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Biochar Amendment Enhances Tomato Resistance to Some Soil Borne Diseases

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ABSTRACT

The present study aimed to determine whether biochar soil amendment can improve tomato resistance against wilt (caused by *Fusarium oxysporum*) and root rot (caused by *Rhizoctonia solani*) diseases under greenhouse conditions. It was also attempted to unravel the physiological and biochemical mechanisms involved in biochar-mediated systemic responses of plant to these phytopathogens. The plants were cultivated into plastic pots, containing soil mixtures (clay and sand; 1: 3 v/v) that was homogenized with or without biochar 5% by weight. The soil mixtures in which the plants grown were either infested with *F. oxysporum*, *R. solani* or left un-inoculated. There were altogether six treatments, each with 10 replicates arranged in split split plot design. In the absence of biochar, fungal pathogen infection adversely influenced the plant growth, reducing the total plant biomass by up to 69% and 55% in *F. oxysporum* and *R. solani*, respectively, compared to the controls. Disease incidences were comparatively higher, being 86.6±5.7% and 80.0±10.0% for *F. oxysporum* and *R. solani*, respectively. Similarly, the disease severities were highest in the absence of biochar, reaching 75.0±3.0% and 67.6±6.8% for *F. oxysporum* and *R. solani*, respectively. Biochar amendment significantly improved plant growth and vigor, an effect that was more pronounced for infected plants. Biochar treatment (5%) led to enhance the total biomass by up to 20% (non-infested controls), 93% (*F. oxysporum* inoculated plants), and 75% (*R. solani* inoculated plants) relative to the respective controls. Plants grown in amended substrate exhibited remarkably higher resistant to *F. oxysporum* and *R. solani* (indicated by 85% and 80% lower diseased incidences and 84% and 80% lower disease severities). This was coincided with an improved water balance, increased phenolic compound and higher PAL and PO activities. Our results revealed that biochar soil utilization could diminish some of the detrimental effects caused by *F. oxysporum* and *R. solani*, conferring higher resistance and survival of tomato plants under constrained conditions.

Keywords: Tomato, Fusarium wilt, Root rot, Biochar, Phenolic compounds, Enzyme activity

Introduction

Solanum lycopersicum L. (Family: Solanaceae), an important agricultural commodity, is the second most consumed vegetable crop globally (Georgé *et al*., 2011), with an annual yield came up to 164 million tonnes from 4.7 million hectares under cultivation in 2013 (FAOSTAT, 2013). The production of this important crop is, however, threatened by major fungal diseases like Fusarium wilt (caused by *Fusarium oxysporum*) and root rot (caused by *Rhizoctonia solani*) (Smith *et al*., 1988; Agrios, 2005). Pathogenic strains of *F. oxysporum* causes wilting via infecting the plant roots and growing internally into the cortex to the stele, thus blocking the xylem vessels (McGovern, 2015). This interferes mainly with the plant ability to take up water, resulting in leaf wilting and yellowing, stunted growth and even plant death under severe infection (Beckman, 1987). Other common diseases caused by *R. solani* in tomato are sudden death syndrome "damping off" and root rot (Sneh *et al*., 1991). Both pathogens, *F. oxysporum* and *R. solani*, are well known as soil-borne and occur wherever tomato is grown, causing substantial economic losses worldwide (Fawzi *et al*., 2009). Controlling soil-borne fungal diseases is a real challenge due to the limited currently-utilized management practices compared to those employed against air borne pathogens. Agricultural chemicals are usually used to control both fusarium wilt and root rot diseases, which become neither satisfactory nor environment-friendly (Fravel *et al*., 2003). Moreover, occurrence of resistance breaking pathogenic strains and the failure of host resistance against pathogens enforces the development of alternative disease management strategies (Takken and Rep, 2010). Implementation of soil organic amendments

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such as biochar seems to be a promising approach to increase plant growth, crop productivity and resistance against several plant diseases (Kammann *et al*., 2015). Biochar is a pyrolytic porous solid byproduct of thermal degradation (at temperature < 700°C) of organic materials (crop residues, wood chips, manure, etc.) in the near absence of oxygen (Kammann *et al*., 2015). Whereas the initial interest in biomass pyrolysis is the value of fuels and distilled gases that could be collected, biochar as a byproduct has garnered much more attention at scientific, commercial and political levels as an eco-sustainable management approach for long-term carbon sequestration to sustain soil fertility enhancement (Lehmann and Joseph, 2009; Ruano-Rosa and Mercado-Blanco, 2015). Biochar is known to enhance many physiochemical and biological characteristics of the soil like bulk density, water holding capacity, nutrient retention, and cation exchange capacity, leading to improve the plant growth and crop productivity (Silber *et al*., 2010; Bonanomi *et al.*, 2014). In addition, available literature demonstrates that biochar can potentially enhance plant resistance against various aerial- and soil-borne pathogens, with evidences that disease severity is biochar dose-dependent (Graber *et al*., 2014; Jaiswal *et al*., 2015). Regarding soil borne pathogens, biochar amendment has been reported to suppress disease incidences and severities in 22 pathosystems (Bonanomi *et al*., 2015). For example, biochar soil application increased the plant root fresh weight of asparagus plants, and markedly reduced the percentage of root lesions caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* compared with the respective controls (Elmer and Pignatello, 2011). Zwart and Kim (2012) also noted that biochar amendment lowered the severity of stem canker caused by *Phytophthora* sp. in red oak and red maple. Recently, it has been found that biochar soil amendment suppressed damping off caused by *R. solani* on cucumber and common bean (Jaiswal *et al.*, 2014) . Despite an increasing interest on the utilization of biochar, specific mechanisms responsible for attenuating disease severity in biochar-amended soils are still widely unclear (Lehmann *et al*., 2011). The suppressive effects of biochar on phytopathogens might be due to a myriad of mechanisms similar to those by which compost is believed to suppress pathogens (Kraus *et al*., 2003). As reported by Datnoff *et al*. (2007), the improved nutrient supply in soils amended with biochar improves the physiological, morphological and histological characters of plant tissues, enabling quick plant responses to invading pathogens. To some extent, the chemical compositions of biochar and its sorption properties may alter the growth, development and activity of the pathogens in the soil or mediate signaling between pathogens and plants (Lehmann *et al*., 2011). Recently, biochar has been reported to potentiate the plant systemic resistance along with both salicylic acid and jasmonate/ethylene defence pathways, triggering the plant's antioxidant enzymatic activities as well as priming for gene expression upon infection by foliar fungal pathogens (Harel *et al*., 2012; Wang *et al*., 2014). Against this background, the general objective of the present study was to determine whether biochar soil amendment can improve tomato resistance against fusarium wilt (*F. oxysporum*) and root rot (*R. solani*) diseases under greenhouse conditions. It was also attempted to shed some light on the physiological and biochemical mechanisms, which may be involved in biochar-mediated systemic responses of plant to these phytopathogens.

Materials and Methods

The present study was carried out at the greenhouse of Plant Pathology Dept., Faculty of Agriculture, Ain Shams University, Qalyubia Governorate, Egypt (30° 06′ 42″ N 31° 14′ 46″ E). Pot experiments were performed during two successive seasons of 2013/2014 and 2014/2015 to investigate the effects of biochar application to potting substrate (5% w/w) on plant performance and disease resistance of tomato plants to fusarium wilt and root rot.

Source of inocula, isolation and identification of the causal agents

Fusarium sp. and *Rhizoctonia* sp. were isolated from naturally infected tomato plants collected from different commercial fields in El-Giza, Monufia and Qalyubia Governorates, Egypt. The roots of diseased-plants tap were initially rinsed with water and surface-sterilized using sodium hypochloride solution (3% v/v) for 2 minutes before they were washed several times with sterilized distilled water. The root segments were then gently dried and transferred into Petri-dishes containing potato dextrose agar medium (PDA). The plates were then incubated at 25±2°C for 5 days. The fungal cultures were

purified using hyphal tip technique cultures as described by Barnett and Hunter (1986) and identified based on their cultural, morphological and microscopical characteristics according to the identification keys of Summerell *et al*. (2003) and Sneh *et al*. (1991) for *Fusarium* and *Rhizoctonia*, respectively.

Pathogenicity test

The pathogenic potentials of the isolated fungal strains were evaluated as described by Karima and Nadia (2012) to determine the most aggressive isolates. Inocula of *F. oxysporum* and *R. solani* isolates were prepared by incubating each isolate in sterilized bottles containing sterilized sorghum grain at 25±2°C for 15 days. Sterilized pots (10 cm diameter) were filled with disinfested loamy sandy soil (clay: sand, 1:3 w/w). The soil mixture was mixed with the inoculum of each fungal isolate at rate of 2% (w/w). Tomato seeds (*S. lycopersicum* cv. *Super strain B*) were surface-sterilized using 1.5% NaOCl solution for 1 min and rinsed three times with sterile water. Then seeds were sown into a plastic multi-pot tray containing disinfected potting mixture (clay, sand and peat moss, 1: 1: 1 by volume) and kept on a bench in the greenhouse at 25±2 ºC daytime and 15±2 ºC night time temperatures for a photoperiod of 16 h. Tomato young seedlings were transplanted into the infested plastic pots (2 seedlings per pot). The disease severity was determined, 45 days after transplanting, by recording the percentage of dead seedlings. The most aggressive isolates of both *F. oxysporum* and *R. solani* were used in the present study to assess the potentialities of biochar soil amendment in reducing disease severity caused by these fungal strains in tomato.

Experimental setup, growth conditions and challenge inoculation

One-liter plastic pots (15 cm diameter) were filled with soil mixtures of clay and sand (1: 3 v/v) was previously homogenized with or without biochar 5% by weight. Biochar used in the present study was produced mainly from Salix wood chips (*Salix babylonica*), which was pyrolyzed for 1 hour at temperature between 350 and 550 °C using a lab-scale pyrolysis reactor. The biochar was then ground into powder, sieved to obtain particle sizes less than 2 mm prior to use. This biochar had a pH value of 9.64, $EC_{(1:10)}$ value of about 3.61, a bulk density of 0.26 g cm^{-3} and contained 18.0% moisture, 16.4% ash, 83.6% carbon (C), 0.56% nitrogen (N), 0.55% phosphorus (P), 1.36% potassium (K). The pots were then separated into two groups (each of 30 pots), depending on whether the potting soil is biochar-amended or not. Each group was then subdivided into three groups (each of 10 seedlings), where they either infested with fresh inoculum of *F. oxysporum*, *R. solani* (at a rate of 2% w/w of the soil weight) or left un-inoculated to serve as controls. There were altogether six treatments, each with 10 replicates. Tomato seedlings (twenty-five-days old) of uniform size were transplanted individually into the pots and maintained in the greenhouse under ambient temperatures of 25/23◦C day/night, photoperiod of 16 h, light intensity of 600 – 800 lux, and relative humidity of 65±5% throughout the entire experiment. The pots were arranged in a split split plot design and irrigated as needed and fertilized as usual.

Disease resistance evaluation

Disease incidence and severity were recorded weekly after inoculation for a total period of five weeks post-inoculation. The percentage of disease incidence was estimated according to Song *et al*. (2004) by dividing the number of symptomatic plants over the total number of plants of the corresponding treatment. Disease severities were assessed as described by Abdou *et al*. (2001) using a rating scale from 0 – 5 based on the degree of root discoloration or leaf yellowing as follow:

0 = neither root discoloration nor leaf yellowing
1= 1-25% root discoloration or one leaf yellowing
2= 26-50% root discoloration or more than one leaf yellowing
3= 51-75% root discoloration with one wilted leaf
4= more than 76% root discoloration with more than one wilted leaf
5= dead seedlings

Disease severity indices were calculated according to Song *et al*. (2004) using the following formula:

$$\text{DSI \%} = \sum \left(\frac{\text{d} * \text{number of plants in that grade}}{\text{dmax} * \text{n}} \right) * 100$$

Where: d= disease rating of each plant
d max = the maximum disease rating
n = the total number of plants evaluated in each replicate.

Agronomical traits

Five weeks after inoculation, five plants (replicates) from each treatment were destructively harvested and their height, fresh weight, leaves fresh weight and number of branches/plant were immediately recorded. The root segments were then washed thoroughly with running water, blotted on tissue paper and their fresh weighs were determined. Representative samples from the roots as well as leaves were then oven dried at 70 °C for 72 h or until constant weight for the determination of dry weight and water content.

Determination of total phenolic compounds and enzyme activity

Total phenolic compounds (TPC)

Total phenolic compounds (TPC) were extracted and quantified in the roots and leaves spectrophotometrically as described by Swain and Hillis (1955). A sample of 1 g of fresh materials was mixed with 15 ml ethanol 80% and stored in dark bottles at 4 °C for 72 h. Ethanol was daily changed and all extracts were combined and filtered. Thereafter, 1 ml of ethanolic extract was mixed with 0.25 ml concentrated HCl, heated in water bath at 100 °C for 10 min, and then cooled. The mixture was then mixed with 1 ml Folin-Denis reagent and 6 ml 20% sodium carbonate solution. After 30 minutes, the mixture was diluted to 10 ml with bedsit water. The optical density of the chromophore was measured at 725 nm and the concentration of total phenols was quantified by comparing sample absorbance with a standard catechol curve.

Assay of phenylalanine ammonia-lyase (PAL)

Phenylalanine ammonia-lyase (PAL) activity was determined following the method of Solecka and Kacperska (2003). A sample of 1 g fresh materials was extracted in 2 ml of 50 mM borate buffer (pH 8.8). The extracts were then centrifuged at 12000 rpm for 10 min at 4 ºC and 1 ml of the supernatant was mixed with 2 ml sodium borate buffer (pH 8.8) and 1 ml of 10^{-2} M of L-phenylalanine. The reaction mixture was incubated for 1 h at 30 ºC and the reaction was terminated by adding 500 µl HCl (6N). The reaction mixture was then centrifuged for 10 min at 12000 rpm. Enzyme activity was expressed as trans-cinnamic acid produced at 290 nm by using UV/Vis spectrophotometer (Unico-2100, United Products & Instruments, Inc. Dayton, NJ).

Assay of peroxidases (PO)

The activity of PO was assessed according to the methods described by Biles and Martyn (1993). Peroxidase activity in the crude enzyme extracts was directly determined by adding 100 µl of the crude enzyme extract to a reaction mixture consisted of 2.9 ml of 100 mM sodium phosphate buffer (pH 6.0) containing 0.25% (v/v) guaiacol (2-methoxy-phenol) and 100 mM H_2O_2. The change in absorbance was recorded spectrophotometrically for 3 min at 470 nm. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} fresh weight.

Statistical analysis

Combined analysis for the raw data of both experimental years was statistically analyzed using CoStat Software to evaluate the differences between non-inoculated and inoculated tomato plants grown either in the absence or presence of biochar amendment. The differences between mean values were assessed using Duncan's multiple range test ($P \leq 0.05$).

Results

Effect of biochar on disease incidence and disease severity index

The influence of biochar soil application on tomato resistance to fusarium wilt and root rot diseases was evaluated five weeks post-inoculation. Infested plants grown either in potting medium non-amended or amended with 5% biochar were infected, showed disease symptoms of various degrees. However, disease incidence was significantly ($P \leq 0.05$) reduced in response to biochar treatment by up to 85% and 80% for fusarium wilt and root rot, respectively, as compared with the non-amended controls (Fig. 1A). Additionally, biochar soil amendment resulted in significant ($P \leq 0.05$) reductions of about 84% and 80% in the severity of fusarium wilting and root rot, respectively, compared to the corresponding controls (Fig. 1B).

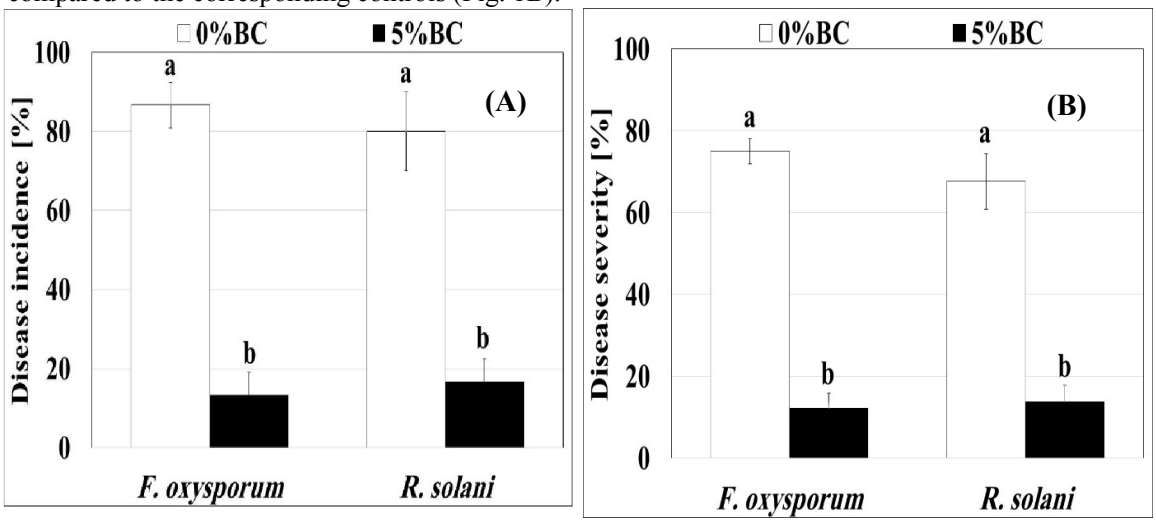

Fig. 1: Disease incidence (A) and disease severity (B) of *F. oxysporum* and *R. solani* in tomato plants as affected by biochar soil application. Significant differences ($P \leq 0.05$) between infected and not-infected plants (within each pathogen treatment) are indicated by different letters as evaluated by Duncan's multiple range test.

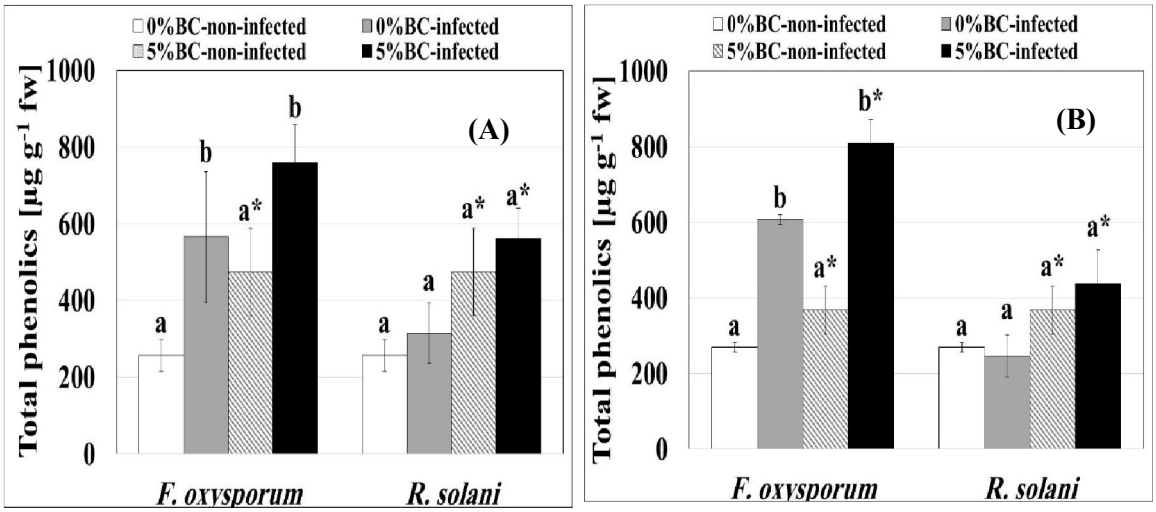

Fig. 2: Effect of biochar soil amendment on the phenolic compounds in the root (A) and leaves (B) of non-infected, *F. oxysporum*-infected and *R. solani*-infected tomato plants. Significant differences ($P \leq 0.05$) between infected and not-infected plants (within each pathogen treatment) are indicated by different letters, while significant differences between biochar treatments (within each pathogen treatment) are indicated by an asterisk

Effects of biochar on some agronomical traits

Fungal inoculation with *F. oxysporum* and *R. solani* negatively affected the overall growth of tomato plants. Plant height was distinctly declined by 43% and 32% in response to infection with *F. oxysporum* and *R. solani*, respectively, compared to the controls (Table 1). The fresh weights of infected plants were also significantly ($P \leq 0.05$) reduced, reached only 39% and 45% of the controls in wilt- and root rot-infected plants, respectively (Table 1). This was coincided with a marked reduction in the leaf number per plant in both wilt- and root rot-infested plants (Table 1). Root fresh weight was much more affected when compared to those of the shoots, leading consequently to increase the shoot:root fresh weigh ratio from 1.77±0.38 (controls) to 3.09±0.59 (wilt-diseased plants) and 2.61±0.39 (root rot-diseased plants) (Table 1). While fungal infection led to substantial increases of about 1.6 folds (*F. oxysporum*) and 1.8 folds (*R. solani*) in the root dry weight, it did not significantly alter the dry weight of the shoots (Table 1). As a general trend, biochar soil amendment has promoted the overall growth and biomass accumulation of tomato plants both in non-infested and infested soils. Biochar significantly ($P \leq 0.05$) enhanced the plant height by 20% (healthy controls), 93% (*F. oxysporum*-infested plants), and 75% (*R. solani*-infested plants), compared to their respective non-amended counterparts (Table 1). Plant biomass was also substantially increased in response to biochar application, being 17%, 140% and 105% higher for non-infested, *F. oxysporum*-infested and *R. solani*-infested plants, respectively, compared to the respective controls (Table 1). This was again positively correlated with the leaf number per plant, which increased by 20% (healthy controls), 99% (wilt-diseased plants), and 66% (root rot-diseased plants), relative to the respective controls (Table 1). Although biochar did not alter the ratio of shoot:root fresh weight in the absence of pathogen inoculation, it reduced this ratio from 3.09±0.59 to 1.80±0.16 in wilt-diseased plants and from 2.61±0.39 to 1.65±0.12 in root rot-diseased plants (Table 1). Root dry weight of healthy plants were slightly (statistically not significantly) increased, while those of wilt- and root rot-diseased plants were progressively decreased by roughly 66% upon biochar treatment (Table 1). No significant changes were observed in the shoot dry weight between amended and non-amended substrates (Table 1).

Table 1: Effect of biochar soil amendment on some agronomic traits of tomato plants upon infection with *F. oxysporum* and *R. solani*. Ln, leaf number per plant; Ph, plant height; PFW, plant fresh weight; RDW, root dry weight as % of fresh weight; ShDW, shoot dry weight as % of fresh weight

Treatments	Pathogen	Ph [cm]	Ln per plant	PFW [g]	Shoot/Root ratio	RDW	ShDW
0%BC	Non-infected	34.667 ^{a} ±1.528	8.000^{a} ±1.000	14.153 ^{a} ±0.295	1.770 ^{a} ±0.379	10.093 ^{a} ±3.702	13.709 ^{a} ±2.325
	F. oxysporum	19.667 ^{b} ±3.215	4.667 ^{b} ±0.577	5.543 ^{b} ±0.519	3.089 ^{b} 0.594	26.182 ^{b} ±4.125	14.227 ^{a} ±3.673
	R. solani	23.333 ^{b} ±2.887	5.000 ^{b} ±1.000	6.317 ^{b} ±0.611	2.606 ^{b} ±0.392	28.583 ^{b} ±1.243	11.338 ^{a} ±1.600
5% BC	Non-infected	41.667 ^{a*} ±1.528	9.667 ^{a} ±0.577	16.583 ^{a*} ±1.156	1.798 ^{a} ±0.161	13.114 ^{a} ±0.647	15.627 ^{a} ±0.856
	F. oxysporum	38.000 ^{a*} ±2.646	9.333 ^{a*} ±0.577	13.317 ^{b*} ±0.939	1.897 ^{a*} ±0.164	10.187 ^{b*} ±1.708	17.312 ^{a} ±3.648
	R. solani	41.000 ^{a*} ±1.000	8.333 ^{a*} ±1.528	12.987 ^{c*} ±0.359	1.647 ^{a*} ±0.124	10.240 ^{b*} ±0.032	15.928 ^{a} ±0.768

Values represent mean ± SD for ten replicates per treatment. Different letter indicates significant ($P \leq 0.05$) differences between pathogen treatments (within the same biochar treatment), while asterisk indicates a significant ($P \leq 0.05$) difference between biochar treatments (within the same pathogen).

Effect of biochar on some biochemical traits related to the induction of resistance

Total phenolic compounds (TPC)

In the absence of biochar, TPC of both roots and leaves increased progressively upon *F. oxysporum* infection, but did not significantly change upon infection with *R. solani* (Fig. 2A & B).

Significant ($P \leq 0.05$) increases of about 120% (roots) and 125% (leaves) in the contents of TPC were observed in fusarium wilt-diseased plants (Fig. 2A & B). Biochar soil amendment enhanced the TPC of the roots and leaves in both non-infected and infected plants, the effect that was more obvious for infected ones (Fig. 2A & B). It led to significant progressive increases in TPC by more than 3 folds (*F. oxysporum*-inoculated plants), and 1.6 – 2 folds (*R. solani*-infested plants).

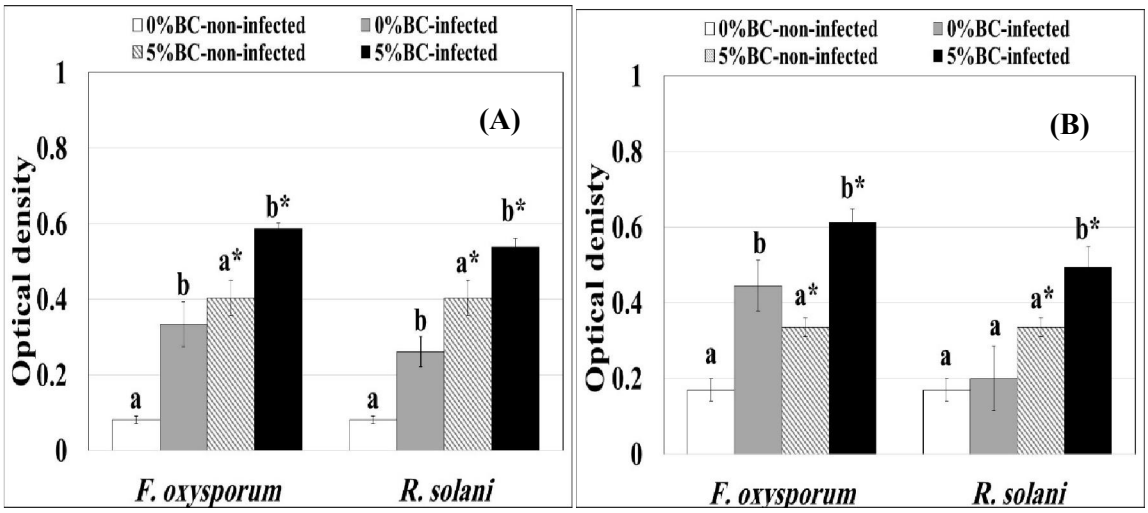

Fig. 3: Effect of biochar soil amendment on PAL activity in the root (A) and leaves (B) of non-infected, *F. oxysporum*-infected and *R. solani*-infected tomato plants. Significant differences ($P \leq 0.05$) between infected and not-infected plants (within each pathogen treatment) are indicated by different letters, while significant differences between biochar treatments (within each pathogen treatment) are indicated by an asterisk.

Enzyme activity

The activity of phenylalanine ammonia-lyase (PAL) was considerably enhanced in the roots and leaves of both wilt- and root rot-diseased plants (Fig. 3A & B). fusarium-wilt led to enhance PAL activity by roughly three folds (roots) and 1.5 folds (leaves), compared with the healthy controls (Fig. 3A & B). Similarly, root rot-infected plants exhibited 226% and 17% higher PAL activities for the roots and leaves, respectively, compared with the healthy controls (Fig. 3A & B). Biochar had a positive effect on the activity of PAL, especially in the presence of fungal infection (Fig. 3A & B). In fusarium-wilt diseased plants, PAL activity was distinctly increased by 6 and 2 folds in the roots and leaves, respectively, compared to the healthy controls grown in non-amended soils (Fig 3A & B). Root rot-diseased plants grown in biochar-amended soils exhibited 5.6-folds and 1.8-folds higher PAL activity in their roots and leaves, respectively, relative to healthy plants that grown in non-amended soils (Fig. 3 A & B).

Peroxidase (PO) activity in both the roots and leaves was also profoundly altered in tomato plants upon fungal infection (Fig. 4A & B). fusarium-wilt significantly ($P \leq 0.05$) increased PO activity of the root and leaves by 34% and 45%, respectively, compared to the healthy controls. These disease-induced increments were only 16% (roots) and 26% (leaves) for plants grown in root rot-infested soils (Fig. 4A & B). Incorporation of biochar in the soil significantly ($P \leq 0.05$) enhanced PO activity in both the roots and leaves of infected and non-infected plants, the effect which was more pronounced in infected ones (Fig. 4A & B). It led to conspicuous increases of about 78% (roots) and 54% (leaves) in PO activity in fusarium wilt-diseased plants, compared with those healthy grown in non-amended soils (Fig. 4A & B). Root rot-diseased plants grown in biochar amended soil exhibited 74% and 40% higher PO activity for the roots and leaves, respectively, compared to those healthy grown in non-amended soils (Fig. 4A & B).

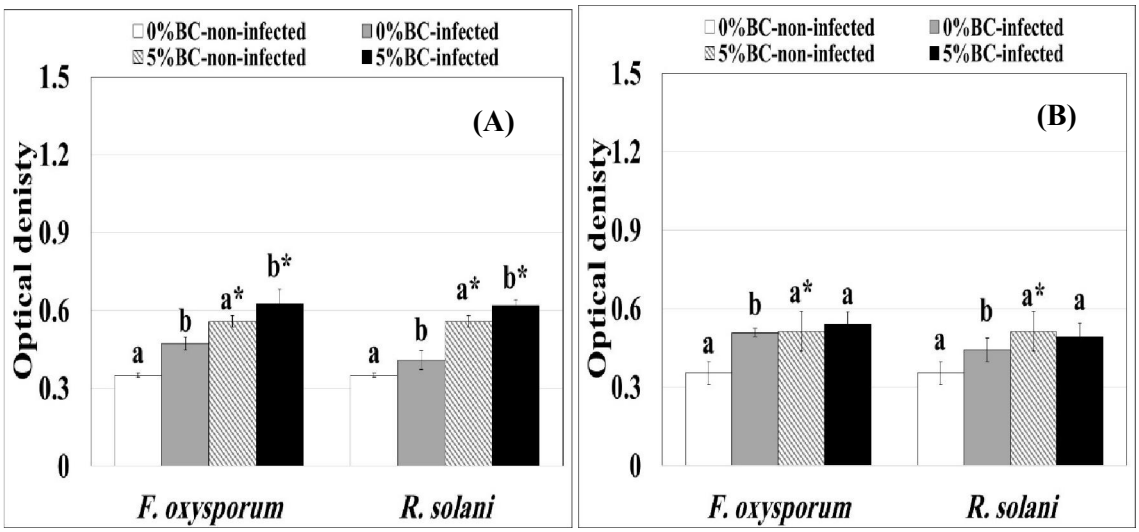

Fig. 4: Effect of biochar soil amendment on PO activity in the root (A) and leaves (B) of non-infected, *F. oxysporum*-infected and *R. solani*-infected tomato plants. Significant differences ($P \leq 0.05$) between infected and not-infected plants (within each pathogen treatment) are indicated by different letters, while significant differences between biochar treatments (within each pathogen treatment) are indicated by an asterisk

Discussion

Sustainable utilization of organic amendments such as biochar could be a promising approach to sustain soil fertility and enhance plant productivity (Ruano-Rosa and Mercado-Blanco, 2015). In accordance with previous studies (Graber *et al*., 2010; Agegnehu *et al*., 2015), biochar soil amendment markedly stimulated the overall plant growth (total biomass, plant height, leaf number/plant) of tomato grown in pathogen-free medium (Table 1). Biochar-induced plant growth promotion might be attributed either to direct effect via biochar-supplied nutrients (Silber *et al.*, 2010) or many other indirect effects. These include: an improved nutrient retention and soil cation exchange capacity (Chan and Xu, 2009; Novak *et al.*, 2009), altered soil pH (Steiner *et al.*, 2007), improved soil tilth and water retention (Novak *et al.*, 2009), neutralization of phytotoxic compounds in soil (Wardle *et al.*, 1998), enhanced soil microbial populations and functions (Graber *et al.*, 2010; Kolton *et al.*, 2011), and stimulation of plant genes like those regulating plant growth hormones and photosynthetic machinery (Viger *et al*., 2014). Biochar soil amendment not only promotes the plant growth, but also has a suppressive effect against several foliar and soil borne fungal diseases (Graber *et al*., 2014; Jaiswal *et al*., 2015). This is also shown by our data: Biochar soil application delayed *F. oxysporum* and *R. solani* development, hence significantly reduced their disease incidences by up to 85% and 80%, respectively (Fig. 1A). Moreover, the disease severities of fusarium wilt and root rot were progressively lowered by as much as 84% and 80%, respectively, in tomato plants treated by biochar (Fig. 1B). These results are in qualitative agreement with several earlier reports on the suppressive effects of biochar against various soil-borne pathogens like *F. oxysporum* f. sp. *asparagi* (Matsubara *et al*., 2002; Elmer and Pignatello, 2011), *Ralstonia solanacearum* (bacterial wilt) (Nerome *et al*., 2005), *Phytophthora cactorum* and *P. cinnamomi* (Zwart and Kim, 2012), *Pythium aphanidermatum* and *R. solani* (Jaiswal *et al*., 2014, 2015). Nonetheless, results from previous studies investigating biochar impacts on soil-borne diseases are inconsistent and contradictory. Biochar soil amendment had no effect on the suppression of *Pythium ultimum* in lettuce, sweet pepper and some herbal plants (Gravel *et al*., 2013). In contrast with the data presented here, some reports showed that incorporation of biochar increased the disease incidence of *F. oxysporum* f. sp. *lycopersici* in tomato (Akhter *et al*., 2015). This was, however, not surprisingly since biochars prepared from various feedstock types under different pyrolysis conditions vary in their production parameters as well as physicochemical and biological properties (Lehmann and Joseph, 2009). These would inevitably alter all biochar subsequent impacts on soil quality, plant performance and disease resistance (Graber *et al*. 2014;

Jaiswal *et al*. 2015). Additionally, the effects of biochar might also vary depending on the pathosystems, inoculum source, soil characteristics, climatic conditions and nutrient availability (Copley *et al*., 2015). The specific mechanisms by which biochar amendment attenuate soil-borne pathogens and hence enhance plant disease resistance are still obscure. According to Graber *et al*. (2014), nutrients supplied or made more available by biochar addition could enhance the plant vigor, enabling quick plant responses to pathogen attack. In this study, infected tomato plants grown at 5% biochar were more vigorous, exhibiting significant increases of about 139% (fusarium wilt-diseased) and 100% (root rot-diseased) in their total fresh weight, respectively, compared to the respective controls (Table 1). In accordance with Graber *et al*. (2014), higher disease resistance of tomato against both *F. oxysporum* and *R. solani* observed in this study upon biochar treatment might be attributed to an improved nutrient supply. This would enhance morphological, histological and biochemical characteristics of plant tissues and hence the overall plant vigor (indicated by greater plant height, higher leaf number per plant and substantial increases in the total plant biomass), enabling thereby quick plant responses to pathogen invasion. Intriguingly, in both *F. oxysporum* and *R. solani* infested plants, biochar-induced growth promotion was much more pronounced for the roots, leading to reduce the shoot: root fresh weight ratio (Table 1). In addition, improved root growth of infested plants upon biochar addition would support prompt water uptake, so that some of the detrimental effects of fusarium wilt and root rot on plant water balance and hence CO_2 assimilation capacity could be negated. This interpretation is further supported by the trends of water contents, particularly of the roots, which considerably increased by 21.5% and 25.6% in *F. oxysporum* and *R. solani* diseased plants, respectively, upon biochar treatment (data not shown). Although, biochar is initially sterile (no consortium of microorganisms), its soil incorporation leads to increase microbial taxon and functional diversity, microbial activities as well as the abundance of several groups related to biocontrol and plant growth promotion (Graber *et al*., 2014; Jaiswal *et al*., 2015). Beneficial microbiota can also compete with soil pathogens for space and nutrients or produce antimicrobial agents, improving thereby the plant performance (Berendsen *et al*., 2012). In line with several earlier studies (Kloss *et al*., 2014), biochar-elicited increase in soil pH might suppress the incidence of fusarium wilt in tomato plants due to a reduction of nutrients availability. Furthermore, biochar, as a strong adsorbent may alter the mobility and activity of pathogens or modify signaling between pathogens and plants (Lehmann *et al*., 2011). It may also adsorb extracellular degrading enzymes (Lammirato *et al*., 2011) and other phytotoxins produced by the soil pathogens, hence reducing their contact with root cell and protecting therefore the plant to some extent (Graber *et al*., 2014). Besides, organic compounds associated with the labile fraction of biochar can suppress disease-causing microorganisms in the rhizosphere (Graber *et al*., 2014). Recently, it has been documented that biochar induced systemic plant resistance response, with elicitors being biochar-borne chemicals, biochar-induced changes in rhizospheric microbiota or both (Harel *et al*., 2012). As well-known, induction of resistance involves the activation of a wide variety of general defense reactions including the oxidative burst, structural cell wall modifications, and production of defense-related compounds (Shetty *et al*., 2008). Data presented in Figure (2A and B) showed clearly that biochar treatment resulted in substantial increases in the total phenolic compounds in the roots and leaves of both infected and non-infected plants. Phenolic compounds may suppress disease development by inhibiting the extracellular fungal enzymes (cellulases, pectinases, lactase and xylanase), inhibition of fungal oxidative phosphorylation, nutrient deprivation, inhibition of both spore germination and mycelial growth and antioxidant activity in plant tissues (Raghvendra *et al*., 2007). This may explain, at least in part, the higher disease resistance of tomato plants observed in this study upon biochar treatment. Higher accumulation of phenolic substances is usually associated with higher lignification rate and known to occur in induced resistance plant response (Oven and Torelli, 1994). This would contribute to strengthen the cell walls, the first line of defense against pathogen invasion, enhancing thereby the plant disease resistance (Wuyts *et al*., 2006). Enhanced lignification is usually accompanied by an increased activity of the some key enzymes of the phenylpropanoid pathway such as phenylalanine ammonia-lyase (PAL), and peroxidases (PO) (Nicholson and Hammerschmidt, 1992). Our results indicate clearly that PAL activity was markedly enhanced in both root and leaves upon biochar amendment in both infected and non-infected tomato plants (Fig. 3A & B). Induction of PAL and subsequent increase in the content of phenolic compounds has been previously documented as a general response associated with disease resistance (Nagarathna *et al*., 1993). Exactly how PAL

contributes to enhanced plant disease resistance has been the focus of several research. Principally, PAL, an important enzyme of the phenylpropanoid pathway, catalyzing the transformation of L-phenyalanine into trans-cinnamic acid, providing the phenyl propane carbon skeleton for the synthesis of flavonoids, phenolic, phenyl propanes and lignin, all of which play key roles in a range of plant-pathogen interactions (Wuyts *et al*., 2006). It is involved in the synthesis of phytoalexins, which acts as antimicrobial agent, implicated in the biosynthesis of lignin and the salicylic acid (Dixon *et al*., 2002). The latter is considered as another defense-related compound and a key signaling component required for the activation of pathogen related genes, catalases, receptor-like protein kinases and transcription factors (Mould *et al*., 2003).

The activity of peroxidase (PO), another defense enzyme, was induced in this study upon biochar in both roots and leaves of tomato plants. This effect was however, more obvious for *F. oxysporum* and *R. solani* infected plants compared to non-infected ones (Fig. 4 A & B). Similarly, Mydlarz and Harvell (2006) observed that pathogen infection led to an induction of peroxidase activity in plant tissues and a higher increase was observed in resistant plants compared to the susceptible ones. Peroxidases participate in the cell wall forming processes such as oxidation of phenols, suberization and lignification of host plant cell walls during the defense reaction against pathogenic agents (Carvalho *et al*., 2006; Hücklehoven, 2007). H_2O_2 produced by some peroxidases can either serve as a substrate for other PO, or act as antimicrobial agents, triggering self-defense responses at the site of pathogen ingress (Bolwell *et al*., 2002).

Taken together, these data indicate that biochar soil application improved considerably the growth of tomato plants, with total biomass enhanced by about 20% (non-infested controls), 93% (*F. oxysporum* inoculated plants), and 75% (*R. solani* inoculated plants) relative to the respective controls. Our results show clearly that plants grown in amended substrate were obviously more resistant to *F. oxysporum* and *R. solani* (indicated by 85% and 80% lower diseased incidences and 84% and 80% lower disease severities). Higher disease resistance in response to biochar was associated with enhanced overall plant growth, particularly the root, improved water balance, increased phenolic compound and higher PAL and PO activities. Results of this study justify the beneficial role of biochar in elevating plant disease resistance against soil borne pathogens and is a step forward in its utilization as a promising disease management strategy for sustainable crop production.

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