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Effects of biochar treatments on the elemental composition of tobacco (*Nicotiana tabacum* L.) leaves based on the priming period

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ABSTRACT

This study determined the effects of different doses of biochars (B) on Virginia tobacco (*Nicotiana tabacum* L.) cultivar, on first and second harvest dependent change in plant nutrients (N, P, K, Ca, Mg, Cl, Zn, Fe, Mn, Cu, and B), leaf color parameters (L*, a*, and b*), chlorophyll value (SPAD), electrolyte leakage (EL), crude ash, number of leaves, and plant height. Pot experiments were conducted with biochar treatments of 10 tons ha^{-1} (B1), 20 tons ha^{-1} (B2), 40 tons ha^{-1} (B3), and 80 tons ha^{-1} (B4). Tobacco leaf macroelement (N, P, K, Ca, and Mg) levels increased with increasing biochar doses. The highest values were obtained for B4 treatments (80 tons ha^{-1}) and the lowest for control (B0) treatments. Microelements (Fe, Zn, Mn, and B) exhibited a non linear change, while Cl and Cu exhibited a linear change. Color parameters (L*, a*, and b*) for the first and second priming showed the highest L* and b* values for B2 and B3 treatments, respectively, and the highest a* values for the B2 treatment. Leaf SPAD values increased with increasing biochar doses; further, the obtained SPAD values were ordered as B4 > B3 > B2 > B1 > B0. Leaf electrolyte leakage values were 25.90 %–37.25 % in the first priming and 26.90 %–40.59 % in the second priming. For both the primings, the highest crude ash values (21.94 % and 19.05 %) were observed for the B4 treatments, whereas the lowest values (17.89 % and 17.01 %) were observed for the B0 treatments. the tallest plant height (121.9 cm) and the highest number of leaves (45.3) were determined in B4 applications. Overall, considering the nutrition and quality of tobacco, B2 application is recommended.

1. Introduction

Various food and agricultural wastes can be used as soil conditioners to improve soil health and plant production [1]. Industrial and agricultural processes generate large quantities of waste and these wastes, originating from plants and animals, exert severe environmental pressure, resulting in surface and groundwater pollution [2]. However, the direct utilization of such wastes as soil conditioners may result in various risks on soil health, especially on chemical and microbiological characteristics [3]. Therefore, these wastes should be converted into non-hazardous materials through appropriate methods.

Recycling waste materials reduces environmental risks, ensures reliable disposal, and enables final product utilization for sustainable soil fertility [4]. Converting these wastes into biochar (rich in carbon) by pyrolysis is an effective agricultural waste recycling

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method [5]. Biochar improves soil health and increases soil fertility and crop yields [6]. Waste management through biochar has been reported as a possible method for carbon sequestration, reduction of greenhouse gas emissions [7], the adsorption of potentially toxic elements, and climate change mitigation [8]. Biochar modulates soil conditions with its large surface area, porous nature, high cation exchange capacity, and functional groups [9]. Further, it is used as a suitable soil conditioner to improve plant growth and development [10].

Tobacco (*Nicotiana tabacum* L.) plants require high quantities of macronutrients, such as N, P, and K and as well as, such as Ca, and Mg for high yield and quality [11]. Although micronutrients, including B, Mn, Cu, Fe, and Zn, are also required for the quality of tobacco leaves [12]. Chlorine (Cl) is an essential plant micronutrient that affects osmotic and stomatal regulation, oxygen development during photosynthesis, and disease resistance and tolerance [13]. Although it is classified as a micronutrient, it has been stated that the Cl value in the leaf of tobacco is close to the macronutrient element [14]. It has been suggested that the Cl concentration in the leaf is a suitable indicator for the burning rate and fire holding capacity [15].

Electrolyte leakage (EL) is the hallmark of the stress response in intact plant cells [16], it is widely used as a measure of plant stress tolerance used to predict plant membrane integrity against various sources of stress, environmental stresses, aging, senescence, fruit ripening, etc. Electrolyte leakage is ubiquitous between different species, tissues and cell types and can be triggered by all major stress factors [17]. Electrolyte leakage from tissues is one of the central reactions of the plant organism to stress. It is observed under almost all types of stress, both abiotic and biotic. Loss of key electrolytes can lead to significant changes in metabolism and, in some cases, death of cells or the whole organism. For a long time it was believed that electrolyte leakage is associated with disruption of cell integrity and disruption of plasma membranes, and this is an unregulated process. However, in recent years there has been much evidence that electrolyte leakage is prevented and reversible by ion channel blockers in most cases [18].

Tobacco (*Nicotiana tabacum* L.) is classified based on its chemical composition, such as flue-cured, oriental, burley, Maryland, sun-cured, and cigar tobacco. There is a significant difference in the internal quality and chemical composition of these tobacco types [19]. The chemical composition of tobacco leaves is vital in assessing tobacco quality. The absolute and relative quantities of plant nutrients, leaf color parameters (L*, a*, and b*), chlorophyll value (SPAD), Electrolyte leakage (EL), crude ash, number of leaves, and plant height of Virginia tobacco cultivar. These components depend on crop varieties and maturity, soil and climate conditions, the drying process, as well as the optimum mineral nutrition of tobacco plants [20]. The physicochemical properties of soil contribute to tobacco growth and quality [21]. Normal growth and maturation of tobacco are closely related to the physical and chemical properties of the soil in which tobacco is cultivated. In addition, tobacco also requires sufficient amounts of various nutrients from the soil. Tobacco (*Nicotiana tabacum* L.) is economically valuable and is among the most important cultivated plants in the world [22].

This study determines the effects of different doses of biochar, obtained through slow pyrolysis (500 °C) on harvest period (first and second harvest) dependent change in plant nutrients (N, P, K, Ca, Mg, Cl, Zn, Fe, Mn, Cu, and B), color parameters (L*, a*, and b*), SPAD values, electrolyte leakage (EL), crude ash, number of leaves, and plant height of Virginia tobacco cultivar.

2. Materials and methods

2.1. Pot experiments and layout

Pot experiments were initiated on the May 16, 2022, under greenhouse conditions in Bornova district, Izmir province (38°27′12.5″ N, 27°13′40.2″ E). The physical and chemical characteristics of the soils are provided in Table 1. Following air drying, soil samples were passed through a 4-mm sieve. Experimental soils were classified as Typic Xerofluvent [23]. Virginia tobacco cultivar LCV K-326 was used as the plant material.

Post-harvest residues of tomato plants (stalks) were used as feedstock for biochar. These stalks were converted to biochar through slow pyrolysis at 500 °C. Biochar physicochemical properties are presented in Table 2. For plantation, plastic pots were filled with 5 kg

Table 1
Soil physico-chemical properties.

pH[a] (1:2.5)	7.53	K[e] (mg kg^{-1})	128.53
E.C[b] (dS m^{-1})	0.26	Ca[e] (mg kg^{-1})	2362.71
$CaCO_3$ (%)	5.42	Mg[e] (mg kg^{-1})	125.85
Sand (%)	68.96	Na[e] (mg kg^{-1})	54.17
Silt (%)	18.00	Fe[f] (mg kg^{-1})	3.41
Clay (%)	13.04	Zn[f] (mg kg^{-1})	0.48
Texture	Sandy loam	Mn[f] (mg kg^{-1})	3.83
Organic matter (%)	1.63	Cu[f] (mg kg^{-1})	0.34
N[c] (%)	0.062	B[g] (mg kg^{-1})	0.42
P[d] (mg kg^{-1})	5.41		

[a] w:v, 1:2.5 water.
[b] Electrical conductivity w:v, 1:2.5 water.
[c] Total kjeldahl.
[d] available olsen.
[e] available 1 N NH_4OAc extract.
[f] available DTPA extract.
[g] hot water extract were determined azometine-H methods. Each value is the mean of three replicates.

of soil. Biochar treatments were arranged as 1-control (B0), 2-biochar 10 tons ha^{-1} (B1), biochar 20 tons ha^{-1} (B2), biochar 40 tons ha^{-1} (B3), and biochar 80 tons ha^{-1} (B4). Ammonium sulfate ($NH_4(SO_4)_2$, diammonium phosphate (DAP), and potassium sulfate (K_2SO_4) were used as the source of N, P, and K, respectively. All pots were supplied with 150 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5, and 100 kg ha^{-1} K_2O. Initially, tobacco seedlings were grown in a nursery, and two seedlings were planted in each pot on 16 May 2022. Then, the number of plants was thinned to one in each pot. Throughout the experiments, irrigation was performed based on the soil field capacity. Pots were watered every four days with deionized water to maintain soils close to 70 % of water holding capacity (WHC) throughout the experiment duration. The leakage water was put into the pots to prevent losses. Chemicals were applied against louse aphids and pests. The first harvest was made on July 7, 2022 and the second harvest on August 25, 2022.

2.2. Data collection

Harvested tobacco leaves were dried under controlled conditions according to curing can be divided into three distinct stages: yellowing, leaf drying, and stem drying. The first step air temperature in the barn is maintained between 30 and 40 °C, with relative humidity of 80–95 %, for about 48 hour until the leaves turn yellow. In the second stage, air temperature in the barn is increased gradually to 50 or 60 °C, while relative humidity is lowered to allow more rapid moisture removal. This stage lasts for 36–72 h. The last stage (stem drying) generally requires 36–48 h. Air temperature is increased to 74 °C with further decrease of relative humidity to permit rapid drying of the midrib [24]. Plant samples were rinsed with distilled water and dried at 65°C-70 °C for 48 h. Then, the obtained samples were ground for analysis. Total nitrogen (N) analysis was performed using the modified Kjeldahl method [25]. After wet digestion (using HNO_3 and $HClO_4$ in the ratio of 4:1 (v/v) [26], K ve Ca concentrations were determined using a flame photometer (Eppendorf Geratebau), and Mg, Fe, Zn, Cu, and Mn contents were measured using an atomic absorption spectrophotometer (Varian AA 220 FS) [27,28].

Total phosphorus was determined spectrophotometrically by the vanadomolibdophosphoric yellow color method [29]. Following dry ashing, the concentration of boron (B) was estimated spectrophotometrically by the azomethine-H method [30]. Crude ash content was determined gravimetric as described by Nelson [31]. Chlorine content was determined using standard AOAC [32] methodology. Further, the surface color was measured for ten tobacco leaf cubes using a colorimeter (CR-300, Minolta Co., Osaka, Japan). The colorimeter had an 8-mm diameter viewing area and was calibrated with a white tile ($L^* = 97.26$, $a^* = +0.13$, $b^* = +1.71$). Measurements were recorded in L* (lightness), +a* (redness), +b* (yellowness), and CIE (Commission Internationale de I'Eclairage) color coordinates [33]. Fresh green leaf chlorophyll content was measured using a Minolta Chlorophyll Meter (SPAD-502). Plants were selected randomly to determine chlorophyll content. Measurements were performed on both sides of five expanded leaves per plant (from the top of the plant), and the average of these readings was considered. Chlorophyll content was calculated using the SPAD values [34]. Electrolyte leakage (EL) was measured twice at the first and second priming. Leaf disks were excised from five of the youngest, fully expanded, and disease-free plant leaves to measure the EL. Electrical conductivity (EC) meter was used to measure the initial electrical conductivity (EC1) of the solution. Further, the samples were boiled for 1 h using a water bath and were allowed to cool down to room temperature for final EC (EC2) measurements. EL was estimated using the following formula: $EL (\%) = (EC1/EC2) \times 100$ [35]. Tobacco height was measured as the distance from the soil surface to the tip of the plant, and the number of leaves per plant was counted.

2.3. Statistical analyses

Experiments were conducted in a randomized plot design with three replications. The analysis of variance (ANOVA) for the experimental data was performed using the JMP (version 5.0) software, and the significant means were compared using the Tukey's test.

Table 2
Biochar physico-chemical properties.

pH[a] (1:10)	9.90	Ca[e] (%)	5.45
E.C[b] (dS m^{-1})	13.79	Mg[e] (%)	1.38
O. Madde (%)	68.22	Na[e] (mg kg^{-1})	1895.20
C/N	18.14	Fe[e] (mg kg^{-1})	3328.04
O.C[c] (%)	38.46	Zn[e] (mg kg^{-1})	84.98
N[d] (%)	2.12	Mn[e] (mg kg^{-1})	173.02
P[e] (%)	0.27	Cu[e] (mg kg^{-1})	63.01
K[e] (%)	3.46	B[f] (mg kg^{-1})	45.38

[a] 1:2.5 water extract.
[b] Electrical conductivity, w:v, 1:5 water.
[c] Organic carbon.
[d] kjeldahl.
[e] total (HNO_3+$HClO_4$)extract.
[f] ash were determined azometine-H methods.Each value is the mean of three replicates and on an oven-dry (105 °C) basis.

3. Results and discussion

3.1. Results

Increasing the biochar doses increased N concentrations and showed linear trend (Table 3) at higher doses. The data showed that the applied biochar increased first priming and second priming, nitrogen values were determined in 2.07 %–3.84 % in first priming samples and 2.58 % to 4.15 in second priming leaf samples according to primings times. When the biohar dose was increased from B0 (2.07 %) to B4 (3.84 %), the nitrogen value increased by 85.5 % in the first priming and biohar dose was increased from B0 (2.58 %) to B4 (4.15 %), the nitrogen value increased by 60.8 % in the second priming. The data for P content showed that biochar significantly affected P content in tobacco leaves (Table 3). The data ranged from 0.41 % to 0.77 % P in first priming and second priming 0.38 %–0.66 % P, the highest value P (0.77 %) was observed B4 biochar doses, and the lowest P value was 0.38 % recorded B0 applied in first priming period. Furthermore, the level of B4 doses was accumulated the highest P content in the tobacco leaves. Similarly, biochar treatments had significant effects on the P contents, the leaf P contents were higher at the first priming than at the second priming leaf samples. Biochar application rate was increased from B0 to B4 phosphor content in first and second primings increased by 87.8 %, and 73.6 %, respectively. The data revealed that biochar has a positive effect on P concentration in tobacco leaves. The results regarding the potassium content showed significant differences according to the applications. The firs priming data ranged from 2.43 % to 3.17 8 % and second priming 3.22 %–4.41 % respectively (Table 3). The maximum potassium concentration was observed with 4.41 % K of the B4 biochar application dose and the minimum concentration was recorded with 2.58 % K in the control plants. The resuls of potassium indicated that biochar application on second primings were higher than first primings.

The results revealed that Ca levels increased linearly and significantly with increasing biochar doses. Experimental treatments had significant effects on plant leaf Ca concentration in firs priming the greatest Ca (%) concentration (4.25 %) was obtained from B4 and the lowest (3.13 %) from the control treatments. In second priming plants, the greatest value of Ca (3.79 %) was obtained from B4 treatments and the lowest (3.15 %) from the control treatments (Table 3). The Ca concentration in the first priming samples are listed as > second priming time Ca concentrations. Results regarding magnesium content varied between 0.42 and 0.68 % in the first priming time and between 0.51 and 0.80 % in the second priming plant leaves. Magnesium concentration showed a linear increase with the application dose of biochar. The highest Mg contents were observed for B4 treatments and the lowest for B0 treatments (Table 3). The highest amount of magnesium was reached in the second priming at the B4 dose. It was found that the magnesium contents increased in parallel with the increasing biochar dose. Data on chlorine (Cl) content showed that biochar significantly affected the Cl concentration in tobacco leaves (Table 4). The effects of biochar treatments on leaf Cl values were also significant in both harvest periods. In both priming periods, the lowest values (0.35 % and 0.38 %) were obtained for B0 treatments, and the highest values (1.18 % and 1.25 %) were obtained for B4 treatments. Cl concentrations also increased with increasing biochar doses. The first priming samples exhibited lighter colors as compared to the second priming samples. At the first priming, leaf iron (Fe) concentration obtained for the B0 treatment 112.42 mg kg^{-1} increased to 127.19 mg kg^{-1}, 162.80 mg kg^{-1}, 150.65 mg kg^{-1} and 138.73 mg kg^{-1} for B1, B2, B3 and B4 treatments respectively, while decreased to B3 and B4 (downward 150.65 mg kg^{-1} to 138.73 mg kg^{-1}) treatment. Thus, at the first priming, leaf Fe contents were ordered as B2 > B3 > B4 > B1 > B0. Similarly, at the second priming, leaf Fe contents were ordered as B2 > B3 > B4 > B1 > B0. Concentration of Fe showed a decreasing trend in B3 and B4 (downward 184.24 mg kg^{-1} to 165.69 mg kg^{-1}) treatment applications compared to control. The Fe concentration also increased but goes downward at higher levels (B3 and B4) in both times priming. Zinc (Zn) concentration differed significantly according to the application doses and priming time. Firs priming Zn concentration varied between 63.56 and 86.77 mg kg^{-1} and with the greatest value of Zn (86.77 mg kg^{-1}) in B2 treatments and the lowest value (63.56 mg kg^{-1}) was calculeted in the control treatments. Zn change in the second priming samples was similar to the first priming samples and the maximum and minimum values were obtained with 84.98 mg kg^{-1} and 50.55 mg kg^{-1} in B2 and B0 applications. Based on treatment doses, firs priming copper (Cu) concentrations 13.78–16.96 mg kg^{-1} and second priming 13.54–18.11 mg kg^{-1} were calculated. Results show that In first priming minimum Cu value were obtained from the control (13.78 mg kg^{-1}) treatments and the maximum values (16.96 mg kg^{-1}) were obtained from B4 treatment. The second priming Cu value were similar to the firs priming Cu concentration, maximum and minimum values were determined in B4 (18.11 mg kg^{-1}) and B0 (13.54 mg kg^{-1})

Table 3
Effects of biochar on micro-nutrients based on harvest times.

	Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg(%)
First priming	B0	2.07 d	0.41 d	2.43 d	3.13 d	0.42 d
	B1	2.18 d	0.47 cd	2.58 cd	3.29 cd	0.49 cd
	B2	2.95 c	0.55 bc	2.72 bc	3.47 c	0.54 bc
	B3	3.56 b	0.63 b	2.95 ab	3.83 b	0.62 ab
	B4	3.84 a	0.77 a	3.17 a	4.25 a	0.68 a
p value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Second priming	B0	2.58 c	0.38 c	3.22 b	3.15 c	0.51 c
	B1	3.06 bc	0.44 bc	3.57 ab	3.38 bc	0.57 c
	B2	3.27 abc	0.53 abc	3.98 ab	3.52 ab	0.64 bc
	B3	3.63 ab	0.58 ab	4.17 a	3.60 ab	0.77 ab
	B4	4.15 a	0.66 a	4.41 a	3.79 a	0.80 a
p value		0.0019	0.0014	0.0089	0.0022	0.0002

(Different letters indicate significant differences).

Table 4
Effects of biochar treatments on micro elements based on priming periods.

	Treatments	Cl (%)	Fe	Zn	Cu (mg/kg)	Mn	B
First priming	B0	0.35 d	112.42 d	63.56 c	13.78 b	37.89 b	37.45 c
	B1	0.44 d	127.19 cd	72.30 b	14.32 b	42.13 ab	42.62 bc
	B2	0.57 c	162.80 a	86.77 a	14.65 b	44.35 ab	45.75 ab
	B3	0.71 b	150.65 ab	83.01 a	15.22 ab	50.73 a	49.48 a
	B4	1.18 a	138.73 bc	79.08 ab	16.96 a	46.68 ab	46.91 ab
	p value	<0.0001	<0.0001	<0.0001	0.0056	0.0685	0.0016
Second priming	B0	0.38 e	144.65 b	50.55 d	13.54 c	39.40 c	37.55 c
	B1	0.47 d	160.63 ab	58.96 cd	13.75 c	46.45 b	41.23 bc
	B2	0.63 c	193.61 a	84.98 a	15.16 bc	47.59 b	47.68 ab
	B3	0.84 b	184.24 a	80.86 ab	16.70 ab	59.32 a	56.32 a
	B4	1.25 a	165.69 ab	68.22 bc	18.11 a	50.33 b	49.82 ab
	p value	<0.0001	0.0068	<0.0001	<0.0001	<0.0001	0.0005

(Different letters indicate significant differences).

treatments (Table 4). Tobacco leaves manganese (Mn) contents significantly increased with increasing biochar doses except for B4 application, as compared to the control plots. Maximum and minimum Mn values in the first priming B3 (50.73 mg kg^{-1}), B0 (37.89 mg kg^{-1}) and second priming B3 (59.32 mg kg^{-1}), B0 (39.40 mg kg^{-1}) were observed in biochar applications. Plant boron (B) concentration increased with biochar rates from 37.45 mg kg^{-1} in the control plots to 49.48 mg kg^{-1} with the B3 application rate in the first priming period. Similarly, maximum and minimum boron values B3 (56.32 mg kg^{-1}) and B0 (37.55 mg kg^{-1}) were obtained from in second priming period. Table 5 presents the color (L*, a*, and b*) values of tobacco leaves. At the first priming, the highest L* value of 60.37 was obtained for B2 treatments. At the second priming, the highest L* value of 39.03 was obtained for B3 treatments and the lowest for B0 treatments. The a* values of tobacco leaves varied between −4.68 and 6.91 at the first priming. At the second priming, a* values showed a similar range for lower leaves but varied between −6.09 and 6.35 for upper leaves. The highest a* values were obtained for B2 (6.91 and 6.35) treatments in both priming periods. At the first priming, b* values varied between 33.22 and 48.83, with the highest value for B2 treatments and the lowest for B0 treatments (Fig. 1A). At the second priming, b* values varied between 19.69 and 30.57, with the highest value for B3 and the lowest for B0 treatments (Fig. 1B). The SPAD value in first and second priming time with B4 application reached its peak, 54.42 and 53.39 respectively higher than in the control (35.96 and 34.31) group (Table 5). The SPAD value could directly reflect the chlorophyll content, and B4 could promote the accumulation of chlorophyll in tobacco. Leaf SPAD values (relative chlorophyll value) were significantly higher in both priming periods than in the control group. Leaf SPAD values were ordered as B4 > B3 > B2 > B1 > B0 in both primings time. SPAD values increased with increasing biochar doses. Electrolyte leakage (EL) values differed based on the priming periods. Leaf EL values varied between 25.90 % and 37.25 % at the first priming and between 26.90 % and 40.59 % at the second priming. In both priming periods EL values decreased as B0 > B1 > B2 > B3 > B4, the maximum EL values were obtained from B0 treatments and the minimum from B4 treatments. The crude ash amount in the first priming samples varied between 17.89 % and 21.94 % according to the biochar application doses. The minimum crude ash content (17.01 %) was observed for the B0 treatment and maximum crude ash content calculated from (21.94 %) with B4 biochar treatment dose. Second priming samples crude ash varied between 17.01 and 19.05 % according to the biochar application doses. The minimum crude ash content (17.01 %) was observed from B0 treatment and maximum crude ash content calculated from (19.05 %) with B4 biochar application. The results confirmed that the crude ash content decreases from the plants lower leaves to upper leaves (Table 5). According to the applications, the number of leaves per plant was determined as 34.3–45.3, the higher the biochar addition rate, the higher the number of leaves was obtained. Increases rate were determined in B1 (12.8 %), B2 (21.5 %), B3 (25.3 %) and B4 (32.1 %) respectively, compared to the control. The number of plant leaves was obtained at the addition of B4 and reached with 43.0 value. The tobacco plant height increased in the order B4 (121.9 cm) > B3 (108.1 cm) > B2 (86.6 cm) > B1 (68.2 cm) > B0 (66.0 cm) biochar applications. The highest plant height was obtained in the B4 (121.9 cm) and the smallest plant height in the B0 (66.0 cm) bichar

Table 5
Effects of biochar on some parameters based on priming periods.

	Treatments	L*	a*	b*	SPAD	E.L (%)	Crude ash (%)
First priming	B0	43.95 b	5.01 a	33.22 b	35.96 d	37.25 a	17.89 b
	B1	48.76 ab	6.23 a	38.67 b	42.72 c	34.67 b	18.30 b
	B2	60.37 a	6.91 a	48.83 a	45.80 bc	31.56 c	20.52 ab
	B3	52.79 ab	−2.53b	40.19 ab	48.27 b	28.64 d	18.08 b
	B4	50.65 ab	−4.68 b	35.04 b	54.42 a	25.90 d	21.94 a
	p value	0.0153	<0.0001	0.0025	<0.0001	<0.0001	0.0020
Second priming	B0	37.59	4.56 a	19.69 b	34.31	40.59 a	17.01
	B1	38.94	5.14 a	20.43 b	38.24	36.67 b	17.21
	B2	33.81	6.35 a	23.04 b	44.28	32.56 c	17.86
	B3	39.03	−4.01 b	30.57 a	46.63	28.96 d	18.82
	B4	36.32	−6.09 c	25.29 ab	53.39	26.90 d	19.05
	p value	ns	<0.0001	0.0014	<0.0001	<0.0001	ns

(Different letters indicate significant differences).

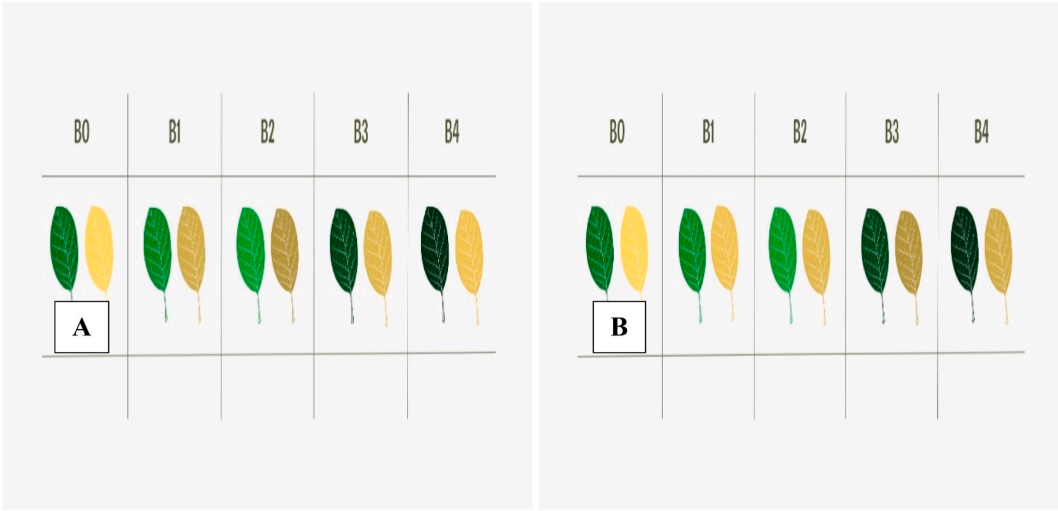

Fig. 1. Change in color parameters based on priming periods: A (1st priming) and B (2nd priming). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

application doses (Fig. 2).

3.2. Discussion

Biochar treatments showed significant effects on the nitrogen, Phosphorus, potassium, calcium and magnesium (N, P, K, Ca and Mg) contents of the tobacco leaves. A previous research has shown that biochar improves the availability of soil nutrients and the efficiency of plant nutrient absorption [36]. Further, the N contents of the tobacco leaves varied between 2 % and 5 % and deficiency symptoms were observed at N contents of <1.5 % [37]. Nitrogen losses occur due to differences in the drying method. Nitrogen is lost during flue-curing of tobacco leaves [38]. The nitrogen metabolism is the most basic metabolism process of tobaccos [39]. Losses in total nitrogen content in air-cured burley, flue-cured, and oriental tobacco were 0.31 %, 0.40 %, and 0.27 %, respectively [40]. Nitrogen exhibits the greatest influence on the growth and quality of flue-cured tobacco [41]. The excessive or insufficient application of nitrogen can affect the yield and quality of flue-cured tobacco. Further, it significantly impacts the quality, aroma, and taste of leaf blades, and excess nitrogen results in an unpleasant taste [42]. Excess nitrogen produces strong and bitter flavors associated with high nicotine content [11]. The data revealed that biochar has a positive effect on N concentration in tobacco leaves, N level in both priming leaf samples was included in the adequate classification. Phosphorus is essential for tobacco root development as well as improving the color and quality of leaves. It was determined that it has sufficient concentration (0.1–1.0 %) recommended [37], for tobacco leaf.

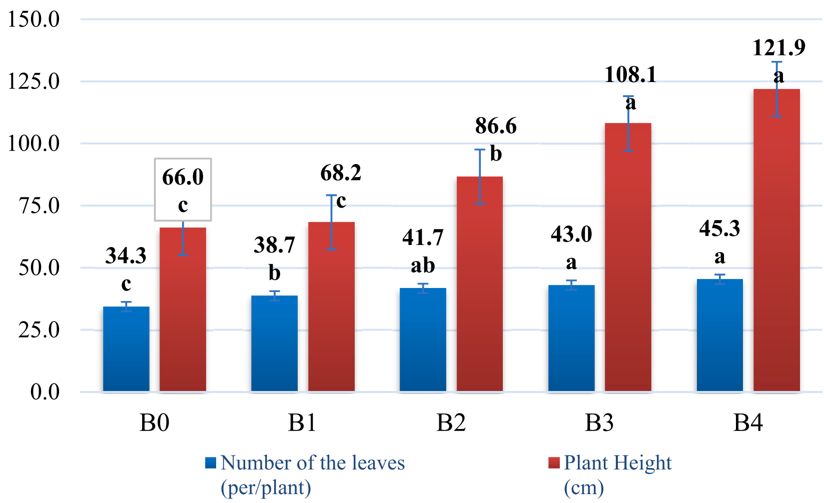

Fig. 2. Effects of biochar treatments on plant height and number of leaves (Different letters indicate significant differences).

However, excess phosphorous content reduces quality, causes dryness, and results in coarse, uneven leaves, producing black crude ash instead of white [41]. The first priming and second priming leaf samples were at a sufficient level in terms of P values. Potassium affects the leaf yield and quality of tobacco [43]. It is observed that the sufficient level for tobacco is at a sufficient level according to Brayson and Mills [44] the K range (1.6 %–4.1 %). Concentrations of K tobacco leaves generally vary between 2 % and 8 %, and it plays a key role in controlling important quality parameters [45]. Potassium is another essential mineral required for the growth and quality (color, texture, sugar content, nicotine, and flammability) of tobacco leaves [46]. High K concentrations in cured leaves improve tobacco quality by increasing the burning rate and heat holding capacity, whereas low K concentrations (<2 %) reduce quality. K concentrations >2 % of dry weight are generally required to produce upper leaves [47]. Potassium element has an effect on increasing the combustibility and hygroscopicity of tobacco leaves and increasing the color and characteristic properties of tobacco leaves [48]. Potassium contents increased with increasing biochar doses, implying that biochar positively affected the K concentrations of tobacco leaves. The increased K content indicates the quality of tobacco because it improves burning and increases smoking and ashing qualities [49]. Potassium concentrations of leaves were significantly increased with biochar additions. Hoyos et al. [11] reported that K had the highest accumulation in tobacco leaves, followed by N and Ca elements. Calcium has a strong influence on biomass and dry matter production in the tobacco plant. It is involved in the development of the cell wall, plasma membrane, cell growth, and enzyme secretion [50]. Calcium is a mineral element that the tobacco plant requires much more than potassium, and Ca content of cured leaves ranges between 1.5 % and 2.0 % [51]. According to the recommended [44] limit values for sufficient leaf Ca concentration (1.5 % and 3.5 %), biochar applications were effective at a sufficient level of Ca oncentration. Mg is a component of the chlorophyll molecule, a co-factor of all enzymes involved in the phosphorylation reaction [46]. According to the recommended [44] limit values for sufficient leaf Mg concentration (0.2 %–0.85 %), biochar applications were effective at a sufficient level. The increased Mg content of tobacco leaves (>2 %) increases the flammability and appearance (color and texture) and results in the formation of porous, loose, and light-colored ash. On the contrary, Mg deficiency reduces leaf quality, results in dark and irregular coloration of dried leaves, and increases ash quantities [41]. Similar findings were obtained herein regarding the K, N, and Ca contents. The most important elements for the production and quality (color, texture, sugar and nicotine contents, flammability, and smoke flavor) of flue-cured tobacco were identified to be N, P, K, Ca, and Mg. Comparisons of leaf concentrations in biochar applied and non applied tobacco results indicated that leaf concentrations for N, P, K, Ca, and Mg increased. The significant increase of these nutrients in leaves of fertilized plants is due to the applied biochar doses. When evaluated according to the limit values specified by Bryson and Mills [44] in tobacco leaves, present findings were found to be suitable for Fe (50–200 mg kg^{-1}), Zn (17–110 mg kg^{-1}), Cu (10–60 mg kg^{-1}), Mn (26–400 mg kg^{-1}), and B (14–50 mg kg^{-1}), the microelement results obtained were included in the sufficient group specified as a reference value. Further, it was observed that biochar treatments increased Fe, Zn, Cu, Mn, and B concentrations in tobacco leaves. Tobacco plants synthesize nicotine through their roots in the soil. Consequently, biochar increased the solubility and thus the content of micronutrients in tobacco leaves [52]. Zn, Mn, and Fe concentrations in tobacco leaves increased with increasing soil acidity [53]. The biochar used herein was alkaline (pH = 9.90) and therefore increased the soil pH level after application [54]. Soil pH is a dominant factor controlling tobacco element uptake, and the bioavailability of metals increases with decreasing soil pH levels [55]. Decreased Fe, Zn, Mn, and B levels, particularly in B4 treatments, can be attributed to the basic nature of biochar and increased soil pH levels. No visual macro and microelement deficiency symptoms were observed herein. Chari [56] indicated the threshold Cl level of tobacco leaf as <1.50 % because large amounts slowed the burning process. Accordingly, the Cl value was below this threshold herein. Chlorine (Cl) is closely related to the flammability, moisture level, and flexibility of tobacco leaves. Extremely high or low Cl levels affect their quality. Based on specified optimum Cl levels of 0.30 %–0.80 % for dried tobacco leaves [53], B2 treatment can be considered an ideal biochar dose. Present Cl levels were well within the acceptable limits of good quality (<1.50 %) [57].

The L* value, which expresses the lightness and darkness of tobacco leaves, differed. The L* value in the first priming samples are listed as > second priming time L* color parameter. The a* values of tobacco leaves represent (+) red and (−) green on the horizontal axis. The a* color parameter showed a change from positive value to negative value (B3 and B4 doses) with the amount of biochar application rate. The average b* value of tobacco leaves in the first priming group was higher than the average b* value of lower tobacco leaves. The b* value represents (+) yellow and (−) blue on the vertical axis. This indicates that the lower leaves had lighter colors than the upper leaves, indicating a partially dominant green tone of the leaves. Biochar provides nutrients to the soil slowly and continuously by reducing the dissolution and washing of nutrient ions, thus increasing soil fertility [58]. SPAD values increased with increasing biochar doses, which can be attributed to the increased N uptake of flue-cured tobacco [59]. The present findings agree with the results obtained by Li et al. [60], which reveal that SPAD values increase with increasing biochar doses. Furthermore, the observed SPAD values were similar to those obtained by Ren et al. [61], which determined SPAD values ranging 29.82–52.93, indicating that the effects of biochar on SPAD values can directly reflect the chlorophyll content and promote chlorophyll accumulation in tobacco leaves. Nitrogen application rate and SPAD are often correlated in tobacco production [62]. The increase in the SPAD values from B0 to B1, B2, B3, and B4 resulted from the chemical composition of the biochar. The increase in SPAD value with Biochar can be explained by the porous structure of biochar and its strong adsorption properties, providing a suitable environment for soil microorganisms and having the capacity to retain nutrients [63]. The organic matter content of biochar is high (68.22 %), and the C/N ratio (18.14) is within ideal limits, further allowing the tobacco to absorb elements, particularly N. Accordingly, Karaivazoglou et al. [64] reported that nitrogen fertilization, particularly the higher doses of N, substantially affects tobacco plant height. Song et al. [65] have emphasized that organic fertilizers with different concentrations affect the morphological characteristics of tobacco. Plant height positively correlated with the number of leaves per plant [66]. Biochar reduces the dissolution and transport of water-soluble nutrients, providing a slow and continuous flow of nutrients thus preventing nutrient leaching, increasing soil fertility, and promoting plant root growth [58]. This provides sufficient water and mineral nutrients to leaves, increasing leaf chlorophyll content [67]. EL occurs in response to stress-induced damage in plant tissues [16]. Biochar exhibits high adsorption capacity, which helps to reduce the harmful effect of salinity by minimizing Na uptake [68]. Therefore, plant tissues accumulate less salt and this causes less EL from the leaves [69]. Lashari et al. [70] indicated that applying biochar in salt-affected soil reduced EL in maize. The increase in plant heights with increasing biochar doses could be attributed to the greater availability of nutrients, nitrogen, and suitable conditions for stem elongation [71]. Similar findings were reported by Haghighi et al. [37]. Biochar treatments reduced ion transport to out of the cell. Crude ash content decreased for all biochar treatments (except for B3) of the second harvest. High ash content is not desired in Virginia tobacco since it reduces the product quality. Ash content in dry tobacco leaves varies from 8 % to 30 %. It is directly linked to the material properties of leaves and the curing method [72]. The results related to ash were within the range of specified values and showed similarity. Biochar treatments significantly affected the number of leaves. The highest number of leaves (45.3) was obtained for the B4 treatments and the lowest (34.3) for the B0 treatments (Fig. 2). Further, a positive correlation between plant height and the number of leaves was observed. The increase in stem heights due to the increased consumption of nitrogen fertilizer resulted in increased number of leaves. The plant heights observed herein were similar to those obtained by Kurt and Ayan [73] (136.04–159.57 cm). In agreement with the reports by Camas et al. [74], a linear increase in plant height up to a certain biochar dose (B2) was observed herein, and this rate changed to cubic form for the next doses (B3 and B4). Similarly, Usman et al. [75] reported increased plant heights in tomatoes due to biochar treatments and Akhtar et al. [76] reported the same for wheat. Based on these results, we can conclude that an appropriate dose of biochar can promote tobacco growth.

4. Conclusion

The effects of biochar treatments on the physicochemical characteristics of tobacco leaves varied based on priming first and second harvest (priming) periods. Leaf macro and micronutrients were all included in sufficient groups. No deficiency symptoms were observed in tobacco leaves. Cl levels, responsible for slow-burning, were below the threshold value. Increasing the biochar doses increased the leaf color parameters (L*, a*, and b*), SPAD values, plant height, and the number of leaves. In terms of the specified fertilizer-use efficiency, B2 (20 tons ha^{-1}) treatment was identified as the most effective and economical method for reliable and good-quality tobacco production. The physicochemical properties of tobacco leaves varied with different biochar treatments and with the priming period. According to the results of this study, biochar is a good alternative for tobacco growers to reduce the cost of using mineral fertilizers and will also help them increase productivity per unit area. One of the promising options to achieve the goal of zero solid waste is the conversion of waste into biochar. Biochar is among the applications aimed at encouraging the reuse and recycling of organic waste in different forms.

Data availability statement

The data used to support the findings of this study are included within the article.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Mahmut Tepecik: Writing - review & editing, Writing - original draft, Visualization, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Sıdıka Ekren:** Writing - review & editing, Writing - original draft, Visualization, Supervision, Formal analysis, Data curation. **Ali Rıza Ongun:** Writing - review & editing, Writing - original draft, Resources, Methodology, Formal analysis. **Nazlı Boke Sarikahya:** Writing - review & editing, Writing - original draft, Supervision, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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