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ORIGINAL ARTICLE

Characterization of different biochars and their impacts on infectivity of entomopathogenic nematode *Heterorhabditis bacteriophora*

Elif Yaman[1] · Tufan Can Ulu[2] · Nurgül Özbay[3]

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Abstract

Entomopathogenic nematodes (EPNs) are important biological control agents that are endoparasites of many types of insects. Improving soil health has potential to provide more suitable conditions and increase effectiveness of EPNs. For this purpose, effects of feedstock type (walnut shell, fir wood sawdust, rice husk, einkorn wheat husk and corn waste from field) and sand to biochar proportion (100:0; 90:10; 80:20 and 60:40 by weight) on infectivity of entomopathogenic nematode was investigated in this study. Based on characterization results, the selected biomass samples are suitable raw materials for the production of biochar by pyrolysis method. With regard to our results, except for walnut shell biochar, all biochar samples have significantly reduced EPN infection at 60:40 mixtures compared to control. Walnut shell biochar had no negative effect on EPNs and infection rate was 100% for all proportions. The macroporous structure of the walnut shell biochar contains moisture that can be adsorbed in the pores. When the oxygen contained in the air dissolved in adsorbed water, it was a source of life for EPNs that provided their oxygen demand from dissolved oxygen in water. However, application of corn waste biochar with high ash content was detrimental to EPN survival as a result of an increased salinity of the soil. The study concluded that biochar application may have detrimental or indifferent impacts on some beneficial nematode species.

Keywords Biochar · Carbonization · Pyrolysis · Characterization · Entomopathogenic nematodes · Biological control

1 Introduction

It is estimated that the world population will reach approximately 9.7 billion in 2050. With the increasing population, both food and energy demand will also increase globally [1]. The rising requirements have exerted tremendous pressure on the agricultural sector. Therefore, the need for different chemical or biological soil amendments and control agents to achieve soil fertility will never end [2].

Sustainable agricultural research and applications also become necessary in this global cycle. In recent years, studies in soil applications with biochar have increased considerably. Biochar is the solid product of pyrolysis that is the thermal decomposition process of biomass by increasing temperature in no- or limited-oxygen conditions [3]. The superior properties of biochar can be listed as being thermally stable, carbon-rich and highly porous [1]. In addition to these properties, biochar is a sustainable source since biomass is sustainable and renewable.

Another approach for sustainable agriculture is biological control of target pests, which is one of the best alternatives and mitigation to rampant chemical applications in agriculture. There are numerous biocontrol agents and entomopathogenic nematodes (EPNs) are an important alternative among them. EPNs are soil-dwelling microscopic roundworms belonging to Heterorhabditidae and Steinernematidae (Nematoda: Rhabditida) [4]. EPNs need an insect host to complete their life cycle, making them a suitable agent for insect management. Infective juveniles (IJs) are developmentally arrested forms of third-stage juveniles of EPNs, and IJs are the only stage living outside the host [5]. IJs can seek their hosts in the soil. They can enter the host through natural openings (anus, spiracle, mouth etc.) and kill their

✉ Elif Yaman
elif.yaman@bilecik.edu.tr

[1] Central Research Laboratory, Bilecik Şeyh Edebali University, Bilecik, Turkey

[2] Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Bilecik Şeyh Edebali University, Bilecik, Turkey

[3] Faculty of Engineering, Department of Chemical Engineering, Bilecik Şeyh Edebali University, Bilecik, Turkey

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host within 24–36 h [6]. EPNs carry a symbiotic bacterium in their gut. After penetrating the host, IJs reach hemocoel and release their symbiotic bacterium to the host hemolymph. The bacterium starts reproducing inside the host and producing various enzymes, which digest host tissues and lead to death [7]. In addition to killing the host, the bacterium also converts it to a nutrient-rich food source called biomass. Inside the host, IJs start feeding with biomass and continue their life cycle to later biological stages [8]. After several generations in the host, IJs leave the cadaver and emerge through the soil to find new hosts.

EPNs have many advantages. They are safe to the environment, they have low toxicity to non-target organisms, they can be applied easily with standard sprayers, they can be mass-produced in fermenters and they have a broad host range [9–11]. However, they need a high initial setup cost for commercial mass production and their shelf life is short compared to chemicals. They also need to be shipped in an isolated box or cold chain. Due to these obstacles, commercial EPN products are relatively expensive and cannot compete with chemical pesticides. Thus, many studies focused on improving mass production yield, longevity, shelf life, effectiveness etc. [12–15]. EPNs should also be applied efficiently. There are many novel EPN application techniques, which aimed to improve their success in the field. Even though EPNs are primarily effective against soil-dwelling insects, new application techniques make EPNs effective against several above-ground insect pests [16–18].

EPNs have a close relationship with soil texture and structure. EPNs prefer sandy soils with bigger particles, and they need a thin layer of water to move. Their distribution is also related to the soil water potential [19]. Although EPNs have a primitive nervous system, they can perceive host cues, humidity, magnetic fields, gravity, pH, salinity etc. [11, 20–25]. For instance, EPNs show low infectivity under saline conditions, which may be closely related to reduced mobility due to osmotic stress [26]. Optimal pH for the symbiotic bacterium and the EPNs ranges between 7 and 8.5, and higher pH values have a detrimental effect on EPNs [27, 28].

It is known that biochar improves soil properties and nutritional status and increases plant productivity [1, 29]; reduces CO_2 and N_2O emissions from a tropical soil and enhances nutrient availability with combined use of earthworms [3, 30]; reduces water evaporation from the soil surface [31]; and mitigates methane emissions via microbial activities from paddy fields and salt-affected soils [32]. The combined use of biochar and biosolids in the soil decreases the change in the chemical properties of the soil, such as pH and mineral nitrogen, thus maintaining the stability of the microbial community [33]. The porous structure of biochar provides a habitat for soil microorganisms. Studies in the literature indicate that the composition and abundance of soil biological community also changed by applying biochar to soil [34].

Few studies have examined the influence of the addition of biochar on soil-inhabiting nematodes. Mondal et al. [35] evaluated the efficacy of some organic amendments, including biochar, on rice root-knot nematode management. They revealed that higher concentrations of biochar applications reduce the reproduction rate of nematodes. Domene et al. [36] and Liu et al. [37] found that biochar application affects soil nematode community, but the effect varies among herbivorous and beneficial nematodes. Ibrahim et al. [38] have specified the effect of biochar on the performance of tomatoes under nematode-inoculated soils. According to the results, nematode gall formation on the roots was decreased by increasing biochar dosage. Ebrahimi et al. [39] reported that biochar using has no effect on neither viability nor reproduction of potato cyst nematodes. Marra et al. [40] examined the impact of biochar application on the survival of nematode *Meloidogyne incognita*. Biochar produced at low temperature (300 °C) inhibits the growth of nematodes, while the toxicity decreases as the biochar production temperature higher. The main idea of these studies was to reveal the relationship between biochar application and soil nematode fauna. However, we still do not have any information about the effect of biochar application on EPNs and this will be the first study to evaluate this relationship.

This study aims to specify the effect of feedstock type and biochar amount on infectivity of entomopathogenic nematode *Heterorhabditis bacteriophora*. For this purpose, the physicochemical and morphological properties of the different biochar samples were characterized in detail. After characterization, the infectivity of the *H. bacteriophora* was tested in different biochar types and sand:biochar proportions.

2 Materials and methods

The biomass and nematode *H. bacteriophora* used in this study are described in this section, as well as the techniques used for biochar preparation and characterization. Also, application studies on the infectivity of *H. bacteriophora* have been explained in detail.

2.1 Biomass samples

Biomass sources grown in abundance in Turkey such as walnut shell (WS), fir wood sawdust (FW), rice husk (RH), einkorn wheat husk (WH) and corn waste from the field (CW) were selected as biomass feedstock. Air-dried (20 ± 2 °C) biomass samples were ground and sieved. The particle size of the used biomass samples was 224–425 µm.

2.2 Biochar production

Biomass samples that have reduced particle size (224–425 µm) were placed in a tightly closed pot with a volume of 50 cm^3. The pot was put in a muffle furnace raised to 500 °C with a heating rate of 20 °C/min, and the sample was kept in the oven at 500 °C for 15 min [41]. As the temperature of the biomass sample is increased, with the initiation of primary pyrolysis reactions, volatile components begin to release from the biomass structure and porous char formation occurs. After the carbonization process, biochar samples were ready for characterization with the codded names of WSB, FWB, RHB, WHB and CWB, respectively.

2.3 Characterization of biomass and biochar samples

A moisture analyser (Sartorious, MA 150) was used to measure weight percent (wt%) of moisture content of biomass samples. Two American Standard Test Methods ASTM E 897–82 [42] and ASTM D 1102–84 [43] were also used to specify wt% of ash and volatile matter content of biomass samples. True density determination was applied to both biomass and biochar samples using helium gas pycnometer (Micromeritics, AccuPyc II 1340). The ASTM E 873–82 [44] method was used to calculate the bulk density of biomass and biochar samples. The functional groups of the biomass and biochar were determined by using a Fourier-transform infrared spectrometer (FT-IR) (Perkin Elmer, Spectrum 100) with a wavelength range of 4000–380 cm^{-1}, a resolution of 0.4 cm^{-1} and attenuated total reflectance (ATR) module. Scanning electron microscopy (SEM) (ZEISS, SUPRA 40VP) was used to specify the morphological characteristics of the biomass and biochar samples. Coating processes were applied for 1 min to provide conductivity of samples under the Au/Pd source in the sputter coater (Quorum, Q300 model). The coating thickness was approximately 100 nm. During the SEM analyses, 15 kV acceleration voltage (EHT), ~ 10 mm working distance (WD) and secondary electron (SE) detector were used. Inorganic contents of biochar samples were identified by applying the energy-dispersive x-ray spectroscopy (EDX) technique (Bruker, EDX detector). Multi-point Brunauer–Emmett–Teller (BET) surface area, pore size, specific micropore volume and specific meso-/macro-pore volume of the biochar samples were determined by the nitrogen (N_2) adsorption isotherm BET method (Micromeritics, ASAP2020).

2.4 pH and electrical conductivity determination of sand:biochar samples

To determine the effect of biochar amount on the soil pH and electrical conductivity (EC), sand:biochar samples were prepared in four different proportions: 100:0 (control); 90:10; 80:20 and 60:40. Before the pH and EC measurements, 20 mL of distilled water was added to 1 g of soil-biochar mixtures and then kept for 24 h. After 24 h, the pH and EC values of the prepared samples were measured using pH meter (Mettler Toledo-Seven Compact) and electrical conductivity meter (Hanna Instruments-Dist 4).

2.5 Entomopathogenic nematode

Commercial strain of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) was used for trials. Commercial product (e-Nema GmbH) was provided by a local distributor. *H. bacteriophora* is one of the most abundant EPN species globally. It has "cruising" host-seeking behaviour [45], and it has relatively small body dimensions among the other EPN species. Maximum body diameters of 30 infective juveniles (IJs) were individually measured using Zeiss Primovert Inverted microscope and analysed in Zeiss Zen v3.3 software.

2.6 Infectivity trials

Infectivity tests were performed in 9 cm diameter plastic Petri dishes. Four different sand:biochar mixtures were used as infection medium: 100:0 (control), 90:10, 80:20, 60:40. Each Petri dish is fully filled with different sand:biochar mixture. Four last instar *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae used as test insect for each Petri dish. The water content of the mixtures was adjusted to 10% before inoculation. In mixtures with 200 µl water, 1500 IJs were applied. This dose was selected based on the suggested commercial dose (50 IJs/cm^2). Inoculated Petri dishes were sealed with parafilm and incubated at 24 °C for 4 days. After incubation, dead larvae were dissected and checked for nematode infection and larva mortality was recorded. Trials were replicated three times.

2.7 Statistical analyses

The effects of different sand:biochar mixtures on EPN infectivity, pH and EC values of soil samples were evaluated with analysis of variance (ANOVA). Means of the different mixture proportions, pH and EC values were compared with LSD test (0.05). The relationship of EC, pH and larva mortality was assessed using the Pearson correlation test at $P \leq 0.05$. All statistical analyses were performed using JMP 13 and Minitab 19 software.

3 Results and discussion

In this section, results from the characterization of the biomass feedstock and biochar samples, as well as the infectivity trials, are presented and discussed.

3.1 Characterization results of biomass and biochar samples

The results of the proximate analysis performed to determine whether the selected biomass samples are suitable for biochar conversion by pyrolysis method are given in Table 1. Lignocellulosic biomass is physically and chemically heterogeneous, and these properties alter according to the biomass type [46]. Therefore, the products produced by the pyrolysis method have also some qualitative and quantitative differences.

Pyrolysis method is a thermochemical process in which the heat given to the raw material is primarily used to evaporate the moisture in the biomass structure [47]. Thus, lower moisture content is preferred to convert biomass into useful products by thermochemical methods to the conservation of energy. Comparing the moisture content of the selected biomasses, the most suitable example for biochar production is RH with the lowest moisture content (2.01 wt.%). In addition, another desired property for the production of porous materials from biomass is the high volatile matter content in the raw material. FW, WS and CW, which have the higher volatile matter content, are suitable for biochar production by pyrolysis.

The biochar yield depends on the fixed carbon amount of the raw material. The raw material with the highest fixed carbon amount (21.14 wt.%) and therefore has a high biochar yield is WH. It was determined that each selected biomass has different properties that are required to produce biochar by pyrolysis method. Therefore, biochar production from these raw materials and the utilization of biochar in agricultural applications have been appropriate.

After the pyrolysis process, it was observed that the moisture content of the biochar produced from each biomass sample is decreased (Table 2). During the process, it was determined that the amount of ash increased in all biochar samples, as volatile components were removed and the inorganic components that constitute the ash content remained in the biochar [48]. The high ash content of RHB, WHB and CWB reveals that a significant portion of the nutrient salts sequestered in the biomass samples was concentrated during the volatilization process [49]. While taking into account that EPNs show low infectivity under saline conditions [26], it can be said that biochar samples with high ash content are not suitable for the habitats of EPNs.

True and bulk density results of the biomass and biochar samples are given in Table 3. Bulk density is an important feature of biomass raw materials that influences conversion processes, storage and logistics [50]. The difference between true density and bulk density gives information about the porosity of the material. While a few increases were observed in the true density values of each biomass sample, it was determined that the bulk densities were decreased at the end of the pyrolysis process. This confirms that the porosity increased with the pyrolysis process and porous material was produced [51]. According to the studies in the literature, as the porosity develops during the pyrolysis process, the bulk density decreases while the true density increases [52]. The relationship between these two density values was explained by Gua and Lua [53] by demonstrating that true densities increased from 8.3 to 24% with the development of pores at pyrolysis temperatures up to 800 °C. The results obtained in this study are in agreement with similar studies in the literature.

FT-IR spectra of biomass and biochar samples are given in Fig. 1 (wavelength vs. transmittance%). The O–H

Table 1 Proximate analysis results of biomass samples

Biomass type	Moisture (%)	Ash (%)	Volatile matter (%)	Fixed carbon* (%)
WS	8.06	0.33	76.38	15.21
FW	7.01	0.21	80.74	12.04
RH	2.01	14.98	63.48	19.53
WH	3.46	10.26	65.14	21.14
CW	6.87	3.11	79.52	10.50

*Estimated by difference

Table 2 Moisture and ash contents of biochar samples

Biochar type	Moisture (%)	Ash (%)
WSB	1.95	7.62
FWB	1.21	8.54
RHB	3.22	20.45
WHB	2.90	19.87
CWB	1.80	17.80

Table 3 True and bulk density results of biomass and biochar samples

Biomass/biochar type	True density (g/cm^3)	Bulk density (g/cm^3)
WS	2.50	0.93
WSB	2.38	0.74
FW	1.39	0.79
FWB	1.41	0.55
RH	1.51	0.67
RHB	1.64	0.45
WH	1.49	0.80
WHB	1.59	0.61
CW	1.48	0.69
CWB	1.55	0.54

stretching vibration in the 3400 cm^{-1} is evidence of the presence of alcohol, phenol and water in the biomass structure [54]. The intensity of this peak, which exists in the WS, has decreased with the carbonization process. The presence of hydrocarbons, methane and alkyl groups in WS was shown by the peak of 2900 cm^{-1} band related to C-H stretching vibration. While the C-O and C = O stretching peaks belonging to the carbon monoxide and carbon dioxide in the 2204 cm^{-1} region were seen in WSB, there was no intense peak in this region in WS. The peak of C = C stretching vibrations, which was not a significant peak in the biomass structure, was seen in the area of 1594 cm^{-1} in WSB. The presence of this peak showed that aromatic structures were formed by carbonization. The most intense peak in the WS structure was in the 1036 cm^{-1} region belonged to C-O stretching and it indicated the presence of alcohol, ester and ether in the structure. This intense peak which disappeared with the carbonization process was not seen in WSB.

While the O–H peak intensity seen in ~ 3300 cm^{-1}, which indicates the alcohol, phenol and water compound, decreased in FWB, WHB and CWB, it completely disappeared in the sample of RHB [55, 56]. The band at 2927 cm^{-1}, 2919 cm^{-1}

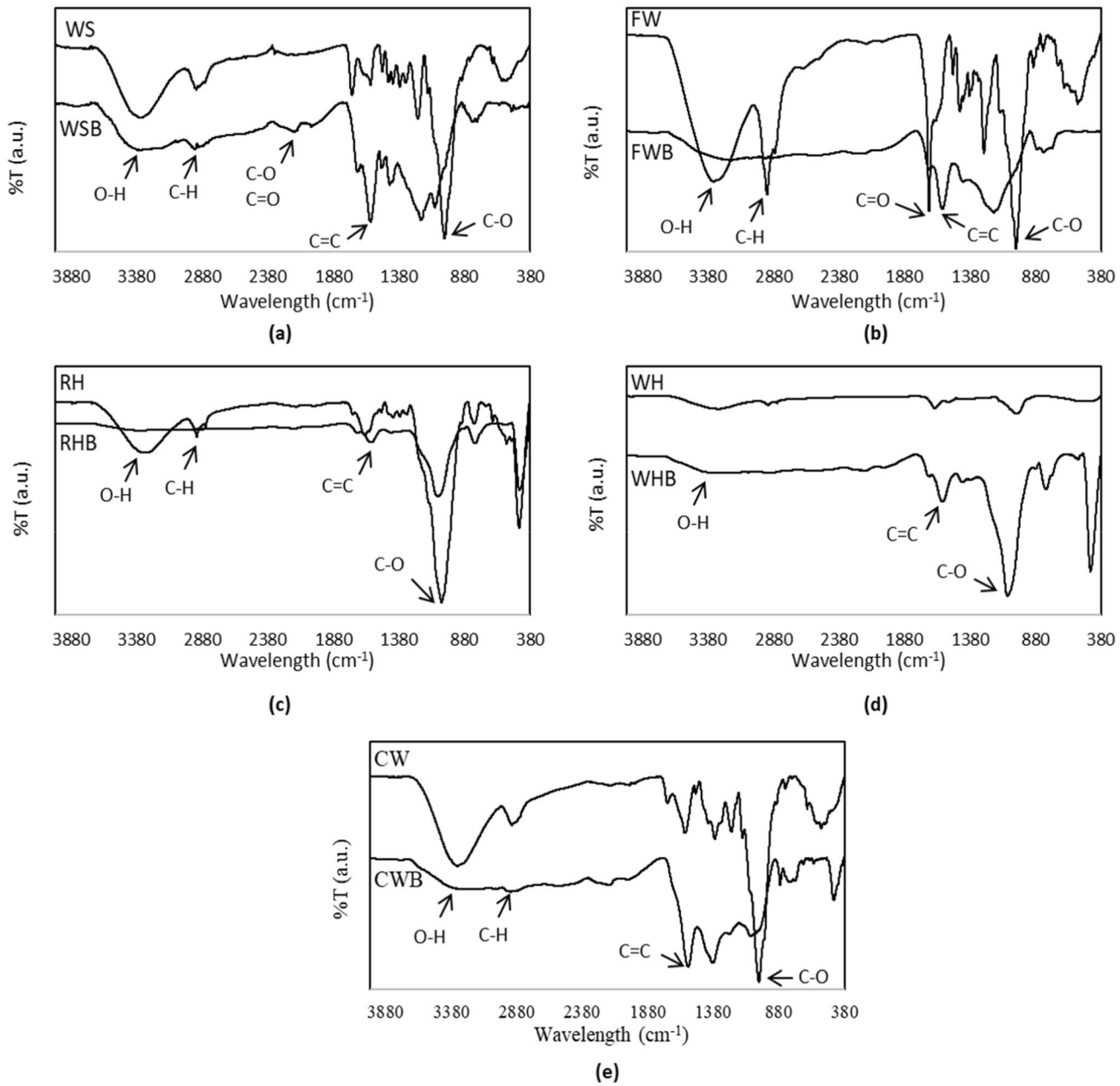

Fig. 1 Comparative FT-IR spectrum results of **a** WS and WSB, **b** FW and FWB, **c** RH and RHB, **d** WH and WHB and **e** CW and CWB

and 2917 cm^{-1} for FW, RH, and CW, respectively, proved C-H stretching vibration in the alkyl groups in biomass structure. This aliphatic chain is one of the main structural elements of lignocellulosic biomass [54]. After the pyrolysis process, these peaks disappeared in the FWB, RHB and CWB samples.

The high-intensity band at 1693 cm^{-1} was ascribable to C = O stretching for ketone, aldehyde, carboxylic acid and ester compounds, as seen in FW, while the intensity of the peak was decreased sharply in FWB [57]. As in the case of WSB, FWB, RHB, WHB and CWB samples have peaks C = C stretching vibration at about 1580 cm^{-1} region, proving that the aromatic ring structure was formed by the pyrolysis process. The WS sample has the peak with the highest intensity which indicates that the aromaticity of this biochar is higher. The high aromaticity of biochar indicates its degradability in the soil, affecting biological activities in the soil [58]. The peak of C-O stretching vibration, which intensity decreased or disappeared in all other biochar samples, showed an opposite trend in the WHB sample. When the FT-IR analysis results were examined, it was determined that the structure of the biomass samples generally contained single-bonded alkyl components, while the biochar samples obtained after the pyrolysis process had double-bond and aromatic ring structures. The fact that some of the results compared according to the biomass type differ from each other showed that the chemical structure of the biochar depends on the type of raw biomass.

SEM images showing the morphological structures of biomass and biochar samples are given in Fig. 2. The porous structure of the biochar could be an excellent habitat for soil microfauna such as nematode, bacteria, fungi, protozoa etc. [58, 59]. Due to moisture that can be adsorbed in the pores, the porous structure of biochar could be an excellent habitat for nematodes [60]. While WS and FW had a layered structure, the natural pore structure of WH and CW is in the form of channels. Despite RH was completely non-porous morphology, due to its rough surface it had a large surface area. After the pyrolysis process, the pores were opened in the biochar samples with layered structure (Fig. 2b and d) [1], the channel diameters were widened in the biochar samples with channel structure (Fig. 2h and j), in the RH sample, the surface morphology was completely altered and it was seen that the particle size decreased (Fig. 2f). The porosity characteristics like size and fraction of pores were depended on the physicochemical and surface properties of feedstock [61]. The average maximum body diameter of *H. bacteriophora* populations was measured as 25 µm ($n = 30$) (Fig. 3). Nematodes have flexible hydrostatic skeleton; they can move through microsoil pores [60]. Among BC samples, moisture adsorbed in 3-dimensional expanding pores of WSB provides a more suitable living environment for EPNs that need a thin layer of water to move [62]. It was seen that from all of the SEM images of raw biomass samples, accumulation and inorganic granules in the pores existed. The pyrolysis process provided to "clean up" the biochar, resulting in a smoother surface and clearer pore structure, with similar results [63, 64].

The results of EDX analysis performed to determine the amount of inorganic components contained in the biochar samples are given in Fig. 4. Oxygen content was highest in RHB (35.90 wt%), followed by WSB (25.85 wt%), WHB (19.29 wt%), CWB (16.23 wt%) and FWB (9.80 wt%). The Si content of the RHB sample with the highest ash content was determined as 41.30 wt%, while the other biochar had Si contents ranging between 7.94 wt% and 0 wt%. It was observed that each of the biochar samples had K in the range of 9.68–0.39 wt%. Other inorganic components included in biomass samples are Ca, Mg and Cl, and their amounts vary according to the type of biomass. The amount of inorganic substances contained in biochar could be ordered as follows: RHB > CWB > WHB > FWB > WSB. The inorganic substance content of biochar samples was related to the salinity of the charosphere (the soil surrounding the biochar, which is directly affected by the physicochemical characteristics of the biochar) [65]. Under saline conditions as a result of higher inorganic content, EPN infectivity is likely reduced. For this reason, the WSB sample with the lowest inorganic content among all biochar samples was more likely suitable for the habitats of EPNs. The results obtained from EDX analyses also confirmed the indications of differences in physicochemical and morphological properties of biochar as influenced by the type of feedstock.

Porosity and surface area is an essential property of soil due to its effect on the functions of fertility containing moisture, air, microbial activity and nutrient cycling. Soil types with low surface area also have low water holding capacity [52]. Coarse sands and fine sands have very low specific surface area values of 0.01 m^2/g and 0.1 m^2/g, respectively [66]. The addition of porous and high surface area materials into soil both increases the water holding capacity and provides aeration of the soil [67]. Specific BET surface area, pore size, specific micropore volume and specific meso-/macro-pore volume values of all biochar samples are listed in Table 4. When the specific surface area values of different biomass samples were compared, it was seen that these values varied in a wide range of 1.113–79.627 m^2/g, depending on the type of biomass. Biochar has nanoporosity (Table 4) as well as macroporosity (Fig. 2). The nanopore size change in biochar samples was determined as WSB > CWB > WHB > FWB > RHB. WSB had largest pore size and lowest surface area value. The low micropore volume and wide pore diameter in the WSB sample showed that its macroporosity was higher. The high macroporosity provides aeration of the soil. When the oxygen contained in the air dissolves in water, it becomes a source of life for

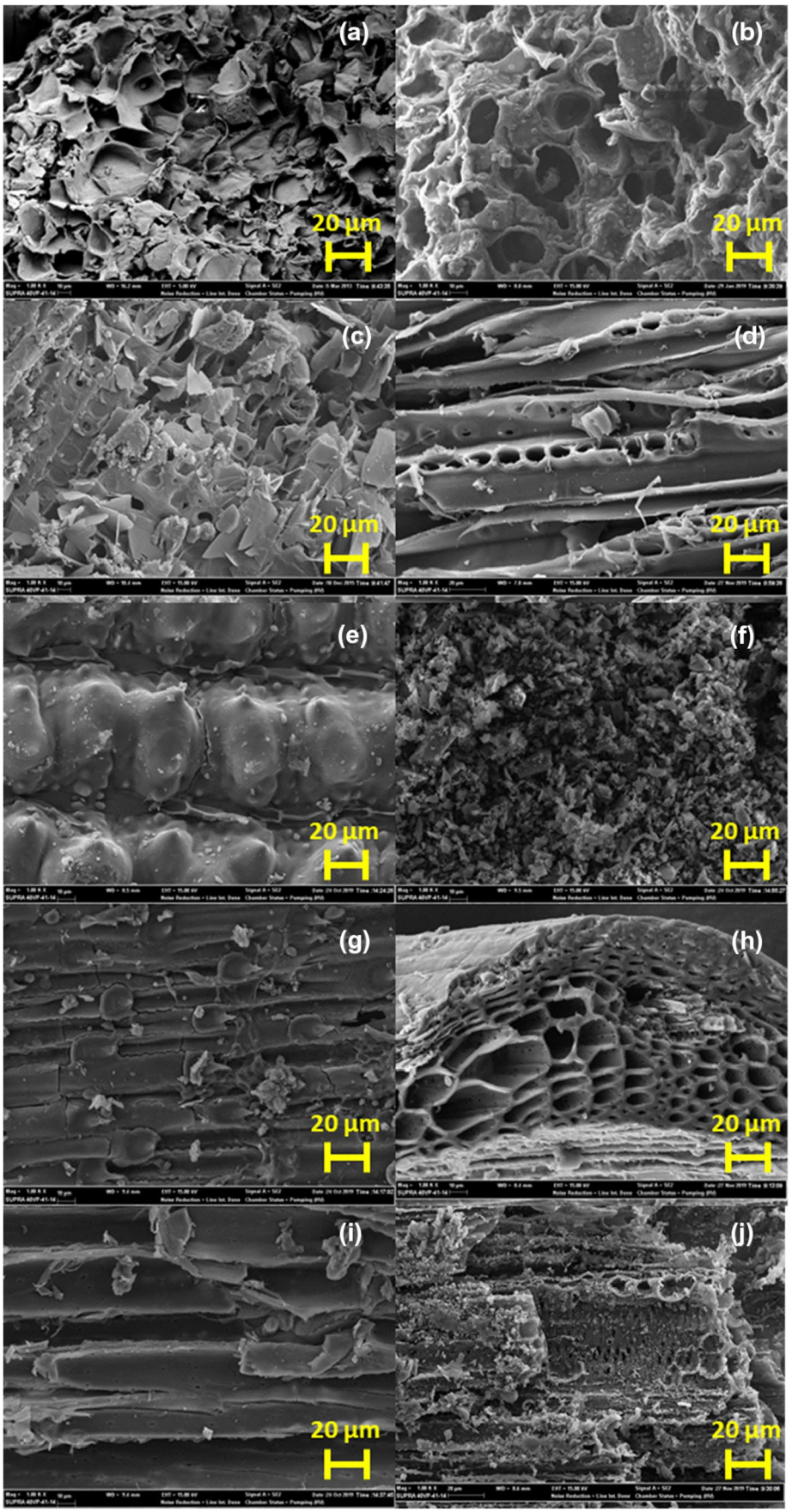

Fig. 2 SEM images of **a** WS, **b** WSB, **c** FW, **d** FWB, **e** RH, **f** RHB, **g** WH, **h** WHB, **i** CW and **j** CWB

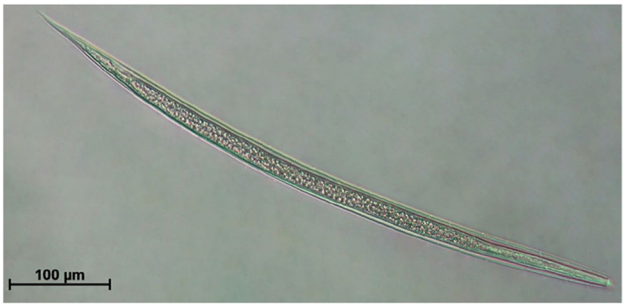

Fig. 3 Infective juvenile of *Heterorhabditis bacteriophora*

EPNs that provide their oxygen demand from dissolved oxygen in water (Neher, 2010). Thus, it can be said that WSB with high macroporosity is a suitable porous material for the survival of EPNs. It was specified that RHB, which had the highest surface area with 79.627 m^2/g, also had the highest V_{micro} and $V_{meso/macro}$ values. As a result, the specific surface area and pore size values of WSB and FWB, which were determined to have similar morphologic structures from SEM images, were different from each other. This behaviour showed that the chemical structure of biomass in the volatilization process had more effectiveness on solid product (biochar) porosity. Similar results were observed for WHB and CWB samples. The results obtained were in agreement with other studies in the literature showing that biochar porosity character depended on the type of raw material [68–70].

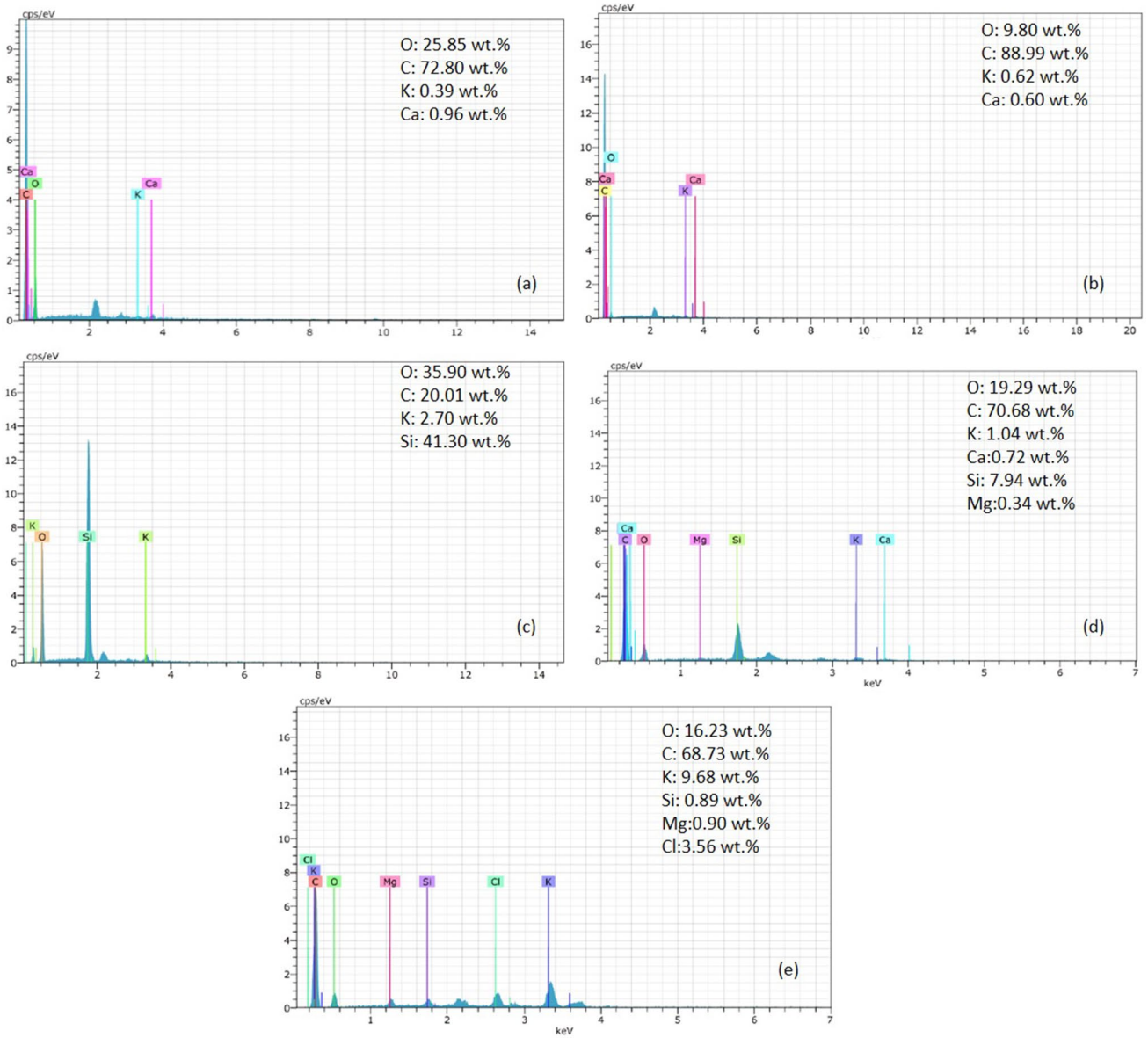

Fig. 4 EDX results of **a** WSB, **b** FWB, **c** RHB, **d** WHB and **e** CWB

Table 4 Porosity characteristics of biochar samples (N_2 adsorption)

Biochar type	S_{BET}[†] (m^2/g)	Pore size[‡] (nm)	V_{micro}[§] (cm^3/g)	$V_{meso/macro}$[#] (cm^3/g)
WSB	1.113	31.17	0.0004	0.0018
FWB	36.140	6.99	0.0125	0.0072
RHB	79.627	5.07	0.0233	0.0319
WHB	48.742	7.79	0.0152	0.0181
CWB	4.957	14.82	0.0013	0.0065

[†]Multi-point BET surface area

[‡]Determined by BJH method from nitrogen adsorption data

[§]From V-t plot analysis

[#]From the difference of total pore volume at P/P_0=0.99 minus the micropore volume

S_{BET}: specific BET surface area (m^2/g)

V_{micro}: specific micropore volume (cm^3/g)

$V_{meso/macro}$: specific meso-/macro-pore volume (cm^3/g)

3.2 Results of infectivity trials

The results of the infectivity tests showed that different biochar types and different mixture proportions have a significant effect on EPN infectivity. There was a negative correlation between sand:biochar mixture and larva mortality. Except for WSB, all biochar samples have significantly reduced EPN infection at 60:40 mixtures compared to control. Among all biochar samples, CW had the most negative effect on larva mortality even at low biochar proportions. On the other hand, WSB had no significant impact on IJ infectivity for all proportions compared to control treatments (Fig. 5).

Almost all biochars significantly decreased EPN infectivity. Interestingly, WSB shows opposite results compared to other biochar samples. To understand the background of these effects, it was hypothesized that pH and EC values of mixtures might have a relation with the results (Fig. 6). For a better comparison, results of the 60:40 mixture proportions were selected as the larva mortalities significantly differ among biochar samples.

When the EC values of the mixtures were examined, there was a negative correlation between EC values and larva mortalities of the mixtures ($R = -0.70$). CWB had the highest EC while it had the lowest larva mortality. Similar relation can be specified for WSB; hence, it has the lowest EC and highest larva mortality. Likewise, pH values were also negatively correlated with larva mortality ($R = -0.60$). We examined some replicates with lower infectivity, and we found that EPNs are mostly dead but their body composition remained the same. We think that EPNs simply could not survive under detrimental pH and salinity levels.

There is limited information about the effect of biochar application on nematodes. Several researchers focused on understanding this relationship, but the results were sparse. One of the most recent studies by Domene et al. [36] aimed to assess the impact of biochar application on the soil nematode community. They found that biochar application did not affect total nematode abundance, but they observed shifts between different nematode communities. For instance, low biochar application rates increased predator and plant-parasitic nematodes, while high application rates promote fungivore and bacterivores. In contrast, Liu et al. [37] found that high biochar application rates positively affected herbivorous nematodes. Mondal et al. [35] revealed that lower biochar doses had no significant effect on mortality and infectivity of plant-parasitic nematodes, but higher biochar doses reduced the reproduction capacity. Similar results were also obtained from other studies [40, 71–73]. Current results supported the idea that even the same biochar source may have a different impact on different nematode species.

According to our results, WSB showed no significant impact on the infectivity of *H. bacteriophora.* We suggest that this result may be related to the high aromaticity of WSB. The high aromaticity of biochar increases its stability in the soil. The high stability of biochar delays the release of toxic degradation product components such as NH_4^+, which are nematicidal to plant-parasitic nematodes [38]. Another significant result was found for CWB, which reduced the infectivity significantly at higher biochar proportions. CWB had the highest EC and pH values among all biochars. As mentioned before, this can be related to its high amount of inorganic substances. High salinity and pH are known to be detrimental for some EPN species [74, 75]. We infer that the high content of inorganic components and consequently high salinity value caused high mortality of *H. bacteriophora* and thus reducing its infectivity.

4 Conclusion

In this study, different biochar samples were investigated comprehensively for their physical and chemical properties to determine the materials' potential application on

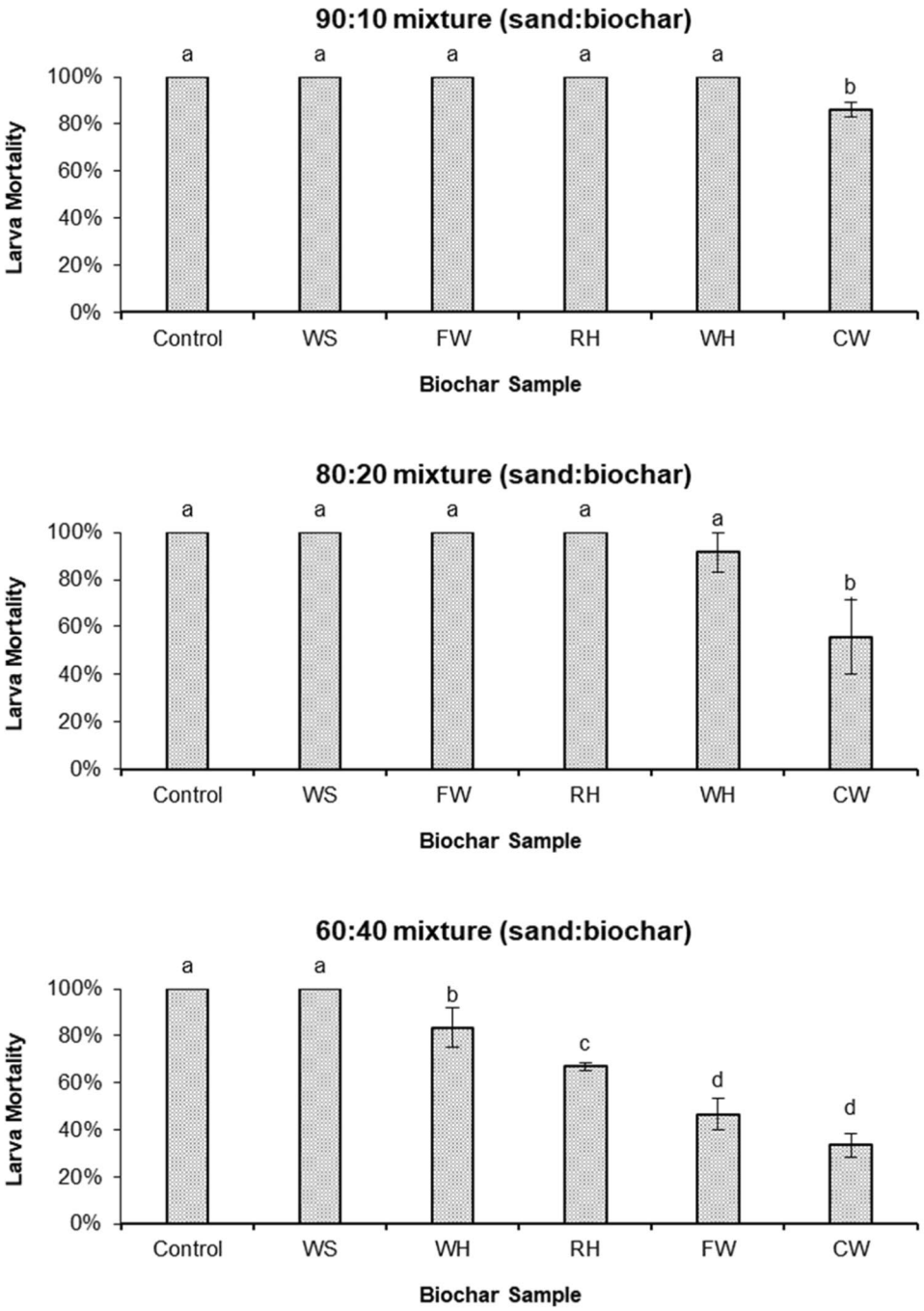

Fig. 5 Effects of different biochar samples on larva mortality at different mixture proportions

infectivity of entomopathogenic nematodes. Walnut shell (WS), fir wood sawdust (FW), rice husk (RH), einkorn wheat husk (WH) and corn waste from field (CW) were selected as five different biomass sources. While selecting raw material, it was considered that the biomass to be utilized is waste biomass that has no food value. In addition to that, the selected biomass samples are grown in abundance in Turkey. The use of waste biomass in this study has also contributed to sustainable agriculture studies. Proximate analyses of biomass samples were carried out in order to determine whether they were appropriate to volatilize by the pyrolysis process. According to the results, these raw materials with low moisture and high volatile content were suitable for use in the pyrolysis process. Biomass samples were carbonized by the pyrolysis process under constant reaction conditions (550 °C and 15 min) and the biochar obtained as pyrolysis solid products was characterized by different characterization methods. It was determined that the amount of moisture content in the obtained biochar samples decreased. As a result of density measurement and SEM analysis, it was designated that

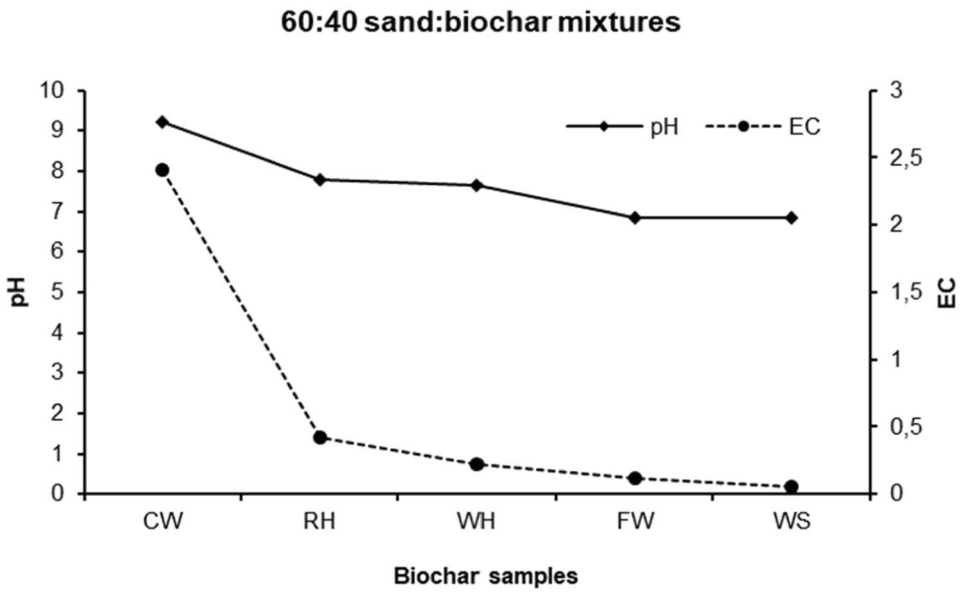

Fig. 6 EC and pH values of 60:40 sand:biochar mixtures

the porosity of biomasses increased with heat treatment by pyrolysis. FT-IR analysis has shown that a more aromatic structure is obtained with biochar production and WSB has the highest aromaticity. This is an important property as the aromaticity of the material affects its degradability in the soil. From the EDX analysis results, it was seen that the biochar with the highest inorganic matter and oxygen content was RHB. Inorganic components in the structure influenced the amount of ash in the biochar. Therefore, these results are confirmed by the fact that the sample with the highest ash content is RHB. Porosity characteristics showed that the WSB sample with the lowest specific surface area had the highest pore diameter in all biochar samples. When the pore volume values were examined, it was determined that the WSB has more macropores and RHB had more micropores. Effects of feedstock types of biochars on larva mortality (EPN infectivity) were significant. Except for WSB, EPN infectivity was significantly reduced, especially at higher biochar proportions. Our study suggests that biochar application as an environmentally friendly soil amendment method may have detrimental or promoting impacts on some beneficial nematode species. We think that biochar sources should be considered if biochar and beneficial nematodes are to be applied simultaneously. Further investigations are also needed to understand the effects of biochars on soil communities and more accurate results in the field.

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Author contribution EY: designed the research, produced and characterized biochar samples and wrote the manuscript. TCU: co-designed the research, conducted infectivity trials, statistically analysed the data and wrote the manuscript. NÖ: co-designed the research. All authors have read and approved the manuscript.

Data availability Data will be available upon acceptance of the manuscript.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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