

Effect of Mycorrhizal Inoculation on the Release of fixed Phosphorus in Soil Colloid for Growth and Effective Nodulation of Cowpea (*Vigna unguiculata*)

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Abstract: Cowpea (*Vigna unguiculata* (L)) cultivation serves many purposes, which include fixation of atmospheric nitrogen to meet its nitrogen requirement for tissue building, for the needs of companion and subsequent cereal crop and production of proteinous grains for human consumption and fodder for animals. Reduced availability of phosphorus nutrient is known to limit cowpea potentials for optimum production on acidic tropical farmland. Application of mycorrhizal fungus has been postulated to complement roots in sourcing for phosphorus nutrient (both inherent or applied) which is important for effective nodulation. This study was conducted in Babcock University Teaching and Research Farm to assess the effects of P application and mycorrhizal fungus inoculation on the ability of cowpea to fix atmospheric nitrogen (N₂) on a marginal fertility soil (Utiols). Two factors namely: P with two levels 0 and 40 kg/ha and mycorrhizal fungus (*Glomus deserticola*) applied at four levels 0, 2.5, 5.0, and 7.5 g/hill were assessed on performance of cowpea variety, Ife Brown. The eight treatment combination obtained were replicated four times in a randomized complete block design (RCBD). Variables such as plant height, growths, nodulation (nodule count, dry weight and size), percent tissue N and P, and percent of nodules with leghaemoglobin and seed yield were assessed. Results show that there were no interaction effects of the factors studied in this work on cowpea variety Ife brown's variables like plant growth and nodulation assessed. The effects of separate application of phosphorus and mycorrhizal fungus inoculation on the variables became marked mostly at the reproductive growth phase of the crop. For instance, phosphorus application promoted plant height and nodulation notably; crown nodule number relative to no-P application significantly impacted percent tissue P and seed yield respectively. Erratic increases due to increased inoculation rates on nodule size relative to the control resulting from mycorrhizal fungus inoculation were recorded during the trial. Somewhat higher shoot tissue N and P respectively were obtained through mycorrhizal fungus inoculation. For most of the variables, assessed mycorrhizal inoculant application at 2.5 g/hill was observed to be a threshold rate.

Keywords: Cowpea, Phosphorus fertilization, Mycorrhizal inoculation, Nodulation and Tissue N and P.

1. INTRODUCTION

Cowpea is important as a source of protein of high quality used for humans and farm animal nutrition. According to Bressani (1985), Cowpea consumption can significantly help alleviate malnutrition because it contains about 25% protein that is easily digestible and 64% carbohydrate. It is known generally that the consumption of cowpea seeds go a long way in balancing the amino acids composition of rural diets by supplying needed amino acids like lysine to complement the amino acids from cereal and tuber crops. The addition of cowpea to local food preparation also prevent infection and

promotes health of rural people. In Tanzania, cowpea is consumed as an important vegetable (Hallensleben *et al.*, 2009). Cowpea being an important proteinous grain legume nodulates freely with native strains of Bradyrhizobium or Rhizobium for biological nitrogen fixation in tropical soil. However cowpea yields have been very low on farmers fields compared to the average yield in the farms as reported by Ndiakindemi *et al* 2011. The authors observed that on the average the average the yield of cowpea on various tropical African countries ranges between 50 – 300kg/ha. This low cowpea productivity has been attributed to poor cultural practices which lead to poor soil nutrients management and to its poor nitrogen fixation ability and hence poor grain yield (Cooper *et al.*, 1996).

Many cultural practices had been reported to have improved the yield and quality of soybean and cowpea in different countries including application of starter N dose, P and K. Phosphorus plays an important role in the energy metabolism of all organisms and since nitrogen fixation is a high energy consuming in particular, N₂ fixation requires sufficient amount of phosphorus nutrition (Dilworth, 1994). It is a general knowledge that phosphorus is vital for root formation. Therefore, the way in which Phosphorus deficiency or excess affect nodule ammonium metabolism and energy status is relevant to its function. Nitrogen fixation in most legumes has been found to be influenced by availability of phosphorus and it is one of several elements which affects N₂ fixation and along with N, it is a major yield-limiting nutrient in many regions of the world (Pereira and Bliss, 1989). Phosphorus deficiency is widely spread in many Tropical Agricultural regions and this causes substantial economic losses (Singh and Sale, 2000; Sinclair, 2002). Specifically, in a study in Isreal it was found that legume growth and effective nodulation demands for high phosphorus nutrition (Israel, 1987). According to Rotaru (2003), dry weight of soybean nodules was reduced by two times under adequate moisture regime when phosphorus is deficient. Leguminous plants such as cowpea that biologically fixes nitrogen needs more Phosphorus when compared to any other crops for development of extensive rooting and nodulation (Cassman, 1990). Unfortunately phosphate around root zones can easily be depleted due to crop uptake and the immobile nature of Phosphorus in soil (Hinsinger *et al.*, 2005) while there may be a large deposite of Phosphorus nutrient just a few distance away; thereby negatively affecting the growth and development. The use of Mycorrhizal Inoculants on cowpea may apparently enhance Phosphorus nutrition of cowpea.

Most Tropical soils are heavily weathered therefore fragile and nutrient poor hence nutrient use efficiency of plant is generally low. This is because of high concentration of Al³⁺ and Fe²⁺ ions which fixes the phosphorus that are needed in most plants for root development (Irene and Thomas, 2006). This is further stressed by Sanchez (1987) that humid soils are nutritionally poor (Udisols) with attendant severe acidity and Phosphorus deficiency problems. Stressing the importance of Phosphorus, Irene and Thomas (2006) noted that low or non-availability of P nutrient due to fixation, constitutes a constraint to biological fixation. Fixed P is usually immobile and as a result unless the problems are alleviated through soil amerioration with external nutrient sources and cultural method namely inoculation of Mycorrhizae, the growth and yield of conventional grain legumes will be appearantly hampered (Jackson and Masson 1984). Mycorrhizal fungus has been credited with ability to solubilize fixed P and transfer it to crop roots thus alleviating the problem of poor P ion use efficiency (Lin *et al.*, 1991). Phosphorus is very vital for legumes and especially during its physiological function of fixing atmospheric N₂ (Lambert and Weidensaul, 1991). In many tropical agroecosystem soils, P nutrients are not readily available for absorption and uptake by crops even when the nutrient and materials that can supply it as part of soil constituents. Its deficiency is considered by some Agronomists as the main biophysical constraint that limits food production in most farm land in sub humid and semi arid Africa (Bationo *et al.*, 1986).

Mycorrhizal fungi association with most tropical plants is a symbiotic association. However Mycorrhizal fungi are capable of solubilizing immobile phosphorus beyond rhizosphere as they proliferate into the non-rhizosphere soil. The sourced P is made available to plant roots. Thus, mycelia hyphae act as root extension. In another development however Swift *et al.* (1994) pointed out that fungi are very important because of their efficiency to scavenge for P in a low soil. Also according to Dodd (2000) Mycorrhizae also performs other roles beside the transferring of immobile nutrients to the crop roots, such roles include soil aggregation (ie formation of soil structure), protection of roots against infection and reducing drought on the stress crops. Biofertilization through soil of cultured organisms like Rhizobium and Arbuscular Mycorrhizae fungus are microsymbiont in different symbiotic system involving arable crops and soil microbes. The role of rhizobia in symbiotic system involving symbiotic nitrogen fixation of legumes and ability of mycorrhizae fungus to scavenge for phosphorus in low P soil are well documented. It is generally known that Nitrogen and Phosphorus are limiting minerals in crop production. In another study conducted by Stancheval *et al.* (2006), the activity of Mycorrhizae species in Phosphorus scavenging and transfer to plant root in low – phosphorus level was demonstrated. Bargali and Bargali (2009) asserted that the use of Arbuscular Mycorrhizal fungus as a fertilizer was crucial in cropping nitrogen and

non – nitrogen fixing plant on degraded soil. In previous works done in this regard, Nwoko and Sanginga (1999) reported that shoot weight, nodule weight and number, phosphorus uptake were improved in soybean and some herbaceous legumes – *Mucuna pruriens* following colonization of the crop roots by Arbuscular Mycorrhizal Fungus. Plants inoculated with Arbuscular Mycorrhizal fungi usually have higher nutrient content and growth rate than non- Mycorrhizal crops (Janos *et al.*,2001). Ahiabor and Hirata 1994, stressed that there was increased shoot growth and uptake of nutrients like P,K and Ca in cowpea, groundnut and pigeon pea with Arbuscular Mycorrhizal inoculation. This study was conducted in Babcock University Teaching and Research Farm with the aim of assessing the effects of P application and mycorrhizal fungus inoculation on the ability of cowpea to fix atmospheric nitrogen (N_2) on a marginal fertility soil (Udisols) .

2. MATERIALS AND METHODS

Soil Property Analysis: The field trial was conducted on 0.018ha size plot at the Teaching and Research farm of the School of Agriculture and Industrial Technology, Babcock University Ilishan-Remo Ogun state Nigeria. The site lies within the rainforest ecological zone with annual rainfall of 2000 mm and minimum and maximum temperature of 24⁰C and 30⁰C respectively. Maize seeds were broadcasted on the experimental site to mop up native nitrogen in order to obtain a low-N soil status; a situation which will stimulate effective legume nodulation. A pre-cropping soil analysis was carried out at the analytical laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan Nigeria for the determination of soil particle size (Bouyoucos, 1962), soil pH, % carbon, available phosphorus and bases like Na⁺, Ca²⁺, Mg²⁺ and K⁺ using mellich 3 soil test technique.

Test Crop: A line of early-maturing cowpea namely Ife brown was obtained from the seed store of the Institute of Agricultural Research and Training (IAR&T) Moor Plantation, Ibadan. A soil culture of mycorrhizal (*Glomus deserticoli* fungus) inoculants which was obtained from the department of Microbiology Ibadan was produced as follows: Two kilogrammes of soil sample was sterilized at a range of 180⁰C to 210⁰C for 3 – 4 hours. The sterilized soil was then taken to the green house. Sterilized seeds of maize was planted in the soil; then a pure isolate of the vesicular arbuscular mycorrhizal fungus was seeded into the soil for multiplication. The maize was allowed to grow for two months. Watering was done as required using sterile water. After two months, the soil together with maize root was processed by grinding to small particles which was then stored in sterilized bags ready for use.

Treatment Combinations: Two factors namely P fertilization with two levels of phosphorus denoted with P₀ and P₁ and mycorrhizal inoculation with four levels denoted

- a. No mycorrhizal inoculation (control) – I₀
- b. Inoculation with *Glomus deserticoli* at a rate of 2.5g/planting hill – I₁
- c. Inoculation with *Glomus deserticoli* at a rate of 5.0g/planting hill – I₂
- d. Inoculation with *Glomus deserticoli* at a rate of 7.5g/planting hill – I₃

Eight treatment combinations were obtained as follows:

P₀I₀ – A

P₀I_{2.5} – B

P₀I_{5.0} – C

P₀I_{7.5} – D

P₁I₀ – E

P₁I_{2.5} – F

P₁I_{5.0} – G

P₁I_{7.5} – H

Basal Fertilizer Application: Potassium at 30 kg/ha as potassium chloride (52.3% K), molybdenum at 20 kg/ha as ammonium molybdate (49% Mo) and starter N at 25 kg/ha as urea (46%N) and Zn as zinc chloride (47.8%Zn) were applied.

Data Collection: Plant height, dry matter weights of root, shoot and nodules, nodulation (crown and total) and nodule size were assessed at 2, 4, 6, 8 and 10 weeks after planting (WAP). The percent nodules with leghaemoglobin, percent tissue N and P and seed weight were determined at 8 and 10 WAP. The data collected were subjected to Analysis of Variance; and Duncan's Multiple Range Tests (D.M.R.T.) was used to separate the significant means (SAS, 1999).

3. RESULTS AND DISCUSSION

The results of the pre-crop soil properties analysis are presented in Table 1. The present organic carbon (O.C %) was 0.71 which is an equivalent of 1.3% organic matter an indication that the soil is very low in organic matter. The soil used was slightly acidic (Table 1) with very high proportion of sand loam (Textural triangle). Both the low soil organic matter of 1.3% a known store-house of both major and minor nutrient elements and a soil physical properties improver (Plaster, 1992). The acidic nature of soil could have contributed to the low N and P status of the soil through possible impairment of nitrification and chemical immobilization of P. Juxtaposed with the recommended optimum soil inherent P status of 15ppm for legume nodulation (Ajeigbe *et al.*, 2010) it became very evident that the P status of the soil used was very low. The exchanged bases were low (Table 1) apparently in consonance with the slightly acid soil with low organic matter while the Mn^{2+} and Fe^{2+} concentration were high in tandem with the slightly acid reaction of the soil. Based on the soil analysis results (with particular reference to nutrient and organic carbon matter) detailed in Table 1, the soil could be classified as a low nutrient, degraded soil belonging to the order; utisols (Brady and Weil, 2004).

Plant height as affected by P application and mycorrhizal inoculant rates:

The results of plant height response to P fertilization are shown in Table 2. Irrespective of P application, plant growth in height increased with age. There were however only marginal increases in height resulting from P application. These are expected because phosphorus nutrition is known above-ground vegetative growth. These findings corroborate the findings of Singh and Sale (2000) who obtained stimulatory effects of P only on roots. The mean plant height as influenced by Mycorrhizal inoculant rates are presented in Table 3. Taller plants were observed as the plants aged irrespective of whether or not Mycorrhizal fungus was applied. Mycorrhizal appeared to have stressed the plant growth in height at the early vegetative growth phase of between 2-6WAP (Table 3) but increases in plant height thereafter (8-10WAP) became evident with increased rate of Mycorrhizal inoculant relative to the control. The result demonstrate the transient nature of the postulated stress due to the inoculation but also showed that mycorrhizal inoculation had an apparent positive influence on plant. The inoculated plant height ranges from 132cm to 140cm compared to the control that has 101.75cm at 8WAP and 140cm to 172cm compared to the control with 135.49cm at 10WAP. At the 8WAP plant with the highest inoculant rate has the highest height.

Root and shoot dry matter weight response to P application:

Table 4 and Fig 1 show the result of root and shoot dry matter weight responses to P application respectively. At the early vegetative growth phase (about one month after planting) there was no significantly positive root dry weight response to P application (Table 4), apparently because the seedlings were able to subsist on the low soil nutrient content (Table 1). The late vegetative growth phase (6WAP) to the reproductive phase (8-10WAP) increases in root dry weight compared to the control treatment of between 15% - 18% became evident though not significant. The results showed that as the plants grew bigger the soil native nutrients were apparently no longer adequate to meet their P nutrition requirement. Therefore, a positive root growth response of cowpea (*Vigna unguiculata*) to P application was recorded; similar to the result obtained by Olivera *et al.* (2004) with common bean (*Phaseolus vulgaris*) as test crop. Also, Ufot *et al.* (2003) indicated that the major function of P is stimulation of root development and proliferation for enhanced nutrient use efficiency. Results of shoot dry matter weight as influenced by P application are presented in figure 1. There was increase in shoot dry matter weight as the plant aged (with or without P application). However there was 1.1% increase in shoot dry weight at 6WAP rising to 31% increase in shoot dry matter each at 8WAP and 10WAP (reproductive growth phase with P application), the plant with P application is significantly higher in dry weight compared to control. The result understood the apparent positive response of the cowpea to P application as it grow older (fig 1).

Root and shoot dry matter weight response to mycorrhizal inoculant rates:

Results of roots and dry matter weight response to mycorrhizal inoculant rates are presented in Table 5 and Figure 2 respectively. The inoculation of the fungus on the plant were apparently stressful and showed on the root development at the seedling growth phase. However, higher root dry weight became consistent for plant with Mycorrhizal inoculant rates

relative to the control treatment at the reproduction phase of 8 and 10 WAP depicting that the stress was transient (Table 5). At the reproductive stage of the cowpea it was observed that the mantle of fungal mycelia might have enveloped roots which is expressed as the increase in the dry weight of root recorded at the reproductive growth phase of the cowpea. Also at 8WAP inoculant rate of 2.5g/hill gave highest root dry weight suggesting that it was the threshold rate, because beyond the rate, reduced root dry weight were recorded (Table 5). The shoot dry matter weight per plant increased with increase in this age of the plant and generally with mycorrhizal inoculant application were recorded with each inoculant rate recording substantial increase in shoot weight compared to the control (Fig 2). Highest rates of inoculation beyond 2.5g/hill resulted in substantially depressed dry shoot weight suggesting further that the 2.5g/hill was a threshold rate of application in this study.

Plant nodulation (crown and total) response to P fertilization and mycorrhizal inoculant rates:

Nodulation (Crown and total) variables as influenced by P application are presented in Table 6. Expectedly, nodulation generally increased as cowpea aged; up to late vegetative growth phase (6WAP) but thereafter; total nodule count dropped markedly by almost 60% (8WAP) and 52% (10WAP) on the average irrespective of whether P was applied or not (Table 6). The decline in total nodulation was most probably due to nodule senescence and decay over the root infectible sites at the reproductive phase (8 and 10 WAP) of cowpea (grain legume) without new nodule flushes as replacements for the decay nodule on the same infectible sites. This is in contrast to what may be obtained in forage legumes. However application of P promoted higher nodulation notably crown nodulation and the promotion became significantly higher (5% level) with P application compared to control treatment at the reproductive phase (8 and 10WAP) in tandem with higher root mass observed during the phase (Table 4) prompted by P application and with attendant increase in availability of infectible sites for nodule initiation. This is in agreement with Carsky *et al.* (2002), who reported that P plays prominent role in nodule formation and effectiveness.

Nodulation (crown and total) response to mycorrhizal inoculation rates:

Nodulation (crown and total) response to mycorrhizal inoculation rates is presented in Table 7. Crown nodulation is an index of how soon after planting a legume commences nodulation for fixation of atmosphere N_2 in order to meet its N nutritional demand. There was no significant differences in both crown and total nodulation (Table 7) irrespective of mycorrhizal rates applied compared with the control throughout the four sampling periods. Crown and total nodule counts were highest with the highest mycorrhizal inoculant rate of 7.5g/hill probably because the massive fungal hyphae provided by the rate would have explored largest soil volume for a possible corresponding concentration of phosphorus for enhance plant rooting and subsequent highest nodulation recorded (Table 7). The fact that total nodulation with 2.5g/hill inoculate dropped when the rate doubled (5.0g/hill) apparently marked out 2.5g inoculant/hill as a threshold rate of application. Generally, for every mycorrhizal inoculant rate, there were progressive increases in crown and total nodulation respectively up to late vegetative growth phase 6WAP when the nodulation peak reduced sharply at (8 WAP); only for the two categories of nodulation to appreciate in number at (10WAP). The reduced crown and total nodule count obtained at the onset of reproductive phase (8WAP) could be attributed to nodule senescence and decay while the increase in nodulation at 10WAP may be due apparently to development of a new set of roots.

Nodule dry weight and size responses to P application and mycorrhizal inoculant rates:

Nodule dry weight and size responses to P application are presented in Fig 3 and Table 8 respectively. Irrespective of P application, there were increases in nodule weight of the plant expected as plants aged, during which period photosynthate production would have been enhanced correspondingly. It appeared that the carbohydrate must have been translocated into the nodules as one of the natural sinks for it. Therefore nodule weight increases could be accounted for possibly by the photosynthate translocated to it in addition to its bacteriod (modified rhizobia) population which are both essential for fixation of atmosphere N_2 in a low-N soil like the one used for the trial (Table 1) nodule weight is usually taken as an index of nodule effectiveness or ability to fix N_2 . This findings corroborates earlier findings Oliver *et al.* (2004) who working with common bean as test crop obtained increased nodule dry weight with P application. The general lower nodule dry weight of cowpea with no-P application probably underscored the low P status of the status of the soil used for the study (Table 1). The findings in this study demonstrate the importance of P application for nodule weight and therefore nodules effectiveness in nitrogen fixation. The findings was similar to that of Rotaru (2004) which showed reduced soybean nodule dry weight when P was deficient. An earlier worker, Israel (1997) noted that legume nodule effectiveness required high P nutrition and Carsky *et al.* (2002) stressed the importance of P in biological N_2 fixation in

legumes. Also P nutrient is used in energy reaction required for nitrogen synthesis (Brady and Weil, 2004). Increase in nodule size were recorded at both 4 and 6 WAP with P application compared (No-P application) with the P application promoting significantly (5% level) bigger nodules at 6 WAP. In contrast, however nodule size were similar at the reproductive growth phase of the plant (cowpea) even with P application (Table 8). A sag in nodules size was recorded at 8 WAP compared to 6WAP even with P application (Table 8). A slightly increase of 13% in nodule size at 10 WAP ie two weeks later was obtained probably because of increased photosynthate translocation into the nodules to boost the size.

The weight and size as influenced by mycorrhizal inoculant rate are presented in Figure 4 and Table 9 respectively. As expected the plant aged nodule dry matter increased with or without mycorrhizal inoculation (Fig 4). During both the vegetative growth phase (4-6 WAP) and the reproductive growth phase (8-10 WAP), irregular increases in nodule dry weight due to increased mycorrhizal rates were recorded (Fig 4). However, at the early vegetative growth phase (4WAP) there were obvious reductions in nodule weight with the two highest 5.0 and 7.5g/hill inoculant rates relative to control and with the 2.5g inoculant rate/hill which gave the highest weight at that sampling period of 4WAP. In contrast, however, the significantly higher nodule weight were obtained with the highest rate at the reproductive growth (8 and 10 WAP). The fact that the mycorrhizal fungus-root symbiosis must have been fully established to source for higher P most of which might have ended up in the nodules and that more photosynthate might have been translocated into the nodules could account for the result. Hart (1989) classified nodules as a strong sinks for photosynthate assimilate. However, the inoculant rate of 2.5g/hill appeared to be an economic threshold rate in this trial because throughout the sampling period of 8 and 10 WAP, application of even a double rate (5.0g/hill) resulted in a reduced nodule weight and similar nodule was obtained weight with the highest inoculants rate of 7.5g/hill (Fig. 4). Except for the no-inoculant application, nodule size increases with inoculant rates were erratic (Table 9). For example, the progressive increases in nodule size observed due to each inoculant rate between 4 and 6 WAP reduced at 8WAP to appreciate at 10WAP. This result appeared natural or common and can be attributed to possible increased translocation of both phosphorus and photosynthate into the nodules by the 10 WAP sampling period. Except at the 8 WAP sampling period each higher rate of mycorrhizal inoculant applied led to somewhat increase in nodule size compared to control during the other sampling periods (Table 9). In respect of the other sampling periods, bigger-sized nodules were recorded with 2.5g inoculant/hill compared to any of the other rates. This result further emphasized 2.5g inoculant/hill as the economic threshold application rate for obtaining plumber nodule size.

Percentages of cowpea tissue N and P nodule with leghaemoglobin and seed yield per hectare:

The results of response of cowpea percent tissue N and P, nodule with leghaemoglobin and grain yield to P application are presented in Table 10. Application of 40kg/ha resulted in significantly higher tissue N concentration over no P (control) at 5% levels at 8WAP. Carsky *et al.* (2002) found that adequate P nutrition determined soybean tissue N concentration. However P application was found to promote percent P by only 100% over the control (Table 10) suggesting some response of plant to P application in terms of % P. The percent nodule with leghaemoglobin cowpea was very high, an average of 98% with or without P application an indication that the nodules are functional. Leghaemoglobin is the pink pigment observable in an incised nodule; the function of which is to mop up excess oxygen nodule oxygen capable of destroying nitrogenase enzyme that facilitates reduction of N₂ to NH₃ (Postgate, 1998) during symbiotic N fixation. Though slight (7%) cowpea grain yield was higher with P application compared to the control. Earlier researchers namely Pereira and Bliss (1989) suggested that P is a principal crop yield limiting nutrient in many regions of the world. The percent N and P, nodule with leghaemoglobin and seed yield as influenced by mycorrhizal fungi inoculant rates are presented in Table 11. Generally mycorrhizal fungi inoculation markedly (5% level) increased both N and P, tissue concentration of the cowpea relative to the control treatment. Increases in percent tissue P over the control were progressive and in consonance with increased inoculant application rate expectedly (Table 11). It is apparent that the higher the inoculant rate, the more massive the hyphae available as prospect for immobile nutrients notably P which is subsequently translocated to the plant system through the roots.

Apart from the control (No-inoculant), percent tissue N concentration were statistically similar for the three mycorrhizal inoculant rates namely 2.5, 5 and 7.5g/hill (Table 11). The for control has 3.10%, 2.5g/hill has 3.86% which is the highest, 5.0g/hill has 3.52% and 7.5g/hill has 3.84%. However, 2.5g/hill rate with 3.86% appearing to be the economic threshold application rate beyond which there were decreases in % tissue N. As with nodule dry weight percent tissue N of cowpea is a reliable index of percent N derived from the atmosphere (%Ndfa) in any low -N soil like that was used for the trial (Table 1). Except with 7.5g/hill rate, mycorrhizal inoculation showed no influence on % percent nodule with

leghaemoglobin compared to the control. Also the influence of mycorrhizal was only slightly higher compared to the control in respect of seed yield. Undoubtedly because it is apparent that the amount of photosynthate sunk into the grain would largely be an obvious determinant factor of such an economic yield.

4. CONCLUSION

There were only slight effects of phosphorus fertilization and mycorrhizal inoculation rates on plant shoot height in the early stage of crop growth, but the effects became significant on plant height at later growth phase. Application of mycorrhizal inoculation and P fertilization respectively affected root weight rather than the shoot dry weight positively though not significantly at the early growth phase; however root weight was significantly affected during the reproductive growth phase. Apparently at the reproductive growth phase 2.5g/hill inoculant rate was the original for maximum root tissue weight of the cowpea variety, Ife brown. Phosphorus application promoted higher nodulation relative to control, this became significantly higher for crown nodulation at the reproductive growth phase. Throughout the sampling periods noticeable effects of mycorrhizal inoculation was recorded on nodulation (Crown and total) and the 2.5g inoculation/hill was found to be a threshold rate for the variables. Likewise, there were increases in size of nodule as a result of mycorrhizal inoculation although, the increase over the sampling period were erratic in relation to the inoculant size compared to the control was however recorded with 2.5g/hill inoculant rate at the late vegetative growth phase. Neither P application nor mycorrhizal inoculation has marked effects on nodule pigment content (leghaemoglobin). Phosphorus fertilization has a significantly positive effect on perfect tissue N, but not on tissue P and seed yield respectively. Generally the mycorrhizal inoculation resulted in a moderately higher percent tissue N and P respectively, compared to control; and only a minimal effects on seed yield compared to the control. Separate applications of phosphorus fertilizer at a rate of 40kg/ha and mycorrhizal fungus (*Glomus deserticoli*) inoculation at 2.5g per hill to cowpea improved growth variable notably: nodule weight are recommended. It is pertinent to carry out further studies on the effects of mycorrhizal inoculation and P fertilization on other legumes, grain and forage.

REFERENCES

- [1] Abayomi, Y.A. and Abidoye T.O., (2009). Evaluation of cowpea genotypes for soil moisture stress under screen house conditions. *Africa Journal of Plant Science*, 3(10): 229-237.
- [2] Abercio V. Rotor, P., Catabian D. (2010) Mycorrhizal association, n fertilization and biocide application on the efficacy of bio-N on corn (zea mays L.) growth and productivity *E-International Scientific Research Journal ISSN: 2094-1749 Volume: 2 (3) 46-54*.
- [3] Adams J. F. (1997). Yield response to molybdenum by field and horticultural crops. In: Gupta U.C, ed. Molybdenum in agriculture. *Cambridge University Press*. Pg 251-253
- [4] Adebayo A. (1994). Nitrogen fixation and management practices for its enhancement in tropical agriculture. *Recent Development in Biological Nitrogen Fixation Research in Africa*. Pg 362-308.
- [5] Adediran J.A. Taiwo L. B. Akande M.O. and Banjoko V.A. (2006). Recycling of farm wastes for sustainable organic Agriculture. A paper presented at the 2nd National Conference on organic Agriculture in Nigeria held Nov 27th to December 1st 2006 in the Fac. of Agriculture and Forestry, Univ. Of Ibadan, Ibadan, Nigeria.
- [6] Adedoyin, S.F. (1995). An assessment of the linkage in the delivery of technologies on rehabilitation and management of soils. *All African soil science conference. University of Ibadan*. Pg 19-26.
- [7] AdeOluwa, O. O. Adeoye, G. O. And Sridhar M .K.C. (2006). Adoption of oil palm *Elaeis guineensis* jaca .empty fruits bunch (EFB) as organic fertilizer in oil palm plantation. A paper presented at the 2nd National conference on organic Agriculture in Nigeria held on 27th of November to December 1st in the faculty of Agriculture and Forestry. *Univ of Ibadan, Ibadan, Nigeria*.
- [8] Ahiabor, B.D. and Hirata, H. (1994). Characteristic responses of three tropical legumes to the inoculation of two species of VAM fungi in Andosol soils with different fertilities. *Mychorriza*. 1994. 5(1). 63-70. *Copyright CAB Internatinal Abstract*.
- [9] Ajeigbe, H.A., Singh, B.B., Adeosun, J.O. and Ezeaku, I.E. (2010.) Participatory on-farm evaluation of improved legumes- cereals cropping system for crop –livestock farmers: Maize double cowpea in northern guinea Savanna Zone Nigeria. *African Journal Research Vol 5(16), pp 2080-2088*.

- [10] Akanbi, W.B., Adediran, J.A., Togun, A.O. and Sobulo R.A. (2001). Organic base fertilizer on the growth, yield and storage life of tomato (*Lycopersicon esculentum* Mill). *Bioscience Research Communication* 12: 14-18.
- [11] Albrecht, S. L., Bennett J.M., and Boote K.J. (1994). Relationship of nitrogenase activity to plant water stress in field grown soybeans. *Field Crop Res.* 8:61-71.
- [12] Anon G .L. (1990) Literature review on soil fertility investigation in Nigeria. Federal Ministry of agriculture, Lagos. Vol 3 :109-200.
- [13] Bagyaraj, D.J. and Manjunath, A. (1980). Selection of a suitable host for mass production of VA mycorrhizal inoculum. *Plant and Soil* 55: 495- 498.
- [14] Baldani, V. L. D., James, E. K., Baldani, J. I. and Dobereiner, J. (1993). Colonization of rice by the nitrogen fixing bacteria *Herbaspirillum* spp. and *Azospirillum brasilense*. In *New Horizons in Nitrogen Fixation* (eds Palacios, R. *et al.*), Kluwer Academic Publishers, The Netherlands, , p. 705.
- [15] Bationo A., Mughogho S.K. and Mokwunye A.U., (1986). Agronomic evaluation of fertility in tropical phosphate in tropical Africa. P283-318 In Mokwunye A.U. and Vlek P.L.G (eds) *Management of nitrogen and phosphorus fertilizers in sub Sahara Africa*. Martinus nijhoff, Dordrecht, the Netherlands
- [16] Bargali K. and Bargali S.S. (2009). Effects of phosphorus nutrition on growth and mycorrhizal dependency of *Coriaria nepalensis* seedlings. *Nature and Science, ISSN 1545-0740*
- [17] Barker R and Chapman D (1988) *The Economics of Sustainable Agricultural Systems in developing Countries*. Mimeo, Cornell University, Ithaca, NY. 85: 45-49..
- [18] Blumenthal, J.M., Russelle M.P., and Lamb J.F.S. (1999). Subsoil nitrate and bromide uptake by contrasting alfalfa entries. *Agron. J.* 91:269-275.
- [19] Boddey, R.M., de Oliveira O.C., Urquiaga S., Reis, V.M., de Olivares E.L., Baldani, V.L.D. and Dobereiner J. (1995). Biological nitrogen fixation associated with sugar cane and rice. Contributions and prospects for improvement. *Plant and Soil* 174: 195-209
- [20] Bohlool B. B, Ladha J.K., Garrity D.P. and Goerge T. (1992) Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant and soil* 141: 1-11.
- [21] Bordeleau, L. M., and Prevost D. (1994). Nodulation and nitrogen fixation in extreme environments. *Plant and Soil* 161:115-124.
- [22] Bouyoucos G. J. (1962). Hydrometer method improved for making particle size analysis of soil. *Agron. J.* 54 464-465.
- [23] Brady N C and Weil R.R (2004) . *Elements of the nature and properties of soil* 2nd edition. Prentice Hall Upper Saddle River New Jersey 606 p
- [24] Bremner, J.M., Mulvaney, C.S. (1982). Nitrogen-Total. In A.L. Page, R.H. Miller, and D.R. Keeney (Eds.) *Methods of soil analysis. Part 2, Agronomy* 9, 595-624
- [25] Bressani, R. (1985). Nutritive value of cowpea. Pages 353-360 In *Cowpea: Research, production and utilization*, edited by S.R. Singh and K.O. Rachie. John Wiley & Sons, New York, USA.
- [26] Brockwell, J., Bottomley, P.J., and Thies, J.E. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant Soil* 174:143-180.
- [27] Bunch R. (1999). More productivity with fewer external inputs: Central American case studies of agro-ecological development and their broader implications. *Environment, Development and Sustainability, v.1, p.219-233, 1999*.
- [28] Buresh R.J., Smithson P.C., and Hellums C., (1997)s .Building phosphorus capital in Africa p. 111-150. In Buresh R.J., Sanchez P.A., and Calhoun. *Replenishing soil fertility in Africa. Soil sci. Soc of A merican, Madison, Wisconsin, USA*.
- [29] Carsky R.J., Abaidoo R., Dashiell K.E., Sanginga N., (1997). Effect of soybean on subsequent maize grain yield in Guinea savanna of West Africa. *Afr. Crop Sci. J.*, 5 : 31-39..
- [30] Carsky R.J., Singh B.B., Oyewole B., (2001). Contribution of early season cowpea to late season maize in the savanna zone of West Africa. *Biological Agriculture and Horticulture*, 18 : 303-315.

- [31] Carsky R.J, Oyewole B., Tian G., (2001). Effect of phosphorus application in legume cover crop rotation on subsequent maize in the savanna zone of West Africa. *Nut. Cycl. Agroecosyst.*, 59 : 151-159.
- [32] Carsky R.J., Vanlauwe B., Lyaase O.,(2002). Cowpea rotation as a resource management technology in savanna cereal-based systems in West Africa. In *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria, 4–8 September, 2000. IITA, Ibadan, Nigeria., 252-266.
- [33] Cassman K.G., Whitney A.S. and Fox R. L.(1981). Phosphorus Requirement of Soybean and cowpea as affected by Mode of N nutrition. *Agronomy journal*. Vol. 73 Jan: , p.17-22.
- [34] Cassman K. C. (1990) The role of soil fertility research in developing sustainable food production systems. *Better Crops With Plant Food* 74. 16-19.
- [35] Cocking, E. C., Srivastava, J. S., Kothari, S. L. and Davey, H. R.,(1992) Invasion of non-legume plants by diazotrophic bacteria. In *Nodulation and Nitrogen Fixation in Rice: Potential and Prospects*, International Rice Research Institute, Philippines, 119– 121.
- [36] Cooper, P. J.M, Leakey R.R.B, Rao M.R. and Reynolds. L. (1996) Agroforestry and the mitigation of land degradation in the humid and sub-humid tropics of Africa. *Exp Agric* 32: 235-290
- [37] Cummings S.P, Humphry D.R., Santos S.R., M. Andrews .M, James E.K. (2006). The potential and pitfalls of exploiting nitrogen fixing bacteria in agricultural soils as a substitute for inorganic fertiliser. *Environmental Biotechnology* 2 (1) 2006, 1-10
- [38] Dilworth, M.J., (1974). Dinitrogen fixation, *Annu. Rev. Plant Physiol.*, 25, 181-214.
- [39] Dobereiner, J., Primental, J. P., Olivares, F. L. and Urquiaga, S. (1990) Bacteria diazotrofica podem ser endofitas e ou fitopatogenicas. *An. Acad. Bras. Cienc.*, 62, 319.
- [40] Dodd J.C.,(2000). The role of arbuscular mycorrhizal fungi in agro- and natural ecosystems. *Outlook on Agriculture* 29(1) 55-62 In Elderidge D.J. and Strokes S(eds). Copyright: CAB Internationals.
- [41] Dogan, E., Kirnak, H. and Copur, O. (2007) Deficit irrigations during soybean reproductive stages and CROPGRO-soybean simulations under semi-arid climatic conditions. *Field Crops Res.* 103: 154-159.
- [42] Ehlers, J. D., and Hall A.E.(1997): Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Res.* 53, 187–204.
- [43] Enwezar W.O., Udo E. J., Ayotade K. A, Adepetu J.A. and Chude V. O.(1998). Literature review on Nigeria (5 volumes) for federal ministry of Agric. Published by bobman publishers. Ibadan 281 pp
- [44] Ferreira, A. C., Cozzolino, K., Carvalho, A. R. V., and Dobereiner, J. (1995). Isolation and characterization of diazotrophic bacteria in oil palm trees. In *International Symposium on Sustainable Agriculture for the Tropics - The role of Biological Nitrogen Fixation* pp. 210 211. Boddey, R. M. and Resende, A. S., (eds). EMBRAPA, Rio de Janeiro.
- [45] Fraga, V. Da. S. and Salcedo, I.H. (2004) Declines of organic nutrient pools in tropical semi-arid soils under subsistence farming. *Soil Science Society of America Journal*, vol.68, p.215-224, 2004.
- [46] Franson, R. L. ,Brown M.S., and Bethlenfalvay G.J. (1991). The *Glycine-Glomus-Bradyrhizobium* symbiosis. XI. Nodule gas exchange and efficiency as a function of soil and root water status in mycorrhizal soybean. *Physiol. Plant.* 83:476-482.
- [47] Freeden, A.L., Rao I.M., and Terry N., (1989) – *Influence of phosphorus nutrition on growth and carbon Plant and Soil*, v. 207, p. 91-95.
- [48] George T, Ladha J K, Buresh R J and Garrity D P (1992) Managing native and legume-fixed nitrogen in lowland rice-based cropping systems. *Plant and Soil* 141, 69-91.
- [49] Graham, P. H., K. Draeger, M. L. Ferrey, M. J. Conroy, B. E. Hammer, E. Martinez, S. R. Naarons, and C. Quinto. (1994). Acid pH tolerance in strains of Rhizobium and Bradyrhizobium, and initial studies on the basis for acid tolerance of Rhizobium tropici UMR1899. *Can. J. Microbiol.* 40:198-207.

- [50] Gopalaswamy, G. and Kannaiyan, S.,(2000) The xylem of rice is colonized by *Azorhizobium caulinodans*. *Proc. R. Soc. London, Ser. B*, 2000, 267, 103–107.
- [51] Hale KL, McGrath SP, Lombi E, Stack M.S, Terry N, Pickering IJ, Graham, NG, Pilon-Smits, EAH. (2001). Molybdenum Sequestration in *Brassica* Species. A Role for Anthocyanins? *Plant Physiology* 126: 1391-1402.
- [52] Hallensleben.M, Polreich. S, Joachim Heller, Brigitte L. M. (2009).. Assessment of the importance and utilization of cowpea (*Vigna unguiculata* L. Walp.) as leafy vegetable in small-scale farm households in Tanzania – East Africa. Tropentag (2009) University of Hamburg, October 6-8, 2009 Conference on International Research on Food Security, Natural Resource Management and Rural Development.
- [53] Hart, A. L. (1989). Nodule phosphorus and nodule activity in white clover New Zealand *Journal Agric. Res.* 32:145-149.
- [54] Hayman D.S.,(1981). Influence of soil and fertility on activity and survival of arbuscular mycorrhizal fungi. *Phytopathological* 72:119-1125.
- [55] Hinsinger , P Gobran, G.R. Gregory P.J., Wenzel, W.W. (2005). Rhizosphere geometry and heterogeneity arising from root mediated physical and chemical processes. *New Phytologist* 168:293-303
- [56] Hseih, S and Hseih, C.F.(1990). The use of organic manure in crop production in Extesion bulletin 315(ASPAC) Food and Fertility Technology centre, Taiwan pp 19
- [57] Irene M. C and Thomas W. K. (2006). Mycorrhizas and tropical soil fertility. *Agriculture, Ecosystems and Environment* 116 (2006) 72-82
- [58] Jackson, R.M. and Masson, P.A. (1984). Mycorrhiza. Edward Arnold, Ltd.. London, pp;60, ISBN 0-7131-2876-836.
- [59] Israel, D.W.,(1987) - Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiol.* v. 84, 294-300.
- [60] Janos D.P., Schroeder. M.S., Schaffer B. and Crane J. H.,(2001). Inoculation with mychorrhizal fungi enhances growth of Litchi chinensis Sonn tree after propagation by air layering. *Plant and Soil*, 233: 85-94.
- [61] Kaiser N.B, Gridler K .L, Ngair B.J, Phillips.T, Tyerman S.D. (2005). The role of molybdenum in agricultural plant Production. *Annals of Botany* 96: 745-754.
- [62] Keyser, H. H., P. Somasegaran, and B. B. Bohlool. (1993). Rhizobial ecology and technology, p. 205-226. In F. Blaine Metting (ed.), *Soil microbial ecology: applications in agricultural and environmental management*. Marcel Dekker, Inc., New York, N.Y
- [63] Keyser H .H. and Li F.(1992). Potential for increasing biological fixation soybean. *Plant and Soil* 141: 119-13
- [64] Kitts, C. L. and Ludwig, R. A. (1994). *Azorhizobium caulinodans* respire with at least four terminal oxidases. *J. Bacteriol.*, 176, 886– 895.
- [65] LaFavre, A. K., and Eaglesham A.R.J. (1986). The effects of high temperature on soybean nodulation and growth with different strains of bradyrhizobia. *Can. J. Microbiol.* 32:22-27.
- [66] LaFavre, A. K., and Eaglesham A.R.J. (1987). Effects of high soil temperature and starter nitrogen on the growth and nodulation of soybean. *Crop Sci.* 27:742-745
- [67] Lambers H, Chapin F.S, Pons T.L. (1998). *Plant Physiology Ecology* Springer. New York. 540: 56- 59.
- [68] Lambert, D.H.and Weidensaul T.C. (1991). Element uptake by mychorrhizal soybean from sewage sludge –treated soil. *Soil. Sci. Soc Am. J.*, 55:393-902.
- [69] Lin li X. George, E. and Marschner (1991). Extension of the phosphorus depletion zone in VA-mychorrhizal white clover in a calcareous soil. *Plant and soil* 136: 41-48.
- [70] Magalhães, F. M. and Dobereiner, J. (1984). Ocorrência de *Azospirillum amazonense* em alguns ecossistemas da Amazônia. *Microbiologia* 15: 246-252.
- [71] Mathimaran N. Ruh R., Vullioud P., Frossard E.and Jansa J.(2005). *Glomus intraradices* dominates arbuscular mychorrhizal communities in a heavy textured agricultural soil. *Mycorrhizal* 16(1),61-66 doi10.1007/s00572-005-0014-9

- [72] Mendel R.R, Hänsch R.(2002). Molybdoenzymes and molybdenum co-factor in plants. *Journal of Experimental Botany* 53: 1689-1698.
- [73] Michiels, J., Verreth.C., and Vanderleyden J. (1994). Effects of temperature stress on bean nodulating *Rhizobium* strains. *Appl. Environ. Microbiol.* 60:1206-1212
- [74] Muleba N., (1986). Agronomie du niébé. In SAFGRAD Annual Report for 1986. SAFGRAD/IITA, Ouagadougou, Burkina Faso., p. E-1 to E-63.
- [75] Munevar, F., and Wollum A.G. (1982). Response of soybean plants to high root temperature as affected by plant cultivar and *Rhizobium* strain. *Agron. J.* 74:138-142.
- [76] Munns, D. N. (1986). Acid soils tolerance in legumes and rhizobia. *Adv. Plant Nutr.* 2:63-91. National Research Council. (NRC). 1994. Biological nitrogen fixation research challenges -A review of research grants funded by the U.S. Agency for International Development. National Research Council, Washington, D.C.: National Academy Press. 27- 31.
- [77] National Fertilizer Company of Nigeria (NAFCON) (1995).Annual reports on the sale and use of fertilizer in Nigeria for 1994. Sales highlights, 1995 : 8-10.
- [78] Ndakidemi P. A, Joachim H.J., Makoi R. (2011) Changes in plant growth, nutrient dynamics and accumulation of flavonoids and anthocyanins by manipulating the cropping systems involving legumes and cereals- a review. *Australia Journal of Agricultural Engineering.* 2(3):56-65.
- [79] Nwoko H and Sanginga N. (1999). Dependence of promiscuous soybean and herbaceous legumes on arbuscular mycorrhizal fungi and their response to bradyrhizobial inoculation in low P soils. *Applied soil ecology* 13 :251-258.
- [80] Odum E P (1989) Input management of production systems. *Science* 243, 177-182
- [81] Ogoke I.J., Carsky R J, Togun A.O., Dashiell K., (2001). Maize yield following phosphorus fertilized soybean in the Nigerian guinea savanna. In Impact, Challenges and Prospects of Maize Research and Development in West and Central Africa. edited by B. Badu-Apraku, M.A.B. Fakorede, M. Ouedraogo, and R.J. Carsky. Proceedings of a Regional Maize Workshop 4-7 May, 1999, Cotonou, Benin. IITA, Ibadan, Nigeria., p. 205-213.
- [82] Okito A, Bruno J. Rodrigues A., Segundo U. and Robert M. B.(2004) Nitrogen fixation by groundnut and velvet bean and residual benefit to a subsequent maize crop. *Pesq. agropec. bras., Brasília, v.39, (.12), .1183-1190, dez.*
- [83] Okogun, J. A. and Sanginga N. (2003). Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria? *Bio Ferti. Soils.* 38: 26-31.
- [84] Olivera, M., Tejera. N, Iribarne C, Ocana A, Liuch C, (2004). Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus, *Physiol. Plant.*, 121, 498-505.
- [85] Orchard, V. A., and Cook F.G. (1983). Relation between soil respiration and soil moisture. *Soil Biol. Biochem.* 15:447-453.
- [86] Parker M. B. and Harris H.B. (1977). Yield and leaf nitrogen of nodulating and non-nodulating soybeans as affected by nitrogen and molybdenum. *Agronomy Journal* 69: 551-554.
- [87] Pereira, P. A. A., and F. A. Bliss. (1989). Selection of common bean (*Phaseolus vulgaris* L.) for N₂ fixation at different levels of available phosphorus under field and environmentally-controlled conditions. *Plant Soil* 115:75-82.
- [88] Peoples, M.B and Craswell. E. (1992). Biological nitrogen fixation: Investment, expenditure and actual contribution to agriculture. *Plant and Soil*, 141: 13-39.
- [89] Peoples, M.B., Herridge, D.F., and Lahda, J.K. (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* 174:34-38.
- [90] Prayitno, J. et al., (1999). Interactions of rice seedlings with bacteria isolated from rice roots. *Aust. J. Plant Physiol.*, 26, 521-535.
- [91] Plaster E.J (1992). Soil science and management. 2nd edition. Delmar publisher Inc NY. Pg 514
- [92] Plucknett D. L. and Smith N. J. H.(1986) Sustaining agricultural yields. *Bioscience* 36, 40-45.

- [93] Pingali P L, Moya P F and Velasco L E (1990) The post-green revolution blues in asian rice production-The diminished gap between experiment station and farmer yields. IRRI Social Science Division paper No. 90-01.
- [94] Postgate, J. (1998). *Nitrogen Fixation, 3rd Edition*. Cambridge University Press, Cambridge UK.pp 55-57.
- [95] Quin, F. M., (1997): Advances in cowpea research. Singh B.B., Mohan Raj D.R., Dashiell K.E, and Jackai L.E.N. ,eds. *Advances in Cowpea Research*, pp. ix—xv. Co-publication of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and Japan International Research Centre for Agricultural Sciences(JIRCAS), Sayce Publishing, Devon, UK.
- [96] Radin, J.W., and Eidenbock M.P, (1984) – Hydraulic conductance as a factor limiting leaf expansion of phosphorus–deficit cotton plants. *Plant Physiol.* v. 75, p. 372-377.
- [97] Rotaru V. (2003). The Effects Of Phosphorus Application On Soybean Plants Under Suboptimal Moisture Conditions. *Lucrări științifice - , Nr. 2/2010, seria Agronomie vol. 53: 72-76*
- [98] Saif S.R.(1986). Vesicular-arbuscula mychorizal in tropical forage species as influenced by season, texture. Fertilizers, and host species. *Angew. Bot* 60:125-139.
- [99] Saikia, S. P., Jain, Vanita and Srivastava, G. C.2003 Effect of *Azospirillum* and *Azorhizobium* on maize yield. *Indian J. Plant Physiol.* 6: 539–544.
- [100] Sanchez P. A. (1980) Management considerations for acid soils with high phosphorus fixation capacity. *In The Role of Phosphorus in Agriculture.* pp 471-514. American Society of Agronomy, Madison, WI. Pp 471-544.
- [101] Sanchez P.A., Shepherd K.D., Soule M.J., Place F.M., Buresh R.J., Izac A.N., Mkwunye A.U.,Kwesiga F.R.,Ndiritu C. G W and Woomer P.L.(1997). Soil Fertility replenishment in Africa: An investment in natural resource capital, p1-45 In Buresh R.J Sanchez P.A., and Calhoun. Replenishing soil fertility in Africa. Soil sci. Soc of A merican, Madison, Wisconsin, USA.
- [102] Sanginga, N. Dashiell, K., Okogun, J. A. and Thottapilly, G (1997).. Nitrogen fixation and Nitrogen contribution in promiscuous soybeans in Southern Guinea Savanna of Nigeria. *Plant Soil* 195: 257-266.
- [103] Sanginga N., Lyasse O., Singh B.B., (2000). Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant and Soil*, 220 : 119-128.
- [104] SAS 1999. Statistics Analysis software. System for windows, SAS users' Guide statistics, version 9.1 SAS Institute Inc. Cary NC. U.S.A. 102 p
- [105] Schroder, J.L., Zhan H, Richards J.R., and M.E. M.E.. (2010). An inter-laboratory validation of Mehlich 3 as a universal extractant for plant nutrients. *J. AOAC International* (accepted).
- [106] Sessitsch,A J., Howieson.J.G., Perret,X., Antoun, H. and . Martínez-Romero E. (2002) Advances in Rhizobium Research. *Critical Reviews in Plant Sciences*, 21(4):323–378
- [107] Shanmugasundaram S. (1988) Seed production and management of mungbean and soybean. *In Sustainable Agriculture: Green Manure in Rice Farming.* pp 359-376. The Int. Rice Research Inst., Los Banos, Philippines.
- [108] Shamsuddin, M. M., Rohani, O., Roslina, A. W., Ho, C. N., Noraza, H. Chalk, P. M. (1995)Nitrogen fixation by *Azospirillum* in association with oil palm (*Elaeis guineensis* Jaquim.). *In International Symposium on Sustainable Agriculture for the Tropics - The role of Biological Nitrogen Fixation* pp. 232-233. Boddey, R. M. and Resende, A. S., Eds. EMBRAPA, Rio de Janeiro.
- [109] Shiferaw B., Bantilan M.C.S and R Serraj R. (2004). Harnessing the Potentials of BNF for Poor Farmers: Technological, Policy and Institutional Constraints and Research Needs. Overview of LegumeSymbiotic nitrogen fixation (SNF) Research Achievements in IARC. Pp 3 -27.
- [110] Silvester M.B. (1983) Analysis of nitroger. fixarion In Gordon JC an6 Wheelcr CT ed Biological nitrogen fixation in forest ecosystems: foundations and applications. pp 173-2 I 5 The Hague. NijhoffJunk.
- [111] Sinclair, T R and Wit, C T de (1975) Photosynthate and nitrogen requirements for seed production by various crops. *Science* 189. 565-567.
- [112] Sinclair, T.R. and Vadez, V., (2002) - Physiological traits for crop yield improvement in low N and P environments. *Plant and Soil*, v. 245, p. 1-12.

- [113] Singh, B.B., Asante, S.K., Florini, D., Jackai, L .E.N., Fatokun, C, and Wydra, K.. (1997). Breeding for multiple disease and insect resistance. IITA Annual Report. 1997. Project 11. 22.p
- [114] Singh B.B.(2002) Recent genetics studies in cowpea. In; Fatokun C.A., Tarawali S.A., Singh B.B., Kormawa P.M.,Tamo M (eds) Challenges and opportunity for enhancing sustainable cowpea production. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp. 3-13
- [115] Singh,A, Baoule, A.L. Ahmed, H.G., Dikko, A. U., Aliyu U.,Sokoto,.M.B., Alhassan J., Musa.M., Haliru B.. (2011). Influence of phosphorus on the performance of cowpea (*Vigna unguiculata* (L) Walp.) varieties in the Sudan savannah of Nigeria. *Agricultural sciences*, Vol.2, No.3, 313-317.
- [116] Singh, D.K. and Sale, P.W.G., (2000) - Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agronomy J.*, v. 92, p. 868-874.
- [117] Singh, H .B (2002). The role of manures and fertilizer in crop production.I.F. D. C D.I.A.M.N.A. projects (Developing information Bulletin. I.F .D .C. office Garki Abuja, Nigeria pp 71.
- [118] Smaling, E.M.A.; Nandwa, S.M.; Janssen, B.H.(1997). Soil fertility in Africa is at stake. In: Buresh, R.J.; Sanchez, P.A.; Calhoun, F. (ed.). Replenishing soil fertility in Africa. Madison, WI: Soil Science Society of America, 1997. p.47-62. (Special Publication, 51).
- [119] Smith K.S., Balistrieri L.S., Smith S.S., Severson R.C. (1997). Distribution and mobility of molybdenum in the terrestrial environment. In molybdenum in agriculture (Gupta UC Ed.), Cambridge: CUP.
- [120] Soheil K ., Keyvan S. and Siros E. (2011) Soybean nodulation and chlorophyll concentration (SPAD value) affected by some of micronutrients. *Annals of Biological Research*, 2011, 2 (2) : 414-422
- [121] Sprent J. 1. and Minchin F. R. (1983) Environmental factors on the physiology of nodulation and nitrogen fixation In Temperate Legumes: Physiology. Genetics and Nodulation. Eds. G Jones and O.R Davies pp 269-317. Pitman Advance Publishing Program. Boston
- [122] Stancheva I, Geneva M., Zehirov G., Tsvetkova G., Hristozkova M., Georgiev G., (2006) Effects of combined inoculation of pea plants with arbuscular mycorrhizal fungi and rhizobium on nodule formation and nitrogen fixing activity. *General applied plant physiology special issue*, pp 61-66
- [123] Swift, M.J., Dvorak, K.A., Mulongoy, K., Musoko,. M.,Sanginga, N., and Tian, G., (1994). The role of soil organisms in the sustainability of tropical cropping systems In:Syers J.K. and Rimmer(eds) D.L. Soil Science and Sustainable Land Management in the Tropics pp155-170. CAB International, Cambridge University Press UK.
- [124] Tate, R. L. (1995). Soil microbiology (symbiotic nitrogen fixation), John Wiley& Sons, Inc., New York, N.Y p 307-333.
- [125] Taylor, R. W., Williams M.L., and Sistani KR. (1991). Nitrogen fixation by soybean-Bradyrhizobium combinations under acidity, low P and high Al stresses. *Plant and Soil* 131:293-300
- [126] Thibaund G.R. (2005). Molybdenum relationships in soils and plants. KwaZulu-Natal Department of Agriculture and Environmental Affairs, Cedara College, Private Bag X9059, Pietermaritzburg, 3200,South Africa. Available online:
- [127] Thomas, R. J., Askawa N.M., Rondon M .A., and Alarcon H.F. (1997). Nitrogen fixation by three tropical forage legumes in an acid soil savanna of Colombia. *Soil Biol. Biochem.* 29:801-808.
- [128] Tian, G.; Hauser, S.; Koutika, L.-S.; Ishida, F.;and Chianu, J.N. (2001). Pueraria cover crop fallow systems: Benefits and applicability. In: Tian, G.; Ishida, F.; Keatinge, D. (Ed.). Sustaining soil fertility in West Africa. Madison,WI: Soil Science Society of America; American Society of Agronomy, . .137-155.
- [129] Tu, J. C. (1981). Effect of salinity on Rhizobium-root hair interaction, nodulation and growth of soybean. *Can. J. Plant Sci.* 61:231-239.
- [130] Ufot, U.D.,Awe O.A. and Chukwu G. O. (2003) Effects of phosphorus on basal and apical internodes of maize (*Zea mays*) on Hapic Acrisoil. Proceedings of the Annual Conference of soil science society of Nigeria: 103- 106
- [131] Vance . C.P. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. *New phytologist* 157:423-447.

- [132] Vanlauwe B, Aihou K, Iwuafor ENO, Houngnandan P, Diels J, Manyong V. M. and Sanginga N:(2003). Recent developments in soil fertility management of maize-based systems. The role of legumes in N and P nutrition of maize in the moist savanna zone of West Africa *Agro-Chemicals Report*.3 (3):17-26.
- [133] Vanlauwe, B.; Diels J.; Sanginga, N.; Merckx. R(2002). Integrated plant nutrient management in Sub-Saharan Africa: from concept to practice. Wallingford, Oxon: CABI Publications, 2002. 352p.
- [134] Van Rossum, D., Muyotcha A., De Hope B.M., Van Verseveld H.W., Stouthamer A.H., and Boogerd F.C..(1994). Soil acidity in relation to groundnut-Bradyrhizobium symbiotic performance. *Plant and Soil* 163:165-175.
- [135] Vincent J. M. (1980) Factors controlling the legume-Rhizobium symbiosis. *In Nitrogen Fixation*. S.Vol. 2. Eds.W E Newton and W H Orme-Johnson. pp 103-129. University Park Press Baltimore. MD.
- [136] Vitousek, P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W. H., and Tilman D.G .. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol. Appl.* 7:737-750.
- [137] Wadisirisuk, P., S. Danso K A., Hardarson G., and G. D. Bowen G.D. (1989). Influence of Bradyrhizobium japonicum location and movement on nodulation and nitrogen fixation in soybeans. *Appl. Environ. Microbiol.* 35:1711-1716.
- [138] Walley, F.,G.Fu,J.W. van Groenigen, and Kessel C van. (2001). Short-range spatial variability of nitrogen fixation by field-grown chickpea. *Soil Sci. Soc. Am. J.* 65:1717-1722.
- [139] Walsh, K. B. (1995). Physiology of the legume nodule and its response to stress. *Soil Biol. Biochem.* 27:637-655.
- [140] Westermann D. T. (2005). Nutritional requirements of potatoes. *American Journal of Potato Research* 82: 301-307.
- [141] Williams RJP, Frausto da Silva J.R. (2002). The involvement of molybdenum in life *Biochemistry and Biophysical Research Communication* 292(2): 293-299.
- [142] Zhang, H., S. Kariuki, J. Schroder, M. Payton, and . Focht. C.(2009). Inter-laboratory validation of the Mehlich 3 for extraction of plant-available phosphorus. *J. AOAC International* Vol. 92 (1): 91-102.

APPENDIX - A

Table 1. Pre- crop Soil Property Analysis

SOIL PROPERTIES	VALUES
Soil pH (1:1 soil /H ₂ O)	6.0
C	0.71 %
N	0.07 %
Available P	3.54 ppm
Zn ²⁺	18.3ppm
Cu ²⁺	0.91ppm
Mn ²⁺	73.25ppm
Fe ²⁺	52.21ppm
Soil physical properties	Percent soil composition
Sand	82%
Silt	3%
Clay	16%
Textural class	Sandy loam
Exchangeable bases	Centimol/kg
Ca ²⁺	1.83
Mg ²⁺	0.61
K ⁺	0.17
Na ⁺	0.09

Table 2. Plant Growth Response in Height(cm) as Affected by P Fertilization

Kg P/ha	2WAP	4WAP	6WAP	8WAP	10WAP
0	10.64a	11.17a	101.44a	129.10a	148.02a
40	11.77a	16.97a	101.69a	127.03a	148.69a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

Table 3. The Mean Plant Height (cm) as Influenced by Mycorrhizal Inoculant Rates

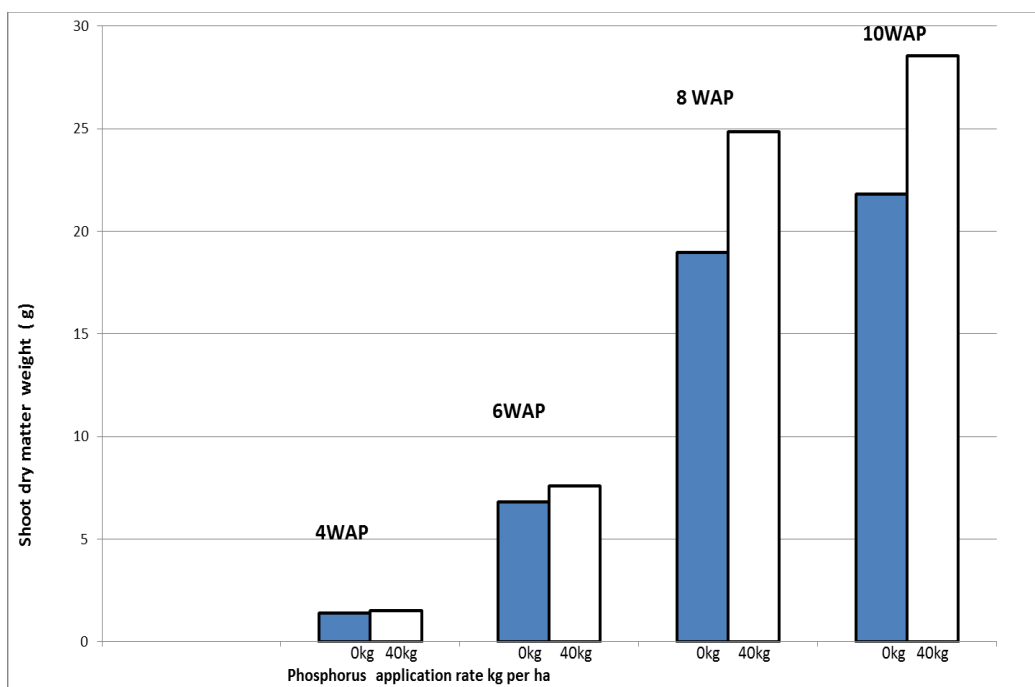
Inoculant Rates (g/hill)	2WAP	4WAP	6WAP	8WAP	10WAP
0.0	11.71a	17.66a	109.31a	101.75a	135.49a
2.5	11.33a	17.19a	106.75a	132.38a	144.94a
5.0	10.88a	17.75a	99.00a	141.88a	140.88a
7.5	10.91a	15.63a	91.19a	140.63a	172.11a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

Table 4. Root Dry Matter Weight Response to P Application at Four Sampling Periods

Kg P/ha	4WAP	6WAP	8WAP	10WAP
0	0.26a	0.66a	1.63a	1.86a
40	0.24a	0.76a	1.93a	2.24a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

**Fig. 1. Plant shoot dry matter weight response to P application at four sampling periods****Table 5. The Influence of Mycorrhizal Inoculant Rates on Root Dry Matter Weight**

Inoculant rates (g/hill)	4WAP	6WAP	8WAP	10WAP
0	0.25ba	0.78a	1.58a	1.80a
2.5	0.29a	0.70a	1.98a	2.25a
5.0	0.23ba	0.63a	1.76a	2.09a
7.5	0.22b	0.72a	1.79a	2.06a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

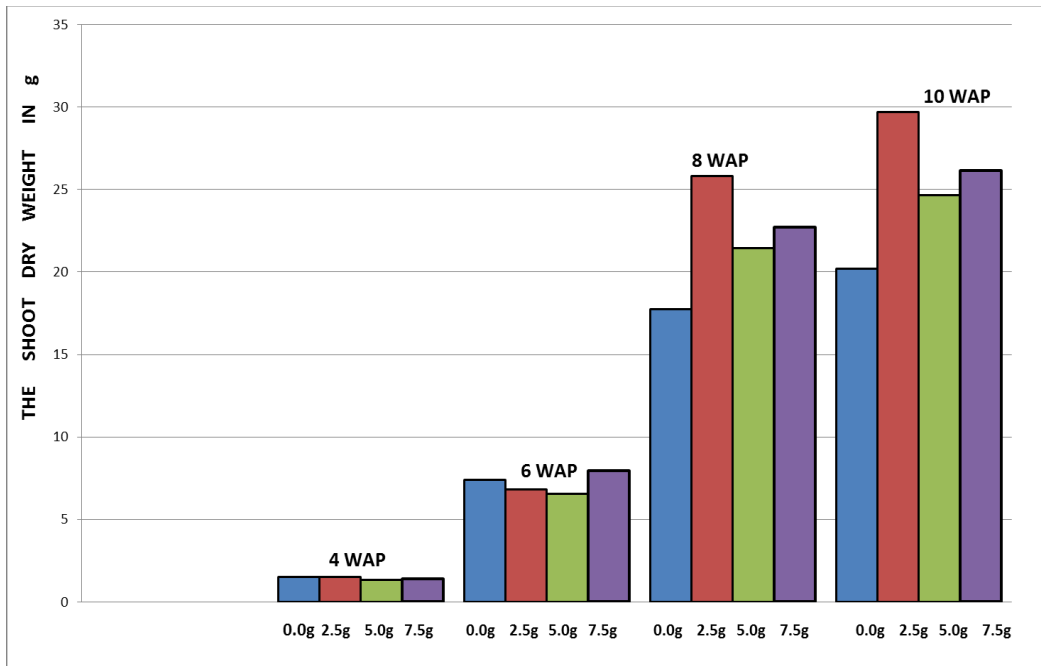


Fig 2. Response of the shoot dry weight to mycorrhizal inoculant rates at four sampling periods.

Table 6. Crown and Total nodulation Variables as Influenced by P fertilization

P Kg/ha	4WAP		6WAP		8WAP		10WAP	
	Crown nod	Total nod	Crown nod	Total nod	Crown nod	Total nod	Crown nod	Total nod
0	1.50a	5.20a	1.60a	11.20a	0.30b	4.60a	0.69b	5.60a
40	2.20a	7.10a	3.44a	13.10a	1.44a	5.10a	2.60a	6.00a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

Table 7. Plant crown and total Nodulation response to Mycorrhizal Inoculant Rates at Four Sampling periods

Inoculantrates g/hill	4WAP		6WAP		8WAP		10WAP	
	Crown nod	Total nod	Crown nod	Total nod	Crown nod	Total nod	Crown nod	Total nod
0.0	1.60a	5.62a	1.63a	12.40a	0.81a	4.75a	1.62a	5.90a
2.5	1.40a	5.69a	2.60a	11.50a	0.60a	4.69a	1.20a	6.00a
5.0	2.00a	6.56a	2.63a	11.44a	0.40a	4.44a	1.31a	4.94a
7.5	2.40a	6.63a	3.50a	13.30a	1.70a	5.50a	2.44a	6.31a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

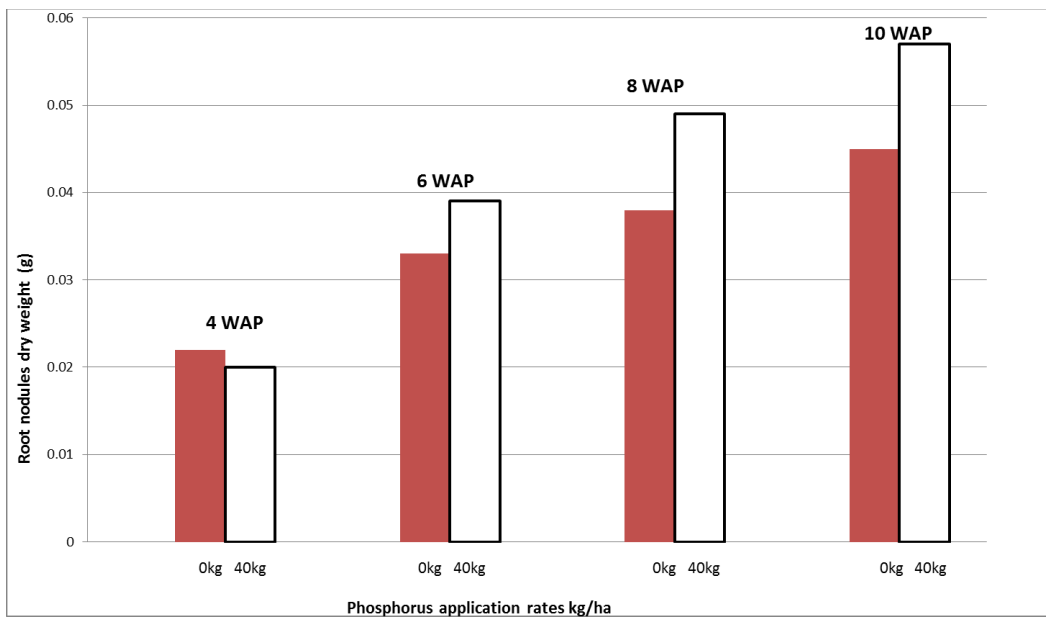


Fig. 3. Influence of P Fertilization Rates on Nodule Dry Matter Weight at Four Sampling Periods

Table 8. The Influence of P Application on Nodule Size at Four Sampling Periods

Kg P/hill	4WAP	6WAP	8WAP	10WAP
0	1.90a	3.00b	3.20a	3.60a
40	2.30a	4.10a	3.20a	3.61a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

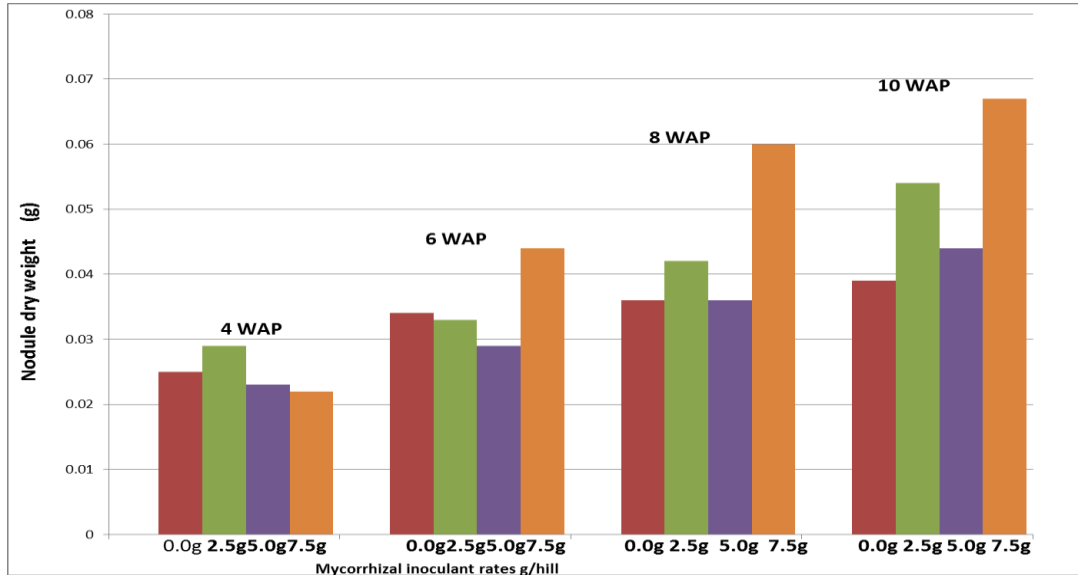


Fig. 4. Nodule Dry Matter Weight as Influenced by Mycorrhizal Inoculant rates at Four sampling periods

Table 9. Nodule Size in (mm) Response to Mycorrhizal Inoculants rates at Four Sampling periods

Inoculant rates g/hill	4WAP	6WAP	8WAP	10WAP
0.0	1.90a	3.11b	3.22a	3.33a
2.5	2.16a	3.99a	3.30a	3.86a
5.0	2.03a	3.84a	3.15a	3.54a
7.5	2.30a	3.30ab	3.03a	3.64a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

Table10. Percentage of Tissue Nitrogen and Phosphorus, Nodules with leghaemoglobin at 8 WAP and Seed Yield (10 WAP) as affected by P Application

<u>kgP/ha</u>	<u>% Tissue N</u> <u>per plant</u>	<u>% Tissue P</u> <u>per plant</u>	<u>% Nodule with</u> <u>leghaemoglobin</u>	<u>Seed yield(kg/ ha)</u>
0	3.25a	1.56a	98.90a	590a
40	3.92b	1.69a	97.81a	630a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)

Table 11. Percentages of Tissue Nitrogen , Phosphorus, Nodules with leghaemoglobin at 8WAP and Seed Yield at 10 WAP as Affected by mycorrhizal inoculant rates Application

<u>Inoculants rates</u> <u>(g/hill)</u>	<u>% Tissue/ plt</u>	<u>% Tissue/ plt</u>	<u>% Nodule with</u> <u>legh</u>	<u>Seed yield kg/ha</u>
0.0	3.10b	1.15b	98.313a	600a
2.5	3.86a	1.68a	97.625a	612a
5.0	3.52ab	1.79a	98.125a	618a
7.5	3.84a	1.89a	99.375a	610a

The means (column-wise) with the same alphabet are not significantly different (D.M.R.T. 5% level)