains with *Rhizobium* enhances yield and other advantageous outcomes. *Rhizobium* also serves as a crucial bio-control agent [33]. *Rhizobium* biofertilizers enhance grain yields, particularly in leguminous crops, through root growth stimulation and enhanced nutrient uptake [38]. Application increases the quantity and quality of vital enzymes and hormones, including auxins, essential for plant growth [41]. Optimal outcomes sometimes result from *Rhizobium* application with other microbial strains including *Pseudomonas* and *Bacillus* [38]. Co-inoculation proves superior to single inoculation with rhizobia, improving legume productivity across various agronomic and environmental conditions [37]. Thus, *Rhizobium* biofertilizer application with other mic-robial strains enhances efficiency [38].

3.4.Gluconacetobacter (formerly Acetobacter)

Gluconacetobacter (formerly classified as *Acetobacter*) is Gram-negative and appears as oval or rod-shaped cells. This genus belongs to *Aceto-bacteraceae*, classified as motile, aerobic, and capable of incomplete oxidation of sugars and alcohols to organic acids as metabolic end products. These organisms thrive in highly acidic environments (pH 3.0-3.5), with optimal pH 5.0-6.5. *Gluconacetobacter* accelerates plant growth through growth hormone production [42]. *Gluconacetobacter* has one impor-tant species, *Gluconacetobacter diazotrophicus* (ori-ginally described as *Acetobacter diazotrophicus*), capable of growing on methanol, ethanol, acetate, amino acids, and producing H_2S. This species was discovered in 1988 within Brazilian sugarcane plants and reclassified from *Acetobacter* to *Gluco-nacetobacter* based on 16S rRNA analysis. It oxidizes acetate and lactate to carbon dioxide and water. As an obligate aerobic and nitrogen-fixing bacterium, *G. diazotrophicus* requires oxygen to produce high ATP amounts for nitrogen fixation [42].

Gluconacetobacter diazotrophicus is an endo-phyte, prokaryotes associated with plants through internal tissues [43]. This species does not utilize tricarboxylic acids for growth and adapts to high osmolarity and sucrose content (10-30%). Cultivation with high sugar content reveals that ammonium addition only partially inhibits nitrogenase activity. It exhibits high acid tolerance, effectively fixes nitrogen in nitrate concentrations exceeding 10 mM, and reduces oxygen's detrimental effects on nitrogenase through oxidative metabolism in the periplasmic space at the membrane level [42]. A second nitrogen-fixing species, *Acetobacter nit-rogenifigens* (now considered within *Gluco-nacetobacter*), was isolated from Kombucha tea in India. This species exhibits polar flagella resembling those of *Gluconobacter* and produces brown pigment and γ-pyrene compounds, indicating potential for 2-ketogluconate and 2,5-diketogluconate synthesis as documented in *Gluconobacter* and *Gluconaceto-bacter* research [44]. *Gluconacetobacter diazotro-phicus* produces gibberellic acid and indole acetic acid, aiding root proliferation, nutrient and water uptake, and phosphate solubilization, benefiting sugarcane growth and sugar recovery [12]. Suman et al. [45] applied one *G. diazotrophicus* strain (IS100) with *Azospirillum brasilense* and *Azotobacter chroococcum* to evaluate nitrogen efficiency in Indian sugarcane. *G. diazotrophicus* demonstrated optimal yield results, with combinations showing notable pro-ductivity enhancement. De la Cruz et al. [46] evaluated *G. diazotrophicus* application on sugarcane stem piece germination in the Philippines, testing different cell densities (10^8, 10^{10}, 10^{12} cells/ml) and application methods (spray, 2-hour immersion, 2-minute dipping). Inoculation increased plant survival, height, and shoot/root biomass compared to controls at 45 days after planting. The 10^{12} cells/ml immersion treatment produced the greatest height, biomass, and root mass.

4. EFFECTS OF NITROGEN-FIXING BACTERIAL BIOFERTILIZERS ON AGRICULTURAL PRODUCTIVITY

Recent studies have emphasized the importance of nitrogen-fixing bacterial biofertilizers for increasing agricultural yields. Mondal et al. [47] found that *Rhizobium meliloti* involved in N_2 fixation produced chitinase and increased peanut yields. Alfalfa-*Rhizo-bium* symbiosis enhances nitrogen fixation, phytohormone production, and plant growth [48]. Mfilinge et al. [49] showed that soybean inoculation with *Rhizobium* strains improved growth and yield components, including branches with pods per plant, total pods, and seeds per pod. *Rhizobium legumino-*

sarum inoculation on pea and lentil seeds increased nodulation, shoot and root weight, and seed yield [50]. Variations in rhizosphere microbial communities and their interactions with bradyrhizobia influence soybean symbiotic efficiency [51]. Quality control analysis of *Rhizobium* biofertilizer using coal ash and mulatani mitti as inert solid carriers showed that coal ash supported favorable growth for approximately two months compared to mulatani mitti [52]. Abdallah et al. [53] analyzed co-inoculant attri-butes using charcoal-based carriers stored at ambient temperature (25-30°C) or refrigeration (4°C). Via-bility testing after three months showed sustained counts for both rhizobia and phosphate-solubilizing bacteria. Co-inoculation of *Rhizobium* and phos-phate-solubilizing bacteria enhanced legume yield and seed quality [54]. A global meta-analysis demon-strated that co-inoculation of rhizobia and PGPR increased yields of common bean (+34%), chickpea (+19%), and soybean (+2%), with *Pseudomonas* and *Bacillus* as the most effective genera, followed by *Azo-spirillum*. Microbial consortia also exhibited satisfactory shelf life without antagonistic effects [55]. Sangwan et al. [56] reported that *Bacillus* strain SYB 101 increased wheat (variety WH 711) seed yield by 32% and variety Raj 3765 by 23%, while *Azo-tobacter chroococcum* inoculation caused only a 7.5% increase in Raj 3765. Chaudhary et al. [57] observed that salinity-tolerant *Azotobacter* strains significantly increased total nitrogen, biomass, and grain yield in saline soil under pot conditions, with strain ST 24 at 120 kg N/ha showing the greatest effect. Wang et al. [58] studied *Azotobacter chroococcum* nitrogen fixa-tion, noting a 158% increase when a carbon source was applied at 4 µg/ml due to altered nitrogenase structure. Yousefi and Barzegar [59] reported that combined *Azotobacter* and *Pseudomonas* inoculation increased wheat grain yield, harvest index, biomass, and protein content by 34, 7, 12, &13%, respectively.

Azotobacter inoculation under various conditions has increased crop productivity by 10-12%. Different *Azotobacter* species fix 20-40 kg N/ha/year, and selected strains improve germination, seedling vigour, and wheat grain yield [60]. Jain et al. [60] found that all 24 *Azotobacter* strains produced siderophore, HCN, and ammonia, with genetic analysis revealing high diversity and clustering into two major groups at a 0.64 similarity coefficient. *Azotobacter* inoculation significantly enhanced maize growth parameters in pot studies.

Galindo et al. [61] demonstrated that *Azospirillum brasilense* reduces nitrogen fertilization, enhances plant nutrition, and increases wheat biomass and grain yield. Alen'kina and Nikitina [62] evaluated lectins from *Azospirillum brasilense* strains Sp7 and Sp245 on wheat germination under abiotic stress. Lectins neutralized negative effects of heavy metals, hypo- and hyperthermic stress, salinization, and drought to varying degrees. Heavy metal exposure produced the most significant germination effects. Lectins stimulated wheat seedling root length and number, with Sp245 lectin showing higher efficiency.

Dual inoculation strategies sometimes yield greater plant growth stimulation and yield increases than single inoculation [63]. Combined *Bradyrhizobium* spp. and *Azospirillum brasilense* inoculation significantly increased soybean yield compared to single Bradyrhizobium inoculation in field experiments [64]. Rhizobia with *Anabaena laxa* and *Trichoderma* sp. promoted nodulation, nitrogen fixation, and yields in pea, chickpea, and lentil [65]. Kavadia et al. [66] evaluated symbiotic associations on cowpea in pot-gnotobiotic trials with or without *Sinorhizobium meliloti* and three mycorrhizal fungal species (*Dominikia disticha*, *Claroideoglomus etunicatum*, *Rhizophagus irregularis*). Both mycorrhizal fungi and nitrogen-fixing bacteria increased above-ground biomass, though effects depended on fungal species. Single mycorrhizal inoculation improved cowpea growth and phosphorus uptake, but nitrogen-fixing bacteria were needed to address nitrogen deficiency. Cowpea nitrogen content increased with both symbionts, while phosphorus content was minimally affected. Table 2 shows that recent studies have focused on using biofertilizers enriched with nitrogen-fixing bacteria to increase agricultural efficiency.

Table 2. Using biofertilizers enriched with nitrogen-fixing bacteria to increase agricultural efficiency

Biofertilizer	N-fixing bacteria	Crop	Experimental scale	Environment	Biofertilizer reduction	Main outcome	Mechanism	Ref
N-enriched P- and K-rock biofertilizer	*Beijerinckia indica* NFB10001	Sugarcane	Field split-plot; 4 replicates; 2 years	Brazil; Ultisol, low P/K	Not quantified	Comparable nutrient uptake to soluble fertilizer	BNF + *Acidithiobacillus*-mediated P/K release	[40]
Azospirillum-based inoculant	*A. lipoferum* B1, B2, B3	Wheat	Pot experiment; drought stress	Controlled water stress (80, 50, 25% FC)	Not evaluated	Strain B3: 43-109% yield increase under drought	N fixation + auxin + P solubilisation + ACC-deaminase	[67]
Liquid biofertilizer	*A. brasilense*	Wheat	Two field trials; 4 replicates	Brazil, humid mesothermal	50% (50 vs 100 kg N/ha)	Favourable agronomic performance	Associative N fixation + root stimulation	[68]
Seed inoculant + bioregulator	*A. brasilense*	Maize	Field trial	Brazil	50%	Increased plant height and grain yield	N fixation + phytohormonal stimulation	[69]
N supplementation strategy	N-fixing bacteria	Various	Field/lab studies	Various;	Improved NUE	Enhanced N retention, reduced losses	Biochar + BNF + nitrification inhibition	[70]
Biofertilizer	N-fixing bacteria	Bell pepper	Greenhouse	Controlled	Reduced chemical fertilizer	Improved production and quality	BNF + phytohormone production	[71]
Diazotrophic inoculant	*G. diazotrophicus, Burkholderia, Herbaspirillum*	Sugarcane	Greenhouse/field	Various	Reduced N	Improved rooting and growth	Endophytic N fixation	[72]
Mycorrhizae + endophyte	*H. seropedicae*	Rice	Field	Various	Reduced N/P	Improved nutrient management	Mycorrhizal symbiosis + endophytic N fixation	[73]
Mixed inoculant	*Burkholderia, Herbaspirillum*	Sorghum	Field, increasing N levels	Various	Variable	Enhanced growth	Associative N fixation	[74]
Endophytic *Azotobacter*	*Azotobacter* sp. Avi2	Rice	Greenhouse/field	Various	Reduced N	Increased yield	Endophytic N fixation + growth promotion	[75]
Azotobacter-enriched manures	*Azotobacter* spp.	Various	Field	Various	Reduced N	Increased N fixation and productivity	BNF + organic matter mineralization	[76]
A. chroococcum	*Azotobacter chroococcum*	Cotton	Field	Various	Reduced N	Effective N reduction	BNF + growth promotion	[77]
Diazotrophs + chitosan	Diazotrophic bacteria	Melon	Field	Various	Alternative to conventional	Improved characteristics and uptake	BNF + chitosan-mediated defence	[78]
N-enriched biofertilizer	*B. indica*	Sugarcane	Field, Ultisol	Various	Replacement of soluble	Effective alternative	BNF + rock P/K solubilisation	[79]
G. diazotrophicus	*G. diazotrophicus*	Rice	Field	Various	Reduced N	Improved yield and growth	Endophytic N fixation	[80]
Azospirillum spp.	*Azospirillum* sp.	Potato	Field	Various	Improved NUE	Acts as liming source	BNF + phytohormones + NUE	[81]
A. brasilense	*A. brasilense*	Maize	Field, multiple genotypes	Various	Improved NUE	Promotes growth and NUE	BNF + growth promotion	[82]

BNF :biological nitrogen fixation; N :nitrogen; NUE :nitrogen use efficiency; PK :phosphorus and potassium

5.COMMERCIALISATION CHALLENGES AND REGULATORY FRAMEWORKS

Despite demonstrated benefits, widespread comer-cialization and adoption of nitrogen-fixing bacterial biofertilizers face significant challenges. The global biofertilizer market is projected to reach $5.02 billion by 2030, with cereals and grains accounting for 41.6% of the market share in 2024. However, except for a few successful cases, commercialization of PGPR-based inoculants remains limited, primarily due to inconsistent field efficacy [83].

5.1 Formulation and production challenges

The lack of effective formulation technologies that maintain microbial inoculant viability during storage, transport, and field application is a key driver of Nevertheless inconsistent biofertilizer efficacy. Technology readiness levels for PGPR-based biofertilizers remain low (phases 1–4; laboratory environment). High manufacturing costs, poor quality control, ineffective formulation, short shelf life, and limited awareness further impede wider adoption and commercial success [83].

5.2 Regulatory Frameworks

The term "biofertilizer" lacks a universally accepted definition. The European Union, Brazil, India, and China have made regulatory progress, but the US market (projected to exceed $1 billion by 2029) lacks clear production and sale guidelines. Regulators are tightening quality controls and formalising consistent data requirements [6]. India's Ministry of Agriculture and Farmers Welfare notified the Fertilizer (Inorganic, Organic or Mixed) (Control) Fourth Amendment Order, codifying standards for biostimulants, microbial formulations, and bio-chemical fertilizers, clarifying categories, ingredient specifications, and documentation requirements. The East African Standard DEAS 1176:2023 on Bio-fertilizers - Specifications has been adopted by Uganda [38].

5.3 Paths Forward

Enhancing global biofertilizer adoption requires new innovations based on transdisciplinary approaches. A robust quality control system at all stages is essential. Active partnership between academic and industry stakeholders will pave the way for enhanced global adoption. Future research should focus on modes of action, specific claims, and robust regulations [83].

6. CONCLUSION

Excessive chemical fertilizer applications over recent decades, driven by population growth and rising food demand, has created significant environmental concerns and human health risks. Biofertilizer inoculation offers considerable potential to enhance crop yields while reducing chemical fertilizer use. Bio-fertilizer manufacturing is relatively straightforward and time-efficient, with enhanced shelf life, increased yields, and maintained soil fertility supporting environmentally sustainable agricultural models.

Several plant growth-promoting microbes characterized for beneficial traits play significant roles in improving nutrient availability (particularly nitrogen), modulating phytohormones, suppressing plant diseases, and alleviating abiotic stresses. Inoculation with individual beneficial microorganisms or consortia enhances plant biomass and crop yield under both greenhouse and field conditions. Recent meta-analyses provide quantitative evidence for nitrogen-fixing bacterial inoculation efficacy, with overall positive effects on crop yield (Hedges' g = 1.263). *Azospirillum* shows particular efficacy in wheat (Hedges' g = 1.312) and maize; *Rhizobium* improves wheat yields (Hedges' g = 0.883); *Azotobacter* significantly benefits chickpea (Hedges' g = 0.835). Co-inoculation strategies prove superior to single inoculation, increasing common bean yields by 34%, chickpea by 19%, and soybean by 2%. Current application of organic biofertilizers containing nitrogen-fixing bacteria improves plant health parameters, crop productivity, and soil health. Developing new biotechnological applications requires expanding knowledge and exploiting genomic potential for adaptation, competition, and survival. Introducing species to diverse plants and environmental conditions requires systematic evaluation. Further studies should develop genetically modified nitrogen-fixing bacteria adapted to field conditions, including competition, stress, and microbiota interactions. Addressing formulation challenges, improving quality control, and establishing robust regulatory frameworks are essential for widespread biofertilizer adoption in sustainable agriculture.

7. STATEMENTS & DECLARATIONS

7.1. Acknowledgment

The authors would like to acknowledge the support of Islamic Azad University.

7.2. Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

7.3. Conflict of Interest

There are no competing interests between the authors.

7.4. Ethics

Consent to Participate declaration

This study doesn't have any human or animal samples.

Clinical Trial Number

Not applicable

Human Ethics &Consent to Participate declarations

This study did not involve human participants or animal experiments. Therefore, ethical approval and informed consent were not required.

7.5. Author Contributions

All authors contributed to the study conception and design, data collection and analysis were performed Equally. All authors read and approved the final manuscript."

7.6. Data Deposition Information

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

7.7. Using Artificial Intelligent chatbots

No AI chatbot has been used in this study.

8. REFERENCES

1. Zhang J, Cook J, Nearing JT, Zhang J, Raudonis R, Glick BR, Langille MGI, Cheng Z. Harnessing the plant microbiome to promote the growth of agricultural crops. Microbiol Res. 2021; 245: 126690.

2. Xu J, Li Y, Li L. A comprehensive review of the effects of organic amendments on soil health and fertility: Mechanisms, greenhouse gas emissions, and implications for sustainable agriculture. *Agronomy*. 2025; *15*(12): 2705. https://doi.org/10.3390/agronomy15122705

3. Jote C. The impacts of using inorganic chemical fertilizers on the environment and human health. Organic Med Chem Int J. 2023; 13: 008. https://doi.org/10.19080/OMCIJ.2023.13.555864

4. Chaudhary P, Singh S, Chaudhary A, Sharma A, Kumar G. Overview of biofertilizers in crop production and stress management for sustainable agriculture. Front Plant Sci. 2022; 13: 930340. https://doi.org/10.3389/fpls.2022.930340

5. Daniel AI, Fadaka AO, Gokul A, Bakare OO, Aina O, Fisher S, et al. Biofertilizer: the future of food security and food safety. Microorganisms. 2022; 10(6): 1220. https://doi.org/10.3390/microorganisms10061220

6. Santos F, Melkani S, Oliveira-Paiva C, Bini D, Pavuluri K, Gatiboni L, et al. Biofertilizer use in the United States: definition, regulation, and pros-pects. Appl Microbiol Biotechnol. 2024; 108(1): 511. https://doi.org/10.1007/s00253-024-13347-4

7. Aguilar-Paredes A, Valdés G, Araneda N, Valdebenito E, Hansen F, Nuti M. Microbial community in the composting process and its positive impact on the soil biota in sustainable agriculture. *Agronomy*, 2023; *13*(2): 542. https://doi.org/10.3390/agronomy13020542

8. Zhao X, Li J, Che Z, Xue L. Succession of the bacterial communities and functional characteristics in sheep manure composting. *Biology*. 2022; *11*(8): 1181. https://doi.org/10.3390/biology11081181

9. Mahmoudi M, Asbai Z, Foughal T, Mabicka Obame RG, Ognalaga M, Maloughou Bounangnan JD, et al. Biofertilizers for sustainable and environment-friendly agriculture. Nat Built Social Environ

Health. 2025; 1(5): 49–83.
https://doi.org/10.63095/NBSEH.25.934721

10. Aasfar A, Bargaz A, Yaakoubi K, Hilali A, Bennis I, Zeroual Y, Meftah Kadmiri I. Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. Front Microbiol. 2021; 12: 628379.
https://doi.org/10.3389/fmicb.2021.628379

11. Ma X, Wang L, Xue Z, Wang Q, Qian Y, Yan A, Cai L. The combined application of organic fertilizer and chemical fertilizer increases alfalfa yield, enhances soil nutrient availability, and improves soil biological properties. *Agronomy*. 2026; *16*(8): 795.
https://doi.org/10.3390/agronomy16080795

12. Kumar S, Diksha, Sindhu SS, Kumar R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Curr Res Microb Sci. 2022; 3: 100094.
https://doi.org/10.1016/j.crmicr.2021.100094

13. Lopez-Arredondo EJ, Rodríguez-Zaragoza S, Cabirol N, Perales-Vela HV, Ledesma-Medina KY. Nitrogen-fixing bacterial inoculation: a meta-analysis of its effects on crop productivity and methodological considerations. Cogent Food Agric. 2025; 11(1).
https://doi.org/10.1080/23311932.2025.2448326

14. Sulaiman KH, Al-Barakah FN, Assaeedi AM, Muhammad Dari BA. Isolation and identification of *Azospirillum* and *Azotobacter* species from *Acacia* spp. at Riyadh, Saudi Arabia. Bangladesh J Bot. 2019; 48(2): 239-251.
https://doi.org/10.3329/bjb.v48i2.47546

15. Alom K, Akbar D, Xu CY, Dong TH. Assessing environment impacts of chemical fertilizers consumption in Australia: State-level evidence. Environ Sustain Indicators. 2025; 28: 101053.
https://doi.org/10.1016/j.indic.2025.101053.

16. Ahmed T, Noman M, Qi Y, Shahid M, Hussain S, Masood HA, et al. Fertilization of microbial composts: A technology for improving stress resilience in plants. Plants. 2023; 12(20): 3550.
https://doi.org/10.3390/plants12203550

17. Shahzad M, Hayat R, Mujtaba G, Rehman WU, Nadeem M. Biofertilizers in sustainable agriculture: mechanisms, applications, and future prospects. Discov Agric. 2025; 3: 224.
https://doi.org/10.1007/s44279-025-00318-0

18. Carro L, Nouioui I. Taxonomy and systematics of plant probiotic bacteria in the genomic era. AIMS Microbiol. 2017; 3(3): 383-412.
https://doi.org/10.3934/microbiol.2017.3.383

19. Mgolozeli S, Nciizah AD, Wakindiki IIC, Mudau FN. Nitrogen mineralization of selected organic materials and their combined effects with nitrogen fertilizer on spinach yield. *Plants*. 2024; 13 (14): 1974.
https://doi.org/10.3390/plants13141974

20. Yang H, Li Y, Cao Y, Shi W, Xie E, Mu N, et al. Nitrogen nutrition contributes to plant fertility by affecting meiosis initiation. Nat Commun. 2022; 13: 485. https://doi.org/10.1038/s41467-022-28173-3

21. Kristy B, Davidson J, Evans SE, Tiemann LK. Reduced soil diazotroph diversity decreases nitrogen fixation rates, but depends on land management. Appl Soil Ecol. 2025; 211: 106152.
https://doi.org/10.1016/j.apsoil.2025.106152.

22. Chen SL, Tsai MK, Huang YM, Huang CH. Diversity and characterization of *Azotobacter* isolates obtained from rice rhizosphere soils in Taiwan. Ann Microbiol. 2018; 68: 17–26.

23. Sumbul A, Ansari RA, Rizvi R, Mahmood I. *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci. 2020; 27 (12): 3634-3640.
https://doi.org/10.1016/j.sjbs.2020.08.004

24. Haris M, Hussain T, Tauseef A, Khan A, Khan AA. Application of microbial inoculants as an alternative to chemical products for decomposition of organic wastes. In: Developments in Applied Microbiology and Biotechnology, Microbial Inoculants. Editor(s): Sharma VK, Kumar A, Zambrano Passarini MR, Parmar S, Singh VK. Academic Press. 2023; pp. 29-52. https://doi.org/10.1016/B978-0-323-99043-1.00006-2

25. Wani SA, Chand S, Wani MA, Ramzan M, Hakeem KR. *Azotobacter chroococcum* – A potential biofertilizer in agriculture: An overview. In: Soil Science: Agricultural and Environmental Prospectives. Editor(s): Hakeem K, Akhtar J, Sabir M. Springer. 2016. https://doi.org/10.1007/978-3-319-34451-5_15

26. Kosem N, Ohsaki Y, Watanabe M, Inada M, Song JT, Ishihara T. Exploring *Azotobacter*: A nitrogen-fixing microorganism as a powerhouse for sustainable and green ammonia synthesis. J Appl Microbiol. 2026; 137(4); lxag083. https://doi.org/10.1093/jambio/lxag083

27. Figiel S, Rusek P, Ryszko U, Brodowska MS. Microbially enhanced biofertilizers: Technologies, mechanisms of action, and agricultural applications. *Agronomy*. 2025; *15*(5): 1191. https://doi.org/10.3390/agronomy15051191

28. Suryatmana P, Handayani S, Bang S, Hindersah R. Screening and profiling of mercury-resistant *Azotobacter* isolated from gold mine tailing in Pongkor, West Java. J Degrad Mining Lands Manag. 2024; 11(2): 5287-5300. https://doi.org/10.15243/jdmlm.2024.112.5287

29. Raffi MM, Charyulu PB. *Azospirillum*-biofertilizer for sustainable cereal crop production: Current status. In: Recent Developments in Applied Microbiology and Biochemistry. Editor(s): Viswanath B. Academic Press. 2021; Pp. 193-209. https://doi.org/10.1016/B978-0-12-821406-0.00018-7

30. Fukami J, Cerezini P, Hungria M. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express. 2018; 8: 73. https://doi.org/10.1186/s13568-018-0608-1

31. Yasuda M, Dastogeer KMG, Sarkodee-Addo E, Tokiwa C, Isawa T, Shinozaki S, et al. Impact of *Azospirillum* sp. B510 on the rhizosphere microbiome of rice under field conditions. Agronomy. 2022; 12: 1367. https://doi.org/10.3390/agronomy12061367

32. Amat D, Thakur JK, Mandal A, Sahu A, Reddy KKK. Production and utilization of legume inoculants (*Rhizobium*) in India. Harit Dhara. 2020; 3(2): 28–31.

33. Maitra S, Praharaj S, Brestic M, Sahoo RK, Sagar L, Shankar T, et al. *Rhizobium* as biotechnological tools for green solutions: An environment-friendly approach for sustainable crop production in the modern era of climate change. Curr Microbiol. 2023; 80(7): 219. https://doi.org/10.1007/s00284-023-03317-w

34. Pereira de Moraes Carvalho C, Oliveira AF, Rouws LFM, dos Santos Dourado F, Reed Rodrigues Coelho M, Alves BJR, Zilli JÉ. Genetic Diversity and Nodulation Potential of *Bradyrhizobium* Strains in Cowpea and Soybean. *Plants*. 2025; *14*(24): 3857. https://doi.org/10.3390/plants14243857

35. Goyal RK, Mattoo AK, Schmidt MA. Rhizobial host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. Front Microbiol. 2021; 12: 669404. https://doi.org/10.3389/fmicb.2021.669404.

36. Duan HX, Luo CL, Wang X, Batool A, et al. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi under abiotic stresses: A global meta-analysis. Agronomy. 2024; 14(11): 2597. https://doi.org/10.3390/agronomy14112564

37. da Silva FB, Barbosa JZ, Tiecher T, Borin JBM, Treichel B, de Sá ELS. Species-dependent effect of rhizobacteria co-inoculation in legume plants: A global meta-analysis. Rhizosphere. 2024; 30: 100869. https://doi.org/10.1016/j.rhisph.2024.100869

38. Bahuguna V, Matura R, Farswan AS, Naqvi SS, Sharma N, Chaudhary M. *Rhizobium* as a potential biofertilizer and its quality control analysis for sustainable agriculture. J Appl Biol Biotech. 2025; 13(3): 97-105. http://doi.org/10.7324/JABB.2025.197428

39. Thirumal G, Reddy RS, Triveni S, Damodarachari K, Bhavya K. Evaluate the shelf life of *Rhizobium* carrier based biofertilizer stored at different temperatures at different intervals. Int J Curr Microbiol Appl Sci. 2017; 6(7): 753-759. https://doi.org/10.20546/ijcmas.2017.607.094

40. Oliveira ALM, Santos OJAP, Marcelino PRF, Milani KML, Zuluaga MYA, Zucareli C, Gonçalves LSA. Maize inoculation with *Azospirillum brasilense* Ab-V5 cells enriched with exopolysaccharides and polyhydroxybutyrate results in high productivity under low N fertilizer input. Front Microbiol. 2017; 8: 1873. https://doi.org/10.3389/fmicb.2017.01873

41. Mnalku A, Demise N, Muleta D, Abera Y, Mitiku G. Manual for rhizobial inoculant development and management. Ethiopian Inst Agric Res. 2020. https://doi.org/10.13140/RG.2.2.28906.67521

42. Reis VM, Teixeira KR. Nitrogen fixing bacteria in the family *Acetobacteraceae* and their role in

agriculture. J Basic Microbiol. 2015; 55(8): 931-49. https://doi.org/10.1002/jobm.201400898

43. Ouattara YA, Soro D, Fossou KR, Yebouet ACI, Cissé IA, Akissi DM, et al. Isolation and characterization of indigenous *Acetobacter* strains from cashew apple and their potential use in vinegar production. *Fermentation*. 2025; *11*(1): 38. https://doi.org/10.3390/fermentation11010038

44. Dutta D, Gachhui R. Novel nitrogen-fixing *Acetobacter nitrogenifigens* sp. nov., isolated from Kombucha tea. Int J Syst Evol Microbiol. 2006; 56: 1899–1903.

45. Suman A, Singh P, Lal M. Effects of diverse habitat biofertilizers on yield and nitrogen balance in plant-ratoon crop cycle of sugarcane in Subtropics. Sugar Tech. 2013; 15: 36–43.

46. De la Cruz CPP, Bird CO, Isulat MD. Sprouting, survival and growth of young sugarcane (*Saccharum officinarum L.*) treated with diazotrophic bacteria (*Gluconacetobacter diazotrophicus*). Philipp Agric Sci. 2012; 95: 106–111.

47. Mondal M, Skalicky M, Garai S, Hossain A, Sarkar S, et al. Supplementing nitrogen in combination with *Rhizobium* inoculation and soil mulch in peanut (*Arachis hypogaea L.*) production system: Part II. Effect on phenology, growth, yield attributes, pod quality, profitability and nitrogen use efficiency. Agronomy. 2020; 10: 1513. https://doi.org/10.3390/agronomy10101513

48. Fang L, Ju W, Yang C, Jin X, Zhang C. Exogenous application of signaling molecules to enhance the resistance of legume rhizobium symbiosis in Pb/Cd-contaminated soils. Environ Pollut. 2020; 365: 114744. https://doi.org/10.1016/j.envpol.2020.114744

49. Mfilinge A, Mtei K, Ndakidemi P. Effect of *Rhizobium* inoculation and supplementation with phosphorus and potassium on growth and total leaf chlorophyll (Chl) content of bush bean *Phaseolus vulgaris L.* Agricul Sci. 2014; 5: 1413–1419. https://doi.org/10.4236/as.2014.514152

50. Bourion V, Heulin-Gotty K, Aubert V, Tisseyre P, Chabert-Martinello M, Pervent M, et al. Coinoculation of pea core-collection with diverse rhizobial strains shows competitiveness for nodulation and efficiency of nitrogen fixation are distinct traits in interaction. Front Plant Sci. 2017; 8: 2249–2263. https://doi.org/10.4236/as.2014.514152

51. Han Q, Ma Q, Chen Y, Tian B, Xu L, Bai Y, et al. Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. ISME J. 2020; 14: 1915–1928. https://doi.org/10.1038/s41396-020-0648-9

52. Singh AK, Deepuraj, Masih H, Kumar Y, Peter JK, Mishra SK, et al. Optimization of production parameters and evaluation of shelf life of *Rhizobium* biofertilizers. Elixir Bio Tech. 2014; 67: 21787–21795.

53. Abdallah HA, Elsalahi RH, Mohamed SE. Quality attributes of co-inoculants based on rhizobia and phosphate solubilizing bacteria under different storage conditions. J Agric Vet Sci. 2019; 12(10): 1–7.

54. Osman AG, Rugheim AME, Elsoni EM. Effects of biofertilization on nodulation, nitrogen and phosphorous content and yield of pigeon pea (*Cajanus Cajan*). Adv Environ Biol. 2011; 5(9): 2742–2749. https://doi.org/10.3923/pjn.2011.978.981

55. Sangeetha D, Stella D. Survival of plant growth promoting bacterial inoculants in different carrier materials. Int J Pharm Biol Arch. 2012; 3(1): 170–178.

56. Sangwan VP, Sindhu SS, Dahiya OS, Kharb RPS. Improvement of wheat (*Triticum aestivum L.*) yield inder field conditions by inoculation of microbial strains. Microbiol J. 2012; 2(3): 86–95. https://doi.org/10.3923/mj.2012.86.95

57. Chaudhary D, Narula N, Sindhu SS, Behl RK. Plant growth stimulation of wheat (*Triticum aestivum L.*) by inoculation of salinity tolerant Azotobacter strains. Physiol Mol Biol Plants. 2013; 19: 515–519. https://doi.org/10.1007/s12298-013-0178-2

58. Wang H, Li H, Zhang M, Song Y, Huang J, Huang H. Carbon dots enhance the nitrogen fixation activity of *Azotobacter chroococcum*. ACS Appl Mater Interf. 2018; 10: 16308–16314. https://doi.org/10.1021/acsami.8b03758

59. Yousefi AA, Barzegar AR. Effect of *Azotobacter* and *Pseudomonas* bacteria inoculation on wheat yield under field condition. Int J Agric Crop Sci. 2014; 7: 616.

60. Jain D, Sharma J, Kaur G, Bhojiya AA, Chauhan S, Sharma V, et al. Phenetic and molecular

diversity of nitrogenfixing plant growth-promoting *Azotobacter* isolated from semiarid regions of India. Hindawi BioMed Res Int. 2021; 2021: 6686283. https://doi.org/10.1155/2021/6686283

61. Galindo FS, Pagliari PH, Fernandes GC, Rodrigues WL, Boleta EHM, Jalal A, et al. Improving sustainable field-grown wheat production with *Azospirillum brasilense* under tropical conditions: a potential tool for improving nitrogen management. Front Environ Sci. 2022; 10: 821628. https://doi.org/10.3389/fenvs.2022.821628

62. Alen'kina SA, Nikitina VE. Stimulating effect from lectins of associative bacteria of the genus *Azospirillum* on the germination and morphometric characteristics of spring wheat sprouts in simulated abiotic stress. Russ J Plant Physiol. 2021; 68: 315–321. https://doi.org/10.1134/S1021443721010027

63. Chaudhary SR, Sindhu SS. Growth stimulation of clusterbean (*Cyamopsis tetragonoloba*) by coinoculation with rhizosphere bacteria and *Rhizobium*. Legume Res. 2016; 39(6): 1003–1012. https://doi.org/10.18805/lr.voiOF.8605

64. Hungria M, Nogueira MA, Araujo RS. Soybean seed coinoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. Am J Plant Sci. 2015; 6(6): 8–11. https://doi.org/10.4236/ajps.2015.66087

65. Babu AN, Jogaiah S, Ito SI, Nagaraj AK, Tran LSP. Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant Sci. 2015; 231: 62–73. https://doi.org/10.1016/j.plantsci.2014.11.006

66. Kavadia A, Omirou M, Fasoula DA, Louka F, Ehaliotis C, Ioannides IM. Co-inoculations with rhizobia and arbuscular mycorrhizal fungi alters mycorrhizal composition and lead to synergistic growth effects in cowpea that are fungal combination-dependent. Appl Soil Ecol. 2021; 167: 104013 https://doi.org/10.1016/j.apsoil.2021.104013

67. Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. World J Microbiol Biotechnol. 2011; 27: 197–205. https://doi.org/10.1007/s11274-010-0444-1

68. Piccinin GG, Dan LG, Braccini ALE, Mariano DC, Okumura RS, Bazo GL, Ricci TT. Agronomic efficiency of *Azospirillum brasilense* in physiological parameters and yield components in wheat crop. J Agronom. 2011; 10(4): 132–135. https://doi.org/10.3923/ja.2011.132.135

69. Braccini AL, Dan LGM, Piccinin GG, Albrecht LP, Barbosa MC, Ortiz AHT. Seed inoculation with *Azospirillum brasilense*, associated with the use of bioregulators in maize. Revista Caatinga. 2012; 25(2): 58-64.

70. Su Y, Wang Y, Liu G, Zhang Z, Li X, Chen G, et al. Nitrogen (N) "supplementation, slow release, and retention" strategy improves N use efficiency via the synergistic effect of biochar, nitrogen-fixing bacteria, and dicyandiamide. Sci Total Environ. 2024; 908: 168518. https://doi.org/10.1016/j.scitotenv.2023.168518

71. Nejati Sini H, Barzegar R, Soodaee Mashaee S, Ghasemi Ghahsare M, Mousavi-Fard S, Mozafarian M. Effects of biofertilizer on the production of bell pepper (*Capsicum annuum L.*) in greenhouse. J Agric Food Res. 2024; 16: 101060. https://doi.org/10.1016/j.jafr.2024.101060

72. dos Santos SG, Chaves VA, da Silva Ribeiro F, Alves GC, Reis VM. Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria. Appl Soil Ecol. 2019; 133: 12–23. https://doi.org/10.1016/j.apsoil.2018.08.015

73. Hoseinzade H, Ardakani MR, Shahdi A, Asadi Rahmani H, Noormohammadi G, Miransari M. Rice (*Oryza sativa L.*) nutrient management using mycorrhizal fungi and endophytic *Herbaspirillum seropedicae*. J Integrative Agric. 2016; 15(6): 1385–1394. https://doi.org/10.1016/S2095-3119(15)61241-2

74. dos Santos CLR, Alves GC, de Matos Macedo AV, Giori FG, Pereira W, Urquiaga S, Reis VM. Contribution of a mixed inoculant containing strains of *Burkholderia* spp. and *Herbaspirillum* spp. to the growth of three sorghum genotypes under increased nitrogen fertilization levels. Appl Soil Ecol. 2017; 113:

96–106. https://doi.org/10.1016/j.apsoil.2017.02.008

75. Banik A, Dash GK, Swain P, Kumar U, Mukhopadhyay SK, Dangar TK. Application of rice (*Oryza sativa L.*) root endophytic diazotrophic *Azotobacter* sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. Microbiol Res. 2019; 219: 56–65. https://doi.org/10.1016/j.micres.2018.11.004

76. Rodrigues MÂ, Ladeira LC, Arrobas M. *Azotobacter*-enriched organic manures to increase nitrogen fixation and crop productivity. Eur J Agronom. 2018; 93: 88–94. https://doi.org/10.1016/j.eja.2018.01.002

77. Romero-Perdomo F, Abril J, Camelo M, Moreno-Galván A, Pastrana I, Rojas-Tapias D, Bonilla R. *Azotobacter chroococcum* as a potentially useful bacterial biofertilizer for cotton (*Gossypium hirsutum*): Effect in reducing N fertilization. Revista Argentina de Microbiol. 2017; 49(4): 377-383. https://doi.org/10.1016/j.ram.2017.04.006

78. Silva WO, Stamford NP, Silva EVN, Santos CERS, Freitas ADS, Silva MV. The impact of biofertilizers with diazotrophic bacteria and fungi chitosan on melon characteristics and nutrient uptake as an alternative for conventional fertilizers. Sci Horticul. 2016; 209: 236–240. https://doi.org/10.1016/j.scienta.2016.06.027

79. Oliveira NFL, Oliveira WS, Stamford NP, Silva EVN, Santos CERS, Freitas ADS. Effectiveness of biofertilizer enriched in N by *Beijerinckia indica* on sugarcane grown on an Ultisol and the interactive effects between biofertilizer and sugarcane filter cake. J Soil Sci Plant Nutr. 2017; 17(4): 1040–1057. https://doi.org/10.4067/S0718-95162017000400015

80. Dung TV, Tan DB, Khanh TH, Gale D, Long VV. Effect of *Gluconacetobacter diazotrophicus* inoculation and reduced nitrogen fertilizer on yield and growth parameters of rice varieties. J Seed Sci. 2021; 43: e202143029. https://doi.org/10.1590/2317-1545v43253229

81. Naqqash T, Malik KA, Imran A, Hameed S, Shahid M, Hanif MK, et al. Inoculation with *Azospirillum* spp. acts as the liming source for improving growth and nitrogen use efficiency of potato. Front Plant Sci. 2022; 13: 929114. https://doi.org/10.3389/fpls.2022.929114

82. Zeffa DM, Perini LJ, Silva MB, de Sousa NV, Scapim CA, de Oliveira ALM, et al. *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. PLoS ONE. 2019; 14(4): e0215332. https://doi.org/10.1371/journal.pone.0215332

83. Fadiji A, Xiong C, Egidi E, Singh B. Formulation challenges associated with microbial biofertilizers in sustainable agriculture and paths forward. J Sustain Agric Environ. 2024; 3(3). https://doi.org/10.1002/sae2.70005