



Effects of Biochar and Rhizobium Inoculation on Nodulation and Growth of Groundnut in Sokoto State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAY, IM and NGH designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MMS and IYT managed the literature searches, analyses of the study and performed the laboratory analysis. Authors MAM, GAA and SAL managed the experimental process. Author AMH sourced and supplied both the rhizobium inoculants and the groundnut seeds. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/27297

Editor(s):

(1) Martin Koller, University of Graz, Research Management and Service, c/o Institute of Chemistry, Austria.

Reviewers:

(1) Magda Mahmoud ElAraby, Ain Shams University, Egypt.

(2) David Burke, Boyce Thompson Institute for Plant Research, USA.

(3) Ipsita Das, Orissa University of Agriculture and Technology, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history/16823>

Original Research Article

Received 27th May 2016
Accepted 27th September 2016
Published 7th November 2016

ABSTRACT

An experiment was conducted in 2015 at the screenhouse of Teaching and Research Farm, Usmanu Danfodiyo University, Sokoto, to determine the effects of biochar and rhizobium inoculation (HISTICK) on nodulation and growth of groundnut plant (SAMNUT-24). Two factors were used for this experiment; Biochar rates and rhizobium inoculation. Biochar was applied at the rates of 0 t/ha, 10 t/ha and 20 t/ha while 5 g of rhizobia was inoculated to 1 kg of groundnut seeds

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(inoculated [+]). The experiment was laid out in a completely randomized design and replicated three times. Data were collected on growth parameters and nodulation and were analyzed by ANOVA and means were separated using Duncan's Multiple Range Test at 5% level of significance. The results showed that 10 t/ha and 20 t/ha of biochar significantly ($P < 0.05$) increased number of effective nodules (11.33 and 14.0 respectively) and shoot dry matter yield (7.95 kg/ha and 8.67 kg/ha respectively) when compared with control (0 t/ha). No significant differences were observed in number of leaves, heights of plants, root dry matter yield and number of non-effective nodules among biochar rates. Rhizobium inoculation had significantly ($P < 0.05$) increases the number of effective nodules (12.33), shoot (8.14 kg/ha) and root (2.2 kg/ha) dry matter yields. In conclusion rhizobium could be inoculated in biochar amended soil to improve nodulation and growth parameters of groundnut plant. 10 t/ha could be used to improve growth parameter of groundnut plant.

Keywords: Biochar; rhizobia; inoculation; nodulation; SAMNUT-24.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the world's most popular crops cultivated throughout the tropical and subtropical areas. Groundnut also referred to as peanut is known by many other local names such as earthnut, goober pea, monkey nut, pygmy nut and pignut. Groundnut seeds contain about 50% edible oil, 20% carbohydrate with average yield that ranges between 1271.2-1520 kg/ha [1,2]. Groundnut as a legume plant is relatively high in protein, which can be directly attributed to its ability to supply most of its own nitrogen needs with the help of symbiotic bacteria (rhizobia) living in their roots [3]. The bacteria produce ammonia (NH_3) using the hydrogen acquired from the plant's carbohydrates and nitrogen from the air, which then serves a source of nitrogen for plant to growth; the bacteria also use the carbon from the carbohydrates as the energy source to grow and carry out physiological processes including nitrogen-fixation- this symbiotic relationship between bacteria and legume allows them both to flourish and produce high-protein seeds or forage crops [3]. Inoculation of legumes with rhizobium was found to increase number of nodule and nitrogen fixing activity of the crop [4]. When inoculated with the proper strain of bacteria, legumes can supply up to 90% of their own nitrogen [3]. Similarly, [5] reported that inoculation with rhizobium brings about significant increases in all the growth and yield parameters than when not inoculated; Biochar addition to soil increases soil nutrient concentrations and microbial activity leading to development of plant growth [6].

Biochar is a carbon-rich product that is obtained through pyrolysis- which is a thermal decomposition of organic material under limited

oxygen supply [7]. It is also used as a soil amendment for improving soil fertility, enhancing plant growth, reducing soil greenhouse gas emissions and sequestering carbon [7,8,9]; Biochar can alter soil physical and chemical properties, improve soil quality by increasing nutrient availability thereby increasing plant biomass production [10,11]. While data on biochar effects on nodulation and plant growth are accumulating, there are several important gaps in our knowledge of these effects. So far, results on the effects of biochar on nodulation and plant growth have been contradictory some authors observed no or little effect whereas, other authors found remarkable changes [12]. Therefore, this study aims to broaden the information base concerning how biochar amendment influences rhizobium nodulation and growth performance of groundnut plant.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted during the 2015 rainy season in the Screenhouse at Teaching Research Farm of Usmanu Danfodiyo University, Sokoto. The farm is located at Latitude $13^{\circ} 01' \text{N}$ and Longitude $5^{\circ} 15' \text{E}$, with average annual rainfall of 557.4 mm per annum and air temperature that varies from 34.7°C in July to 30.6°C in August to 32.0°C in September and 34.0°C in October [13]. The soil is predominantly sandy in nature and classified as Entisols [14].

2.2 Biochar Production

The biochar was produced using maize cob (shelled cob) collected from Gada Local Government Area, Sokoto State. The biochar was produced using pyrolysis method [15] and

achieved by loading the maize cob in the drum covered with a metal sheet at the top. The maize cobs were burnt in the presence of little oxygen. The burnt cobs were then ground and sieved with 2 mm sieve and used as biochar. The chemical compositions (such as organic carbon, N, P, K, Mg, Ca, Na, CEC, and pH) of biochar were determined.

2.3 Experimental Design and Layout

The experiment was laid out in a 2 x 3 factorial arrangement in a completely randomized design and replicated 3 times. The experiment consists of two factors; rhizobium inoculation (inoculated [+] and uninoculated [-]) and biochar application rates (0 t/ha, 10 t/ha and 20 t/ha). Hence a total of 18 plastic buckets were used for this experiment.

2.4 Biochar Incorporation and Rhizobium Inoculation

A corresponding quantity of biochar was mixed thoroughly in a rubber bucket containing 10 kg of sterilized top soil 2 weeks before planting. Five grams (5 g) of rhizobium inoculants (HISTICK) was applied to 1 kg seeds of groundnut (SAMNUT-24). The presence of black specks on the seeds indicates the inoculums and was done under aseptic condition. Both the rhizobium inoculants and the groundnut variety were sourced from Institute for Agricultural Research (IAR) Samaru, Zaria.

2.5 Sowing, Fertilization and Weeding

The seeds were planted by dibbling method. Holes of 3-5 cm depth in each bucket were dug and one seed was sown per hole. Note that, the

uninoculated seeds were sown first to avoid contamination. 10 kg N, 54 kg P₂O₅ and 25 kg K₂O were applied to both control and treated buckets as starter dose. Weeds were manually controlled by hand pulling.

2.6 Data Collection

Data were collected on the following parameters; plant height at 3 and 6 weeks after sowing (WAS) using meter rule, number of leaves at 3 and 6 WAS and were counted manually, the shoot and root biomasses at 8 WAS which were air dried and then oven dried at a temperature of 60°C for 24 hours and the weights were extrapolated in kg/ha, and the number of effective and non-effective nodules at 8 weeks WAS i.e. when the groundnut plants reached 50% flowering and nodulation was at its peak-where the groundnut plants were gently uprooted and the roots were washed with tap water and the number of effective and non-effective nodules were counted.

2.7 Data Analysis

The data collected were subjected to analysis of variance (ANOVA) techniques [16] using computer package. The treatment means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Number of Leaves and Plant Height

The biochar was found to have strongly alkaline pH, high OC and K, very low phosphorus level, N, Ca and Mg (Table 1). Figs. 1, 2, 3 and 4 showed that, there were no significant effect in

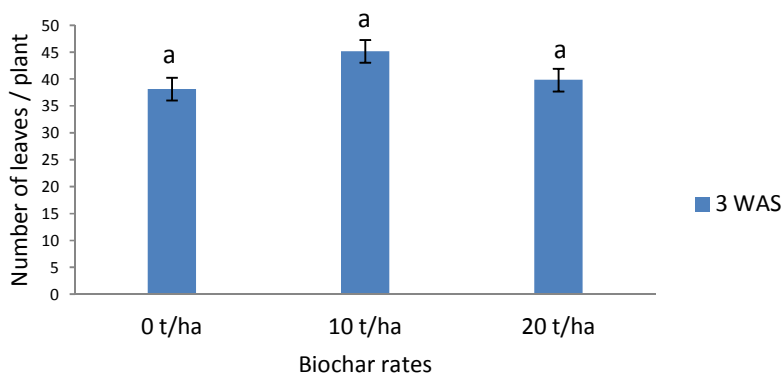


Fig. 1. Effects of biochar on number of leaves of groundnut plants at 3 WAS

Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

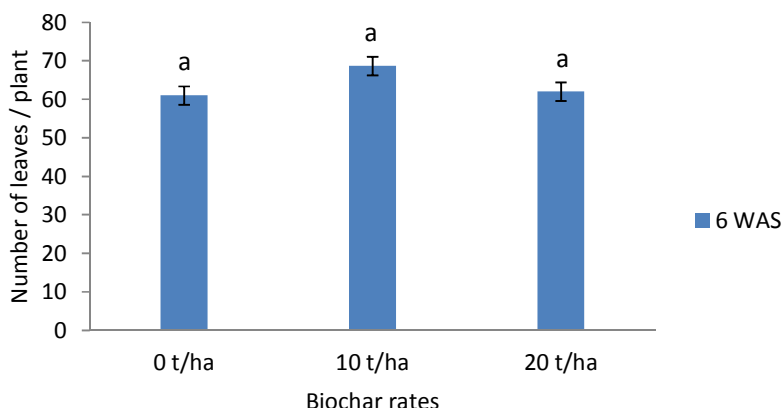


Fig. 2. Effects of biochar on number of leaves of groundnut plants at 6 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

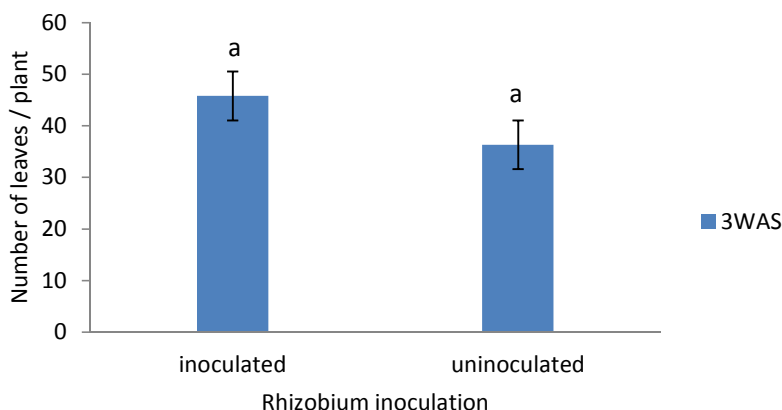


Fig. 3. Effects of rhizobium inoculation on number of leaves of groundnut plants at 3 WAS
 Bars with the same letters are not significantly different according to Duncan's New Multiple Range Test at ($P < 0.05$)

Table 1. Composition of biochar used for the experiment

Property	pH (H ₂ O)	OC (%)	Total N (%)	Total P (mg/kg)	Exchangeable bases (cmol/kg)				CEC (cmol/kg)
					Ca	Mg	K	Na	
Biochar	10.4	40.7	0.053	1.47	0.65	0.20	3.64	3.3	9.1

number of leaves and heights of plants among biochar rates at 3 and 6 WAS, though 10 t/ha recorded highest value (45.17 / plant and 68.67 cm/ plant respectively) followed by 20 t/ha and lastly 0 t/ha. According to [17] biochar treatments were found to have increased the number of leaves and plant height of lettuce plant in comparison to no biochar treatments. However, the insignificant difference among biochar rates as observed in our result could be attributed to the slow nature of biochar in releasing nutrients.

Rhizobium inoculation also showed no significant effect on the number of leaves (Figs. 5 and 6) and plant height (Figs. 7 and 8) at 3 and 6 WAS, though higher values were obtained with inoculated plants than un-inoculated ones. Higher values obtained in inoculated plants may be attributed to the symbiotic relationship of rhizobium bacteria with the roots of the leguminous crops, which fix the atmospheric nitrogen into the roots of groundnut and thus the number of leaves was increased [5]. Biochar and rhizobium interactions in number of leaves and

plant heights at 3 and 6 WAS were not significant.

3.2 Number of Effective and Non-effective Nodules, Shoots and Roots Dry Weights

There was significant increase ($P < 0.05$) in effective nodules (Fig. 9) and shoot dry weight (Table 2) as a result of the application of biochar with 10 t/ha (11.33 / plant and 7.95 kg/ha) and 20 t/ha (40.00 / plant and 8.67 kg/ha) producing higher values than the control. Similar finding was reported by [18] that addition of biochar to soils resulted on average in increased aboveground productivity, crop yield, soil microbial biomass, rhizobia nodulation compared with control conditions. No significant differences were observed in non-effective nodules and root dry weights among biochar application rates

though non-effective nodules decreased with increasing biochar rate.

Number of effective nodules (12.33 / plant) in Fig. 10, shoot (8.14 kg/ha) and root (2.20 kg/ha) dry weights (Table 2) were significantly higher ($P < 0.05$) with inoculated plants than uninoculated ones. Concomitantly, this finding has agreed with [5] who reported an increase in number of nodules and shoot per plant with plants inoculated with rhizobia than un-inoculated plants. However, increase in number of nodules could be attributed to inoculation of rhizobia, which increased the number of bacteria and hence more nodules per plant were produced. No significant difference was observed in number of non-effective nodules between inoculated and uninoculated ones. Biochar and rhizobium interactions in number of effective and non-effective nodules, shoots and roots dry weights were not significant.

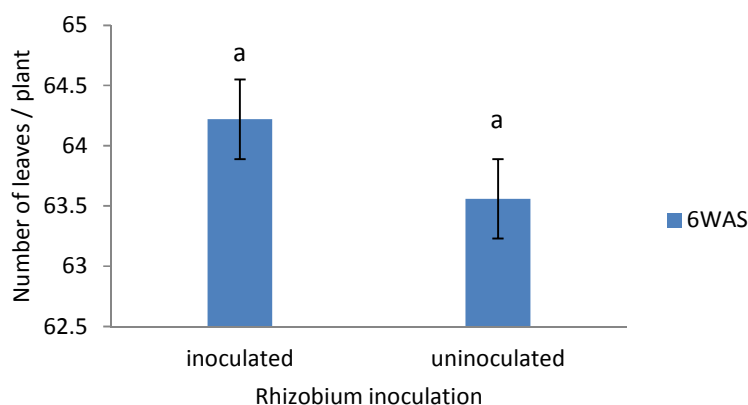


Fig. 4. Effects of rhizobium inoculation on number of leaves of groundnut plants at 6 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

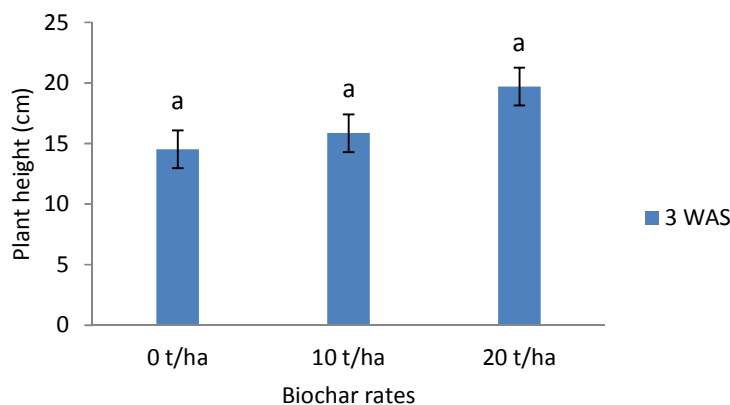


Fig. 5. Effects of biochar on heights of groundnut plants at 3 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

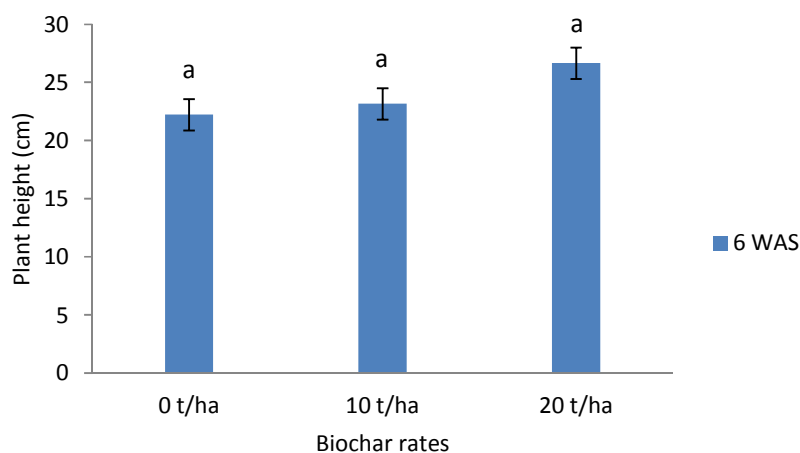


Fig. 6. Effects of biochar on heights of groundnut plants at 6 WAS

Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

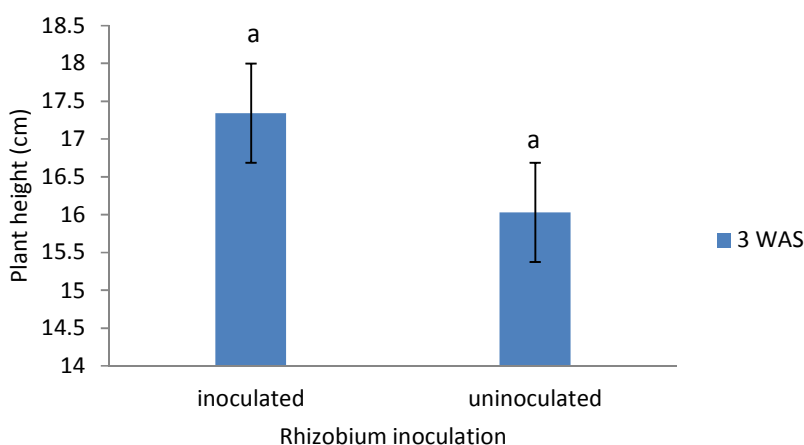


Fig. 7. Effects of rhizobium inoculation on heights of groundnut plants at 3 WAS

Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

Table 2. Effects of biochar and rhizobium inoculation on number of effective and non-effective nodules, shoot and root dry biomasses

Treatment/ parameters	Dry shoot weight (kg/ha)	Dry root weight (kg/ha)
Biochar rate (B) (t/ha)		
0	3.42 ^b	0.83
10	7.95 ^a	1.68
20	8.67 ^a	1.98
SE±	0.61	0.53
Rhizobium inoculation(R)		
+	8.14 ^a	2.20 ^a
-	5.21 ^b	0.80 ^b
SE±	0.50	0.43
Interaction		
B x R	ns	ns

Means within the same column with the same letters are not significantly different according to Duncan's New Multiple Range Test at ($P < 0.05$). ns, not significant; +, inoculated; -, uninoculated; B, biochar rate; R, rhizobium inoculation

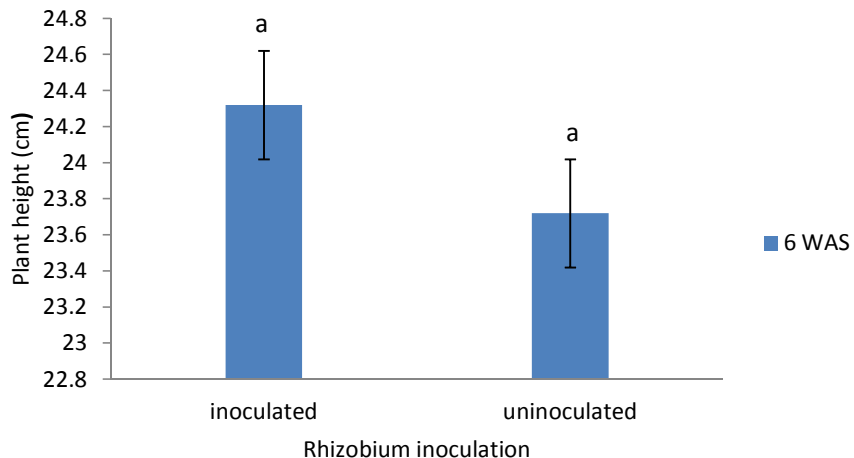


Fig. 8. Effects of rhizobium inoculation on heights of groundnut plants at 6 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

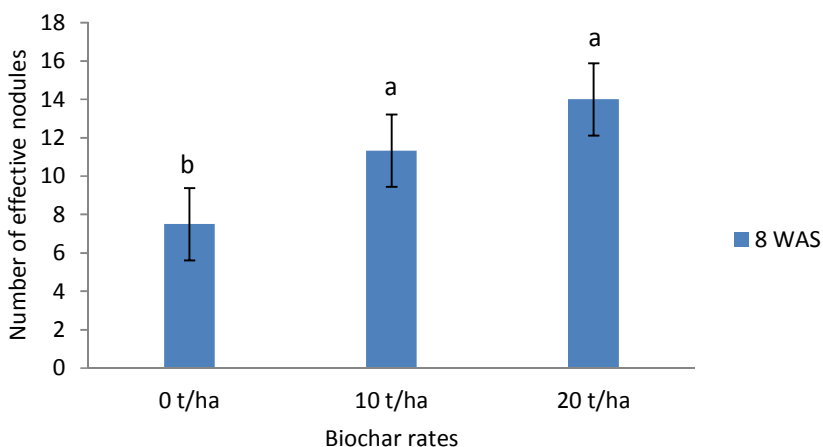


Fig. 9. Effects of biochar on number of effective nodules of groundnut plants at 8 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

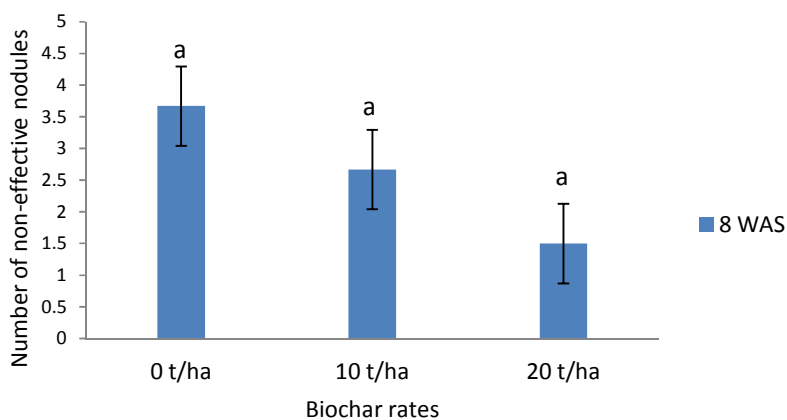


Fig. 10. Effects of biochar on number non-effective nodules of groundnut plants at 8 WAS
 Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

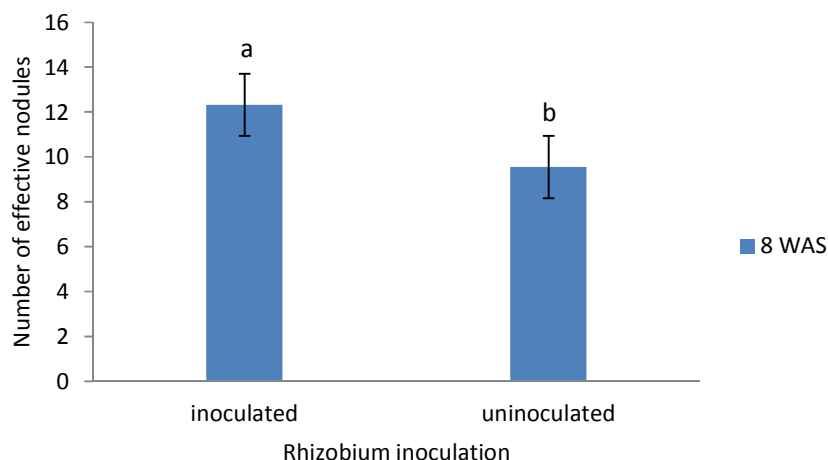


Fig. 11. Effects of rhizobium inoculation on number of effective nodules of groundnut plants at 8 WAS

Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

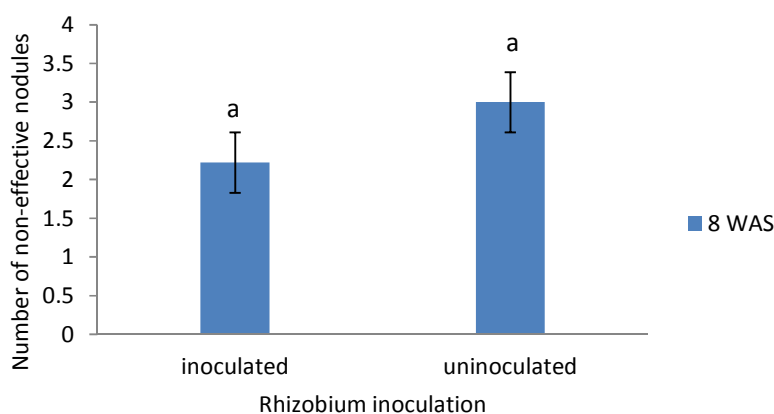


Fig. 12. Effects of rhizobium inoculation on number non-effective nodules of groundnut plants at 8 WAS

Bars with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P < 0.05$)

4. CONCLUSION

Application of biochar and rhizobium inoculation on groundnut plants was found to increase number of effective nodules, shoot and roots dry weights. However, the interaction between biochar and rhizobium inoculation in all the growth performances and nodulation was mutually exclusive. 10 t/ha of biochar could be applied to groundnut plant to improve growth performance and rhizobium could be inoculated in biochar amended soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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