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Effect of Some Nutrients and Rhizobium on the Nodulation of Cowpea *Vigna unguiculata* (L) Walp in Some Nigerian Soils.

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Abstract: This study was conducted using soils from nine selected locations (Agege, Amassoma, Bakolori, Nsukka, Onne, Olupano, the polytechnic Ibadan, University of Ibadan and Zaria) to determine the effect of some nutrients and rhizobium on the nodulation of cowpea [*Vigna unguiculata* (L.) Walp]. Four greenhouse experiments were conducted. The treatments consisted of control, boron, zinc, copper, managanese, combination of micronutrients, combination of micronutrients + Nitrogen (all with inoculant) and Combination of micronutrients – inoculant. The control for the third greenhouse experiment was without micronutrients and inoculant. The fourth greenhouse experiment was on effect of NPK on nodulation and growth of cowpea. The greenhouse experiments were laid out in Completely Randomized Design and replicated four times. The results revealed that the inoculant had no significant effect generally on nodulation in all the soils both in the greenhouse. Agege soil had the highest number of nodules, nodule weight and nodule volume while Bakolori recorded the least ($p>0.05$). The treatment Combinations without the inoculant had the highest nodule weight (0.69 g/plt), zinc (1.29 g/plt) and boron (1.92 g/plt). After seven weeks of four greenhouse experiments, the nodulation was very low despite additions of nutrients.

Keywords: Greenhouse, micronutrients, nodulation, organic matter, treatments.

Introduction: Nigeria's population growth and food production are not moving in tandem. The increasing mouths have to be fed without depending on food imports. Food insecurity and malnourishment has assumed an alarming level globally and more seriously in Nigeria. At the root of all these food problems is the soil. Soil is the natural capital asset upon which the agricultural system is based. Soil quality is the capacity of a soil to sustain ecological productivity, maintain environmental quality and promote plant and animal health (Ogunkunle, 2015). The inherent quality of Nigerian soils is generally low (Agboola, 1978). The soils are easily degraded in their physical, chemical and biological properties as soon as they are opened up for cultivation. In recent decades, global warming has caused climate change resulting in increased flooding, leading to erosion, landslides, leaching losses of nutrient elements, longer dry periods, shrinking water supplies (as in Lake Chad), desertification and unpredictable and changing seasonal weather resulting in reduced crop yields and lower farm production (Obigbesan, 2015). The government of Nigeria and Scientists need to put on their thinking caps to attempt to bridge the gap between population growth and food production. Nitrogen is the key to plant growth (Isirimah, 2001). The soils are low in organic matter and consequently Nitrogen. The inorganic and organic fertilizers applied to meet N needs are washed or leached away due to the heavy down pour and flooding common in the humid tropics. There are also the problems of ground and surface water pollution from the leachates and run off. Yet, N is needed principally, in addition to other nutrient elements to make the crops grow and yield bountifully to feed the teeming population.

The main aim of this study was to determine the effect of some nutrients and Rhizobium (Nitragin strain) on the Nodulation of Cowpea (*Vigna unguiculata (L) Walp)* in Some Nigerian Soils.: Assess the effect of Nitragin strain of Rhizobium on growth, nodulation of cowpea.; To see the effect of micronutrients singly and in combination on nodulation of cowpea.; To see the effects of addition of nitrogen to the micronutrients on the nodulation of cowpea.

Materials and Methods: Study Area**:** This study was conducted in nine selected locations (Agege, Bakolori, Nsukka, Onne, Olupona, University of Ibadan. The Polytechnic Ibadan. Amasoma and Zaria). The coordinates of the project sites are shown in Table 2 in Appendix

Collection of Materials

(i.) Bulk soil samples were collected from Umudike, Umudike, Mokwa, Ikenne, Onne, Nsukka, Zaria, Bakolori, Agege, Olupona, University of Ibadan, and the Polytechnic, Ibadan. One hundred kg of top soil, 0-15cm was collected from each site. Clean plastic bags

were used to avoid micronutrient contamination.
These soils were air-dried, ground, sieved through a 2mm sieve and stored in well labelled polythene bags. Soils from 5 of these sites were used at a time. 160 plastic pots were filled with 2.5kg of the soils. 32 for each site.

(ii.) **Seeds**
Ife brown cowpea variety was obtained from the National Seed Service Head Office at Ibadan.

(iii.) Inoculant (Nitragin 176 A 22) was obtained from NIFTAL strains from the University of Georgia for the source of Rhizobium.

(iv.) Nutrients (main treatments)
B at 0.5 mg/kg, Zn at 4.0 mg/kg, Cu at 2.0 mg/kg, Mn at 5.0 mg/kg. Uniform soil addictions of 0.25 mg/kg Mo, P (30 mg/Kg), and Mg (15 mg/kg) were supplied as KH_2P (30 mg/kg), K(50 mg/kg), $MgSO_4$ (reagent grade) respectively.

(v.) Distilled water

Experiment 1

2.5 kg of soil from each of five sites, Agege, Bekolori, Nsukka, Onne and Zaria were put into 160 pots (32 pots per site).

There were 8 treatments for each soil replication four times as follows:

1. Micronutrients control
2. +B at 0.5 mg/kg
3. +Zn at 4.0 mg/kg
4. +Cu at 2.0 mg/kg
5. +Mn at 5.0 mg/kg
6. +B, +Zn, +Cu, +Mn all at the above rates
7. Same as No.6 except add 100 g/kg N as NH_4NO_3
8. Inoculant control- same as No.6, but no inoculant.

The uniform soil additions earlier mentioned were added to all soils. These materials were dissolved in distilled water and equal portions were put into each of the 160 pots

Planting Procedure: Ten Ife Brown cowpea seeds were planted in each pot. After emergence, they were thinned to 5 uniform plants per pot. One gram by volume of inoculant was distributed over the seeds except treatment No.8, which is the inoculant control (combination of micronutrient less inoculant)**'** The plants were watered with distilled water throughout the experiment. The 32 pots for each site were arranged on a rack in the greenhouse in a Completely Randomized Design.

Nodulation Data: Roots were carefully removed from each pot to minimize nodule loss. Water was used to tease out the soil. The nodules were removed from the roots, counted, volume determined and the fresh weights were taken.

Results: Mean Nodule Production: Table 1 show cowpea dry matter and nodule production: Agege had the highest nodule number and least number of Nodules was obtained in Zaria. The highest nodule weight (0.825 g) was recorded in Agege while the lowest nodule weight (0.119 g) was recorded in Zaria. Agege had the highest nodule volume while Zaira had the least

Effect of Treatments on Nodule Production.: Table 3 shows the cowpea dry matter and nodule production. Combination-inoculant had the highest nodule volume with a mean of 0.581 cm^3 while zinc had the least nodule volume with a mean of 0.247cm^3

Effect of Treatments on Nodule Weight (g) of Cowpea: The results of the nodule weight as shown in table 6. In Olupona the check had the highest nodule weight of 0.72 g and copper had the least nodule weight of 0.35g. The check had the highest nodule weight of 0.58 g in Bakolori while boron was the least at 0.34 g. The highest nodule weight was recorded in check with a mean of 0.48 g while copper had the least nodule weight of 0.11 g in Zaria. In University of Ibadan soil, the highest nodule weight was in Combination, with a mean of 0.62 G and the least nodule weight was in copper with a mean of 0.30 g. The treatment with combination was recorded the highest nodule weight of 0.53 g in poly Ibadan while copper had the least nodule weight of 0.21 g.

Effect of Treatments on Nodule Volume (cm^3) of Cowpea.: Table 4 show the effect of the treatment on the nodule volume of cowpea in the different site. In Olupona the highest nodule volume was recorded in check with a mean of 0.76 cm^3 while the lowest nodule volume was obtained in copper with a mean of 0.35 cm^3. The check in Bakori was recorded the highest nodule volume of 0.56cm^3 and combination-inoculant was recorded the least nodule volume of 0.35 cm^3. In Zaria the check had the highest nodule volume in University of Ibadan was obtained from the treatment with combination and Combination + N with a mean of 0.60cm^3. Combination + N had the highest nodule volume of 0.51cm^3 in Poly Ibadan while copper had the least nodule volume of 0.22cm^3.

Discussion: According Javaid and Mahmood (2010) *B. Japonicum* inoculation had significant effect on plant growth plant growth, nodulation, yield and dry weight of soyabeans. Daza *et al.*, (2003) also reported that the beans seed inoculation and microelement application in vetch increased nodule dry weight. In Olupona control had the highest nodule weight 0.72 g/plt while copper had the least 0.35 g/plt. The control had the highest nodule weight of 0.58 g/plt

in Bakolori while boron had the least 0.34 g/plt. The highest nodule weight was in the control with a mean of 0.48 g/plt while copper had the least 0.11 g/plt in Zaria. That the control had the highest nodule weight in these locations means that the indigenous rhizobium is more effective than the introduced one. Fening and Danso (2002), reported that the control treatment had a higher nodule number, nodule weight than inoculated treatment for some of the soils, this may be an indication of the presence of native Rhizobia in the soil that was efficient and more competitive. In the University of Ibadan soil, the highest nodule weight was in the treatment combination0.62 g/plt while the least was in copper 0.30 g/plt. The treatment with combination recorded the highest nodule weight of 0.53g/plt in Poly, Ibadan while copper had the least 0.21 g/plt. Giller (2001), reported that cowpea and some other tropical legumes have rarely been found not to respond to inoculation unless they are grown in a soil where the conditions are not conducive for survival of rhizobia. The effect of the treatment on nodule volume was significantly different (P>0.05). In Olupona the highest nodule volume was in control with a mean of 0.76 cm^3 while the lowest was in copper with mean of 0.35$cm^{3.}$ The control in Bakolori had the highest nodule volume of 0.56cm^3 while the combination-inoculant had the least 0.35cm^3. In Zaria the control had the highest nodule volume of 0.45 cm^3 while copper had the least 0.14 cm^3. That the control had the highest nodule volume in these locations means that the indigenous rhizobium are more effective than the introduced rhizobium. Again, Fening and Danso (2002), reported that the control treatment having a higher nodule volume than inoculated treatments for some of the soils may be an indication of the presence of native Rhizobia in the soil that were efficient and more competitive. The highest nodule volume in the University of Ibadan soil was in combination and combination +N (0.60cm^3) while copper had the least 0,28cm^3. Combination + N had the hight nodule volume of 0.51cm^3 in poly Ibadan soil while copper had the least 0.22 cm^3. Shibru and Mitiku (2000) reported that inoculating grain legumes with strains of rhizobium is widely reported to increase the number, mass and volume of nodules.

Conclusion: Rhizobia inoculation on cowpea has a great potential for cowpea productivity in Nigeria. Nigeria has a great market for cowpea within and across West Africa. While soils in Agege and Ibadan have large presence of rhizobium, others like Amassoma have very sparse population. Rhizobium bred for each ecological zone will satisfy the need for cowpea inoculation.There is no need for foreign inoculum. Cowpea grows from the coast of the Niger Deita to the Sahel of Sokoto, Daura and Maiduguri and if well harnessed, cowpea production with rhizobium inoculation will add a lot of in situ nitrogen into the soil. This will drastically reduce nitrogen needs for which a lot of money is spent and the attendant pollution effects. Even on fallows, cowpea can be inoculated and sown for its fodder value and soil fertility improvement.
Phosphorus incorporated in cowpea production did not have significant advantage.
Nitrogen addition to the rhizobium cowpea system can have advantage where the organic matter level is low. Place as Agege, Ibadan, Amassoma, Onne did not benefit from the added nitrogen but Bakolori and Zaria benefited from the added nitrogen to boost the nodulation process leading to nitrogen fixation. Micronutrient deficiency is surfacing very seriously in Nigeria soils. The addition of micronutrients in soils as in Nsukka was of great advantage in boosting cowpea growth.
Generally, the combination of Zn, B, Cu, Mn, (Zinc, Boron, Copper and Manganese) was of greater advantage than the single micronutrient additions. Even in this, the need is more acute in some particular soils so that there cannot be any blanket recommendation. Each zone will need to be tested what is limiting yield and treated at such Zone/site specific experimentation is advocated in soil research.
Recommendations: That indigenous rhizobia be bred and applied at the different ecological zones to boost nodulation and hence nitrogen fixation. We do not need one foreign rhizobium inoculum to meet the needs of the whole country; To eliminate or reduce soil and water pollution with nitrogenous fertilizers the use of rhizobium inoculum should be encouraged; To reduce or eliminate the prohibitive cost of nitrogenous fertilizers for the rural farmers, the use of home bred rhizobium inoculant is recommended.

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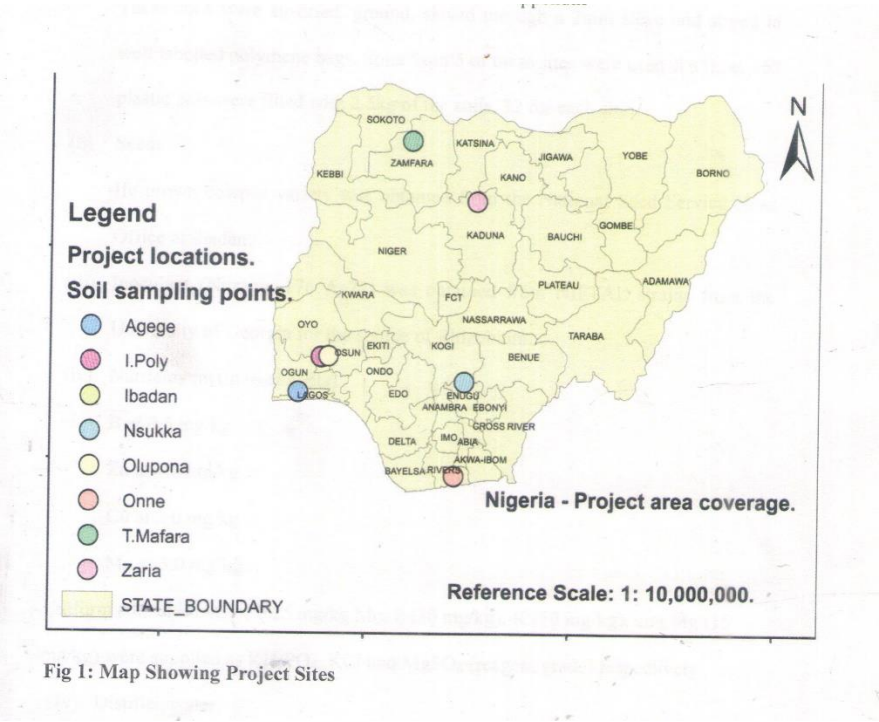

Fig 1: Map Showing Project Sites

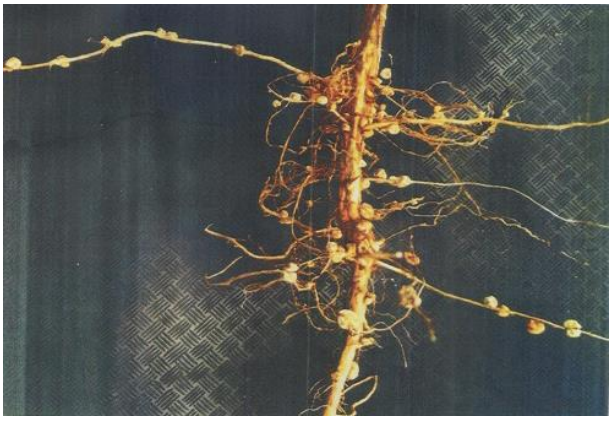

Plate 1 &2: Nodules on Cowpea Roots (Sources: Prof I.A Ogboghodo, 2017)

Table 3: Effect of Treatments on Mean Dry Matter and Nodule Production

Treatment	Leaf dry weight	Dry weight residue	Dry weight total	Nodule weight	Number of Nodules	Volume Nodule
	← g →					(cm^3)
Control	0.728d	1.576bc	2.303cd	0.444ab	21.45ab	0.433ab
B + inoculant	0.699d	1.286c	1.984de	0.300b	16.45b	0.293b
Zn + inoculant	0.700d	1.285c	1.918e	0.251b	18.00b	0.247b
Mn + inoculant	1.004c	1.718bc	2.721bcd	0.286b	18.15b	0.276b
Cu + inoculant	0.689d	1.270c	1.957de	0.275b	17.60	0.262b
Combination	1.152bc	1.886b	3.038bc	0.408b	18.80	0.394b
Combination + N	1.303ab	2.054b	3.339b	0.445ab	27.35a	0.425ab
Combination - inoculant	1.506a	2.635a	4.140a	0.597a	22.55ab	0.581a

Means followed by the same letter(s) in the same column are not significantly different from one another at 5%level of probability using Duncan Multiple Range Test (DMRT), B = Boron, Zn = Zinc, Mn = Manganese, Cu = copper and N = Nitrogen

Table 7 Effect of Treatments on Mean Nodule Weight (g) of Cowpea

Treatment	Olupona	Bakolori	Zaria	University of Ibadan	Poly Ibadan
Control	0.72a	0.58a	0.48a	0.38b	o.33bc
B + inoculant	0.63ab	0.34a	0.26Bcd	0.38b	o.23c
Zn + inoculant	0.63ab	0.40a	0.28bc	0.43ab	o.27c
Mn + inoculant	0.52abc	0.52a	o.37abc	0.35b	o.31bc
Cu + inoculant	0.35c	0.49a	o.11d	0.30b	o.21c
Combination	0.64ab	0.50a	o.33abc	0.62a	o.53a
Combination + N 100kg/ha	0.62ab	0.53a	o.41ab	0.60	o.52a
Combination-inoculant	0.42bc	0.37a	o.22cd	0.46ab	o.46ab

Means followed by the same letter(s) in the same column are not significantly different from one another at 5%level of probability using Duncan Multiple Range Test (DMRT), B = Boron, Zn = Zinc, Mn = Manganese, Cu = copper and N = Nitrogen

Table 8 Effect of Treatments on Nodule Volume (cm^3) of Cowpea

Treatment	Olupona	Bakolori	Zaria	University of Ibadan	Poly Ibadan
Control	0.76a	0.56a	0.45a	0.34b	0.30b
B + inoculant	0.64ab	0.38a	0.26bc	0.39b	0.24b
Zn + inoculant	0.58abc	0.41a	o.30ab	0.43ab	0.26b
Mn + inoculant	0.52bcd	0.53a	o.35ab	0.36b	0.30b
Cu + inoculant	0.35d	0.48a	o.14c	0.28b	0.22b
Combination	0.64ab	0.50a	o.33ab	0.60a	0.50a
Combination + N	0.62abc	0.55a	o.43a	0.60a	0.51a
Combination-inoculant	0.41cd	0.35a	o.21bc	0.43ab	0.38ab

Means followed by the same letter(s) in the same column are not significantly different from one another at 5%level of probability using Duncan Multiple Range Test (DMRT). B = Boron, Zn = Zinc, Mn = Manganese, Cu = copper and N = Nitrogen