



(RESEARCH ARTICLE)



Response of soybean (*Glycine max*) to Rhizobia inoculation, mineral nitrogen and inoculation amendment at the two agro ecological zones of northern Nigerian savannah

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Abstract

For sustainable agriculture nitrogen must be adequately supplied because it is the most limiting major nutrient required by all living plants. This experiment was conducted at the two ecological zones, Sudan savannah in Kano State and Guinea savannah in Bauchi state of Nigeria during the 2016 cropping season. The design of the experiment was Randomized Complete Block Design (RCBD) with seven treatments and four replications in each site. The treatments were; legume fix, alosca, 50 kg N ha⁻¹, cattle manure, legume fix + cattle manure, alosca + cattle manure and control. The result showed that Legume fix had the highest grain N uptake (90.51 kg N ha⁻¹) which differed significantly ($P \leq 0.001$) from the rest of the treatments. At Guinea savannah legume fix gave the highest N uptake value (95.53 kg N ha⁻¹) which was significantly different from all treatments except legume fix + cattle manure. However, 50 kg N ha⁻¹ gave the least (48.64 kg N ha⁻¹) even lower than the control and differs significantly with the rest of treatments ($P \leq 0.001$). Legume fix gave the highest P uptake value (9.66 kg P ha⁻¹) which significantly differed from all other treatments. At Sudan savannah, significant differences ($P \leq 0.001$) occurred between some of the treatments. Alosca + cattle manure recorded the highest K uptake (49.99 kg K ha⁻¹). Result for the fixed N in the Sudan and Guinea savannahs soybean field revealed a significant difference between the treatments and the control.

Keywords: Nodulation; Rhizobium; Inoculants; Biological nitrogen fixation; Grain uptake; soybean

1. Introduction

Biological nitrogen fixation is a complex phenomenon which could be affected by many factors both biotic and abiotic. The presence of effective and highly competitive *rhizobia* is very vital for the success of nodulation and subsequent nitrogen fixation to occur. There are contradicting reports on the role of mineral nitrogen fertilizer in nodule formation. Some workers reported negative influence of nitrogen fertilizer on nitrogen fixation. [1], [2] it has been discovered that when plant is left with a choice between the nitrogen applied and fixed nitrogen, plant prepares the former, as such they will be relax in fixing the nitrogen [2]. Some workers reported that there is a need for reasonable amount of nitrogen for the plant to establish itself before nodule formation [3] to [5]. Other researchers have reported the importance of supplementing mineral nitrogen in the establishment of vigorous plant biomass and general crop performance before nodulation commences [3], [4], [6] to [8].

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Manure application result in availability of nutrients to the plants and also help in the enhancement of uptake of nutrients by the growing plants through the enhancement of soil structure and soil moisture retention [9]. Moreover, apart from these benefits it add nutrient including the micronutrients to the growing plants which assist in enhancing the uptake of other plant nutrient especially nitrogen. In an experiment in northern Ghana Molybdenum was found to enhance nutrient uptake potentials of soybean [10].

There exists a wide gap in yield of various leguminous crops in many countries. The yield gap is very wide especially between developing and developed nations. There is need to reduce this gap through improved management practice [11]. Although yield increment has been reported due to use of inoculants, there is still potential for further yield increase when additional avenues are explored [12].

This study was therefore conducted to evaluate the influence of microbial inoculants and complementary treatments on enhancement of Biological Nitrogen Fixation (BNF) potentials and major nutrients uptake in soybean crop in two agroecological zones of northern Nigeria savannahs (Guinea savannah and Sudan savannah).

2. Material and methods

2.1. Study site and soil characteristics

The research was conducted at two locations: Kano University Science and Technology Research farm at Bagauda (Sudan savannah) located latitude 11° 37. 409'N and longitude 08° 22. 994'E, altitude 481 meters (or about 1580 feet) above sea level and Abubakar Tafawa Balewa University Research farm at Gubi (Guinea savannah) located latitude 10°27. 985'N and longitude 9°49. 768'E. The area is situated at about 666.5 m above sea level. The study was conducted during 2016 cropping season.

Initial soil sample was done by collecting twelve core soil samples from depth of 0-15 cm from each block from the two experimental sites following a 'W' design before planting. Composite sample was obtained after careful mix of the soil and then air dried before sieving through a 2 mm mesh sieve. The collected samples were leveled and packed in a clean polythene bags before being taken to the laboratory for selected chemical and physical analyses. Particle size distribution of soil sampled was determined using the Bouyoucos hydrometer method [11]. Soil pH was determined according to [14]. The modified Walkey and Black procedure as described by [15] was used to determine organic carbon. The method involved chemical or wet oxidation then followed by the measurement of expelled CO₂. Soil total nitrogen content was determined using the Macro - kjeldahl method. The method involved digestion, distillation and titration as described by [16]. Available phosphorus was determined through Bray No. 1 method to extract the readily acid soluble forms of phosphorus from the soil as described by [17]. Exchangeable bases (K, Ca, Mg and Na) were determined by 1.0M ammonium acetate (NH₄OAc) extract. The exchangeable acidity was determined using titration method as described by [13].

2.2. Experimental layout

Field experiment were carried out in two agro ecological zones of Nigerian savannahs Bagauda (Sudan savannah) in Kano and Gubi (Guinea savannah) in Bauchi States of Nigeria in 2015 cropping season. The design of the experiment was Randomized complete block design (RCBD) replicated four times. Land preparations were done before planting the seeds. After clearing and land preparations, experimental plots were demarcated. Plot size was 4 by 4.5m. There were seven treatments replicated four times. Soybean crop were used for the experiment. The major nutrient uptake was determined in each plot. BNF was also computed for each plot using N difference method. Maize was used as a reference crop for estimating the BNF in kg ha⁻¹ using N difference method.

The parameters measured were N, P and K uptake for soybean and BNF. The formulas used during computation were as follows:

$$N, P, K \text{ uptake (kg/ha)} = \text{dry matter (kg/ha)} \times \% N, P, K/100 \text{ [14]}$$

$$\text{BNF (kg/ha)} = N \text{ uptake in Legumes} - N \text{ uptake reference Crop [15]}$$

1.3. Analysis of cattle manure

Cattle manure was analyzed in the laboratory for N, P, K, Mg and OC using standard protocols. Lignin and polyphenol was determined using protocol ascribed to [16].

Calculation:

Polyphenol (mg kg^{-1}) = graph reading \times dilution of aliquot \times dilution of sample

Where,

$$\text{Sample dilution} = \frac{\text{final volume of sample}}{\text{weight}} = 50/1 \text{ [16]}$$

3. Results and discussion**3.1. Chemical analysis of cattle manure**

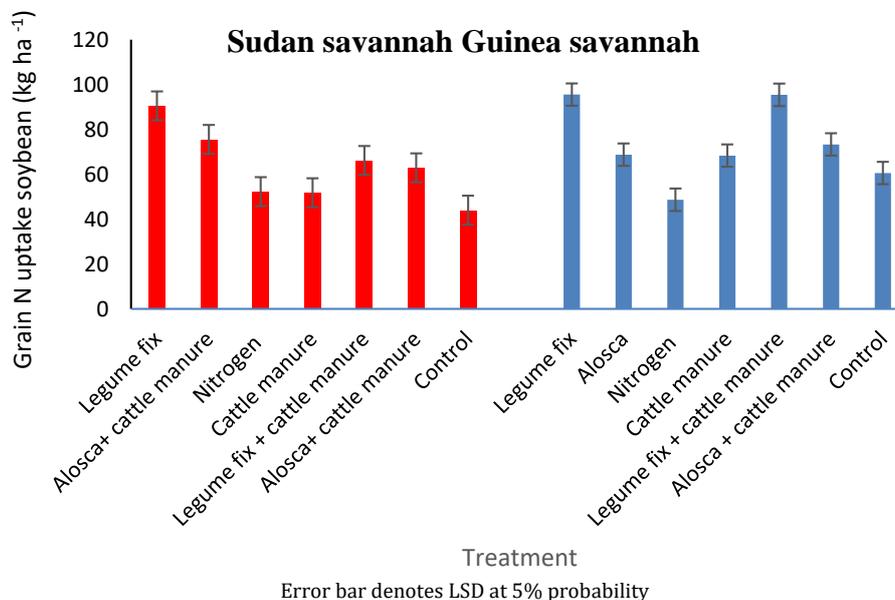
Analysis of the lignin and polyphenol contents of the cattle manure used revealed that the contents of these two important parameters were within the safe limit as could be seen in (Table 1). Lignin content was 10.73% and the polyphenol was 2.97%. It was opined by [17] that organic materials having lignin and polyphenol contents of $< 15\%$ and $< 4\%$ respectively could be use as soil amendments.

Table 1: Chemical analysis of cattle manure

Parameters	(%)		p	%				C/N ratio	(mg kg ⁻¹)	
	Lignin	Polyphenol		N	P	K	C		Fe	Mn
Values	10.73	2.97	7.1	1.35	0.48	0.73	26.95	19.96	2.82	1.27

3.2. Grain N uptake of soybean at Sudan and Guinea savannahs

Results for grain N uptake of soybean in Sudan and Guinea savannahs is as shown in Figure 1. Legume fix had the highest grain N uptake ($90.51 \text{ kg N ha}^{-1}$) which differed significantly ($P \leq 0.001$) from the rest of the treatments. An increase of 106, 72, 50 and 43% over the control were obtained for legume fix, alosca, legume fix + cattle manure and alosca + cattle manure respectively. Treatment of 50 kg N ha^{-1} and cattle manure produced increases of 19% and 17% over the control respectively.

**Figure 1** Grain nitrogen uptake of soybean at Sudan and Guinea savannahs.

At Guinea savannah legume fix gave the highest N uptake value ($95.53 \text{ kg N ha}^{-1}$) which was significantly different from all treatments except legume fix + cattle manure. Grain N uptake increases of 58, 57, 21, 14 and 13% over the control were observed in legume fix, legume fix + cattle manure, alosca + cattle manure, alosca and cattle manure respectively. However, 50 kg N ha^{-1} gave the least ($48.64 \text{ kg N ha}^{-1}$) even lower than the control and differs significantly with the rest of treatments ($P \leq 0.001$).

3.3. Grain P uptake of soybean at Sudan and Guinea savannahs

Results for soybean grain P uptake are presented in Figure 2. Significant differences were observed among some treatments ($P \leq 0.001$). Alosca + cattle manure gave the highest P uptake ($11.18 \text{ kg P ha}^{-1}$) while the control recorded the least ($6.34 \text{ kg P ha}^{-1}$). Increases in P uptake of 76, 66, 49 and 34% over the control were observed under alosca + cattle manure, legume fix + cattle manure and alosca respectively. Furthermore, these treatments differ significantly with control ($P \leq 0.001$).

Also, cattle manure and 50 kg N ha^{-1} treatments recorded higher P uptake values 11% and 10% respectively over the control (Figure 2).

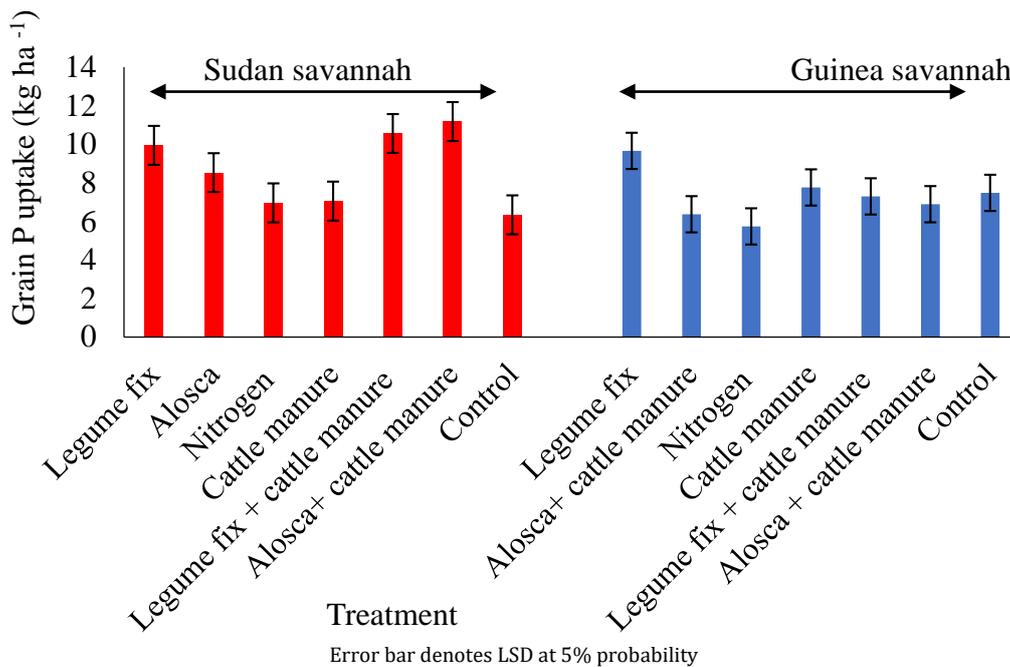
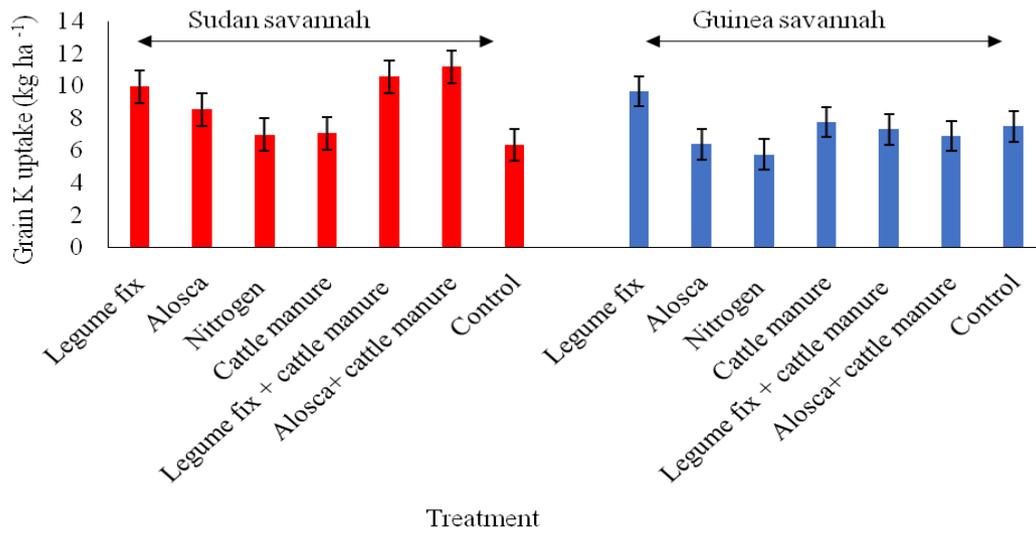


Figure 2 Grain P uptake of soybean at Sudan and Guinea savannahs

At Guinea savannah there are significant differences among some of the treatments ($P \leq 0.001$). Legume fix (in Sudan savannah alosca + cattle manure is the best) gave the highest P uptake value ($9.66 \text{ kg P ha}^{-1}$) which significantly differed from all other treatments. However, all other treatments did not differ significantly from the control.

3.4. Grain K uptake of soybean at Sudan and Guinea savannahs

Results of soybean K uptake as shown presented in Figure 3. At Sudan savannah, significant differences ($P \leq 0.001$) occurred between some of the treatments. Alosca + cattle manure recorded the highest K uptake ($49.99 \text{ kg K ha}^{-1}$) which was statistically at par with legume fix. The control recorded the least K uptake ($29.01 \text{ kg K ha}^{-1}$) which was not significantly different from cattle manure and 50 kg N ha^{-1} treatments. Significant K uptake increments of 72.14, 46.16, 40.47 and 33.92% over the control were recorded for alosca + cattle manure, legume fix, legume fix + cattle manure and alosca respectively.



Error bar denotes LSD at 5% probability
Figure 3 Grain K uptake soybean at Sudan and Guinea savannahs

At Guinea savannah, legume fix recorded the highest grain K uptake (41.32 kg K ha⁻¹) which was not significantly different from legume fix + cattle manure and sole cattle manure. Grain K uptake increments of 49.28, 48.99, 37.93, 11.27 and 10.33% were obtained over the control for legume fix, legume fix + cattle manure, cattle manure, alosca+ cattle manure and alosca respectively.

3.5. Nitrogen fixation for soybean at Sudan and Guinea savannahs

Results of nitrogen fixation for soybean at the two study locations are presented in (Table2). The results at Sudan savannah revealed significant differences ($P \leq 0.001$) between the treatments and the control. Legume fix recorded the highest nitrogen fixation (56.67 kg ha⁻¹) while the control recorded the least (20.69 kg K ha⁻¹). In Sudan savannah increase of 173.90, 101.64, 56.16 and 40.40% for 50 kg N ha⁻¹ fixation were obtained for legume fix, alosca, legume fix + cattle manure and alosca + cattle manure compared to control. Furthermore, the control only differed significantly from legume fix and alosca treatments.

Table 2 Nitrogen fixation in soybean

Treatments	Nitrogen fixation (kg ha ⁻¹)	
	Sudan savannah	Guinea savannah
Legume fix	56.67 ^a	53.66 ^a
Alosca	41.72 ^b	33.57 ^b
Nitrogen (50 kg N ha ⁻¹)	21.67 ^c	17.34 ^c
Cattle manure	21.88 ^c	31.43 ^b
Legume fix + cattle manure	32.31 ^{bc}	58.53 ^a
Alosca + cattle manure	29.05 ^c	36.42 ^b
Control	20.69 ^c	22.23 ^{cd}
F pr.	<0.001	<0.001
Lsd (5%)	11.70	10.86
CV (%)	15.5	7.2

*Means in the same column with the same superscript are not significantly different ($P \geq 0.05$).

At Guinea savannah, legume fix gave the highest nitrogen fixation (53.66 kg K ha⁻¹). All treatments were significantly higher than the control except 50 kg N ha⁻¹ which recorded the least nitrogen fixation (17.34 kg K ha⁻¹). Nitrogen fixation increments of 163.29, 141.38, 63.83, 50.38 and 41.38% were obtained for legume fix + cattle manure, legume fix, alosca + cattle manure, alosca and cattle manure respectively.

Analysis of the lignin and polyphenol contents of the cattle manure used was shown in Table 1. Lignin and polyphenol contents were 10.73% and 2.97% respectively. This is within the safe limit (10-15% and 3-4% for lignin and polyphenol respectively). It was opined by [22] that organic materials having lignin and polyphenol contents of < 15% and < 4% respectively could be use as soil amendments. The lignin content of the cattle manure corroborate with what [18] found (10.1 %) in cattle manure while working at Maradi (Niger).

Result for grain N uptake of soybean in the Sudan and Guinea savannah study locations (Figure 1) showed that legume fix had the highest uptake value (90.51kg ha⁻¹) which differed significantly from the control which recorded the least uptake value (44 kg ha⁻¹). Grain N uptake increases of 58, 57, 21, 14 and 13% over the control was observed in legume fix, legume fix + cattle manure, alosca + cattle manure, alosca and cattle manure respectively. The same trend was also observed in the soybean field in the Guinea savannah [14] while working on soybean in vertisol soils under rainfed condition reported significant N uptake when soybean was treated with biofertilizer. However, a higher N uptake (57.98 kg ha⁻¹) was achieved when combined application of biofertilizer and green manure was used [14]. Legume fix + cattle manure and alosca + cattle manure was also higher in N uptake than the control treatment in both studied locations. Inoculation with effective strains of rhizobia and P supplementation was an effective way of enhancing the growth of soybean [15]. Rhizobial inoculation and combined treatment of rhizobia inoculant and cattle manure enhanced N uptake more than the mineral fertilizer or cattle manure alone. This is in line with the reports of other researchers who found significant increment in uptake of major nutrients when inoculants were used in combination with cattle manure or other organic manure than inoculants or mineral fertilizer alone [17, 23, 16]. Another reason could be the high-quality organic manure that was used during the field experiment (Table 1). The lignin content of the cattle manure corroborates with that of [18] who found (10.1 %) in cattle manure while working at Maradi (Niger). Organic manure with lignin and phelophenol contents of 10-15% and 3-4% respectively have also been reported to be of good quality by [17]-[27]. In this study, as could be seen under Table 1, lignin and polyphenol contents of 10.73% and 2.97% respectively are indications that good quality cattle manure was used.

Result for grain P uptake of soybean in the Sudan and Guinea savannahs is shown in Figure 2. It was observed that there was significant difference between treatments and control. Legume fix and alosca inoculants enhanced P uptake relative to the control in the Sudan savannah. The alosca + cattle manure and legume fix + cattle manure treatments produced the highest uptake values [24] reported that *Bradyrhizobium* and Arbuscular Mycorriza Fungi (AMF) positively affected peanut plant growth, nutrients uptake and yield in both greenhouse and field experiments than sole inoculants.

However, in the Guinea savannah study location, the control treatment performed better than alosca + cattle manure, legume fix + cattle manure, alosca and 50 kg N ha⁻¹ alone even though the difference was not significant. Plant growth, nodule formation and nitrogen fixation need a lot of energy due to enzymatic activities, as such P is necessary [25]. The high grain P uptake obtained under legume fix and alosca in the Sudan and Guinea savannah agro-ecological zones was not surprising because earlier studies in Ethiopia reported that high grain P uptake was influenced by inoculation with *rhizobium* strains than uninoculated treatment [26] also reported that, microbial activity in N fixation highly correlated with P uptake. This is probably why under this current study; high P uptake was observed under inoculated plots relative to un-inoculated. It was also noted that treatments that gave the highest grain P uptake produced higher grain yield. This confirmed the finding of [27] who reported that, adequate supply of P enhanced grain yield of legumes.

Result for the fixed N in the Sudan savannah soybean field revealed a significant difference between the treatments and control (Table 2). Legume fix inoculant recorded the highest fixed N (56.67 kg ha⁻¹) whereas the control recorded the least N (20.69 kg ha⁻¹). In the Sudan savannah, increased values of 173.90, 101.64, 56.16 and 40.40% were obtained respectively for legume fix, alosca, legume fix + cattle manure and alosca + cattle manure over the control. Also, it is worth noting that, inoculation and cattle manure treatments enhanced nitrogen fixation significantly over the control. This could be due to the contribution of the nutrients present in cattle manure (Table 1). Several workers have reported that, organic manure influences soil microbial performance due to the availability of carbon within the soil [31, 29,23]. The nitrogen fixed by soybean in the Sudan savannah followed the same trend except that the 50 kg N ha⁻¹ treatment performed below than control. A study conducted in Southern Spain also confirmed that addition of N fertilizer to inoculated soybean plants (50 kg N ha⁻¹) did not increase seed yield in comparison with treatments that were only inoculated with *Bradyrhizobia* [25]. Other workers in Ethiopia also found that mineral nitrogen suppressed biological nitrogen in faba beans [23]. On the contrary, a study conducted in India on the effect of inoculation on soybean reported that application of starter nitrogen (20 kg N ha⁻¹) enhanced nodulation and yield of soybean [27]. Another study on

rhizobium inoculation and N and P fertilization in Pakistan confirmed that rhizobium inoculation and P fertilization with low level of starter N fertilization increased nodulation and soybean seed yield [28].

It is difficult to draw general conclusions on the response of soybean to N fertilization due to conflicting reported results [29]. The genetic components of a crop and compatibility with nitrogen fixing organisms could also influence BNF capability of a crop [30], [31]

Results for grain K uptake of soybean in the Sudan savannah indicated that alosca + cattle manure recorded highest grain K uptake (49.4 kg ha⁻¹). The least K uptake value was observed under the control treatment (29.01 kg ha⁻¹) Potassium uptake increased in all treatments over the control (Figure 3). In the Guinea savannah, soybean field (Figure 3), results followed the same trend in which soil inoculation and combined application of inoculants and cattle manure treatments enhanced K uptake more than the control. The only exception was 50 kg N ha⁻¹ which recorded an uptake value less than the control. The P and K contents of cattle manure could be the reason behind the relative better performance under the combined inoculant and cattle manure amended plots. This corroborates with the findings of [32] who outlined that significant increase in N fixation, nutrients uptake and grain yield of treated plots was observed over the control. It was reported that, effective rhizobia strains enhanced the growth and uptake of macronutrients in soybean [21, 23].

4. Conclusion

From the result of the experiment it was discovered that inoculation and cattle manure treatments influence the grain uptake of nitrogen, phosphorus and potassium in soybean when compared to control in both Sudan and Guinea savannah agro ecological zones. The combination of inoculants and cattle manure performance was also higher as to the sole nitrogen application in both locations. So, it concluded that the inoculants used contained highly and effective rhizobia and also the cattle manure used influenced yield performance due to its good quality. Therefore, cultivation of soybean inoculated with compatible rhizobia strains with cattle manure amendments will improve soil N fertility and in turns reduces the usage of high cost mineral nitrogen. Nitrogen fixation was also enhanced by inoculation in both study locations as compared to control plots.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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