



## Effect of Soil Proximity to Homestead, Nitrogen Source on Nodulation and Nodule Activity of some Cowpea (*Vigna unguiculata* (L.) Walp) Varieties

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### ABSTRACT

Integration of Bradyrhizobial inoculation with improved cowpea varieties is one viable option to enhance cowpea nodulation and nitrogen fixation, resulting in sustainable cowpea production. This study was conducted in a screen house to determine the effects of soil proximity to the homestead, nitrogen source on nodulation, and nodule activity of some cowpea varieties. This study involved the factorial combination of soil proximity to homestead: close and far; nitrogen source: control (no nitrogen application), application of urea (100 kg N ha<sup>-1</sup>), inoculation with *Bradyrhizobium* strain BR 3262, *Bradyrhizobium* strain BR 3267, and cowpea varieties: Kanannado, IT93K-452-1, IT97K-499-35, and IT90K-277-2, arranged in a completely randomized design and replicated three times. Results revealed that the Most Probable Number (MPN) count of the cultivated soils to cowpea varieties ranged from (9.26 - 12.27 x 10<sup>6</sup>). Results show that the field's proximity to the homestead did not significantly ( $P > 0.05$ ) differ from that away from the homestead. Inoculation with BR 3262 significantly ( $P < 0.05$ ) improved nodule number and dry weight by 62% and 66%. Kanannado significantly increased nodulation compared to the improved varieties. Inoculation with BR 3267 had more nodules than un-inoculated control and N treatment. The result indicates that using *Bradyrhizobium* strain BR 3262 or BR 3267 and Kanannado variety could improve cowpea

nodulation. Further study should, however, be carried out under field conditions to affirm this study.

**KEYWORDS:** *Bradyrhizobium* inoculants, Cowpea, Nitrogen, Rhizobia, Soil proximity, Varieties

## INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is a leading grain legume crop that is grown on an estimated 12.3 million ha in Africa, with West Africa accounting for the bulk of the production on 10.6 million ha of farmlands (Kyei-Boahan *et al.*, 2017). In West Africa, Niger, Nigeria, Burkina Faso, Mali, and Senegal are the leading producers of cowpea (Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT, 2016). In Nigeria, it is grown on an estimated land area of over 2.8 million ha with an average yield of 914 Kg ha<sup>-1</sup>(FAOSTSAT, 2018). It is mostly grown as sole or intercrop with cereals like millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*) and to a lesser extent with maize (*Zea mays*) (Horn and Shimelis, 2020). It is widely grown as a major source of cheap and quality protein (food) for both rural and urban dwellers and fodder. Also, it is an essential component of the cropping systems of most farmlands in the semi-arid regions of sub-Saharan Africa (Kyei-Boahan *et al.*, 2017).

The gap between the average and potential yield of cowpeas in Nigeria is mostly due to several factors. This includes poor planting arrangement in intercrop situations that leads to shading of companion crops, low plant population, low soil fertility, inappropriate planting time, the use of traditional cowpea cultivars with low yielding potential, pest and disease attack, lack of inputs use (Kyei-Boahan *et al.*, 2017). Besides, continuous cropping of the land with no external inputs has resulted in a progressive decline in the yield of cowpeas due to the mining of the soil of its nutrients (Sprent and James, 2007; Woldeyohannes *et al.*, 2007; Dube and Fanadzo, 2013; Kyei-Boahen *et al.*, 2017).

One feasible and effective method that can be adopted by farmers to increase cowpea yield is to adopt a sustainable intensification approach. This involves the integration of growing more drought-tolerant cultivars, the use of improved crop management such as time of planting and plant population, residue management, tillage, and inputs, such as crop protection chemicals, mineral fertilizers, and use of *Rhizobium* inoculants (Kyei-Boahen *et al.*, 2017).

Application of nitrate and ammonium through the addition of nitrogen fertilizers or Biological Nitrogen Fixation (BNF) fertilizers are necessary inputs that can contribute significantly to the yield of cowpeas. Biological Nitrogen Fixation (BNF) is an economically viable and environmentally pleasant N source. Besides, some soils may not have an adequate number of native rhizobia in terms of quantity, quality, or effectiveness to enhance biological nitrogen fixation (BNF). Thus, Biological fixed N via rhizobia - legumes symbiosis accounts for the largest amounts of nitrogen to agriculture. For example, Herridge *et al.* (2008) reported that more than 20 million tonnes of N are fixed by grain legumes to agriculture each year. Cowpea, similar to the majority of leguminous crops, establishes a mutually beneficial relationship with native rhizobia in intercropping setups. This association leads to the nitrogen-rich aboveground portion of the plant, comparable to approximately 55-96 kg of nitrogen fertilizer per hectare per season (Cong *et al.*, 2015). Cowpeas can fix about 240 kg ha<sup>-1</sup> of atmospheric nitrogen and make available about 60-70 kg ha<sup>-1</sup> nitrogen available for succeeding crops grown in rotation with it (Aikins and Afuakwa, 2008). Although cowpeas can meet all or part of their N requirement through BNF (Hungria and Kaschuk, 2014). In a previous study, it has been shown that the crop may benefit from Rhizobial inoculation with specific strains of Rhizobia (Fening and Danso, 2002). Many soils, however, do not have an adequate amount of native Rhizobia in terms of quantity, quality, or effectiveness to enhance biological nitrogen fixation (BNF) (FAO, 2012).

This situation suggests the use of Rhizobia inoculant. Inoculation is the manipulation of Rhizobia populations to the amount that could have been naturally fixed for improved nodulation and crop yield. Inoculation of cowpea with *Bradyrhizobium* strains resulted in a significant increase in cowpea nodulation and yield (Koskey *et al.*, 2017 Yoseph *et al.*, 2017). An increase in the yield of cowpeas due to inoculation with *Bradyrhizobium* strains BR 3267 and BR 3262 has been reported in Brazil (Martins *et al.*, 2003).

One other way to improve the yield of cowpeas is by using improved varieties. In Nigeria, cowpea varieties, IT90K-277-2, IT97K-499-35, IT98K-131-2, and IT89KD-288 resulted in significantly higher grain yield compared to the local and other varieties tested (Kamai *et al.*, 2014). In Nigeria, there is no information on whether inoculating some available cowpea varieties in Nigeria with *Bradyrhizobium* strains BR3267 and BR3262 could exhibit similar yield responses in soils of a Nigerian Savanna location. The question we seek to answer is whether inoculating cowpea varieties with *Bradyrhizobium* strains can address the problem of

low soil N and increase BNF and cowpea production. Hence, the present study had the following objectives;

1. To evaluate the effect of soil proximity to homestead, nitrogen source, and cowpea varieties on nodulation.
2. To assess the nodule activity of cowpea varieties as affected soil proximity to homestead and nitrogen source.

## METHODOLOGY

**Experimental Site, Soil Sample Collection, and Preparation:** A screen house experiment was conducted in 2016 at the Federal University of Technology, Gidan Kwano, Minna; located in the Southern Guinea Savannah (SGS) of Nigeria within longitudes 90° 30' and 90° 40' E and latitudes 6° 30' and 6° 35' N, and at an altitude of 258.5m above sea level. Soil samples for laboratory analysis and screen house study were collected from Paiko, Niger State. Based on cropping history, the field has been put into continuous cultivation with different mixtures of yam, maize, and cowpea between the years 2014 and 2015.

Soil samples were collected randomly across the field using sterilized soil auger and bulked to form composite samples. The sampling depth was (0-20 cm) for both physical and chemical analysis and screen house experiments. The composite samples were taken to the laboratory. Part of the soil samples were air dried and passed through a 2 mm mesh for routine analysis while the remaining sample was prepared moist and kept in the refrigerator for Most Probable Number (MPN) determination.

### Application rates of the treatments and experimental layout:

All treatments received a basal dressing of (P, K, Mg, Zn, Mo, and B) at the rate of 372.6 mg per pot which was calculated based on 2.5 Kg soil per pot, nutrient added was thoroughly mixed. N (urea 100 Kg Nha-1) was split applied as 25% of the (81.60 mg) at sowing and the remaining 75% N (244.80 mg) at two weeks after planting. The factorial combination was used to assign each treatment and treatment combinations to their respective pots. The test crop was cowpea (*Vigna unguiculata*(L.) Walp) varieties: Kanannado, IT93K-452-1, IT97K-499-35, and IT90K-277-2. Cowpea seeds were inoculated before planting with a peat-based inoculum of *Bradyrhizobium* sp. strain (BR 3262 or BR 3267) at the rate of 5 g per kilogram of seed using the slurry method as described by Woomer *et al.* (1994).

### **Laboratory Analysis**

Physical and chemical analyses were carried out by standard methods (IITA, 1989). Soil particle size was determined by the hydrometer method. Soil pH was determined using a pH meter in water (soil solution ratio 1:2.5). Soil organic carbon was determined by the Walkley and Black Oxidation method. Exchangeable bases were extracted with 1 N ammonium acetate ( $\text{NH}_4\text{OAc}$ ) solution at pH 7.0. Potassium in the extract was measured using a Gallen Kamp flame photometer. Calcium and magnesium were determined by titration. Exchangeable acidity ( $\text{H}^+$ ,  $\text{Al}^{3+}$ ) was determined by titrimetric method after extraction with 1N KCl. Total nitrogen was determined by the Kjeldahl method. Available phosphorus was extracted using the Bray P1 method and was estimated colorimetrically. Effective Cation Exchange Capacity (ECEC) was determined by the summation of exchangeable bases ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ) and exchangeable acidity ( $\text{H}^+$ ,  $\text{Al}^{3+}$ ).

**Sowing:** Before planting, the seeds were first surface sterilized by immersing them in 70% ethanol for 10 seconds after which it was drained; the seeds were then submerged in 1.5% sodium hypochlorite for 3 minutes and then drained and then rinsed six times with sterile distilled water. The plants were watered daily with Sandsman's N-free nutrient solution 200 ml (at field capacity of the moist soil) per pot for the first four weeks and later one-quarter strength of the solution or just sterile distilled water. Three seeds per pot were sown and later thinned to one seedling per pot a week after sowing.

### **Most Probable Number (MPN) Method**

MPN was used to estimate the number of viable rhizobia present in the experimental soil. The assay was conducted using a modified Leonard jar method using coarse sand as the potting medium (Howieson and Ballard, 2014). The coarse sand was washed several times with tap water to remove all traces of dissolved nutrients, rinsed with sterile distilled water, sun-dried, and autoclaved before pot filling. Plants were inoculated with 1 ml aliquots of the soil suspensions made from the serial dilution of the soil samples. The soil serial dilution was a 20-fold six-step dilution (20-1 to 20-6) by adding 10 g of soil in 190 ml of sterile distilled water (Woomer, 1994). The presence of nodules on the root of the cowpea at the end of seven weeks was used as an indication of the presence of rhizobia in the soil. Rhizobia count was determined using an MPN table with a confidential interval of  $P < 0.05$  (Somasegaran and Hoben, 1985).

### Inoculation

Cowpea seeds were inoculated before planting with a peat-based inoculum of *Bradyrhizobium* sp. strain (BR 3262 or BR 3267) at the rate of 5 g per kilogram of seed using the slurry method as described by Woomer *et al.* (1994).

### Treatments and Experimental Design

The treatment was a factorial combination of soil proximity to homestead: close and far, nitrogen source: control (no nitrogen application), application of urea (100 kg N ha<sup>-1</sup>), inoculation with *Bradyrhizobium* strain BR 3262, *Bradyrhizobium* strain BR 3267, and cowpea varieties: Kanannado, IT93K-452-1, IT97K-499-35, and IT90K-277-2, arranged in a completely randomized design and replicated three times. The cowpea varieties were IT93K-452-1 (extra-early Maturing), IT97K-499-35 (Early Maturing), IT90K-277-2 (Medium Maturing), and Kanannando (Late Maturing).

### Data Analysis

The data obtained were subjected to analysis of variance (ANOVA) using MINITAB 17.0. Means were separated using the Tukey Test at a 5 % significance level ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

This study reveals that the experimental soil had an adequate number of native rhizobia ( $>103$  cells g<sup>-1</sup> of soil) for cowpea nodulation (Table 1). The findings of Amarger (2001) show that rhizobia is prevalent in tropical soils due to the natural distribution and cultivation of legumes. Indigenous rhizobia population density, effectiveness in forming nodules, and ability to fix N<sub>2</sub> can be characterized functionally for N<sub>2</sub> fixation potential (Singleton and Travers, 1986).

Soil proximity of field to homestead did not significantly affect nodulation in this study, this result does not affirm the observation that fertile plots are often closest to homesteads, as a result of continuous accumulation of organic amendment and household waste applied directly surrounding the settlements (Zingore *et al.*, 2007).

Despite the relatively high population density of native rhizobia in the study location. Inoculation with BR 3262 improved nodule number and dry weight by 62% and 66% respectively compared to the un-inoculated treatment. This implies that strains BR 3262 inoculant used for this experiment have a competitive ability and may have performed a greater role in nodule occupancy and nitrogen fixation. Sanginga *et al.* (1996) and Hougnandan *et al.*

(2000) reported that response to inoculation may occur when the native rhizobia population is (< 5 or 10 cells g-1 of soil). Contrary to this finding despite the high number of native rhizobia responses to inoculation was observed in this study. Meaning that the native populations may be sufficient in number but not effective enough to impede significant response. More so, other factors aside from the native rhizobia population may have reduced the symbiotic performance of the native strains. Giller (2001) also observed that the presence of a large population density of compatible rhizobia does not, however, preclude the possibility that responses to inoculation can be obtained if competitive and highly effective strains are introduced in high-quality inoculants.

The success of *Rhizobium* inoculation primarily depends on the rhizobial strain, the legume genotype, the environmental conditions, and crop management (Woomer *et al.*, 2014). Nodulation in the non-inoculated control and N treatment suggested that the indigenous strains were effective in forming nodules, although the inoculant strain was superior. These did not only show that cowpeas responded to inoculation but also indicated that the introduced strains were highly viable, especially with BR 3262 thereby aggravated a substantial level of increase in nodule number and dry weight. This result conforms to the findings of Martins *et al.* (2003) who reported a significant increase in the nodule number of cowpea after inoculation with *Rhizobium* inoculant. More so, nodule dry weight is very important in strain assessment as it serves as an indicator of symbiotic proficiency (Graham *et al.*, 2004). The N-treated plant recorded the least nodule number and dry weight but was at par with the uninoculated control. More so research has recognized that plants that received mineral nitrogen at a rate of 100 kg N ha-1 recorded the least nodulation. Such results were expected because high levels of nitrogen have been affirmed to affect rhizobia activity in the soil by hindering the host plant from producing lectin which attracts the rhizobia to infect the roots. more so, the use of N at the rate of 100 kg N ha-1 as a control reveals an ideal situation when N is not a limiting factor in cowpea growth.

Nodule number and dry weight were significantly ( $p<0.05$ ) affected by the cowpea varieties in this study although, in some cases, there were marginal differences among the varieties used. Nodulation is an important indicator for evaluating symbiotic potential among cowpea varieties. The highest nodule number and nodule dry weight was produced by kanannando (local variety) compared to the improved varieties used in this study, this could be attributed to the genetic makeup of the local variety and hence will set up more nitrogen in the system

compared to the improved variety. More so, as a local variety, Kanannado is more adapted to the environmental condition and thus has the best symbiotic potential compared to the improved variety. Ayodele and Oso (2014) also observed that significant variation in nodulation per variety could be attributed to differences in the genetic makeup of the individual varieties. Integrating the Kanannado variety into the cropping system because of its nodule weight may have a positive effect on biological nitrogen fixation. Also, Moharram *et al.* (1992), and Bell *et al.* (1994) reported that there is a relationship between nodulation and nitrogen fixation, the local variety will set more N<sub>2</sub> in the system than the improved varieties. IT90K-277-2 and IT97K-499-35 were observed to be low-yielding due to their production of fewer nodules. Low nodule production means less nitrogen will be fixed by the varieties.

The fewer nodule number and dry weight observed with IT90K-277-2 compared to Kanannado when inoculated with BR 3267 in the interaction between Nitrogen sources and varieties could be attributed to differences in the genetic makeup of the varieties. Varietal differences account for nodule differences since the pattern of nodulation, most often reflects the physical distribution of the root system in the soil. Fall *et al.* (2003) also reported that the differences in nodulation and nitrogen fixation could be attributed to the genetic structure of the different varieties. More so, the observed significant difference with plants among varieties in this study is consistent with the earlier work done by Terao *et al.*, (1995) who observed that cowpea varieties with spreading growth habits collected more light than those with erect growth habits and consequently produced more leaves which resulted in more nodules.

The production of more nodule number and dry weight with Kanannado variety when inoculated with BR 3267 for soils sampled away from homestead in the interaction between proximity of field to homestead, Nitrogen sources, and varieties. Indicating that the effectiveness of BR 3267 inoculant in this study cannot be underestimated.

Also, plants that received mineral nitrogen at a rate of 100kgNha<sup>-1</sup> recorded the least nodulation. Such results were expected because high levels of nitrogen have been reported to affect rhizobia activity in the soil by inhibiting legume host production of lectin which attracts the rhizobia to infect the roots. In this study, cowpea modulation can be improved by the use of the kanannado variety with BR 3267 inoculant.

### Characteristics of Soil used in the Study

The results obtained from physical and chemical analysis of the experimental soil (Table 1) showed that soils from both close and far proximities to the homestead were slightly acidic and loamy sandy in texture. Organic carbon (3.60 - 4.35 g kg<sup>-1</sup>), total nitrogen (0.11 - 0.18 g kg<sup>-1</sup>), and available P (6.00 - 7.00 mg kg<sup>-1</sup>) were low, exchangeable cations (0.19 - 5.04 C mol kg<sup>-1</sup>), and effective cation exchange capacity (6.71- 6.76 C mol kg<sup>-1</sup>). The result of ECEC reveals that the experimental soil was low in nutrients.

### MPN count of the Indigenous rhizobia

The MPN count of indigenous rhizobia in the soil using four cowpea varieties with respect to proximities to homestead showed native rhizobia level for Kanannado and IT93K-452-1 was similar ( $12.27 \times 10^6$  -  $11.28 \times 10^6$  cell g<sup>-1</sup> of soil), IT97K-499-35 ( $9.26 \times 10^6$  -  $12.27 \times 10^6$  cell g<sup>-1</sup> of soil), and IT90K-277-2 ( $10.94 \times 10^6$  cell g<sup>-1</sup> of soil) these result reveals that the native rhizobia strains had high fixing ability for cowpea.

**Table 1: Soil characteristics of the study site**

Parameters	Values (Close Proximity)	Values (Far Proximity)
Sand (g kg <sup>-1</sup> )	857	847
Silt (g kg <sup>-1</sup> )	80	80
Clay (g kg <sup>-1</sup> )	63	73
Textural Class	Loamy Sand	Loamy Sand
pH (CaCl)	5.36	5.80
Organic Carbon (g kg <sup>-1</sup> )	3.60	4.35
Total Nitrogen (g kg <sup>-1</sup> )	0.11	0.18
Available Phosphorus (mg kg <sup>-1</sup> )	6.0	7.0
Sodium (C mol kg <sup>-1</sup> )	0.50	0.49
Potassium (C mol kg <sup>-1</sup> )	0.19	0.22
Calcium (C mol kg <sup>-1</sup> )	4.80	5.04
Magnesium (C mol kg <sup>-1</sup> )	1.12	0.80
Exchangeable Acidity (C mol kg <sup>-1</sup> )	0.10	0.21
ECEC	6.71	6.76

**Table 2: MPN count of soils cultivated to cowpea varieties for both proximities to homestead**

Varieties	MPN (Cell g <sup>-1</sup> of soil) (Close proximity)	MPN (Cell g <sup>-1</sup> of soil) (Far proximity)
Kanannado	$12.27 \times 10^6$	$11.28 \times 10^6$
IT93K-452-1	$12.27 \times 10^6$	$11.28 \times 10^6$
IT97K-499-35	$9.26 \times 10^6$	$12.22 \times 10^6$
IT90K-277-2	$10.94 \times 10^6$	$10.94 \times 10^6$

### **Cowpea Nodulation**

Cowpea nodulation, i.e., nodule number and dry weight, were not significantly different under soil proximities to the homestead (Table 3). However, nitrogen sources significantly ( $p<0.05$ ) affected the cowpea nodulation (Table 3). Nodule number and nodule dry weight increased substantially with the BR 3262 inoculant strain, which was at par with the BR 3267 inoculant strain for nodule dry weight compared to the other nitrogen sources. The Kanannado variety recorded more and heavier nodules compared to the IT93K-452-1, IT97K-499-35, and IT90K-277-2 varieties. The use of variety IT97K-499-35 and IT90K-277-2 had similar least and lighter cowpea nodules in this study. In addition, a significant interaction was observed between Nitrogen sources and varieties on nodulation and the combination of proximity to homestead, Nitrogen sources, and varieties on nodule number and nodule dry weight of cowpea (Table 3).

### **Interaction Effects between Nitrogen Sources and cowpea varieties on cowpea Nodulation**

The interaction between nitrogen sources and varieties (Table 4) revealed that irrespective of the varieties used in this study, inoculation with BR 3262 improved the nodule number and cowpea's dry weight, respectively. Although the highest number of nodules was found in the kanannado variety with BR 3267 inoculant strain, it was not significantly ( $p>0.05$ ) different from nodules obtained using un-amended control and BR 3262. A similar trend occurred in nodule dry weight (Table 5). The kannanado variety had the heaviest nodule dry weight, which was at par with that from other treatments except for the urea treatment.

### **Interactive effect of Nitrogen sources, Soil proximity, and Varieties on nodulation of cowpea**

A significant interaction was observed between the proximity of the field to the homestead, nitrogen sources, and varieties on nodule number (Table 6). Heavier nodules were produced with BR 3267 inoculant using kanannado followed by IT93K-452-1 variety for soils sampled further away from the homestead. Similar nodules were produced by plant inoculated with BR 3262 using the four cowpea varieties except for kanannado and IT97K-499-35 under proximity to the homestead. However, they were significantly at par with the control treatment using kanannado for proximity close to the homestead.

Significant interactions were also recorded among the combination of proximities, nitrogen sources, and varieties on nodule dry weight of cowpeas (Table 7). Inoculation with BR 3267 using the kanannado variety resulted in the highest nodule dry weight though not significantly ( $p<0.05$ ) different from the nodule dry weight gotten from IT93K-452-1 for soils sampled away from the homestead and BR 3262 using the four cowpea varieties except for Kanannado and IT97K-499-35 using soils away from the homestead. Similar nodule dry weight was also observed with the un-inoculated control with kanannado variety for soils sampled near the homestead.

**Table 3: Effect of Nitrogen sources, Soil proximity to homestead and Varieties on growth parameters of cowpea**

Nitrogen sources	Varieties			
	Kanannado	IT93K-452-1	IT97K-499-35	IT90K-277-2
Control	0.09abc	0.07bcd	0.04bcd	0.05bcd
Urea	0.05bcd	0.04bcd	0.04bcd	0.04bcd
BR3262	0.09abc	0.11ab	0.09abc	0.10abc
BR3267	0.15a	0.10abc	0.07bcd	0.02d
<b>SE±</b>			<b>0.01</b>	

Means with the same letters in a treatment column are not significantly different according to Tukey test at  $P\leq 0.05$

\*Significant at ( $P\leq 0.05$ ), NS (not significant)

**Table 4: Interaction effect between nitrogen sources and cowpea varieties on nodule number (count plant<sup>-1</sup>) of cowpea**

Treatment	Nodule Number (Count plant <sup>-1</sup> )	Nodule dry Weight (g plant <sup>-1</sup> )
<b>Proximity (P)</b>		
Close	14.31a	0.07a
Far	15.75a	0.07a
SE±	0.87	0.00
<b>Nitrogen sources (N)</b>		
Control	13.08c	0.06b
Urea (100 kg N ha <sup>-1</sup> )	8.50d	0.04b
BR 3262	21.25a	0.10a
BR 3267	17.29b	0.08ab
SE±	1.24	0.01
<b>Varieties (V)</b>		
Kanannado	19.71a	0.10a
IT93K-452-1	15.75b	0.08ab
IT97K-499-35	13.13bc	0.06b
IT90K-277-2	11.54c	0.05b
SE±	1.24	0.01
<b>Interaction</b>		
P × N	<b>NS</b>	<b>NS</b>
P × V	<b>NS</b>	<b>NS</b>
N × V	*	*
P × N × V	*	*

a,b,c,d Means with the same letters are not statistically different ( $P>0.05$ )

**Table 5: Interaction effect between nitrogen sources and cowpea varieties on nodule dry weight (gplant<sup>-1</sup>) of cowpea**

Nitrogen sources	Varieties			
	Kanannado	IT93K-452-1	IT97K-499-35	IT90K-277-2
Control	18.83a-e	13.00b-f	9.83c-f	10.67b-f
Urea	10.67b-f	7.00ef	9.00def	7.33ef
BR3262	20.50a-d	22.50ab	20.17 a-d	21.83abc
BR3267	28.83a	20.50a-d	13.50b-f	6.33f
<b>SE±</b>			<b>2.48</b>	

a,b,c,d Means with the same letters are not statistically different (P>0.05)

This study reveals that the experimental soil had an adequate number of native rhizobia (>103 cells g<sup>-1</sup> of soil) for cowpea nodulation (Table 2). This finding is significant as it supports Amarger's (2001) findings that rhizobia is prevalent in tropical soils due to the natural distribution and cultivation of legumes. The population density of indigenous rhizobia, their effectiveness in forming nodules, and their ability to fix N<sub>2</sub> can be functionally characterized for N<sub>2</sub> fixation potential (Singleton and Travers, 1986). The soil proximity of the field to the homestead did not significantly (p>0.05) affect nodulation in this study (Table 3). This result did not affirm the observation that fertile plots are often closest to homesteads due to the continuous accumulation of organic amendment and household waste applied directly surrounding the settlements (Zingore *et al.*, 2007).

Despite the relatively high population density of native rhizobia in the study location. Compared to the un-inoculated treatment, inoculation with BR 3262 improved nodule number and dry weight by 62% and 66%, respectively. This implies that strains of BR 3262 inoculant used for this experiment have a competitive ability and may have performed a more significant role in nodule occupancy and nitrogen fixation. Singinga *et al.* (1996) and Hougnandan *et al.* (2000) reported that response to inoculation might occur when the native rhizobia population is (< 5 or 10 cells g<sup>-1</sup> of soil). Contrary to this finding, despite the high number of native rhizobia responses to inoculation, it was observed in this study. This means that the native populations may be sufficient in number but not adequate to impede significant response. Other factors aside from the native rhizobia population may have reduced the symbiotic performance of the native strains. Giller (2001) also observed that a large population density of compatible rhizobia does not preclude the possibility that responses to inoculation can be obtained if competitive and highly effective strains are introduced in high-quality inoculants.

The success of *Rhizobium* inoculation is a complex process that primarily depends on the rhizobial strain, the legume genotype, and importantly, the environmental conditions and crop management (Woomer *et al.*, 2014). This understanding underscores the intricate nature of agricultural practices and the need for careful management. Nodulation in the non-inoculated control and N treatment suggested that the indigenous strains were effective in forming nodules, although the inoculant strain was superior. These showed that cowpeas responded to inoculation and indicated that the introduced strains were highly viable, especially with BR 3262, thereby aggravating a substantial increase in nodule number and dry weight. This result conforms to the findings of Martins *et al.* (2003), who reported a significant increase in nodule number of cowpea after inoculation with *Rhizobium* inoculant. More so, nodule dry weight is substantial in strain assessment as it indicates symbiotic proficiency (Graham *et al.*, 2004). The N-treated plant recorded the least nodule number and dry weight but was at par with the uninoculated control. More so, research has recognized that plants that received mineral nitrogen at a rate of 100 kg N ha<sup>-1</sup> recorded the least nodulation. However, such results were expected because high levels of nitrogen have been affirmed to affect rhizobia activity in the soil by hindering the host plant from producing lectin, which attracts the rhizobia to infect the roots. Using N at the rate of 100 kg N ha<sup>-1</sup> as a control reveals an ideal situation when N is not a limiting factor in cowpea growth.

Nodule number and dry weight were significantly ( $p \leq 0.05$ ) affected by the cowpea varieties in this study, although, in some cases, there were marginal differences among the varieties used. Nodulation is an essential indicator for evaluating symbiotic potential among cowpea varieties. The highest nodule number and nodule dry weight were produced by kanannando (local variety) compared to the improved varieties used in this study; this could be attributed to the genetic makeup of the local variety and hence will set up more nitrogen in the system compared to the improved variety. As a local variety, Kanannando is more adapted to the environmental condition and thus has the best symbiotic potential compared to the enhanced variety. Ayodele and Oso (2014) also observed that significant variation in nodulation per variety could be attributed to differences in the genetic makeup of the individual varieties. Integrating the Kanannando variety into the cropping system because of its nodule weight may positively affect biological nitrogen fixation. Also, Moharram *et al.* (1992) and Bell *et al.* (1994) reported a relationship between nodulation and nitrogen fixation; the local variety will set more N<sub>2</sub> in the system than the improved varieties. IT90K-277-2 and IT97K-499-35 were

observed to be low-yielding due to their production of fewer nodules. Low nodule production means the varieties will fix less nitrogen.

The fewer nodule number and dry weight observed with IT90K-277-2 compared to Kanannado when inoculated with BR 3267 in the interaction between Nitrogen sources and varieties (Tables 4 and 5) could be attributed to differences in the genetic makeup of the varieties. Varietal differences account for nodule differences since the pattern of nodulation most often reflects the physical distribution of the root system in the soil. More so, the observed significant difference with plants among varieties in this study is consistent with the earlier work done by Terao *et al.* (1995), who observed that cowpea varieties with spreading growth habits collected enough sunlight than those with erect growth habits and consequently produced more leaves which resulted in more nodules.

The production of more nodule number and dry weight with Kanannado variety when inoculated with BR 3267 for soils sampled away from homestead in the interaction between proximity of field to homestead (Tables 6 and 7), Nitrogen sources, and varieties, indicating that the effectiveness of BR 3267 inoculant in this study cannot be underestimated. Also, plants that received mineral nitrogen at a rate of 100kgNha<sup>-1</sup> recorded the least nodulation. Such results were expected because high nitrogen levels have been reported to affect rhizobia activity in the soil by inhibiting legume host production of lectin, which attracts the rhizobia and infects the roots. In this study, cowpea

The findings of this study suggest a promising avenue for improving nodulation. By using the kanannado variety with BR 3267 inoculant, significant improvements in nodulation can be achieved, offering a hopeful prospect for future agricultural practices.

**Table 6. Interactive effects of nitrogen sources, proximity to homestead and cowpea varieties on Nodule Number (count plant<sup>-1</sup>)**

Proximity	Nitrogen sources	Varieties			
		Kanannado	IT93K-452-1	IT97K-499-35	IT90K-277-2
Close	Control	19.33a-d	13.33bcd	7.67cd	9.33cd
		18.33bcd	12.67bcd	12.00bcd	12.00bcd
Close	Urea	9.33cd	7.33cd	6.67cd	6.33cd
		12.00bcd	6.67cd	11.33bcd	8.33cd
Close	BR3262	23.67abc	22.33a-d	22.00a-d	23.00abc
		17.33bcd	22.67a-d	18.33bcd	20.67a-d
Close	BR3267	19.00bcd	11.33bcd	19.00bcd	9.33cd
		38.67a	29.67ab	8.00cd	3.33d

a,b,c,d Means with the same letters are not statistically different (P>0.05)

**Table 7. Interactive effects of nitrogen sources, proximity to homestead and varieties on Nodule dry weight (g plant<sup>-1</sup>) of cowpea**

Proximity	Nitrogen sources	Varieties			
		Kanannado	IT93K-452-1	IT97K-499-35	IT90K-277-2
Close	Control	0.10a-d	0.08bcd	0.03cd	0.05bcd
		0.08bcd	0.06bcd	0.05bcd	0.06bcd
Close	Urea	0.05bcd	0.06bcd	0.04cd	0.03cd
		0.06bcd	0.03cd	0.04cd	0.04cd
Close	BR3262	0.11abc	0.11abc	0.10a-d	0.11abc
		0.08bcd	0.11abc	0.08bcd	0.10a-d
Close	BR3267	0.09a-d	0.06bcd	0.10a-d	0.04cd
		0.20a	0.15abc	0.04cd	0.00d

a,b,c,d Means with the same letters are not statistically different (P>0.05)

## CONCLUSION AND RECOMMENDATIONS

The results obtained in this study suggest that the proximity of the field to the homestead did not significantly affect nodulation. Also, the *bradyrhizobia* strain BR 3262 was more effective on cowpea nodulation and could be recommended for cowpea cultivation in soils with a high native rhizobia population. This study also revealed that the local variety had better nodulation compared to the improved varieties. It's important to note that further studies will be required for validation under field conditions, demonstrating the thoroughness of our research.

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