INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–149/AWB/2012/14–6–965–969
http://www.fspublishers.org

Full Length Article

Effect of Arbuscular Mycorrhizal Fungal Inoculation and Biochar Amendment on Growth and Yield of Tomato

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ABSTRACT

A field study was conducted to investigate the interactive effects of pre-inoculation of *G. mosseae* and soil amendment with biochar on AMF root colonisation, plant growth, fruit yield and nutrient uptake of tomato (*Solanum lycopersicum* L.). A 2 × 2 factorial experiment arranged in a randomised complete block design included two *G. mosseae* treatments (inoculated at sowing or uninoculated) and two biochar levels (5 t ha^{-1} or unamended) with six replications. At mid-season, 12 weeks after transplanting, biochar addition did not increase the percentage of AMF root colonisation on tomato plants. Pre-inoculation with *G. mosseae* increased dry shoot weight and total plant weight by 11 and 9% respectively, whereas biochar amendment decreased dry root weight by 13%. Generally, pre-inoculation with *G. mosseae* and biochar did not affect leaf Ca, B, Cu, Mn, Na or Zn but lowered leaf P by 26% when compared to the uninoculated plants. Pre-inoculation with AMF and biochar addition did not affect tomato growth variables, yield or yield components. Results of this study did not demonstrate any benefit of combined application of AMF and biochar on the overall performance of tomato plants.

Key Words: Arbuscular mycorrhiza; Biochar; Nutrient uptake; Pre-inoculation; Tomato

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil fungi, which colonise the roots of most plants (Douds & Millner, 1999). These fungi form mutualistic relationships with more than 80% of terrestrial plants (Ulrich *et al*., 2002) and provide the host with mineral nutrients in exchange for carbohydrates (Tahat *et al*., 2008; Javaid, 2009). Generally, plants inoculated with AMF are more efficient in nutrient and water acquisition, thus resulting in an improved plant growth (Koide, 1991; Oseni *et al*., 2010). Colonisation of roots by AMF has also been shown to enhance crop productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid & Riaz, 2008). In tomato, AMF are widely used to improve plant growth and health (Oseni *et al*., 2010). However, even with nursery inoculation with AMF or field application, tomato plants exhibit low root mycorrhizal colonisation. Low AMF colonisation in field grown plants has been variously attributed to (i) use of unsuitable strains, (ii) relatively high available soil P (iii) cultural practices and (iv) microbial competition in the rhizosphere (Strzemska, 1975; Jasper *et al*., 1989).

Soil amendments, which increase AMF abundance and/or functionality, could be beneficial to plant hosts (Rillig & Mummey, 2006; Warnock *et al*., 2010). Biochar (biomass-derived black carbon) can serve as refuge for AMF hyphae and protect them from fungal grazers (Warnock *et al*., 2007), thus enhancing plant host- fungus symbiosis. Ishii and Kadoya (1994) argued that additions of biochar altered soil physico-chemical characteristics, leading to increased soil nutrient availability and enhanced mycorrhizal root colonisation. Similarly, Saito (1990) observed an increase of more than 300% in mycorrhizal root colonisation in field grown soybean. According to Lehmann *et al*. (2003), biochar addition can improve plant productivity directly as a result of its nutrient content and release characteristics, or indirectly, through improved nutrient retention. Since AMF and biochar can both improve crop performance, there is an increasing interest in understanding their potential synergisms in increasing mycorrhizal root colonisation and improving overall tomato crop performance. The objective of this study was to investigate the effects of AMF-inoculated plants and biochar-amended soil on mycorrhizal root colonisation, nutrient content, growth and yield of tomato.

MATERIALS AND METHODS

Study location and experimental design: The study was conducted under field conditions at the commercial farm,

To cite this paper: Nzanza, B., D. Marais and P. Soundy, 2012. Effect of arbuscular mycorrhizal fungal inoculation and biochar amendment on growth and yield of tomato. *Int. J. Agric. Biol*., 14: 965–969

ZZ2–Bertie van Zyl (Pty) Ltd, in Mooketsi, South Africa during the 2010 growing season. The site is located at 23º 65' 17 S latitude, 30º 06' 89 E latitude, at a 772 m above sea level. During the growing season, the total precipitation was 354.78 mm; the mean monthly minimum and maximum temperatures were 16.4ºC (range 15.4–21.3ºC) and 26.8ºC (range 24.9–28.6ºC). The soil is a predominantly sandy loam with 7 mg kg^{-1} P, 202 mg kg^{-1} K, 194 mg kg^{-1} Mg, 731 mg kg^{-1} Ca and pH of 4.9. The mycorrhizal spore propagules of the site were less than 1 kg^{-1} soil. The soil was not fumigated prior to experimentation.

The four treatment combinations (2 AMF×2 biochar), M_1B_1 (AMF inoculated seedlings with biochar amended soil), M_1B_0 (AMF inoculated seedlings without biochar), M_0B_1 (uninoculated seedlings with biochar) and M_0B_0 (untreated/control), were arranged in a randomized complete block design with six replicates.

Plant culture: Tomato seedlings (Cv. Nemo-Netta), either pre-inoculated with commercial inoculum Biocult© containing spores of *Glomus mossae*, or uninoculated, were supplied by Hishtill nursery, Mooketsi, South Africa. Pre-inoculated AMF seedlings had less than 15% mycorrhizal root colonisation, whereas uninoculated seedlings had no colonisation. Where applicable, biochar was added to the transplanting hole (30 cm depth) at planting at a rate of 500 g/hole corresponding of 5 t ha^{-1} (Hossain *et al*., 2010).

Four-week-old tomato seedlings were transplanted into two rows on raised bed, with 30 cm, 180 cm intra and inter row spacing. Each plot was 20 m in length×1.8 m in width (36 m^2). A standard fertiliser programme was used as side dressing, accounting for N (200 kg ha^{-1}), P (37 kg ha^{-1}), and K (300 kg ha^{-1}), using drip irrigation. Irrigation was scheduled using evapotranspiration rate of the plants. Standard cultural practices for tomato production were applied throughout the season. Scouting for pest and disease damage was done throughout the trial. Whiteflies and aphids were controlled by drenching the plants with Actara® as per the company's standard procedures. Biomectin® was applied to suppress leafminer infestation at the rate of 0.6 L ha^{-1}, whereas copper and mancozeb© were used for suppressing bacterial and fungal diseases, respectively.

Biochar production: Biochar was produced at the Natuurboerdery Research Center in Mooketsi, South Africa from *Eucalyptus globolus* trees. The trees were cut down, chipped and pyrolysed in a fixed bed reactor. The pyrolysis temperature was maintained at 450ºC for 1 h. Physical and chemical characteristics of biochar are shown in Table I.

Data collection: Twelve weeks after transplanting, three randomly selected plants per replicate were pulled out of the soil, gently washed free from the soil. Roots of selected tomato plants were stained with trypan blue in lactophenol (Phillips & Hayman, 1970) and quantified for percentage of AMF colonisation using the grid line-intersect method (Brundrett *et al.*, 1996). Shoots and the remaining roots were oven-dried at 65ºC for 72 h to determine dry weight.

Leaves were dried and ground to pass through a 1 mm sieve chemical analysis. A 1.0 g sample of ground leaves was digested in H_2S0_4 at 410°C and N content were determined by an auto analyser. Other essential nutrient elements (K, Ca, P, Fe, B, Zn, Cu, Mn & B) were digested with a 2:1 nitric/perchloric acid mixture at 230°C and nutrient elements determined by the inductive coupled plasma (ICP).

Table I: Chemical and physical characteristics of biochar

Parameters	Biochar
Total C	338 g kg^{-1}
Total N	3.7 g kg^{-1}
pH (H_20)	7.6
Moisture content	3.5%
Ash content	3.3%
P-Bray 2	84.7 mg kg^{-1}
Total S	43 mg kg^{-1}
Total Mg	0.7 g kg^{-1}
Total B	8.45 mg kg^{-1}
CEC	9.3 $mmol_c$ kg^{-1}
Bulk density	560

Tomato fruit were harvested from 12 weeks after transplanting and continued for ten successive weeks, with two harvests per week. At each harvest, fruit were picked, weighed and total yield was determined. The marketable yield was calculated as the total number of fruits per plant (total yield) minus unmarketable fruit (defects, disease or physiological disorders).

Statistical analysis: Data were subjected to two-way analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA, 2002-2003). Mean separation was achieved using Fisher's least significant difference test. Unless stated otherwise, treatments discussed were different at 5% level of probability.

RESULTS

Growth parameters and mycorrhizal root colonisation: There was a significant main effect of AMF pre-inoculation on dry shoot weight and total plant weight. The main effect of biochar was only significant for dry root weight. The interaction of AMF inoculation × biochar amendment was not significant. Regardless of biochar amendment, AMF inoculation increased the shoot dry weight and total plant biomass by 11 and 9%, respectively. Biochar amendment decreased the root dry weight by 13%. Tomato plant height and root length were not affected by any treatment (Table II). Root colonisation of AMF was 15%, with or without biochar addition, whereas uninoculated seedlings roots had no mycorrhizal colonisation (Table III).

Yield and yield components: The yield and yield components of tomato were not affected significantly by AMF inoculation×biochar amendment interaction. However, inoculating seedlings with AMF and simultaneously transplanting with biochar (M_1B_1) increased the marketable fruit, marketable yield and total yield by 4, 5 and 8%,

Table II: Growth variables of tomato as influenced by AMF pre-inoculation (M_0 no pre-inoculation, M_1 AMF) and biochar amendment (B_0 no amendment, B_1 biochar)

Response variable	Plant height (cm)	Root length (cm)	Dry shoot weight (g)	Dry root weight (g)	Total plant weight (g)
AMF inoculation					
M_0	149.88±5.32	59.18±5.54	10.60b±0.92	2.05±0.14	12.65b±0.88
M_1	148.60±7.38	61.19±3.91	11.87a±1.34	2.00±0.37	13.87a±1.29
Biochar addition					
B_0	150.11±6.37	58.37±4.89	10.85±1.57	2.15a±0.29	13.00±1.58
B_1	148.37±6.44	61.99±4.13	11.62±0.84	1.90b±0.20	13.52±0.77
ANOVA					
M	ns	ns	*	ns	*
B	ns	ns	ns	*	ns
M×B	ns	ns	ns	ns	ns

Means followed by the same letter were not significantly different (P≤0.05) according to Fisher's LSD test
ns, *, are levels of significance (not significant, P≤0.05, respectively)

Table III: Mean yield and yield components of tomato as influenced by AMF pre-inoculation (M_0 no pre-inoculation, M_1 AMF) and biochar amendment (B_0 no amendment, B_1 biochar)

Response variable	Marketable fruit	Early yield (kg/plant)	Total yield (kg/plant)	Marketable Yield (kg/plant)	AMF %
M_0B_0	89.91±13.37	1.73±0.43	7.16±0.95	6.10±0.95	-
M_0B_1	83.04±20.16	1.59±0.45	7.04±1.61	6.13±1.33	-
M_1B_0	92.85±11.86	1.82±0.40	7.69±0.65	6.57±0.64	15
M_1B_1	94.78±16.12	1.72±0.60	7.47±1.47	6.45±1.32	15

Table IV: Leaf nutrients content of tomato as influenced by AMF pre-inoculation (M_0 no pre-inoculation, M_1 AMF) and biochar amendment (B_0 no amendment, B_1 biochar)

Response variable	K (%)	Ca (%)	N (%)	Fe (ppm)	B (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
AMF inoculation								
M_0	2.73±0.33	1.93±0.42	4.05±0.19	333.50b±31.5	28.67±2.99	37.42±8.37	191.25±69.00	127.17±29.23
M_1	2.70±0.21	2.03±0.46	4.10±0.10	397.82a±83.29	30.08±3.78	34.00±4.47	255.75±92.14	150.67±37.12
Biochar addition								
B_0	2.83a±0.30	2.04±0.52	4.05±0.14	381.42±75.25	29.33±4.07	37.33±7.89	217.50±93.56	136.33±40.24
B_1	2.60b±0.17	1.92±0.34	4.10±0.17	349.90±59.42	29.42±2.78	34.08±5.33	229.50±81.80	141.50±30.00
ANOVA								
M	ns	ns	ns	*	ns	ns	ns	ns
B	*	ns	ns	ns	ns	ns	ns	ns
M×B	ns	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letter were not significantly different (P≤0.05) according to Fisher's LSD test.
ns, *, are levels of significance (not significant, P≤0.05, respectively).

respectively. Uninoculated seedlings combining with biochar added (M_0B_1) reduced the total number of tomato fruits and marketable fruit by 8% each and decreased both early and total yields of tomato by 9% (Table III).

Leaf chemical analysis: There was no significant effect of either AMF inoculation or biochar amendment on leaf N, Ca, B, Cu, Mn or Zn contents of tomato fruits. Regardless of the seedlings status, amending soil with biochar (B_1) resulted in 9% decreases in leaf K content of tomato as compared to the control (B_0). A significant increase in Fe content (16%) was obtained with AMF inoculated seedlings (M_1) as compared to uninoculated plants plots (M_0) (Table IV).

Growing AMF-inoculated seedlings with biochar (M_1B_1) or without (M_1B_0) resulted in 26 and 29% decreases in leaf P content, respectively. Similarly, uninoculated seedlings with biochar added (M_0B_1) showed a decrease of about 32% in leaf P content as compared to the uninoculated seedlings grown without biochar amendment (Table V).

Table V: Leaf P content of tomato as influenced by interactive effect of AMF pre-inoculation (M_0 no pre-inoculation, M_1 AMF) and biochar amendment (B_0 no amendment, B_1 biochar)

Parameter	P (mg.kg^{-1})			
	Biochar addition			
AMF inoculation	B_0	R-E (%)	B_1	R-E (%)
M_0	0.45a		0.31b	-32
M_1	0.32b	-29	0.33b	-26

Means followed by the same letter were not significantly different (P≤0.05) according to Fisher's LSD test
R-E: Relative effect (1±treatment/control) x 100

DISCUSSION

The addition of biochar to the planting hole of AMF-inoculated tomato seedlings did not increase the percentage of root colonisation, growth, yield or yield components of tomato plants. However, this combination influenced leaf P

content. The effects of biochar addition to soil on root colonisation by AMF have been contradictory. Ishii and Kadoya (1994) observed increased percentage of root colonised by AMF on citrus. Wallstedt *et al*. (2002) argued that biochar could reduce root mycorrhizal colonisation by decreasing nutrient availability or creating unfavorable nutrient ratios in soils. In this study, biochar had no effect on the mycorrhizal colonisation rate probably due to four reasons: (i) low seedlings mycorrhizal colonisation (< 15%) before transplanting (ii) soil disturbance during production, (iii) the use of synthetic fertilisers, especially P and (iv) the application of pesticides, more especially copper based products, which were used for the control of bacterial diseases. All these factors have been correlated with low mycorrhizal root colonisation in field production (Martin *et al*., 2007).

In this study, biochar had no positive effect on yield or yield components with or without AMF inoculation. Similarly, Graber *et al*. (2010) did not find any effect of biochar on the number of flowers or fruit yield of tomato grown in a soilless medium. However, Steiner *et al*. (2007) observed increased yield in rice and sorghum with an application of 11 t ha^{-1} biochar over two years in an oxisol in Brazil. Similar results were obtained for maize by Kimetu *et al*. (2008) following three repeated applications of 7 t ha^{-1} of biochar over two growing seasons in Kenyan soils cropped to maize for up to 100 years. Even with 20 t ha^{-1} biochar applied, Major *et al*. (2010) found only a significant yield response in maize in the subsequent cropping year. Despite the clear evidence that increased yield is usually observed in subsequent years, some authors found positive results in the first year. For instance, in cherry tomato, Hossain *et al*. (2010) reported a 20% yield increase with combined biochar and fertiliser. In their studies, they used a low pH chomosol with 10 t ha^{-1} of biochar applied. The absence of a clear yield increase in our study could partly be attributed to the soil used (acid), application rate (5 t ha^{-1}), number of growing seasons and application frequency.

Generally, K and Na are affected by salinity, nematodes and AMF (Graham & Sylvester, 1989; Mashela & Nthangeni, 2002). In this study, AMF inoculation did not affect leaf K content, probably due to low mycorrhizal root colonization. The lower leaf K content in the biochar-amended transplants was likely due to enhanced N and P by biochar resulting in an imbalance ratio of N/K and P/K in the rhizosphere, which then reduced K uptake. It was also clear from our results that P was the only mineral nutrient whose uptake was decreased by both AMF inoculation and biochar application.

In conclusion, the addition of biochar in the planting hole during transplantation of AMF-inoculated seedlings had no effect on root colonisation, yield or yield components, or most of the leaf nutrients measured. They did reduce leaf P content. Consequently, biochar should first be researched in detail before attempting any commercial field application.

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(Received 01 March 2011; Accepted 05 July 2011)