RESPONSE OF FOUR COWPEA VARIETIES TO BRADYRHIZOBIAL INOCULATION IN SOME SOILS OF NORTHERN GUINEA SAVANNA ZONE OF NIGERIA

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ABSTRACT

Yield of cowpea in savanna soils of sub-sahara Africa has remained low as a result of less effective native rhizobia, necessitating the use of elite inoculant strains to improve Biological Nitrogen Fixation. The main objective of this study was to observe the response of four varieties of cowpea to inoculation by two elite strains of bradyrhizobia in some soils of Northern Guinea Savanna (NGS) zone of Nigeria. Two greenhouse experiments were carried out at the Teaching and Research Farm of the Federal University of Technology Minna. To determine (1) the size of the native rhizobial population in the soils using the Most Propable Number (MPN) technique and (2) The Need to Inoculate cowpea in the soils under study. The treatments for the MPN experiment consisted of (i) soils in area of close proximity to homestead (< 50 m), and those far from homestead (> 250 m) and (ii) cowpea varieties (Kanannado, IT93K-452-1, IT97K-499-35, and IT90K-277-2). The experiment was laid out in a 2 x 4 factorial in a completely randomised design (CRD) replicated four times. Treatment for the need to inoculate experiment consisted of (i) five locations (Rijau, Kontagora, Magama, Mashegu, Mariga,),in the NGS zone of Nigeria (ii) four nitrogen sources (plants inoculated with *Bradyrhizobium* sp. strain BR 3262, *Bradyrhizobium* sp. strain BR 3267, control (neither N nor inoculant rhizobia applied), and plants treated with N in form of urea at the rate of 100 kg N ha^{-1}, (iii) four cowpea varieties, (iv) two levels of proximities to homestead. Proximity to homestead and cowpea varieties were the same as in the MPN experiment. The experiment was laid out in a 5 x 4 x 4 x 2 factorial fitted to a completely Randomised block design (CRD) replicated four times. In general the MPN value ranged from 4.61 x 10^6 to 7.19 x 10^7 cells g^{-1} of soil. MPN value for IT90K-277-2 variety (9.27 x 10^6 cells g^{-1}) was significantly lower than that estimated by the other varieties Kanannado (3.31 x 10^7 cells g^{-1}), IT93K-452-1 (1.76 x1 0^7 cells g^{-1}), and IT97K-499-35 (1.42 x 10^7 cells g^{-1}). In spite of the high population of the native rhizobia in the soils, the cowpea varieties responded to inoculation suggesting the effectiveness of the elite bradyrhizobia strains used. Proximity of sampling site close to homestead had significantly (P < 005) higher Shoot dry weight than those away from homestead. The results from this experiment showed that the main effects of Nitrogen sources and varieties had significant effect on shoot dry weight of cowpea in the all locations. The shoot dry weight of plants that received 100 kg N ha^{-1} was marginally higher than those of the inoculated plants, indicating that elite rhizobia strain only partially met the N need of the cowpea crops. Further studies are suggested to evaluate larger number of bradyrhizobial on this environment.

INTRODUCTION

The most frequently limiting nutrient in soils of the Nigerian Savanna is nitrogen, due to its continuous depletion from the soil pool by processes such as volatilization, leaching and removal of nitrogen containing crop residues from the soil (Aliyu *et al.*, 2013). Legumes such as cowpea that biologically fix nitrogen are integrated as part of the cropping systems to optimize the supply of this nutrient in agricultural systems. Legumes are able to meet part of their nitrogen requirement through biological nitrogen fixation (BNF) (Bagayoko *et al.*, 2000).

This is however not the case when the native rhizobia are absent, small in number, or the presence of incompatible or less effective

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indigenous rhizobia strains in soil. Under this situation, BNF can be improved through inoculation of soils with effective and compatible elite rhizobial strains (Abaidoo *et al*., 2007). The presence of large population density of compatible rhizobia does not however, impede the possibility that responses to inoculation can be obtained if competitive and highly effective strains are introduced in high quality inoculants (Giller, 2001). Inoculating cowpea with rhizobia has been used to achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels over this period. These gains are usually highest when the inoculated legumes are grown in devoid of rhizobia or low-rhizobia soils, but marginal in soils already containing high number of compatible rhizobia (GRDC, 2013).

Inoculation is not a common practice in Nigeria, although results from field trials involving cowpea often varied, with some showing significant increases in yield Giller *et al*. (2005) and others showing little or marginal differences between inoculated and uninoculated crops. Results from Southern Africa, shows variability in soil fertility as farmers preferably apply nutrient resources to fields closest to homesteads leading to gradients of decreasing soil fertility with proximity away from homesteads (Zingore *et al.,* 2007). Information on weather soils in the Nigerian Savanna exhibit similar gradients in fertility is lacking. Additionally, few studies have been conducted on the varietal response of cowpea to inoculation in soils of Nigerian Savanna. In view of this, a need to inoculate trial was carried out in the teaching and research farm of the federal university of Technology Minna to determine the following

(i) Population of native rhizobia present in some soils of Northern Guinea Savanna.

(ii) Response of four cowpea varieties (Kanannado, IT93K-452-1, IT97K-499-35, and IT90K-277-2) to *Bradyrhizobium* inoculation.

(iii) To investigate if there exist a relationship between cowpea response to inoculation and proximity of field to homestead.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in the screen house of the Federal University of Technology Minna. The study area is located in the Southern Guinea Savanna zone of Nigeria which lies between longitudes 90 30' and 90 40' E and latitudes 6° 30' and 6° 35' N at an elevation of about 258.5 m above sea level.

Soil Sampling and Analysis

Soil samples were collected from 5 locations in the Northern Guinea Savanna (NGS) (Rijau, Kontagora, Magama, Mashegu, and Mariga,) of Niger state. At each location, soils were collected from sites close to homesteads (0-50 m) and soils sampled further away from homesteads (at least 250 m away) and bulked to form composites sample using sterilized shovel before and after sampling. In order to characterize the soil, part of the soil samples was taken to the laboratory, air-dried gently crushed and passed through a 2 mm sieve for physical and chemical analyses by standard methods (IITA, 1989). The remaining samples were prepared moist for greenhouse study.

Treatments and Experimental Design

The treatments for the MPN experiment were (i) 2 proximity of field to homestead soils in area of close proximity to homestead (< 50 m), and those far from homestead (> 250 m) (ii) 4 cowpea varieties (Kanannando, IT93K-452-1, IT97K- 499-35, IT90K-277-2). The experiment was arranged in a 2 x 4 factorial fitted to a completely randomized design (CRD) replicated four times. While for the need to inoculate trial the treatments were (i) 5 locations (Rijau, Kontagora, Magama, Mashegu, and Mariga). (ii) 4 nitrogen sources (urea at the rate of 100 kg ha^{-1}, plant inoculated with *Bradyrhizobium* sp. strain BR 3262 or *Bradyrhizobium* sp. strain BR 3267, and control (Neither N nor inoculant rhizobia applied), (iii) 4 cowpea varieties (iv) 2 proximity (varieties and

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proximity to homestead was the same as that of MPN). The experiment was a 5 x 4 x 4 x 2 factorial, fitted to a completely randomised design (CRD) and replicated four times.

Greenhouse study

MPN Determination

The assay was conducted using modified Leonard jar method in Southern Australia (Howieson *et al*., 2014) using coarse sand as the potting medium. Coarse sand was washed thoroughly with water to remove all traces of dissolved nutrients before sun drying. 2.5 kg of the sand was transferred to a 3 litre pot and 200 ml of calcium solution was added directly to the sand. Cowpea seeds were sown at the rate of 4 seeds per pot and thinned to 1 per pot a week after planting. Plants were watered daily using sandsman's nitrogen free nutrient solution (Sandsman, 1970). Inoculated was done a week after planting with 1 ml aliquots of the soil suspensions made from serial dilution of soils sampled. Each soil sample was initially diluted 20-fold by adding 10 g of soil in 190 ml of sterile distilled water. The resulting suspension was thoroughly mixed and then used to prepare a 10-fold serial dilution (Woomer, 1994). Each dilution level was replicated four times resulting in a total of 24 pots per each soil sample. Harvesting was done in a period of seven weeks after planting to observe the patterns of nodule appearances. The (+) sign was used to indicate presence of at least one nodule, while (–) denoted absence of nodules (Vincent, 1970).

Need to Inoculate Trial

Soil samples were added to 2.5 litre pot at 2 kg per pot. To each pot was added 372.6 mg of P, K, Mg, Zn, Mo, and B fertilizer and thoroughly mixed. Cowpea seeds were planted at 2 per pot and thinned to one seedling per pot one week after emergence. N treated plants were supplied with nitrogen and was split applied to the N treatment at first week (81.60 mg) and second week (244.80 mg) after planting. Inoculants were applied to the seeds before planting in inoculated treatment. The plants were watered daily with sandsman's N free nutrient solution 200 ml per pot for the first four weeks and later sterile distill water was used, plants were harvested 7 weeks after planting.

Data Analysis

The data was subjected to statistical analysis using MINITAB 17.0. Analysis of variance (ANOVA) of the general linear model was used to check for significant effects and significant means were separated using Fisher least significant difference (L.S.D) method.

RESULTS

Physical and chemical characteristics of the experimental soil

The physical and chemical properties of soils used for this study are presented in (Table 1). Soils of the experimental site from both close and far proximities to homestead were either sandy loamy or loamy sandy, except for Mashegu close which was sandy soil. Soil pH for both proximities to homestead in the 5 locations was slightly acidic to near neutral ranging from 5.31-6.79 but a moderate acidity of 4.77 was observed for soils sampled near homestead in Kontagora. Total nitrogen was low for all locations and proximities to homestead (0.06 g kg^{-1} - 0.22 g kg^{-1}), also organic carbon was low in the 5 locations but a moderately high value was observed with Kontagora close to homestead (12.45 g kg^{-1}). Available P ranges from low to moderately low (18.0 mg kg^{-1}) in Kontagora close to homestead and very high (31.0 mg kg^{-1}) in Mariga away from homestead. The result of Effective cation exchange capacity (ECEC) ranges between 5.79 cmol kg^{-1} – 8.25 cmol kg^{-1} respectively.

Estimate for rhizobial population of four varieties of cowpea in NGS of Niger State

The rhizobial populations as estimated using four (4) cowpea varieties as trap host are presented in (Table 2). The soils of the study sites had high numbers of indigenous rhizobia (>10^3 rhizobia cells g^{-1} of soil). However, soils sampled close to homestead in Mashegu, and for those away from

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homestead in Mariga and Magama had the highest population (71.84 x 10^6 cell g^{-1} of soil) using Kanannado variety while the least native populations (4.61 x 10^6 cell g^{-1} of soil) were obtained using IT93K-452-1 variety for Kontagora and Rijau using soils sampled close to homestead.

Effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea

The main effect of nitrogen sources significantly ($P<0.05$) affected shoot dry weight of cowpea in the 5 locations (Table 3). The Urea treated plants produced the highest shoot dry weight of cowpea in all locations while the control plants had the smallest shoot biomass. There was a significant response of cowpea to rhizobia inoculation, plant inoculated with either or both of the inoculant strains produced the greater shoot dry weight than the control in all soils.

The main effect of proximity to homestead on shoot dry weight of cowpea was significant ($P<0.05$) for all locations except in Magama, and Rijau (Table 3). Soils sampled close to homestead supported higher cowpea shoot dry matter than soils further away from homestead.

The varietal effect on shoot dry weight was significant in the five locations (Table 3). The Kanannado variety produced the highest shoot dry weight while IT90K-277-2 variety had the smallest dry weight but the reverse was the case with IT93K-452-1 in Mashegu. IT93K-452-1 and IT97K-499-35 produced similar shoot biomass at four out of the five locations. The interactive effect among nitrogen source, proximity to homestead, and varieties significantly affected the shoot dry weight of cowpea in all location.

Table 1. Physico-chemical properties of soils used for this study

Location	Proximity	pH CaCl	OC	N	Avail.P	Textural Class	Ca	ECEC
			g kg^{-1}		(mg kg^{-1})		(cmol kg^{-1})	
Kontagora	Close	4.77	12.45	0.20	18.0	LS	5.20	7.64
	Far	5.31	2.55	0.10	6.0	LS	4.40	6.13
Mashegu	Close	5.84	3.75	0.10	6.0	S	4.64	6.34
	Far	5.97	4.95	0.08	5.0	SL	5.60	7.43
Rijau	Close	6.57	4.80	0.06	8.0	LS	4.64	6.17
	Far	5.99	6.15	0.15	9.0	SL	4.88	6.49
Mariga	Close	5.78	6.45	0.22	6.0	LS	6.40	8.25
	Far	5.85	8.25	0.10	31.0	LS	5.20	7.53
Magama	Close	5.47	2.70	0.14	6.0	SL	4.56	5.79
	Far	5.57	3.75	0.20	7.0	LS	5.44	7.18

***SL-sandy loamy *LS-loamy sand *S-sandy**

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Table 2 **Estimate of rhizobial population for four varieties of cowpea at the 5 locations in ($x10^6$ cell g^{-1} of soil)**

Location	Proximity	Kanannado	IT93K-452-1	IT97K-499-35	IT90K-277-2	Means
Kontagora	Close	10.94	4.61	11.24	7.19	**8.50**
	Far	22.48	31.85	12.27	11.24	**19.46**
Mashegu	Close	71.89	22.48	11.24	7.19	**28.2**
	Far	12.27	22.48	11.24	7.19	**13.30**
Rijau	Close	12.27	4.61	9.26	12.27	**9.60**
	Far	12.27	12.27	9.26	10.94	**11.19**
Mariga	Close	22.48	42.59	11.24	7.19	**20.88**
	Far	71.89	12.27	12.22	11.24	**26.91**
Magama	Close	22.48	10.94	42.59	7.19	**20.80**
	Far	71.89	12.27	11.24	11.24	**26.66**
Means		**33.06**	**17.64**	**14.18**	**9.29**	

Table 3 **Effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight (g plant-¹) of cowpea**

Treatment	Kontagora	Mashegu	Rijau	Mariga	Magama
Nitrogen sources(N)					
Control	1.27c	1.16c	1.39c	1.52b	1.13c
Urea	1.87a	1.86a	2.13a	2.06a	1.75a
BR 3262	1.54b	1.48b	1.36c	1.50b	1.27bc
BR 3267	1.22c	1.80a	1.65b	1.91a	1.30b
Significance	**	**	**	**	**
LSD (0.05)	0.17	0.17	0.23	0.25	0.16
Proximity (P)					
Close	1.55a	1.70a	1.63a	1.85a	1.33a
Far	1.40b	1.45b	1.63a	1.64b	1.40a
Significance	*	*	NS	*	NS
LSD (0.05)	0.12	0.12	0.16	0.17	0.11
Varieties (V)					
Kanannado	1.95a	1.88a	2.01a	2.02a	1.67a
IT93K-452-1	1.39b	1.34c	1.65b	1.89ab	1.17c
IT97K-499-35	1.42b	1.49bc	1.72b	1.71b	1.38b
IT90K-277-2	1.14c	1.58b	1.16c	1.37c	1.23bc
Significance	**	**	**	**	**
LSD (0.05)	0.17	0.17	0.23	0.25	0.16
Interaction					
N X P	NS	*	*	NS	NS
N X V	**	**	**	**	NS
P X V	NS	*	NS	**	NS
N X P X V	*	*	*	*	*

Means with the same letters in a column under a treatment are not statistically different (P>0.05). Significant at (P<0.05), ** highly Significant at (P< 0.01), NS (not significant)

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Interactive effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Mashegu

Kanannado variety treated with nitrogen in soils sampled further away from homestead had the heaviest shoot dry weight. These were however similar to the shoot dry weight produced by the same variety treated with BR 3262 or BR 3267 for soils close to homestead using IT90K-277-2 (Fig 1).

The smallest shoot biomass was produced by IT93K-452-1 plant in soils further away from homestead treated with BR 3262. A similar trend was observed using the same inoculant and proximity to homestead for IT90K-277-2 also IT93K-452-1, IT90K-277-2, and IT97K-499-35 that were either not treated with N or inoculated or those treated with either inoculant strains in soils sampled further away from homestead.

Interaction of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Mariga

Fig 2 shows that nitrogen sources, proximity to homestead, and varieties of cowpea had a significant ($P<0.05$) positive effect on shoot dry weight of cowpea. The N – treated kanannado plants grown on soils sampled close to homestead produced the largest biomass yield. The N – treated kanannado plants in soils further away from homestead had similar yield to those of BR 3267 inoculated IT97K-499-35 plant using soils sampled away from homestead. The least biomass was produced generally by the various varieties with no amendments.

Interactive effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Kontagora

Kanannado plant irrespective of proximity to homestead treated with nitrogen had the heaviest shoot dry weight (Fig 3). These were however similar to the yield produced by the N-treated IT90K-277-2 plant using soils sampled close to homestead and BR 3262 inoculated Kanannado plant in soils sampled from both proximities to homestead. The least biomass was produced generally by IT90K-277-2 plant in soils of both proximities to homestead that were treated with inoculants or the unamendment plant. These was however not the case for the urea treated plant where IT97K-499-35 plant had the lowest shoot dry weight.

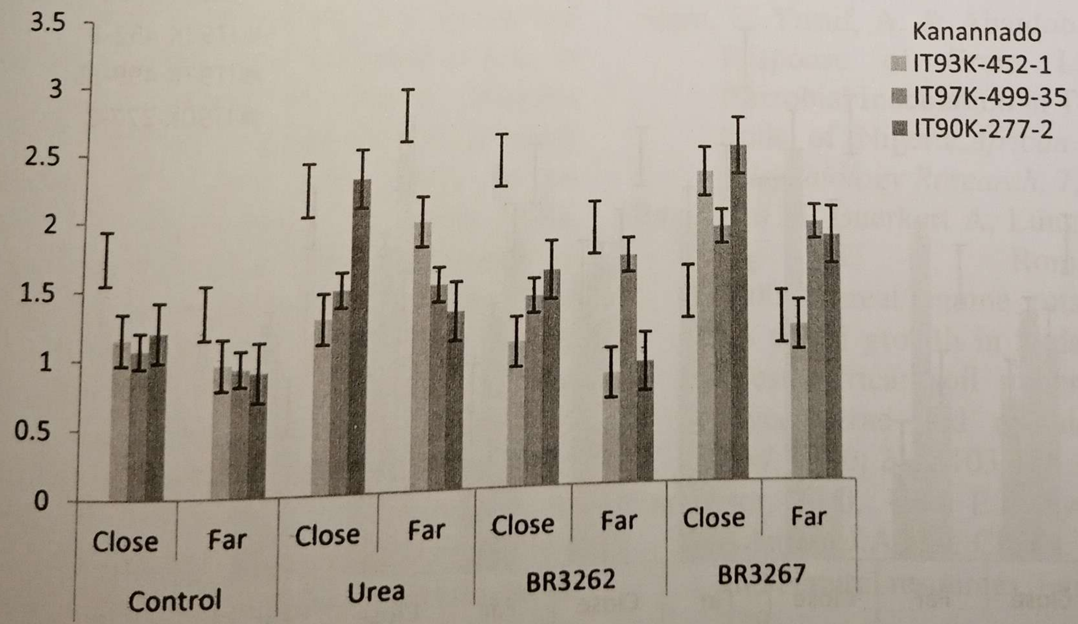

Fig 1 **Interaction effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Mashegu**

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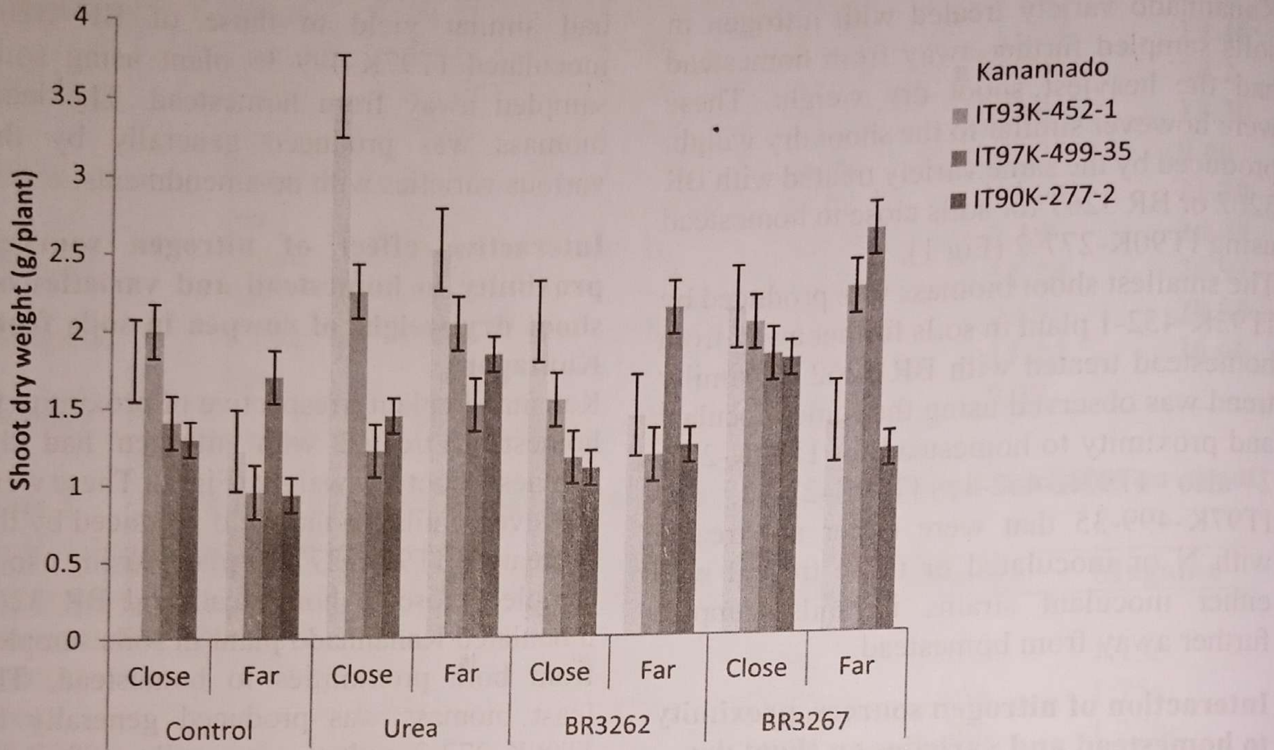

Fig 2 **Interaction effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Mariga**

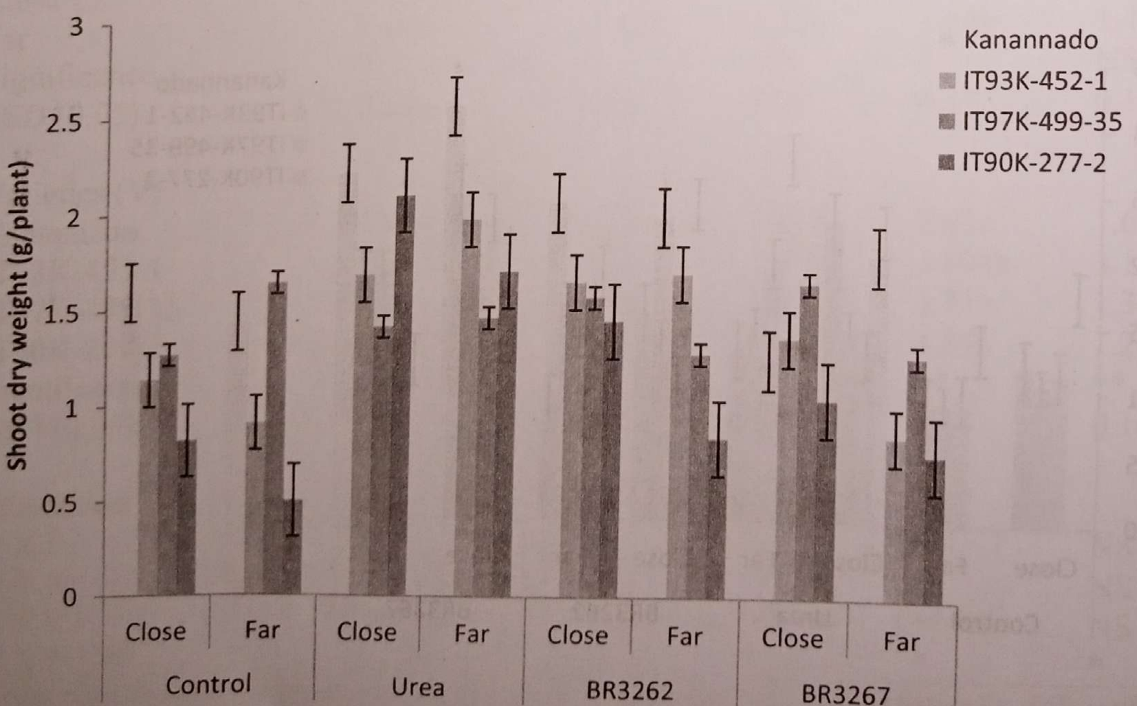

Fig 3 **Interaction effect of nitrogen sources, proximity to homestead and varieties on shoot dry weight of cowpea in soils from Kontagora**

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DISCUSSION

Organic carbon of the experimental soil was low also the nitrogen content was much lower than the critical level of 15 g Kg^{-1} (Okalebo *et al.*, 1993).The available phosphorus result indicate that Kontagora far away from homestead and Mariga close to homestead soils fall within the optimum available P concentration that would help enhance nodulation and Nitrogen fixation in the grain legume (Enwezor, 1990). Results of ECEC confirm that the experimental soil is low in fertility.

In this study, proximity of fields close to homesteads had significantly higher shoot dry weight than those away from homestead similar reports was made by Zingore *et al.* (2007) whose study shows that the fertile plots is often closest to homesteads, as a result of continous accumulation of organic ammendment including all kinds of manure and household waste applied directly surrounding the villages.

The success of rhizobia inoculation primarily depends on rhizobial strain, the legume genotype, environmental conditions, and the crop management (Woomer *et al.*, 2014). Although, there are two main situations where there is likely to be a response to rhizobia inoculation; where compatible rhizobia of the host legume are absent and where native rhizobia population is low. In this study, counts of native rhizobia population were very high (>10 cells g^{-1} soil). Suggesting that there may likely be no response to inoculation in these soils. Contrary to this observation, response to inoculation was observed in this study. This result agrees with the findings of Giller (2001) who report in his findings that the 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. Similar results were obtained which could be due to the effectiveness of the elite strain introduced.

This study shows that Kanannado variety had better response to inoculation than the other varieties used. Also, Interaction between nitrogen sources, proximity to homestead and varieties significantly affect the shoot dry weight.

Result from this study suggests that the two elite inoculant strains used for this study could establish effective symbiosis with cowpea in soils of the study area. Thereby, increasing the yield of cowpea plants. However, in spite of the beneficial effect of elite rhizobia strains, shoot dry weight of the N-treated plant was marginally higher than those of the elite strains thus unable to meet the optimum nitrogen requirement of the plant. Further study could however be carried out to evaluate greater number of inoculant strains to further get to the conclusion on the efficacy of inoculation on cowpea which could serve as a cheaper alternative to inorganic N-fertilizer application.

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