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Full Length Article

Biochar Application from Different Feedstocks Enhances Plant Growth and Resistance against *Meloidogyne incognita* in Tomato

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Abstract

Activated charcoal (biochar) is well known as carbon sequestering compound which acts as plant growth promoter and suppressor of soil borne pathogens. Tomato plant is affected by number of microbial pathogens including root-knot nematodes (RKNs) which belongs to genus *Meloidogyne*. *Meloidogyne incognita* is one of the most devastating pests infecting tomato roots and causes significant losses in quality and quantity. To manage these losses new strategies are required to be developed and adopted to strengthen plant growth and control RKNs populations in the soil. Current study was designed with the hypothesis that biochars from different feedstocks may lead to increased resistance against *M. incognita* in tomato. For this purpose, biochars from three different feedstocks *i.e*., wheat straw (WS), rice husk (RH) and sugarcane bagasse (SCB) were evaluated for their potential against *M. incognita* under *in vitro* and pot trials. Surface structure analysis of all the biochars was done using Scanning Electron Microscope (SEM) and substance structure analysis through x-rays diffraction (XRD). Five different concentrations of exudates (0.3, 0.5, 1.2, 2.3 and 3%) from all three biochars were assessed under *in vitro* conditions for their nematicidal efficacy. The exudates of the selected biochars at different levels were directly applied on freshly hatched juveniles but there were no direct toxic effects of biochar exudates on nematodes mortality observed. Similarly, three different concentrations *viz*., 2, 3 and 5% along with positive control (+Nematode) and negative control (-Nematode) were added to the tomato plants in pot experiment. Among all concentrations 3% RH biochar exhibit significant improvement in plant morphological traits like shoot length, shoot fresh weight and dry weight. Similarly, number of galls, egg masses and number of females were also reduced maximum in 3% RH treated plants as compared to control. While root length, root fresh weight and root dry weight were increased in 5% SCB. The results demonstrate the significance of biochar application for controlling RKNs in tomato and other crop plants.

Keywords: Biochar; *Meloidogyne incognita*; Growth promotion; Soil amendment; Tomato

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a short duration, high yielding crop as compare to others and due to its high profit, it has become more popular and the area under its cultivation is increasing day by day (Naika *et al*. 2005). Tomato crop is attacked by a large number of plant pathogens; however, plant parasitic nematodes are major pests of tomato crop worldwide.

Plant-parasitic nematodes are the obligate, microscopic, bio-trophic organisms causing destructive yield loss in vast range of crops such as potato, chickpea, tomato, wheat, sugar beet *etc*. Worldwide the total annual yields losses due to plant-parasitic nematodes occur in various crops have been estimated over $157 billion (Abad *et al*. 2008). Plant-parasitic nematodes can cause yield losses up to 20% and they cast disturbing effect on farmer in developing countries (Atkinson *et al*. 1995). RKNs feed inside the root system and form galls which consist of numerous giant cells on the vasculature of the plant roots. These giant cells are the only source of nutrition for the sedentary stages of RKNs (Jones and Payne 1978; Ali *et al*. 2015). Among a huge number of RKNs species, *M. incognita* is the most dangerous specie of nematodes which can parasitize almost all the cultivated crop plants including tomato (Abad *et al*. 2008).

M. incognita results in significant losses in both quality and quantitiy of the tomato produce and some potential nematode strategy must be developed to minimize these losses. Various strategies have been employed for controlling parasitic nematodes in plants (Ali *et al*. 2017). Synthetic chemicals have been widely used in controlling parasitic nematodes in the field which have ultimate health and environment hazards. Soil amendments with different organic

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and inorganic material have been largely used. Recently several scientists experimented the application of biochar for controlling various pathogens in different crop plants.

Biochar (BC) is a charcoal like material which has excessive amount of carbons and its derivatives. It is acquired by the burning of organic biomass at very high temperature in a special type of furnace. It can be helpful for the sequestration of carbon by adding huge quantity of carbon or burned organic compounds into the soils which are hard to decompose (El-Naggar *et al*. 2019). Biochar remains in the soil for a very long duration and can capture atmospheric carbon for a prolonged period of time (Elad *et al*. 2010; Vaccari *et al*. 2011). It is reported that biochars obtained from different feedstocks led to increase in plant biomass and economic yields in different crop plants (Hussain *et al*. 2017). For instance, Kammann *et al*. (2012) observed a significant increase in biomass in biochar amended soils as compared to controls in perennial ryegrass (*Lolium perenne* L.). Similarly, Rondon *et al.* (2007) displayed that addition of biochar to a tropical, low-fertility soil led to 22% enhanced nitrogen fixation in bean plants (*Phaseolus vulgaris*) in addition to significantly improved biomass production and bean yield. Graber *et al.* (2010) reported that improved plant growth and fruit yield in pepper and tomato due to biochar amendment may shift the microbial community structure towards beneficial plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF) in pepper.

The literature displays the promising effects of biochar to induce systemic resistance against many plant pathogens (Elad *et al.* 2010; Vaccari *et al.* 2011; Zwart and Kim 2012). For instance, biochar application in the soil led induced resistance in two tree species (*Quercus rubra* and *Acer rubrum*) against stem lesions caused by *Phytophthora* spp. (Zwart and Kim 2012). Likewise, soil amendment with biochar reduced the susceptibility of pepper and tomato plants against some foliar fungal pathogens like *Leveillula taurica* and *Botrytis cinerea* in addition to reduced attack of broad mites in pepper plant (Elad *et al*. 2010). Moreover, application of biochar in potting material under different conditions led to enhanced resistance in rice against root knot nematode, *M. graminicola* (Huang *et al*. 2015). By keeping these reports in mind, the present study was designed to control RKN and improve plant growth and development through the application of biochar in tomato. For this purpose, the potential of biochar from different feedstocks was assessed against RKN *M. incognita* in tomato under *in vitro* and pot experiments.

Materials and Methods

Pyrolysis of feedstocks for biochar production

Three feed stocks *i.e.*, sugarcane bagasse, rice husk and wheat straw were used for the production of biochar. Wheat straw was collected from the agricultural farm of department of plant Pathology, University of Agriculture, Faisalabad while rice husk and sugarcane bagasse were taken from rice dehusking and sugar mills, respectively. These feed stocks were sun dried to retain moisture up to 15% before biochar preparation.

The feedstocks were pyrolyzed at temperature 450°C with retention time of 30 min after a gradual rise of 10°C min^{-1} for production of biochars in a laboratory setup Muffle furnace (Gallonhop, England) following the protocol described by Sanchez *et al*. (2009). The biochar was ground and sieved before further experimentation and analysis.

Scanning electron microscopy (SEM) & X-rays diffraction (XRD)

The structural and surface analysis of the particles of three biochars was visualized by mean of SEM. For this purpose, the samples were observed under 30KV Scanning Electron Microscope (JSM5910, JEOL, Japan) at 500,1000,5000 and 10,000x. SEM was performed to examine the structural properties of the samples. The XRD pattern was developed through XRD spectroscopy using a X-ray Diffractometer (Philips PANalytical X'Pert Powder) with CuKα source radiation (λ = 1.5405 Å) over the angular range $20° \leq 2\theta \leq 80°$, operating at 30 kV and 10 mA. The scanning peaks were collected in the range of 20~80°, then the analysis of the data was done through MDI Jade 6.0.

Extraction and identification of nematodes

Infected roots of tomato were collected from the field area of Department of Plant Pathology. These infected roots were washed to clean the debris. The roots were cut into small pieces and put into a jar. 10% solution of NaOCl was prepared and added into the jar and jar was shaken well for 4 min. Three sieves were arranged in order from top to bottom (150, 250, 400 mesh. After that jar solution including roots was poured on the upper sieve and washed enough to remove NaOCl. Eggs were collected from the last (400 mesh) sieve and placed them on filter paper which touched the water surface of a tray. The filter paper was covered and incubated for 48 hours at 23°C. Freshly hatched *Meloidogyne incognita* juveniles were identified under automated upright microscope (LEICA DM5500 B) using software name (LAS V4.12). 2^{nd} stage juveniles (J_2) were identified on the basis of body length (346–463 μm), tail length (40–60 μm), (Dorsal esophageal gland orifices) DGO to stylet base (2–3 μm) and tail shape by Karssen (1996). Larvae counting was done using stereoscope.

In-vitro experiment

Suspension of 1 mL freshly hatched juvenile (200 J2) was incubated with 1 mL of biochar exudate taken from various concentration of biochar (0.3, 0.5, 1.2, 2.3 and 3% from each of the 3 feed stocks *i.e.*, wheat-straw, rice husk and

sugarcane bagasse) in 3.5 cm span wells on a 6 well culture plates. These exudates and their concentrations were prepared and used according the method reported by Huang *et al*. (2015). After 24 h, 48 h and 72 h, 1 N NaOH was added into the suspensions, and nematode that retorted to NaOH by change their bodies shapes within 3 min were consider alive, however nematodes which failed to move after adding NaOH were consider as dead according to Chen and Dickson (2000). This experiment was performed in 6 replications for each feedstock to acquire reliable results.

Growing and transfer of tomato nursery

Tomato cultivar *i.e*., Money Maker, was grown in the pots in the sandy loam soil with the percentage of sand =75%, silt=23% and clay=7%. Three biochars with three concentrations 2, 3 and 5%, respectively were used for the amendment into the potting soil. Each concentration was mixed in 20 g, 30 g and 50 g w/w in the pot capacity of 1000g soil to make concentrations. Before this, soil was properly sterilized with formalin @ 1:5 for a week to minimize the soil nematodes population. After 3 weeks of sowing, tomato seedlings were transplanted in earthen pots (1 kg capacity) with 3 treatments, 3 concentrations, 4 replications and leaving two controls of each which had neither biochar no-inoculum as negative control and the other was +ve control which had only nematode inoculum in completely randomized design fashion (CRD). Plants were irrigated regularly along with appropriate fertilization.

Nematode inoculation

Two-week-old tomato nursery was inoculated with newly hatched juveniles (J2) of *M. incognita* at the rate of 1000 larvae per plant. Nematodes were inoculated in pot by making three holes with a glass rod around the stem of the plant in soil near root zone without damaging root system.

Effect of various biochar treatments on plant growth parameters under nematode infection

Plant growth parameters like plant height (cm), fresh shoot weight (g), dry shoot weight (g) were recorded after 6 weeks of inoculation. Similarly, below ground parameters like root length (cm), root fresh and dry weights (g) were measured. Additionally, disease parameters were also recorded at the termination of experiment after 8 weeks of inoculation. To measure dry weights, samples were dried at 80°C in dry oven for 48 h.

Assessment of nematode development on plant roots in various treatments

The data regarding various parameters like galling index, egg mass index, number of females were evaluated after 8 weeks post inoculation in different treatments of biochar application. Plants were uprooted and taken to the lab and the roots rinsed with tap water to remove debris. Galls were evaluated by the scale of 0–10 described by Bridge and Page (1980) Egg masses were stained using phloxine B (Holbrook *et al*. 1983) and quantified using a scale of 1–9 where: 1 = no egg masses, 2 = 1–5, 3 = 6–10, 4 = 11–20, 5 = 21–30, 6 = 31–50, 7 = 51–70, 8 = 71–100, and 9 = >100 egg masses per root system (Sharma *et al*. 1994). Number of females was counted visually by using stereomicroscope in a root system.

Statistical analysis

The collected data were subjected to factorial analysis of variance (ANOVA) under completely randomized design (CRD) using Statistix 8.1. The means of various treatments were compared using least significant difference (LSD) test at 95% level of confidence ($P \leq 0.05$). Moreover, percentage decrease was calculated from control to assess the efficacy of various treatments for nematode control in tomato.

Results

SEM and XRD of biochars from different feedstocks

The formation of a bundle shaped tube on RH biochar created uneven sized pores having a diameter range between 50 µm when visualized at four different magnifications, pores were well distributed they may have capacity to provide habitat to a number of beneficial microorganisms shown in (Fig. 1b). On the other hand, the pores of SCB were seemed rough at 500x while under 1000x and above, the pores looked like in an arranged manner (Fig. 1c). Compared with biochar from WS under same magnifications appeared tiny carbon slices or ash particles were clearly adhered with each other in scattered form (Fig. 1a).

X-rays diffraction (XRD) is a procedure by which we can analyze the structure of a substance and the abundance of elements present in it. The X-Ray diffraction patterns of various biochars from different sources are displayed in Fig. 2. It is obvious that the only high peak in the RH derived biochar at 2θ=72.3° shows the presence of high amorphous silica present in it while the other peaks show no substantial height. It means the substance which was made on temperature 450° is sub-crystalline in nature shown in (Fig. 2B). However, in case of SCB biochar XRD analysis, broad peaks were located between 23.3–40.4° which indicates the amorphous nature of a substance and some inorganic compounds like (C-200, K_2SO_4, Ca $(OH)_2$, MnO_2 and SiO_2) where the highest peak was again silica diffraction observed at 72.5° has shown in (Fig. 2C). On the other hand, WS showed more crystalline nature than the other two biochars. The small sharp peaks are clearly obvious between the 27.9 –68.3° and mostly represent the inorganic compounds which exist in oxide form (SiO_2, MnO_2, Al_2O_3) and some other compounds like (K_2SO_4, Ca $(OH)_2$, and KCl) are also present in wheat straw biochar. The highest peak was observed at 26.5° for SiO_2 (Fig. 2A).

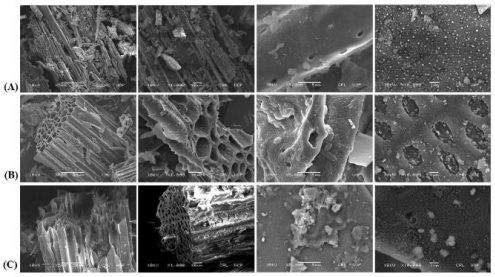

Fig. 1: Images of different biochars under scanned electron microscope (**A**) WS at the magnification of 500, 1000, 5000 and 10,000x (from left to right) (**B**, **C**) RH and SCB with the same magnification respectively

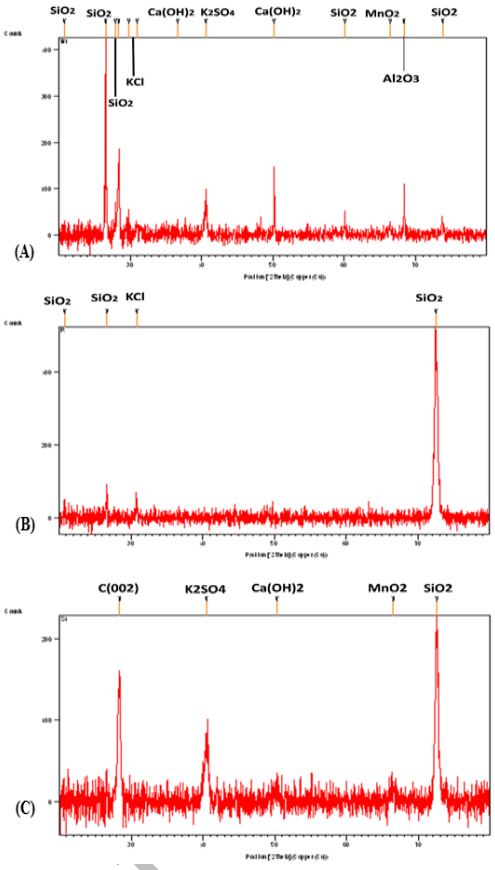

Fig. 2: X-rays diffraction analysis of three biochar to check the structural nature of biochars. Where **A**: XRD of WS, **B**: XRD of RH and **C**: XRD of SCB

Nematicidal potential of different biochars through *in vitro* experiment

To assess the toxic effects of biochars on *M. incognita*, freshly hatched 2nd stage juveniles (J2) were incubated with the different concentrations (0.3, 0.5, 1.2, 2.3 and 3%) of exudates from three different biochar and nematodes incubated in distilled water were kept as control (Fig. 3). After 24, 48 and 72 h, significant

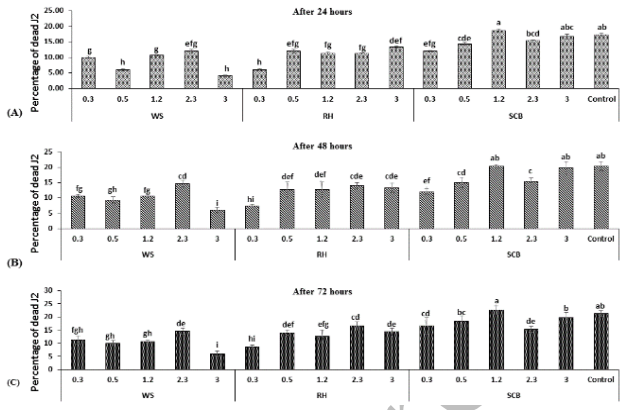

Fig. 3: Percentage of dead nematodes showed in different concentrations (0.3, 0.5, 1.2, 2.3 and 3% of WS, RH and SCB along with control (nematodes incubated in only water) (**A**) After 24 h (**B**) After 48 hours (**C**) After 72 h

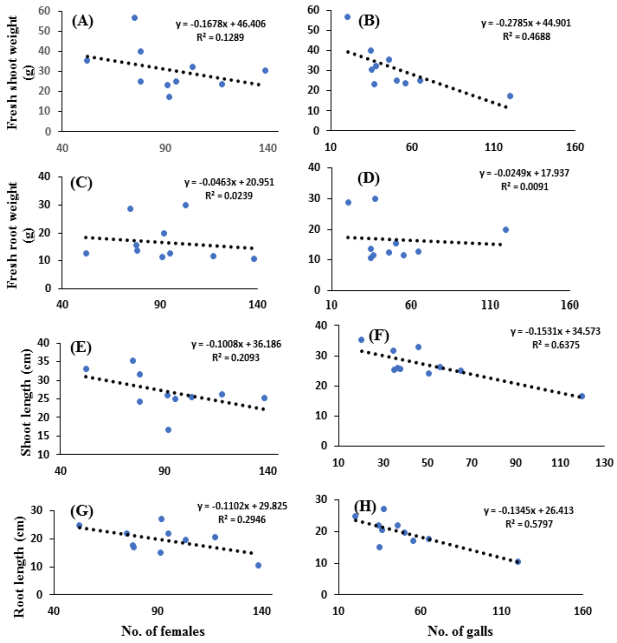

Fig. 4: Regression analysis between No. of females and No. of galls with (**A**, **B**) Fresh shoot weight (g); (**C**, **D**) Fresh root weight (g); (**E**, **F**) Shoot length (cm); (G, H) Root length (cm) during the treatments of three biochars

nematode mortality was found in WS, RH and SCB exudates of above-mentioned concentrations. Very minute difference in mortality was observed in treated and control plates. After 24 h maximum dead juveniles were evaluated in (1.2% SCB, control and 3% SCB) respectively shown in (Fig. 4A). Similarly, the maximum death count after 48 h was observed in same plates mentioned earlier (Fig. 4B). While after 72 h maximum mortality of nematodes were observed in 1.2% SCB and control plates shown in (Fig. 4C) So, the data revealed that biochars have somehow statistically significant

Table 1: Effect of biochar concentrations on RKN population and plant biomass parameters

Treatments	SL*	RL*	FSW*	DSW*	FRW*	DRW*
WS 2%	25.3 b	15.0 de	30.3 cde	16.6 bc	10.8 c	2.7 d
WS 3%	26.0 b	20.6 bc	23.1 f	12.8 bc	11.5 c	4.5 bc
WS 5%	26.3 b	17.0 cd	23.9 f	15.0 bc	11.7 c	3.2 cd
RH 2%	31.6 a	22.0 abc	39.9 b	16.2 bc	13.5 c	3.8 bcd
RH 3%	35.3 a	25.0 ab	56.7 a	22.4 a	28.7 a	7.9 a
RH 5%	33.0 a	22.0 abc	35.6 bc	17.1 b	12.6 c	2.9 cd
SCB 2%	25.0 b	17.6 cd	24.9 f	12.6 c	12.7 c	2.5 d
SCB 3%	24.3 b	19.6 bcd	25.1 ef	13.2 bc	15.5 bc	3.5 cd
SCB 5%	25.6 b	27.3 a	32.5 cd	16.6 bc	30.1 a	9.3 a
Positive control	16.6 c	10.6 e	17.4 g	6.4 d	19.8 b	5.5 b
Negative control	23.0 b	15.0 de	27.3 def	13.0 bc	15.3 bc	4.6 bc
LSD	4.82	5.58	5.32	4.4	5.6	1.83

Values are mean of four replicated plots. Means followed by different letter(s) within a column are significantly different using LSD at $P = 0.05$
*SL= Shoot length, *RL= Root length, *FSW= Fresh shoot weight, *DSW= Dry shoot weight, *FRW= Fresh root weight, *DRW= Dry root weight

Table 2: Effect of different biochar's concentrations on RKN population

Treatments	Females/root system	Decrease over control % (females)	Egg Masses/root system	EMI*	Decrease over control % (egg masses)	Galls/root system	GI*	Decrease over control % (galls)
WS 2%	91.3 cd	33.9	68.0 f	7.3 c	60.1	35.0 e	4.0 cd	70.8
WS 3%	117.6 b	14.9	100.3 cde	8.3 ab	41.2	36.6 de	3.6 de	69.5
WS 5%	78.3 de	43.3	120.6 b	9.0 a	29.3	55.6 bc	5.0 bc	53.6
RH 2%	75.0 e	45.7	71.0 f	7.6 bc	58.3	34.6 ef	2.6 ef	71.1
RH 3%	52.3 f	62.1	38.3 g	6.0 d	77.5	20.3 f	2.3 f	83.1
RH 5%	95.3 c	31.1	89.3 e	8.0 bc	47.6	46.0 cde	3.3 def	61.6
SCB 2%	78.0 de	43.6	108.6 bc	8.0 bc	36.3	65.0 b	5.3 b	45.8
SCB 3%	103.3 bc	26.3	102.3 cd	8.3 ab	40.1	50.6 bcd	4.3 bcd	57.8
SCB 5%	92.0 cd	32.7	93.0 de	8.0 bc	45.4	37.6 de	3.6 de	68.6
Positive control	138.3 a	0	170.6 a	9.0 a	0	120.0 a	6.6 a	0
Negative control	-	-	-	-	-	-	-	-
LSD	16.25	-	12.38	0.82	-	14.63	1.16	-

Values are mean of four replicated plots. Means followed by different letter(s) within a column are significantly different using LSD at $P = 0.05$ *EMI= Egg mass index, *GI= Galling index
Reduction % = [(Cf (control) - Cf (treatment)/Cf (+ control)] × 100

effects on nematode mortality but not efficiently reduced as compare to control.

Incorporation of biochar in potting material leads to enhanced plant growth

The data regarding the effect of different concentration of various biochars on plant growth parameters are given in Table 1. The results demonstrated that shoot length was significantly ($P < 0.05$) increased in all of the RH treatments including 3, 5 and 2% concentrations of this biochar. While, the lowest value of shoot length was recorded in positive control (16.6 cm) where only nematodes were applied. For the FSW and DSW the 3% of RH found best with maximum mean values of 56.7 g and 22.4 g respectively. However, the least values in FSW and DSW after positive control were found in 3% of WS and 2% SCB respectively. Similarly, for the root system parameters like RL, FRW and DRW demonstrated significant results at 95% level of confidence for different treatments of biochars. Significantly highest values of RL, FRW and DRW (27.3 cm, 30.1 and 9.3 g respectively) were observed in 5% of SCB. Likewise, 3% RH showed the increasing value in RL, FRW and DRW after 5% SCB with mean values (25.0, 28.7 and 7.9 g). However, the FRW and DRW in positive control demonstrated minimum values (19.8 and 5.5) respectively due to root abbreviation and heavy galling on the roots. Moreover, the lowest values after positive control were found in 2% WS with 15.0 cm, 10.8 g and 2.7 g beside this in negative control plants having no biochar and no inoculum showed overlapping values with some treatments of biochars (Table 1).

Addition of biochars led to enhanced resistance against *M. incognita* in tomato

Various nematode reproduction and establishment attributes were studied in response to different concentrations of biochars (Table 2). Number of galls, number of egg masses per root system, galling index, egg mass index and number of females were significantly ($P < 0.05$) different in all the treatments. The lowest number of females, galls, galling index, egg masses and egg mass index were found in 3% RH treatment. Minimum number of females (52.3) was examined in 3% RH while the highest number of egg masses were recorded in 5% WS which were (120.6) while the nematode control treatment showed maximum value for egg masses (170.6). In addition, highest number of females among all the biochar treatments were recorded in 3% WS with mean value (117.6). Likewise, maximum

number of galls in treated plants was recorded in 2% SCB. Beside this the highest number of nematode establishment based on number of females, egg masses and number of galls in root system with values (138.3, 170.6 and 120) respectively was examined in positive control; however, the negative control displayed no infection of nematodes due to absence of nematode inoculum. Percentage of decrease of nematode parameters was determined and number of females, egg masses and galls were 62.1, 77.5 and 83.1% decreased, respectively as compared to positive in 3% RH treatment. Response of various growth parameters was correlated with different nematode development parameters. Fresh shoot weight, fresh root weight, shoot length and root length showed a decreasing trend in response to increasing number of females and number of galls in tomato (Fig. 4). Although, fresh root weight showed little effect, it increased in females and galls (Fig. 4C–D).

Discussion

Surface and structural assessment of various biochars was done using SEM and XRD techniques. SEM and XRD analyses demonstrated that the wheat biochar displayed sub-crystalline nature and exhibited more inorganic compounds as compare to other two biochars. While in rice husk biochar the silica was present in high concentration which might be responsible for improving plant biomass when it absorbs into the soil which highly favors the findings in the present study. This could be due the reason that formation of phytolith in plants is highly dependent on availability of silica (Nawaz *et al*. 2019). Moreover, the silica compounds would not loss after pyrolysis process (Houben *et al*. 2013). Our findings showed that other miscellaneous inorganic compounds were absent in rice husk biochar which were present in other biochars. Sub-crystalline nature of WS biochar developed at high pyrolysis temperature displayed high peaks for silica and K_2SO_4 which is in accordance with the results of Yuan *et al*. (2011). Most of the compounds present in SCB and WS biochar were in oxide form. These results were comparable with some recent findings based on XRD analysis of different biochars regarding the possible abundance of different compounds at different theta angles (Trubetskaya *et al*. 2016: Mohan *et al*. 2018).

Plant pathogenic nematodes are notorious pests of economically important crop plants (Ali *et al*. 2015). Various management practices have been used to control the nematode population below the damaging levels including cultural, chemical, biological and transgenic approaches (Ali *et al*. 2017). To investigate the potential of biochar as a significant nematode control strategy, we conducted a series of experiments including *in vitro* nematicidal assay and pot experiments. To check the nematicidal effect of different biochars, the exudates of biochar were obtained and assessed for *in vitro* nematicidal effects on J2s of *M. incognita*. The result of this experiment concluded that there are no such chemicals present in the tested biochar, which can have direct effects on nematode mortality. Although, very minute difference in nematode mortality has been found between treated and control treatments. Similar experiment was designed by the Huang *et al*. (2015) who also reported no direct effect of biochar exudates on *M. graminicola* at different concentrations. Moreover, very recently *in vitro* treatments of biochar did not show any direct toxicity or suppression in mycelia of two fungi pathogenic to tomato plant (Jaiswal *et al*. 2018).

Real time efficacy of various biochars with different concentrations was investigated through a pot experiment based on soil application of the biochars. Soil amendments have largely been used for the purpose of pest and disease control in different crop plants. Amendment of soil with biochar led to enhanced plant growth due to sequestration of carbon into soil which not only improves soil fertility but also plays vital role in suppressing the soil borne pathogen populations (Lehmann and Joseph 2009; Zimmerman 2010). Our results demonstrated that biochar amendment led to significant improvement in the plant growth and development. Previously, application of biochar has increased plant growth in various plant species like pine and alder (Robertson *et al*. 2012), peanut (Agegnehu *et al*. 2015), tomato (Vaccari *et al*. 2015), wheat (Akhtar *et al*. 2015) and soybean (Sanvong and Nathewet 2014). However, contrastingly, some studies which demonstrated no significant effect of biochar on plant growth (Chan *et al*. 2007; Zwieten *et al*. 2010). Another study revealed that the biochar amendment in the soil can enhance the grapevines biomass in addition to reduction in nematode population by accumulating beneficial microbes in the soil (Rahman *et al*. 2014). A study conducted by Abrishamkesh *et al*. (2015) found that amendment of RH biochar can increase the above-ground and below-ground biomass of lentil plants which supports our results where RH biochar showed significant results in promoting overall plant growth. Moreover, increase in root length in tomato by adding biochar is also described by Vaccari *et al*. (2015). Another study reported enhanced root biomass in maize after biochar application (Yamato *et al*. 2006).

Our findings show that among different concentration of various biochar applied to the potting soil, 3% biochar concentration displayed the most promising results in reducing the nematode attack on plants. Bonanomi *et al*. (2015) used several concentrations of biochar against few soil borne diseases and found 3% as the most conducive treatment to control the studies plant diseases. However, in another study from Huang *et al*. (2015), 1.2% was concluded the best concentration in potting medium for controlling *M. graminicola* in rice plants. Recently, addition of nutshell biochar displayed reduction of *Meloidogyne* spp. population, egg masses and galls in addition to a significant increase in the overall performance of tomato plant (Ibrahim *et al*. 2018) and these findings support our results.

Our results showed that number of galls and females in roots were higher and plants showed partial wilting in

positive control (only nematodes) and they exhibited strongly negative correlation as shown in (Fig. 4E–F). This was in accordance with the results of Mitkowski and Abawi (2003) who reported stunted growth in lettuces due to dense galling by *Meloidogyne* spp. Our results showed that while increasing in number of females and galls had reduced the fresh shoot and root weight of the plant (Table 1) and (Fig. 4C–D) also agreed with the findings of Maleita *et al*. (2012) who concluded that plants which receive heavy inoculum exhibits stunted growth and yield. All three biochars treatments had significantly reduced number of egg masses as compare to control. Regression analysis showed (Fig. 4G) that number of females and root length were negatively correlated and it was in accordance with the report of Sharma and Sharma (2015), who indicated decrease in root length as a result of RKN infection in tomato plants. It has been studied (Anwar and Gundy 1993), that root weight increased due to penetration and formation of huge galls on the roots (Fig. 4C–D), which conforms to the present study in which root weight increased due to increasing the number of galls.

Enhancement of nematode resistance in biochar treatments could be due to number possible reasons. For instance, biochar amendment may increase soil pH that could lead to reduce the nematode population (Novak *et al*. 2009). Moreover, increase in soil pH led to the reduction of number of galls and egg masses with increasing the rate of biochar in potting medium (Ibrahim *et al*. 2018). Similarly, addition of biochar may accumulate beneficial microbes which could be helping to enhance the plant growth in addition to nematode control through antagonistic effects of these microbes against nematodes. Another important explanation could be the formation of phytoliths in plant roots due to high concentration of silica in all of the biochars. This phytolith formation provides physical barrier in the form of depositions in the cell wall matrix to hinder the entry of nematodes into the plant roots (Alhousari and Greger 2018). Moreover, defense response could be induced by silicon present in biochars to produce several enzymes which possibly can inhibit nematode establishment on the plant roots as observed in cucumber against *Pythium* spp.

Conclusion

Potential of biochar in management of root knot nematode was confirmed by overall increment in plant biomass and reduction in nematode attack in biochar amended soil. Whereas the study revealed that the appropriate biochar among the three is 3% w/w Rice husk biochar in comprising the plant growth and reduction in nematode infection. Moreover, biochar has no direct nematicidal effect on root knot nematode. Reduction in nematode infection through the plant root system is may be due to changing pH of the medium or by producing ammonia compound in soil as result of degradation of biochar (Ibrahim *et al.* 2018). Through SEM analysis it has analyzed that the biochar surface is the best habitat for rhizosphere organisms (mostly beneficial) they may involve direct or indirectly to inhibit the nematode invasion in root zone. Biochar particles may resist in the locomotion of fast-moving nematodes between the soil particles. Moreover, the presence of silica compounds in biochar's may directly involve inhibiting the nematode penetration in root system. Although, SCB biochar showed no promising results in the management of root knot nematodes but somehow it contributed in enhancing overall plant growth.

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