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Influence of Mycorrhizal Inocula on Cowpea Root Colonization and Soil Improvement in Kano, Nigeria

Surayya AM*, Safianu R, Halima MR and Bala ZM

Department of Biological Sciences, Bayero University Kano, P.M.B. 3011, Kano 700241, Nigeria

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Abstract

This research was conducted to investigate the influence of three (3) different Arbuscular mycorrhizal fungi (AMF) inocula (*Glomus mossae, G. etunicatum* and *Acaulospora kentinensis)* from the International Institute of Tropical Agriculture (IITA) Ibadan, singly and in combination inoculation on cowpea root colonization and soil improvement in Kano. Soil samples were collected from BUK new campus and used for the screen house experiment. Cowpea seeds (IT99K-573-1-1) were obtained from the International Institute of Tropical Agriculture (IITA) Kano. Ten grams of Mycorrhizal inocula were inserted into each planting hole. The study results showed that cowpea plants treated with *Glomus etunicatum* had the highest mycorrhizal root colonization (15%) while cowpea plants treated with *Acaulospora kentinensis* had the least root colonization (10%). AMF present in the inocula colonized host plant in greater levels compared to control cowpea plants. This study showed significant ($P < 0.0001$) effect of different treatments on mycorrhizal root colonization, and there were significant ($P < 0.0001$) differences of the various treatments on the soil physicochemical parameters (pH, phosphorus, nitrogen, potassium, organic carbon and moisture content). The results showed that AMF can significantly contribute to soil improvement. However, more research should be further explored using AMF as an option for chemical fertilization to improve soil fertility in Nigeria.

Keywords – Arbuscular mycorrhizal fungi – inoculation – soil parameters

Introduction

Mycorrhiza is the symbiotic association between soils fungi with the roots of vascular plants (Sieverding 1991). There are two major groups of mycorrhizal fungi, ectomycorrhizae and endomycorrhizae fungi. Among the types of endomycorrhizal fungi, arbuscular mycorrhizal (AM) fungi are the most prevalent in soils. Arbuscular mycorrhizal fungi (AMF), (formerly VAM) are a type of mycorrhizae characterized by forming arbuscules, hyphae and vesicles within root cortex cells (Brundrett 1991). Mycorrhizal fungi colonize about 90% of terrestrial plants species. Arbuscular mycorrhizal fungi are significant in increasing the ability of the host plant to absorb immobile or fixed ions such as phosphorus, zinc and copper in acidic or alkaline soils as well as in the absorption of water and nitrogen (Sharif et al. 2009). In Kano state, due to manmade deforestation, cattle pressure and improper water management, fertile soil turned out to be wasteland (Kotia & Kumar 2001); the introduction of AMF can improve soil structure and

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Corresponding Author: Surayya A.M. – e-mail – malalasurayya@gmail.com

contribute to maintain soil fertility, which indirectly affects plant growth (Jeffries et al. 2003, Wu et al. 2014). Glomalin, secreted by AMF can glue small soil particles into a diameter of >0.25 mm macroaggregates, further forming the large polymers (Lovelock et al. 2004). The formation of soil aggregate improves soil physical condition and increases soil stability (Bever et al. 2001, Chaudhary et al. 2009). The aim of this research was to investigate the influence of mycorrhizal inocula on cowpea root colonization and on soil improvement in Kano, Nigeria.

Methodology

Study Area

The soil sample used in the screen house experiment was sampled from the New Campus Farm Bayero University Kano. The geographical location of the area was 11^{o}58'51.7"North Latitude and 008 ^{o}24'52.3"East Longitude.

Soil Sample Collection

The soil was collected at a 0-15 cm depth from the site, and transferred into a polythene bag. The soil sample was further sterilized and analyzed for different properties, these are: Nitrogen, phosphorous, potassium, pH, moisture content, and organic carbon, at the Soil Science Laboratory before inoculation with AMF treatments.

Selected Host Plant

Cowpea (IT99K-573-1-1) was selected as the host plant for this study. The variety was used because it was multiple disease resistance, especially Fusarium wilt, drought tolerance, Striga and Alectra resistance (Singh et al. 2011). Cowpea Seeds were obtained from the International Institute of Tropical Agriculture (IITA) Kano.

Source of AMF

Three strains of soil base AMF were obtained from International Institute of Tropical Agriculture (IITA) Ibadan and were used as the inocula. These are: *Glomus mossae, Glomus etunicatum* and *Acaulospora kentinensis*.

Pre-Planting

The experiment was laid out in a completely randomized design using a single factor (improved cowpea variety IT99K-573-1-1), with 7 levels of treatments and control in 3 replicates. The treatments were: T1 (*Glomus mossae)*, T2 (*Glomus etunicatum),* T3 *(Acaulospora kentinensis)*, T4 (*G. mossae + G. etunicatum),* T5 (*G. mossae + A. kentinensis),* T6 (*G. etunicatum + A. kentinensis)*, T7 (*G. mossae +G. etunicatum + A. kentinensis)*, and C (Control). Cowpea seeds were surface sterilized using methods described by (Oyebanji 2009). Briefly, the seeds in a container were washed and rinsed 3 times in sterile distilled water in the biosafety cabinet. One percent sodium hypo chloride solution was prepared and were added to cover the seeds and stirred for 2 minutes. The sterilizing agent was then decanted, and the seeds were rinsed thoroughly three times with sterile distilled water. Finally, Seeds were placed on sterile tissue to dry before planting. Rubber Pots were also sterilized by washing with distilled water and sodium hypo chloride solution. Pots were taken and filled with 7 kg of sterilized soil. Ten grams of mycorrhizal inocula were inserted into each planting hole (Miyasaka et al. 2003).

Planting and Agronomical Practices

Three cowpea seeds were sown in each pot and thinned to two. The plants were maintained with regular watering, and weeding was done by hand. Pots of different treatments were rotated weekly to different bench positions to minimized positional effects. Infestation was first observed in the control pot. Spraying was done at the fourth week using Imiforce (imidachloprid) at 40 mls per 16 L of water. Destructive sampling was conducted starting from two weeks after planting for

root colonization assessments. There were seven collections for the cultivation period, root samples were taken at weekly basis.

Data Collection

Percentage Roots Length Colonization Data

Root samples were collected from each pot for microscopic determination of arbuscular mycorrhizal fungi root length colonization. Roots were taken at 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th weeks after planting; respectively. Roots processing was done by the modified procedure of Phillips & Hayman (1970) as reported by Bala & Safianu (2020). At each harvest, the root samples were washed thoroughly with tap water to remove the adhering soil particles and cut into pieces of 2 cm in length. Ten (10) grams of root material were heated to 90°C in 3% KOH for 30 minutes to soften the root, and were then bleached in alkaline H_2O_2 for 30 minutes. The roots were acidified with 0.1ml HCl overnight and heated to 90°C with lactoglycerol trypan blue stain for 30 minutes; this was be followed by destaining in lactoglycerol for 24 hours. Stained roots were placed and examined over glass slide for both external and internal hyphal structures under compound microscope (Leica CME). Each slide examined contained 5 root pieces of 2 cm long, for each pot, 5 slides were prepared, such that 25 root pieces and 50 cm of root were examined per pot. All fungal structures were observed and recorded.

The percentage of mycorrhizal colonization was estimated by following formula;

$$\text{Colonization (\%)} = \frac{\text{Number of root segment with Amf}}{\text{Total number of root segments examined}} \times 100$$

Soil Analysis

For the physicochemical analysis of rhizosphere pH, Nitrogen, Phosphorus contents, potassium, organic carbon, and moisture content were analyzed at Soil Science Department Bayero University Kano.

Soil Moisture content was determined by drying sample in an oven over night at 105℃, soil pH was determined using pH meter, Nitrogen was determined by weighing sample into macro-Kjeldahl flask, Phosphorus determination in the Soil is by Bray No. 1 method, determination of Potassium is by using atomic absorption spectrophotometer, and Organic Carbon was determined by Walkley-Black Modified Method (Francisco et al. 2009).

Results

Percentage AMF Root Colonization of Cowpea Plant Assessment

Table 1 showed that cowpea plants treated with T2 (*G. etunicatum*) had the highest mycorrhizal root colonization of 5.26 ± 0.30 mm (15%) while cowpea plants treated with T3 (*A. kentinensis*) had the least mycorrhizal root colonization of 3.52 ± 0.26 mm (10%). However, the mycorrhizal root colonization of cowpea plants under other AMF treatments were T1 (4.47 ± 0.25 mm), T4 (3.72 ± 0.24 mm), T5 (4.90 ± 0.23 mm), T6 (3.99 ± 0.25 mm), T7 (4.59 ± 0.22 mm) and C (control) (4.58 ± 0.23 mm) with the percentages of 12.8%, 10.6%, 14%, 11.4%, 13.1% and 13.1%, respectively.

Table 2 the Single-Factor Anova result shows significant effect of the various treatments on Mycorrhizal root colonization of cowpea plants ($F_{7,11689} = 5.75$, $P < 0.0001$).

Physicochemical Analyses of Soil before and After Inoculation (At Harvest) with AMF

Table 3 showed the results of the physicochemical analyses of the selected soil samples from new campus farm before inoculation with AMF isolates. The Two-Factor with replication result shows a significant difference in the soil physicochemical parameters before the various treatments ($F_{7,51} = 2.9$, $P = 0.013$). The result revealed that the soil samples were acidic in nature with a pH

range of 5.05 ± 0.07 and 5.90 ± 0.14. The percentage organic carbon (OC) content of the soil samples revealed that S1 had the highest % OC of 0.59 ± 0.01 while S8 had the least % OC of 0.24 ± 0.03. The result also showed that potassium (K) content of the soil samples to be S1 (0.34 ± 0.02 mg/kg), S2 (0.25 ± 0.00 mg/kg), S3 (0.29 ± 0.01 mg/kg), S4 (0.30 ± 0.02 mg/kg), S5 (0.30 ± 0.01 mg/kg), S6 (0.18 ± 0.02 mg/kg), S7 (0.23 ± 0.03 mg/kg) and S8 (0.11 ± 0.01 mg/kg). The result showed that phosphorus (P) contents of the soil samples were 14.38 ± 1.81, 15.92 ± 1.06, 20.40 ± 1.46, 10.98 ± 2.08, 11.31 ± 5.54, 13.73 ± 0.85, 14.91 ± 1.26 and 13.16 ± 2.59 mg/kg. The concentration of phosphorus was in the order S3>S2>S7>S1>S6>S8>S5>S4. Percentage nitrogen (N) content of the soil samples was maximum in S5 (0.55 ± 0.63%) and minimum in S1 and S3 (0.13 ± 0.03%) soil samples. The moisture content (MC) of the soil samples ranged between 0.19 ± 0.01% and 0.43 ± 0.04%.

Table 1 Mycorrhizal Root Colonization (%) of Cowpea Plants. 20th October 2019

3	Root Colonization (mm)	% Colonization
T1	4.47±0.25[a]	12.8
T2	5.26±0.30[b]	15.0
T3	3.52±0.26[c]	10.0
T4	3.72±0.24[c]	10.6
T5	4.90±0.23[ab]	14.0
T6	3.99±0.25[c]	11.4
T7	4.59±0.22[a]	13.1
C (control)	4.58±0.23[a]	13.1

Key: T1 = Treatment with *G. mossae*, T2 = treatment with *G. etunicatum*, T3 = treatment with *A. kentinensis*, T4 = treatment with *G. mossae* + *G. etunicatum*, T5 = treatment with *G. mossae* + *A. kentinensis*, T6 = treatment with *G. etunicatum* + *A. kentinensis*, T7 = *G. mossae* + *G. etunicatum* + *A. kentinensis*, C = control sample. Values are Mean ± SD of three replicates. Means followed by different superscript letters along the column are significantly different at $P < 0.05$ (Tukey HSD Test).

Table 2 Anova (Single-Factor) of (%) Mycorrhizal Root Colonization of Cowpea

Source	Type III Sum of Squares	*Df*	Mean Square	*F*	*P*
Corrected Model	384.418[a]	7	54.917	5.751	0.000
Intercept	23865.500	1	23865.500	2.499E3	0.000
Treatment	384.418	7	54.917	5.751	0.000
Error	11689.101	1224	9.550		
Total	35918.000	1232			
Corrected Total	12073.519	1231			

[a] R Squared = 0.032 (Adjusted R Squared = 0.026)

Table 3 Physicochemical Properties of Soil Samples from New Campus Farm before and After Inoculation (at harvest) with AMF Isolates

Soil ID/ Treatment	Treatment Application	pH (H_2O)	O.C (%)	P (mg/kg)	N (%)	M.C (%)	K (cmol/kg)
S1/T1	BT	5.23 ± 0.25	0.59 ± 0.01	14.38 ± 1.81	0.13 ± 0.03	0.19 ± 0.01	0.34 ± 0.02
	AT	6.42 ± 0.01	0.68 ± 0.11	19.30 ± 0.14	0.15 ± 0.01	0.46 ± 0.06	0.81 ± 0.01
S2/T2	BT	5.10 ± 0.17	0.57 ± 0.01	15.92 ± 1.06	0.20 ± 0.08	0.27 ± 0.05	0.25 ± 0.00
	AT	6.03 ± 0.04	0.85 ± 0.05	19.98 ± 0.03	0.23 ± 0.03	0.29 ± 0.02	0.38 ± 0.04
S3/T3	BT	5.90 ± 0.14	0.50 ± 0.00	20.40 ± 1.46	0.13 ± 0.03	0.21 ± 0.02	0.29 ± 0.01
	AT	6.00 ± 0.01	0.74 ± 0.02	24.39 ± 0.16	0.15 ± 0.01	0.39 ± 0.01	0.38 ± 0.03
S4/T4	BT	5.44 ± 0.15	0.45 ± 0.01	10.98 ± 2.08	0.20 ± 0.14	0.28 ± 0.03	0.30 ± 0.02
	AT	6.36 ± 0.06	0.85 ± 0.01	18.01 ± 0.01	0.31 ± 0.01	0.28 ± 0.03	0.19 ± 0.01
S5/T5	BT	5.07 ± 0.05	0.40 ± 0.00	11.31 ± 5.54	0.55 ± 0.63	0.43 ± 0.04	0.30 ± 0.01
	AT	6.26 ± 0.06	0.87 ± 0.02	19.23 ± 0.32	1.03 ± 0.04	0.41 ± 0.01	0.22 ± 0.02

Table 3 Continued.

Soil ID/ Treatment	Treatment Application	pH (H_2O)	O.C (%)	P (mg/kg)	N (%)	M.C (%)	K (cmol/kg)
S6/T6	BT	5.05±0.07	0.38±0.03	13.73±0.85	0.14±0.01	0.31±0.01	0.18±0.02
	AT	5.75±0.07	0.88±0.02	18.49±0.02	0.21±0.01	0.48±0.03	0.19±0.01
S7/T7	BT	5.15±0.08	0.48±0.03	14.91±1.26	0.16±0.06	0.25±0.00	0.23±0.03
	AT	6.21±0.15	0.80±0.01	17.92±0.11	0.19±0.01	0.48±0.03	0.30±0.00
S8/C	BP	5.09±0.05	0.24±0.03	13.16±2.59	0.20±0.00	0.31±0.01	0.11±0.01
(control)	AH	5.04±0.05	0.83±0.02	15.29±0.73	0.13±0.01	0.33±0.01	0.10±0.00

Keys: OC = Organic carbon; P = Phosphorus; N = Nitrogen; MC = Moisture content; K = Potassium. Values are Mean±SD of two replicates. T1 = Treatment with *G. mossae*, T2 = treatment with *G. etunicatum*, T3 = treatment with *A. kentinensis*, T4 = treatment with *G. mossae*+ *G. etunicatum*, T5 = treatment with *G. mossae* + *A. kentinensis*, T6 = treatment with *G. etunicatum* + *A. kentinensis*, T7 = *G. mossae* + *G. etunicatum* + *A. kentinensis*, C = control sample. Values are Mean ± SD of three replicates. BT = before treatment, AT = after treatment, BP = before planting, AH = at harvest.

Soil Physicochemical Analyses after Inoculation (At Harvest) With AMF Isolates

Table 3 also showed the results of the physicochemical analyses of the eight soil samples after inoculation with AMF isolates with the pH range between 5.04 ± 0.05 and 6.42 ± 0.01. The percentage organic carbon (OC) content of the soil samples revealed that T6 had the highest % OC of 0.88 ± 0.02 while T1 had the least % OC of 0.68 ± 0.11. The result showed that phosphorus (P) contents of the soil samples were 19.30 ± 0.14, 19.98 ± 0.03, 24.39 ± 0.16, 18.01 ± 0.01, 19.23 ± 0.32, 18.49 ± 0.02, 17.92 ± 0.11, 15.29 ± 0.73 mg/kg. The concentration of phosphorus was in the order T3>T2>T1>T5>T6>T4>T7>C. Percentage nitrogen (N) content of the soil samples was maximum in T5 (1.03 ± 0.04%) and minimum in C (0.13 ± 0.01%) soil samples. The moisture content (MC) of the soil samples ranged between 0.29 ± 0.02% and 0.48 ± 0.03%. T6 and T7 soil samples both had the highest moisture contents of 0.48 ± 0.03% followed by T1 (0.46 ± 0.06%), T5 (0.41 ± 0.01%), T3 (0.39 ± 0.01%), C (0.33 ± 0.01%), T2 (0.29 ± 0.02%) and the least moisture content was observed in T4 (0.28 ± 0.03%). The result also showed that potassium (K) content of the soil samples to be T1 (0.81 ± 0.01 mg/kg), T2 (0.38 ± 0.04 mg/kg), T3 (0.38 ± 0.03 mg/kg), T4 (0.19 ± 0.01 mg/kg), T5 (0.22 ± 0.02 mg/kg), T6 (0.19 ± 0.01 mg/kg), T7 (0.30 ± 0.00 mg/kg) and C (0.10 ± 0.00 mg/kg).The Two-Factor with replication result shows a significant difference between the various treatments on the soil physicochemical parameters ($F_{7,81.3}$ = 148.13, P< 0.0001).

Discussion

Cowpea plant treated with *G. etunicatum* had the highest mycorrhizal root colonization; this is in line with the study of Kumar et al. (2012), who found higher AMF colonization in leguminous than in graminaceous crops. Thus, as a legume, cowpea plant probably favored mycorrhizal colonization to assist the symbioses with *Rhizobium* (Bethlenfalvay & Newton 1991). Perhaps, Diop et al. (2015), found the genus *Glomus* to be the most diverse with six species, representing 40 % of all the species found in roots of cowpea. The predominance of this genus in most ecosystems like Senegal (Manga et al. 2007, Ndoye et al. 2012), China (Wang et al. 2010), Burkina Faso (Bâ et al. 1996) and Central Europe (Oehl et al. 2003) suggests a better adaptation of the genus to adverse conditions such as warm, cold, drought, salinity and other environmental stress (Blaszkowski et al. 2002). This indicates that roots colonization may not necessarily be a function of spore number in the soil but a reflection of the response of the host plant to the inocula.

At harvest (In AMF inoculated soil samples), organic carbon, phosphorus, nitrogen and potassium contents were greater than the non-inoculated soils (before inoculation) and control. Such increases in soil nutrient contents in response to mycorrhizal effects were highly associated, with the level of each mycorrhizal infection (Solaiman et al. 2010). AMF treatments influenced soil quality compared to before inoculation and in the control plants. Such an increase in carbon pool is due to the strong influence of mycorrhizal fungi on the release of compounds from living roots

because these fungi can affect plant carbon metabolism while representing a sizeable sink for plant derived carbon (Averill & Finzi 2014). It has been shown that AMF treatments drastically increased the concentration of P in the soils by releasing extracellular phosphatase enzymes (Koide & Kabir 2000, Hamel 2004). Phosphorous concentration was correspondingly highest in cowpea plants treated with *A. kentinensis*. Nitrogen was not, however as high as in phosphorus. This could have possibly resulted from the increased amount extracted from the soil by the abundant AMF colonization and a possible explanation for this could be the need for protein formation in the seeds of the cowpea plant and most of it might have been stored in the leaves where plenty of chlorophyll was probably being built up. In a study, the external hyphae of AMF can deliver up to 80% of plant P, 25% of plant N and 10% of plant K (Marschner & Dell 1994). The role of AMF as a bio-fertilizer can potentially strengthen plants' adaptability to changing environments and improve plant nutrition by increasing the availability as well as translocation of various nutrients (Rouphael et al. 2015).

Conclusions

This study has shown that there was significant ($F_{7,11689}$ = 5.75, P< 0.0001) effect of different treatments on mycorrhizal root colonization. There was significant ($F_{7,81.3}$ = 148.13, P < 0.0001) difference between the effect of the various treatments on the soil physicochemical parameters (pH, phosphorus, nitrogen, potassium, organic carbon and moisture content).
Furthermore, the results demonstrate that AMF can significantly contribute to nutrient uptake in Kano State.

Conflict of Interest

There was no conflict of interest on the findings of this study.

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