**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 that can improve 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 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-1), 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 obtained revealed that Most Probable Number (MPN) count of the soils cultivated to cowpea varieties ranged from (9.26 - 12.27 x 106). Results obtained show close proximity of field to homestead did not significantly (P > 0.05) differ from that away from homestead. Inoculation with BR 3262 significantly (P < 0.05) improved nodule number and dry weight by 62% and 66%. Nodulation was significantly increased by Kanannado compared to the improved varieties. Inoculation with BR 3267 had more nodules compared to the un inoculated control and N treatment. The result indicates that the use of Bradyrhizobium strain BR 3262 or BR 3267 and Kanannado variety could improve cowpea nodulation. Further study should however be carried out under field condition in order 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 a 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 Agricultural 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-1(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](https://cabiagbio.biomedcentral.com/articles/10.1186/s43170-023-00159-1#ref-CR30)). 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 average and potential yield of cowpea in Nigeria is mostly due to a number of factors. This includes poor planting arrangement in intercrop situation 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 the progressive decline in the yield of cowpea 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 the farmers to increase cowpea yield is to adopt sustainable intensification approach. This involves the integration of growing more drought tolerant cultivars, 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) fertilizer are necessary inputs that can contribute significantly to the yield of cowpea. 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 is fixed by grain legume 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). Cowpea can fix about 240 kg ha-1 of atmospheric nitrogen and make available about 60-70 kg ha-1 nitrogen available for succeeding crops grown in rotation with it (Aikins and Afuakwa, 2008). Although cowpea can meet all or part of its 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 howweever, do not have 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 yield of cowpea due to inoculation with *Bradyrhizobium* strains BR 3267and BR 3262 has been reported in Brazil (Martins *et al*., 2003).

One other way to improve the yield of cowpea 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 are no information on whether inoculating some available cowpea varieties in Nigeria with *Bradyrhizobium* strains BR3267and BR3262 could exhibit similar yield response 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. On the basis of cropping history, the field has been put into continuous cultivation with different mixture of yam, maize, cowpea between the year 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 experiment. 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 on the basis of 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. 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 prior to planting with a peat - based inoculum of *Bradyrhizobium* sp. strain (BR 3262 or BR 3267)at the rate of 5 g per a kilogram of seed using the slurry method as described by Woomer *et al*. (1994).

**Laboratory Analysis**

Physical and chemical analysis were carried out by standard methods (IITA, 1989). Soil particle size was determined by the hydrometer method. Soil pH was determine using a pH-meter in water (soil solution ratio 1:2.5). Soil organic carbon was determined by Walkley and Black Oxidation method. Exchangeable bases were extracted with 1 N ammonium acetate (NH4OAc) solution at pH 7.0. Potassium in the extract was measured using Gallen Kamp flame photometer. Calcium and magnesium were determined by titration. Exchangeable acidity (H+, Al3+) was determined by titrimetric method after extraction with 1N KCl). Total nitrogen was determined by the Kjeldahl method. Available phosphorus was extracted using Bray P1 method and was estimated colorimetrically. Effective Cation Exchange Capacity (ECEC) was determined by summation of exchangeable bases (Ca2+, Mg2+ K+, Na+) and exchangeable acidity (H+, Al3+).

**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 3minutes 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 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 cowpea at the end of seven weeks was used as an indication for the presence of rhizobia in the soil. Rhizobia count was determined using MPN table with confidential interval P < 0.05 (Somasegaran and Hoben, 1985).

**Inoculation**

Cowpea seeds were inoculated prior to planting with a peat - based inoculum of *Bradyrhizobium* sp. strain (BR 3262 or BR 3267)at the rate of 5 g per a 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-1), 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), Kanannando (Late Maturing).

**Data Analysis**

The data obtained were subjected to analysis of variance (ANOVA) using MINITAB 17.0 Means were separated using Tukey Test at 5 % significance level (P < 0.05).

**RESULTS AND DISCUSSION**

This study reveals that the experimental soil had adequate number of native rhizobia (>103 cells g-1 of soil) for cowpea nodulation (Table 1). The findings of Amarger (2001) shows that rhizobia are prevalent in tropical soils due to the natural distribution and cultivation of legumes. Indigenous rhizobia populations density, eﬀectiveness in forming nodules and ability to fix N2 can be characterized functionally for N2 fixation potential(Singleton and Travers, 1986).

Soil proximity of field to homestead did not significantly affected nodulation in this study, this result doesnot affirmed the observation that fertile plots is often closest to homesteads, as a result of continous accumulation of organic ammendment 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. Which implies that strains BR 3262 inoculant used for this experiment have a competitive ability and may have perform a greater role in nodule occupancy and nitrogen fixation. Sanginga *et al*. (1996) and Houngnandan *et al.* (2000) reported that response to inoculation may occur when the native rhizobia population is (< 5or 10 cells g-1 of soil). Contrary to this finding in spite of the high number of native rhizobia response 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 native rhizobia population may have reduced the symbiotic performance of the native strains. Giller (2001) also observed that presence of 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 Rhizobiuminoculation 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 in N treatment suggested that the indigenous strains were eﬀective in forming nodules, although the inoculant strain was superior.

These did not only show that cowpea responded to inoculation but indicate that the introduced strains were highly viable especially with BR 3262 thereby aggravated 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 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 for 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. Although 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 production of 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 is to reveal 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 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 varieties could be attributed to difference in the genetic makeup of the individual varieties. Integrating Kanannado variety into cropping system because of its nodule weight may have positive effect on biological nitrogen fixation. Also Muharram *et al*. (1994), Bell *et al*. (1994) reported that there is a relationship between nodulation and nitrogen fixation, the local variety will set more N2 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 genetic makeup of the varieties. Varietal differences account for nodule differences since the pattern of nodulation, most often reflect 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 plant among varieties in this study in 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 habit 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-1 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 nodulation can be improved by the use of kanannado variety with BR 3267 inoculant.

**Characteristics of Soil used in the Stud***y*

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

3.2 MPN count of the indigenous rhizobia

The MPN count of indigenous rhizobia in the soil using four cowpea varieties in respect to proximities to homestead showed native rhizobia level for Kanannado and IT93K-452-1 was similar (12.27 × 106 - 11.28 × 106 cell g-1 of soil), IT97K-499-35 (9.26 × 106 - 12.27 × 106 cell g-1 of soil), and IT90K-277-2 (10.94 × 106 cell g-1 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-1) | 857 | 847 |
| Silt (g kg-1) | 80 | 80 |
| Clay (g kg-1) | 63 | 73 |
| Textural Class | Loamy Sand | Loamy Sand |
| pH (CaCl) | 5.36 | 5.80 |
| Organic Carbon (g kg-1) | 3.60 | 4.35 |
| Total Nitrogen (g kg-1) | 0.11 | 0.18 |
| Available Phosphorus (mg kg-1) | 6.0 | 7.0 |
| Sodium (C mol kg-1) | 0.50 | 0.49 |
| Potassium (C mol kg-1) | 0.19 | 0.22 |
| Calcium (C mol kg-1) | 4.80 | 5.04 |
| Magnessium (C mol kg-1) | 1.12 | 0.80 |
| Exchangeable Acidity (C mol kg-1) | 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-1 of soil)(Close proximity) | MPN (Cell g-1 of soil)(Far proximity) |
| Kanannado | 12.27 × 106 | 11.28 × 106 |
| IT93K-452-1 | 12.27 × 106 | 11.28 × 106 |
| IT97K-499-35 | 9.26 × 106 | 12.22 × 106 |
| IT90K-277-2 | 10.94 × 106 | 10.94 × 106 |

**Cowpea Nodulation**

Cowpea nodulation i.e. nodule number and dry weight was not significantly different under soil proximities to homestead (Table 3). However, nitrogen sources significantly (P <0.05) affected the cowpea nodulation (Table 3). Nodule number and nodule dry weight were significantly increased with the application of BR 3262 inoculant strain, was at par with BR 3267 inoculant strain for nodule dry weight compared to the other nitrogen sources.

Kanannado variety recorded more and heavier nodules compared to IT93K-452-1, IT97K-499-35, 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 as well as 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 nodule number and dry weight of cowpea respectively. Although the highest number of nodules was recorded using kanannado variety with BR 3267 inoculant strain though not significantly (P > 0.05) different from nodules gotten using un-amended control and BR 3262. A similar trend was also observed in nodule dry weight (Table 5) kannanado variety had the heaviest nodule dry weight which was at par with that gotten from other treatment except for the urea treatment.

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

|  |  |  |
| --- | --- | --- |
| Treatment | Nodule Number(Count plant-1) | Nodule dry Weight(g plant-1) |
| **Proximity (P)** |  |  |
| Close | 14.31a | 0.07a |
| Far | 15.75a | 0.07a |
| SE± | 0.87 | 0.00 |
| **Nitrogen sources (N)** |  |  |
| Control | 13.08c | 0.06b |
| Urea (100 kg N ha-1) | 8.50d | 0.04b |
| BR 3262 | 21.25a | 0.10a |
| BR 3267 | 17.29b | 0.08ab |
| SE± | 1.24 | 0.01 |
| **Varieties (V)** |  |  |
| 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 |
| **Interaction**  |  |  |
| P × N | **NS** | **NS** |
| P × V | **NS** | **NS** |
| N × V | **\*** | **\*** |
| P × N × V | **\*** | **\*** |

Means with the same letters in a treatment column are not significantly different according to Tukey test at P≤0.05 \*Significant at (P<0.05), NS (not significant)

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

A significant interaction was observed between proximity of field to 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 homestead. Similar nodules were produced by plant inoculated with BR 3262 using the four cowpea varieties except for kanannado and IT97K-499-35 under close proximity to homestead, though were significantly at par with the control treatment using kanannado for proximity close to homestead.

Significant interactions were also obtained among the combination of proximities, nitrogen sources, and varieties on nodule dry weight of cowpea (Table 7). Inoculation with BR 3267 using 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 homestead and BR 3262 using the four cowpea varieties except for Kanannado and IT97K-499-35 using soils away from homestead. Similar nodule dry weight was also observed with the un-inoculated control with kanannado variety for soils sampled close to homestead.

**Table 4: Interaction effect between nitrogen sources and cowpea varieties on nodule**

**number (count plant-1) of cowpea**

|  |
| --- |
| Varieties |
| Nitrogen sources |  | 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**SE±** |  | 0.15a | 0.10abc | 0.07bcd**0.01** | 0.02d |

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-1) of cowpea**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Varieties |  |  |
| Nitrogen sources | 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 |
| **SE±** |  |  | **2.48** |  |

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

This study reveals that the experimental soil had adequate number of native rhizobia (>103 cells g-1 of soil) for cowpea nodulation (Table 2). The findings of Amarger (2001) shows that rhizobia are prevalent in tropical soils due to the natural distribution and cultivation of legumes. Indigenous rhizobia populations density, eﬀectiveness in forming nodules and ability to fix N2 can be characterized functionally for N2 fixation potential(Singleton and Travers, 1986).

Soil proximity of field to homestead did not significantly affected nodulation in this study (Table 3), this result doesnot affirmed the observation that fertile plots is often closest to homesteads, as a result of continous accumulation of organic ammendment 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. Which implies that strains BR 3262 inoculant used for this experiment have a competitive ability and may have perform a greater role in nodule occupancy and nitrogen fixation. Sanginga *et al*. (1996) and Houngnandan *et al.* (2000) reported that response to inoculation may occur when the native rhizobia population is (< 5or 10 cells g-1 of soil). Contrary to this finding, in spite of the high number of native rhizobia response 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 native rhizobia population may have reduced the symbiotic performance of the native strains. Giller (2001) also observed that presence of 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 Rhizobiuminoculation 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 in N treatment suggested that the indigenous strains were eﬀective in forming nodules, although the inoculant strain was superior.

These did not only show that cowpea responded to inoculation but indicate that the introduced strains were highly viable especially with BR 3262 thereby aggravated 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 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 for 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. Although 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 production of 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 is to reveal 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 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 varieties could be attributed to difference in the genetic makeup of the individual varieties. Integrating Kanannado variety into cropping system because of its nodule weight may have positive effect on biological nitrogen fixation. Also, Moharram *et al*. (1992), Bell *et al*. (1994) reported that there is a relationship between nodulation and nitrogen fixation, the local variety will set more N2 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 (Table 4 and 5) could be attributed to differences in genetic makeup of the varieties. Varietal differences account for nodule differences since the pattern of nodulation, most often reflect physical distribution of the root system in the soil. More so, the observed significant difference with plant among varieties in this study in 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 habit 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 (Table 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-1 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

nodulation can be improved by the use of kanannado variety with BR 3267 inoculant.

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

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

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

From the results obtained in this study, it could be concluded that proximity of field to homestead did not have significant effect on nodulation. Also, *bradyrhizobia* strain BR 3262 was more effective on cowpea nodulation and could be recommended for cowpea cultivation in soils with high native rhizobia population. It was glaring in this study that the local variety had better nodulation compared to the improved varieties. However, further studies will be required for validation under field conditions.

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