

Rhizobium-Based Biofertilizer for Rational Use of Mineral Nitrogen Fertilizers: Case Study of Common Bean Cultivation in Senegal

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Abstract

Synthetic fertilizers are widely used to address the urgent challenge of ensuring food supplies for a growing world population in the context of climate change. However, their industrial production and use in agriculture have a negative impact on the environment and consequently on human health. While chemical fertilizers may not have to be abandoned in agricultural production systems, limiting their use could help to make agriculture sustainable and resilient to climate change. In Senegal, the level of mineral fertilizers used in market gardening has become alarming in the Niayes area. As a result, microbial biotechnologies have been promoted for biofertilizer production of common bean (*Phaseolus vulgaris* L.) cultivation. Rhizobial inoculums have thus been used to reduce the rate of chemical nitrogen fertilizers being applied in cropping systems. Several investigations in the laboratory, on experimental stations and in the field have shown a possibility of a significant reduction in the use of nitrogen fertilizers in common bean production. Conventional mineral fertilization use can be reduced from over 120 kg N/ha to 20 kg N/ha. This contributes both to a very significant reduction in the application rate with the same level of yield and to an improvement in the standard of living. In addition, the environmental impact of using chemical fertilizers can be mitigated. This study is a contribution to the promotion of biofertilizers adoption in agricultural systems.

Keywords

Sustainable Agriculture, Mineral Fertilizer, Inoculation, Rhizobium, Common Bean

1. Introduction

Increasing land degradation is a major issue for global agriculture [1]. Thereby, the loss of fertility in arable land is a major contributor to crop yield drop. To cope with this situation, agricultural production systems are being intensified to boost crop yields. The use of chemical fertilizers has become essential to compensate for the low nutrient content of arable lands. However, the farming systems adopted have shown their vulnerability, with risks of worsening land degradation and threats to ecosystems and the environment.

Since agricultural yields are intimately related to plant nitrogen nutrition, the manufacture of chemical nitrogen fertilizers using industrial processes has expanded rapidly worldwide [2]. However, this industrial synthesis of nitrogen fertilizers leads directly to environmental damage linked to greenhouse gas emissions [3]. Moreover, their use in agriculture has a number of devastating side-effects, such as water and air pollution and biodiversity erosion, with adverse consequences for public health [4] [5]. Meanwhile, the management of natural microbial resources in their diversity can address the challenge of quantitative and qualitative agricultural production while preserving the environment. Hence, the relationship between plant production and beneficial soil microorganisms remains a promising approach to exploring high-performance and sustainable agricultural systems.

A strategy for adapting crops to various biotic and abiotic constraints based on exploiting the biodiversity of soil microorganisms is conceivable. Indeed, the presence of microorganisms in the plant rhizosphere enhances the performance of cropping systems by strengthening their resistance to various types of stress [6] [7].

Horticultural crops are primarily planted in the peri-urban regions of major coastal cities in Senegal. A lot of strain is being placed on arable land, which is no longer enough to sustain horticultural production, in order to meet the rising demand for market gardening goods for both domestic and foreign markets. Farmers, who are compelled to use excessive amounts of chemical fertilizers, are very interested in producing the common bean (*Phaseolus vulgaris* L.), an off-season cash crop, in this complicated circumstance.

Microbial biofertilizers have been used for common bean cultivation in an effort to ensure sustainable output that conforms with current phytosanitary regulations [8]. Therefore, a technology package is recommended to rationalize chemical nitrogen fertilizer application rates. It entails formulating an inoculum based on nitrogen-fixing bacteria known as rhizobia. This proven biotechnology, which has been extensively used globally, was long tested in Senegal [9]. It has been suggested to farmers as a substitute for the misuse of mineral nitrogen fertilizers. Because it lessens the harmful environmental impact of chemical fertilizers in agriculture, it is therefore an effective and sustainable method of fertilization. This article aims to evaluate the main results of inoculating common bean with rhizobia and its impact on the use of mineral nitrogen in Senegal.

2. Common Bean Production in Senegal

2.1. Socio-Economic Importance of Bean Growing

Beans are a staple food in many countries around the world. The seeds can be eaten fresh or dried. Its wide popularity is due to the quality of its nutritious content. In fact, it provides a wide range of dietary fiber, vitamin, protein and mineral contents [10] [11]. Accordingly, common beans are regarded as a source of a number of minerals, including calcium, zinc, iodine, magnesium, potassium, phosphorus, and iron. As a result, it can be very helpful to tackle food insecurity.

Domestic bean consumption in Senegal is mainly restricted to cities. A significant source of foreign exchange for the nation, more than 80% of harvest is intended for export to the European market. A sizable amount of off-season horticulture product exports is made up of beans. Bean yields are between 6 and 12 T/ha for the most widely used bean varieties, and they can reach 20 T/ha worldwide [12]. This wide variability is mainly linked to crop varieties, cropping systems and environmental factors.

2.2. Conventional Bean Cultivation

The common bean is a seed legume with a wide morphological variability. It is grown in both temperate and tropical zones at optimum growing temperatures of between 20°C and 25°C. In Senegal, these conditions are obtained during the Senegalese dry season from November to April, particularly in the agroecological Niayes zone.

To ensure high yields, farming systems need to be intensified, while the already limited arable land continues to be depleted. Paradoxically, fresh manure poorly decomposed used as organic fertilizer poses a phytosanitary danger, including damping-off. In this context, some farmers are attracted by the excessive use of chemical fertilizers to improve crop yields. Recommended mineral nitrogen fertilizer inputs of over 300 kg urea/ha can account for an average of 23% of total operating costs [13]. This is out of reach for those involved in the market gardening sector.

2.3. Specificity of the Cropping Agroecological Zone

In Senegal, a particular area known as Niayes is an agroecological zone characterized by the presence of a typical ecosystem (**Figure 1**) [14]. This area benefits from soil, climate and hydrographic conditions that are highly favourable to market gardening [15]. It centralizes most of the country's vegetable production. A growing population and proximity to major urban centres mean that production can be more easily sold for local consumption and exported to the European market.

The natural region of Niayes stretches along the long coast north of Dakar. It corresponds to a coastal band on the western of sub-Saharan Africa. Its proximity to the Atlantic Ocean ensures moderate average temperatures and fairly high relative humidity. The soils are characterized by very high sand contents in the topsoil horizon (**Table 1**). Like the dune sands of the Sahel, the soil's water retention

capacity is very low. Because of its texture, the very high hydraulic conductivity leads to very rapid internal drainage. Generally, the low natural fertility of these dune soils is linked to their low organic matter and total nitrogen content. In this zone, due to the relatively shallow depth of the water table, water is hardly a limiting factor for agricultural production, even in the dry season. However, the rigorous availability of water resources and quality management in this locality remains crucial if the vital needs of the population for water and healthy agricultural produce are to be met [16].

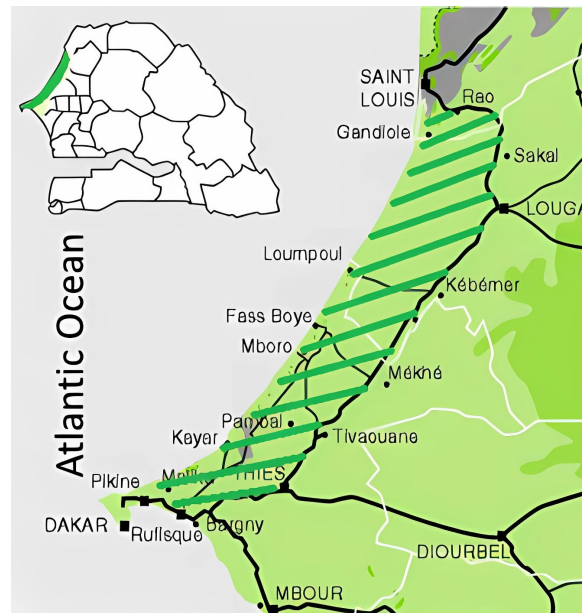


Figure 1. Location of agroecological Niayes zone in Senegal [14].

Table 1. Soil physical and chemical characteristics of main sites of common bean experimentation in pots and fields in Niayes zone.

Characteristics	Bayakh	Bel Air	K Massar	K Moussa	Kounoune	Bambilor	Gorom I	Km 50
Sand (%)	91.2	94.1	96.5	95	92.7	91.2	96.5	70.1
Silt & clay (%)	8.8	5.9	3.5	5	7.3	8.8	3.5	29.9
pH (KCl)	7.5	7	6.8	7.5	7.4	7.5	6.8	7.3
C (‰)	3.3	4.0	2.7	3.1	4.5	3.3	2.7	9.0
N (‰)	0.2	0.3	0.2	0.2	0.4	0.2	0.2	0.8
P _{av} (ppm)	073	280	151	190	350	73.0	150.0	278.0

P_{av} = available P (ppm).

Particularly suitable conditions for agriculture have made it the area of choice for market gardening. This has led to intense development of peri-urban agricultural activities. As a result, the over-exploitation of the land by continuous and inappropriate cropping systems has led to the deterioration of arable land fertility and water resource quality.

3. Chemical Fertilizers

Chemical fertilizers are mineral products applied to soils to improve their fertilization and productivity. They provide crops with essential nutrients that are often absent or deficient in the soil.

3.1. Synthesis and Agronomic Importance of Mineral Fertilizers

The fundamental component for all chemical nitrogen fertilizers is ammonia, synthesized by industrial processes. Formerly, this discovery was a huge success worldwide, as fertilizers played a vital role in improving agricultural yields. Mineral nitrogen fertilizers come in a variety of simple or compound forms. Solid forms of granulated fertilizers, composed of nitrogen (N), phosphorus (P) and potassium (K), are the most commonly used in Senegal.

Nitrogen is an essential constituent of amino acids and proteins. It is therefore an essential mineral element for all living organisms. Although it makes up more than three-quarter of the air, nitrogen cannot be assimilated directly in its atmospheric form by animals and plants. Its transformation and integration into organic molecules are essentially only possible through the intervention of free-living or symbiotic microorganisms associated with plants. Plants generally assimilate nitrogen directly from the soil in the form of nitrates. Other forms of nitrogen are transformed into nitrates by soil microorganisms and made available to plants. However, the lack of return of mineral elements in the soil, exported by crop products, is a contributing cause to the impoverishment of farmed land. So, to satisfy the mineral requirements of a new crop, mineral or organic fertilizers are applied to the crops.

The considerable increase in crop yields is often attributed to the use of nitrogen fertilizers [17]. However, this is only the case when the fertilizer is applied in the right quantity at the right stage of vegetative development. Optimizing application rates is essential for maximum profitability, and can only be achieved by taking into account the crop's demand for nitrogen. Dello [18] reports that in the context of sustainable agriculture, it is essential to improve crop nitrogen use efficiency to fields. For these reasons, soil mineral status must be determined in advance to avoid excessive and inappropriate nitrogen fertilization.

3.2. Environmental Impact of Chemical Nitrogen Fertilizers

The production and use of nitrogen fertilizers to improve crop yields have a multifaceted impact on the environment [5] [19]. Indeed, the industrial synthesis of chemical nitrogen fertilizers emits large quantities of greenhouse gases, in this case carbon dioxide (CO₂), in the atmosphere. In addition, agricultural applications of these fertilizers release nitrous oxide (N₂O), a more dangerous greenhouse gas than CO₂ [11] [20]. Nitrous oxide provides the atmosphere with a global warming potential greater than CO₂ [21]. In addition, it is a long-life greenhouse gas, making it a powerful destroyer of the atmospheric ozone layer.

In addition, other environmental damage and health risks linked to uncontrolled

agricultural applications of chemical nitrogen fertilizers are often noted [3]. Excess nitrate from nitrogen fertilizers easily pollutes groundwater and surface water through infiltration and runoff respectively. Moreover, surface waters are contaminated in this way, containing excess nutrients. As a result, they are exposed to eutrophication, which is considered to be a major factor in the degradation of biodiversity [11]. From a health perspective, the absorption of nitrates by humans represents a danger due to their capacity to be transformed into nitrites, which are harmful to human health.

4. Potentiality and Production of Biofertilizers

For sustainable intensification of agricultural systems, nitrogen biofixers offer an alternative to the plant mineral fertilizers supply.

4.1. Importance of Soil Beneficial Microbial Communities

Thanks to its great diversity, the microbiological component of the soil plays an essential role in soil biogeochemical cycles. The microbial population is involved in the transformation of carbon, nitrogen and phosphorus, making it an essential factor in maintaining soil agricultural quality. Moreover, the microbiome involved in these phenomena plays a part in soil-plant relationships, either directly through mineral nutrition and plant protection or indirectly through the mineralization of organic matter such as crop residues, mulch, manures, compost, etc. Rhizospheric microorganisms such as bacteria and fungi are abundantly represented in their taxonomic diversity in cultivated soils. They also represent an enormous functional potential, acting naturally as biofertilizers, phytostimulators and/or phytoprotectors for plants [6] [7]. In this way, they play a part in improving hydromineral plant nutrition, particularly the biosynthesis of growth factors, the bioavailability of essential nutrients and plant health protection.

4.2. Nitrogen-Fixing Bacteria

Nitrogen in its molecular form N_2 cannot be assimilated by higher organisms. Its transformation and incorporation into organic molecules are essentially the work of soil microorganisms. This phenomenon is known as biological nitrogen fixation (BNF). In this way, a large amount of nitrogen is naturally fixed. Bacteria are the most representative and diverse group of soil microorganisms, with free-living and symbiotic forms. Rhizobia establish symbiotic relationships with plants of the *Leguminosae* family (*Fabaceae*). When environmental conditions are favourable, this relationship enables the plant to recover the nitrogen it needs for its development. Through this association, beans can satisfy their nutritional demand for nitrogen in poor soils, without the need for mineral nitrogen fertilizers. In addition to the direct benefits derived from BNF, these nitrogen-fixing plants enrich the soil with nitrogen that is beneficial to the cultivation of non-fixing plants such as cereals. Enriched in this way, the soil can satisfy an essential part of the nitrogen requirements of subsequent crops.

4.3. Production of Rhizobial Inoculum

A rhizobial inoculum is a microbiological preparation containing one or more rhizobial germs. To ensure the availability of the product to farmers, it must be packaged in solid form. For this purpose, a bacterial suspension in a Yest Extract Mannitol broth is produced at a concentration of around 10^8 cells/ml, sufficient to induce nodulation of the crop to be inoculated [8]. A peat-based substrate is used as a solid support for the inoculum. Preparation involves introducing a suitable volume of rhizobium suspension into peat packaged in plastic bags under sterile conditions. The quality of the inoculum is later checked by authentication and estimation of the number of rhizobium strains capable of inducing bean nodulation. The application rate of an inoculum in the field is related to seed size.

5. Biofertilizers Use on Common Bean Crops in Senegal

Inoculation involves supplying plants with efficient symbiotic microorganisms capable of naturally satisfying their specific nutrient requirements. For successful field inoculation, a selection of high-performance microbial strains must first be carried out.

5.1. Selection of Agronomic Interest Rhizobium Strains

Rhizobial inoculations have been tested in view of the many challenges facing the common bean cultivation. Among the various varieties of bean used by growers, elite rhizobium-bean associations were selected to ensure the success of the experiment. According to the work of Pastor-Bueis *et al.* [22], the selection of indigenous rhizobia is a key factor in the success of bean inoculation in the field. Thus, a collection of indigenous rhizobial strains isolated from soil sampled from different agroecological zones in Senegal was established for bean cultivation [23]. Strains with high nitrogen-fixing potential were selected under laboratory and greenhouse conditions. On the basis of these preliminary results, the more efficient symbiotic associations were selected for field trials. Indeed, the inoculation of bean with rhizobium strains showed a very significant increase in the amount of total nitrogen in plant shoot (+180%), compared to non-inoculated plants [24]. Hungria *et al.* [25] reported that to ensure successful inoculation in beans, rhizobium strains that are more efficient than native strains for nodulation and nitrogen fixation are required.

With a view to successfully transferring inoculation technology to farmers, agricultural itineraries and soil biological conditions are taken into account. These include soil nitrate levels and the competitiveness of native rhizobia with inoculated strains. Thus, induction of bean nodulation in this area was only possible thanks to the proven competitiveness of inoculated strains against the native rhizobia population. The strain was tagged using the Gus-gene transfer technique [26]. Results showed that the percentage of nodule occupancy of the inoculated strain varied from one soil to another, depending on the investigated sites. Nodulation induction performance ranged from 90% to 98% at four of the five sites

studied, while it was only 41% at the last site.

The innovation consisted in inoculating common bean culture at the experimental station with decreasing doses of nitrogen fertilizer, ranging from 80 to 20 kg N/ha. Dello [18] reported that reducing nitrogen inputs to field is essential to improve crop N use efficiency. Rhizobial strain used in this work was highly competitive and N-fix efficient. As a result, higher nitrogen fixation was noted when plants were fertilized with 20 kg N/ha compared to 0 and 80 kg N/ha (Table 2). When rhizobium inoculation was applied to bean crop fertilized with 20 kg N/ha mineral nitrogen fertilizer, +77% increase in pod yield was obtained compared to the non-inoculated and non-fertilized control treatment (Table 2). Rhizobium inoculation in presence of 20 kg N/ha increased pod production by 42,65% compared to 80 kg N/ha as nitrogen fertilization. The highest mineral nitrogen fertilization tested (80 kg N/ha) inhibited bean N-fixed compared with the inoculated treatment in the presence of 20 kg N/ha of mineral fertilizer. In fact, the presence of large quantities of nitrate in cultivated soils is reported to limit biological nitrogen fixation by inhibiting plant nodulation. Abaidoo *et al.* [27] found that soil nitrogen inputs can limit the presence and activity of soil microorganisms. This relatively low mineral fertilization dose (20 kg N/ha) can be considered a starter dose for stimulating rhizobium strain activity in the Niayes zone.

Table 2. Amount of fixed nitrogen (Ndfa) and pod yield of common bean (*Phaseolus vulgaris*) inoculated with elite rhizobium strain and supplied with N fertilizer at field experimental station.

Inoculation	N fertilizer (kg N/ha)	Ndfa (kg N/ha)	Pod yield (kg/ha)
Non inoculated	0 kg N/ha	39.6 a	2730 b
	20 kg N/ha	49.6 a	3540 ab
	80 kg N/ha	27.5 b	3384 ab
<i>Rhizobium</i> strain	0 kg N/ha	65.2 b	3384 b
	20 kg N/ha	140.4 a	4836 a
	80 kg N/ha	42.2 c	4890 a
CV %		13.8	18.6

In each column, values followed by the same letter do not differ significantly at $p = 0.05$.

5.2. Impact of Rhizobial Inoculation on Common Bean Production

In Niayes zone, soils contain a rhizobial population of around 10^3 cells/g of soil. This very low size of the indigenous bacterial population is considered incapable of satisfying the bean's natural nitrogen requirements. Thies *et al.* [28] reported that the success of inoculation in the field depends on the size of the native rhizobia population in the soil. Thereby, this local situation augurs well for the success of crop inoculation when competitive rhizobium strains with high nitrogen-fixing potential are applied.

Field inoculation of beans was carried out in collaboration with producers at

three sites in Niayes zone. The farmers' conventional cropping itineraries, consisting of high mineral nitrogen fertilization (+120 kg N/ha), were taken into account as positive control.

At all experimental sites, a comparative analysis between the different treatments showed a positive effect of inoculation and mineral fertilization compared to the control without chemical fertilizers or inoculation. In these multi-site trials, the quantities of nitrogen fixed by crops subjected to rhizobium inoculation and fertilizer at 20 kg N/ha, were largely more important compared to non-inoculated and N fertilizer plants (**Table 3**).

Table 3. Nitrogen yield, proportion (%Ndfa) and amount (Ndfa) of nitrogen derived from the atmosphere of shoot and pod yield of field-grown common bean (*Phaseolus vulgaris*) cultivated at three sites in Niayes zone under rhizobial inoculation and nitrogen fertilizer.

Site	Treatment	N fertilizer kg N/ha	Total N (kg/ha)	% Ndfa	Ndfa (kg/ha)	Pod yield (kg/ha)
Bambilor	Non inoculated	0	17.0 b	3.7 c	0.6 c	3105 b
	Inoculated	20	49.3 a	69.4 a	39.7 a	5110 a
	N fertilizer	120	44.7 a	21.5 b	10.2 b	4809 a
Gorom	Non inoculated	0	16.7 b	2.8 c	0.4 b	2250 b
	Inoculated	20	44.9 a	68.6 b	35.2 a	4939 a
	N fertilizer	120	48.0 a	20.9 a	11.6 a	5062 a
Bayakh	Non inoculated	0	16.0 b	3.4 c	0.5 c	2050 b
	Inoculated	20	49.8 a	69.6 a	34.0 a	5078 a
	N fertilizer	120	42.3 a	21.9 b	9.8 b	4727 a
CV %			21.2	18.7	14.8	20.4

In each column and for each site, values followed by the same letter do not differ significantly at $p = 0.05$.

Equivalent pod yields between conventional farmers' practice and rhizobial inoculation combined with 20 kg/ha fertilization were noted (**Table 3**). Yields in the non-inoculated control plots were very low compared to those of inoculated plants. In average, in presence of 20 kg N/ha, rhizobium inoculation induced a high increase (+104%) in bean pod yield compared to non-inoculated plants without mineral fertilizer. As a result, common bean N-fix and yield were enhanced by the application of low doses of mineral nitrogen fertilizers in combination with biofertilizers. This result could be explained by the efficiency of the native rhizobium strains inoculated and their adaptation to environmental conditions. Thus, synergistic effects of the low size of the native rhizobium population in the area, the high competitiveness of the inoculated strains and the low nitrogen content of soil could therefore be taken as responsible for the performance of rhizobial inoculation [8] [26]. This significant reduction in the quantities of synthetic fertilizers used can make a significant contribution to reducing production costs for growers. Use of rhizobium inoculum can, therefore lead to a reduction in crop production costs, with a +84% saving in the quantities of chemical fertilizers to be

applied.

In addition to the economic importance of the use of biofertilizers, the environmental aspects related to the cultivation practice in this area with high population density have been analysed.

Indeed, the use of nitrogen fertilizers to boost crop yields has become a universal practice. Naturally, crop growth and productivity in agroecosystems are governed by the availability of soil nutrients. Among these nutrients, nitrogen is the most important chemical element determining plant growth and production. According to Danso and Eskew [17] up to 75% of the increase in crop yields may depend on nitrogen. Moreover, for these authors, only 30% to 50% of synthetic nitrogen fertilizers applied to crops are used by the plants and the rest is lost through leaching, volatilization and denitrification. A paradigm shift is therefore necessary to meet the challenge of establishing sustainable agricultural production systems. However, the complexity of the issue remains problematic. Unfortunately, even if agroecological practices are perceived as the future of agriculture, expensive and polluting mineral nitrogen fertilizers are still necessary to meet future food needs.

Despite nitrogen's strong contribution to crop production and consequently to carbon sequestration, uncontrolled mineral fertilization of crops is having a dangerous impact on the environment. Adedoyin *et al.* [29] consider modern agriculture to be the main cause of environmental pollution. Intensive agricultural use of chemical fertilizers leads to the alteration of water resources, soil and environmental quality through the emission of nitrogen protoxides [11] [19]. Consequently, this situation generates health risks for local populations.

In order to ensure large-scale vegetable production in Senegal, traditional cultivation methods are being distorted by the excessive introduction of chemical inputs into farming itineraries. Conventional common bean cropping systems, for example, rely heavily on mineral nitrogen fertilizers. Unfortunately, the physico-chemical properties of the sandy soils in the cultivation zone and the nature of the hydrographic network are highly unfavourable to such practices, which are reported to disrupt the natural functioning of ecosystem services [28]. Thus, given the difficulty of getting local producers to adopt the concept of regenerative agriculture in the practical sense of sustainable intensification, an approach aimed at reducing the quantities of synthetic fertilizers applied should be promoted as a priority.

6. Conclusion

In the Niayes zone, chemical fertilization is usually applied to increase the productivity of already very poor and overexploited farmland. In this way, common bean yields are improved by growers' abusive use of mineral nitrogen fertilizers, despite their harmful effects on the environment and human health. However, exploiting the soil's microbiological potential may offer an alternative to the use of chemical fertilizers to support common bean production. Improved nitrogen nutrition of

bean can be achieved by inoculating the soil with high-performance rhizobium strains. Its introduction into farmers' cropping systems has shown that it is possible to reduce the conventional doses of chemical fertilizers applied without significantly affecting crop yields. Extension documents are produced in the form of technical data sheets for local farmers to promote inoculation. However, strategies to disseminate technical innovation are necessary, and they can only be achieved by setting up well-controlled quality inoculum production units. This would make a very significant contribution to the integration of inoculation into bean cultivation itineraries and its large-scale adoption. In addition, promoting this practice could thus help mitigate the drastic and multi-faceted consequences of chemical fertilizers over-use. The use of rhizobium-based biofertilizers preserves soil quality and maintains crop yields and producers' incomes at satisfactory levels. It, therefore, contributes to the sustainability and preservation of agroecosystems and the environment.

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Conflicts of Interest

The author declares that there isn't any type of conflict of interest for this research.

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