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BIOCHAR-SUPPORTED IN VITRO CULTURES OF *Lavandula officinalis L.*

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Abstract

Plants are the sources of valuable biomass that are being currently used in many areas. It is important to produce high biomass for efficient commercial production. Amongst the many factors that affect *in vitro* propagation of plants, changing or enriching the media composition is one of the commonly used techniques in micropropagation of plants. Biochar is a solid product obtained from organic wastes and because of its rich composition, it has many beneficial effects on plants. In our study, *Lavandula officinalis* plantlets were subjected to two types of biochars (Geocharged biochar and Biorfe biochar) at 0.5 and 2 g/L concentrations and their effects were investigated by means of plant growth, biomass accumulation and biochemical composition. The results showed that 0.5 g/L concentration of biochar had better effects than 2 g/L concentration and except for biochemical composition, biochar type had no significant effect on plant growth and biomass accumulation. Mean root dry weights and multiple shoot formations/explant enhanced up to 3.7 and 4.17 times higher than the control at 0.5 g/L concentration. Explant browning was also detected lower in biochar-applied media. The differences between biochemical accumulations of different media were also found statistically significant. The total concentrations of phenolics and flavonoids and radical scavenging activities were detected lower when biochars were applied. The total antioxidant concentration was higher in the control group. These findings showed that biochars lowered the negative effects of the culture conditions for *L. officinalis* plantlets.

Keywords

Lavandula officinalis,
Micropropagation,
Biochar,
Biomass and biochemical accumulations

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1. INTRODUCTION

Plant tissue cultures offer different options for the higher production of plant biomass. It is possible to trigger the regeneration of shoots and roots with the addition of different materials to culture media. Generally, cytokinins and auxins have been used for the induction of biomass accumulations. These growth regulators are effective as a result of their physiological effects [1-3]. However, they are expensive and push up the cost of commercial productions. Therefore, alternative products have been tested to increase the growth parameters of *in vitro*-grown plantlets.

Biochar is the solid by-product of biomass (plant, manure, organic wastes, animal bones, etc.) pyrolisis performed under high temperatures (200-900ºC) and in the absence of oxygen. Its positive effects on soil quality and crop improvement have been known since ancient times. Its use in agriculture is beneficial because of the improved physicochemical and biological properties of soil and enhanced

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sequestration of atmospheric carbon. It is also known that biochar is effective in disease suppression of pathogens and very enduring because of its resistance to microbial degradation caused by its wide C-to-N ratio [4-7]. Chang et al. [8] reported that root length, number of root forks and crossings of *Vitis rotundifolia* were improved by adding 20% pinewood-based biochar into pure sandy soil. Biochar addition also enhanced the fine root and total mass of *Phragmites australis* [9] and shoot biomass of tomato seedlings [10]. It was also reported that biochar can balance and alleviate endogenous phytohormone concentration under stress conditions [11].

Lavandula officinalis L. is a valuable medicinal plant that is native to the Mediterranean region and has multiple pharmacological effects. Its essential oils, primarily composed of monoterpenes and sesquiterpenes, are frequently used in the perfume and cosmetic industry**.** Because of its wide range of uses, the large-scale production of this medicinal plant is important. In order to enhance production, it is not a preferable option to use agrochemicals due to their possible adverse effects on humans and the environment [12, 13]. Therefore, it is important to apply an appropriate biomass enhancer, especially environment-friendly organic-based products.

Biochar, obtained from different organic sources, is used in agronomic studies as stated above. However, only a few *in vitro* studies were conducted about the utilization of biochar in plant tissue cultures. Because of their rich organic content, biochar may be an alternative for highly-prized plant growth regulators and can be used to obtain high-quality plantlets in *in vitro* conditions. In this perspective, in this study, we used two biochars, Geocharged biochar and Biorfe biochar, at 0.5 and 2 g/L concentrations to trigger higher biomass production and better growth in *in vitro* cultures of *L. officinalis*.

2. MATERIALS AND METHODS

Geocharged biochar (T) and Biorfe biochar (P) were used in this study. Nodal explants of *L. officinalis*, grown in Woody Plant Medium (WPM) containing 6 g/L agar and 30 g/L [14] were used as explant sources. The subcultures of the cultures were done with intervals of four weeks and the explant was incised from the *L. officinalis* plantlets grown in these conditions.

The nodal segments (0.5-1 cm long) were incised and put into semi-solid WPM supplemented with 0.5 and 2 g/L of biochar. Basal WPM was determined as the control group. The media were coded as T0, T5 and T20 for Geocharged biochar and P0, P5 and P20 for Biorfe biochar. The pH of all the media used in this study was set to 5.8. All the media were sterilized for 15 minutes at 121ºC and 1.2 kg/cm^3 pressure in an autoclave. The cultivation duration of the nodal explants was determined as four weeks, they were cultured at 4000 lux and 6 h photoperiod. The temperature was kept constant at 23±1°C. The roots and bottom parts were washed carefully with distilled water and the excess water was moved with the help of a napkin.

Root, node and shoot numbers/explant were calculated by dividing the total number of plant parts (shoots, nodes and roots) by the total number of explants. Their fresh weights (shoots – SFW, roots - RFW) were also determined and recorded. The biomass was dried overnight in an oven (50°C) in order to specify their dry weights. Their growth parameters were stated as; average shoot length (SL) (cm), node (NN), root (RN), shoot (SN) numbers, internode length (IL) (cm), multiple shoot numbers (MSN), explant browning (EB) percentage (%). Biomass accumulations were also specified (shoot fresh weights – SFW (g), shoot dry weights - SDW (g), root fresh weights – RFW (g), root dry weights - RDW (g)).

Shoot and root samples (0.025 g) were mixed with 5 mL ethanol and the mixture was sonicated and heated (50ºC) for 1h (Bandelin Sonorex) with a frequency of 35 kHz. The extracts were centrifugated (5000 rpm) for 15 min and the supernatants were distinguished from precipitate. The determinations of total phenolic concentrations (TPC) were performed according to Stoica et al [15] method. 250 µL extract, 9 mL distilled water and 250 µL of Folin&Ciocalteu's phenol reagent were put together and

agitated. 2.5 mL 7% Na_2CO_3 was added after 5 mins and agitated again. 1.25 mL double distilled water was added and mixed once again. After 90 mins, the absorbance of each sample was read in a spectrophotometer at 750 nm (Shimadzu UV-1201 V). As the standard, Gallic acid (GA) was employed and TPCs were given as mg gallic acid equivalents (mg GAE/g). DPPH scavenging activity were performed according to Desta and Cherie [16] method. 4 ml of ethanolic solution of DPPH (0.004%) was mixed with 2 mL of extract. They were agitated for 10 s and kept for 30 min in dark conditions. The absorbances of the samples were measured at 517 nm. The radical scavenging activities (RSA) (%) of the samples were decided using the equation given below: (A_0 = Absorbance of the control, A_1 = Absorbance of the sample)

$$\% \text{ RSA} = [(A_0-A_1)/A_0]x100$$

The determinations of total flavonoid concentrations (TFC) were performed according to Aluminum chloride assay [17]. 4 ml of 2% $AlCl_3$ and 4 mL of the extracts were mixed. After 10 mins, the absorbances of these mixtures were measured at 415 nm. As the standard, quercetin was employed and TFCs were given as mg quercetin equivalents (mg QE/g). The determination of total antioxidant capacity (TAC) was performed according to Phosphomolybdate method [17,18]. Phosphomolybdate reagent was prepared by mixing 50 milliliters of 0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$. 0.3 mL of this reagent and the extracts were mixed and incubated for 90 min at 95ºC. The absorbances of the samples were determined at 695 nm. As the standard, ascorbic acid was employed and TACs were expressed as ascorbic acid equivalents (mg AAE/g).

All experiments were performed in three replications. In each replication, 15 explants were cultivated. Experiments were set to a factorial randomized plots design. All the data were analyzed with ANOVA and for posthoc tests, Tukey tests (p<0.05) were deployed.

3. RESULTS

Biomass accumulation of shoots and roots of *L. officinalis* plantlets were not statistically affected by biochar applications, except mean RDW values (p<0.05). SFW values varied between 0.5 g (T20) and 1.02 g (P5) (**Table 1a**). The values showed an increase in T5 and P5 media when compared to control media. In T20 and P20, the values detected were lower than control media. These results were also verified by mean SFW values. The highest mean SFW was detected in 0.5 g/L biochar-containing media (0.87 g) and this increase was 32% higher than the control (0.66 g) (**Table 1b**). The total SFW of T (0.75 g) was 21% higher than P (0.62 g) (**Table 1c**). In SDW values, increasing biochar concentrations decreased biomass accumulations gradually (**Table 1a**). The total SDW values were found almost the same (0.11 g for T and 0.10 g for P) (**Table 1c**). RFW values were in accordance with SFW values. The values varied between 0.025 g (P20) and 0.105 g (P5), and showed an increase in T5 (0.071 g) and P5 (0.105 g) which were 1.97 and 2.92 times higher than the control (0.036 g), respectively (**Table 1a**). The highest mean RFW was detected in 0.5 g biochar concentration (0.09 g) and T had a higher biomass accumulation than P concerning total RFW (**Table 1b and 1c**). Mean RDW values were significantly affected by biochar concentrations (**Table 1b**); 0.5 g biochar was in the first group (A group) with 0.015 g biomass accumulation and a sharp decrease was observed in 2 g biochar concentration (AB group). However, both concentrations had higher biomass accumulations than the control group (B group).

In the growth parameters of *L. officinalis* plantlets, only mean MSN values were significantly affected by biochar concentrations. All the other parameters were found statistically insignificant (p>0.05). Lengths of shoots varied between 2.44 cm (T0 and P0) and 4.36 cm (P5) (**Table 2a**). The highest SLs were detected in T5 and P5, but as the biochar concentrations enhanced to 2 g/L, SL values decreased. However, all the values of biochar applications were higher than the control group. The mean SL value was higher at 0.5 g/L biochar concentration (**Table 2b**) and P (3.59 cm) had higher SL than T (2.86 cm) (**Table 2c**). NNs varied between 5.24 (T20) and 7.31 (P5) and the highest values were obtained in 0.5 g/L biochar concentration (T5 and P5) (**Table 2a**). This result was also verified by the mean NN values;

the highest mean NN was detected at 0.5 g/L biochar concentration (**Table 2b**). Total NN of P (6.30) was detected higher than T (6.00) (**Table 2c**). The lengths of internodes were higher in biochar-applied plantlets than in the control groups (**Table 2a**). In 2 g/L biochar-containing media, the plantlets had the highest mean IL (0.59 cm) (**Table 2b**). Total IL of P (0.57 cm) was higher than T (0.49 cm) (**Table 2c**). The number of roots per explant decreased in media supplemented with biochar (**Table 2a**). Similar to the other growth parameters, the total RN of P (1.23) was also found higher than T (1.04) (**Table 2c**).

Table 1. (a) Shoot fresh (SFW) and dry (SDW) weights and root fresh (RFW) and dry (RDW) weights of *L. officinalis* plantlets, and their mean and total weights with regard to **(b)** biochar concentrations and **(c)** biochar types.

Table 1a

Media	SFW (g)	RFW (g)	SDW (g)	RDW (g)
T0	0.656 ± 0.150	0.036 ± 0.009	0.120 ± 0.027	0.004 ± 0.001
T5	0.703 ± 0.179	0.071 ± 0.008	0.103 ± 0.020	0.013 ±0.003
T20	0.500 ± 0.075	0.041 ± 0.025	0.085 ± 0.014	0.009 ±0.005
P0	0.656 ± 0.150	0.036 ± 0.009	0.120 ± 0.027	0.004 ± 0.001
P5	1.020 ± 0.308	0.105 ± 0.046	0.113 ± 0.028	0.017 ± 0.008
P20	0.562 ± 0.078	0.025 ± 0.002	0.094 ± 0.019	0.004 ± 0.001

Table 1b

Conc.(g/L)	Mean SFW (g)	Mean RFW (g)	Mean SDW (g)	Mean RDW (g)
0	0.66	0.04	0.12	0.004 **B**
0.5	0.87	0.09	0.11	0.015 **A**
2.0	0.54	0.03	0.09	0.006 **AB**

Table 1c

Biochar Type	Total SFW (g)	Total RFW (g)	Total SDW (g)	Total RDW (g)
T	0.75	0.06	0.11	0.008
P	0.62	0.05	0.10	0.008

(The values with different letters are statistically significant according to Tukey test)

Table 2. (a) Shoot lenghts (SL), node numbers (NN), internode lengths (IL) and root numbers (RN) of *L. officinalis* plantlets, and their mean and total values with regard to **(b)** biochar concentrations and **(c)** biochar types.

Table 2a

Media	SL (cm)	NN	IL (cm)	RN
T0	2.44 ± 0.73	5.42 ± 1.47	0.44 ± 0.02	1.36 ± 0.55
T5	3.45 ± 0.60	7.29 ± 2.03	0.51 ± 0.11	0.84 ± 0.24
T20	2.68 ± 0.32	5.24 ± 0.45	0.52 ± 0.08	0.93 ± 0.07
P0	2.44 ± 0.73	5.42 ± 1.47	0.44 ± 0.02	1.36 ± 0.55
P5	4.36 ± 0.38	7.31 ± 0.95	0.63 ±0.12	1.31 ± 0.12
P20	3.97 ± 0.41	6.16 ± 1.04	0.66 ±0.04	1.02 ± 0.09

Table 2b

Conc. (g/L)	Mean SL (cm)	Mean NN	Mean IL (cm)	Mean RN
0	2.44	5.42	0.44	1.36
0.5	3.90	7.30	0.57	1.08
2.0	3.33	5.70	0.59	0.98

Table 2c

Biochar Type	Total SL (cm)	Total NN	Total IL (cm)	Total RN
T	2.86	6.00	0.49	1.04
P	3.59	6.30	0.57	1.23

SNs of *L. officinalis* were affected by the addition of biochar, however, except for mean MSN values, they were not found statistically significant ($p>0.05$). SNs varied between 1.78 (control – T0 and P0) and 2.31 (T5). In T5 and T20, as the result of the ascending biochar concentration, SN showed a decrease. However, both SN values were higher than T0 (**Table 3a**). In mean SNs, it was detected that 0.5 g/L and 2 g/L biochar applications had 1.25 and 1.13 times higher values than the control (**Table 3b**). The total SN of P was higher than T (**Table 3c**). In MSN values, remarkable results were observed. The values ranged between 0.47 (control) and 2.11 (P20). The addition of 0.5 g/L and 2 g/L T into the media, MSN values enhanced 4.34 and 3.49 times, respectively. The addition of P at 0.5 g/L and 2 g/L concentrations also enhanced MSN values approximately by 4 and 4.5 times, respectively (**Table 3a**). Mean MSN values were found statistically significant ($p<0.05$); 0.5 g/L concentration of biochar had the highest MSN (1.96) and in the same statistical group with 2 g/L biochar application (1.88). These MSN values were 4.17 and 4 times higher than the control (0.47) (group B) (**Table 3b**). Total MSN values were 1.39 and 1.48 in T and P, respectively (**Table 3c**). Browning percentages of the node explants were all found lower in biochar-added media than in the control (26.67%), as expected**.** The lowest browning was observed in P20 (2.22%) (**Table 3a**). EB decreased gradually as the concentration of biochar increased (**Table 3b**). Total EB of P was detected as 17.77%, whereas 22.22% was observed in T (**Table 3c**).

Table 3. (a) Shoot number (SN), multiple shoot number (MSN) and explant browing (EB) percentages of *L. officinalis* plantlets and their mean and total values with regard to **(b)** biochar concentrations and **(c)** biochar types.

Table 3a

Media	SN	MSN	EB (%)
T0	1.78 ± 0.53	0.47 ± 0.10	26.67 ± 9.11
T5	2.31 ±0.35	2.04 ± 0.36	17.78 ± 5.89
T20	1.80 ± 0.10	1.64 ± 0.11	22.22 ± 2.22
P0	1.78 ± 0.53	0.47 ± 0.10	26.67 ± 9.11
P5	2.13 ± 0.10	1.87 ± 0.10	24.44 ± 5.89
P20	2.22 ± 0.30	2.11 ± 0.26	2.22 ± 2.22

Table 3b

Conc. (g/L)	Mean SN	Mean MSN	Mean EB (%)
0	1.78	0.47 **B**	26.67
0.5	2.23	1.96 **A**	21.11
2.0	2.02	1.88 **A**	12.22

Table 3c

Biochar Type	Total SN	Total MSN	Total EB (%)
T	1.78	1.39	22.22
P	2.02	1.48	17.77

(The values with different letters are statistically significant according to Tukey test)

TPC values of the shoot and root parts of *L. officinalis* were observed in the range of 38.35-83.35 mg GAE/g (**Table 4a**). The effect of biochar concentrations, plant parts, interactions between biochar types*biochar concentrations and biochar concentrations*explant types were found statistically significant ($p<0.05$). Mean TPCs decreased contrary to enhancing biochar concentrations (**Table 4b**). Biochar type did not have a distinct effect on TPCs (**Table 4c**). Shoot parts had higher TPCs than root parts of the plantlets (**Table 4d**). TACs were detected in the range of 45.55-114.69 mg AAE/g.The interaction between biochar types*biochar concentrations*plant parts was found statistically significant ($p<0.05$). Shoot parts were detected superior to root parts (**Table 4d**); the highest TACs were in P20 and P5 (Group A), and T5, T20, T0 and P0 followed (Group B). The lowest TAC was detected in root parts obtained from T20 (**Table 4a**). Mean TACs were increased in parallel with biochar concentrations (**Table 4b**) and P was found superior to T (**Table 4c**). TFC values ranged between 5.70-13.80 mg QE/g

in shoot parts and 1.41-2.12 mg QE/g in root parts (**Table 4a**). The interaction between biochar types*biochar concentrations*plant parts was found statistically significant ($p<0.05$). The highest TFCs were obtained from the control group and the addition of biochars lessened TFC values (**Table 4b**). P was detected superior to T (7.19 mg QE/g and 5.54 mg QE/g, respectively) and shoot parts had higher TFCs than roots, distinctly (**Table 4c and 4d**). RSA percentages were observed between 91.73-92.53% in shoot parts and 68.87-85.63% in root parts of *L. officinalis* (**Table 4a**). The interaction between biochar types*biochar concentrations*plant parts was also found statistically significant in RSA percentages ($p<0.05$). Similar to TFC values, mean RSA percentages decreased when biochars were applied (**Table 4b**). Biochar type was found effective on mean RSA percentages and P was more effective than T (84.92% and 81.08%, respectively) (**Table 4c**). Similar to TPC, TAC and TFC results, total RSA percentages were found higher in shoot parts (92.06%) than in root parts (73.95%) (**Table 4d**).

Table 4. (a) Total phenolic (TPC), antioxidant capacity (TAC), flavonoid (TFC) and radical scavenging activity (RSA) of *L. officinalis* plantlet in response to biochar concentrations **(b)** Mean TPC, TAC, TFA and RSA in response to biochar concentrations **(c)** Mean TPC, TAC, TFA and RSA in response to biochar type **(d)** Mean TPC, TAC, TFA and RSA in response to plant parts.

Table 4a

Plant Part	Media	TPC (mg GAE/g)	TAC (mg AAE/g)	TFC (mg QE /g)	RSA (%)
Shoot	T0	83.35± 5.77	90.46±6.00 **B**	13.80±0.16 **A**	91.87± 0.00
	T5	64.85±10.97	94.31±0.67 **B**	5,70±0.02 **E**	91.80±1.02
	T20	38.85±2.45	93.92±0.44 **B**	8.72±0.20 **D**	91.87±0.19
	P0	83.35± 5.77	90.46±6.00 **B**	13.80±0.16 **A**	91.87± 0.00
	P5	69.10±2.31	113.15±0.89 **A**	10.86±0.04 **C**	92.53±0.34
	P20	49.85±2.74	114.69±1.33 **A**	12.53±0.17 **B**	91.73±0.19
Root	T0	45.73±1.52	49.69±0.22 **CD**	2.12±0.01 **F**	78.81±1.26
	T5	54.85±0.00	49.69±0.22 **CD**	1.41±0.01 **G**	70.54±3.77
	T20	38.35±2.89	45.85±0.67 **D**	1.49±0.13 **G**	61.03±2.05
	P0	45.73±1.52	49.69±0.22 **CD**	2.12±0.01 **F**	78.81±1.26
	P5	47.85±2.31	49.31±0.89 **CD**	1.70±0.01 **FG**	68.87±0.54
	P20	54.23±0.22	59.69±0.67 **C**	2.12±0.11 **F**	85.63±0.25

Table 4b

Conc. (g/L)	Mean TPC (mg GAE/g)	Mean TAC (mg AAE/g)	Mean TFC (mg QE /g)	Mean RSA (%)
0	64.54 **A**	70.01 **B**	7.96 **A**	85.34 **A**
0.5	59.16 **A**	76.62 **A**	4.92 **C**	81.10 **B**
2.0	45.32 **B**	78.54 **A**	6.22 **B**	82.57 **B**

Table 4c

Biochar Type	Mean TPC (mg GAE/g)	Mean TAC (mg AAE/g)	Mean TFC (mg QE /g)	Mean RSA (%)
T	58.35	70.66 **B**	5.54 **B**	81.08 **B**
P	54.33	79.50 **A**	7.19 **A**	84.92 **A**

Table 4d

Plant Part	Total TPC (mg GAE/g)	Total TAC (mg AAE/g)	Total TFC (mg QE /g)	Total RSA (%)
Shoot	64.90 **A**	99.50 **A**	10.90 **A**	92.06 **A**
Root	47.79 **B**	50.65 **B**	1.83 **B**	73.95 **B**

(The values with different letters are statistically significant according to Tukey test)

4. DISCUSSION

Biochar is an interesting and cost-effective product to be used as a plant growth promotor agent, because of its ethylene-inhibiting and growth hormones-promoting effects on plant tissues [11,19]. Biochar was also investigated for its effects on soil biota [20,21]. Despite its beneficial effects, most studies were conducted in this field but only a few *in vitro* studies were reported.

In our study on *in vitro*-grown *L. officinalis* plantlets, fresh weights of shoots and roots, and dry weights of shoots were not statistically affected by the biochar addition to media. SFWs, RDWs and RDWs showed an increase at 0.5 g/L concentrations but decreased under the control at 2 g/L concentration (**Table 1**). In SDW, both concentrations lowered the dry weight when compared to the control. The only significant effect was detected in mean RDW; 0.5 g/L concentration of biochars enhanced the dry weight 3.74 times more than control. Root growth-promoting effects of biochars were previously reported. **Di Lonardo et al.** [19] studied the effects of biochars on *Populus alba* L. clones and reported that dry biomass of roots and root numbers/shoots increased at 0.5 and 1.5 g/L biochar applications. **Hammer et al.** [21] reported that total plant biomass and root biomass were enhanced when *Lactuca sativa* plants were exposed to biochar application. Shoot and root biomass of *L. officinalis* plants grown in greenhouse conditions were detected higher [22]. **Miclea et al. (2020)** [13] reported that shoots of *L. angustifolia* grew taller when activated charcoal was added to the plant growth medium.

SL, NN, IL and RN were also not significantly affected by biochar applications at 0.5 and 2 g/L concentrations. However, at both concentrations, SL, NN and IL values were detected higher than the control. SL and NN values were enhanced higher by 0.5 g/L concentration than 2 g/L concentration. RN values were lower than the control at both concentrations for both biochar types (**Table 2**). **Di Lonardo et al.** [19] reported a higher elongation rate in *Populus alba* L. clones when biochar was used and this effect was attributed to the absorption of ethylene molecules by biochar.

Multiple shoot formation enhances not only shoot biomass but also plantlets' numbers in *in vitro* propagation. Therefore, it is an important parameter for micropropagation studies. In our study, in both concentrations of two biochars, SNs and MSNs were higher than the control (**Table 3a**). Biochar concentration was found statistically significant on mean MSN and 0.5 g/L concentration of biochars enhanced mean MSN by 4.17 times higher than the control (**Table 3b**). This result may be attributed to the ethylene inhibition and growth-promoting effects of biochars and indicate that biochar can be used instead of non-natural growth regulators to stimulate plant growth.

Prevention of explant from browning is a predictable effect of biochars because of their absorption capacity, similar to activated charcoal [23]. Di Lonardo et al. [19] used biochar instead of activated charcoal successfully. In our study, both concentrations inhibited explant browning. Although there was no statistical significance, rising concentrations of biochars decreased browning. The highest browning-preventing effect was detected in P20 (2.22%) which was approximately 12 times lower than the control (**Table 3a**).

Biochemical compositions of plant biomass change when different chemicals are applied to plant cells and tissues and secondary metabolites are the defense system of plants [24-25]. TPC, TAC, TFC and RSA values of *L. officinalis* plantlets were strongly affected by the utilized concentrations of biochars in this study. TPC, TFC and RSA values were detected as lower than the control in biochar-applied biomass, however, the control had higher TAC values (**Table 4c**). These results confirmed that phenolic and flavonoid contents of *in vitro*-grown *L. officinalis* plantlets were lowered by biochar applications. Similar to our findings, **Kul et al.** [26] reported biochar applications at 2.5% and 5% concentrations decreased the antioxidant activities and gave the bean plantlets strength towards stress conditions. Although the two main secondary metabolite groups (phenolics and flavonoids) productions were detected lower in biochar-supplemented media, antioxidant capacities were higher than in the control

group. Moreover, explant brownings were also lower when biochars were applied. These findings revealed that biochar applications can help plant tissues fight against the stress of *in vitro* conditions.

5. CONCLUSION

Biochar can be produced from different organic wastes and has beneficial effects on plant growth, biomass accumulation and biochemical content. It has also proved that different types of biochars may boost the *in vitro* production of plants. Consequently, biochars derived from different sources should be used and their utilization should be optimized. Therefore, in order to reveal their protective effects, more studies about biochar applications on different plant types and culture types should be conducted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CRediT AUTHOR STATEMENT

Pınar Nartop: Laboratory experiments, Data collection, Data Analysis, Investigation, Conceptualization, Writing – Original draft preparation, Supervision. **Sena Özdil Şener:** Laboratory experiments, Data collection. **Seray Begüm Gök:** Laboratory experiments, Data collection.

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