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Biochar Soil Amendment Induced Resistance in Tomato against Tobacco Mosaic Virus

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ABSTRACT

Tobacco mosaic virus (TMV) is one of the most serious pathogens for tomato cultivation, causing considerable economic losses. Biochar soil application is thought to convey several benefits to plants including improved plant growth and induction of systemic resistance against several plant pathogens. Accordingly, this study aimed to verify whether biochar soil amendment could induce resistance against TMV and to determine how this resistance is related to the amount of biochar added. Tomato seedlings were transplanted individually into one-liter plastic pots, containing sterilized soil mixtures (clay and sand; 1: 3 v/v) that was previously homogenized with biochar (0, 2.5 and 5% by weight). Two weeks after transplanting, the plants of each biochar group were subdivided in two groups (each of 12 plants), where they either mechanically inoculated with TMV or left un-inoculated. In TMV-inoculated plants, initial symptoms of discoloration and mosaic were first observed on inoculated leaves 7 and 10 dpi in un-amended and biochar-treated plants, respectively, suggesting that biochar could suppress TMV development. This was supported by the trends of disease incidences, which were markedly reduced by 16.7 and 33.3% upon treatment with 2.5 and 5% biochar, 30 dpi, respectively. TMV severity was also declined in biochar-treated plants, both at 15 and 30 dpi, the effect that was more pronounced for plants treated with 5% biochar, 30 dpi. Biochar-induced TMV inhibition was associated with higher leaf total phenolic compounds as well as peroxidase activity, particularly at 5% biochar treatment. Together, these results indicate that biochar addition has greatly delayed TMV development in tomatoes, enhancing therefore their resistance. This conclusion is further confirmed by DAS-ELISA analysis, which showed that 2.5 and 5% biochar distinctly declined the relative TMV concentrations by 46.4 and 60.4%, respectively, at 30 dpi. Finally, this study delivered, for the first time, evidences that biochar was able to suppress TMV infection in tomato plants. It could be a step forward towards biochar utilization as a promising environmental-friendly disease management approach.

Keywords: Tomato, TMV, Viral diseases, Biochar, Phenolic compounds, ELISA, Enzyme activity

Introduction

Tomato, *Solanum lycopersicum* Mill. (Solanaceae) is one of the most important vegetable commodity in the Mediterranean region, particularly in Egypt. Owing to its high nutritive value, it garners worldwide attention for both fresh market and processed foods and pharmaceutical industries (Barba *et al*., 2006; Georgé *et al*., 2011). In 2016, cultivated area under tomato in Egypt was 199712 hectares, produced 7943285 tons (FAOSTAT, 2016). Tomato production is severely constrained by several viral diseases, causing substantial damage and huge economic losses (Balogun, 2008; Blancard, 2012). *Tobacco mosaic virus* (TMV), a type member of the genus *Tobamovirus*, infects more than 500 plant species including tomato, seriously threatening its growth, development, yield and fruit quality (Moreira *et al*., 2001). Infected plants are often stunted and dwarfed, with various discoloration occur on their leaves, including vein clearing, mottling, mosaic, with patches of various shades of green and yellow (Agrios, 2005). The expanding leaves may be distorted, typically twisted with the leaflets become much narrowed and develop a fern-like “shoestring” appearance. The fruits are usually small and misshaped, mature unevenly and show yellow discoloration, sometimes in rings, as well as internal localized brown necrotic areas (MacNab *et al*., 1983; Conti *et al*., 1996;

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Cerkauskas, 2004). Traditionally, the control of viral diseases is solely dependent on preventive measures and vector management using insecticides, physical barriers and reflective mulches (Hilje *et al*., 2001). Besides, proper agricultural practices including virus-free transplants, crop-free periods, weed control and removal of infected plants are commonly used (Navas-Castillo *et al*., 2011). These tactics, however, have not been efficient, and no curative treatment is yet available (Wang *et al*., 2012). Hence, implementation of environment-friendly control measures that can induce plant innate immunity against viral diseases is of great interest (Cantrell *et al*., 2012; Thakur and Sohal, 2013). As a part of an ongoing research to screen biologically active agents, considerable attention has been directed to soil organic amendments like biochar. Biochar is generated by thermal degradation in anaerobic conditions (Kammann *et al*., 2015). Biochar has been reported to sustain soil fertility, enhance crop productivity and elicit plant defenses against a broad spectrum of phytopathogens (Sohi *et al*., 2010; Biederman and Harpole, 2013). In tomato plants, biochar soil application reduced the incidence and severity of diseases caused by *Fusarium oxysporum* and *Rhizochtonia solani* (Khalifa and Thabet, 2015). Similarly, diseases severities of powdery mildew (*Oidiopsis taurica*) and grey mould (*Botrytis cinerea*) in both tomato and pepper plants were declined in response to biochar soil application (Elad *et al*., 2010). Biochar significantly suppressed diseases caused by *F. oxysporum* f. sp. *asparagi* in asparagus (Elmer and Pignatello, 2011), and *R. solani* in cucumber (Jaiswal *et al*., 2014) and bean (Jaiswal *et al*., 2015). In strawberry, biochar amendment significantly decreased disease severity of *B. cinerea*, *Colletotrichum acutatum*, and *Podosphaera apahanis*, and led to a distinct up-regulation of several defense-related genes upon infection (Harel *et al*., 2012). Mechanisms by which biochar may alter diseases are numerous and varied (Lehmann *et al*., 2011). It is proposed that biochar improves the plant health by changing the soil microbial populations (Lehmann *et al*., 2011) and/or by direct induction (priming) of plant defences (Graber *et al*., 2010; Harel *et al*., 2012; Wang *et al*., 2014). Although much has been written during the last decades on the potentials of biochar and its effects on the basal resistance to bacterial and fungal pathogens of plants, studies regarding its antiviral activity are scarce. Therefore, major motivations of this study was initially to 1) verify whether biochar soil amendment could induce resistance against TMV in tomato seedlings under greenhouse conditions; 2) determine how this resistance is related to the amount of biochar amendment and 3) if any, which mechanism is responsible for this induced resistance.

Materials and Methods

The present work 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 (two successive seasons) were performed during 2016/2017 to study the influence of different biochar levels (0, 2.5% and 5% w/w) on plant growth, development and resistance in TMV-tomato pathosystem.

Plant materials, growth conditions and experimental set up

Tomato (*Solanum lycopersicum* cv. *Super strain B*) seedlings (twenty-five-days old) of uniform size were transplanted individually into one-liter plastic pots, containing sterilized soil mixtures of clay and sand (1: 3 v/v) that was previously homogenized with biochar (0, 2.5 and 5% by weight). The plants were arranged in three different groups, each of 24 plants. Biochar (BC) that was used in this investigation was produced from Salix (*Salix babylonica*) wood chips that pyrolyzed for 1 h, at temperature between 350 and 550 °C using a lab-scale pyrolysis reactor. The pyrolysed materials were ground into powder and sieved to get particles less than 2 mm. The obtained biochar had bulk density of 0.26 g cm^{-3}, pH value of 9.64 and $EC_{(1:10)}$ of about 3.61, and contained 18.0% moisture, 16.4% ash, 0.56% nitrogen (N), 83.6% carbon (C), 1.36% potassium (K), 0.55% phosphorus (P). The plants were maintained in the greenhouse under temperatures of 25/23°C day/night, photoperiod of 16 h, light intensity of 600 – 800 lux and relative humidity of 65±5%. The plants were irrigated as needed and fertilized as usual. Two weeks after transplanting, the plants in each biochar group were subdivided in two groups (each of 12 plants), where they either artificially infected with TMV or left un-inoculated (mock controls). Hence, there were all together six different experimental groups that were arranged in a completely randomized block design (CRD).

TMV- inoculation

TMV was kindly provided by the Plant Virus Lab., Department of Plant Pathology, Faculty of Agriculture, Ain Shams University. The virus was propagated on *Nicotiana tabacum*. Inoculum consisted of symptomatic leaves of TMV-infected tobacco were ground (1/3 w/v) in 0.1 M phosphate buffer pH 7.2 containing 0.002 M EDTA and carborundum (Garciacano *et al*., 2006). Tomato plants were mechanically inoculated with TMV, while mock plant were dusted with carborandum and treated with a buffer solution.

Plant growth and morphological traits

The plants were harvested 30 dpi and some morphological parameters such as plant height, leaf number per leaf, leaf area per leaf, fresh weight of all plant organs as well as the shoot: root fresh weight ratio were assessed.

Assessment of disease resistance

The level of TMV resistance was evaluated based on disease incidence and severity of symptoms. Disease incidence expressed as the percentage of plants that showing TMV symptoms was determined 30 dpi. The disease severity was assessed by visual observation of the viral symptoms using an arbitrary scale according to Wang *et al*. (2009b) where 0= no symptoms observed; 1=light mottling and a few thin yellow veins; 2=mottling and vein clearing unevenly distributed on the leaf; 3=mottling, leaf distortion, and stunting and 4=severe mottling, leaf curling, and stunting. The severity index was estimated for the different set of plants using the following formula as described by Raupach *et al*. (1996).

$$\textbf{Disease severity index (\%)} = \sum \left(\frac{\textbf{disease grade} * \textbf{number of plants in that grade}}{\textbf{total number of plants} * \textbf{the highest disease grade}} \right) * \mathbf{100}$$

Determination of relative virus concentration using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

Relative TMV accumulation in the leaves was determined by DAS-ELISA (modified after Clark and Adams, 1977) using commercially available polyclonal antibodies (Bioreba AG, Switzerland) according to the provided instructions. Samples were considered positive for the presence of TMV if the ELISA absorbance value was greater than twice for comparable negative control samples (Cordoba-Selles *et al*., 2007).

Total phenolic compounds (TPC)

TPC were determined in the plant leaves of all treatments spectrophotometrically according to the methods described by Swain and Hillis (1955). The optical density (OD) of the formed chromophore was measured at 725 nm and the concentration of total phenols was quantified using a standard catechol curve.

Assay of peroxidases (POXs)

POXs activity was determined as described by Biles and Martyn (1993). The changes in absorbance were measured spectrophotometrically for 3 min at 470 nm. The activity of POXs was expressed as the increase in absorbance min^{-1} g^{-1} fresh weight.

Statistical analysis

All data sets were subjected to two-way analysis of variance (ANOVA) using SPSS (version 20) in order to determine if significant differences were found among means. Duncan's multiple range

test was employed to determine if significant ($P \leq 0.05$) differences occurred between individual treatments.

Results

Plant growth, and morphological parameters

While non-inoculated plants did not show any TMV symptoms, discoloration and mosaic symptoms were first observed on the leaves of TMV-infected plants 7 dpi in un-amended pots. These symptoms started to occur on the leaves 10 dpi for plants treated with BC. In the absence of BC, TMV-inoculated plants were dwarf with smaller and narrower leaves (Fig. 1). The infected plants showed substantial reductions of 12.6 and 30.0% in their height and fresh weights, respectively, compared to mock controls (Table 1). Whereas TMV infection did not alter leaf number per plant, it led to reduce the leaf area by 42% (Table 1). The leaves, particularly the newly emerged, were deformed, showing a fern-like appearance to various degrees (Fig. 2). Biochar has generally stimulated the growth of both non-infected and TMV-inoculated plants, with maximal effect at 5% BC (Fig. 1). In absence of pathogen, BC applied at 2.5 and 5% significantly ($P \leq 0.05$) increased the plant height by 50 and 77%, respectively, as compared with respective controls (Table 1). Similarly, plant fresh weights were markedly improved by 13.2 and 53.2% in response to 2.5 and 5% BC treatments, respectively, compared to the respective controls (Table 1). This was accompanied by significant reductions in the shoot: root fresh weight ratio, which decreased from 4.06 to 2.05 and 2.72 in plants treated with 2.5 and 5% BC, respectively (Table 1). Biochar increased also the leaf number by about 23.5 and 35.3% for plant grown at 2.5 and 5% BC, respectively (Table 1). This was associated with significant increases of about 46.9 and 66.1% in leaf area of the plants treated with 2.5 and 5% BC, respectively (Table 1). TMV symptoms were less severe in plants treated with biochar, particularly 5% BC (Fig. 1 & 2). At 5% BC, infected plants were generally healthier, taller and more vigorous, having greater number of leaves with higher surface area compared to those grown in non-amended pots (Fig. 1 & 2). Biochar incorporation at 2.5 and 5% increased significantly ($P \leq 0.05$) the height of TMV-inoculated plants by 36.7 and 82.3%, respectively, compared to un-amended plants (Table 1). Similarly, the fresh weights of infected plants enhanced considerably by 46.4 and 81.5% upon treatment with 2.5 and 5% BC, respectively (Table 1). TMV-infected plants grown at both 2.5 and 5% BC exhibited higher leaf number and greater leaf area as compared with those grown at 0% BC. Leaf number was significantly increased by 27.3 and 32.7% in response to 2.5 and 5% BC application, respectively, compared to the respective controls (Table 1). Similarly, 2.5 and 5% BC caused substantial increases of about 1.6% and 96.5%, respectively, in the leaf area of TMV-infected plants compared to the un-amended controls (Table 1).

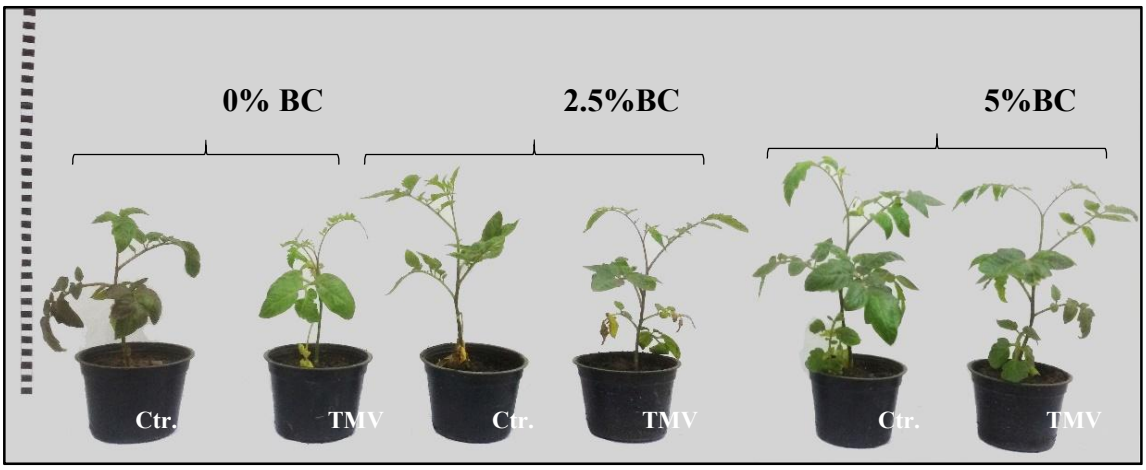

Fig. 1: Growth and development of tomato plants in response to TMV infection when grown under different level of biochar soil amendment.

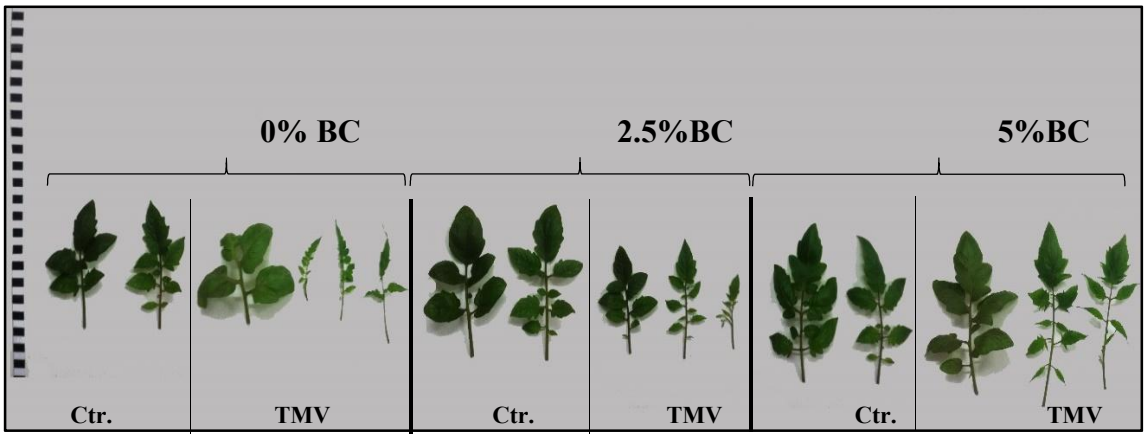

Fig. 2: Effects of TMV infection on the leaf morphology of tomato plants grown under different rate of biochar soil application.

TMV incidence and severity

Un-amended controls have depicted the maximum TMV-incidence at 30 dpi, being 100 % (Fig. 3). TMV incidence was significantly ($P \leq 0.05$) decreased by 16.7 and 33.3% upon biochar treatment with 2.5 and 5%, respectively (Fig. 3). Disease severity was obviously lower for plants grown in biochar-amended soil, both at 15 and 30 dpi (Fig. 4). At 15 dpi, TMV severities were 16.4 and 33.5% lower in plants treated with 2.5 and 5% BC, respectively, compared to the controls (Fig. 4).

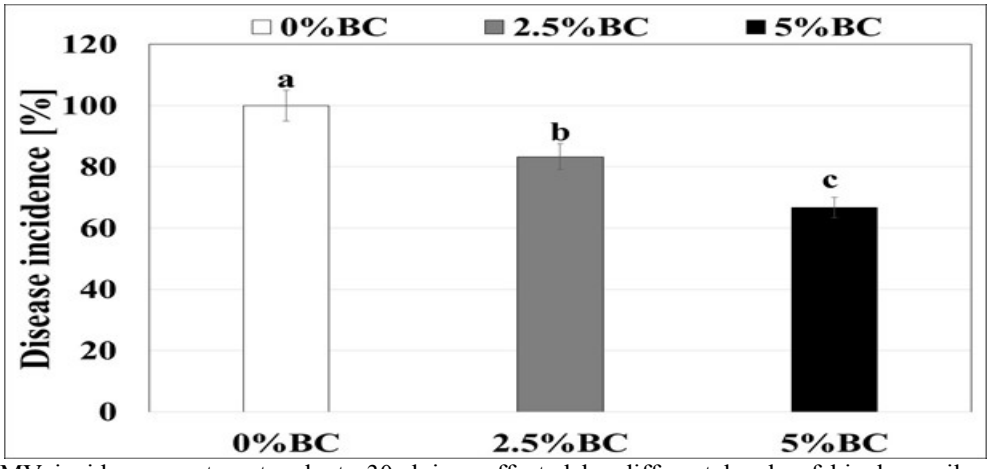

Fig. 3: TMV incidence on tomato plants 30 dpi as affected by different levels of biochar soil application. Significant differences ($P \leq 0.05$) between biochar treatments are indicated by different letters as evaluated by Duncan's multiple range test.

Table 1: Effect of biochar soil application on some morphological traits of tomato plants upon infection with TMV. Ph, plant height; PFW, plant fresh weight; Ln, leaf number per plant and La, Leaf area.

Treatments		Ph [cm]	PFW	Shoot/root ratio	Ln per plant	La [cm^2]
Non-infected	0% BC	18.000^{a} ±2.646	8.967^{a} ±0.0643	4.056^{a} ±0.471	5.667^{a} ±0.577	28.203^{a} ±4.590
	2.5% BC	27.000^{b} ±1.000	10.150^{b} ±2.192	2.050_{b} ±0.552	7.000^{b} ±0.000	41.421^{b} ±3.892
	5% BC	32.333^{c} ±2.517	13.740^{b} ±4.573	2.720^{b} ±0.547	7.667^{b} ±0.577	46.843^{b} ±11.244
TMV-infected	0% BC	15.723^{a*} 1.109	6.263^{a*} ±1.421	3.926^{a} ±0.244	5.500^{a} ±0.707	16.118^{a*} ±4.473
	2.5% BC	21.5000^{b*} ±0.707	9.167^{b} ±0.231	3.668^{a*} ±0.708	7.000^{b} ±1.000	16.390^{a*} ±1.273
	5% BC	28.667^{c*} ±1.528	11.367^{c} ±1.739	4.887^{a*} ±0.729	7.333^{b} ±0.577	31.680^{b*} ±6.765

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

The same trend was also found at 30 dpi, where plants amended with 2.5 and 5% BC exhibited 38.0 and 52.5% reductions in disease severity (Fig. 4).

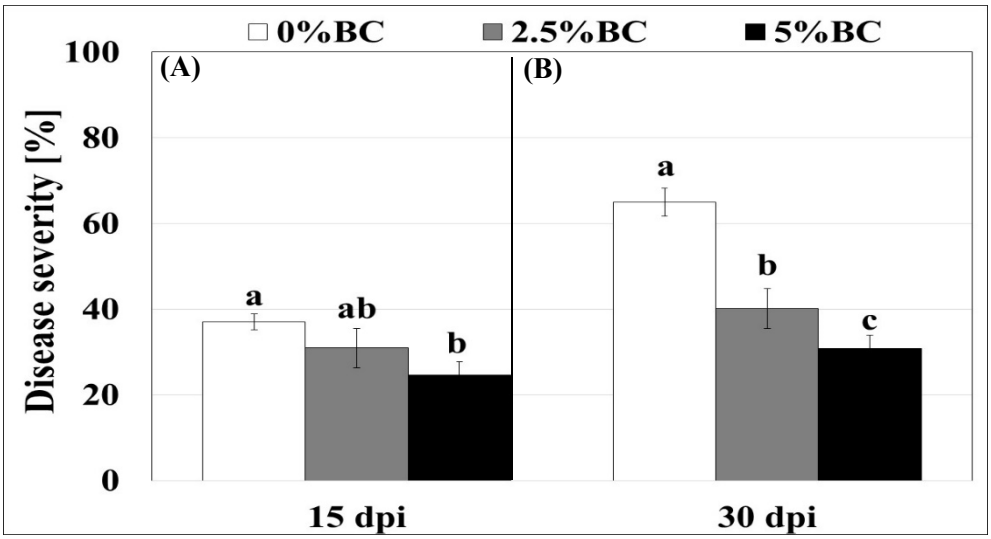

Fig. 4: TMV severity on tomato plants at 15 dpi (A), and 30 dpi (B) in response to different levels of biochar soil application. Significant differences ($P \leq 0.05$) between biochar treatments are indicated by different letters as evaluated by Duncan's multiple range test

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA)

Without biochar, the amounts of TMV coat protein in infected leaves were 4-folds and 9-folds higher at 15 and 30 dpi, respectively, compared to healthy controls (Fig. 5). Treatment of 2.5% BC distinctly declined TMV concentrations by 24.3 and 46.4% at 15 and 30 dpi, respectively, as compared to the respective controls (Fig. 5). Likewise, biochar applied at 5% significantly reduced TMV concentrations by 41.1 and 60.4% at 15 and 30 dpi, respectively (Fig. 5).

Total phenolic compounds (TPC)

TMV infection significantly ($P \leq 0.05$) increased TPC by 35.0% in the leaves of plants grown without biochar (Fig. 6). TPC concentrations were significantly enhanced as soil BC level rose, both

in non-inoculated and TMV-infected plants, with more obvious effects on infected ones. In absence of pathogen, TPC contents were 22.6 and 93.0% higher in plants treated with 2.5 and 5% BC, respectively (Fig. 6). TMV-infected plants grown at 2.5 and 5% BC showed 79.6 and 43.6% higher TPC concentration in their leaves compared to non-amended healthy controls (Fig. 6).

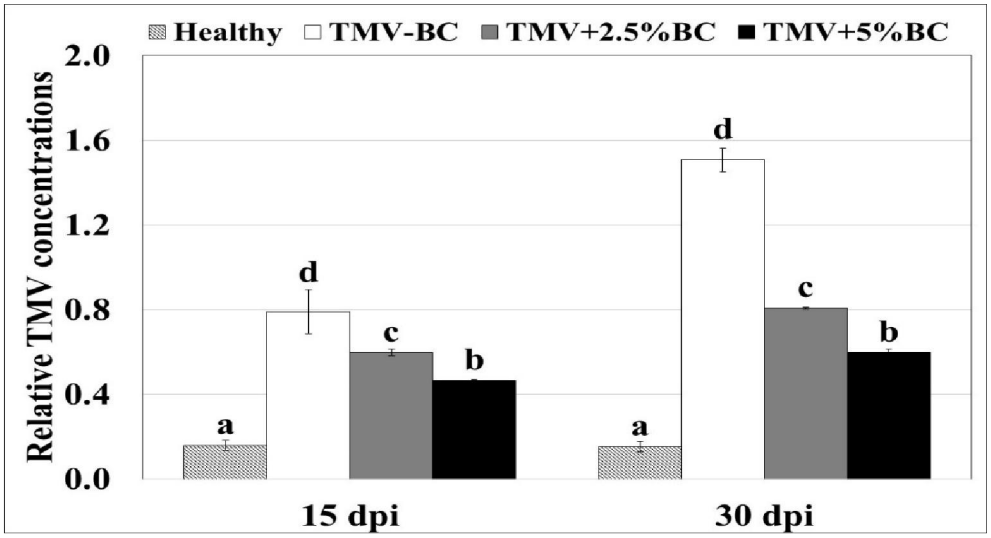

Fig. 5: Relative TMV concentrations at 15 dpi (A), and 30 dpi (B) in the leaves of tomato plants grown under different levels of biochar soil applications. Significant differences ($P \leq 0.05$) between biochar treatments are indicated by different letters as evaluated by Duncan's multiple range test.

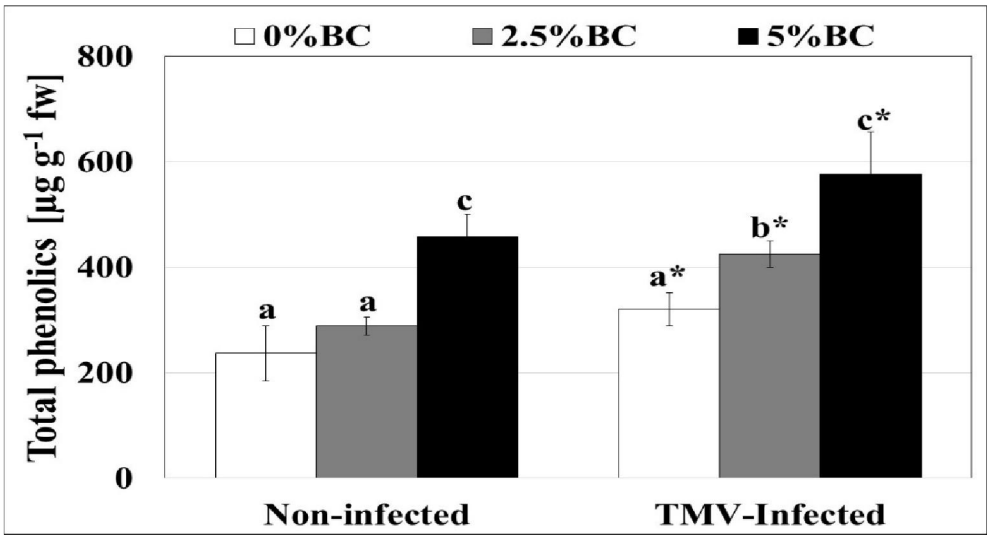

Fig. 6: Effect of biochar soil amendment on the total phenolic compounds in the leaves of non-infected and TMV-infected tomato plants. Significant differences ($P \leq 0.05$) between biochar treatments are indicated by different letters as evaluated by Duncan's multiple range test.

Peroxidase (POX) activity

Activity of POX in the leaves was significantly increased by 48.8% in response to TMV infection (30 dpi), as compared to controls (Fig. 7). The same trend of enhanced activity was observed upon BC addition for both non-infected and TMV-infected plants. Non-infected plants treated with 2.5 and 5% BC showed 58.1 and 75.2% higher POX activity, relative to their corresponding controls (Fig. 7). Furthermore, TMV-infected plants exhibited significant increases of about 33.2 and 61.0% in POX activity when grown at 2.5 and 5% BC, respectively, compared with respective controls (Fig. 7).

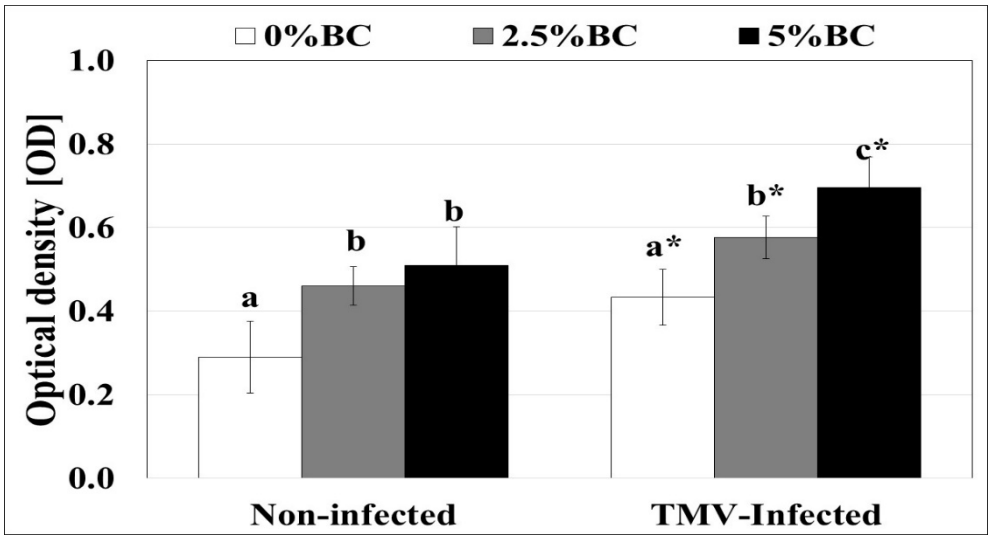

Fig. 7: Effect of biochar soil amendment on PO activity in the leaves of non-infected, and TMV-infected tomato plants. Significant differences ($P \leq 0.05$) between biochar treatments are indicated by different letters as evaluated by Duncan's multiple range test.

Discussion

This study delivers, for the first time, evidence that biochar is able to reduce TMV incidence and severity in tomato plants. Based on the visible symptoms, the initial symptoms of discoloration and mosaic were observed on the leaves of inoculated plants 7 dpi in un-amended pots. However, these symptoms occurred 10 dpi in biochar-amended plants, suggesting that biochar amendment, both at 2.5 and 5%, could delay TMV development. This was supported by the trends of disease incidences, which were markedly reduced by 16.7 and 33.3% upon treatment with 2.5 and 5% BC, respectively. Furthermore, TMV severity was declined in biochar-treated plants, both at 15 and 30 dpi, the effect that was more pronounced for plants treated with 5% BC, 30 dpi (Fig. 4). This biochar level resulted in substantial reductions of 33.5 and 52.5% in disease severity at 15 and 30 dpi, respectively, compared to non-amended plants. Together, these results indicate that biochar addition has greatly suppressed TMV development in tomatoes, enhancing therefore their resistance. This conclusion is further confirmed by DAS-ELISA analysis, which showed that 2.5 and 5% BC distinctly declined the relative TMV concentrations by 24.3 and 41.1%, respectively, at 15 dpi. This effect was more obvious at 30 dpi, where 2.5% and 5% BC reduced TMV concentrations by 46.4 and 60.4%, respectively. Similarly, in several other plant-pathogen interactions, biochar suppressive effect was found to be dose-dependent (Harel *et al*., 2012). Despite the overwhelming interest in biochar as a promising approach to enhance disease resistance, the exact mechanisms underlying its contribution are poorly understood. Several mechanisms operating in tandem or synergistically both at cellular and whole-plant levels might account for biochar-suppressive effects (Graber *et al*., 2010). Considering that biochar was soil-implemented and its inhibitory effects against TMV were observed on the leaves, one can speculate that biochar was able to induce systemic resistance response in tomato plants. Induced systemic resistance (ISR), a physiological state of enhanced defensive capacity elicited by specific stimuli, is reported to be effective against a wide range of pathogens (Vallad and Goodman, 2004; Jones and Dangl, 2006). A number of potential ways has been proposed to explain how biochar can induce systemic plant defenses (Graber *et al*., 2010; Elad *et al*., 2010; Harel *et al*., 2012; Khalifa and Thabet, 2015). According to Graber *et al*. (2014), nutrients delivered or became more available by biochar addition could enhance plant growth and help maintaining higher level of inhibitory compounds within plant tissues, thus enabling higher resistance to pathogen attack. As shown in Figures (1 and 2) and Table 1, biochar application improved all growth parameters of both healthy and TMV-infected plants (i.e. plant height, plant fresh weight, leaf number, leaf area) when compared to un-treated controls. High biochar level (5%) led to significant ($P \leq 0.05$) increases of up to 82.3%, 81.5%, 32.7% and 96.5% in plant height, fresh weight, leaves number and leaf area, respectively (Table 1). Consistent with previous observations on the efficacy of biochar on some soil-

borne fungi (Khalifa and Thabet, 2015), biochar-induced growth stimulation might alter the ability of TMV to infect the plant, hence improving plant resistance. In agreement with several researchers (i.e. Lehmann *et al*., 2011; Quilliam *et al*., 2013; Dai *et al*., 2017), the porous structure of biochar, its high surface area and ability to adsorb nutrients and water might affect the microbial activities in the rhizosphere, as well as the abundance of several groups closely related to biocontrol. This assertion is also supported by the findings of Wang *et al*. (2009a) who reported that some *Bacillus* spp. strains, well-known as plant growth-promoting rhizobacteria (PGPR), markedly improved the plant height and fresh weight, while reducing the TMV severity and its relative concentration in tomato plants. ISR involves also the activation of wide variety of biochemical pathways responsible for the accumulation of different defense-related secondary metabolites and oxidative bursts (Asselbergh *et al*., 2008).

Here, TPC concentrations were substantially increased with increasing biochar level, both in non-inoculated and TMV-infected plants. Furthermore, TPC contents were significantly highest in TMV- infected plants treated with 5% BC (Fig. 6). These results are in agreement with earlier reports demonstrated that phenolic contents were increased in different plants after infection with pathogens (Rai *et al*., 2010; Siddique *et al*., 2014). Biochar-induced increase in TPC is suggested to accelerate the phenol synthesizing pathways involved in the mechanical strength of host cell walls, leading, in turn, to block the spread of pathogens (Ngadze *et al*., 2012; Singh *et al*., 2014). Enhanced lignification is generally associated with higher activity of some key enzymes such as peroxidase (POX), one of the first enzymes responding and providing fast defense against plant pathogens (Nicholson and Hammerschmidt, 1992; Sulman *et al*., 2001). Here, POX activity was significantly enhanced upon biochar treatment both in non-inoculated and TMV-inoculated plants, the effect which was much more obvious for TMV-inoculated plants grown at 5%BC. Induction of POXs in response to pathogen inoculation has been reported in several pathosystems and a higher increase was observed in resistant plants compared to susceptible ones (Mydlarz and Harvell, 2006; Houterman *et al*., 2007; Khalifa and Thabet, 2015). In plant-virus interactions, POXs activity was also increased in tobacco - TMV (Lagrimini and Rothstein, 1987; Ye *et al*., 1990), beans - white clover mosaic potexvirus (Clarke *et al*., 2002) and pumpkin - cucumber mosaic virus and zucchini yellow mosaic virus (Radwan *et al*., 2007). POXs is known to participate in many reactions such as polysaccharide bonds, oxidation of phenols, suberization and lignification of cell walls during the defense reaction (Carvalho *et al*., 2006; Maksimov *et al*., 2014). Hence, the higher activity of POX in biochar-amended plants observed in this study might contribute to lignification process, which is considered as a resistance mechanism against pathogen attack.

In brief, results of the present study delivered, for the first time, evidence that biochar was able to suppress TMV infection in tomato plants, the effect which was obvious for plant amended with 5%. The suppressive effect of biochar might be attributed to an induced systemic resistance (indicated by higher TPC concentrations and higher POX activity in the leaves of tomato plants treated with 5%BC). Finally, it should be mentioned that this study could be a step forward in biochar utilization as a promising environmental-friendly disease management approach. However, further studies are needed to unravel the individual mechanisms responsible for biochar induced systemic resistance TMV-tomato pathosystem as well as other phytopathosystems.

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