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Potential Use of Eco-Friendly *Rhizobium* Biofertilizer Isolated from *Uraria rufescens* for Sustainable Agricultural Practices: A Review

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ABSTRACT

A bioformulation that contains live organisms is known as biofertilizer. For the final consumer, biofertilizer quality is crucial and needs to be checked at several points. *Rhizobium*, a member of the Rhizobiaceae family, is one such biofertilizer that infects leguminous plant root nodules and converts atmospheric nitrogen to ammonia so that the plants may use it. The root of the well-known medicinal plant *Uraria rufescens* (DC.)Schindl., which grows in the Northeast, is utilized as an ingredient in the ayurvedic remedy "dasamoola." The isolation and potential application of *Rhizobium* from this medicinal plant have not been the subject of any such research. The colony-forming unit count of the plate at various time intervals may be used to assess the viability of cells within the biofertilizer. Pre-culture, broth, and peat tests are used to evaluate the quality of *Rhizobium* biofertilizer. The use and advantages of *Rhizobium* as a biofertilizer, which can be separated from *Uraria rufescens* (DC.)Schindl., for the socioeconomic advancement of this area are highlighted in this paper.
Keywords: *Uraria rufescens* (DC.)Schindl, *Rhizobium* sp. chemical fertilizers, ayurvedic formulation, biofertilizer. sustainable agriculture

INTRODUCTION

The economic and environmental problems brought on by overuse of artificial fertilizers have made sustainable agricultural development a global priority. Nitrogen is one of the most important plant elements that limits crop yield, yet fixing it industrially with chemical fertilizers uses a lot of energy and harms the environment (Purwaningsih, *et al*.,2021). By transforming atmospheric nitrogen into bioavailable forms through microbial activity, biological nitrogen fixation (BNF) provides a sustainable and natural substitute that lessens reliance on artificial nitrogen fertilizers and improves soil health (Kumar *et al*.,2024) .Because of their symbiotic relationship with leguminous plants, *Rhizobium* spp. are among the nitrogen-fixing bacteria that have been investigated the most. These Gram-negative bacteria infect the root hairs of legumes and cause the development of root nodules, which are specialized structures where the nitrogenase enzyme complex converts atmospheric nitrogen (N_2) to ammonia (Nuc and Olejnik, 2025). A mutually beneficial interaction that is necessary for sustainable nitrogen cycling in agroecosystems is formed when the bacteria obtain carbon compounds and a protected microenvironment while the host plant absorbs the ammonia generated (Zhang *et al*., 2024).Recent studies have gone beyond nitrogen fixation to identify rhizobia as multifunctional rhizobacteria that promote plant growth (PGPR). Rhizobial strains can produce phytohormones like indole-3-acetic acid (IAA), improve phosphate solubilization, release siderophores for iron uptake, and provide systemic resistance against plant diseases, according to numerous research (Bagga, *et al*., 2024). Under both favorable and unfavorable climatic circumstances, these extra characteristics greatly improve root architecture, nutrient uptake, and stress tolerance in host plants, increasing overall crop output (Barquero, *et al*., 2022).The selection of effective and regionally adapted strains is a major factor in the efficacy of rhizobial biofertilizers. Native rhizobial populations are often better suited to the soil, climate, and host plant genotypes in their area (Megu, *et al*.,2024). Thanks to new ways of characterizing *Rhizobium* strains, such 16S rRNA gene sequencing and functional characteristic analysis, it is now easier to find and choose the right strains for making biofertilizers (Hakim, *et al*., 2024). Wild and underutilized legumes are a significant but little-researched source of rhizobial diversity. Studies on wild legumes have shown distinct rhizobial strains with enhanced stress tolerance and nutrient-mobilizing capacities, which may make them superior to strains obtained from intensively cultivated crops (Chellem, *et al*.,2024). Tropical and subtropical regions, mainly in Southeast Asia and India, the leguminous plants are easily found which are belongs to the genus *Uraria*, it is member of the Fabaceae family. Because *Uraria* species are members of the legume family, they may naturally establish symbiotic relationships with bacteria that help to fix nitrogen (Chamkhi, *et al*., 2022). Special bacteria found in *Uraria* plants function as natural plant nourishment. Finding these microorganisms might provide farmers with an affordable, environmentally friendly fertilizer which can help in growing crops in poor soil (Navarro, *et al*., 2025). Furthermore, biofertilizers derived from native rhizobia can maintain long-term soil fertility,it will boost soil microbial diversity, and encourage nutrient cycling without having the negative ecological consequences of artificial fertilizers (Madhukar,2021). In order to maintain cell viability and shelf life, *rhizobium*-based biofertilizers are created by mass-producing certain strains and combining them with suitable carrier materials. Recent advancements in carrier-based and liquid biofertilizer technologies have made rhizobial inoculants more stable and efficient in field settings (Shukri, *et al*.,2025).

The isolation of *Rhizobium* from leguminous host plants is a crucial initial step in understanding legume-microbe symbiosis and developing efficient biofertilizer formulations (Bustos-Segura, *et al*., 2024). The root nodules of leguminous plants operate as natural microhabitats for nitrogen-fixing bacteria in a symbiotic manner. *Uraria* rufescens, a wild leguminous plant in the Fabaceae family with the natural capacity to establish symbiotic interactions with rhizobia, is a potential source for the isolation of native *Rhizobium* strains (Mathesius, 2022). Surface sterilization of root nodules is a crucial step in eliminating surface-associated bacteria and ensuring that only endophytic rhizobia are separated. The selected nodules are first cleansed with sterile distilled water and briefly immersed in 70% ethanol to remove surface lipids before being treated for a specific period of time with a sterilizing agent, such as sodium hypochlorite or mercuric chloride (Mataranyika, *et al*.,2024). A loopful of the nodule extract is streaked onto Yeast Extract Mannitol Agar (YEMA), a selective medium commonly used for the isolation of *Rhizobium* species due to its favorable carbon and nitrogen content (Ilangumaran, *et al*.,2024). Proper preservation is essential for maintaining isolates' viability and functional traits for additional characterisation investigations, including morphological, biochemical, and molecular analysis (Sheu, *et al*.,2016). The method Isolating *Rhizobium* from *Uraria rufescens* is essential because wild legumes are often exposed to shifting environmental conditions such nutrients to poor soils, moisture stress, and temperature variations. Rhizobial strains associated with such hosts may have better adaptability, stress tolerance, and nitrogen-fixing efficacy than bacteria obtained from highly controlled agricultural settings (Ahluwalia, *et al*.,2021). These indigenous strains may be more suitable for producing biofertilizers that are safe for the environment and appropriate for local agroecosystems. These studies provide valuable insights into the development of legume–*Rhizobium* symbiosis and expand the pool of beneficial microbial resources available for sustainable agriculture and soil fertility management (Prasad, *et al*., 2026).

Morphological and Biochemical Characterization of *Rhizobium* Strains:

Following separation, *rhizobium* strain characterisation is crucial because it confirms the isolates' identities and provides crucial information about their physiological, metabolic, and behavioral traits. Morphological and biochemical characterisation is the primary and most used technique for rhizobia identification before more involved molecular studies (Haftu, *et al*.,2025). These conventional techniques are reliable, affordable, and provide valuable insights into the diversity and functional potential of rhizobial populations associated with leguminous hosts. The primary approach of morphological characterization is to examine the colony and cellular characteristics of the isolated *Rhizobium* strains grown on selective medium such as Yeast Extract Mannitol Agar (YEMA). When examining colony morphology, factors including size, shape, color, margin, elevation, surface roughness, opacity, and mucilage production are all taken into consideration (Venado, *et al*.,2025). Typical *Rhizobium* colonies are spherical, smooth, convex, transparent to milky white, and mucilaginous due to the release of extracellular polysaccharides (EPS), which are essential for root hair attachment, infection thread development, and nodulation processes (Astuti and Fauzi,2021). The growth pattern of rhizobial isolates on YEMA treated with Congo red dye is a crucial diagnostic feature. Most *Rhizobium* strains are classified as sluggish to moderately growing bacteria; at 28-30°C, they typically take 3-7 days to produce observable colonies on YEMA (Begom, *et al*.,2021). The difference between this growth pattern and that of other rhizospheric bacteria, which often develop rapidly within 24 to 48 hours, is another requirement for presumed identification. Under a microscope, *Rhizobium* strains appear pink and are invariably Gram-negative. Gram staining is commonly used to detect characteristics of cell walls (Mahmood, *et al*.,2023).

Rhizobium isolates' physiological responses, metabolic capacity, and enzymatic activities are evaluated using a variety of procedures in biochemical characterization. These tests are essential for differentiating rhizobia from closely similar bacterial species and understanding their functional diversity (Hegazy, *et al*.,2025). The catalase test is widely used to determine if the catalase enzyme, which breaks down hydrogen peroxide into oxygen and water, is present. Most *Rhizobium* isolates have a positive catalase response, indicating their ability to tolerate oxidative stress throughout both symbiotic and free-living stages (Hawkins and Oresnik, 2022). Similarly, *Rhizobium* typically shows a positive oxidase test, confirming an aerobic respiratory metabolism and suggesting the presence of cytochrome c oxidase (Banjare, *et al*.,2023). Carbohydrate use tests are crucial for biochemical characterization. Mannitol is the primary carbon source in YEMA, and *Rhizobium* species are well-known for their efficient mannitol consumption (El-Zamik and Wright,1987).Other sugars including glucose, sucrose, lactose, maltose, and fructose may be examined to determine carbohydrate usage patterns, which often vary between species and strains and can provide taxonomic clues (Almihyawi, *et al*., 2022). The citrate utilization test is used to find out if isolates can use citrate as their only carbon source. Many *Rhizobium* strains are either weakly positive or citrate-negative, making it difficult to distinguish them from Pseudomonas and other citrate-positive soil bacteria (Tyagi, *et al*.,2017). The urease test is also often employed because certain rhizobial strains exhibit urease activity that helps with nitrogen metabolism and survival in soil conditions (Teresa, *et al*.,2021). Nitrate reduction tests evaluate isolates' capacity to convert nitrate to nitrite or even to gaseous nitrogen molecules. Many *Rhizobium* strains show positive nitrate reduction, indicating their ecological significance in soil cycle nutrients and their involvement in nitrogen transformation pathways other than symbiotic nitrogen fixation (Chaudhary, *et al*.,2023). Starch hydrolysis and gelatin liquefaction assays typically provide negative results for *Rhizobium* because these bacteria typically lack the enzymes amylase and gelatinase (Mir, *et al*.,2024). By searching for negative findings in these tests, rhizobia can be differentiated from other heterotrophic soil bacteria with strong hydrolytic enzyme activity. Physiological investigations, such as growth response to different pH levels and salt concentrations, are frequently included in biochemical characterization (Shrivastava and Kumar, 2015; Riseh, *et al*., 2024).

Importance and Advantages of *Rhizobium* Biofertilizer:

Based on visual and biochemical characterization, *Rhizobium* strains are methodically identified and categorized. These methods provide vital baseline data that guides the selection of possible isolates for testing symbiotic efficiency, assessing plant growth-promoting characteristics, and improving molecular identification (Das, et. al., 2024). Furthermore, understanding the phenotypic variation among rhizobial isolates facilitates the identification of strains with increased adaptability, competitiveness, and biofertilizer potential (Ebrahimi, *et al*., 2022). *Rhizobium* biofertilizer has several agronomic, environmental, and economical advantages, making it a crucial component of sustainable agricultural systems. One of its greatest advantages is its ability to enhance biological nitrogen fixing in leguminous crops through symbiotic root nodule development. By generating substances like cytokinins, gibberellins, and indole-3-acetic acid, *Rhizobium* biofertilizer not only fixes nitrogen but also enhances plant growth and development (Soumare, *et al*., 2020). These phytohormones promote root growth, enhance nutrient and water uptake, and increase overall plant health. These effects benefit both non-leguminous plants and leguminous crops through associative interactions in the rhizosphere (Shahzad, *et al*., 2022). Another important advantage of *Rhizobium* biofertilizer is that it increases soil fertility and microbial activity. Applying beneficial *Rhizobium* strains enhances soil biological health by increasing microbial diversity, encouraging nutrient cycling, and improving organic matter decomposition. These changes enhance the agricultural soils' long-term sustainability, nutrient availability, and soil structure (Wei,*et al*., 2023) *Rhizobium* biofertilizer is also required to increase the availability of essential nutrients like phosphate and micronutrients. Many *Rhizobium* strains can solubilize insoluble phosphates and create siderophores that chelate micronutrients like iron. Increased nutrient absorption efficiency and support for essential physiological processes like photosynthesis and enzyme activity lead to improved crop growth and yield (Alzate, *et al*., 2024). Using *Rhizobium* biofertilizer reduces greenhouse gas emissions associated with the production and excessive use of chemical fertilizers. By lowering nitrate leaching and soil degradation, it preserves ecosystems and promotes climate-resilient agriculture. Furthermore, *Rhizobium* biofertilizers are non-toxic, biodegradable, and safe for humans, animals, and beneficial soil organisms (Pimpalse,2025). Farmers can afford and get *Rhizobium* biofertilizer, particularly in resource-poor areas. By reducing the cost of fertilizer inputs and raising crop productivity and quality, it boosts farm profitability and encourages sustainable living. Therefore, including *Rhizobium* biofertilizer into integrated nutrient management systems is a practical and ecologically conscious approach to modern agriculture (Fasusi, *et al*.,2021).

Effects of Eco-Friendly *Rhizobium* Biofertilizer Isolated from *Uraria rufescens* on:

a) Soybean (*Glycine max*)

The growth, nodulation, and productivity of soybeans (*Glycine max*) have been demonstrated to be significantly improved by the use of environmentally benign *Rhizobium* biofertilizer obtained from *Uraria rufescens*. By infecting root hairs and creating specialized structures called root nodules, where atmospheric nitrogen is physiologically converted into ammonia *Rhizobium* species build a symbiotic relationship with leguminous plants. Inoculating soybeans with effective *Rhizobium* strains increases the number, size, and dry weight of nodules, which improves biological nitrogen fixation and increases the host plant's access to nitrogen (Adinurani, *et al*., 2021). Particularly during nitrogen-demanding growth stages, this improved nitrogen feeding is essential for maintaining vegetative growth and reproductive development (Vincent, 1970;Tomo, *et al*., 2025).*Rhizobium* strains derived from *Uraria rufescens* are used to inoculate soybean plants, which show better vegetative development, including increased plant height, leaf area, root length, and total biomass. These effects are linked to both increased nitrogen fixation and the bacterial isolates' synthesis of compounds that promote plant growth, like indole-3-acetic acid (IAA). IAA improves the efficiency of nutrient and water intake via influencing root elongation and branching. In comparison to uninoculated plants, enhanced root formation promotes improved rhizosphere colonization and nutrient mobilization, leading to strong plant growth (Lebrazi, *et al*., 2020).Research has shown that soybean plants inoculated with efficient *Rhizobium* strains exhibit enhanced photosynthetic activity and overall metabolic efficiency, which eventually results in a higher potential yield (Jaiswal and Dakora,2025).*Rhizobium* biofertilizer made from *Uraria rufescens* enhances soil fertility and health while also promoting plant development. These isolates frequently have other characteristics that promote plant growth, such as phosphate solubilization, siderophore synthesis, and increased soil microbial activity(Öğütcü and Avsar, 2020).Soybean production and yield-attributing characteristics, such as number of pods per plant, seeds per pod, and seed weight, are greatly increased by the use of *Rhizobium* biofertilizer which maintains or increases the crop output by lowering reliance on artificial nitrogen fertilizers (Bahuguna,*et al*.,2025). *Rhizobium* biofertilizer can be derived from *Uraria rufescens* to provide a sustainable substitute for synthetic fertilizers from both an economic and environmental standpoint. It decreases input costs for farmers, minimizes environmental contamination, and cuts greenhouse gas emissions linked to the manufacture of chemical fertilizers. As a result, including these environmentally acceptable biofertilizers into leguminous plants farming systems promotes both economically and ecologically sound farming methods (Chaudhary, *et al*., 2022; O'Callaghan, *et al*., 2022).

b) On Cowpea (*Vigna unguiculata*):

The development, nodulation, and productivity of cowpea (*Vigna unguiculata*), a widely grown legume prized for its nutritional and agronomic significance, are greatly enhanced by the administration of environmentally benign *Rhizobium* biofertilizer derived from *Uraria rufescens*. *Rhizobium* develops a symbiotic association with cowpea roots through the formation of nitrogen-fixing nodules, where ambient nitrogen is converted into ammonia and then absorbed by the plant (Nkot, *et al*.,2025). Vegetative growth indicators such as plant height, leaf count, root development, and total biomass demonstrate notable advantages when cowpea plants are fed *Rhizobium* biofertilizer derived from *Uraria* rufescens. These gains are caused by increased nitrogen availability and the bacterial isolates' production of substances like indole-3-acetic acid (IAA) that stimulate plant development.Hormonal stimulation improves root architecture, which boosts the effectiveness of food and water intake and supports better plant establishment and vigor throughout the growth season (Adeola and Chinwe,2024). Cowpea physiological performance is also enhanced by *rhizobium* injection. Improved nitrogen fixation increases assimilate production and photosynthetic efficiency, leading to a higher concentration of chlorophyll. Improved transfer of photosynthates to developing pods and seeds and faster growth are supported by increased photosynthesis(Allam, *et al*.,2021).Additional characteristics such as promote plant growth, phosphate solubilization, siderophore synthesis, and the expansion of beneficial microbial communities in the soil are improved by the addition of biofertilizer for creating a suitable soil environment for cowpea production and sustain soil productivity over time by increasing the availability of vital minerals and improving nutrient cycling (Kenasa, *et al*.,).Following inoculation with environmentally benign *Rhizobium* biofertilizer, cowpea yield and yield-contributing characteristics, such as number of pods per plant, seeds per pod, and seed weight, are greatly increased. Increased nitrogen availability throughout the blooming and pod-filling stages encourages protein synthesis and seed development, which leads to higher grain yield and improved seed quality. Thus, utilizing *Rhizobium* biofertilizer in sustainable agricultural systems reduces dependency on artificial nitrogen fertilizers while maintaining or increasing cowpea output (Girija, *et al*.,). By lowering the use of artificial fertilizers, *Rhizobium* biofertilizer derived from *Uraria rufescens* can be a cost-effective and ecologically advantageous way to grow cowpeas. This will enhance the long-term soil health against greenhouse gas emissions and soil and water pollution. Therefore, using these biofertilizers into cowpea-based cropping systems encourages sustainable agriculture and enhances food security in resource-constrained places (Zhang,*et al*.,2024).

On Promoting Growth of Non-Leguminous Plants:

Numerous investigations have demonstrated that *Rhizobium* strains obtained from wild legumes, such as *Uraria* rufescens, may also promote the development of non-leguminous plants through rhizospheric and associative connections, even though *Rhizobium* species have historically been associated with symbiotic nitrogen fixing in leguminous plants. In non-legume crops, *Rhizobium* can successfully colonize the rhizosphere and behave as a plant growth-promoting rhizobacterium (PGPR), However, it doesn't result in the development of nodules. By affecting nutrient availability, root development, and soil biological activity, *rhizobium* indirectly encourages plant growth (Bashan, and de-Bashan,2005, Cangioli,*et al*.,2025) . Enhancing the rhizosphere's nitrogen availability is one of the primary methods Non-leguminous plants benefit from *rhizobium* biofertilizer. Certain *Rhizobium* strains that can fix atmospheric nitrogen in a free-living or associative manner can supply small but ecologically significant amounts of biologically fixed nitrogen to the soil. This nitrogen is made available to non-leguminous plants through microbial turnover and root absorption, enhancing growth and reducing the requirement for synthetic nitrogen fertilizers (Pereira,*et al*.,2024). In addition to nitrogen enrichment, *Rhizobium* isolates from *Uraria rufescens* often exhibit traits that support plant growth, such as the production of phytohormones, particularly indole-3-acetic acid (IAA). IAA is necessary for the growth of root hair, lateral root formation, and root elongation in non-leguminous plants. The efficiency of water and nutrient absorption is increased by improved root architecture, which is especially beneficial in low-nutrient soil (Spaepen,*et al*., 2007). Furthermore, *Rhizobium* biofertilizer increases phosphate and micronutrient availability in non-leguminous crops. Many strains can solubilize insoluble phosphate complexes by generating organic acids and mobilize micronutrients like iron by generating siderophores. These strategies enhance nutrient accessibility and promote healthy plant development by promoting essential physiological processes like photosynthesis and enzyme activity (Richardson, *et al*.,2009). *Rhizobium* biofertilizer also improves soil microbial diversity and the overall health of the rhizosphere. Beneficial bacteria improve nutrient cycling, organic matter decomposition, and soil structureThese alterations in soil biological properties promote the growth of non-leguminous plants and improve long-term soil fertility and sustainability (Biswas, *et al*.,2024). *Rhizobium* strains derived from wild legumes, like *Uraria* rufescens, can help non-leguminous plants endure abiotic problems including drought, nutrient shortages, and soil degradation. These strains are often well suited to harsh situations. By improving root development, nutrient absorption efficiency, and hormonal balance, these bacteria boost plant resistance and productivity in less than optimal growing conditions (Vessey,2003, Du, *et al*., 2025,Hamdi, *et al.,*2026).

CONCLUSION

Rhizobium biofertilizer is a sustainable and versatile microbial input that may benefit both leguminous and non-leguminous plants, as the current synthesis clearly demonstrates. By means of root nodule formation , *Rhizobium* and legumes have a highly developed symbiotic association that leads to efficient biological nitrogen fixation, which significantly enhances plant growth, yield, and soil nitrogen status. This combination reduces the need for artificial nitrogen fertilizers while increasing crop output and soil fertility. Despite the lack of conventional nodulation, *Rhizobium* colonizes the rhizosphere and affects plant development by associative nitrogen fixation, phytohormone synthesis, nutrient solubilization, and increased soil microbial activity in non-leguminous plants. These indirect mechanisms enhance photosynthetic efficiency, root development, nutrient absorption, and stress tolerance in non-legume crops. Recent studies have shown that *Rhizobium* strains derived from wild legumes, such as *Uraria* rufescens, are very desired due to their adaptability, metabolic flexibility, and multifunctional plant growth-promoting traits. Their use encourages improved soil health, enhanced nutrient cycling, and long-term agricultural sustainability. Therefore, *Rhizobium* biofertilizers show a lot of potential as ecologically acceptable inputs in integrated nutrient management systems for a range of cropping patterns, including non-legumes and legumesIncreased usage of these biofertilizers supports global efforts for sustainable food production, encourages environmentally conscious farming, and reduces dependency on chemical fertilizers.

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