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Role of Rhizobium and Non-Symbiotic Plant Growth Promoting Rhizobacteria in Nitrogen Fixation and Growth of *Arachis hypogea*: A Review

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

This review aims to synthesize current knowledge on the role of *Rhizobium* and plant growth-promoting rhizobacteria (PGPR) in enhancing nitrogen fixation and growth in *Arachis hypogaea*. The environmental and agronomic factors influencing microbial effectiveness, highlights recent advances in biotechnological tools and strain improvement, and discusses challenges related to inoculant application and adoption. By integrating data from laboratory, greenhouse, and field studies, this work aims to provide a comprehensive perspective on leveraging microbial solutions for sustainable peanut production. *Rhizobium* and PGPR play a vital role in enhancing nitrogen fixation and improving the growth and productivity of *Arachis hypogaea* (peanut), a globally important legume crop. As an alternative to synthetic nitrogen fertilizers, these soil microorganisms contribute to sustainable agriculture by promoting plant nutrition, improving soil fertility, and supporting environmental health. *Rhizobium*, particularly *Bradyrhizobium* spp., forms symbiotic relationships with peanut roots, resulting in the formation of root nodules that fix atmospheric nitrogen. PGPR, including strains of *Azospirillum*, *Pseudomonas*, and *Bacillus*, support plant growth through various mechanisms such as phosphate solubilization, phytohormone production, siderophore secretion, and induction of systemic resistance. Co-inoculation of *Rhizobium* with PGPR has demonstrated synergistic effects, leading to improved root development, higher nodulation efficiency, increased nitrogen uptake, and enhanced yields under diverse agro-climatic conditions. Despite promising results, several factors limit the consistency and effectiveness of these microbial inoculants, including soil pH, nutrient status, temperature, microbial competition, and host genotype interactions. Issues related to inoculant viability, formulation, delivery, and farmer adoption remain significant constraints. Recent advances in biotechnology, such as genetic manipulation, marker-assisted selection, and omics-based tools, offer new opportunities for strain improvement and a better understanding of plant-microbe interactions. Developing region-specific microbial consortia, improving formulation technologies, and strengthening regulatory frameworks are critical to expanding the use of bioinoculants in legume farming.

Keywords: Arachis hypogaea; rhizobium; PGPR; nitrogen fixation; sustainable agriculture; biofertilizer.

1. INTRODUCTION

For sustainable agriculture, it is essential to efficiently utilize and manage available resources to ensure its long-term viability. This includes proper utilization of the soil, water, and other natural resources for a balanced system, as well as efficient management practices that ensure the good utilization of these resources. The use of rhizobia with non-legume plants is a relatively new area of research that aims at exploring the potential benefits of establishing symbiotic relationships between non-legume plants and these groups of soil bacteria. The idea behind the use of rhizobia with non-legume plants is to extend the benefits of rhizobia to a wider range of crops, which could help to improve soil fertility, increase crop yields, and come up with a synergetic effect with synthetic fertilizers (González-López and Ruano-Rosa, 2020). Plant growth promoting rhizobacteria (PGPR) are rhizobacteria (soil bacteria on the root surface and surrounding) that support plant growth through their various activities, including nitrogen fixation, phosphate solubilization, synthesizing phytohormones such as indole-3-acetic acid (IAA), as well as synthesizing lyses enzymes, such as proteases (Wulandari et al., 2024).

A. *Arachis hypogaea* (peanut) as a Legume Crop

Arachis hypogaea, commonly known as peanut or groundnut, is a geocarpic legume of significant agronomic and economic importance (Akram et al., 2018). Belonging to the family Fabaceae and subfamily Papilionoideae, peanuts are native to South America and are now cultivated in tropical and subtropical regions worldwide. It is a tetraploid species (2n = 4x = 40) believed to have originated from a hybridization event between *Arachis duranensis* and *Arachis ipaensis*. Peanut is primarily grown for their high oil and protein content. The seeds contain about 45–50% oil and 25–30% protein, making them a valuable source of edible oil and dietary protein for human consumption. The crop is also an excellent rotational component in cereal-based systems due to its capacity to fix atmospheric nitrogen and improve soil fertility. Global production of peanuts reached approximately 53 million metric tons in 2022, with major producers including China, Nigeria, Sudan, and the United States.

B. Importance of Nitrogen in Plant Growth and Productivity

Nitrogen is an essential macronutrient involved in the synthesis of nucleic acids, amino acids, chlorophyll, and numerous metabolic compounds. It directly influences vegetative growth, photosynthetic capacity, and yield formation. In legumes such as *Arachis hypogaea*, nitrogen is especially critical during stages of rapid biomass accumulation and seed filling. Nitrogen deficiency in peanut plants leads to symptoms such as chlorosis, stunted growth, reduced leaf area, and lower pod yield. Studies have shown that nitrogen uptake in peanuts can range from 100 to 200 kg N ha^{-1}, depending on the variety, soil fertility, and environmental conditions. Maintaining sufficient nitrogen availability is therefore essential to achieve optimal crop performance.

C. Challenges in Nitrogen Availability and Sustainability in Agriculture

Despite the essential role of nitrogen in agriculture, its management remains one of the most pressing challenges (Liu et al*.,* 2020). The application of synthetic nitrogen fertilizers has significantly increased crop yields over the past century, yet has also led to widespread environmental issues such as nitrate leaching, soil acidification, greenhouse gas emissions, and eutrophication of water bodies. Peanut production often takes place in sandy or low-organic matter soils that are prone to nitrogen losses. Such soils also have poor water and nutrient-holding capacities, exacerbating the inefficiency of fertilizer use. Nitrogen use efficiency (NUE) in agricultural systems is estimated at only 30–50%, indicating that a substantial portion of applied nitrogen is lost to the environment rather than being absorbed by plants. In smallholder farming systems, limited access to chemical inputs and high fertilizer costs further restricts nitrogen use, resulting in low yields and soil fertility decline. These challenges highlight the need for more sustainable nitrogen management strategies.

D. Biological Nitrogen Fixation as an Eco-Friendly Alternative to Synthetic Fertilizers

Biological nitrogen fixation (BNF) offers a natural and renewable solution to nitrogen limitations in agriculture (Bohlool et al*.,* 1992). It involves the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3) through the enzymatic action of nitrogenase, primarily carried out by symbiotic bacteria associated with legumes. In the case of *Arachis hypogaea*, BNF occurs through symbiosis with slow-growing rhizobia of the genus *Bradyrhizobium*. Under optimal conditions, peanut plants can derive 60–80% of their nitrogen needs through symbiotic fixation, equivalent to 70–150 kg N ha^{-1} per growing season. This not only reduces dependence on synthetic nitrogen fertilizers but also improves soil nitrogen balance and benefits subsequent crops in rotation. The environmental advantages of BNF include lower greenhouse gas emissions, reduced nitrate leaching, and long-term improvement in soil microbial diversity and structure. Harnessing this natural process is therefore a cornerstone of sustainable legume-based agriculture.

E. Objective and Scope of the Review

This review aims to synthesize current knowledge on the role of *Rhizobium* and plant growth-promoting rhizobacteria (PGPR) in enhancing nitrogen fixation and growth in *Arachis hypogaea* (Palai et al*.,* 2021). It explores the biological mechanisms underlying symbiosis and microbial interactions, identifies key microbial strains associated with peanut cultivation, and evaluates their impact on root development, nutrient uptake, and yield enhancement. The environmental and agronomic factors influencing microbial effectiveness, highlights recent advances in biotechnological tools and strain improvement, and discusses challenges related to inoculant application and adoption. By integrating data from laboratory, greenhouse, and field studies, this work aims to provide a comprehensive perspective on leveraging microbial solutions for sustainable peanut production.

2. *Arachis hypogaea* (PEANUT)

A. Botanical Description and Taxonomy

The genus Arachis belongs to the family Fabaceae, subfamily Faboideae, and tribe Aeschynomeneae. *Arachis hypogaea L.*, commonly referred to as peanut or groundnut, is an annual legume crop that is geocarpic in nature meaning its fruits develop below the soil surface after flowering above ground. Peanut is an allotetraploid species (2n = 4x = 40) believed to have originated through hybridization between two diploid wild species, *Arachis duranensis* and *Arachis ipaensis* (Bhat et al*.,* 2021). The cultivated varieties are classified into two main subspecies: *A. hypogaea* subsp. *hypogaea* (typically the runner and Virginia types) and *A. hypogaea* subsp. *fastigiata* (including Spanish and Valencia types). Each subspecies shows distinct growth habits, pod characteristics, and maturation periods.

B. Economic and Nutritional Importance

Peanut is one of the most important oilseed crops in the world, ranking fourth globally after soybean, rapeseed, and sunflower. In 2023, global peanut production was approximately 53 million metric tons, with major producers including China, Nigeria, and the United States. Nutritionally, peanuts are rich in protein (25–30%), oil (45–50%), and are a significant source of essential vitamins and minerals. The oil extracted is high in monounsaturated fatty acids, particularly oleic acid, which contributes to cardiovascular health. They also contain bioactive compounds such as resveratrol, flavonoids, and phytosterols, which exhibit antioxidant and anti-inflammatory properties. Peanuts are also a valuable component of crop rotations, contributing to soil health through nitrogen fixation and organic matter improvement (Sun et al*.,* 2025).

C. Growth Requirements and Soil Preferences

Climate: Peanuts are typically grown in tropical and subtropical regions with well-defined warm seasons. Optimal growth occurs at temperatures between 25°C to 30°C, with a minimum temperature threshold of around 18°C for germination. Being a short-day plant, peanut flowering and development are sensitive to day length. The crop requires 500–1000 mm of well-distributed rainfall throughout the growing season, but is vulnerable to waterlogging and prolonged drought stress. Ideal soils for peanut cultivation are sandy loam to loamy soils that are well-drained, friable, and rich in calcium. Soil pH between 5.5 to 7.0 is considered optimal. Soil compaction or heavy clay texture impairs pod penetration and development, leading to reduced yield. Peanut plants are particularly sensitive to calcium deficiency during the pegging and pod formation stages. Gypsum (calcium sulfate) is often recommended as a soil amendment to enhance pod filling and prevent issues such as “pops” (empty pods).

D. Nitrogen Needs of Peanut Crop During Development Stages

Although peanuts are legumes and capable of biological nitrogen fixation (BNF), their nitrogen demands vary significantly across growth stages and depend on nodulation efficiency (Liu et al*.,* 2023). During the first 30 days after sowing, nitrogen uptake is primarily soil-derived as nodulation is not yet fully established. Early application of starter nitrogen (around 15–20 kg N ha^{-1}) is sometimes recommended in nitrogen-deficient soils to promote initial vegetative growth. From 30–70 days after sowing, the peak period of nodulation and BNF occurs. Efficient strains of *Bradyrhizobium* or *Rhizobium* can fix 50–200 kg N ha^{-1} over the crop cycle, depending on host genotype and environmental conditions. During pod filling, nitrogen demand increases once more. If BNF is constrained due to drought or suboptimal inoculation, the crop may exhibit nitrogen deficiency, leading to poor kernel development. A study indicated that peanut can meet 60–80% of their total nitrogen requirement via symbiotic fixation under ideal conditions, with the rest supplemented by soil reserves or organic inputs. Inoculated crops consistently outperform uninoculated ones, particularly in nitrogen-deficient soils.

3. BIOLOGICAL NITROGEN FIXATION (BNF) IN LEGUMES

A. Definition and Mechanism of BNF

Biological Nitrogen Fixation (BNF) is the process through which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) by the action of microorganisms, making nitrogen available in a form that can be utilized by plants (Saha et al*.,* 2017). Unlike chemical nitrogen fixation, which requires high energy inputs, BNF is mediated under ambient environmental conditions and is critical for sustainable agriculture. BNF primarily occurs in leguminous plants through a symbiotic association with diazotrophic bacteria such as *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium*. These bacteria possess the nitrogenase enzyme complex, which catalyzes the conversion of N_2 to NH_3 under anaerobic conditions. The overall reaction is represented by:$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$. The fixed ammonia is incorporated into amino acids like glutamine and transported throughout the plant (Hirel et al., 2001). This process significantly reduces the need for synthetic nitrogen fertilizers and enhances soil fertility.

B. Symbiotic Relationships Between Legumes and Nitrogen-Fixing Bacteria

Legume roots exude flavonoids, which attract compatible rhizobia in the rhizosphere. These molecules induce the expression of nodulation (nod) genes in rhizobia, resulting in the synthesis of Nod factors. These signalling molecules initiate root hair curling and the formation of infection threads. Infection threads guide the rhizobia into the cortical cells of the root, where they differentiate into bacteroids inside the newly formed nodules. Within these specialized structures, the bacteria fix nitrogen and supply it to the plant in exchange for photosynthates (sugars). This mutualistic relationship forms the basis for high-efficiency nitrogen input into cropping systems. The symbiotic association is highly specific. For example, *Arachis hypogaea* typically associates with *Bradyrhizobium* species. The efficiency of nitrogen fixation is dependent on the compatibility between host and bacterial strain, with some combinations resulting in ineffective nodulation (Mus et al., 2016).

C. Role of Leghemoglobin, Nitrogenase, and Nodulation

Leghemoglobin is a hemoprotein found in the cytoplasm of nodule cells. It binds oxygen and maintains a low oxygen concentration inside the nodule to protect the oxygen-sensitive nitrogenase enzyme. At the same time, it supplies sufficient oxygen for the respiration of bacteroids. This dual role ensures efficient nitrogen fixation while preventing enzyme inactivation. The nitrogenase complex is composed of two main proteins: dinitrogenase reductase (Fe protein) and dinitrogenase (MoFe protein). It is highly sensitive to oxygen and requires large amounts of ATP to function. The presence and activity of this enzyme are the biochemical foundation of nitrogen fixation. Nodule development involves the activation of plant genes such as *NIN*, *ENOD*, and *SYM*, and requires hormonal signaling, particularly by auxins and cytokinins. Nodule formation follows a tightly regulated developmental process, which includes initiation, infection, and maturation phases (Verma et al., 1992).

D. Environmental and Genetic Factors Influencing BNF

Soil pH, texture, moisture, and nutrient availability play crucial roles in determining BNF efficiency. Acidic soils (<5.5 pH) often inhibit rhizobial survival and nodulation, while alkaline conditions may affect the availability of essential micronutrients like iron and molybdenum needed for nitrogenase activity. Optimal temperatures for rhizobial activity and nodule development range from 25–30°C. Extremely high or low temperatures reduce bacterial viability and enzyme efficiency, thus lowering nitrogen fixation rates. Host genetic variation determines the ability to form effective nodules and support nitrogen fixation. Some cultivars have been identified to harbor greater nodule numbers and higher nitrogen fixation rates. The selection of responsive legume varieties can enhance overall nitrogen input. Rhizobial strain quality and competitiveness in the rhizosphere are key determinants of successful symbiosis. Effective strains can fix up to 200–300 kg N ha^{-1}, depending on host and environmental factors. Microbial diversity in the rhizosphere, including the presence of antagonistic or synergistic microorganisms (such as PGPR), may either inhibit or enhance nodulation and nitrogen fixation (Wang et al., 2021). Co-inoculation with beneficial microbes has shown promising results in optimizing BNF under field conditions.

4. ROLE OF *Rhizobium* in NITROGEN FIXATION OF *Arachis hypogaea*

A. Specific Strains of Rhizobium Associated with Peanuts

Peanut plants (*Arachis hypogaea*) establish nitrogen-fixing symbioses primarily with members of the genus *Bradyrhizobium*, which are slow-growing rhizobia (Table 1). The most widely recognized species associated with peanuts include *Bradyrhizobium japonicum*, *Bradyrhizobium yuanmingense*, and *Bradyrhizobium elkanii*. These strains are adapted to the peanut rhizosphere and form effective nodules in a host-specific manner. Several isolates of *Bradyrhizobium arachidis*, a more recently described species, have also been identified from peanut-growing regions in diverse environments. The relationship between specific strains and peanut genotypes influences nodulation efficiency, nitrogenase activity, and overall plant productivity. Host-strain compatibility is a major determinant of successful nitrogen fixation, as certain peanut cultivars respond better to indigenous strains than to commercial inoculants. Field evidence suggests that native *Bradyrhizobium* strains are often better adapted to local soil and climatic conditions than introduced or laboratory-isolated strains, leading to higher biological nitrogen fixation (Karimi et al., 2017).

B. Infection Process and Nodule Formation

Peanuts differ from most legumes in their unique mode of rhizobial infection. Instead of root hair invasion, *A. hypogaea* utilizes a "crack entry" mechanism where *Bradyrhizobium* enters through natural fissures or cracks formed near the lateral root junctions. This method of infection bypasses the need for infection threads and is particularly suited to the geocarpic nature of peanuts. Once inside the root cortex, the rhizobia trigger cell division in the adjacent cortical cells, leading to the formation of nodules. These nodules are typically indeterminate, meaning they have a persistent meristem at the tip and continue to grow as new cells are added. Inside the nodules, rhizobia differentiate into bacteroids and begin fixing atmospheric nitrogen into ammonia through the action of the nitrogenase enzyme complex. The fixed ammonia is then assimilated by the plant into amino acids and other nitrogenous compounds. The nodulation process follows a progression that includes cortical cell activation, nodule primordium formation, bacterial colonization, and nodule maturation. Nodule function peaks between 30 and 60 days after emergence, depending on soil conditions and plant health, and then gradually declines as the plant enters the pod filling stage (Puppo et al., 2005).

C. Effectiveness and Efficiency of Different Rhizobium Strains

The effectiveness of *Bradyrhizobium* strains in nitrogen fixation varies considerably based on both bacterial and host plant genotypes. Efficiency is commonly assessed by measuring nodule number, nodule dry weight, nitrogenase activity (using acetylene reduction assays), and total nitrogen accumulation in plant tissues. Some strains have been reported to fix as much as 150 to 200 kg N ha^{-1} under optimal field conditions. Research has shown that inoculation with *Bradyrhizobium yuanmingense* and *Bradyrhizobium elkanii* results in significant increases in biomass and seed yield. *B. yuanmingense* enhanced shoot nitrogen content by 35% and increased pod yield by over 30% compared to uninoculated controls. Similarly, strain-specific interactions with peanut cultivars have demonstrated that selecting compatible combinations can improve nitrogen fixation by up to 50%, emphasizing the importance of host-strain matching. Efficiency also depends on the ability of the strain to compete with native soil rhizobia. In some soils, ineffective indigenous strains dominate the nodulation sites, leading to reduced nitrogen fixation even when effective inoculants are applied. Therefore, competitive ability and persistence in the rhizosphere are key selection criteria in inoculant development.

Table 1. Role of *Rhizobium* in Nitrogen Fixation of *Arachis hypogaea*

Process Stage	Mechanism	Outcome	Importance
Root Hair Infection	*Rhizobium* attaches to root hairs and enters through infection threads	Initiation of nodule primordia	Establishes symbiosis
Nodule Formation	Cortical cells divide and form nodules housing bacteria	Specialized structures (nodules) develop	Provides micro-aerobic environment
Nitrogenase Activity	*Rhizobium* synthesizes nitrogenase enzyme complex under low oxygen (with leghaemoglobin)	Reduction of atmospheric N_2 into ammonia (NH_3)	Primary step of biological nitrogen fixation
Ammonia Assimilation	Ammonia is converted into amino acids (glutamine, asparagine) within plant tissue	Nitrogen becomes plant-usable form	Directly supports protein and nucleic acid synthesis
Plant-Bacteria Exchange	Plant supplies carbohydrates and minerals to *Rhizobium*; bacteria supply fixed nitrogen	Mutualistic nutrient exchange	Enhances groundnut productivity
Soil Fertility Contribution	Fixed nitrogen enriches soil nitrogen pool beyond plant uptake	Improves fertility for subsequent crops	Reduces dependency on synthetic fertilizers

(Source: Karimi et al., 2017)

D. Factors Affecting Symbiosis: Soil pH, Temperature, Moisture

The efficiency of the peanut–*Bradyrhizobium* symbiosis is significantly influenced by soil environmental conditions (Li et al*.,* 2025). Soil pH is a major determinant, with an optimal range between 5.8 and 6.8. Acidic soils inhibit rhizobial proliferation, reduce nod gene expression, and interfere with nodule initiation. Liming acidic soils to raise pH improves nodulation and nodule function. Temperature also plays a crucial role. The optimal temperature range for *Bradyrhizobium* activity and nodule development lies between 25°C and 30°C. High soil temperatures above 35°C can damage both the rhizobia and nodule structure, while temperatures below 20°C can delay nodulation and reduce nitrogenase activity. Soil moisture is essential for successful root colonization and symbiosis. Drought stress during early growth stages impairs rhizobial infection, nodule initiation, and enzyme activity. In contrast, waterlogging reduces oxygen diffusion into the root zone, leading to nodule senescence due to anaerobic conditions. The delicate oxygen balance required for nitrogenase function is also disrupted under poor drainage conditions. Soil phosphorus levels, essential for energy metabolism, also affect nodule formation and nitrogen fixation.

E. Field Studies and Inoculation Experiments

Field trials conducted across various agro-ecological regions have consistently demonstrated the benefits of *Bradyrhizobium* inoculation on peanut growth and yield (Osei et al*.,* 2020). Inoculated plants not only exhibit higher nodulation and biomass but also greater seed yield and protein content. Peanut inoculation resulted in yield increases ranging from 20% to 45%, with an average biological nitrogen fixation of 80–120 kg N ha^{-1} under rainfed conditions. Several experiments have shown that co-inoculation with phosphorus-solubilizing bacteria or plant growth-promoting rhizobacteria (PGPR) along with *Bradyrhizobium* improves root architecture, nutrient uptake, and nodule performance. Dual inoculation improved seed yield by up to 30% and also enhanced soil nitrogen levels post-harvest, contributing to residual soil fertility. Inoculant delivery methods also influence the success of symbiosis. Seed coating and in-furrowapplication are common practices, with peat-based and lignite-based carriers showing higher microbial survival rates compared to other formulations. Shelf-life, cell viability, and microbial compatibility are important considerations in field-scale application of rhizobial inoculants. Yield improvements following inoculation depend on the absence or inefficiency of native rhizobia. In soils with few compatible strains, inoculation can completely transform the nitrogen economy of the crop. Long-term adoption of rhizobial inoculants contributes not only to productivity gains but also to reduced dependency on chemical nitrogen fertilizers and improved sustainability of legume-based cropping systems (Ananda et al*.,* 2022).

5. INTRODUCTION TO PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

A. PGPR

Plant Growth Promoting Rhizobacteria (PGPR) are a diverse group of soil bacteria that colonize the rhizosphere and enhance plant development through a wide range of direct and indirect mechanisms. The term "PGPR" was first introduced in 1978, describing beneficial rhizobacteria that stimulate plant growth through processes such as nutrient solubilization, hormone regulation, and protection from pathogens.PGPR can be classified based on its functions or modes of action. From a functional perspective, they are grouped into two major types: (1) Symbiotic PGPR, which form close associations with host plants (e.g., *Rhizobium* with legumes), and (2) Free-living PGPR, which exert their effects without forming specialized structures. Based on taxonomy, common PGPR genera include *Azospirillum*, *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Serratia*. Some PGPR strains are obligate endophytes while others remain in the rhizosphere, forming loose associations with the plant root surface (Santoyo et al*.,* 2021). Their effects are not limited to nutrient enhancement but also include disease suppression, stress tolerance, and signalling regulation in the plant-microbe interface.

B. Mechanisms of Action

1. Biological Nitrogen Fixation

Certain PGPR, particularly free-living diazotrophs such as *Azospirillum brasilense*, *Azotobacter chroococcum*, and *Herbaspirillum seropedicae*, possess the nitrogenase enzyme that allows them to fix atmospheric nitrogen independently. Although their nitrogen contribution is lower than symbiotic rhizobia, these bacteria still supply 20–

40 kg N ha^{-1} yr^{-1} under optimal conditions. In cereal–legume rotations, their presence can enhance overall nitrogen use efficiency and reduce the reliance on synthetic fertilizers. In the rhizosphere of *Arachis hypogaea*, co-inoculation of *Azospirillum* with *Bradyrhizobium* has shown a synergistic effect, improving both nodulation and shoot nitrogen content by up to 30% in field trials.

2. Phosphate Solubilization

Phosphorus is often present in insoluble forms in soils, bound with calcium, iron, or aluminium (Sanyal et al., 1991). PGPR such as *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Burkholderiacepacia* secrete organic acids (e.g., gluconic acid, citric acid) that chelate metal cations and release soluble phosphate. This mechanism is particularly important in phosphorus-deficient soils where plant-available P is a limiting factor.Phosphate-solubilizing PGPR have been reported to enhance P uptake in peanuts by up to 25%, increasing root biomass and seed phosphorus concentration.

3. Phytohormone Production (Auxins, Gibberellins, Cytokinins)

Several PGPR produce plant hormones that directly affect root development and overall plant architecture. Auxins, especially indole-3-acetic acid (IAA), are among the most common hormones produced by rhizobacteria like *Azospirillum* and *Pseudomonas* spp. These hormones stimulate lateral root formation, root hair proliferation, and increased surface area for water and nutrient absorption. Gibberellins and cytokinins produced by PGPR modulate cell elongation and division, influencing shoot development and leaf expansion. Studies have shown that inoculation with IAA-producing *Azospirillum* strains led to a 15–30% increase in peanut shoot height and early flowering under controlled conditions.

4. Siderophore Production

Iron is an essential micronutrient but is often present in forms that are unavailable to plants (Rout et al., 2015). PGPR produce low molecular weight iron-chelating compounds called siderophores, which bind ferric iron (Fe^{3+}) and make it available to the plant. Siderophore-producing PGPR also inhibit the growth of phytopathogens by depriving them of iron. For example, *Pseudomonas putida* produces pyoverdine-type siderophores that enhance iron availability and suppress fungal pathogens such as *Fusarium* spp., improving plant vigor and survival. In peanut fields, inoculation with siderophore-producing PGPR improved seed iron content and reduced disease incidence by 20–35%.

5. Induced Systemic Resistance

PGPR can trigger a plant's internal defence mechanism known as induced systemic resistance (ISR). This form of resistance primes the plant to respond more effectively to pathogen attacks without directly activating the defence pathway, allowing normal growth to continue.ISR is typically mediated by jasmonic acid and ethylene signalling pathways. PGPR like *Bacillus subtilis* and *Pseudomonas fluorescens* elicit ISR by producing secondary metabolites, lipopeptides, and enzymes that simulate plant immune responses. Experimental studies have shown that ISR-inducing PGPR reduce disease severity in *Arachis hypogaea* caused by soilborne pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii* by up to 40%.

C. Differences and Synergies Between Non-Symbiotic PGPR and Rhizobium

While both PGPR and *Rhizobium* are beneficial microbes residing in the rhizosphere, they differ significantly in function, specificity, and mode of action (Fahde et al., 2023). *Rhizobium* is primarily a symbiotic bacterium that forms root nodules in legumes and directly contributes to nitrogen fixation within a tightly regulated host-specific relationship. In contrast, PGPR encompass a broader category of microbes with diverse taxonomic affiliations that promote plant growth through multiple direct and indirect mechanisms, often without forming specialized structures. PGPR are generally non-host specific and can colonize a wide variety of crops. Their influence is multifaceted modulate hormone levels, enhance nutrient availability, and induce systemic defence. *Rhizobium*, though highly efficient at fixing nitrogen, usually performs a single specialized function and depends on compatible legume hosts. There is growing interest in the use of PGPR and *Rhizobium* in combination. Such synergistic applications often result in greater plant performance than either inoculant alone. Co-inoculation of *Arachis hypogaea* with *Bradyrhizobium* and PGPR such as *Azospirillum*, *Pseudomonas*, or *Bacillus* has been shown to enhance nitrogen fixation, increase phosphorus uptake, improve drought tolerance, and boost pod yield by 15–35% across various studies (Ijaz et al*.,* 2023). This synergy arises from the complementary functions of both groupsPGPR improve root health and nutrient availability, while *Rhizobium* ensures nitrogen supply through biological fixation. This integrated microbial strategy forms a core component of biofertilizer development for legume-based cropping systems.

6. PGPR ENHANCING GROWTH AND NITROGEN FIXATION IN Arachis hypogaea

A. Common PGPR Strains Used with Peanuts (e.g., Azospirillum, Pseudomonas, Bacillus)

Multiple species of Plant Growth-Promoting Rhizobacteria (PGPR) have been successfully utilized in *Arachis hypogaea* to improve nutrient availability, stress tolerance, and biological nitrogen fixation (Table 2). Among the most studied genera are *Azospirillum*, *Pseudomonas*, and *Bacillus*. *Azospirillum brasilense* is a free-living diazotroph that has shown notable benefits in cereals and legumes. Its role in enhancing root surface area through auxin production leads to improved nutrient uptake. In peanut crops, inoculation with *A. brasilense* increased chlorophyll content and stimulated vegetative growth in multiple field trials. *Pseudomonas fluorescens* is widely known for its phosphate-solubilizing capability and its role in producing siderophores and antifungal metabolites. It enhances iron availability and reduces incidence of root and collar rot caused by *Sclerotium rolfsii* in peanuts. *Bacillus subtilis* and *Bacillus megaterium* have also been extensively evaluated for their ability to solubilize phosphates, produce phytohormones, and trigger induced systemic resistance (ISR). Studies demonstrated that *B. subtilis* enhances root length and seedling vigor index in peanuts by over 30% under both sterile and field soil conditions (Kumari et al*.,* 2024). These PGPR strains have been deployed either singly or in combination with nitrogen-fixing bacteria such as *Bradyrhizobium* to maximize peanut growth responses.

Table 2. PGPR Enhancing Growth and Nitrogen Fixation in *Arachis hypogaea (Source: Kumari et al., 2024)*

PGPR Group	Mechanism of Action	Effect on Nitrogen Fixation	Impact on Plant Growth	Examples of PGPR Strains
Rhizobium spp.	Symbiotic nodulation and nitrogenase enzyme activity	Direct biological nitrogen fixation (BNF)	Improves protein, oil content, and yield in groundnut	*Rhizobium* sp. TAL 1000, NC92
Azospirillum spp.	Free-living/associative N_2 fixation and phytohormone production (IAA, GA)	Enhances N availability in rhizosphere	Stimulates root elongation, nutrient uptake	*A. brasilense*, *A. lipoferum*
Azotobacter spp.	Nitrogen fixation, vitamin and growth hormone secretion	Increases soil N content for nodulation support	Enhances seed germination and vigor	*A. chroococcum*, *A. vinelandii*
Pseudomonas fluorescens	Phosphate solubilization, siderophore and IAA production	Indirectly boosts nodule activity by better nutrient mobilization	Improves root architecture and disease resistance	*P. fluorescens* Pf-5
Bacillus spp.	Production of phytohormones, ACC deaminase, and biocontrol compounds	Promotes nodule efficiency under stress	Increases tolerance to drought/salinity	*B. subtilis*, *B. megaterium*
Co-inoculation (PGPR + Rhizobium)	Synergistic interaction, better nutrient cycling	Greater nodule number, size, and nitrogenase activity	Increases pod yield and biomass significantly	*Rhizobium* + *Pseudomonas* or *Azospirillum* consortia

B. Synergistic Effects of Co-Inoculation with Rhizobium and Non-symbiotic PGPR

Co-inoculation refers to the simultaneous application of both nitrogen-fixing rhizobia and non-symbiotic PGPR strains to peanut roots or seeds. This strategy has been shown to provide additive or even synergistic effects, enhancing plant growth and nutrient efficiency more than either microbe alone. Experimental results from pot and field studies demonstrated that co-inoculation of *Bradyrhizobium* with *Azospirillum brasilense* significantly increased nodule number, plant dry matter, and shoot nitrogen content. Co-application of *Bradyrhizobium* and *Pseudomonas fluorescens* resulted in enhanced phosphate uptake and better protection against root pathogens. The combined microbial action led to stronger root systems, higher nodulation efficiency, and increased total biomass accumulation. PGPR assist *Rhizobium* symbiosis indirectly through improved nutrient acquisition (notably phosphorus and iron), production of signalling molecules, and hormone modulation that stimulate nodule organogenesis (Jaiswal et al*.,* 2021). These microbes also enhance microbial colonization and biofilm formation on root surfaces, which promotes root–bacteria interactions under both optimal and stress conditions.

C. Impact on Root Architecture and Nodule Formation

Improved root development is one of the most consistent responses to PGPR inoculation in peanuts. The production of indole-3-acetic acid (IAA) by strains such as *Azospirillum* and *Bacillus* influences cell division and elongation, leading to increased root length, surface area, and number of lateral roots. This expanded root network facilitates more efficient water and nutrient absorption and provides greater surface area for microbial colonization and nodule initiation.PGPR enhance nodule number and size by creating a rhizosphere conducive to early rhizobial infection and cortical cell activation. In a controlled study, co-inoculation of *Bradyrhizobium* with *Bacillus megaterium* resulted in a 40% increase in nodule dry weight and higher nitrogenase activity compared to *Bradyrhizobium*-only treatment. Similar effects have been observed in greenhouse trials where peanut plants developed more effective nodules and maintained prolonged nodule activity under limited phosphorus supply (Asante et al*.,* 2020). Root colonization by PGPR also alters the root exudate profile, which positively affects the population dynamics of beneficial microbes and enhances compatibility with rhizobia.

D. Field and Greenhouse Studies on PGPR Inoculation

Several replicated trials conducted under both greenhouse and open-field conditions have confirmed the beneficial role of PGPR in peanut cultivation. In greenhouse experiments, peanut plants inoculated with *Azospirillum* and *Bradyrhizobium* recorded increases in shoot dry weight by 25% and seedling nitrogen content by 20% over controls. Similar results were observed in field trials across sandy loam and clay loam soils. One field study showed that dual inoculation of *Pseudomonas fluorescens* and *Bradyrhizobium* led to increased pod yield (by 28%), total nitrogen content in shoot tissue (by 35%), and soil available nitrogen at harvest. Yield advantages were consistent across different seasons, indicating long-term benefits of microbial inoculation. Experiments under rainfed conditions also demonstrated that PGPR-inoculated plants maintained higher photosynthetic rates and sustained growth during short periods of drought, possibly due to improved root-to-shoot signaling and osmotic adjustment. Peanut crops inoculated with PGPR exhibited improved nutrient use efficiency, which is reflected in higher nutrient uptake per unit of biomass produced (Jat et al*.,* 2023). Trials combining PGPR with integrated nutrient management showed enhanced uptake of nitrogen, phosphorus, and potassium (NPK), improved soil microbial biomass, and reduced reliance on chemical fertilizers.

E. Yield and Nitrogen Uptake Improvements

One of the most economically significant outcomes of PGPR inoculation in *Arachis hypogaea* is the marked increase in yield and nitrogen use efficiency. Across multiple studies, seed yield gains from 15% to 40% have been reported with the use of PGPR, either alone or in combination with rhizobia. Pod yield increases were attributed to enhanced root growth, improved nutrient uptake, better nodulation, and suppression of root pathogens. In terms of nitrogen uptake, co-inoculated treatments consistently outperformed single inoculant and uninoculated controls. A study reported nitrogen uptake increases of 18–35% in peanut plants treated with *Bradyrhizobium* + *Azospirillum* compared to *Bradyrhizobium* alone. The biological nitrogen fixation potential was also enhanced, as indicated by higher acetylene reduction assay values and increased total nitrogen in the shoot. Harvest index, seed protein content, and soil residual nitrogen also showed improvements in PGPR-inoculated plots, pointing toward long-term sustainability. Enhanced nitrogen assimilation not only supports higher yields but also contributes to soil nitrogen replenishment in rotation systems (Chen et al*.,* 2024).

7. FACTORS INFLUENCING THE EFFECTIVENESS OF RHIZOBIUM AND NON-SYMBIOTIC PGPR

A. Soil Characteristics and Nutrient Availability

Soil properties such as texture, structure, pH, organic matter, and nutrient levels significantly influence the activity and colonization efficiency of both *Rhizobium* and PGPR. Optimal soil pH for effective nodulation and microbial survival lies between 6.0 and 7.0. Acidic soils (pH < 5.5) limit the survival of inoculated rhizobia and interfere with nod gene expression. In these conditions, aluminum toxicity and deficiencies in essential nutrients such as calcium, magnesium, and molybdenum can further hinder microbial activity. Sandy soils, while well-aerated, often lack sufficient organic matter and nutrients, leading to poor microbial persistence. Clay-rich soils may support higher microbial populations due to better moisture retention but can create anaerobic zones that inhibit nitrogenase activity. High levels of available soil nitrogen, especially in nitrate form, suppress nodule formation through feedback inhibition, making nitrogen fixation energetically inefficient. Excessive phosphorus deficiency also affects nodule initiation by limiting ATP generation, which is required for nitrogenase function. Micronutrient availability particularly molybdenum and ironis essential for cofactor assembly in nitrogenase enzymes. Iron is also critical for leghemoglobin synthesis within nodules. Deficiency in any of these elements reduces nitrogen fixation potential, even in the presence of compatible bacterial strain.

B. Climatic and Environmental Conditions

Temperature, moisture, and light availability are among the most influential environmental parameters affecting microbial inoculant performance. The optimal temperature range for most *Bradyrhizobium* and PGPR strains lies between 25°C and 30°C. Low temperatures (<20°C) slow microbial metabolism and delay nodulation, while temperatures exceeding 35°C can reduce survival and activity of inoculated strains. Moisture stress has a direct impact on the symbiotic process. Drought during the early stages of crop growth can reduce bacterial colonization and root exudation, limiting early-stage signalling necessary for nodule formation. Severe drought has been shown to decrease nitrogenase activity by over 50%, resulting in nitrogen-deficient plants and reduced yields. Conversely, waterlogging leads to hypoxic soil conditions that inhibit microbial respiration and induce premature nodule senescence. Solar radiation influences photosynthate availability for microbial partners. Lower light intensity reduces carbon flux to the rhizosphere, weakening the mutualistic relationship. Ultraviolet radiation also poses risks to microbial survival on seed surfaces and during early colonization.

C. Host Genotype Interactions

The effectiveness of *Rhizobium* and PGPR inoculation is partly determined by the genetic characteristics of the host plant (Korir et al*.,* 2017). Variability among peanut cultivars in terms of root architecture, exudation profiles, and receptor proteins affects microbial recognition, infection success, and nodule function. Genotypic differences in flavonoid release from roots influence nod gene induction in rhizobia, altering the extent of colonization and nodulation. Some cultivars show greater responsiveness to inoculation due to improved compatibility with specific bacterial strains. These differences are not limited to nodulation but also extend to traits such as hormone responsiveness and defence signalling pathways. Certain peanut lines have demonstrated superior nodulation with *Bradyrhizobium japonicum* strains, while others perform better with *B. yuanmingense*, reflecting the need for host-specific inoculant development. Moreover, plant-microbe communication through signalling molecules like Nod factors and microbial surface polysaccharides is genotype-dependent. Successful interaction requires genetic compatibility between bacterial symbiotic genes and plant receptors such as NFR1 and NFR5.

D. Compatibility and Competitiveness of Introduced Strains

The success of any microbial inoculant depends on its ability to establish in the rhizosphere and compete effectively with indigenous microbial populations (Alori et al., 2017). Even when highly efficient strains are applied, failure to outcompete native bacteria at the infection sites can result in nodulation by ineffective or non-fixing strains. This competitive exclusion is influenced by bacterial growth rate, chemotactic ability, root colonization efficiency, and resistance to environmental stresses. Compatibility between PGPR and *Rhizobium* strains also determines co-inoculation success. Antagonistic interactions between strains may reduce the benefits of multi-microbial formulations. For example, some PGPR produce antibiotics or siderophores that inadvertently inhibit rhizobial populations. Formulations must therefore be tested for microbial compatibility before field application. Survivability of the introduced strains also plays a crucial role. Strains that can form resilient spores, such as *Bacillus subtilis*, or biofilms on root surfaces tend to establish more successfully (Fessia et al., 2022). Persistence in the soil across crop cycles allows for longer-term benefits from a single inoculation event.

E. Inoculant Formulation and Delivery Methods

Inoculant formulation significantly affects microbial viability, shelf life, and field performance. Carriers such as peat, lignite, vermiculite, and charcoal have been extensively used to protect bacterial cells from desiccation and environmental stress. Peat-based inoculants are among the most effective, supporting microbial viability for 6–12 months under proper storage conditions. Liquid inoculants containing protective polymers and nutrient additives are also gaining popularity due to ease of application and uniform coverage. These formulations often include humic acids, glycerol, or alginates that promote microbial adhesion to seeds and roots. Biofilm-based formulations are under development to improve microbial survival and root colonization efficiency. Delivery methods such as seed coating, slurry dipping, and soil drenching influence inoculant performance. Seed coating remains the most common technique for legumes, though its success depends on compatibility with seed treatments such as fungicides. Studies have shown that in-furrow application of liquid inoculants results in better nodulation and higher rhizobial populations in the rhizosphere compared to seed-applied methods (Denton et al., 2017). Adherence of bacteria to seed surfaces, distribution in the root zone, and timing of application relative to planting date all determine final colonization success. Improper handling, UV exposure, or high storage temperatures can dramatically reduce the viability of applied strains, leading to inconsistent field performance.

8. BIOTECHNOLOGICAL APPROACHES AND GENETIC ENGINEERING

A. Strain Improvement Through Genetic Manipulation

Genetic manipulation of rhizobacteria and nitrogen-fixing strains has become an important avenue for enhancing their efficiency, stress tolerance, and symbiotic capabilities. Classical strain selection based on field performance has now evolved to include targeted genetic engineering approaches aimed at optimizing key functional traits. Mutagenesis, transposon tagging, and CRISPR-Cas9 genome editing have been employed to improve nitrogen fixation, root colonization, and competitiveness in *Rhizobium* and PGPR strains. For example, overexpression of the *nif* gene cluster in *Bradyrhizobium* has been shown to significantly increase nitrogenase activity under low-oxygen conditions. Similarly, genetic enhancement of *Azospirillum brasilense* strains with genes regulating indole-3-acetic acid (IAA) synthesis has led to more consistent stimulation of root growth in legumes including *Arachis hypogaea*. Stress-tolerant genes have also been introduced into microbial genomes to improve performance in saline, acidic, or drought-prone soils. Transgenic *Rhizobium* strains engineered with osmoregulatory genes such as *proP* and *betA* demonstrated increased survival and nodulation under osmotic stress (Chen et al., 2024). While genetically modified microbes offer improved functionality, their environmental release is subject to stringent biosafety regulations. Therefore, ongoing research focuses on non-GMO (genetically improved but non-transgenic) approaches using adaptive evolution and hybridization among compatible strains.

B. Development of Biofertilizer Consortia

The use of microbial consortia composed of complementary PGPR and *Rhizobium* strains represents an advanced strategy to improve the multifunctionality and field reliability of biofertilizers (Behera et al., 2021). These consortia often combine bacteria with different modes of action such as nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis to deliver broader agronomic benefits.Studies involving co-inoculation of *Bradyrhizobium japonicum* with *Pseudomonas fluorescens* and *Bacillus subtilis* have shown enhanced nodulation, biomass accumulation, and nutrient uptake in peanut plants. These microbial combinations support one another through nutrient cross-feeding and cooperative colonization of the rhizosphere. Bioformulation of such consortia requires compatibility testing, synchronized growth in carrier matrices, and long shelf life. Use of polymer-based encapsulation and nanomaterials has improved the stability and targeted delivery of consortia-based inoculants. Studies also reported that consortia-based biofertilizers led to an average 20–35% increase in peanut yield and improved soil nitrogen status over single-strain inoculants (Mishra et al.*,* 2016). Multifunctional biofertilizer consortia represent a shift from single-target inputs toward ecosystem-based microbial technologies that enhance productivity and sustainability in legume production systems.

C. Marker-Assisted Selection for Efficient Nodulation

Breeding legume genotypes with superior nodulation potential and microbial responsiveness is a key aspect of improving nitrogen fixation efficiency. Marker-assisted selection (MAS) has enabled the identification of quantitative trait loci (QTLs) and functional markers associated with nodulation traits such as nodule number, size, nitrogenase activity, and shoot nitrogen content. In peanut, studies have identified significant QTLs for nodulation efficiency on linkage groups A05 and B02, which correlate with increased nitrogen uptake and dry matter accumulation. Genomic selection using these markers facilitates the breeding of peanut cultivars that respond better to inoculants, even in marginal environments. Use of gene expression markers, including *NIN* (Nodule Inception), *ENOD40*, and *LEC*, enables early-stage screening of peanut seedlings for nodulation efficiency and compatibility with *Bradyrhizobium* strains. RNA-based markers are also being applied to monitor the expression of plant and bacterial genes involved in signalling pathways and nitrogen assimilation. Integration of MAS in legume breeding programs accelerates the development of dual-purpose varieties that show high yield potential and improved microbial interactions, contributing to reduced nitrogen fertilizer inputs (Duc et al.*,* 2015).

D. Use of Omics Tools (Genomics, Transcriptomics) in Studying Interactions

Omics technologiesparticularly genomics, transcriptomics, proteomics, and metabolomics have revolutionized the understanding of plantmicrobe interactions and are increasingly applied to improve biofertilizer efficiency.Genomics has enabled full sequencing of both host and microbial genomes, leading to identification of key genes involved in symbiosis, stress response, and nutrient cycling. For instance, the genome sequencing of *Bradyrhizobium diazoefficiens* USDA110 has revealed over 8,300 protein-coding genes, including multiple gene clusters for *nif*, *nod*, and *fix* functions crucial for nitrogen fixation. Transcriptomics allows researchers to monitor gene expression profiles during symbiotic interaction. Time-course RNA-sequencing in peanut nodules has shown differential expression of signalling and nutrient transporter genes during co-inoculation with *Rhizobium* and PGPR. Upregulation of flavonoid biosynthesis and hormone signalling genes reflects a tightly regulated host response to microbial colonization. Proteomics studies provide insight into the post-translational regulation of nitrogenase and leghaemoglobin. Comparative proteome analysis between efficient and inefficient symbioses revealed differential expression of oxidative stress enzymes, nodulins, and ATP synthase subunit. Metabolomics is being applied to identify rhizosphere metabolites that mediate microbial communication and root colonization (Jacoby et al.*,* 2019). Specific exudate compounds, such as organic acids and flavonoids, have been identified as key drivers of PGPR attraction and biofilm formation.

9. CHALLENGES AND LIMITATIONS

A. Inconsistency of Results Across Environments

The performance of *Rhizobium* and PGPR inoculants varies widely under different agro-ecological conditions. Field trials that report significant yield improvements under controlled environments often fail to replicate similar outcomes across variable field conditions. This inconsistency is influenced by a complex interplay of soil type, microbial competition, climate, plant genotype, and agronomic practices. Inoculant performance can fluctuate based on microbial survival, rhizosphere interactions, and abiotic stress tolerance (Khan et al*.,* 2021). For instance, high temperatures and drought during early crop establishment stages reduce the colonization efficiency of introduced strains. A study demonstrated that introduced *Bradyrhizobium* strains nodulated only 10–30% of plants in the presence of abundant but less efficient native populations. Such environmental variability challenges the development of “universal” inoculants and highlights the importance of tailoring microbial solutions to specific agro-climatic zones.

B. Limited Adoption by Farmers Due to Awareness or Cost

Despite scientific validation, the adoption rate of microbial inoculants among smallholder farmers remains low in many peanut-growing regions (Gelaye et al*.,* 2024). Barriers include limited awareness of product benefits, lack of technical knowledge, and insufficient access to high-quality formulations. Cost considerations also play a role. Although microbial inoculants are generally inexpensive compared to synthetic fertilizers, perceived uncertainty in performance reduces farmers’ willingness to invest. A survey found that less than 15% of legume farmers in Sub-Saharan Africa and Southeast Asia regularly used rhizobial inoculants, mainly due to limited extension outreach and absence of localized inoculant supply chains. Education and awareness programs tailored to local contexts are essential to increase confidence in biofertilizer technology.

C. Survival and Efficacy of Inoculants in the Field

Survival of microbial inoculants after application is one of the most critical determinants of their effectiveness. Desiccation, ultraviolet radiation, fluctuating soil moisture, and competition with native microbes can all reduce viable counts of applied strains within days of sowing. Field experiments have shown that the population of viable rhizobia on inoculated seed drops by more than 90% within 24 hours if the seed is not sown immediately, especially under high-temperature conditions. Carriers with poor water-holding capacity or inadequate buffering properties further compromise inoculant viability. Even when viable, introduced strains must successfully colonize roots, overcome competition, and form functional associations. Inoculants that fail to dominate nodule occupancy or survive in bulk soil contribute little to nitrogen fixation or plant growth.

D. Regulatory and Quality Control Issues in Biofertilizer Industry

Inconsistencies in regulatory standards, lack of enforcement, and poor-quality control remain major constraints in the biofertilizer industry. Many commercially available inoculants fail to meet basic microbial count thresholds or contain unverified strains with little or no field efficacy. A survey of 79 commercial biofertilizer samples in Brazil revealed that nearly 60% of products did not contain the labelled microbial species or had insufficient colony-forming units (CFU) per gram. In regions with limited oversight, the problem is compounded by the presence of counterfeit or substandard products in local markets. Standardization of microbial inoculant production, certification protocols, and post-market surveillance are necessary to ensure consistent quality and build trust among end-users.

10. FUTURE

A. Need for Region-Specific Strain Development

Microbial inoculants should be developed and tested in the agro-climatic regions where they are intended to be used (Yahya et al*.,* 2023). Native strains adapted to local soils, climatic stresses, and cropping systems often outperform introduced ones in nodulation efficiency and persistence. Selection of elite rhizobial and PGPR strains from native microbial pools enhances competitiveness and compatibility. Collaborative efforts between microbiologists, agronomists, and plant breeders can accelerate the identification of effective strains suited to specific soil conditions, pH ranges, and host plant genotypes. Establishing regional microbial germplasm banks would support future strain development programs.

B. Integrating non- symbiotic PGPR and Rhizobium Use in Sustainable Agriculture Practices

The role of beneficial microbes in sustainable agriculture is pivotal. Combining PGPR with *Rhizobium* in integrated nutrient management (INM) systems reduces chemical fertilizer input, lowers environmental pollution, and promotes long-term soil fertility. Research has demonstrated that co-inoculated peanut crops can achieve comparable yields to fully fertilized systems, while improving nutrient use efficiency. Inclusion of PGPR-*Rhizobium* formulations in conservation agriculture, organic farming, and agroforestry systems enhances soil microbial biodiversity and resilience against environmental stresses. Promotion of microbial consortia as core components in ecological intensification strategies offers both productivity and sustainability benefits.

C. Improving Formulation and Delivery Systems

Advancements in microbial formulation technology are essential to improve product stability, shelf life, and field performance. Carriers with high moisture retention and microbial compatibility, such as vermiculite, charcoal, and polymer-based materials, have shown better inoculant survival rates under stress. Nanotechnology and encapsulation techniques have also been applied to control microbial release, protect against UV radiation, and promote root-targeted delivery. Liquid and gel-based formulations offer ease of application, especially in mechanized systems. Precision application tools such as seed drill-mounted sprayers, drip irrigation-integrated inoculant delivery, and seed pelleting technologies enhance microbial placement and efficacy, particularly in dryland and rainfed areas.

D. Policy Support and Extension Services for Adoption

Policy that promote microbial technologies can accelerate adoption Government-supported programs that provide subsidized biofertilizers, training workshops, and demonstration trials build farmer confidence and create local demand. Extension services should play a central role in bridging the gap between research and farm-level implementation. Development of localized user guides, inoculant recommendation charts, and farmer-led participatory trials increases awareness and technical competency. Regulatory harmonization across regions, mandatory quality standards, and certification of producers ensure consistent supply of effective inoculants. Involving rural cooperatives and agri-input dealers in distribution networks also enhances availability.

11. CONCLUSION

The use of *Rhizobium* and non-symbiotic Plant Growth-Promoting Rhizobacteria (PGPR) offers a sustainable and efficient approach to enhance nitrogen fixation and growth in *Arachis hypogaea*. These beneficial microbes contribute significantly to plant nutrition, root development, and soil health through mechanisms such as biological nitrogen fixation, phosphate solubilization, and hormone production. Co-inoculation strategies have demonstrated synergistic effects, improving nodulation, nutrient uptake, and yield under both controlled and field conditions. Despite the proven benefits, challenges such as inconsistent field performance, low farmer adoption, and limited quality control hinder large-scale application. Advances in biotechnology, strain selection, and omics technologies provide new avenues for improving microbial efficacy and formulation. Promoting region-specific inoculant development, integrated nutrient management, and farmer-oriented extension services will be key to maximizing the potential of microbial solutions in peanut cultivation and achieving long-term agricultural sustainability.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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