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The Roles of Biochar and Arbuscular Mycorrhizal Inoculation on Selected Soil Biological Properties and Tomato Performance

*[a]Yusif, S. A., [b]Dare, M. O. [b]Babalola, O. A., [c]Popoola, A. R., [a]Sharif, M. R. and [d]Habib, M. Y.
[a]Department of Soil Science and Agricultural Engineering, Faculty of Agriculture
Usmanu Danfodiyo University, Sokoto, Nigeria.
[b]Department of Soil Science and Land Management, College of Plant Science and
Crop Production, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.
[c]Department of Crop protection, College of Plant Science and Crop Production
Federal University of Agriculture Abeokuta, Ogun State, Nigeria.
[d]Department of Mechanical Engineering, School of Technology
Kano State Polytechnics, Nigeria.
*Correspondence email: yusif.sunusi@udusok.edu.ng

Abstract

Field experiment was conducted to investigate the effects of biochar application and arbuscular mycorrhizal (AM) inoculation on selected soil biological characteristics, shoot P and fruit yield of two tomato genotypes. Result indicated that mycorrhizal inoculation significantly ($p< 0.05$) increased shoot P (0.72 g P kg^{-1}) and number of AM spores (47.90 spores / 25 g soil) compared to non-mycorrhizal plants, but no significant difference was observed in tomato fruit yield, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). Application of 20 t ha^{-1} of biochar significantly ($p< 0.05$) increased tomato fruit yields and number of AM spores when compared with the control. However, 10 and 15 t ha^{-1} of biochar rates gave comparable performance as 20 t ha^{-1} of biochar in most cases. No significant differences were observed in shoot P, MBC and MBN among biochar rates. Thus, AM inoculation enhanced P nutrition while biochar improved AM spores abundance as well as tomato fruit yields. The 20 t ha^{-1} of biochar could be used to improve AM spores abundance as well as tomato fruit yields.

Keywords*:* Biochar; AM Fungi; Biological Properties; Tomato Performance

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) as social microorganisms which form a symbiotic relationship with majority of plant roots such as tomato and sorghum plants that have high colonization potentials (Mwangi *et al.*, 2011). AMF are specialized soil fungi that benefit many important crops and require an association with vascular plants to survive. Plants allow AMF to live within their roots and provide the fungi with sugars (carbohydrate) that enable them to grow. In exchange, the fungi provide the plant with additional water and nutrients that plant could not otherwise extract from the soil which can lead to improved plant health and yield (Siemering *et al*., 2016). The presence of AMF in soils may be crucial for sustainable agriculture (Gianinazzi *et al*. 2010). Their role in cycling of nutrients,

increase in the efficiency of fertilizers, enhancing plant growth and yield of crops created a ground for AM fungal application as biofertilizer (Bhardwaj *et al*., 2014; Schwartz *et al*., 2006).

AMF inoculation promotes aggregate stability of soils, which in turn increases nutrients and cation exchange capacity of soils due to increased organic matter turn over. This aggregation permits the soil to retain water better and facilitate root penetration. In addition, the aggregates reduce soil erosion and compaction while facilitating root hair adhesion, enhancing nutrient and water uptake by plants (Pal and Pandey, 2014).

Enhanced uptake of P is generally regarded as the most important benefit that AMF provide to their host plant (Smith and Read, 1997). AMF functionality can be enhanced by addition of soil amendments such as compost, poultry droppings and biochar (Warnock *et al*., 2010). Research has examined biochar's effect on arbuscular mycorrhizal (AM) symbioses that enhance biological characteristics, performance and plant nutrient uptake (Warnock *et al.* 2007, 2010).

Soil microbial communities' composition and abundance also change with biochar application (Liang *et al.,* 2010; Jin, 2010), because biochar can serve as refuge for AMF and protect them from fungal grazers (Warnock *et al*., 2007), thus enhancing plant host-fungus symbiosis. Biochar amendments have been shown to increase microbial biomass due to the presence of labile C fractions (Luo *et al.,* 2013).

Despite the potential usefulness of biochar and AMF for soil management applications, there is lack of information about how these materials influence soil biological properties and tomato performance. Therefore, this study aims to determine the roles of biochar and AMF on soil microbial biomass carbon and nitrogen, AM spore abundance, shoot P and fruit yield.

MATERIALS AND METHODS

Experimental Site, Biochar Application and AMF Inoculation in the Field

The experiment was carried out at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (Latitude $7^{\circ}12^{l}$ N and Longitude 3° $20^{,}$ E), Ogun State, Nigeria, in 2013 / 2014. Average monthly temperature ranges between 24.9 $^{\circ}$C and 31.5°C (Oluwole *et al*., 2009) and has mean annual rainfall of 1156 mm (Aladenola and Adeboye, 2010).

The biochar was produced using maize-cob by gasification method with a temperature of about 300 $^{\circ}$C to 400 $^{\circ}$C (Xu *et al*., 2012). It was ground and then sieved with 2 mm mesh diameter and incorporated two weeks before transplant (WAT) at rates of 0, 5, 10, 15, 20 t h^{-1}. The AMF inoculants (*Glomus mosseae*) obtained from IITA, Ibadan was inoculated to the soil during the nursery planting at the rate of 80 g per 5 kg of sterilized top soil and the other left un-inoculated for each of the two tomato genotypes (Ex-Lafia sourced from Lafia and Ex-Lokoja sourced from Lokoja).

The total plot size was 315 m^2 with the sub-sub plot size of 2 m × 1 m, a spacing of 1 m between and within sub-sub plots. The plant spacing was 30 cm between and within the plant stands consisting of eighteen plants per sub-sub plot. The field layout was split- split plots design with two tomato genotypes in the main plots, five biochar rates of application in the sub plots and two levels of AMF inoculation (with and without) in the sub-sub plots. Weeding was done manually at 4 and 8 WAT while cypermethrin (insecticide) was applied at 3, 6, and 9 WAT at the rate of 450 ml of active ingredients to 100 litres of water per hectare of land using knapsack sprayer.

Soil and Shoot Sampling

Soil samples from six different points at 0-20 cm in each sub-sub plot in the field were collected and made into composite samples to evaluate for MBC, MBN and AM spores abundance. The soil samples were stored in the refrigerator at 4°C until processing. Shoots from three tomato plants were collected at 7 WAT, air dried after recording the fresh weight and then oven-dried at 60 °C for 24 hours and the average weights recorded. The samples were then ground for shoot P determination. Fully riped tomato fruits from three tomato plants at the middle row were harvested and average weight recorded in each sub-sub plot.

Laboratory Analyses

Shoot P was determined by the vanadomolybdate-yellow method (Motomizu *et al.*, 1983) at Microbiology Laboratory, Institute of Agricultural Research and Training (IAR & T), Ibadan, Nigeria. The MBC and MBN were determined using a 10 g soil sample sieved to 2 mm particle size by the Chloroform-Fumigation extraction methods (Brookes *et al*., 1985; Vance *et al*., 1987)

Extraction and Identification of AMF Spores from Soil

The AMF spores were extracted from 25 g air dried soil samples using wet sieving and decanting method, followed by sucrose density gradient centrifugation method (Daniels and Skipper, 1982). Soil sample was put over a series of soil sieves arranged in descending order of mesh diameter, i.e. 450, 100, 53 µm mesh sieve sizes. The spores retained on the 100 and 53 µm mesh along with fine soil particles were washed and centrifuged at 2500 rpm for 2 minutes and the supernatant discarded. Sucrose solution (40%) was introduced into the sediment, stirred thoroughly and then centrifuged at 2500 rpm for 2 minutes. The soil particles settled at the bottom and the spores floated at the interface of the sucrose solutions. The clean spores were extracted by placing the sucrose layer into a clean 53 µm mesh sieve and washed several times with distilled water before being transferred into water in a clean Petri-dish. The AMF spores were counted under stereomicroscope at a magnification of x40 as reported by Dare *et al*. (2013) and identified morphologically based on spore colour, shape, hyphal attachment and the number was recorded per 25 g of soil.

Data Analysis

Data obtained from this study were subjected to separate ANOVA (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 AND DISCUSSION

Properties of Soil and Biochar Used For the Study

The chemical properties of the soil (Table 1) showed that pH was neutral with sandy loam soil texture. The MBC and MBN contents were moderate, medium in available P with very low number of AM spores (Enweazor, 1989). In Table 2, biochar was found to have high total P, very high OC, N, with very strongly alkaline pH. Biochar was also observed to have very low and very high exchangeable Mg2+ and K+, respectively (Enweazor, 1989).

Shoot P and Fruit Yields

Mycorrhizal plants recorded significantly ($p<0.05$) higher shoot P compared to non-mycorrhizal plants (Table 3) but had no significant effect on tomato fruit yields. The result agrees with the findings of Al-Karaki *et al.* (1998) who reported higher shoot P with mycorrhizal than non-mycorrhizal wheat plants (*Triticum aestivum* L.). Biochar application rates didn't increase shoot P but fruit yields of tomato plants increased with increasing rate of biochar application (Table 4). The biochar rate of 20 t ha^{-1} produced significantly ($p<0.05$) higher fruit yields when compared to the control and 5 t ha^{-1} of biochar. However, tomato fruit yield due to 20 t ha^{-1} of biochar application rate did not show significant differences with 10 and 15 t ha^{-1} of biochar application rates in fruit yields of tomato. This could be attributed to higher supply of nutrients as a result of higher rates of biochar application. Increases in yield as a result of biochar application were reported by Suppadit *et al*. (2012) and Chan *et al*. (2008) who found that biochar significantly increased yield with increased levels of biochar application. Significant interaction was observed between genotype and biochar in shoot P of tomato plant (Table 5).

AMF Spore, Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN)

Number of AM spores was significantly ($P<0.05$) higher in mycorrhizal plots when compared with the non-mycorrhizal plots (Table 3). This indicated an increase of 77.41 % (47.90) in the number of AM spores by AM inoculated when compared to the initial value of 27 AM spores (Table 1) obtained prior to the experiment. However, MBC and MBN were found not significantly different between mycorrhizal and non-mycorrhizal plots (Table 4). Plots assigned to Ex-Lokoja genotype had significantly higher number of AM spores when compared with plots assigned to Ex-Lafia genotype (Table 5). It was observed that number of AM spores increased with increasing quantity of biochar applied as shown in Table 4. This indicated an increase of 103.07% (54.83) in the number of AM spores by 20 t ha^{-1} of biochar application rate when compared to the initial value of 27 AM spores (Table 1) obtained prior to the experiment. Similar findings have been reported by Harvey *et al.* (1976) who reported that application of biochar have positive effects on abundance and colonization of host plant by AMF and thus promotes plant growth. Higher number of AM spores in biochar amended soils could also be attributed to the high organic carbon content of biochar which is a major substrate for AM fungi. Ishii and Kadoya (1994) also provided evidence that biochar have positive effects on the abundance of mycorrhizal fungi.

CONCLUSION AND RECOMMENDATIONS

AM inoculation enhanced P nutrition while biochar improved AM spores abundance as well as tomato fruit yields. The 20 t ha^{-1} of biochar could be used to improve AM spores abundance as well as tomato fruit yields. It is, therefore, recommended that AMF inoculation in biochar amended soil is helpful particularly for improving P nutrition and AM spore abundance. A long-term evaluation of the effect of biochar and AMF on P nutrition and soil biological characteristics is required for clarity of the effect.

Table 1: Physicochemical and Biological Properties of the Soil Used for the Study

Parameters	Values
pH H_2O(1:1)	6.8
Sand %	77.8
Silt %	8.8
Clay %	13.4
Textural class	Sandy loam
Available P (mg kg^{-1})	10.13
MBC (µg g^{-1})	293.59
MBN (µg g^{-1})	135.75
Number of AM spores per 25 g dry soil	27

Table 2: Chemical Characteristics of Biochar Used for the Study.

Parameters	Values
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

Table 3: Shoot P, Fruit Yields, Number of AM Spore, MBC and MBN in Inoculated and Uninoculated Soils.

Parameters	Shoot P g kg^{-1}	Fruit yield t ha^{-1}	Number of AM spore 25 g	MBC µg g^{-1}	MBN µg g^{-1}
Inoculated	0.72^a	11.69^a	47.90^a	324.08^a	138.97^a
Uninoculated	0.51^b	10.22^a	34.56^b	298.84^a	140.50^a
SE±	0.05	0.93	2.51	20.10	2.91

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P<0.05$), MBC, Microbial Biomass Carbon; MBN, Microbial Biomass Nitrogen; P, phosphorus; SE±, Standard error.

Table 4: Effect of Biochar Application (B) on Shoot P, Fruit Yields, Number of AM Spore, MBC and MBN.

Parameters	Shoot P g kg^{-1}	Fruit yield t ha^{-1}	Number of AM spore 25 g	MBC µg g^{-1}	MBN µg g^{-1}
0 t/ha	0.51^{a}	8.50^{b}	28.08^{c}	335.80^{a}	142.28^{a}
5 t/ha	0.60^{a}	7.93^{b}	33.83^{bc}	273.86^{a}	139.44^{a}
10 t/ha	0.65^{a}	12.27^{ab}	44.18^{ab}	299.14^{a}	145.61^{a}
15 t/ha	0.60^{a}	12.32^{ab}	45.00^{ab}	329.59^{a}	137.15^{a}
20 t/ha	0.71^{a}	13.77^{a}	54.83^{a}	318.91^{a}	134.20^{a}
SE±	0.08	1.47	3.97	31.79	21.11

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at ($P<0.05$), MBC, Microbial Biomass Carbon; MBN, Microbial Biomass Nitrogen; P, phosphorus; SE±, Standard error.

Table 5: Genotype (G) and interaction effect on shoot P, fruit yields, Number of AM spore, MBC and MBN.

Parameters	Shoot P g kg^{-1}	Fruit yield t ha^{-1}	Number of AM spore	MBC µgC g^{-1}	MBN µgN g^{-1}
Genotype (G)					
Ex-lafia	0.54^{a}	10.34^{a}	37.20^{b}	338.97^{a}	138.83^{a}
Ex-lokoja	0.69^{a}	11.57^{a}	45.27^{a}	283.95^{a}	140.64^{a}
SE±	0.05	0.93	2.51	20.10	2.91
Interaction					
G*B	*	ns	ns	ns	ns
G*A	ns	ns	ns	ns	ns
B*A	ns	ns	ns	ns	ns
G*B*A	ns	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 ($P<0.05$), MBC, Microbial Biomass Carbon; MBN, Microbial Biomass Nitrogen; P, Phosphorus; *, Significant and; ns, Not significant at $p<0.05$; SE±, Standard error.

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