



# Effects of Biochar and Rhizobium Inoculation on Selected Soil Chemical Properties, Shoot Nitrogen and Phosphorus of Groundnut Plants (*Arachis hypogaea* L.) 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, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAM and AMH managed the analyses of the study. Author AYF managed the literature searches and other correspondences. All authors read and approved the final manuscript.

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## ABSTRACT

The research was carried out to determine the effects of biochar and rhizobium inoculation on selected soil chemical properties, shoot N and P of groundnut plant. Two factors were used for this experiment; Biochar and rhizobium inoculation. Biochar was applied at the rate of 20 t ha<sup>-1</sup>, 10 t ha<sup>-1</sup> and 0 t ha<sup>-1</sup> while rhizobium was inoculated to groundnut seeds (inoculated [+]) and un-inoculated [-]). The experiment was laid out in completely randomized design and replicated 3 times. Means were separated using Duncan's Multiple Range Test at 5% level of significance. The results showed that 20 t ha<sup>-1</sup> of biochar significantly (p<0.05) produced higher values of soil pH, OC, N, available P

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and Ca, than  $10 \text{ t ha}^{-1}$  and  $0 \text{ t ha}^{-1}$ . No significant differences were observed in shoot P among biochar application rates. Rhizobium inoculation had significantly ( $p < 0.05$ ) increase the number of shoot N, soil N, P, K and Mg when compared with un-inoculated plants. It is recommended that  $20 \text{ t ha}^{-1}$  should be used for improvement of soil chemical properties while inoculation with rhizobia may be more effective in the presence of biochar due to the habitat offered by the biochar.

*Keywords: Biochar; rhizobium; inoculation; groundnut; soil chemical properties.*

## 1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) belongs to the family Leguminosae. It's originated in South America, probably in Brazil, and has been cultivated since ancient times by native Americans [1]. Groundnuts require warm growing season of 120 to 140 days, moderate rainfall and pH 6.0 to 6.5 is more suitable for its cultivation. Groundnut seeds contain 40 to 50 percent oil and 20 to 30 percent protein, and they are an excellent source of B vitamins [2]. High protein content of groundnut can be directly attributed to a legumes ability to supply most of its own nitrogen needs with the help of symbiotic rhizobia bacteria living in their roots [3]. This symbiotic relationship between bacteria and legume allows them both to flourish and produce a high-protein seed or forage crop. Inoculation of legumes with proper strain of rhizobia bacteria can supply up to 90% of their own nitrogen [3]. [4] reported that rhizobium inoculation was found to increase nodule number and nitrogen fixing activity of the crop. Biochar addition to soil leads to an increase in microbial activity and soil nutrient concentrations resulting to an improved plant growth [5].

Biochar is a product of thermal decomposition of biomass feedstock such as plant wastes, forestry and yard wastes and animal manures in the partial or total absence of oxygen [6,7]. The feedstock undergoes a process called pyrolysis or gasification which results in a rearrangement of the biomasses molecules, yielding black biochar and other products [6]. The effect of biochar on soil quality and crop productivity is generally observed to be positive. The chemical composition, surface chemistry, particle and pore size distribution, physical and chemical stabilization mechanisms of biochar in soils, determine the effects of biochar on soil functions [8-10]. Addition of biochar to soil changes important soil chemical qualities such as soil pH, N, available P, exchangeable bases, CEC, organic matter, Mn and decreases  $\text{Al}^+$ , S and Zn toxicity. Soil pH is mostly neutral to basic [11]; typically increased soil CEC [12]; increasing trend of bio-available P and base cations in

biochar applied soils has been reported by [13]. According to [14] application of biochar in the acidic coastal soil increase soil pH, soil organic matter, Mn, Ca and decrease Zn and S toxicity. Despite the potential usefulness of biochar for soil management applications, our knowledge of how these material influence soil chemical and biological properties is limited compared to other soil amendments [15]. This study aims to evaluate the effects of biochar and rhizobium inoculation on selected soil chemical properties, shoot N and P of groundnut plants.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site and Design

The experiment was conducted during the 2015 rainy season in the greenhouse at Botanical Garden, Usmanu Danfodiyo University, Sokoto. Sokoto lies between Latitude  $4^{\circ} 6' 40'' \text{ N}$  and Longitude  $11^{\circ} 30' - 13^{\circ} 50' \text{ E}$  and of altitude of about 350 m above the sea level. Sokoto lies in Sudan Savanna Zone of Nigeria characterized with erratic and scanty rainfall ranging from 500 mm to 1300 mm. Minimum and maximum temperatures ranges between  $23^{\circ}\text{C}$  and  $38^{\circ}\text{C}$  respectively [16]. The soil is predominantly sandy in nature and classified as Entisols [17].

The experiment was laid out in a  $2 \times 3$  factorial arrangement in completely randomized design and replicated 3 times. The experiment consists of two factors; rhizobium inoculation (inoculated [+]) and uninoculated [-]) and biochar application rates ( $0 \text{ t ha}^{-1}$ ,  $10 \text{ t ha}^{-1}$  and  $20 \text{ t ha}^{-1}$ ). Hence a total of 18 plastic buckets were used for this experiment. Seeds were planted by dibbling method. A hole of 3 – 5 cm depth in each bucket was dug and one seed per hole was sown. 10 kg N, 54 kg  $\text{P}_2\text{O}_5$  and 25 kg  $\text{K}_2\text{O}$  was applied to both control and treated buckets as starter dose. Similarly, weeds were controlled manually by hand pulling.

### 2.2 Biochar Production and Incorporation

The biochar was produced using pyrolysis method at a temperature between  $300^{\circ}\text{C}$  to

500°C under partial exclusion of oxygen as reported by [18]. The chemical compositions of biochar were determined such as organic carbon, N, P, K, Mg, Ca, Na, CEC, and pH. The biochar produced was ground and sieved with 2 mm mesh diameter and then mixed thoroughly in a rubber buckets containing 10 kg of top soil collected from the field 2 weeks before planting to allow mineralization of biochar. 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 groundnut variety were sourced from Institute for Agricultural Research (IAR) Samaru, Zaria.

### 2.3 Soil and Plant Tissue Analyses

At 8 weeks after sowing (WAS), the dried shoot biomass was ground using blending machine to determine shoot N and P. Shoot N was determined using Micro-Kjeldahl method while P was determined using Vanado-phosphate molybdate methods. Soil samples were collected at 12 WAS from four different points in the buckets, at a depth of about 15 – 20 cm using hand trowel. Nitrogen was determined using Micro-Kjeldahl method [19], available phosphorus by Bray No.1 method [20], CEC by ammonium acetate saturation method buffered at pH 7 [21], organic carbon by Walkley and Black wet oxidation method [22]. Exchangeable Bases; Ca, Mg, K and Na were determined by EDTA titration method. Soil pH was determined in 1:1 soil to water ratio [23] using electrometric pH meter.

### 2.4 Data Analysis

The data collected were subjected to analysis of variance (ANOVA) techniques using [24] 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 Physical and Chemical Properties of Soil and Chemical Composition of Biochar Used for the Experiment

The results of soil before planting showed that soil used for the experiment before addition of biochar was slightly acidic (6.86) with soil texture of 778 g kg<sup>-1</sup>, 134 g kg<sup>-1</sup> and 88 g kg<sup>-1</sup> for sand,

clay and silt respectively (Table 1). Organic carbon, total nitrogen and available phosphorus were found to be 3.01 g kg<sup>-1</sup>, 0.32 g kg<sup>-1</sup> and 0.49 mg kg<sup>-1</sup> respectively. The exchangeable bases were found to be 0.35 cmol kg<sup>-1</sup>, 0.20 cmol kg<sup>-1</sup> and 0.33 cmol kg<sup>-1</sup> for Ca, Mg and K respectively. This shows that the soil is poorly fertile. The biochar was found to have strongly alkaline pH, high OC, very low phosphorus level, N, Ca, Mg and K.

**Table 1. Initial soil analysis and biochar composition used in this experiment**

Properties	Field soil	Biochar
pH <sub>H<sub>2</sub>O</sub> (1:1)	6.86	10.41
Sand g kg <sup>-1</sup>	778	-
Clay g kg <sup>-1</sup>	134	-
Silt g kg <sup>-1</sup>	88	-
<b>Textural class</b>	<b>Sandy loam</b>	
O C g kg <sup>-1</sup>	3.01	400.72
N g kg <sup>-1</sup>	0.32	0.54
Available P (mg kg <sup>-1</sup> )	0.49	1.42
<b>Exchangeable Bases (cmol kg<sup>-1</sup>)</b>		
Ca	0.35	0.67
Mg	0.20	0.41
K	0.33	3.74
CEC (cmol kg <sup>-1</sup> )	3.28	10.01

### 3.2 Shoot N and P of Groundnut Plant

There was significant difference ( $p < 0.05$ ) in shoot nitrogen (N) among biochar rates with 10 t ha<sup>-1</sup> and 20 t ha<sup>-1</sup> of biochar producing higher values when compared with control (Table 2). This increase could be as a result of biochar addition which may subsequently increase plant N uptake. [25] and [26] reported higher contents of shoot N as a result of biochar addition. No significant difference was observed in shoot P among biochar application rates.

The rhizobium inoculation also showed significant effect on the shoot N (Table 2). Shoot N was significantly higher ( $p < 0.05$ ) with inoculated plants than un-inoculated ones. This is in line with findings of [1] who reported an increase in shoot N of plants inoculated with rhizobia than un-inoculated plants due to increase in the number of bacteria and henceforth more nodules per plant were produced and this entails higher nitrogen fixing activity of the crop. Also, no

significant difference was observed in shoot P between inoculated plants and uninoculated ones. However, biochar and rhizobium interactions in shoot N and P were not significant.

### 3.3 Effects of Biochar and Rhizobium Inoculation on Some Soil Parameters

There were significant differences ( $p < 0.05$ ) in soil pH, organic carbon, nitrogen, phosphorus and Ca among biochar rates with 20 t ha<sup>-1</sup> of biochar producing higher value when compared with 10 t ha<sup>-1</sup> and 0 t ha<sup>-1</sup> (control) in most cases. Higher nutrient availability for plants is the result of both the direct nutrient additions by the biochar and greater nutrient retention [27]. Previous researches have shown that, additions of biochar to soil have shown definite increases in the availability of major cations and phosphorus as well as in total nitrogen concentrations [12,27]. Similarly, [28] reported an increase in soil phosphorus (P), soil potassium (K), total soil nitrogen (N), and total soil carbon (C) compared with control conditions. Soil pH also tended to increase, becoming less acidic, following the addition of biochar.

The rhizobium inoculation had showed significant effect on soil pH and OC (Table 3). Soil pH and OC were significantly higher ( $p < 0.05$ ) with uninoculated plants than inoculated ones. [3] related the lower soil pH with inoculated plants to ability of nodulated legumes to acquire their N from the air as diatomic N rather than from the soil as nitrate, their net effect is to lower the pH

of the soil. In greenhouse studies, alfalfa and soybeans lowered the pH in a clay loam soil by one whole pH unit. Similarly, the decrease in soil OC may be attributed to increase in the consumption of carbon by the increased bacterial populace which may subsequently decrease the quantity of organic matter in the soil. Significant differences were observed in N, P, K and Mg with inoculated plots producing higher values than uninoculated ones. Biochar and rhizobium interactions were significant in soil organic carbon and N, highly significant in soil pH, P, K and Mg and not significant with Ca.

**Table 2. Effects of biochar and rhizobium inoculation on shoot N and P of groundnut plant at 8 WAS**

Treatment/Parameters	N g kg <sup>-1</sup>	P g kg <sup>-1</sup>
<b>Biochar rate (t ha<sup>-1</sup>)</b>		
0	0.021 <sup>b</sup>	0.00005
10	0.061 <sup>a</sup>	0.0001
20	0.068 <sup>a</sup>	0.0003
SE±	0.007	0.0001
<b>Rhizobium inoculation</b>		
+	0.064 <sup>a</sup>	0.0001
-	0.036 <sup>b</sup>	0.0002
SE±	0.006	0.0001
<b>Interaction</b>		
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; WAS, weeks after sowing; B, biochar rate; R, rhizobium inoculation

**Table 3. Effects of biochar and rhizobium inoculation on selected soil parameters**

Treatment/Parameters	pH	OC g kg <sup>-1</sup>	N g kg <sup>-1</sup>	P mg kg <sup>-1</sup>	K cmol kg <sup>-1</sup>	Ca cmol kg <sup>-1</sup>	Mg cmol kg <sup>-1</sup>
<b>Biochar rate (t ha<sup>-1</sup>)</b>							
0	5.95 <sup>c</sup>	5.03 <sup>c</sup>	0.34 <sup>c</sup>	0.76 <sup>b</sup>	0.71 <sup>a</sup>	0.38 <sup>c</sup>	0.52 <sup>a</sup>
10	7.16 <sup>b</sup>	7.10 <sup>b</sup>	0.48 <sup>b</sup>	0.75 <sup>c</sup>	0.70 <sup>b</sup>	0.50 <sup>b</sup>	0.28 <sup>b</sup>
20	7.27 <sup>a</sup>	9.27 <sup>a</sup>	0.70 <sup>a</sup>	0.78 <sup>a</sup>	0.74 <sup>a</sup>	0.60 <sup>a</sup>	0.48 <sup>a</sup>
SE±	0.023	0.54	0.014	0.002	0.010	0.020	0.013
<b>Rhizobium inoculation</b>							
+	6.52 <sup>b</sup>	5.51 <sup>b</sup>	0.53 <sup>a</sup>	0.78 <sup>a</sup>	0.75 <sup>a</sup>	0.50	0.46 <sup>a</sup>
-	7.06 <sup>a</sup>	8.76 <sup>a</sup>	0.48 <sup>b</sup>	0.74 <sup>b</sup>	0.69 <sup>b</sup>	0.48	0.40 <sup>b</sup>
SE±	0.02	0.44	0.012	0.001	0.008	0.017	0.01
<b>Interaction</b>							
B x R	**	*	*	**	**	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; R, rhizobium inoculation; \*\*, highly significant; \*, significant

#### 4. CONCLUSION

From the result obtained, it was observed that biochar improved shoot N, soil OC, pH, N, P, and Ca. while inoculation with rhizobia may be more effective in the presence of biochar due to the habitat offered by the biochar. Biochar rate of 20 t ha<sup>-1</sup> could be applied in soil to improve soil chemical properties.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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