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EFFECTS OF BIOCHAR APPLICATIONS ON GROWTH, NUTRIENT CONTENT AND BIOCHEMICAL PROPERTIES OF *Ocimum basilicum* L.

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ABSTRACT

This study investigated the effects of biochar treatments on the growth, nutrient content and some biochemical properties of basil. Biochars obtained from two different biomasses, rice husk (RBC) and tomato harvest waste (TBC), were applied at a dose of 2% to the growing medium consisting of a 1 : 1 soil and peat mixture. No biochar-added medium (1 : 1 soil and peat) was used as a control. The experiment was established in a completely randomized design with six replications for each treatment. At the end of the study, the height, number of lateral branches, total herb weight, and leaf weight of the plants were measured. In addition, chlorophyll contents in SPAD (Soil Plant Analysis Development), different nutrients and total phenolics contents, and antioxidant activities were analyzed. As a result of the study, the effects of biochar treatments differed depending on the biomass source. RBC significantly increased plant height, total herb weight, and leaf weight while negatively affecting the number of lateral branches. TCB did not cause any significant variation in plant height and number of lateral branches. While RBC provided a slight increase in leaf weight compared to the control, it did not cause a significant change in plant height, total herb weight, and the number of lateral branches. Except for a slight increase in K content due to RBC application, both biochar treatments did not cause a significant increase in leaf nutrient content. While RBC treatment did not cause a significant change in total phenol, it caused an increase in antioxidant activity. TBC application decreased the SPAD value from 22.4 in the control to 20.4.

Key words: antioxidant, basil, biochar, mineral element, phenolics

INTRODUCTION

The substances that come out due to the pyrolysis of organic wastes at 250°C or higher temperatures, in the absence of oxygen, are called biochar [Karhu et al. 2011]. It is claimed that biochar retains toxic elements in the soil and prevents them from being transported to the plant, suppresses soil-borne pathogens [Eo et al. 2018], and reduces the impact of climate change [Lehmann et al. 2006]. In addition, many studies have shown that soil quality and productivity can be amended with biochar application, resulting in increased plant growth [Lehmann et al. 2003]. The positive effects of biochar on plant growth are also associated adding biochar to increase soil water holding capacity, cation-exchange capacity, and specific surface area [Karhu et al. 2011]. Since biochar is a negatively charged substance, it retains water and nutrients [Conte et al. 2013]. It has been demonstrated that biochar applications increase the total C content of the soil and the content of Mg, N, P, K, Ca, and soil enzyme activity [Wang et al. 2014].

As a result of studies carried out on different plants so far, although the effects of biochar varied depending on the characteristics of the soil, the plant species, and the source from which the biochar was obtained,

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in general, it has been determined that these organic matters were effective in increasing plant growth, yield, and nutrient content [Głodowska et al. 2017]. It was reported that biochar obtained from black cherry wood increased plant height by 48%, leaf length by 24%, and leaf width by 27% in basil [Jabborova et al. 2021]. Zhaoxiang et al. [2020] stated that biochar application increased both the root and stem biomass of *Plantago lanceolata* L. compared to the control.

Basil (*Ocimum basilicum* L.) is a medicinal and aromatic plant species belonging to the Lamiaceae family, and its fresh leaves are usually consumed. In addition, the essential oils obtained from this plant are used for many purposes in medicine, thanks to their properties such as pain relieving, urinary tract antiseptic, soothing stomach disorders, diuretic gas--digesting, and sedative [Asımgil 1996, Baytop 1999]. There is increasing interest in the ornamental use of plants, combining features such as smell, taste, food, and visuality. In this respect, basil is seen as one of the plants that will make essential contributions to landscape designs [França et al. 2017].

The study was carried out to determine the effects of different biochar applications on basil plants' development, nutrient, and phytochemical content.

MATERIALS AND METHODS

Material and experimental design. The study was conducted in an unheated greenhouse of Tokat Gaziosmanpaşa University Agricultural Application and Research Center of Tokat, Turkey. The experiment was conducted during the summer when the average temperature was 25–30°C. The biochar materials used in the study were produced by heating the biomass of rice husk or tomato harvest waste at 500°C under anaerobic conditions known as slow pyrolysis [Ronsse et al. 2013]. The C/N ratio of rice husk biochar (RBC) used in the study was 138/1, pH 10.2, specific surface area 212 $m^2 g^{-1}$, total P concentration 0.05 g kg^{-1}, K concentration 38.9 g kg^{-1}. The C/N ratio, pH value, specific surface area, and total P and K concentration of the tomato harvest waste (TBC) biochar were determined as 155/1, 11.6, 209 $m^2 g^{-1}$, 3.69 g kg^{-1}, 34.3 g kg^{-1}, respectively [Günal et al. 2019]. As plant material, *O. basilicum* seedlings grown by seeds sown in seedling trays filled with a 1 : 1 mixture of soil and peat were used. The seeds used in the study were obtained from a private seed company. Two weeks after sowing, healthy, uniform, 4–5 cm tall basil seedlings were transferred into 2-liter pots filled with air dry 1 : 1 ratio of 1800 g soil and peat mixture. As basic fertilization, 90 mg kg^{-1} N in the form of urea, 80 mg kg^{-1} P and 100 mg kg^{-1} K in the form of KH_2PO_4, 2 mg kg^{-1} Fe in the form of Fe-EDTA, and 1 mg kg^{-1} Zn in the form of $ZnSO_4 7H_2O$ were applied to all pots. The RBC or TBC was mixed homogeneously with the soil-peat mixture and basic fertilizers at the beginning of the experiment at a dose of 2%, w/w.

The experiment was carried out in a completely randomized plot design with six replications. Each replication consisted of a pot with a basil seedling in it. The plants were irrigated manually with distilled water, considering their water needs.

Plant growth. Plant height, number of lateral branches, total herb weight, and total leaf weight were determined at the stage of slowing growth before flowering, approximately 40 days after the plants were transferred to the pots. Chlorophyll content expressed as soil plant analysis development (SPAD) value of three randomly selected fully expanded leaves of a plant in each pot was measured with a chlorophyll meter (SPAD-502, Konica Minolta).

Leaf mineral analysis. The collected leaf samples were washed with distilled water, dried in an oven at 70°C for 48 hours, and ground in an agate mill. According to the wet digestion method, the ground plant samples were digested with H_2O_2-HNO_3 in a microwave digestion system (MarsXpress; CEM Corp, Matthews, NC, USA). The resulting solutions were cooled, and the concentrations of P, K, Ca, Mg, S, Fe, Zn, Mn, Cu, and B elements in the solutions were determined by the ICP-OES device (Vista Pro; Varian, Springvale, Australia) [Kaçar and İnal 2008].

Total phenol and antioxidant analysis. Total phenolic content was determined using Folin-Ciocâlteu's chemical, as described by Singleton and Rossi [1965]. The homogenized plant sample was extracted in acetone, water, and acetic acid (70 : 29.5 : 0.5) solution for one day. After filtration, Folin-Ciocâlteu's chemical and distilled water were added to the solution and left for 8 min. Next, 7% sodium carbonate was added. The absorbance value of the solution, which turned bluish after two hours of incubation, was measured at

750 nm using a spectrophotometer (T60U, PG Instruments, UK). Using a curve obtained from gallic acid standards, total phenol contents were calculated and expressed as mg gallic acid equivalents (mg GAE L^{-1}).

Total antioxidant capacity was estimated using the Trolox equivalent antioxidant capacity (TEAC) assays. TEAC solution was prepared by mixing ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic cid) and 2.45 mM potassium bisulfate and kept in the dark for 12–16 h. Then, the mixture was diluted in the acidic medium of 20 mM sodium acetate buffer (pH 4.5) until it reached an absorbance value of 0.700 ±0.01 at a wavelength of 734 nm for more extended stability. Finally, 30 µL of the homogenized plant samples solution and 2.97 mL of the ABTS solution were mixed, and after incubation for 10 min, their absorbance value was measured at 734 nm. By using a curve obtained from the absorbance value of Trolox standards (10–100 µM), total antioxidant capacity was expressed as µM Trolox equivalents per liter [Saracoglu 2018].

Statistical analyzes. One-way analysis of variance (ANOVA) was carried out to determine whether the effects of treatments are statistically significant at a 0.05 probability level. When the variance analysis result was significant, the means of the treatments were separated by Duncan's multiple range test at $p < 0.05$. All statistical analyses were performed using the SAS 9.0 package program (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Adding 2% tomato biochar to the growing medium did not cause a significant change in plant height, while the application of rice husk biochar increased the plant height compared to the control. While the plant height was 16.2 cm in the control treatment, this value reached 19.2 cm in the RBC treatment. In the TBC treatment, the plant height was 16.7 cm (Fig. 1a). Rice husk biochar caused a decrease in the number of lateral branches (Fig. 1b), while there was no significant difference between TBC and control treatments in terms of the number of lateral branches. In previous studies, it has been reported that the effect of biochar on soil fertility or plant growth varied depending on the plant species, doses of the applied biochar, the temperature at which the biochar is obtained, and the properties of the raw material [Jeffrey et al. 2011]. It has been reported that animal manure biochar produced at 400°C significantly increased both stem and root dry matter yield compared to control, whereas animal manure biochar produced at 600°C and kiwifruit wood biochar produced at 1000°C did not affect both stem and root dry matter yield [Subedi et al. 2016]. These results show that the effect of biochar changes depending on the production temperature and the raw material obtained. There have also been studies reporting that biochar applications promoted plant growth [Lin et al. 2015] depending on the raw material used in production, production temperature, and the characteristics of the soil-applied, as well as studies reporting that it was ineffective [Subedi et al. 2016] and even had a negative effect [Nelissen et al. 2015]. Likewise, the effects of two different biochars used in this study on the development of *O. basilicum* seedlings differed. Both biochar treatments caused a significant increase in total basil weight compared to the control, with the RBC treatment more pronounced (Fig. 1c). Rice husk biochar application caused an increase in fresh leaf weight, while the effect of TBC was insignificant (Fig. 1d). Plant herb weight, which was 9.2 g $plant^{-1}$ in the control treatment, increased to 21.4 g $plant^{-1}$ in the RBC treatment and 13.4 g $plant^{-1}$ in the TBC treatment. The fresh leaf weight was 8.4 g $plant^{-1}$ in the control treatment; it increased to 12.5 g $plant^{-1}$ in the RBC treatment.

The positive effect of biochar application on plant development is explained by its improvement of the soil's physical, chemical and biological properties, nutrient elements, and soil water availability [Lehmann et al. 2011]. In this study, the biochar application to the growing medium did not affect the content of macronutrients in basil leaves, except for the K content. RBC and TBC caused significant increases in the K contents of leaves compared to the control (Tab. 1), probably due to the high K concentrations in the biochar used. Similarly, the increase of K uptake in lettuce was observed by Nigussie et al. [2012] in an experiment with maize stalk biochar. Regarding other elements, decreases were observed in the content of Fe and Mn with the application of RBC and in the content of Zn due to both RBC and TBC application (Tab. 2). In the study by Al-Wabel et al. [2014] on maize, it was reported that biochar application increased the Fe content of the leaf while it caused

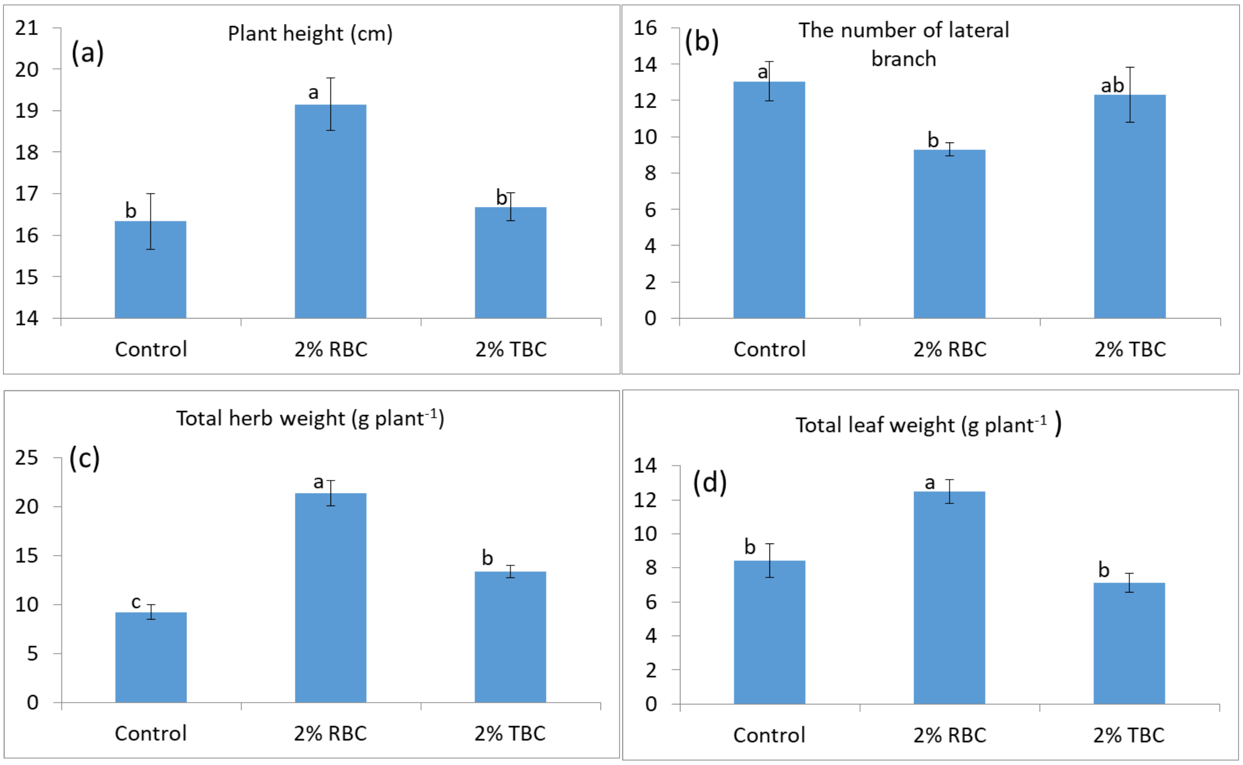

Fig. 1. The effects of rice husk biochar (RBC) and tomato harvest waste biochar (TBC) application on plant height (a), the number of lateral branch (b), total herb weight (c) and total leaf weight (d) of basil plant. The difference between the means denoted by the same letter is not significant $p < 0.05$

a decrease in Mn and Cu contents. Lucchini et al. [2014] reported that biochar application to wheat caused a significant decrease in plant Zn content. The decrease in Mn and Zn concentrations resulting from biochar application might be due to the high charge density of the biochar [Liang et al. 2006] and the corresponding high nutrient holding capacity [Lehman et al. 2003].

Some researchers reported that biochar application increased the chlorophyll content [Jabborova et al. 2021], which was not confirmed by the result of this study. On the contrary, TBC application caused a slight decrease in chlorophyll content (SPAD) due to biochar application (Tab. 3). Netto et al. [2005] reported that the increase in leaf SPAD is most likely linked to better availability of nutrient elements such as K, Fe, Mn, and Zn in the soil, which play an important role in chlorophyll biosynthesis. Biochar applications, which were the subject of this study, only increased the K content of the leaves but did not increase the F, Mn, and Zn content. Perhaps as a plausible consequence of this situation, there was no increase in SPAD values of plants with biochar treatments.

It is known that phenolic compounds with bioactive properties contribute to the nutritional value of plants and play an essential role in the environmental adaptation of the plant and its stress resistance [Lattanzio et al. 2008]. In addition to many other factors, it has been reported that the cultivation system also affects the phenolic content of plants [Borguini et al. 2013]. It has been suggested that the biochar amendment, which is a different cultivation system, may cause changes in the phenolic compound content of the plant by changing the nutrient availability in the medium [Petruccelli et al. 2015]. Jabborova et al. [2021] reported that biochar produced from cherry tree wood increased basil's total flavonoid content and antioxidant activity. The RBC and TBC applications used in this study did not increase the total phenol content; however, TBC caused a decrease (Tab. 3). These conflicting results reveal that biochar's effect on plants' phenolic content may vary depending on the source from which it is produced. Although it did not cause an increase in total phenol content, a significant increase in antioxidant content of RBC application was observed compared to control. This result suggests that applying RBC may have increased the content of antioxidant substances other than phenolics.

Table 1. The effect of biochar applications on *Ocimum basilicum* L. leaf macroelement concentrations

Treatments	Macroelement (%)				
	K	P	Mg	Ca	S
Control	4.15 ±0.23^{b}	0.49 ±0.13^{ns}	0.66 ±0.13^{ns}	3.42 ±1.1^{ns}	0.21 ±0.08^{ns}
RBC	4.70 ±0.22^{a}	0.49 ±15	0.60 ±0.18	3.35 ±1.5	0.22 ±0.07
TBC	4.60 ±0.19^{ab}	0.51 ±19	0.68 ±0.16	3.42 ±1.7	0.21 ±0.11
CV (%)	5.1	4.2	3.7	3.5	4.6

The difference between the means denoted by the same letter is insignificant at $p < 0.05$ (Duncan's test); ns – non-significant, RBC – rice husk biochar, TBC – tomato harvest waste biochar, CV – coefficient of variation

Table 2. The effect of biochar applications on *Ocimum basilicum* L. leaf microelement concentrations

Treatments	Microelement (mg kg^{-1})				
	Fe	Mn	Zn	Cu	B
Control	370 ±31.2^{a}	76.3 ±4.8^{a}	41.0 ±2.3^{a}	42.4 ±2.6^{ns}	29.5 ±3.5^{ns}
RBC	284 ±22.4^{b}	56.3 ±2.2^{b}	36.8 ±1.2^{b}	42.8 ±3.5	29.2 ±2.0
TBC	384 ±35.8^{a}	67.7 ±3.1^{a}	36.7 ±1.1^{b}	43.3 ±2.9	30.0 ±2.9
CV (%)	17.2	15.6	12.6	12.2	5.6

The difference between the means denoted by the same letter is insignificant at $p < 0.05$ (Duncan's test); ns – non-significant, RBC – rice husk biochar, TBC – tomato harvest waste biochar, CV – coefficient of variation

Table 3. The effect of biochar applications on total phenol (TP) content, antioxidant capacity (TEAC), and SPAD values of *Ocimum basilicum* L. plants

Treatments	TP (µg GAE g^{-1} fw)	TEAC (µmol TE g^{-1} fw)	SPAD
Control	3434.1 ±286^{a}	23.18 ±2.1^{b}	22.14 ±0.26^{a}
RBC	3307.0 ±370^{a}	33.89 ±2.7^{a}	21.34 ±0.20^{ab}
TBC	2298.6 ±234^{b}	21.41 ±2.2^{b}	20.39 ±0.59^{b}
CV (%)	22.7	21.3	3.19

The difference between the means denoted by the same letter is insignificant at $p < 0.05$ (Duncan's test); ns – non-significant, RBC – rice husk biochar, TBC – tomato harvest waste biochar, CV – coefficient of variation

CONCLUSION

Biochar applications increased plant growth and did not cause a significant increase in plant nutrient content, except for K in basil. These results indicate that the growth-promoting effect of biochar is not only related to the increase in nutrient intake but also its other features, such as improving the physical properties of the soil and increasing the water holding capacity are also effective in plant development. As a result of this study, it has been determined that the growth of basil plants can be increased significantly by adding biochar to growing substrates. In addition, the results reveal that organic wastes generated from different production systems can be used for a growing media amendment by processing into a biochar. On the other hand, there is a need for further studies to determine the most suitable biochar type and ratio according to the soil type and plant variety.

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