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IMPACT OF BIOCHAR AND ARBUSCULAR MYCORRHIZAL INOCULATION ON ROOT COLONIZATION AND SELECTED SOIL CHEMICAL PROPERTIES IN SOUTH WESTERN NIGERIA

***[1]Yusif, S. A., [2]Habib, M. Y. and [1]Hayatu, N. G.**
[1]Department of Soil Science and Agricultural Engineering, Faculty of Agriculture,
Usmanu Danfodiyo University, Sokoto. P.M.B. 2346, Sokoto, Nigeria.
[2] Department of Mechanical Engineering, School of Technology,
Kano State Polytechnics, P.M.B 3401, Nigeria.
***Corresponding author**: *sunusi.yusif@yahoo.com; yusif.sunusi@udusok.edu.ng*; +2348068969633

ABSTRACT

The use of biochar and arbuscular mycorrhizal fungi (AMF) provide many opportunities for soil improvement, it is, therefore, important to understand their impact on soil and plant development so as to optimally exploit their potentials. Screenhouse experiment was conducted to evaluate the impact of biochar application and arbuscular mycorrhizal (AM) inoculation on root colonization and selected soil chemical properties. The experiment was laid out in a 2×5×2 factorial, fitted into a completely randomized design with three replications. The factors included tomato genotypes (Ex-Lafia and Ex-Lokoja), biochar application rates (0, 5, 10, 15, and 20 t ha^{-1}) and AMF (with and without AMF). Data were subjected to analysis of variance and significant means were separated using Duncan's Multiple Range Test ($p<0.05$). The results showed that AM inoculation significantly ($p<0.05$) increased root colonization (51.33%) when compared with non mycorrhizal plants (10.17%). However, no significant differences were observed in soil pH, organic carbon and available P between mycorrhizal and non mycorrhizal plants. On the other hand, amendment with the 20t ha^{-1} of biochar recorded significantly ($p<0.05$) higher values of AM root colonization (46.25%), soil pH (7.05) and available P (13.93 mg kg^{-1}) when compared to other biochar rates though comparable with 15 t ha^{-1} in soil pH (7.05) and available P (12.26 mg kg^{-1}). It is therefore concluded that AM inoculation in biochar-amended soil improved root colonization while biochar application enhances root colonization, soil pH and available P.

***Keywords*:** Biochar, AM inoculation, soil chemical properties.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil fungi which colonize roots of most plants (Douds and Millner, 1999). Colonization of roots by AMF enhanced crop productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid and Riaz, 2008). Soil amendments, which increase AMF abundance and/or functionality, could be beneficial to plant hosts (Rillig and Mummey, 2006; Warnock *et al*., 2010). There are many soil amendment technologies to improve soil properties such as chemical fertilizers, organic fertilizers and lime. The potential of biochar as a soil amendment in agricultural fields is a recently recognized and yet it is underutilized technology. Biochar is a carbon (C) rich product derived from the pyrolysis of organic material at relatively low temperatures (Lehmann and Joseph, 2009). Biochar addition in soil increase crop nutrient uptake, reverse SOM decline in agricultural soils, improvement of plant nutrients and creates a suitable condition for soil micro-organisms (Glaser et al., 2002; Lehmann *et al*., 2011; Liang *et al*., 2006; Sohi *et al*., 2009). Despite the high potential of biochar in soil improvement, only very limited information exists. Therefore, this study was carried out to investigate the impact of biochar application and arbuscular mycorrhizal inoculation on root colonization and selected soil chemical properties in the screen house.

MATERIALS AND METHODS

Experimental Site

Screenhouse experiment was conducted at Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, in 2013/2014. The area is located in southwestern Nigeria in the transitional zone. Abeokuta lies between Latitudes 7° 10'N and 7° 15'N and Longitudes 3° 17'E and 3° 26'E (Akinyemi *et al*., 2011). Monthly temperature ranges between 24.9 °C and 31.5°C (Oluwole *et al*., 2009) and has mean annual rainfall of 1156 mm (Aladenola and Adeboye, 2010).

Biochar Production

The biochar was produced from the maize-cobs obtained from Dambatta, Kano State and pyrolized at a temperature of about 350°C to 400 °C (Xu *et al*., 2012). After which the product was ground and sieved with 2 mm laboratory mesh diameter for faster reaction and mineralization. Biochar was incorporated into 5 kg sterilized topsoil two (2) weeks before transplant at the rate of 0, 5, 10, 15, 20 t h^{-1}.

AM Inoculation in the Nursery

The AM inoculants (*Glomus mosseae*) obtained from IITA, Ibadan was inoculated to the soil during the nursery planting at the rate of 80 g of inoculants per 5 kg of sterilized topsoil containing two tomato genotypes (Ex-Lafia obtained from Lafia and Ex-Lokoja obtained from Lokoja) in the greenhouse and another same set was left uninoculated with AM fungi. The nursery was maintained for 4 weeks after which the tomato seedlings were transplanted into the greenhouse for the main experiment. The main experiment was conducted in the greenhouse for three months and laid out in a 2×5×2 factorial arrangement fitted in completely randomized design and replicated three times.

Root and Soil Sampling and Analyses

The tomato roots from each bucket were dug out for AM root colonization studies. Another set of soil samples from each bucket were collected at a depth of about 0-20 cm to evaluate for soil pH, organic carbon and available P. The soil pH was determined in 1:1 soil-water suspension (Bates, 1954), organic carbon by the Walkley-Black oxidation method (Juo, 1979), available P by Bray 1 method (Bray and Kurtz, 1966), Particle size analysis was done using Bouyoucos (1962) hydrometer method. All the analyses were carried out at Soil Science and Land Management Laboratory, FUNAAB. The root samples were stored in 50% ethanol until processing.

AM Root Colonization Study

Approximately 20 root hairs of 1 cm length each were chosen randomly from each tomato plant in each of the buckets for AM colonization studies (Phillips and Hayman, 1970). Root samples were rinsed with 50% ethanol thoroughly and then put in 10% KOH and heated in a water bath for 15 minutes and rinsed. The roots were then stained with a mixture of 1:1:1 of glycerol, lactic acid, and distilled water respectively and then 0.05% methyl blue solution was applied and heated for 5 minutes and then rinsed again. 50% Glycerol was added to preserve the root samples and mounted on compound microscope slides to visualize the fungal structure. AM colonization was done on the basis of the presence or absence of arbuscules, hyphae or vesicles (McGonigle *et al*., 1999) and the percentage was calculated as follows:

$$\text{AM root colonization (\%)} = \left(\frac{\text{Number of roots colonised}}{\text{Total number of roots examined}}\right) * 100$$

Data Analysis

Data obtained from this study were subjected to separate ANOVA using SAS (SAS Institute, 2001) to compute mean squares of each of the experimental treatments. Means were separated using Duncan's Multiple Range Test DMRT at 5% level of significance.

RESULTS

Soil and Biochar Characteristics

Table 1 below showed some selected soil properties, where the soil pH of the study area was found to be 6.8 with 778, 134 and 88 g kg^{-1} of sand, clay and silt respectively. The OC content was 1.48% while available P was recorded to be 10.13 mg kg^{-1}. Biochar was found to have 31.00 mg kg^{-1}, 14.4 %, 1.94% and 10.12 of total P, OC, total N and pH respectively. Biochar was also observed to have 0.022 % and 2.29 % of exchangeable Mg^{2+} and K^{+} respectively (Table 2).

Table 1: Physicochemical properties of the soil used for the study

Parameters	Field soil
pH H_2O (1:1)	6.8
Sand g kg^{-1}	778
Clay g kg^{-1}	134
Silt g kg^{-1}	88
Textural class	Sandy Loam
O C%	1.48
Available P (mg kg^{-1})	10.13

Table 2: Chemical characteristics of biochar used for the study.

Parameters	Biochar
pH H_2O (1:1)	10.12
O C%	14.4
N%	1.94
Total P ($mgkg^{-1}$)	31.00
K %	2.29
Mg %	0.022
Fe %	0.13

AM Root Colonization

AM root colonization was significantly ($p<0.05$) higher in plants inoculated with AM than non-mycorrhizal plants (Table 3). Roots were significantly more colonized with 20 t ha^{-1} of biochar (51.33%) when compared to the other biochar rates (15, 10, 5 and 0 t ha^{-1}) (Table 4). No significant differences were observed in root colonization between the two tomato genotypes (Table 5). The interaction among genotypes, biochar, and AMF for the AM root colonization was not significant (Table 5).

Soil pH,

There was no significant ($P > 0.05$) difference in the values of soil pH between mycorrhizal and non-mycorrhizal pots (Table 3). However, higher soil pH was observed with higher rates of biochar application (15 and 20 t ha^{-1}) which were significantly ($p< 0.05$) higher than the control (0 t ha^{-1}) and 5 t ha^{-1} of biochar rates though 15 t ha^{-1} is comparable with 10 t ha^{-1} (Table 4). The pots for the two tomato genotypes showed no significant difference and interaction among the factors in soil pH (Table 5).

Soil Organic Carbon

There was no significant ($P > 0.05$) difference in soil organic carbon between mycorrhizal and non-mycorrhizal pots (Table 3). Similarly, biochar rates did not significantly affect the amount of organic carbon in the pot experiment (Table 4). The pots for the two tomato genotypes showed no significant difference and interaction among the factors in soil organic carbon (Table 5).

Soil Available P

There was no significant ($P > 0.05$) difference in soil available P between mycorrhizal and non-mycorrhizal pots (Table 3). Similarly, the amount of available P was found to increase with increased biochar application rates with 20 t ha^{-1}producing significantly ($p< 0.05$) higher soil available P when compared to 0, 5 and 10 t ha^{-1} of biochar rates but comparable with 15 t ha^{-1} of biochar (Table 4). The pots for the two tomato genotypes showed no significant difference and interaction among the factors in soil available P (Table 5).

Table 3: Effect of AM inoculation on AM root colonization, soil pH, organic carbon, and available P content in the screen house

AM Inoculation	% AM root colonization	Soil $pH_{(H_2O)}$	% OC	Available P (mg kg^1)
AMF (A)				
+	51.33[a]	6.90[a]	1.20[a]	11.19[a]
-	10.17[b]	6.91[a]	1.26[a]	11.65[a]
SE±	2.09	0.03	0.03	0.4

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at (P<0.05) (-) = Uninoculated,(+) = Inoculated, OC = Organic carbon, P = Phosphorous, SE= Standard error, A = Arbuscular mycorrhizal inoculation, AM = Arbuscular Mycorrhiza.

Table 4: Effect of biochar application on AM root colonization, soil pH, organic carbon, and available P content in the screenhouse

Biochar Application Rates	% AM root colonization	Soil $pH_{(H_2O)}$	% OC	Available P (mg kg^1)
Biochar = (B)				
0	20.00[c]	6.80[c]	1.22[a]	9.84[c]
5	32.92[b]	6.80[c]	1.26[a]	9.69[c]
10	26.25[bc]	6.85[bc]	1.24[a]	11.38[bc]
15	28.33[bc]	7.00[ab]	1.27[a]	12.26[ab]
20	46.25[a]	7.05[a]	1.16[a]	13.93[a]
SE±	3.31	0.05	0.05	0.64

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at (P<0.05) OC = Organic carbon, P = Phosphorous, SE= Standard error, B= Biochar application, AM = Arbuscular Mycorrhiza.

Table 5: Genotype and interaction effects on AM root colonization, soil pH, organic carbon, and available P content in the screenhouse

Genotype/Interaction	% AM root colonization	Soil $pH_{(H_2O)}$	% OC	Available P (mg kg^1)
Genotypes (G)				
Ex-Lafia	30.83[a]	6.95[a]	1.26[a]	11.67[a]
Ex-Lokoja	30.67[a]	6.86[a]	1.20[a]	11.16[a]
SE±	2.09	0.03	0.03	0.4
Interaction				
G*B	ns	ns	ns	ns
G*A	ns	ns	ns	ns
B*A	ns	ns	ns	ns
G*B*A	ns	ns	ns	ns

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at (P<0.05). ns, not significant at P<0.05, OC = Organic carbon, P = Phosphorous, SE= Standard error, AM = Arbuscular Mycorrhiza, A = Arbuscular mycorrhizal inoculation, B= Biochar application, G = Genotype effect.

DISCUSSION

Table 1 above indicated that soil pH of the study area was neutral with loamy sand soil texture. The OC content was found to be moderate, while available P was medium (Enweazor *et al*., 1989). Biochar was found to have high total P, very high OC and N, with very strongly alkaline pH. Biochar was also observed to have very low and very high exchangeable Mg^{2+} and K^+ respectively (Table 2). Higher AM root colonization in plants inoculated with AM (Table 3) agrees with the findings of Gupta *et al.* (2002) who reported that higher AM root colonization was observed with mycorrhizal inoculation in *Mentha arvensis* L. than non-mycorrhizal plants. Similarly, the higher AM root colonization observed with 20 t ha^{-1} of biochar (Table 4) could be attributed to the high nutrient

content of biochar which could have attracted the fungi to colonize the plants. Biochar has been shown to increase mycorrhizal root colonisation and create a microhabitat in soil (Warnock *et al.*, 2007). A research conducted in Western Australia reported an increase in colonisation by mycorrhizal fungi due to biochar addition (Blackwell *et al.*, 2007). Solaiman *et al*. (2010) found that AM colonisation increased significantly in the biochar treatment for wheat grown in well-watered and periodic water stressed treatments.

Higher soil pH was observed with higher rates of biochar application. Soil pH increased with increase in biochar application rates but with little influence by mycorrhizal inoculation (Table 4). This could be attributed to the very strongly alkaline pH of the maize-cob biochar and the tendency to raise soil pH was certain. Qadeer *et al*. (2014) and Yusif and Dare (2016) reported that the overall biochar-amended soil showed more obvious shifts in soil pH as compared to the unamended soil. There was no impact on the effect of biochar on soil organic carbon compared to control (Table 4). This could possibly be attributed to the slow mineralization of biochar and the short duration of the experiment for the effect to manifest. A similar finding was reported by Qadeer *et al*. (2014) who reported that though biochar addition raised the soil organic carbon content of soil as compared to the control, however, the rise was not found statistically significant at ($p > 0.05$). Soil available P was found to increase with increased biochar application rates (Table 4). This could be attributed to higher amounts of total P in the biochar and the higher pH of biochar that could help in breaking the bond of Al and Fe complexes with P in the soil thereby releasing more P into soil solution (Niguissie *et al*., 2012). A similar increase in phosphorus was observed in many studies by application of biochar (Cheng *et al.*, 2006; Glaser *et al.*, 2002; Lehmann *et al.*, 2003; Qadeer *et al*., 2014).

Conclusion and Recommendations

It is therefore concluded that AM inoculation in biochar-amended soil improved root colonization while biochar application enhanced AM root colonization, soil pH and available P. However, the shift in soil pH was not high enough to cause havoc on soil properties. It is, therefore, recommended that a field experiment should be conducted to ascertain the interaction effect more effectively.

REFERENCES

Akinyemi, O.D., Bello, R., Ayodeji, A.T., Akanbi, D.E., Ibine M.M. and Popoola, J.A. (2011). Evaluation of water quality in Abeokuta, Southwest Nigeria. *International Journal of Water Resources and Environmental Engineering*, 3(13): 341-369. ISSN 1991-637X. DOI: 10.5897/IJWREE11.099

Aladenola, O.O. and Adeboye, O.B. (2010). Assessing the potential for rainwater harvesting. *Water Resource Management*, 24(10): 2129-2137.

Al-Garni, S.M.S., (2006). Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer Rhizobium. African *J. Biotech*., 5: 133– 142

Bates, R.C. (1954). Electrometric pH determination. John Wiley and Sons Inc., New York. Pp. 35-38.

Blackwell, P., Shea, S., Storer, P., Kerkmans, M. and Stanley, I. (2007). Improving wheat production with deep banded Oil Mallee charcoal in Western Australia. In: 'The 1st International Agriculture Conference, Terringal NSW'.

Bouyoucos, G.S. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal*, 54: 464-465.

Bray, R. and Kurtz, L. T. (1966). Determination of total, organic and available forms of phosphorus in soil. *Soil Science*, 59: 39–45.\

Cheng, C.H., Lehmann, J., Thies, J.E., Burton, S.D. and Engelhard, M.H. (2006). Oxidation of blackcarbon by biotic and abiotic processes. *Org. Geochem*., 37: 1477–1488.

Douds, D.D. and Millner P. (1999). Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture, Ecosystems and Environment*, 74: 77-93.

Glaser, B., Lehmann, J., Steiner, C., Nehls, T., Yousaf, M. and Zech, W. (2002). Potential of pyrolyzed organic matter in soil amelioration. In:*International Soil Conservation*

Organization Conference, Beijing, China, vol. III, pp. 423-527.

Gupta, M.L., Prasad, A., Ram, M. and Kumar, S. (2002). Effect of the vesicular arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition with the crops of different cultivars of menthol mint (Menth *arvensis*) under field condition. *Bioresources Technology*, 81: 77-79.

Enweazor, W.O., Udo, E.J., Usoroh, N.J., Ayotade, K.A., Adepetu, J.A., Chude, V.O., Udegbe CI (eds) (1989). Fertilizer use and management practices for crops in Nigeria. Series no. 2. Federal Ministry of Agric, Water Resources and Rural Development, Lagos. Pp 163.

Javaid, A. and Riaz, T. (2008). Mycorrhizal colonisation in different varieties of Gladiolus and its relation with plant vegetative and reproductive growth. *Int. J. Agric. Biol.*, 10: 278–282.

Juo, A.S.R. (1979). Selected methods of soil and plant analysis, *IITA Manual series* No. 1, 70p.

Khaosaad, T., J.M. Garcia-Garrido, S. Steinkellner and H. Vierheilig, 2007. Take-all disease is systematically reduced in roots of mycorrhizal barley plants. *Soil Biol. Biochem*., 39: 727–734.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. and Crowley, D. (2011). Biochar effects on soil biota – a review. *Soil Biol Biochem*, 43:1812–1836.

Lehmann, J. and Joseph, S. (2009). Biochar for Environmental Management. Science and Technology. Earthscan, London & Sterling, VA. 416p.

Lehmann, J., Pereira da Silva, J., Steiner, C., Nehls, T., Zech, W. and Glaser, B. (2003). Nutrient availability and leaching in an archaeological *Anthrosol* and a *Ferralsol* of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil*,249(2): 343-57.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J. and Neves, E.G. (2006). Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal*, 70:1719-1730.

McGonigle, T.P. and Miller, M.H. (1999). Winter survival of extraradical hyphae and spores of arbuscular mycorrhizal fungi in the field. *Applied Soil Ecology*, 12 (1): 41-50.

Nigussuie, A., Risson, E., Misganaw, M. and Anbanss, G. (2012). Effect of biochar application on soil properties and nutrient uptake of lethieo (*Lactuca sation*) grown in chromium polluted soils. American – *Eurasian Journal of Agricultural and Environmental Sciences*, 12 (3): 369-375.

Oluwole, A.S., Ekpo, U.F., Mafiana, C.F., Adeofun, C.O. and Idowu, O.A. (2009). Preliminary study on temporal variations in biting activity of *Simulium damnosum* S.L. in Abeokuta North LGA, Ogun State Nigeria.*Parasites and Vectors*. DOI: 10.1186/1756-3305-2-55.

Phillip, J. and Hayman, D. (1970). Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55 (1): 158-61.

Qadeer, S., Batool, A., Rashid, A., Khalid, A., Samad, N. and Ghufran, M.A. (2014). Effectiveness of biochar in soil conditioning under simulated ecological conditions. *Soil Environment*, 33(2):149-158.

Rillig, M.C. and Mummey, D.L. (2006). Mycorrhizas and soil structure. *New Phytolo*.171: 41-53.

SAS Institute. (2001). Statistical analysis software (SAS). User's guide. SAS Institute Cary, NC. 85pp.

Sohi, S. P., LoPez-capel, E., Krull, E., and Bol, R. (2009). Biochar's roles in soil and climate change: a review of research needs. Csiro land and water science report 05/09.

Solaiman, Z.M., Sarcheshmehpour, M., Abbott, L.K. and Blackwell, P. (2010). Effect of biochar on arbuscular mycorrhizal colonisation, growth, P nutrition and leaf gas exchange of wheat and clover influenced by different water regimes. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World, 1 – 6 August 2010, Brisbane, Australia.

Warnock, D.D., Lehmann, J., Kuyper, T.W. and Rillig, M.C. (2007). Mycorrhizal responses to biochar in soil – Concepts and mechanisms. *Plant and Soil*, 300:9–20.

Warnock, D.D., Mummey, D.L., McBride, B., Major, J., Lehmann, J. and Rillig, M.C. (2010) Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Appl Soil Ecol*., 46:450–456.

Xu, Z.R., Zhu, W. and Htar, S.H. (2012). Partial oxidative gasification of municipal sludge in subcritical and supercritical water. *Environment Technology*, 33 (10-12): 1217-1223.

Yusif, S.A and Dare, M.O. (2016). Effect of biochar application and arbuscular mycorrhizal inoculation on root colonization and soil chemical properties. International Annals of Science, 1(1): 33-38. DOI: https://doi.org/10.21467/ias.1.1.33-38.