



INVESTIGATING NPK, MOLYBDENUM AND ZINC EFFECTS ON SOYBEAN NODULATION, NITROGEN FIXATION AND YIELD IN NORTHERN GUINEA SAVANNA SOILS

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ABSTRACT

Soybean, like other grain legumes, requires some essential nutrients to accomplish the dual purpose of fixing atmospheric nitrogen and high yielding. A screen house experiment was then setup to investigate the effect of N, P, K, Zn and Mo on nodulation and growth of soybean. Soils from six farmers' fields in Kaya, northern Guinea savanna and seven fertilizer compounds were used in a factorial experiment laid out in completely randomized design. Data collected at 8 weeks after planting on biomass yields, nodulation variables, N₂-fixation, mycorrhizal roots colonization, and N and P uptake were analyzed using SAS. Soils test revealed variation of soils' chemical contents among farmers field, especially for P with 51.7 mg kg⁻¹ in Soil 1 to 2.7 mg kg⁻¹ in Soil 4. Significant effects from soils and fertilizers were observed on biomass yield, nodulation and nutrient uptake. Nitrogen fixation potential of TGX 1448-2E, however, was not increased by soils or fertilizer treatments. Soil x fertilizer effects was significant on P-uptake, shoot and nodule dry weight. It was observed that K application reduced shoot dry weight and P-uptake in soil 1. Owing to these results in response to fertilizer treatments, soybean fertilization should be recommended according to soil inherent fertility.

Indexing terms/Keywords

Soybean, Soil fertility, Fertilizer compounds, Nodulation, Molybdenum, Zinc.

Academic Discipline And Sub-Disciplines

Agriculture and soil microbiology

SUBJECT CLASSIFICATION

Plant nutrition

TYPE (METHOD/APPROACH)

Screenhouse and laboratory experiment



1.0 INTRODUCTION

Continuous soil nutrient mining, resulting from increasing population pressure on land resource is one of the major causes of soil fertility declining in most regions of sub-Saharan Africa [1]. One of its obvious consequences is crop production depressing, as crop yielding potential is severely affected by soil nutrients deficiencies. A leguminous crop like soybean (*Glycine max* L. Merr) is used as dual purpose crops to improve soil fertility and produce cheap and high proteinaceous food for African peasants. Consequently, soybean plays an important role in fighting world hunger and constitutes an important crop to reverse soil nutrient declining. Soybean is a significant component in the cereal based cropping systems of the northern Guinea savanna (NGS) where it may satisfy its N requirement and contribute to soil N pool through biological nitrogen fixation (BNF) for subsequent cereal crops [2]. Keyser and Li [3] stated that, under good conditions, the soybean-Bradyrhizobium symbiosis can fix about 300 kg N ha⁻¹.

However, soybean growth, nodulation, grain yield and BNF ability are often limited by poor soil fertility [3]. Unfortunately, in a series of on-farm studies conducted on farmers' fields in Kaya (Zaria), it was observed that soil fertility varied among peasants' farms in the same agroecological zone of northern Guinea savanna of Nigeria. In fact, soil tests revealed differences in nutrient contents in farmers' fields; P levels were below the critical level in more than 75% of the fields [1]. In addition, it was found that about 30% of the farmers' fields had a low fertility, insufficient to support legume establishment [4]. Summarily, these observations suggest unavoidably a multiform development of soybean on these farms in the same agroecological zone.

In this context where farm-to-farm soil fertility variation is obvious, it could be advantageous to identify the nutrients whose deficiency could be more detrimental to soybean development and BNF activities. Consequently, the need to elaborate different management options arose to meet the challenge of soybean production and demand. This screen house study was then designed to assess the growth, nodulation and grain yield of soybean under different soil types and fertilizer compounds.

2.0 MATERIALS AND METHODS

2.1 Experimental sites

The experiment was carried out in a screenhouse of the International Institute of Tropical Agriculture (IITA), Ibadan (7°13'E, 11°13'N). Soil samples used for this study were collected from six farmers' fields which were selected based on the soil fertility gradient of an existing on-farm trial in Kaya village in the northern Guinea savanna of Nigeria [1]; [5].

2.2 Collections, treatment and analysis of soil samples

A single composite sample was made for each of the 6 selected farmers' fields with 8 representative soil samples randomly taken from a field with a soil auger at a depth of 0-15 cm. After a thorough mixing, subsamples were taken for processing. Subsamples for the determination of pH, particle size, exchangeable cations and available phosphorus were air dried ground and sieved to pass through a 2 mm sieve while subsamples for organic carbon and total nitrogen determination were sieved to pass through a 0.5 mm sieve after air drying. Soil samples were analyzed at the Analytical Service Laboratory of IITA, Ibadan.

Particle size analysis was done using the hydrometer method [6] with sodium hexamataphosphate as the dispersant; soil pH was taken in a 1:1 soil: water ratio was done following IITA [7]. Total N was determined by the macro-kjeldahl method [8] and colorimetric determination on Technicon Autoanalyser. Organic carbon was determined by chromic acid digestion [9], Phosphorus and exchangeable cations was estimated by Mehlich 3 extraction [10]. Phosphorus were determined colorimetrically using the Technicon AAII Auto-analyser, cations were determined using Atomic Absorption spectrophotometer (Model Buck 200A).

2.3 Experimental design and layout

The treatment were laid out as factorial combination in a completely randomized design and replicated three times. The factors were 6 soil samples from different farms and 7 fertilizer compounds using different combinations of N, P, K, Mo and / or Zn. The nutrient included: N as Urea applied at 60 kg N ha⁻¹, P as triple superphosphate applied at 30 kg P ha⁻¹, K as muriate of potash (MOP) applied at 30 kg K ha⁻¹, Mo as sodium molybdate at 5 kg Mo ha⁻¹, and Zn as zinc sulphate at 5 kg Zn ha⁻¹.

The treatments were as follows: T1 = Complete (N, P, K, Mo and Zn), T2 = Complete minus N, T3 = Complete minus P, T4 = Complete minus K, T5 = Complete minus Mo, T6 = Complete minus Zn, T7 = control (no nutrient applied).

Soils of 8 kg weight were filled into each plastic pot. Soybean variety TGx 1448-2E was the test crop. Four seeds were planted in each pot at 2 cm depth. They were thinned to 2 plants per pot 2 weeks after planting (WAP). Watering and weeding were done uniformly as and at when due while the experiment lasted.

2.4 Data collection and analysis

Harvesting was done at 8 WAP from two plants per treatment by cutting each plant's shoot at soil level. The parameters measured were shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, number of nodules, weight of nodules and N and P uptake. Prior to root collection, each pot was emptied onto 4 mm sieve to wash out the soils. Root samples and detached nodules were carefully picked and stored in glass vials. Nodules were counted and their fresh and



dry weights recorded. Sub-samples of fine roots were collected from each treatment for assessment of mycorrhizal root colonization [11]. Fresh roots were oven-dried at 80 °C for 48 hours and their dry weight was recorded. The amount of nitrogen derived from atmosphere was estimated with ureides method [12]; [13]. Soybean stems were oven dried at 60 °C for 24 hours and ground to pass through 1 mm sieve. Samples were extracted with 25 ml boiling water for 2 mins. The extracts were filtrated with hot into a 50 mL volumetric flask and stored at - 15°C to be analyzed later for ureides content.

All data collected were analyzed with Statistical Analysis System version 9.2 [14]. Based on their physical and chemical properties, soils were grouped with PROC TREE using an output from PROC CLUSTER after the data were standardized with PROC STANDARD.

Soybean agronomic parameters collected were submitted to PROC GLM for the analysis of variance (ANOVA). Means were separated using Tukey's studentized range test at $P \leq 0.05$ and orthogonal contrast analysis was conducted to separate the significant interaction means. The relationship between the measured parameters was evaluated using PROC CORR.

3.0 RESULTS

3.1 Soil physicochemical test

The results of soil analysis showed that the soils used for the study were all acidic, with pH ranging from 5.2 to 5.9. Most of these soils were predominantly loamy except Soil 4 which was sandy loamy with a sand content as high as 570 g kg⁻¹ and Soil 6 being silty loamy with silt content as high as 500 g kg⁻¹. Available P content in the soils varied, ranging from (2.7 mg kg⁻¹) in Soil 4 to (51.7 mg kg⁻¹) in Soil 1. The ECEC of these soils were predominantly low (Table1). These discrepancies among soil properties showed, in the cluster analysis result, confirm a non-uniformity of soil nutrients in the peasant farmers' farms (Figure1).

Table 1: Chemical and physical characteristics of soils from northern Guinea savanna

	Soil1	Soil2	Soil3	Soil4	Soil5	Soil6
pH (H ₂ O)	5.9	5.5	5.8	5.6	5.3	5.2
Organic carbon (g kg ⁻¹)	8.8	5.5	7.9	5.2	7.5	10.4
Total N (g kg ⁻¹)	0.07	0.05	0.07	0.05	0.01	0.08
P (mg kg ⁻¹)	51.7	4.4	3.1	2.7	10.5	4.5
Ca (cmol kg ⁻¹)	3.5	2	2.2	1.3	1.8	1.6
Mg (cmol kg ⁻¹)	0.6	0.5	0.8	0.3	0.5	0.5
K (cmol kg ⁻¹)	0.3	0.2	0.2	0.2	0.2	0.2
Na (cmol kg ⁻¹)	0.3	0.2	0.3	0.3	0.3	0.3
Mn (mg kg ⁻¹)	0.1	0.04	0.02	0.1	0.02	0.03
ECEC (cmol kg ⁻¹)	5.4	3.2	3.8	2.4	3.1	3.3
Sand (g kg ⁻¹)	430	470	430	570	450	350
Silt (g kg ⁻¹)	440	400	440	340	460	500
Clay (g kg ⁻¹)	130	130	130	90	90	150
Textural class	Loam	Loam	Loam	Sandy loam	Loam	Silt loam

Soil

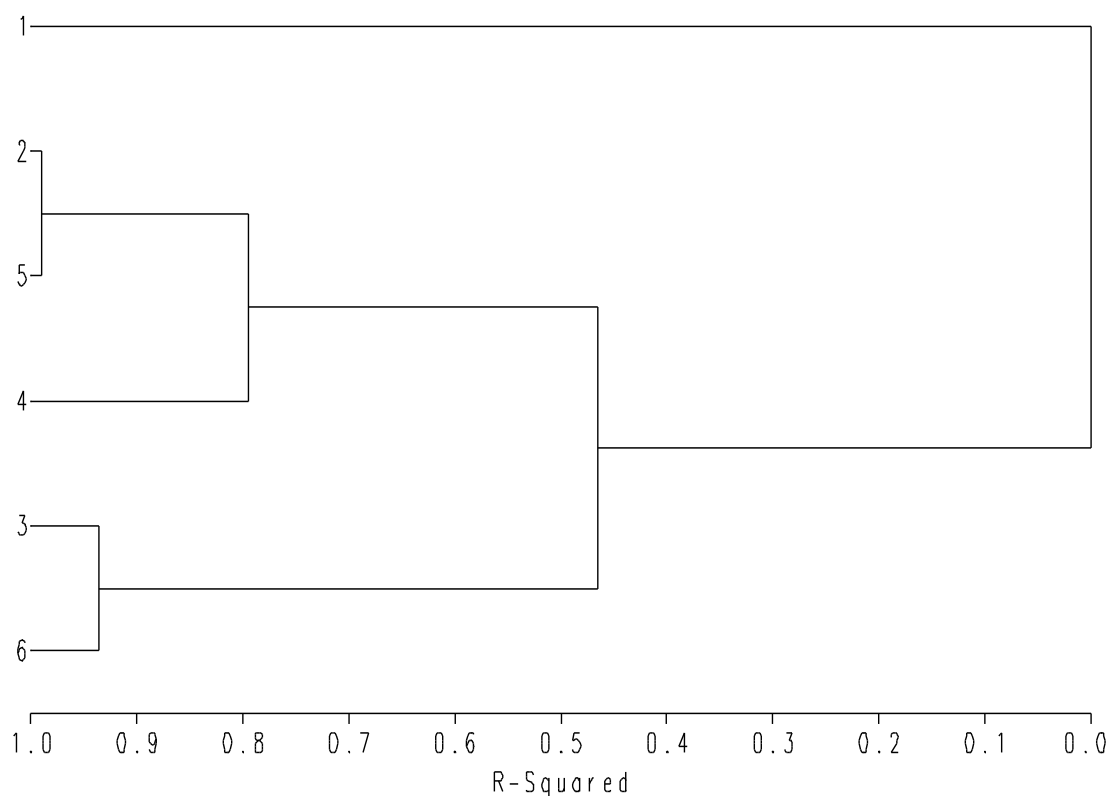


Figure 1: Soils' Clusters based on their physico-chemical contents (Ward's minimum variance cluster analysis)

3.2 Soils and fertilizers effects on soybean growth

As shown in the analysis of variance, most of parameters assessed in this study were significantly ($P \leq 0.01$) influenced by soils and fertilizers treatments. Only root dry weight and %Nfix were not significantly influenced by soil. As shown in Table 2, plants harvested from Soil 1 had the highest mean values for shoot dry weight of $18.28 \text{ g plant}^{-1}$, number of nodules per plant (104), nodule dry weight ($0.64 \text{ g plant}^{-1}$), N uptake (0.27 mg kg^{-1}) and P uptake (1.81 mg kg^{-1}). The performance of Soil 1 in soybean growth and development was confirmed by the Clustering result. In fact, Soil 1 was singled out and not associated to any other soil (Figure 1). The lowest mean values were observed in Soil 3 and Soil 4. Furthermore, the assessment of AMF root colonization showed that root from Soil 3 and Soil 4 had the highest infection of mycorrhizal fungi.

Equally, the application of different types of fertilizers had significant effect on the growth performance of soybean. Omission of P in the applied fertilizer compound (T3) significantly reduced the shoot dry weight, nodules number and dry weight. The combined application of NPK, Mo and Zn (T1) significantly decreased the root colonization by AMF. As indicated in Table 2, there was no significant effect from soils and fertilizers as factors on nitrogen fixation.

The clustering of soils, based on their combined effects on soybean shoot and root dry weight, nodules number and dry weight, root mycorrhizal colonization, percentage N derived from atmosphere (% Nfix), and N and P uptakes, showed as well that Soil 1 was not associable to other soils (Figure 2). Soil 2 and soil 5 on one hand and then 3 and 6 on the other hand, had the same combined effects, respectively. Soil 4 was slightly singled out in this clustering result.



Table 2. Effect of soils and fertilizer compound on soybean biomass, nodulation, nutrient uptake and vesicular mycorrhizal root colonization

	Shtdwt (g plant ⁻¹)	Rootdwt (g plant ⁻¹)	Nonodu (g plant ⁻¹)	Nodudwt (g plant ⁻¹)	N uptake (mg kg ⁻¹)	Nfix (%)	Vam (%)	P uptake (mg kg ⁻¹)
Soils								
Soil 1	18.28 a	3.22 a	104.68 a	0.64 a	0.27 a	36.91 a	43.11 b	0.018 a
Soil 2	13.68 bcd	2.85 ab	69.98 c	0.45 b	0.18 b	34.99 a	31.29 c	0.010 bc
Soil 3	12.47 cd	2.45 b	69.55 c	0.31 c	0.17 bc	36.16 a	54.6 a	0.010 bc
Soil 4	11.38 d	2.89 ab	66.43 c	0.40 cb	0.14 c	40.53 a	56.00 a	0.008 c
Soil 5	14.42 bc	2.98 ab	102.21 ab	0.51 ab	0.2 b	41.05 a	41.91 b	0.011 b
Soil 6	15.01 b	2.98 ab	74.19 bc	0.47 b	0.2 b	41.6 a	41.53 b	0.010 bc
Fertilizer compound								
T1	14.48 abc	2.97 abc	87.58 ab	0.41 bc	0.20 ab	38.08 a	40.47 b	0.012 a
T2	14.64 abc	2.95 abc	93.28 a	0.60 a	0.19 bc	41.13 a	40.93 ab	0.011 ab
T3	11.21 d	2.47 bc	59.06 b	0.33 c	0.15 c	31.31 a	46.08 ab	0.01 ab
T4	17.03 a	3.57 a	90.17 ab	0.47 abc	0.24 a	38.40 a	46.93 ab	0.012 a
T5	15.30 ab	3.27 ab	84.33 ab	0.49 ab	0.22 ab	36.52 a	41.91 ab	0.012 a
T6	14.26 bc	2.76 abc	82.83 ab	0.48 ab	0.20 b	42.08 a	48.25 ab	0.012 a
T7	12.51 cd	2.27 c	70.94 ab	0.47 abc	0.15 c	42.28 a	48.61 a	0.008 b

Shtdwt: shoot dry weight, Rootdwt: root dry weight, Nonodu: number of nodules, Vam: mycorrhizal root colonization percentage, Amount of N derived from atmosphere.

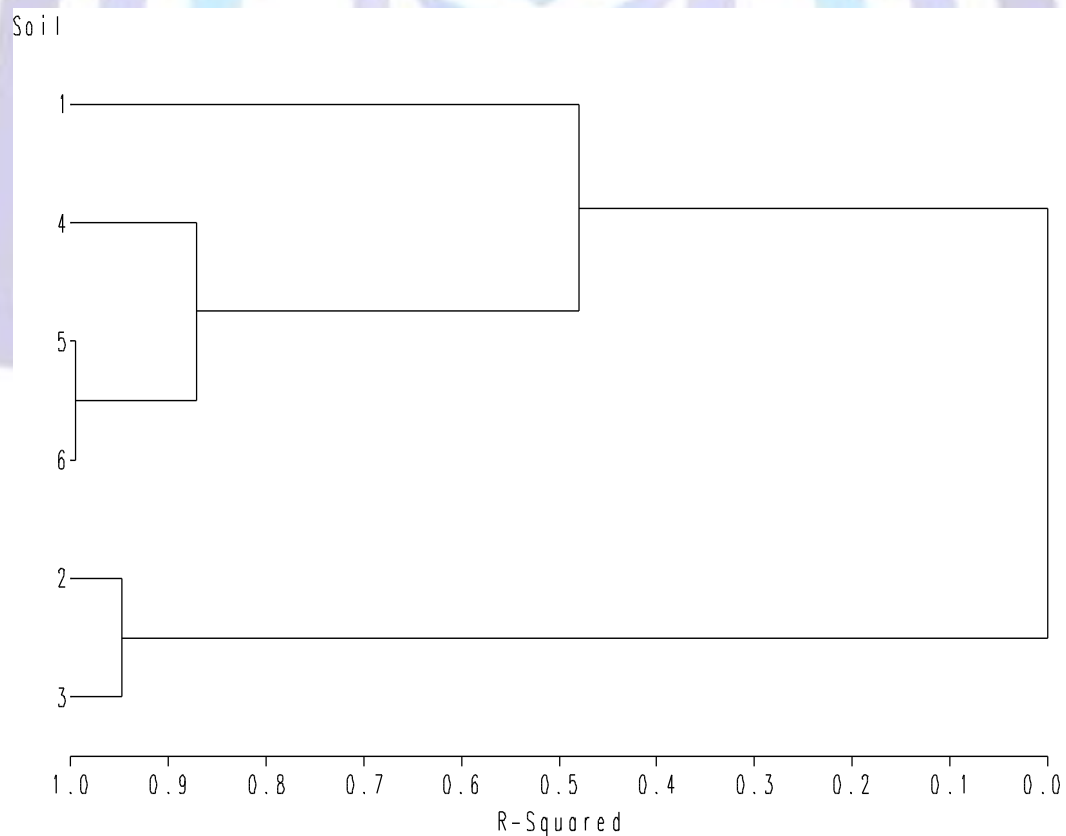


Figure 2: Soils' Clusters based on their combined effects on soybean growth parameters

3.3 Effect soils and fertilizers on the growth soybean

Soil and fertilizer compounds showed positive effect on the assessed parameters. The shoots and nodules dry weights as well as P uptake of soybean were significantly increased. The highest shoot dry weight of 22.15 g plant⁻¹ and P uptake with 0.27 mg kg⁻¹ were recorded under Soil1xT4 interaction while Soil1xT2 produced the highest nodule dry weight of 0.77 g plant⁻¹. Comparison between significant treatments resulting from the interaction between soil and fertilizer compounds was shown in Table 3. In shoot dry weight, highly significant differences existed between T1 and T3 (omission of P), T1 and T4 (omission of K), and T1 and T7 (control). The contrast analysis between T1 and T3 showed that omission of P in the fertilizer compound significantly reduced soybean shoot dry weight. In P uptake, significant differences were shown between T1 and T2, T1 and T3, and T1 and T7 at P ≤ 0.05. The relationship among the seven agronomic variables measured in this study is presented in Table 4. Positive and significant relationships were observed between shoot dry weight and nodule dry weight (r = 0.88), N-uptake (r = 0.98) and P-uptake (r = 0.89). Nodule dry weight was significantly (P ≤ 0.05) positive with number of nodules (r = 0.81), root dry weight (r = 0.96) and N-uptake (r = 0.82). The relationship of N-uptake and P-uptake were positive and highly significant (P ≤ 0.01).

Table 3. Comparison of the treatments using orthogonal contrast in soil*fertilizer interaction

Contrasts	Means squares		
	shoot dry weight (g plant ⁻¹)	Nodules dry weight (g plant ⁻¹)	P uptake (mg kg ⁻¹)
T1 vs others	0.004	0.058	0.00000907
T1 vs T2 (T1-N)	0.256	0.324***	0.00002500*
T1 vs T3 (T1-P)	95.975***	0.060	0.00004444**
T1 vs T4 (T1-K)	58.854***	0.031	0.00000000
T1 vs T5 (T1-Mo)	6.175	0.053	0.00000000
T1 vs T6(T1-Zn)	0.405	0.043	0.00000000
T1 vs Control	34.702*	0.026	0.00013611***

T1 = (N + P + K + Zn + Mo). *, ** and *** are significant levels of 0.05, 0.01 and 0.001 respectively.

Table 4. Correlation coefficient of the growth and nutrient uptake of soybean in Kaya soils

	Shtdwt	Nonodu	Nodwt	Rootdwt	VAM	Nfix	Nuptake
Nonodu	0.76						
Nodwt	0.88*	0.81*					
Rootdwt	0.77	0.69	0.96**				
VAM	-0.53	-0.26	-0.53	-0.42			
Nfix	-0.43	-0.38	-0.76	-0.86*	0.54		
Nuptake	0.98**	0.78	0.82*	0.68	-0.44	-0.28	
Puptake	0.89*	0.72	0.77	0.67	-0.26	-0.23	0.95**

Shtdwt: shoot dry weight, Nonodu: number of nodules, Nodwt: nodules dry weight, Rootdwt: root dry weight, VAM: mycorrhizal root colonization percentage, Nfix: Amount of N derived from atmosphere

* and ** are significant levels of 0.05 and 0.01, respectively

4.0 DISCUSSION

Leguminous plants are very sensitive to soil factors especially to soil acidity effect [15]; [16]. Soils across the farms were acidic. Losses in legume productivity due to soil acidity could lead to impaired plant and rhizobia growth, decreased nodule development and nitrogen fixation [17]. Soil pH has been observed to influence the growth, survival and abundance of rhizobia [18].

There were variations among the treatments for the parameters measured. In this experiment, the highest shoot dry weight was obtained with omission of K in the fertilizer. This observation is contrary to what was observed by some authors [19]; [20]. K levels in the soils were at and or above critical level probably because K fixation is not critical in tropical soils. Thus, the negative response of soybean following K supply could be due to an unbalanced nutritional effect



of the plant. In addition, soybean grain yield often showed no response to K application within NGS agroecological zone as observed by some authors [21]; [22]; [23]; [24].

Contrary to potassium, phosphorus is a major nutrient limiting soybean production. This investigation has persistently demonstrated that soybean responds to P application in the NGS soils of Nigeria. This observation corroborating the findings of [25] might be due to the soil available P being generally below the critical level [26]. The significant low shoot dry weight, nodule dry weight, and nodule number observed in this study for most soils agreed with the studies carried out by [27], [28] and [29] in which the high phosphorus requirement for growth, nodulation and nitrogen fixation of legume were stressed. Moreover, a report has shown 36% reduction in biomass when P was deficient in growth media [30], while another report estimated 28% increase in soybean total plant dry matter resulting from P addition in the soil [31].

Soybean nodulation is limited by high amount of N fertilizer application and this could lead to a decrease in grain yield. It was observed in this study, that non-application of N fertilizer increased soybean nodules dry weight. This supports the remark that nitrogen fertilizer application is inversely related to the nodule formation and activity [32]. Also, nutrient supply appeared to have negatively affected VAM colonization, the control treatment (no nutrient applied) significantly recorded more colonization than the complete (N, P, K, Mo and Zn applied) treatment. The result from this study concurs with the observation stating that the variation in environmental conditions influences VAM colonization [33]. Many reports have shown that VAM increases nutrient uptake particularly P. Under P deficiency, VAM could increase plant growth, improve P nutrition and favor N fixation in legumes [34]; [35]). The results from this work was however contrary to the above finding, as VAM colonization exhibited no correlation with root dry weight, N and P uptake, number of nodules and N-fixation on soybean [35].

5.0 CONCLUSION

In conclusion, owing to the variation of the soils in response to fertilizer treatment in this experiment, meticulous studies are needed before fertilizer recommendation for soil amendments in soybean production. Moreover, since soils from the same cluster do not always give the same effect, more experiments could be helpful to identify soil elements that significantly influence soil combined effects on soybean development.

ACKNOWLEDGMENTS

Our thanks to the experts of the International Institute of Tropical Agriculture who have contributed to the realization of this experiment and laboratory activities.

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