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Efficacy of Biochar Developed from Different Feedstocks at Different Temperatures for Mitigating Root Knot Nematodes in *Lycopersicon esculentum* Mill

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Tomato (*Lycopersicon esculentum* Mill) is a fundamental component of our diet, but it often faces a hidden problem in the soil, root-knot nematodes (*Meloidogyne* spp.). These tiny worms form galls on tomato roots leading to reduced yields and fruits with inferior quality. Usually, farmers use chemical nematicides to get rid of these worms, but these chemicals can harm the environment and even make people sick. So, there's a need to find a better and more sustainable solution. Biochar, a charcoal made from natural byproducts, could be a potential strategy to control nematodes. This study focuses on the utilization of biochar developed from different feedstocks (wheat straw, maize stalks, green waste and sugarcane bagasse) at two different pyrolysis temperatures (300°C and 500°C) to manage root-knot nematodes in tomato. The extracts of these biochars were tested for their nematicidal potential at first but no direct nematicidal activity was found. A pot experiment was launched using a triplicate completely randomized design to test the efficacy of various biochars at different concentrations (1%, 2% and 3% w/w) to combat root-knot nematodes and growth promotion effects. The application of biochar resulted in improved tomato plant growth and a decrease in root-knot nematode development. According to the results, the most effective biochar treatment was sugarcane bagasse biochar (SCB) produced at 300℃, followed by SCB500℃ for both enhancing growth and managing nematodes in tomato. However, further investigation is needed to understand the specific mechanisms through which biochar application promotes growth and controls nematodes in tomato. The number of females, galls and egg masses differed for all treatments. The lowest number of galls, egg masses and females were found in 3% sugarcane bagasse pyrolyzed at 300°C and highest results of nematode parameters were examined in the positive control. The results show that using biochar could be an important strategy for managing root-knot nematodes in tomato and other crops.
Keywords: Biochar; *Meloidogyne incognita*, growth promotion, soil amendment, tomato

INTRODUCTION

Tomato, scientifically known as *Lycopersicon esculentum* Mill. belong to the Solanaceae family. It is widely recognized as one of the most significant and commercially valuable vegetables globally, often ranked second only to potato. Tomato fruits have high nutritional value, and their health benefits are also well recognized (Olaniyi *et al*., 2010; Bhowmik *et al*., 2012).
In Pakistan, tomato crops are vulnerable to various biotic and abiotic factors, which contribute to reduced tomato yield. Among them, RKNs (root-knot nematodes) are a major constraint towards yield, including vegetables (Collange *et al*., 2011; Kiewnick and Sikora, 2006; Zahoor et al., 2023). In tropical and subtropical countries, *Meloidogyne* spp. have a wide host range, and it especially affects vegetable crops (Sikora and Fernandez, 2005).
Root-knot nematodes have a wide host range and are sedentary endoparasites (Ali *et al*., 2015). Furthermore, nematodes are ubiquitously distributed in different types of soil but in Pakistan, the activities and development of nematodes are enhanced in both clayey and sandy soils. In Pakistan, 40% of yield losses have been reported in tomato due to root-knot nematodes.
Controlling RKNs especially the *M. incognita* species is a challenging task due to their wide range of hosts and adaptability to various soil types. Researchers have been trying to develop environment-friendly and effective approaches for their management. In this perspective, the use of organic amendments based on biologicals is highly efficient (Oka, 2010; Arshad et al., 2021; Tahir *et al*., 2023).
Biochar, a charcoal-type substance possessing carbon and derivatives of carbon, is mainly synthesized in the furnace from organic materials at elevated temperature (El-Naggar *et*

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al., 2019). Forestry and agricultural residues, poultry and livestock waste, sewage sludge, and algae are commonly used feedstocks for biochar (Duku *et al*., 2011). The chemical and physical properties of the biochar mainly depend on the pyrolysis conditions and the nature of the feedstock (Arshad *et al.*, 2020).

Previous studies provide evidence of the potential advantages of biochar in triggering systemic resistance against various plant pathogens (Arshad et al., 2021; Elad *et al*., 2010; Vaccari *et al*., 2011; Zwart and Kim, 2012). Similarly, incorporating biochar into the soil reduced the susceptibility of pepper and tomato plants to specific foliar fungal pathogens such as *Leveillula taurica* and *Botrytis cinerea*. Furthermore, it mitigated the infestation of broad mites in pepper plants (Elad *et al*., 2010).

Biochar helps in management of soil borne pathogens especially nematodes by promoting antagonistic microbes, enhancing plant growth or by boosting plant's innate immunity (Arshad et al., 2022; 2023). Zhang *et al*. (2013) investigated the sensitivity of nematodes against biochar. The use of biochar derived from wheat straw had an impact on soil nematode diversity in a microcosm trial. It led to an increase in the population of fungivorous nematodes while decreasing the presence of plant-parasitic nematodes including those from genera like *Coslenchus*, *Hirschmanniella*, *Rotylenchus*, and *Tylenchus* among others (Zhang *et al*., 2013). Similarly, poultry litter biochar application resulted in a significant reduction of *M. javanica*, *T. semipenetrans*, *Helicotylenchus* spp., *Pratylenchus* spp., and *Criconemoid* spp., populations in grapevine because of enhanced diversity in the plant growth promoting microbes (Rahman *et al*., 2014). Likewise, Suthar *et al*. (2018) observed that the application of bamboo biochar enhances both the quality of fruit and the growth of tomato plants with higher vitamin C content, acid, and sugar. Biochar also helps in enhancing the growth of the plant under drought and stressed conditions (Akhtar *et al*., 2014; Ali *et al*., 2017; She *et al*., 2018).

Biochar amendment also results in decreased availability of lead, copper, chromium, and zinc in contaminated soils by chelating these heavy metals and reducing the risk of contamination of groundwater (Cao *et al*., 2018). Regarding plant pathogens, research has shown that lower concentrations of biochar (≤1%) can be beneficial in reducing various plant diseases, while higher concentrations (>3%) are often less effective (Frenkel *et al*., 2017). In this research, we have used different biochars developed from the byproducts of different crops and lawn clippings (green waste) and tested the nematode control potential of these biochars both *in vitro* and *in planta*.

MATERIALS AND METHODS

The current study was carried out in the research facilities of the Department of Plant Pathology at the University of Agriculture, Faisalabad. Completely randomized design (CRD) was used to layout the experiment in both *in vitro* and *in planta*.

Collection of infected root samples: The tomato roots infected with root-knot nematodes were collected from the vegetable area of the Institute of Horticultural Sciences University of Agriculture, Faisalabad. Infected root samples were collected in plastic bags to avoid moisture loss and brought to the lab. The soil and debris were removed by rinsing the samples with running tape water and were preserved in the refrigerator.

Nematodes isolation: Isolation of RKN 2nd stage larvae was done using a modified Hemming tray method. For this purpose, root samples were cleaned with running tap water to wash out the roots completely and then cut them into small pieces. Afterward, the roots were placed into the wet tissue paper present in the perforated tray. The perforated tray was placed in the non-perforated tray which was filled with enough water so that the bottom of the upper perforated tray was touching the water. The upper sieve was covered with another tray to prevent moisture loss. After 24 hours water from the lower tray was collected and poured off excess water from the upper portion gently. The maximum nematode population was obtained from the bottom of the tray. Water suspension with nematodes was left for one hour for settlement of nematodes in the bottom. Then suspension was poured into a beaker and left again for an hour to make nematodes steady and poured off water again from the top until 100 ml was left. Some part of these 2nd stage juveniles was used to evaluate the direct nematicidal potential of biochars produced at different temperatures.

***In vitro nematode control with different concentration of biochars*:** The biochars were produced from selected feedstocks using Muffle furnace (Gallonhop, England). Four feedstocks were used, sugarcane bagasse (SCB), wheat straw (WS), green waste (GW) and maize stalk (MS) for the production of biochar at two pyrolysis temperatures 300℃ and 500°C. The biochar extracts were made according to previously available protocols (Arshad et al., 2020; Huang *et al*., 2015). For evaluation of the nematicidal activity of biochars, 1 ml of newly hatched 2nd stage juveniles (100 juveniles) were incubated in 1 ml of biochar extracts obtained from different concentrations i.e., 0.5, 1, 2, 2.5 and 3% (v/v). After incubating for 24 hours, 48 hours and 72 hours 1 N NaOH solution was added to the mixture. Nematodes that undergo a change in their body shape within 3 minutes after being exposed to NaOH were considered alive. On the other hand, nematodes that remain straight and unresponsive was counted as dead using the approach outlined by Chen and Dickson (2000). This experiment was replicated three times with five Petri plates (4.5cm diameter) in each replicate to ensure the reliability of the results.

***Soil sterilization and filling of pots and biochar application in the soil*:** For the pot experiment, the potting soil was prepared and sterilized following Arshad et al. (2020). Four biochars with three concentrations 1, 2, and 3% (w/w) of soil were used. Biochar was mixed in the pots manually 1 week before transplanting of the nursery. We skipped the 0.5% concentration experiment in this activity because one of the master students did an experiment where it was reported that 0.5% concentration of these biochars does not have any pronounced effect in terms of nematode resistance and growth promotion in tomato (Shahzadi, 2021).

***Raising tomato nursery*:** Tomato seeds of the tomato cv. Rio Grande was sown in trays at the Department of Plant Pathology research area at the University of Agriculture, Faisalabad. After 25 days of sowing, tomato seedlings were transferred into earthen pots that had a capacity of 1000 g soil.

***Transplantation of nursery into pots*:** The nursery of the Rio Grande was transferred into earthen pots with different biochar treatments in the potting soil prepared in the last section. In addition to biochar treatments from various feedstocks, two controls i.e., positive and negative controls were used in 3 replications in a completely randomized design (Table 1). In positive control, only nematodes were applied while in negative control neither biochar nor nematode was applied. Plants were irrigated regularly, and the appropriate amount of fertilizer was applied.

***Inoculation of M. incognita culture*:** Tomato plants were then inoculated with newly hatched juveniles (J_2) at the rate of 1000 2^{nd} stage juveniles of *M. incognita* per pot was applied to plants with different treatments. Nematode inoculum was prepared according to the methods given by Arshad *et al.* (2020). Nematodes were introduced into the pots by creating three holes around the stem of the plant using a glass rod carefully avoiding damage to the root system. The plants received regular irrigation and appropriate fertilization. The experiment continued for 6 weeks following inoculation. At the end of the experiment, the plants were gently uprooted, transported to the laboratory, and rinsed with tap water to remove any debris. Data regarding different plant growth parameters and nematode responses were then recorded for nematode-related parameters.

***Evaluation of biochar's potential for controlling nematodes and promoting plant growth under pot experiment*:** The ability of biochar to control RKNs was assessed by carrying out the pot experiment. Biochars from four feedstocks (Wheat straw, Green waste, Maize stalk, and Sugarcane bagasse) were used. Three concentrations of biochars were used 1.0, 2.0, and 3.0%. The growth and development parameters like (fresh root weight and shoot weight (g), dry root and shoot weight (g), plant growth (NDVI values using Trimble® GreenSeeker® crop sensor), chlorophyll contents (SPAD using Minolta SPAD 502, Japan), plant height (cm), and fruit weight (g)), while nematodes characters include no. of galls, no. of egg masses, no. of females were studied and data were analyzed.

Table 1. Biochar with different pyrolysis temperature and their treatments

Sr.	Treatment	Biochar type	Temperature
1	T1	Green Waste	300°C
2	T2	Maize Stalk	300°C
3	T3	Sugarcane Bagasse	300°C
4	T4	Wheat Straw	300°C
5	T5	Green Waste	500°C
6	T6	Maize Stalk	500°C
7	T7	Sugarcane Bagasse	500°C
8	T8	Wheat Straw	500°C
9	T9	+ve control	
10	T10	-ve control	

***Statistical Analysis (if any)*:** The analysis of variance (ANOVA) under factorial settings using a completely randomized design (CRD) (Steel *et al.*, 1997) was applied to data regarding the nematode and plant growth parameters. The comparison of mean values was done by using the least significant difference test (LSD test) with a 95% level of confidence.

RESULTS

***In vitro experiment to evaluate the nematicidal effectiveness of different biochars against M. incognita*:** To assess the direct nematicidal potential of different biochars developed from different feedstock at two pyrolysis temperatures, an *in vitro* experiment was conducted. To achieve this, newly hatched *M. incognita* 2^{nd} stage juveniles (J2) were subjected to incubation with varying concentrations (0.5%, 1.0%, 2.0%, 2.5%, and 3%) of extracts derived from four distinct biochars. Nematodes incubated in distilled water were used as a control group for comparison. Significant nematode mortality was observed in the exudates of SCB, MS, WS, and GW at the concentrations mentioned after 24, 48, and 72 hours (Fig.1-3). However, there was only a slight and not statistically significant difference in mortality between the treated and control Petri plates. For instance, after 24 hours the highest number of dead juveniles was observed in various concentrations of WS biochar pyrolyzed at 300°C followed by SCB biochar developed at 300°C. However, the increases were statistically non-significant (Fig. 1). Almost similar pattern was observed in the larval mortality after 48h of application of biochar extracts in the larvae suspension (Fig. 2). While after 72 hours maximum mortality of nematodes was observed in 2.0% WS biochar made at 500°C with high deviation from the mean which was statistically non-significant as compared with the control plates (Fig. 3) So, the data revealed that the extracts developed from different biochars have statistically non-significant effects on nematode mortality as compared to control. Overall, the biochars made from different feedstocks at 300°C showed comparatively more mortality than those developed at 500°C.

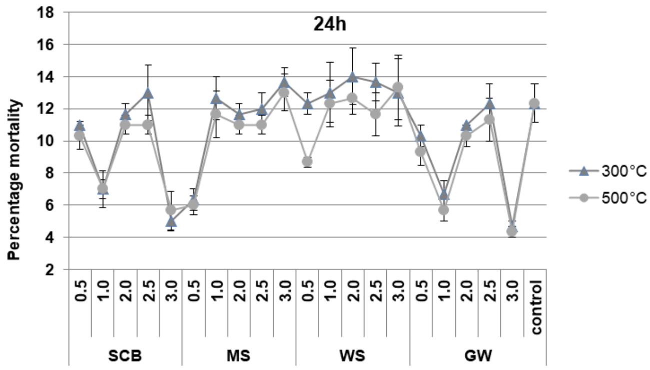

Figure 1. Percentage mortality of 2nd stage juveniles in response to exudates of different biochars with diverse pyrolysis temperatures after 24h.

***Assessing the influence of biochar application on plant growth and development*:** The data relating to the impact of different concentrations of biochars developed from different feedstocks on plant growth parameters was analyzed using ANOVA under a factorial scheme under CRD with two factors i.e., biochars and concentrations (Table 2).

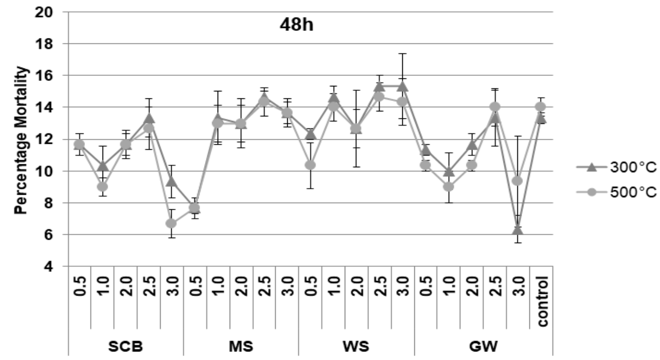

Figure 2. Percentage mortality of 2nd stage juveniles in response to exudates of different biochars with diverse pyrolysis temperatures after 48h.

The ANOVA results displayed that biochar treatments under study were significantly different from each other. However,

Table 2. Mean squares of growth related parameters under biochar treatment

SOV	Df	FSW	FRW	DSW	FPP	FW	DRW	CC	PG	PH
Biochars	9	66.592*	10.664*	7.887*	8.53*	665.43*	2.511*	50.595*	0.0100*	32.62*
Conc.	2	22.622*	1.820^{ns}	4.943^{ns}	10.80*	40.41^{ns}	0.066^{ns}	91.295*	0.0135*	126.56*
Biochar*Conc.	18	20.498*	3.276*	2.088*	3.01*	125.77^{ns}	0.536^{ns}	18.099^{ns}	0.0019*	17.50^{ns}
Error	58	7.135	1.779	1.184	0.88	99.567	0.357	24.049	0.0008	10.558

Whereas SOV= source of variation, df=degree of freedom, FRW=fresh shoot weight, FRW=fresh root weight, DSW=dry shoot weight, FPP=fruits per plant, FW=fruit weight, DRW=dry root weight, CC=chlorophyll Contents, PG=plant growth, PH=plant height, ns=not significant, *=significant as 95% level of confidence

Table 3. Mean comparison of different biochars and concentrations for growth parameters of tomato

Biochars	Fresh shoot weight	Fresh root weight	Dry shoot weight	Dry root weight	Fruits per plant	Average fruit weight	Chlorophyll contents	Plant growth	Plant height
Means of different biochars over various concentrations									
GW300°C	22.00c-f	9.39cd	10.54cde	3.76cde	5.56ab	58.11f	44.14abc	0.372abc	44.38abc
MS300°C	21.78c-f	9.72cd	11.14cd	3.92cd	4.11de	63.67def	40.62bc	0.359bcd	44.96abc
SCB300°C	28.89a	11.50a	12.26ab	4.24bc	5.89a	76.56abc	44.01abc	0.389a	45.77ab
WS300°C	24.00bc	11.17ab	11.24bcd	4.23bc	4.89cd	72.22bcd	40.89bc	0.342de	40.90d
GW500°C	20.11f	10.17bc	10.75cde	3.92cd	5.00bc	82.56a	41.42bc	0.321ef	45.68ab
MS500°C	20.44ef	7.94e	10.29de	3.31e	3.33ef	62.22ef	47.72a	0.352cd	43.88bcd
SCB500°C	23.22cd	10.28abc	11.34bc	4.17bc	5.00bc	80.44ab	42.90bc	0.382ab	46.38ab
WS500°C	20.89def	9.94bc	10.05e	3.38de	3.11f	64.33def	45.03ab	0.289g	43.48bcd
+Control	22.80cde	8.50de	11.50bc	5.08a	4.63cd	68.67cde	40.11c	0.352cd	42.23cd
-Control	26.00b	9.34cd	13.17a	4.55ab	3.33ef	79.00ab	44.00abc	0.302fg	47.02a
LSD	2.62	1.24	1.02	0.56	0.8870	9.41	4.63	0.026	3.06
Means of different concentrations over different biochars									
1%	22.37b	10.07a	10.88b	4.08a	3.80b	69.50a	41.13b	0.323b	42.14b
2%	22.67ab	9.58a	11.13ab	4.09a	4.90a	71.07a	43.66ab	0.351a	45.23a
3%	24.00a	9.73a	11.67a	4.00a	4.77a	71.77a	44.47a	0.365a	46.03a
LSD	1.43	0.68	0.56	0.31	0.49	5.15	2.53	0.015	1.68

Whereas; LSD=least significant difference, the values carrying same letter are statistically similar.

concentrations were significantly different for fresh shoot weight, fruits per plant, chlorophyll contents, growth, and plant height while concentrations were not significantly different from each other for fresh root weight, dry shoot weight, and dry root weight.

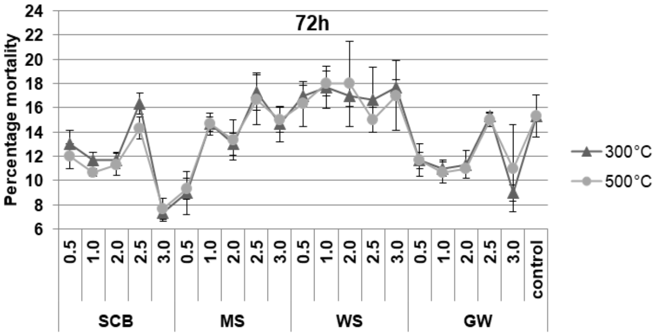

Figure 3. Percentage mortality of 2nd stage juveniles in response to exudates of different biochars with diverse pyrolysis temperatures after 72h.

Biochar treatments were significantly different from each other for fruit weight, while concentrations and interaction were not significant for this parameter. Similarly, biochar x treatment interaction was significantly different from each other for all the characters except dry root weight and chlorophyll contents.

The results of the means of biochars over different concentrations under factorial design are shown in Table 3. The results of biochar means demonstrated that sugarcane bagasse biochar developed at 300°C (SCB300°C) demonstrated the best mean value for fresh shoot weight followed by the negative control and wheat straw biochar pyrolyzed at 300°C (WS300°C). Likewise, fresh root weight was maximum in SCB300°C treatment followed by WS300°C and SCB500°C. The maximum mean value for dry shoot weight was revealed by the negative control which was not significantly different from that of SCB500°C.

On the other hand, the dry root weight for the highest in the positive control followed by the negative control. Similarly, fruits per plant and fruit weight were highest in the SCB300°C

Table 4: Mean comparison of biochars ×concentrations for growth parameters of tomato

Biochar	Conc.	FSW	FRW	DSW	DRW	FPP	FW	CC	PG	PH
GW300°C	1%	22.00f-j	10.50b-e	10.50f-j	4.110b-h	4.00c-f	85.3cd	38.88de	0.388bcd	44.00c-g
	2%	22.33e-j	9.17d-g	10.63e-j	3.667e-i	7.67a	164.93a	49.58ab	0.368c-f	45.58b-g
	3%	21.67f-j	8.50efg	10.48f-j	3.500ghi	5.00bcd	107.8bc	43.97a-e	0.360c-g	43.57d-g
MS300°C	1%	18.67ij	10.50be	10.01hij	4.333b-g	3.00fgh	64.3de	39.42cde	0.350c-h	43.28efg
	2%	21.33g-j	8.17fg	11.45b-h	3.417ghi	4.33c-f	94.07cd	43.58a-e	0.370c-f	46.95a-f
	3%	25.33b-g	10.50b-e	11.97a-g	4.00c-h	5.00bcd	108.77bc	38.85de	0.357c-g	44.63c-g
SCB300°C	1%	27.33bc	10.83a-d	10.77e-j	3.917c-h	5.00bcd	107.93bc	41.7b-e	0.338e-j	40.98gh
	2%	27.00bcd	10.83a-d	12.54a-d	4.000c-h	5.00bcd	108.77bc	43.57a-e	0.392a-d	45.33b-g
	3%	32.33a	12.83a	13.48a	4.800abc	7.67a	166.37a	46.75a-d	0.437a	51.00a
WS300°C	1%	26.00b-f	12.50ab	11.86a-g	4.700a-d	4.00c-f	87.13cd	40.27cde	0.320g-l	40.83gh
	2%	26.67b-e	10.83abcd	9.55ij	4.167b-g	4.67b-e	100.87bc	40.017cde	0.358c-g	41.23gh
	3%	19.33hij	10.17c-f	12.32a-e	3.833c-h	6.00b	130.03b	42.4bcde	0.348d-i	40.63gh
GW500°C	1%	19.67hij	10.17c-f	9.92hij	3.833c-h	5.33bc	116.67bc	38.95de	0.273m	44.28c-g
	2%	20.00hij	11.50abc	10.89d-i	4.333b-g	5.33bc	115.93bc	39.32cde	0.347d-i	45.48b-g
	3%	20.67hij	8.83d-g	11.45b-h	3.583f-i	4.33c-f	94.07cd	45.98a-e	0.342e-i	47.27a-f
MS500°C	1%	20.33hij	7.50g	9.87hij	3.167hi	3.00fgh	64.30de	46.48a-d	0.330f-k	42.10fg
	2%	18.67ij	8.83d-g	10.35g-j	3.583f-i	5.00bcd	108.77bc	46.18a-e	0.372c-f	44.20c-g
	3%	22.33e-j	7.50g	10.65e-j	3.167hi	2.00h	42.90e	50.48a	0.355c-g	45.35b-g
SCB500°C	1%	18.33j	10.17c-f	10.59e-j	3.750d-h	4.00c-f	87.13cd	38.42e	0.350c-h	42.00fgh
	2%	23.67b-h	10.50b-e	12.15a-f	4.583a-e	5.00bcd	108.77bc	46.08a-e	0.378b-e	49.18abc
	3%	27.667b	10.17c-f	11.27c-i	4.167b-g	6.00b	130.40b	44.20a-e	0.418ab	47.97a-e
WS500°C	1%	23.33b-h	10.83a-d	11.26c-i	3.917c-h	2.33gh	50.80e	44.08a-e	0.278lm	45.05b-g
	2%	18.33j	8.17fg	9.11j	3.487ghi	4.00c-f	86.77cd	43.92a-e	0.285klm	41.40gh
	3%	21.00g-j	10.83a-d	9.79hij	2.750i	3.00fgh	65.50de	47.10abc	0.303i-m	43.98c-g
Control	+VE	22.66d-j	8.50efg	11.17c-i	5.410a	4.66b-e	92.70cd	40.66cde	0.295j-m	36.75h
	-VE	26.67b-e	9.16d-g	13.50a	4.80abc	3.66d-g	65.50de	44.30a-e	0.330f-k	50.23ab
LSD		4.535	2.150	1.78	0.976	1.5363	16.297	8.015	0.043	5.037

Whereas; LSD=least significant difference, the values carrying same letter are not-significantly different from each other. FRW=fresh shoot weight, FRW=fresh root weight, DSW=dry shoot weight, FPP=fruits per plant, FW=fruit weight, DRW=dry root weight, CC=chlorophyll Contents, PG=plant growth, PH=plant height. the values carrying same letter are statistically similar.

treatment followed by the plants treated with green waste biochar developed at 300°C (GW300°C). The maize stalk biochar developed at 500°C demonstrated the top mean value for chlorophyll contents that was followed by WS500°C biochar and GW300°C respectively. Plant growth mean value was the best in SCB500°C biochar where GW300°C and SCB500°C biochars were not significantly different from SCB500°C. Maximum growth was shown by the negative control which statistically like the mean values of GW300°C, MS 300°C, SCB300°C and SCB500°C biochars. As far as the mean comparison of concentrations is concerned, 3% concentration outclassed the other two concentrations for all the characters (Table 3). The mean comparison for biochars × concentrations revealed that SCB300°C showed the best results for most of the parameters under study at 3% concentrations with very few exceptions (Table 4).

***Biochar addition reduced M. incognita development in tomato*:** The data related to the influence of different concentrations of various biochars. developed from different feedstocks on nematode-related parameters was analyzed using ANOVA under a factorial scheme under CRD with two factors i.e., biochars and concentrations (Table 5). The ANOVA results displayed that different nematode-related characters i.e., number of galls, number of egg masses, and number of females, were significantly different from each other under biochar, concentration treatments at 95% level of confidence.

The results of the means of biochars over different concentrations under factorial design is shown in Table 6. The results of biochar means demonstrated that sugarcane bagasse biochar developed at 300°C (SCB300°C) was the most efficient biochar for controlling *M. incognita* in tomato as it displayed minimum mean values for number of galls, number of egg masses, and number of females.

Table 5. Mean squares of nematode related parameters under biochar treatment

SOV	Df	No. of galls	No. of egg masses	No. of females
Biochars	9	12512.7*	47751.4*	117478*
Conc.	2	1201.6*	3327.6*	9956*
Biochar*Conc.	18	1126.6*	4204.9*	9585*
Error	58	84.1	205.9	800

Whereas SOV= source of variation, df=degree of freedom, *=significant as 95% level of confidence

The second most efficient biochar treatment was sugarcane bagasse biochar developed at 500°C (SCB500°C) in controlling nematodes in tomato. The mean comparison of different concentrations showed that 3% concentration was the most efficient for nematode control at 95% level of confidence. Similarly, the interaction of biochars and concentrations were studied using mean comparison through LSD test at 5% level of probability (Table 7). The results showed that almost all the concentrations of SCB300°C were effective to manage root knot nematodes in tomato with relatively more pronounced effect of 3% concentration. The 2nd most effective biochar was SCB500°C.

Table 6. Mean comparison of different biochars and concentrations for nematode related parameters in tomato

Biochars	No.of galls	No.of egg masses	No. of females
Means of different biochars over various concentrations			
GW300°C	39.67c	64.33c	106.89cd
MS 300°C	38.67cd	47.44d	78.00e
SCB300°C	12.22e	8.22ef	14.22f
WS 300°C	75.89 b	122.56b	204.33b
GW500°C	43.67c	69.89c	116.78c
MS500°C	18.22e	15.00e	25.11f
SCB500°C	14.00e	13.44ef	22.44f
WS 500°C	30.67 d	37.22 d	82.00de
+Control	127.18a	240.57a	376.67a
-Control	0.00f	0.00 f	0.00f
LSD	8.654	13.54	26.69
Means of different concentrations over different biochars			
1%	47.07a	73.60a	122.83a
2%	38.17b	58.78b	97.67b
3%	34.82b	53.23b	87.43b
LSD	4.741	7.42	14.26

Whereas; LSD=least significant difference, the values carrying same letter are not-significantly different from each other.

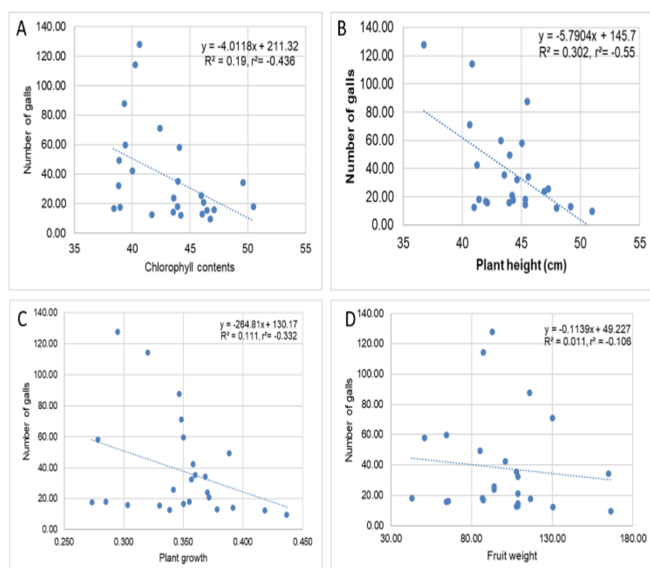

Figure 4. Relationship between number of galls and parameters like chlorophyll contents (A), plant height (B), plant growth (C) and fruit weight (D).

Table 7. Mean comparison of biochars × concentrations for nematode related parameters of tomato

Biochar	Conc.	No. of galls	No. of egg mass	No. of females
GW300°C	1%	49.33de	82.67fg	139.00f
	2%	34.33fg	55.33hi	91.00gh
	3%	35.33efg	55.00hi	90.67gh
MS300°C	1%	59.67cd	96.33ef	159.67ef
	2%	24.00g-j	39.67ij	64.00hij
	3%	32.33fgh	6.33kl	10.33lm
SCB300°C	1%	12.67ijk	10.67kl	16.67klm
	2%	14.33ijk	11.67kl	19.67j-m
	3%	9.67jk	2.33l	6.33lm
WS300°C	1%	114.33a	185.67b	310.00c
	2%	42.33ef	68.33gh	114.67fg
	3%	71.00c	113.67d	188.33de
GW500°C	1%	17.67hij	29.33jk	49.33h-l
	2%	87.67b	139.67c	233.00d
	3%	25.67ghi	40.67ij	68.00hi
MS500°C	1%	15.67ij	22.33jkl	37.67i-m
	2%	21.00g-j	10.33kl	17.00klm
	3%	18.00hij	12.33kl	20.67j-m
SCB500°C	1%	16.67ij	10.67kl	18.00j-m
	2%	13.00ijk	12.00kl	19.67j-m
	3%	12.33ijk	17.67jkl	29.67i-m
WS500°C	1%	58.00cd	94.00ef	157.00ef
	2%	18.00hij	17.67jkl	30.67i-m
	3%	16.00ij	23.25jkl	58.33h-k
Control	+VE	127.88a	284.27a	402.00a
	-VE	0.00k	0.00l	0.00m
LSD		14.986	23.450	46.231

Whereas; LSD=least significant difference, the values carrying same letter are not significantly different from each other.

The relationship between the number of galls and parameters like chlorophyll contents, plant height, plant growth, and fruit weight are given in Fig. 4 where all these parameters showed a negative correlation with the number of galls.

DISCUSSION

Biochar developed from different sources is getting attention of the scientists around the globe (Arshad *et al.*, 2023). In addition to ameliorating plants against biotic and abiotic stresses, biochar has emerged as an important resource to mitigate plant diseases (Arshad, 2024). The present study aimed to evaluate the impact of various concentrations of different biochars for both the direct nematicidal activity and plant growth parameters. These parameters included plant height, fresh shoot weight (FSW), dry shoot weight (DSW), fresh root weight (FRW), and dry root weight (DRW) as well as overall growth, chlorophyll contents, and fruit weight. There are a few studies where scientists have evaluated the direct nematicidal activity of the biochars developed from different feedstocks. For instance, Huang *et al*. (2015) tested the exudates of various biochars on rice root-knot nematode *Meloidogyne graminicola* and found no direct effect on the 2nd stage juveniles' mortality. Similarly, recently we evaluated the biochar extracts on *M. incognita* and reported that various biochars have no direct nematicidal activity on southern root-knot nematode, *M. incognita* (Arshad *et al*., 2020). This suggests that biochar exudates may not have direct nematicidal activity and have some other phenomenon responsible for the resistance in plants against parasitic nematodes.

In the pot experiment, the effect of different biochars with different concentrations was assessed which showed that sugarcane biochar developed at 300℃ is more efficacious than other biochars for improvement of growth-related characteristics of tomato. Several studies have demonstrated this effect of biochar. Incorporating biochar into the soil has the potential to increase the biomass of tomato plants by stimulating beneficial soil organisms, consistent with findings from earlier studies (Suthar *et al*., 2018).

Incorporating biochar into the soil has been proven to boost plant growth, sequester carbon in the soil, improve soil fertility, and play a vital role in reducing the population of soil-borne pathogens (Lehmann and Joseph, 2009). Biochar amendment has been shown to improve morpho-physiological traits in tomato previously (Arshad et al., 2022). The application of biochar has demonstrated positive effects on the growth of various plant species, including pine and alder (Robertson *et al*., 2012), maize (Iqbal *et al*., 2020) and tomato (Vaccari *et al*., 2015), ryegrass (Kammann *et al*., 2012) and bean (Rondon *et al*., 2007). Application of biochar is also helpful in suppressing the population of nematodes by accumulating beneficial microbes (Ikwunagu *et al*., 2019; Zhang *et al*., 2013).

Differences were observed in the number of females, galls, and egg masses among all treatments. The treatment with 3% sugarcane bagasse pyrolyzed at 300°C showed the lowest counts of galls, egg masses, and females while the highest counts were noted in the positive control. Introducing biochar into the soil has the potential to gather beneficial microbes, fostering an environment conducive to enhanced plant growth. Moreover, these microbes can play a role in controlling nematodes through their antagonistic effects.

Another significant factor could be the development of phytoliths in plant roots, a result of the high silica concentration present in all the biochars. The formation of phytoliths creates a physical barrier through depositions in the cell wall matrix, impeding the entry of nematodes into the plant (Alhousari *et al*., 2018). Recent studies indicate that incorporating biochar into the soil can boost tomato plant biomass. Additionally, it has been found to reduce nematode populations or inhibit their invasion of roots by activating other beneficial soil organisms, aligning with findings from previous research (Rahman *et al*., 2014).

Our results showed that there is a negative relation between the number of galls and chlorophyll content, which showed similarity with Lu *et al*. (2014) who demonstrated an association between *M. incognita* infection and a decrease in chlorophyll content in cotton leaves. Similarly, it was also clear from the results that with the increment in the number of galls, there is a reduction in growth and height as shown by Maleita *et al*. (2012). Moreover, our results showed that plants having a severe attack of nematodes showed stunted growth and reduced fruit yield as reported by Mitkowski and Abawi (2003). He reported that dense galling by *Meloidogyne spp.* cause stunted growth in lettuce.

***Conclusion*:** The results concluded that biochar extracts used in different concentrations did not have any direct nematicidal activity. Similarly, the application of biochar led to enhanced plant growth in tomato and reduced development of the root-knot nematode in tomato. The best biochar treatment from the results was sugarcane bagasse biochar developed at 300°C followed by SCB500°C for both growth enhancement and nematode management in tomato. However, it is to be studied that due to particular mechanisms, biochar application leads to growth promotion and nematode control in tomato.

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REFERENCES

Abawi, G.S., J. Chen. 1998. Concomitant pathogen and pest interactions. Plant and nematode interactions 36: 135-158.

Akhtar, S.S., G. Li, M.N. Andersen and F. Liu. 2014. Biochar enhances yield and quality of tomato under reduced irrigation. Agricultural Water Management 138:37-44.

Alhousari, F. and M. Greger. 2018. Silicon and mechanisms of plant resistance to insect pests. Plants 7; Article 33.8.

Ali, M.A., A. Abbas, F. Azeem, N. Javed and H. Bohlmann. 2015. Plant-Nematode Interactions: From Genomics to Metabolomics. International Journal of Agriculture and Biology, 17:1071-1082.

Ali, S., M. Rizwan, M.F. Qayyum, Y.S Ok, M. Ibrahim, M. Riaz, M.S Arif, F. Hafeez, M.I Al-Wabel and A.N. Shahzad. 2017. Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. Environmental Science and Pollution research 24:12700-12712.

Arshad, U., F. Azeem, G. Mustafa, Allah Bakhsh, H. Toktay, M. McGiffen, M.A. Nawaz, M. Naveed, M. A. Ali. 2021. Combined application of biochar and biocontrol agents enhances plant growth and activates resistance against Meloidogyne incognita in tomato. Gesunde Pflazen (Journal of Crop Health) 73: 591-601.

Arshad, U., M. Naveed, N. Javed, M.D Gogi and M.A Ali. 2020. Biochar application from different feedstocks enhances plant growth and resistance against *Meloidogyne incognita* in tomato. International Journal of Agriculture and Biology 24:961-968.

Arshad, U., M.T. Altaf, W. Liaqat, M. Ali, M.N. Shah, M. Jabran, M. A. Ali 2023. Biochar: Black Gold for Sustainable Agriculture and Fortification Against Plant Pathogens—A Review. Gesunde Pflanzen. Published Online at https://doi.org/10.1007/s10343-023-00952-y

Arshad, U., M. Raheel, W. Ashraf, A. Rehman, M. S. Zahid, M. Moustafa, M.A. Ali. 2022. Influence of biochar application on morpho-physiological attributes of tomato (Lycopersicum esculentum Mill) and soil properties. Communications in Soil Science and Plant Analysis 54:515-525.

Bhowmik, D., K.S. Kumar, S. Paswan and S. Srivastava. 2012. Tomato-a natural medicine and its health benefits. Journal of Pharmacognosy and Phytochemistry 1:33-43.

Cao, Y., Y. Gao, Y. Qi and J. Li. 2018. Biochar-enhanced composts reduce the potential leaching of nutrients and 357 heavy metals and suppress plant-parasitic nematodes in excessively fertilized cucumber soils. Environmental Science and Pollution Research 25:7589-7599.

Chen, S.Y. and Dickson, D.W. 2000. A technique for determining live second-stage juveniles of *Heterodera glycines*. Journal of Nematology 32:117.

Collange, B., M. Navarrete, G. Peyre, T. Mateille and M. Tchamitchian. 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. Journal of Crop Protection 30:1251-1262.

Duku, M.H., S. Gu, E. Hagan and E. Ben. 2011. Biochar production potential in Ghana - A review. Renewable and Sustainable Energy Reviews 15:3539-3551.

Elad. Y., D.R David, Y.M. Harel, M. Borenshtein, H.B. Kalifa, A. Silber and E.R Graber. 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Phytopathology 100:913-921.

El-Naggar A., S.S. Lee, J. Rinklebed, M. Farooq, A.K. Sarmah, A.R. Zimmerman, M. Ahmad, S.M. Shaheen and Y.S Ok. 2019. Biochar application to low fertility soils: A review of current status, and future prospects. Geoderma 337:536-554.

Frenkel, O., A.K. Jaiswal, Y. Elad, B. Lew, C. Kammann and E.R. Graber. 2017. The effect of biochar on plant diseases: what should we learn while designing biochar substrates?

Journal of Environmental Engineering and Landscape Management 25:105-113.

Huang, W-k., H-l. Ji, G. Gheysen, J. Debode and T. Kyndt. 2015. Biochar-amended potting medium reduces the susceptibility of rice to root-knot nematode infections. BMC Plant Biology 15:1-15.

Ikwunagu, E.A., C.C. Ononuju and C.C. Orikara. 2019. Nematicidal effects of different biochar sources on root-knot nematode (*Meloidogyne* spp.) egg hatchability and control on mungbean (vigna radiata L.) wilczek IJEN 4:1-14.

Iqbal, M., M. Yasir, A. Javed and S.T Ahmad .2020. Comparative Effects of Different Types of Biochar on Physical Properties of Soil and Growth of Maize. Journal of Bioresource Management 7:2.

Kammann, C., S. Ratering, C. Eckhard and C. Müller. 2012. Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, methane) fluxes from soils. Journal of Environmental Quality 41:1052-1066.

Kiewnick, S. and R. Sikora. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control 38:179-187.

Lehmann, J. and S. Joseph. 2009. Biochar for environmental management: an introduction. Biochar for environmental management: Journal of Science and Technology 1:1-12.

Lu, P., Davis, R.F. Kemerait, R.C. van Iersel, M.W. Scherm H. 2014. Physiological effects of *Meloidogyne incognita* infection on cotton genotypes with differing levels of resistance in the greenhouse. Journal of Nematology 46: 352.

Mitkowski, N.A. and G.S. Abawi. 2003. Reproductive fitness on lettuce of populations of *Meloidogyne hapla* from New York state vegetable fields. Nematology 1:77-83.

Maleita, C.M.N., R.H.C. Curtis, S.J Powers and I.M.O. Abrantes. 2012. Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction and growth of tomato genotypes. Phytopathologia Mediterranea 3:566-576.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments—a review. Applied Soil Ecology 44:101-115.

Olaniyi, J., W. Akanbi, T. Adejumo and O. Akande. 2010. Growth, fruit yield and nutritional quality of tomato varieties. African Journal of Food Science 4:398-402.

Rahman, L., M.A. Whitelaw-Weckert and B. Orchard. 2014. Impact of organic soil amendments, including poultry litter biochar, on nematodes in a Riverina, New South Wales, vineyard. Soil Research 52:604-619.

Robertson, S.J., P.M. Rutherford, J.C. Lopez-Gutierrez and H.B. Massicotte. 2012. Biochar enhances seedling growth and alters root symbioses and properties of sub-boreal forest soils. Canadian Journal of Soil Science 92:329-340.

Rondon, M.A., J. Lehmann, J. Ramírez and M. Hurtado. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biology and fertility of soils 43:699-708.

She, D., X. Sun, A.H.D. Gamareldawla, E.A. Nazar, W. Hu, K. Edith and S. Yu. 2018. Benefits of soil biochar amendments to tomato growth under saline water irrigation. Scientific Reports 8:1-10.

Shahzadi, S. 2021. Efficacy of biochar application from different feed stocks on plant growth and resistance against root-knot nematodes in tomato. MSc (Hons) Thesis. Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

Sikora, R.A. and E. Fernandez. 2005. Nematodes parasites of vegetables. In: Plant parasitic nematodes in subtropical and tropical agriculture. CAB International 319-392.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics. A Biomedical Approach 3:172-177.

Suthar, R.G., C. Wang, M.C.N. Nunes, J. Chen, S.A. Sargent, R.A. Bucklin and B. Gao. 2018. Bamboo biochar pyrolysed at low temperature improves tomato plant growth and fruit quality. Agriculture 8:10-153.

Tahir, M., Ali, M. A., Khan, A., Akram, M. Z., Ullah, S., Faraz, I., Khan, M. S., Abrar, M., Ali, F., & Abbas, Q. (2023). Impact of various soil amendments on root knot nematodes and plant growth attributes of tomato (Solanum lycopersicum L.). Journal of Pure and Applied Agriculture, 8: 30-37.

Vaccari, F.P., A. Maienza, F. Miglietta, S. Baronti, S.D. Lonardo, L. Giagnoni, A. Lagomarsino, A. Pozzi, E. Pusceddu, R. Ranieri and G. Valboa. 2015. Biochar stimulates plant growth but not fruit yield of processing tomato in a fertile soil. Agriculture Ecosystems and Environment 207:163-170.

Vaccari, F., S. Baronti, E. Lugato, L. Genesio, S. Castaldi, F. Fornasier and F. Miglietta .2011. Biochar as a strategy to sequester carbon and increase yield in durum wheat. European Journal of Agronomy 34:231-238.

Zahoor, A., Z. Niaz, M. Tahir-ul-Qamar, and A. M. I. Hawamda. 2023. Computational analysis of 16D10 effector peptides from root-knot nematodes and their interaction with Mi resistance (R) protein through molecular docking . Integrative Plant Biotechnology 01:01-08.

Zhang, X-K., L. Qi, W-J. Liang, M. Zhang, B. Xue-Lian and X. Zu-Bin . 2013. Soil nematode response to biochar addition in a Chinese wheat field. Pedosphere 23:98-103.

Zwart, D.C. and S.H. Kim. 2012. Biochar amendment increases resistance to stem lesions caused by *Phytophthora* spp. in tree seedlings. Horticultural Science 47:1736-1740.