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ORIGINAL PAPER

Exploring the Potential Impact of Biochar Amendments in Promoting Redox Reactions, Agro-Morphological, and Phytochemical Characteristics in *Satureja khuzistanica* Jamzad Under Salt Stress

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Abstract
Salinity stress is one of the most important environmental factors that substantially affects the yield of plants and changes their secondary metabolites worldwide. Biochar is a vital eco-friendly amendment widely used to improve soil health and promote plant productivity under stress conditions. In the present study, the effect of biochar, a carbon-rich organic substance (0, 1, 2, and 3% of the total mass of the pot), on agro-morphological and physiological traits, essential oil and carvacrol percentage, and antioxidant activity of *Satureja khuzistanica* under salt stress conditions (0, 2, 4, and 8 ds m^{-1} NaCl). The plant agro-morphological traits and yield, including plant height, number of main and secondary branches, length and width of leaf, fresh and dry weight of aerial parts, and dry weight of leaves and flowers were decreased with increasing salinity level, but these traits were improved with the application of biochar. The highest yield was observed in the 3% biochar treatment in normal conditions. The highest percentage of essential oil (3.55%) and carvacrol (97.66%) were obtained from the plants under salinity stress (8 ds m^{-1}) treated without and with 3% biochar. With increasing levels of salinity stress, the amount of SPAD decreased, and electrolyte leakage (EL) and the activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) enzymes increased. However, biochar treatments effectively reduced the damage caused by salinity stress, so that the addition of 3% biochar treatment will decrease the destructive effects of salinity stress in the *S. khuzistanica*, so that decreased EL content and the activity of POD, SOD, and CAT enzymes. According to the positive effects of biochar on functional traits, essential oil content, carvacrol percentage, and SPAD index, its application can be suggested as a sustainable strategy to increase the yield of *S. khuzistanica* under salinity stress.

Keywords Biochar soil amendments · Antioxidant enzymes · Carvacrol · Proline · Salinity

1 Introduction

Satureja khuzistanica Jamzad (Lamiaceae) is a perennial medicinal plant that is widely distributed in the southwestern regions of Iran (Jamzad 2011; Khani et al. 2019; Ghorbanpour et al. 2016). The plant is locally utilized as a spice, herbal tea, analgesic, and antiseptic in traditional medicine and pharmaceutical industries (Shariat and Sefidkon 2021) and exhibits antifungal, antimicrobial, antibacterial, analgesic, antidiabetic, antioxidant, anti-inflammatory, and antilipemic properties (Hadian et al. 2011). Based on the results of the previous studies, several drugs such as dentol, saturex, and orthodentol are produced from this plant, and new formulations, like zagrol are prepared for the veterinary industry. Further, plant pomace, after extraction, is used as livestock forage (Hadian et al. 2011; Nooshkam et al. 2017).

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Aerial parts of *S. khuzistanica* have been collected from its natural habitats for medicinal uses during the past two decades. Considering the growing importance of this plant and the need of the pharmaceutical and food industries for plant raw materials, it is necessary to domesticate and comprehensively cultivate these plants. The plant has unique qualities by growing on low-input lime soil and in a dry environment, making it a promising candidate to be cultivated in marginal lands and low-input farming systems (Hadian et al. 2016). Salinity is one of the main limiting factors in marginal lands and low-input farming systems. Salinity is rapidly rising, occupies approximately 20% of water bodies worldwide, and affects 50% of the area under cultivation in the most fertile lands of the world by 2050 (FAO 2017). Irrigation with saline water, improper drainage structures in agriculture, and Earth's global warming are expected to increase the level of saline soil in various regions worldwide (Munns & Gilliham 2015). Salt stress negatively influences plant growth, normal physical-biochemical processes, and nutrient uptake. Furthermore, it disturbs photosystem II (PSII) reactions and indirectly causes molecular damage through reactive oxygen species (ROS) (Farouk et al. 2020; Jiang et al. 2020; Ren et al. 2020; Sheng et al. 2020; El-Banna et al. 2022; Farouk & AL-Huqail 2022). The application of organic amendments, which enhance soil fertility along with modifying soil, is known as one of the methods to remove salt from the soil profile. Given the expansion of saline lands in the world, biological approaches such as various organic materials (e.g., manure, plant residues, and biochar) can be adopted as one of the essential solutions to reduce the adverse effects of salt stress on plants (Xu et al. 2016).

Biochar, called black gold in agriculture, is a carbon-rich organic substance, which is obtained by pyrolyzing biomass or plant residues in the presence or absence of oxygen at 300–1000 °C (Akhtar et al. 2014). The material can affect soil physic-chemical characteristics, leading to a change in its biological yield and better fertility, and consequently, more plant yield (Farrell et al. 2014; El-Gamal et al. 2021; Farouk & AL-Huqail 2022). In addition, biochar results in absorbing and retaining nutrients, raising water holding capacity, increasing cation exchange capacity, improving soil structure, and subsequently providing mineral nutrients (e.g., P, Mg^{2+}, Ca^{2+}, and K^{+}) for plants. The high specific surface area of biochar structure and the presence of many pores lead to decreased nutrients (Clough et al. 2013; Ghezzehei et al. 2014). Accordingly, biochar can enhance plant productivity rate and growth (Jeffery et al. 2011; Kim et al. 2016; El-Gamal et al. 2021; Farouk & AL-Huqail 2022; Farouk et al. 2023). Further, the percentage of biochar effect on plant growth and development depends on several items, including the type of biochar, the availability of nutrients following the use of biochar, plant species, and the texture of soil (Xu et al. 2016). The utilization of biochar retains macro- (P, N^{+}, K^{+}, Ca^{2+}) and micronutrients (Zn^{2+}, Mg^{2+}) for plants. Several researchers demonstrated that biochar decreases sodium ion (Na^{+}) uptake and improves the amount of potassium ion (K^{+}) under salt stress, although the substance fails to influence N and Zn^{+} content (Brantley et al. 2016; Chaganti & Crohn 2015). According to previous studies, biochar enhances soil physicochemical properties (Lv et al. 2023; Ge et al. 2023). It diminishes the effects of salt stress during the growth of *Brassica chinensis* L. (Tang et al. 2020), *Sorghum bicolor* L. (Ibrahim et al. 2013), *Glycyrrhiza uralensis* Fisch. (Egamberdieva et al. 2021), and *Borago officinalis* L. (Farouk et al., 2020).

Little is known about the effects of wood-derived biochar amendments on agro-morphological and physiological traits, essential oil production (content and yield), and the major essential oil constituents (carvacrol) percentage, as well as their antioxidant activity of *S. khuzistanica* under salt stress conditions in a controlled pot experiment. We hypothesized that increasing biochar amendments would increasingly promote beneficial effects on *S. khuzistanica* growth, plant physiological properties, agro-morphological traits, as well as on essential oil percentage/yield and carvacrol content. Thus, the results of the present study can increase the knowledge about the effect of biochar in enhancing growth parameters, physiological, and phytochemical traits as well as essential oil compounds under salinity stress.

2 Materials and Method

2.1 Biochar and Plant Materials

To prepare biochar, the required amount of pomegranate wood (*Punica granatum*) was collected from the pomegranate orchards in the Saveh region, dried, packed in aluminum sheets, and placed in a furnace at 450 °C for 4 h to perform the pyrolysis process. Then, biochar was removed from the furnace and subjected to chemical analysis, the results of which are provided in Table 1. The plant seeds were obtained from Pakan Bazar Company (Isfahan, Iran) and planted in pots with 20 cm diameter and 25 cm depth, which were filled with sandy loam soil (71.43% sand, 20.81% silt, and 7.76% clay). Table 1 summarizes the physicochemical properties of the applied soil.

2.2 Experimental Design

This study was performed based on a factorial, completely randomized design with three replicates at the greenhouse of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University (Tehran, Iran). The experimental treatments included four percentages of biochar (0, 1, 2,

Table 1 Chemical and physical properties of the soil and biochar

Soil		Biochar	
pH	8.2	pH	7.6
Texture	Sandy loam	Ash content	2.1%
Sand (%)	71.43	Moisture content	4.39%
Silt (%)	20.81	Volatile matter	71.5%
Clay (%)	7.76	Organic carbon	65.43%
EC (dS/cm)	3.92	N (%)	0.21
OC (%)	0.81	P (%)	0.18
N (%)	0.067	K (%)	0.58
P (mg/kg)	21.86	Na (%)	0.32
K (mg/kg)	235	Mg (meq/l)	0.28
Ca (meq/l)	15.3		
Mg (meq/l)	11		
Cl (meq/l))	15		

and 3% biochar in potting soil) and four concentrations of NaCl (0, 2, 4, and 8 ds/m). Before filling the pots, biochar was powdered, passed through a sieve with 1–2 mm diameter, and mixed with the soil in desired ratios. The mixtures were incubated for 1 week, followed by transferring *S. khuzistanica* transplants at the four-leaf stage to the pots containing biochar in the spring (10th of May) of 2022. One plant was placed in each pot, to which four salinity levels were applied at the six-leaf stage by adding NaCl to irrigation water. In order to prevent the accumulation of salt in the pots, two holes with a diameter of 1 cm were built at the bottom of them as drainage, and sand was poured at the bottom of each pot to a height of 5 cm. The pots were placed under greenhouse conditions at 17/26 °C (night/day) and 65–80% relative humidity. The soil moisture content was maintained at field capacity, and irrigation time was based on the soil moisture measurement using a time-domain reflectometer (TDR) (Model Sabta Barbara 6050X, USA).

2.3 Evaluation of Agro-Morphological Traits and Yield

Following the treatment application, the plants were grown for 70 days, and four subshrubs were selected from each treatment to assess morphological and functional characteristics. Morphological properties, such as plant height, leaf length and width, as well as the number of main and side branches, were examined at the full flowering stage. After harvesting, the shoot's fresh weight was determined, and then the plant materials were dried at room temperature. The flower and leaf were removed from the shoot, and the dry weight of the shoot, leaf, and flower was measured by using the scale.

2.4 Antioxidant Enzyme Activity Assay

A Powerwave XS Microplate spectrophotometer (Bio-Tek Instruments, Inc., USA) was utilized to evaluate the activity of enzymes. Regarding the antioxidant enzyme activity assay, 0.1 g of fresh leaf samples was mixed with 1 ml of cold 50 mM phosphate buffer (pH = 7.8) and centrifuged at 4 °C for 20 min at 12,000 × g. Catalase (CAT) enzyme activity measurement was performed based on H_2O_2 decomposition rate and reduction of absorbance rate at 240 nm during 3 min. The reaction mixture contained potassium phosphate buffer (50 mM), H_2O_2 (15 mM), and 100 µL of enzymatic extract. Enzyme activity was expressed as U g^{-1} FW unit (Aebi 1984). Peroxidase (POD) activity: 1 g of each leaf sample was separately milled in 5 mL of assay buffer. The homogenates were centrifuged at 12,000 × g for 30 min at 4 °C (Zhang 1992). Five milliliters of the assay buffer for the peroxidase activity contained the following: 125 µM of phosphate buffer, 50 µM of pyrogallol, 50 mM of H2O2, pH 6.8, and 1 mL of the 20 times diluted enzyme extract. This was incubated for 5 min at 25 °C, and subsequently, the reaction was stopped by adding 0.5 mL of 5% (v/v) H2SO4. The amount of purpurogallin was determined by measuring the absorbance at 420 nm. In order to evaluate the superoxidase dismutase (SOD) activity rate, 100 µL of enzymatic extract was added to the reaction mixture (contained potassium phosphate buffer 50 mM, methionine 0.013 M, EDTA 0.1 µM, and riboflavin 2 µM) and then, absorbance rates were recorded at 560 nm. Enzyme activity was expressed as U g^{-1} FW unit (Beauchamp & Fridovich 1971). The extraction and measurement of free proline in leaf tissues were performed according to the acid ninhydrin method described by Bates et al. (1973). Briefly, samples of 100 mg frozen leaves were homogenized with 2 mL of 3% sulphosalicylic acid in a pre-chilled mortar and pestle. The extracts were shaken for 30 min at 750 rpm. The residues were removed by centrifugation at 12,000 × g for 20 min. Supernatants (0.8 mL) were mixed with an equal volume of an acid-ninhydrin reagent (1.25 g ninhydrin, 30 mL of glacial acetic acid, and 20 mL of 2 M orthophosphoric acid) and incubated for 60 min in boiling water. After cooling, the reaction mixture was extracted with 2 mL of toluene, mixed vigorously, and left at room temperature for 20 min until separation of the two phases occurred. The absorbance of the toluene phase was measured at 520 nm using pure toluene as a blank.

2.5 Cell Membrane Damage

The cell membrane leakage was examined by determining electrolyte leakage (EL) according to Lutts et al. (2004). To this end, 0.30 g of fresh leaves were collected, washed three times with distilled water, cut into smaller pieces (length, 1 cm), and transferred to a test tube with distilled water (10

mL). After keeping in room condition for 24 h, primary EL (EL1) was obtained by using an electrical conductivity meter (Inobl, Japan). Then, the samples were incubated at 121 °C for 15 min. Afterward, the solution was cooled at the room condition, the electrical conductivity of which was assessed (EL2). Finally, the rate of cell membrane damage was evaluated by dividing EL1 by EL2 and expressed as EL%.

2.6 SPAD Measurements

The number of 10 leaves of each plant with different ages and colors was randomly chosen to determine leaf chlorophyll content under diffuse lighting (Xiong et al. 2015). Each leaf measurement averaged 5–10 readings using a chlorophyll meter SPAD-502 (Minolta, Japan) (Uddling et al. 2007).

2.7 Essential Oil Extraction and Analysis

To extract the essential oil, about 30 g of dried flowers and leaves were finely chopped and heated in a Clevenger apparatus for 3.5 h, according to the British Pharmacopoeia (1993). The obtained essential oil was dehydrated with dry sodium sulfate, weighed carefully, and stored in a refrigerator (4 °C) until analysis. The amount of essential oil was calculated based on the weight obtained from 100 g of the plant samples (w/w). Then, gas chromatography coupled with mass spectrometry (GC–MS) was applied to identify the essential oil constituents quantitatively and qualitatively. The essential oil was analyzed using an Agilent 7890A gas chromatograph (Agilent Technology, USA) coupled to a mass spectrometer equipped with an HP-5MS column (length, 30 m; internal diameter, 0.25 mm; and thin layer thickness, 0.25 µm). Further, oven temperature increased from 50 to 250 °C at a rate of 4 °C/min and was kept at 250 °C for 10 min. Helium carrier gas with a flow rate of 1 mm/min was utilized, and the ionization energy and ionization source temperature were 70 eV and 270 °C, respectively. Finally, the GC-FID and GC–MS analyses were performed based on the equipment and approach of Hadian et al. (2014) to quantify the carvacrol content of the essential oils.

2.8 Statistical Analysis

Regarding the experimental data analysis, the normality of the data was first checked in Minitab 16 software. Then, the data were subjected to the ANOVA using R 4.0.4 software (https://www.r-project.org), followed by utilizing the least significant difference (LSD) test to compare the means. All diagrams were drawn by using Origin 2021 software.

3 Results

3.1 Agro-Morphological Characteristics

The results suggested decreased morphological and functional properties after salt stress. An increase in the stress level led to a further reduction in the traits so that their minimum was observed in the salinity of 8 ds/m (Table 2). In addition, the use of biochar, especially 3%, significantly improved the morphological and functional characteristics (Table 2). The results of ANOVA revealed the significant interaction effect of biochar amendment and salt stress on the plant height, shoot fresh weight, leaf length and width, as well as the dry weight of the flower, leaf, and shoot, and the number of main and side branches ($P < 0.01$) As shown in Table 2, the plant height is maximized (63.66 cm) following the B_3S_0 treatment (3% biochar + 0 ds/m salinity). In comparison, the lowest height (34.00 cm) is related to the exposure to the salinity of 8 ds/m without biochar (B_0S_3). The plant height increased sharply with increasing levels of biochar 3% treatments, which significantly increased by 18.70% compared with the control. In addition, applying salt reduced the plant height by 41.98% compared to the control.

Applying biochar under the various levels of salinity promoted the number of main and side branches, as well as leaf length and width compared to the control (B_0S_0). The treatment could moderate the effect of salinity and lead to a lower decrease in the properties compared to the control. The highest number of main (36) and side branches (4.66), as well as the largest leaf length (17 cm) and width (11.6 mm), was found after applying the B_3S_0 (Table 2). Biochar treatments (B_3S_0) increased the number of main branches, number of secondary branches, leaf length, and leaf width by 46.38%, 18.75%, 79.45%, and 60.11%, respectively, compared to the control. Further, biochar amendment in salinity conditions was effective on shoot fresh weight, as well as the wet weight of the shoot, flower, and leaf so that the traits were maximized following the B_3S_0 (176.66, 84. 33, and 33 g/plant in order) (Table 2). The application of biochar improved the fresh weight, dry weight, and leaf and flower dry weight by 221.2%, 237.32%, and 205.55%, respectively, compared to the conditions of salinity stress (B_0S_3).

3.2 Essential Oil and Carvacrol Content

The content of essential oil was determined at the full flowering stage. The main and interaction effects of biochar amendment and salt stress on essential oil and carvacrol percentages were significant ($P < 0.01$). Biochar utilization under various salinity levels led to a rise in the two parameters compared to the control. The mean

Table 2 Mean (± standard error) comparison of interaction effects of salinity stress and biochar application on growth traits of *S. khuzistanica*

Treatment	Plant height (centimeters)	Number of main branches	Number of lateral branches	Leaf length (millimeter)	Leaf width (millimeter)	Fresh weight (gr.plant^{-1})	Dry weight (gr.plant^{-1})	Leaf and flower dry weight (gr. plant^{-1})	Essential oil percentage	Essential oil yield (gr. plant^{-1})	Carvacrol percentage
B_0S_0*	53.63 ± 1.3^{d}**	26.00 ± 1.3^{ef}	2.66 ± 0.01^{bcd}	11.66 ± 1.54^{f}	7.63 ± 0.24^{d}	125.00 ± 2.3^{e}	56.33 ± 1.4^{e}	24.60 ± 0.43^{d}	2.18 ± 0.04^{j}	0.54 ± 0.005^{f}	83.00 ± 0.19^{j}
B_0S_1	45.00 ± 1.7^{g}	22.33 ± 1.2^{i}	2.33 ± 0.09^{cd}	9.66 ± 1.03^{g}	6.65 ± 0.19^{e}	92.00 ± 2.1^{j}	42.00 ± 1.1^{h}	18.00 ± 0.14^{h}	2.46 ± 0.06^{i}	0.44 ± 0.003^{gh}	84.00 ± 0.29^{ij}
B_0S_2	39.00 ± 0.8^{h}	18.33 ± 0.9^{k}	2.00 ± 0.07^{d}	7.65 ± 0.94^{h}	5.65 ± 0.14^{fg}	73.33 ± 1.8^{m}	33.66 ± 0.9^{j}	14.00 ± 0.11^{j}	2.96 ± 0.03^{f}	0.41 ± 0.004^{hi}	87.00 ± 0.41^{gh}
B_0S_3	34.00 ± 0.7^{i}	17.33 ± 0.6^{l}	2.00 ± 0.09^{d}	6.70 ± 0.03^{i}	5.00 ± 0.12^{g}	55.00 ± 1.5^{o}	25.00 ± 0.6^{l}	10.80 ± 0.09^{l}	3.55 ± 0.05^{a}	0.38 ± 0.003^{ij}	93.33 ± 0.51^{cd}
B_1S_0	56.83 ± 1.1^{c}	27.33 ± 0.5^{d}	3.00 ± 0.08^{bc}	13.35 ± 1.32^{de}	8.68 ± 0.38^{c}	144.03 ± 3.1^{c}	67.00 ± 1.4^{c}	26.50 ± 0.51^{c}	2.21 ± 0.02^{j}	0.58 ± 0.004^{e}	84.66 ± 0.31^{i}
B_1S_1	52.00 ± 1.2^{de}	25.33 ± 1.3^{fg}	2.66 ± 0.05^{bcd}	11.33 ± 1.14^{f}	7.65 ± 0.27^{d}	115.00 ± 2.4^{g}	54.33 ± 1.2^{f}	21.36 ± 0.18^{e}	2.70 ± 0.05^{h}	0.57 ± 0.005^{ef}	86.66 ± 0.23^{gh}
B_1S_2	48.33 ± 0.6^{f}	22.66 ± 0.7i	2.33 ± 0.04^{cd}	9.00 ± 0.91^{g}	6.60 ± 0.19^{e}	83.00 ± 2.1^{l}	39.76 ± 0.8^{i}	15.83 ± 0.21^{i}	2.92 ± 0.06^{fg}	0.46 ± 0.004^{g}	90.66 ± 0.32^{e}
B_1S_3	45.86 ± 0.9^{g}	19.66 ± 0.4^{j}	2.00 ± 0.03^{d}	7.30 ± 0.73^{hi}	5.65 ± 0.13^{fg}	63.33 ± 1.7^{n}	30.30 ± 0.9^{k}	12.00 ± 0.09^{k}	3.11 ± 0.07^{e}	0.37 ± 0.003^{j}	94.00 ± 0.71^{c}
B_2S_0	60.08 ± 1.2^{b}	30.33 ± 1.4^{b}	3.33 ± 0.09^{b}	16.00 ± 1.63^{b}	9.60 ± 0.43^{b}	155.60 ± 3.8^{b}	73.00 ± 1.8^{b}	28.67 ± 0.63^{b}	2.52 ± 0.04^{i}	0.72 ± 0.008^{c}	86.33 ± 0.53^{i}
B_2S_1	56.00 ± 1.5^{c}	26.66 ± 1.1^{ed}	3.00 ± 0.08^{bc}	13.66 ± 1.29^{d}	8.30 ± 0.32^{cd}	135.00 ± 2.6^{d}	63.66 ± 1.3^{d}	25.74 ± 0.42^{cd}	2.87 ± 0.05^{g}	0.74 ± 0.009^{c}	88.66 ± 0.21^{f}
B_2S_2	51.33 ± 1.4^{e}	25.33 ± 0.9^{fg}	2.66 ± 0.05^{bcd}	11.66 ± 1.05^{f}	6.65 ± 0.27^{e}	103.00 ± 1.9^{h}	49.50 ± 1.2^{g}	20.26 ± 0.31^{fg}	3.10 ± 0.07^{e}	0.62 ± 0.007^{d}	92.66 ± 0.12^{d}
B_2S_3	45.66 ± 1.3^{g}	23.66 ± 0.7^{h}	2.66 ± 0.04^{bcd}	9.33 ± 0.84^{g}	6.00 ± 0.31^{ef}	89.00 ± 1.2^{k}	42.50 ± 0.9^{h}	17.00 ± 0.22^{h}	3.30 ± 0.06^{c}	0.56 ± 0.004^{ef}	95.33 ± 0.41^{b}
B_3S_0	$63.66^{a} \pm 1.9$	32.00 ± 1.4^{a}	4.66 ± 0.11^{a}	17.00 ± 1.53^{a}	11.60 ± 0.75^{a}	176.66 ± 4.3^{a}	84.00 ± 2.1^{a}	33.00 ± 0.47^{a}	2.71 ± 0.04^{h}	0.89 ± 0.013^{a}	87.66 ± 0.13^{fg}
B_3S_1	60.21 ± 1.6^{b}	29.00 ± 0.8^{c}	3.33 ± 0.08^{b}	14.66 ± 1.07^{c}	9.65 ± 0.62^{b}	144.10 ± 3.2^{c}	68.00 ± 1.4^{c}	27.83 ± 0.35^{b}	2.90 ± 0.05^{fg}	0.81 ± 0.009^{b}	91.00 ± 0.34^{e}
B_3S_2	56.29 ± 1.1^{c}	$26.330.9^{e}$	3.00 ± 0.06^{bc}	12.66 ± 1.12^{e}	8.64 ± 0.53^{c}	145.00 ± 3.5^{c}	57.53 ± 1.2^{e}	23.45 ± 0.28^{3}	3.21 ± 0.04^{d}	0.75 ± 0.008^{c}	94.33 ± 0.12^{bc}
B_3S_3	51.00 ± 0.8^{e}	24.66 ± 0.6^{g}	2.66 ± 0.05^{bcd}	11.66 ± 0.98^{f}	7.60 ± 042^{d}	98.00 ± 1.7^{i}	47.66 ± 0.8^{g}	19.40 ± 0.17^{g}	3.41 ± 0.03^{b}	0.66 ± 0.005^{d}	97.66 ± 0.48^{a}
LSD	1.65	0.96	0.75	0.96	0.98	2.13	1.88	1.12	0.06	0.04	1.22
Mean	51.14	24.81	2.77	11.45	7.62	110.81	52.14	21.15	2.88	0.59	89.71

∗*S* salt, S_0 without salt, S_1 2 dS m^{-1}, S_2 4 dS m^{-1}, S_3 8 dS m^{-1} NaCl/, *B* biochar, B_0 non-biochar, B_1 1% of total pot mass, B_2 2% total pot mass, B_3 3% total pot mass

∗∗Values in the same column followed by the same letter(s) are not significantly different at $P < 0.05$ according to the LSD test

comparison results introduced B_0S_3 (0% biochar + 8 ds/m salinity) and B_3S_3 (3% biochar + 8 ds/m salinity) as the treatments leading to the highest essential oil (3.55%) and carvacrol percentage (97.66%), respectively. However, the lowest amounts of the two variables equaled 2.18 and 83.00%, respectively, which were related to the control. At the highest salinity level, the biochar application increased the essential oil and carvacrol percentage by about 56.42% and 17.66%, respectively, compared with control. The results indicated higher levels of essential oil and carvacrol by elevating the concentrations of biochar and salt (Table 2, Fig. 1).

3.3 Physiological Parameters

3.3.1 Leaf Photosynthetic Pigment Content

According to variance analysis, the interaction effect of the various levels of biochar and salinity on the SPAD index was significant ($P < 0.01$). SPAD readings varied from 13.23 to 31.20. Furthermore, SPAD diminished by increasing salinity, while it improved after amending with biochar. The greatest SPAD was 31.20, which was observed following the application of 3% biochar without salt stress, an increase of 18.18% compared to the control (Fig. 2A).

3.3.2 Determination of Membrane Damage

The membrane damage in the plant was significantly affected by the main and interaction effects of the various levels of biochar and salinity ($P < 0.01$). The use of biochar reduced the EL, while a significant rise was detected in the variable by exposing it to more salinity (Fig. 2B). The highest salinity treatment of 8 ds/m significantly increased the EL by 88.12% compared with the control. So that, the B_0S_3 (0% biochar + 8 ds/m salinity) and B_3S_0 (3% biochar + 0 ds/m salinity) treatments led to the maximum (74.12%) and minimum (30.42%) membrane damage, respectively (Fig. 2B).

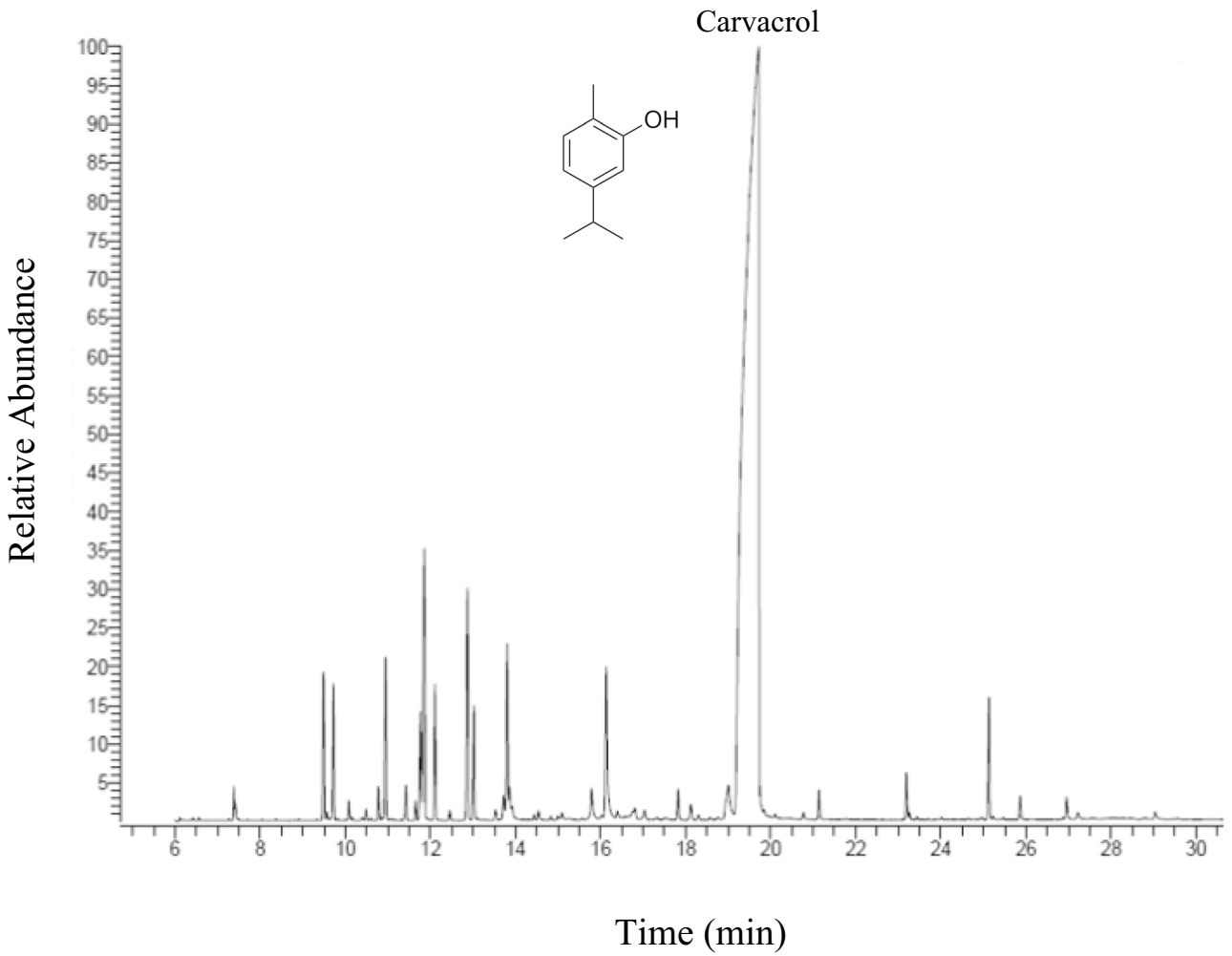

Fig. 1 The GC–MS chromatogram of *S. khuzistanica* essential oil

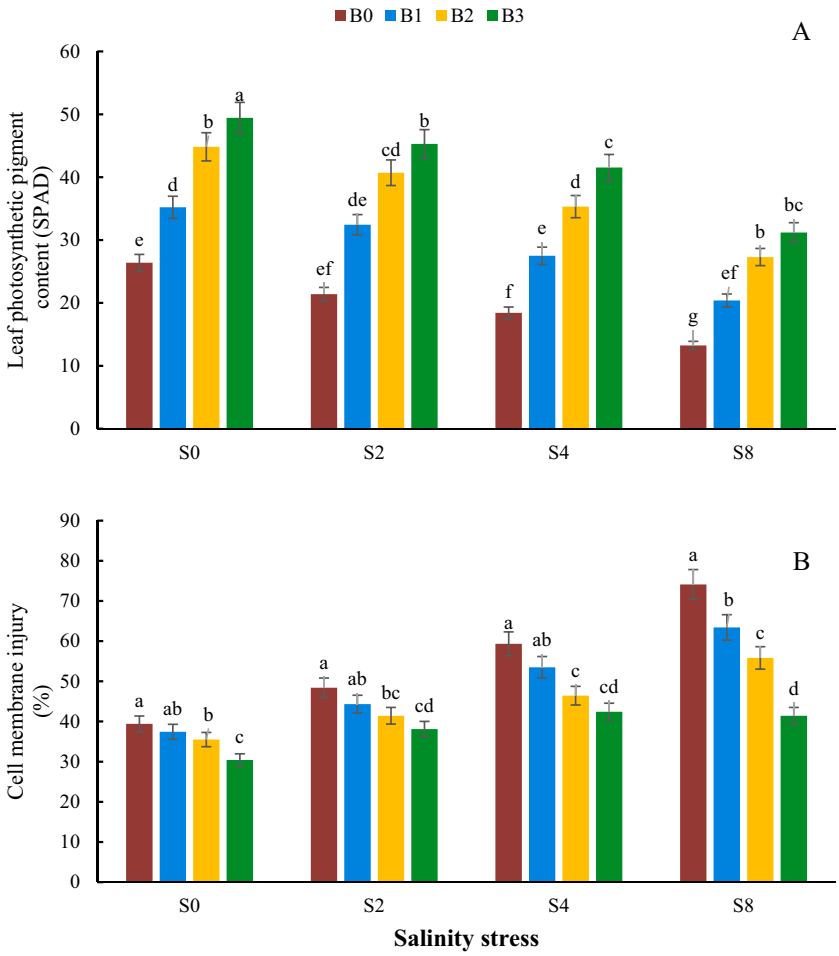

Fig. 2 Effect of salinity levels and biochar application on leaf photosynthetic pigment (**A**) and cell membrane injury (**B**) of *S. khuzistanica*. S, salt; S_0, without salt; S_1, 2 dS m^{-1}; S_2, 4 dS m^{-1}; S_3, 8 dS m^{-1} NaCl/; B, biochar; B_0, non-biochar; B_1, 1% of total pot mass; B_2, 2% total pot mass; B_3, 3% total pot mass. Bars with the same letter(s) are not significantly different at $P < 0.05$ according to the LSD test

3.3.3 Antioxidant Enzyme Activities and Leaf Proline Content

The exposure of the plant to the various concentrations of salt in the absence of biochar improved the activity of POD, SOD, and CAT, although the enzymes exhibited lower activities when biochar was added. Further, the enzyme activities increased by raising salinity, while biochar decreased the stress regulation and enzyme activities. The highest salinity level (8 ds/m) SOD and POD increased about 302.22% and 226.07%, respectively. So, the highest activity of SOD and POD was related to the B_0S_3 treatment (Fig. 3A, B), although the B_3S_8 and control ones resulted in maximizing (with a 135% increase compared to the control) and minimizing CAT activity, respectively (Fig. 3C). Furthermore, proline concentration elevated under salt stress, and the salinity of 8 ds/m significantly increased (345.45%) accumulation in the vegetative tissue compared to the control. However, using biochar at various salinity levels diminished proline content (Fig. 3D).

4 Discussion

Salt stress is one of the main abiotic risks, which limits plant growth and biomass production (Sheng et al. 2020; Yang et al. 2020; Zhang et al. 2021). The results of the present study represented that salt stress decreased morphological and functional properties. An elevation in the stress level led to more reduction in the characteristics so that their lowest amount was related to the salinity 8 ds/m, which is consistent with the results of Taarit et al. (2011) on the growth traits of *Salvia sclarea* under salt stress and those of Mehdizadeh et al. (2020) on *S. hortensis* under stress. Salinity reduces plant growth rate by affecting metabolic pathways and molecular responses associated with the present results. Furthermore, salt stress can disrupt plant ion homeostasis leading to lower photosynthesis activity and membrane stability. Ion toxicity, lower cell water, less osmotic potential, osmotic stress stimulation, and lower dry weight are the other dominant adverse effects of salinity (Ren et al. 2020; Yang et al. 2020; El-Banna et al. 2022). Salinity disrupts chloroplast

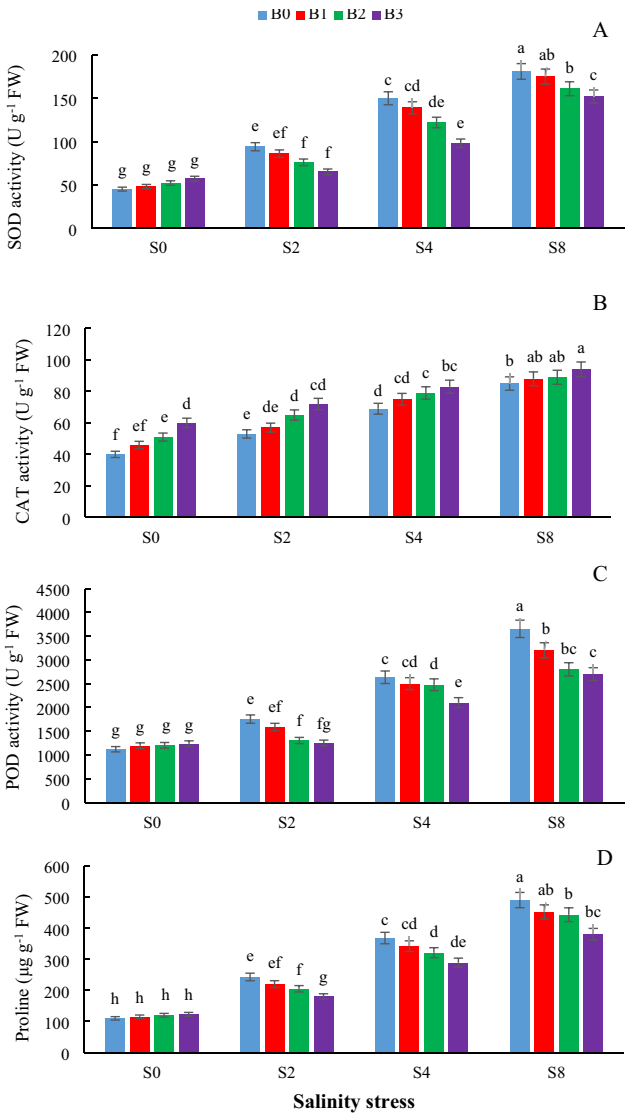

Fig. 3 Effect of salinity levels and biochar application on superoxide dismutase (SOD) (**A**), catalase (CAT) (**B**), peroxidase (POD) (**C**), and proline (**D**) of *S. khuzistanica*. S, salt; S_0, without salt; S_1, 2 dS m^{-1}; S_2, 4 dS m^{-1}; S_3, 8 dS m^{-1} NaCl/; B, biochar; B_0, non-biochar; B_1, 1% of total pot mass; B_2, 2% total pot mass; B_3, 3% total pot mass. Bars with the same letter(s) are not significantly different at $P<0.05$ according to the LSD test

function and reduces cell water potential, resulting in closing stomata and absorbing a limited level of CO_2 after a short time, consequently stopping cell division (Bukhat et al. 2019; Farouk & AL-Huqail 2022). Following the salt stress, ROS accumulation increases, which accelerates oxidative damage, leads to constant oxidative damage by weakening essential cellular compounds, inactivates antioxidant capacity, interrupts plant-water relations, and diminishes nutrient uptake (Ahmad et al. 2021; Bukhat et al. 2019; Farouk et al. 2020; Yang et al. 2020).

Further, a significant improvement was observed in the morphological and functional properties after utilizing biochar, especially 3%. Biochar enhances plant ability to resist environmental stress factors and elevates agricultural productivity (Ran et al. 2019; Farouk & AL-Huqail 2022; Farouk et al. 2023). The substance results promote the growth of *S. hortensis* (Mehdizadeh et al. 2020) and *Solanum tuberosum* (Akhtar et al. 2015) under salt stress, which are in line with the results of the present study. Furthermore, the application of biochar increases shoot weight in maize (Abiven et al. 2015), as well as the biomass of aerial parts and the length of roots in potato crops (Akhtar et al. 2015) under salt stress. Limited information is available concerning the exact mechanism of biochar on plant growth and the interaction between biochar and salinity.

Biochar may stabilize salt ions in saline soils or form non-saline microsites to enhance nutrient uptake (Ghezzehei et al. 2014). However, the enhanced growth rate could be related to increased stomatal conductivity and plant water consumption (Akhtar et al. 2015; Zhang et al. 2013). It seems that a rise in water-holding capacity and ionic solute uptake because of utilizing biochar in saline conditions is associated with lower salt concentration in the soil, leading to less adverse effects of NaCl on plant growth parameters. Additionally, biochar stimulates leaf photosynthetic performance and decreases oxidative stress, causing more biomass and plant yield under salinity conditions (Yang et al. 2020). Some of the most important characteristics of soil, such as pH, enzyme activity, microbial biomass, and nutrient and water holding capacity, elevate after treatment with biochar (Jeffery et al. 2011). In the biochar-amended soil, root sensitivity to osmotic stress diminishes by increasing soil moisture and improving soil properties, as well as Na binding to the biochar structure (Akhtar et al. 2015; Mehdizadeh et al. 2020).

The quantity and quality of the essential oil of medicinal plants can be influenced by environmental stresses and nutrient availability. Despite the reduced plant productivity, different results have been reported regarding the changes in the quantity and quality of medicinal plant essential oil in response to salinity levels. Some researchers have found a rise in the essential oil content of *Coriandrum sativum* (Nefati et al. 2011), *Ocimum basilicum* (Farasaraei et al. 2020), *Mentha spicata* (El-Danasoury et al. 2010), and *Lallemantia iberica* (Heydari & Pirzad 2020) at high salinity level. In some plant species, essential oil accumulation can be ascribed to an increased density of essential oil glands along with the production of more glands during stress (Ghassemi-Golezani & Farhadi 2022), as well as net assimilation or assimilate distribution during growth and differentiation processes (Chrysargyris et al. 2018). Further, a reduction in plant primary metabolism during stress can result in the accumulating of specific products

(intermediates), which can be shifted toward the synthesis of secondary metabolites such as essential oils (Ghassemi-Golezani & Farhadi 2022). In the present study, the essential oil percentage was significantly affected by biochar. The treatments could enhance plant yield and essential oil content under salt stress because of having specific physicochemical traits, minimizing sodium uptake, and maximizing the amounts of available nutrients (Ghassemi-Golezani & Farhadi 2022). The results of several studies have demonstrated different carvacrol biosynthesis and accumulation in response to various environmental factors (Majdi et al. 2017; Mohammadi et al. 2019). Some plant species like mint (Aziz et al. 2008) and basil (Ashraf & Orroj 2006) have less essential oils, especially monoterpenes, following salt stress. However, the stress is associated with the elevated concentrations of essential oil components in some other plant species, such as chamomile (Baghalian et al. 2008). The results of the present study represented a more significant carvacrol percentage in salinity conditions, which is in line with those of Neffati and Marzouk (2008).

Furthermore, SPAD significantly diminished under salt stress, which is in agreement with the results of Taïbi et al. (2016) on *Phaseolus vulgaris* L., Taffouo et al. (2010) on *Vigna subterranean* L., and Farouk et al. (2020) on *Ocimum basilicum* L. The lower photosynthetic pigment level of plants under salt stress is considered a typical sign of oxidative stress and is attributed to the inhibition of chlorophyll synthesis and activation of its degradation by chlorophyllase (Santos 2004; Smirnoff 1996; Taïbi et al. 2016). The declined chlorophyll content caused by slow synthesis or rapid degradation suggests the presence of a light protection mechanism through decreasing light absorption by reducing chlorophyll amount (Elsheery & Cao 2008). Based on the results of the previous studies, a rise in salinity level diminishes the chlorophyll content of basil due to light inhibition, increased chlorophyllase activity, and enhanced ROS production, as well as the instability of the protein structure of pigments (Heidari 2012). In the present study, a higher SPAD was found by applying biochar under salinity conditions. The results of other studies indicated that biochar treatment elevates chlorophyll amount, improves PSII activity, and facilitates electron transfer (Lyu et al. 2016), as well as maximizing the production of chlorophyll, carotenoid, amino acids, and protein in plants (Younis et al. 2015).

Some defense systems, like cell membrane stability, protect plants against the adverse effects of stress. Cell membrane stability and EL refer to the cell damage rate induced by environmental stress in plants, respectively. As for the basil, the EL increases significantly under salt stress because of enhancing membrane permeability, and consequently increasing solute leakage (Hu et al. 2012). Salinity conditions are accompanied by higher membrane damage and lipid peroxidation due to the greater concentrations of Na^+ in plants (Banu et al. 2009). After exposure to salt stress, EL improves in *O. basilicum* (Farasaraei et al. 2020), *S. hortensis* (Mehdizadeh et al. 2020), and *Thymus daenensis* and *T. vulgaris* (Bistagni et al. 2019), which is consistent with the results of the present study. The stress causes nutritional imbalance in plant tissue by elevating the accumulation of specific ions like Na^+, leading to toxic effects on membrane stability and permeability, as well as more EL (Tavakkoli et al. 2010). Further, it increases membrane damage by promoting the levels of cell free radicals in many plants (Farasaraei et al. 2020). Regarding the results of the present study, cell membrane damage was reduced by utilizing biochar, which aligns with the results of Mehdizadeh et al. (2020) on *S. hortensis*. Biochar enhances water-holding capacity in soil, improving soil moisture and lowering salt concentration in soil solution (Akhtar et al. 2015).

Like to other abiotic stresses, salt stress causes excessive ROS production in plants (An et al. 2016). Furthermore, SOD, POD, and CAT are the main components of the antioxidant enzyme system and play a crucial role in removing ROSs. Plant antioxidants are a natural defense system against various stresses (Zulfiqar et al. 2021). The results of the present study revealed that the activities of CAT, SOD, and POD elevated by increasing salinity, while they were modulated after amending with biochar. Some researchers have reported using biochar promotes the systemic resistance of antioxidant enzymes in plants (Quartacci et al. 2017; Rehman et al. 2019; Abideen et al. 2020; Rasool et al. 2021).

Proline, as one of the compatible soluble substances, plays an important role in maintaining cell osmotic balance in plants (Shtereva et al. 2015), the accumulation of which enhances salt resistance (Kishor et al. 2005). In the present study, a rise in salinity level led to a greater proline content in the leaves of *S. khuzistanica*, which is in agreement with those of Mehdizadeh et al. (2020) on summer savory and Huang et al. (2019) on sweet corn. Generally, proline concentration elevates through biosynthesis of protein, or its hydrolysis and oxidative degradation to protect against salinity. It serves as an osmotic protectant, ROS scavenger, and protein stabilizer, and plants can increase the accumulation of the material to deal with osmotic stress (Trovato et al. 2008). Based on the results of the previous studies, biochar differently influences the amount of proline in various plants. Biochar decreases proline content in bean (Farhangi-Abriz & Torabian 2017) and corn seedlings (Lashari et al. 2014), which is inconsistent with the results of the present study. However, some researchers found a greater proline concentration in summer savory after the treatment (Mehdizadeh et al. 2020), which is in line with the results of the present study.

5 Conclusion

The results of the present study revealed that salt stress reduced the growth of *S. khuzistanica*, and biochar amendment could moderate the adverse effects of salinity. Biochar amendment may effectively reduce salinity stress on plants by increasing the soil cation exchange capacity, total porosity, soluble and exchangeable K^+, and fertilities, decreasing soil electrical conductivity, soluble Na^+, and Cl^- contents, and water evaporation, reducing the shoot Na^+ accumulation, and relative electrical leakage; increasing the shoot K^+ accumulation, improving leaf water status, increasing chlorophyll content, and increasing N use efficiency. The current study suggests that biochar advances salinity tolerance and may be considered a potential regulator of agricultural production under salt stress. This makes a significant contribution to the production of *S. khuzistanica* in salty and low-yielding lands. Thus, we conclude that further work on specific interactions between biochar type and rates, and cultivation measures is needed and quite promising.

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Declarations

Competing Interest The authors declare no competing interests.

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