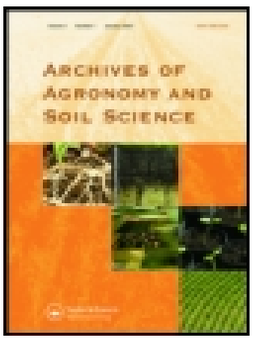

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Response of summer savory at two different growth stages to biochar amendment under NaCl stress

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ABSTRACT

Salinity stress leads to various biochemical changes in plants. Biochar (BC) is a soil amendment that is derived from pyrolyzed organic materials. The aim of this study was to investigate the effect of BC on growth and some biochemical characteristics of summer savory (*Satureja hortensis* L.) under NaCl stress at two different growth stages. So, a pot factorial experiment based on completely randomized design was performed that comprised three levels of BC (0, 1 and 2% w/w of soil) and four NaCl levels (0, 40, 80 and 120 mM) with four replications. According to the results, by increasing the NaCl concentration chlorophyll a, b, total chlorophyll, carotenoid and polyphenol oxidase (PPO) decreased, whereas antioxidant activity, total soluble sugar and phenolic contents increased. The use of BC (especially 2% w/w of soil) under NaCl stress had the greatest effects on studied traits at the vegetative and flowering stages and significantly increased chlorophyll a, b, total chlorophyll, carotenoid and PPO activity. The results of this experiment confirmed the view that each stage of growth responses differently to NaCl stress and the use of BC due to sorption of NaCl and increasing osmotic adjustment can lead to summer savory protection against NaCl stress.

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Satureja hortensis L.; phenol; antioxidant activity; soluble sugar; chlorophyll

Introduction

The genus *Satureja* belongs to the family Lamiaceae and 30 species of this genus generally distributed in Mediterranean region, moreover spread out to Irano-Turanian phytogeographical region. Sixteen species of this genus are existed in Iran which are found in north, northwest, west, northeast and central parts of the country and eight of them are endemic to Iran (Jamzad 2010). *Satureja hortensis* L. (summer savory) is one of the most important Iranian species that widely dispersed in different parts of Iran. The aerial parts of summer savory used in pharmaceutical and cosmetic industries and applied as a flavor ingredient in foods. Furthermore, in traditional medicine, it is used as a muscle pain reliever and treatment for stomach and intestinal diseases (Zargari 1990).

The medicinal and aromatic plants production is reduced due to salinity stress (Ben Taârit et al. 2012). Salt stress has different harmful effects on chlorophyll content, plasma membrane permeability and other metabolic disturbances (Gupta and Huang 2014). Salt stress reduces leaf water content and produces reactive oxygen species (ROS) that leads to oxidative stress in plants (Gupta and Huang 2014; Noman et al. 2015). Under salinity condition, plants use enzymatic and non-enzymatic antioxidant mechanisms in order to protect themselves against oxidative damages caused by ROS (Abogadallah 2010; Acosta-Motos et al. 2017). Plants that are

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at risk of high salinity levels show different physiological and biochemical modifications like antioxidant activity and osmotic adjustment that leads to a decline in plant growth and several changes in the organization and function of cell membranes (Ben Taârit et al. 2012). Among non-enzymatic antioxidants, plant tissues contain a network of low-molecular mass antioxidants such as phenolic compounds that their production could be induced by environmental stresses such as salinity (Maisuthisakul et al. 2007). The halophyte plants have the capacity to adapt to salinity stress by adjusting the osmotic potential of their internal tissues (Aghaleh et al. 2009). There are several valuable secondary metabolites in plants like phenolic compounds. Plants activate biochemical and molecular mechanisms to deal with salt stress such as induction of compatible solutes formation (Khan et al. 2011). The plants are adapted to salinity due to the accumulation of osmoregulator solutions like soluble sugars which prevent the loss of water and ion toxicity (Khan et al. 2011).

Several techniques are available to reduce the harmful and toxic influences of salinity in plants. Pyrolysis of waste organic substances by thermo chemical conversion under the lack of oxygen supply resulted in production of porous carbon-rich material, known as biochar (BC) (Clough et al. 2013; Ok et al. 2015; Rizwan et al. 2016). Using BC as a sustainable and useful soil amendment currently have gained considerable attention worldwide due to its ability to improve soil properties and functions such as enhancing soil water holding capacity, improving soil quality, boosting soil fertility, raising carbon sequestration, increasing soil pH, improving cation exchange capacity (CEC) and immobilization of organic and inorganic contaminants (Ok et al. 2015; Rajapaksha et al. 2016; Rizwan et al. 2016; Subedi et al. 2017). The variation among BC properties are related to their physical and chemical characteristics like porosity and particular surface area, the type of applied materials and pyrolysis conditions, the rate and the type of methods (Baronti et al. 2014). Also, BC has been identified as an amendment that applied for plant production under abiotic and biotic stresses (Rizwan et al. 2016; Ali et al. 2017). Based on the previous study, BC cause to decrease soil salinity in wheat (Lashari et al. 2013).

Subsequently, there are few reports that concerning the influence of BC amendment on biochemical characteristics of medicinal plants such as *S. hortensis* under salt stress. Therefore, we performed an experiment to examine the effect of BC on growth, some biochemical and physiological characteristics of *S. hortensis* under NaCl stress for the first time. The objective of this experiment was to investigate the effect and mechanism of BC on reducing Na^+ negative effects on summer savory as a salt sensitive plant.

Materials and methods

Plant materials

Plastic pots with a top diameter of 20 cm and a depth of 25 cm were filled with sieved sandy loam soil consisted of sand (73.4%), silt (18.3%) and clay (8.3%). Seeds were prepared from Anbari Company, Iran. In spring 2017, seeds were directly sown in each pot and after four weeks were thinned to five healthy seedlings per pot. The plants were grown in greenhouse with controlled conditions (25/18°C day/night temperature, and 70–85% relative humidity). During the period of seed germination and seedling established, the plants were irrigated with tap water every other days and the water content of the soil kept near the field capacity (FC). To supply saline treatments, the NaCl was added to irrigation water at four leaves stage.

Experimental design and treatments

The experiment was arranged as a 4 × 3 × 4 factorial in a complete randomized design (CRD) with four replications. Four salinity regimes (0, 40, 80, and 120 mM NaCl) were applied by adding NaCl into irrigation water and three BC levels (included 0, 1 and 2% w/w of soil) which were mixed with soil before planting.

Biochar (BC) amendment

BC was prepared from mulberry (*Morus alba* L.) woods pruning collected from the landscape trees that planted in the campus of Ferdowsi University of Mashhad, Iran. The woods were collected, air-dried and chopped into small pieces with the same size. Then they were pyrolyzed at 530°C temperature for 14 h under anaerobic conditions produced BC. After preparing the materials, they were passed through a 2 mm sieve, mixed thoroughly to obtain uniform sized particles. The basic BC properties include: pH 9.7, CEC 5.2 cmol(+) kg^{-1} and EC 6.8 dS m^{-1}, organic carbon 3.51%, N 0.97%, P 0.43%, K 1.23% and Na 0.19%.

Plant assay

The first examination was done four weeks after treatments applied at vegetative stage. The samples were selected from the leaves to measure some biochemical characteristics. The second examination was managed at flowering stage. Since in this stage of harvest, the plants were damaged at the highest NaCl concentration (120 mM), so, we just examined the plants under three levels of salinity.

Total, aboveground and underground biomasses

Three plants were chosen from each pot at the end of the experiment and aboveground and underground biomasses were harvested, oven-dried at 72°C for 48 hours, and weighed.

Methanolic extraction procedure

300 mg of fresh leaves were grinded, then 3 mL of 99% methanol put into the 15 mL falcon tubes. The mixture was centrifuged at 1245 × *g* at room temperature for 10 min. The insoluble portions were then separated by filtration. In the next step, the prepared extracts were stored at 4°C in refrigerator for 24 h.

Chlorophyll and carotenoid content

The chlorophyll content of samples were measured by Wellburn (1994) method. The absorbance of extracts was determined by a spectrophotometer (Bio Quest C2502) at 653, 666 and 470 nm. The results were described in mg per gram of fresh weight (mg g^{-1}FW).

Total soluble sugar content

Total sugar content was determined by Mc Cready et al. (1950) method with anthrone reagent by spectrophotometry. In order to prepare anthrone reagent, 0.15 g of anthrone was solved at 100 mL 72% H_2SO_4. Then the mixture reactions were ready by adding 3 mL of prepared anthrone reagent to 100 µL methanolic extract and put the test tube in bath for 10 min at 100°C. They were then put at room temperature to cool and optical density was measured by spectrophotometer at 630 nm.

Total phenolic content

Folin–Ciocalteu (F–C) reagent was used to examine total phenolic content of leaf extracts, by Singleton and Rosi (1965) method with slightly modification. The reaction mixture included 100 µL methanolic extract, 200 µL 50% F–C and 100 µL distilled water. 7.5% sodium carbonate was added after 3 minutes and the solution was put in darkness for 60 min. Then, the absorbance versus prepared blank was read at 765 nm. Total phenolic content of leaf extracts was expressed as mg

gallic acid equivalents (GAE) per gram of fresh weight (mg GAE g^{-1} FW) through a calibration curve (0–400 μgmL^{-1}) with gallic acid.

Antioxidant activity

The 2,2-diphenylpicrylhydrazyl radical (DPPH.) scavenging activity was determined by Hanato et al. (1988) method. The leaf extracts were diluted in absolute methanol in 1:10 ratio, and subsequently 1 mL of DPPH 500 mM was added into the leaf methanolic extracts. The mixture was shaken vigorously and was left standing in the darkness at room temperature for 30 minutes, then the absorbance was measured at 517 nm. Finally, the inhibition percentage of free radical DPPH (IP%) was calculated as following:

$$IP\% = \left[\left(A_{blank-}A_{sample}\right)/A_{blank}\right]\times 100$$

Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract.

Preparation of enzyme extraction

For preparing enzyme extraction, 300 mg of fresh leave samples was homogenized in 3 mL of 50 mM potassium phosphate buffer (pH = 7.5) containing 1 mM EDTA, 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 1537 × *g* and 4°C for 20 min (Gapińska et al. 2008).

Polyphenol oxidase (PPO) activity

PPO activity was measured by Raymond et al. (1993) method. Reaction mixture composed of 2.5 mL of 50 μM potassium phosphate buffer (pH = 7), 0.2 mL of 20 mM pyrogallol and 0.1 mL of enzyme extract. The absorbance was determined at 420 nm. The PPO activity was quantified as pyrogallol oxidized after 3 minutes per mg protein [Unit mg^{-1} (protein)].

Statistical analysis

All analyses were performed in four replications and the results were expressed as mean values ± Standard Error (SE). The data were subjected to statistical analysis by statistical program package Minitab 17. The combined effects of BC and salinity were analyzed by a two-way ANOVA. The Bonferroni's test was applied as post-ANOVA test. The differences between individual means were deemed to be significant at $P < 0.05$. The figures were drawn by Microsoft excel software.

Results

Total, aboveground and underground biomasses

The results of this study showed that total, aboveground and underground biomasses of salt-stressed plants decreased by increasing NaCl concentration. The highest total biomass (1.86 g DW $plant^{-1}$), aboveground biomass (1.51 g DW $plant^{-1}$) and underground biomass (0.35 DW $plant^{-1}$) were obtained at the treatment of 2% BC without salinity (Figure 1(a–c)). By using BC total, aboveground and underground biomasses increased especially at 2% BC treatments (Figure 1(a–c)).

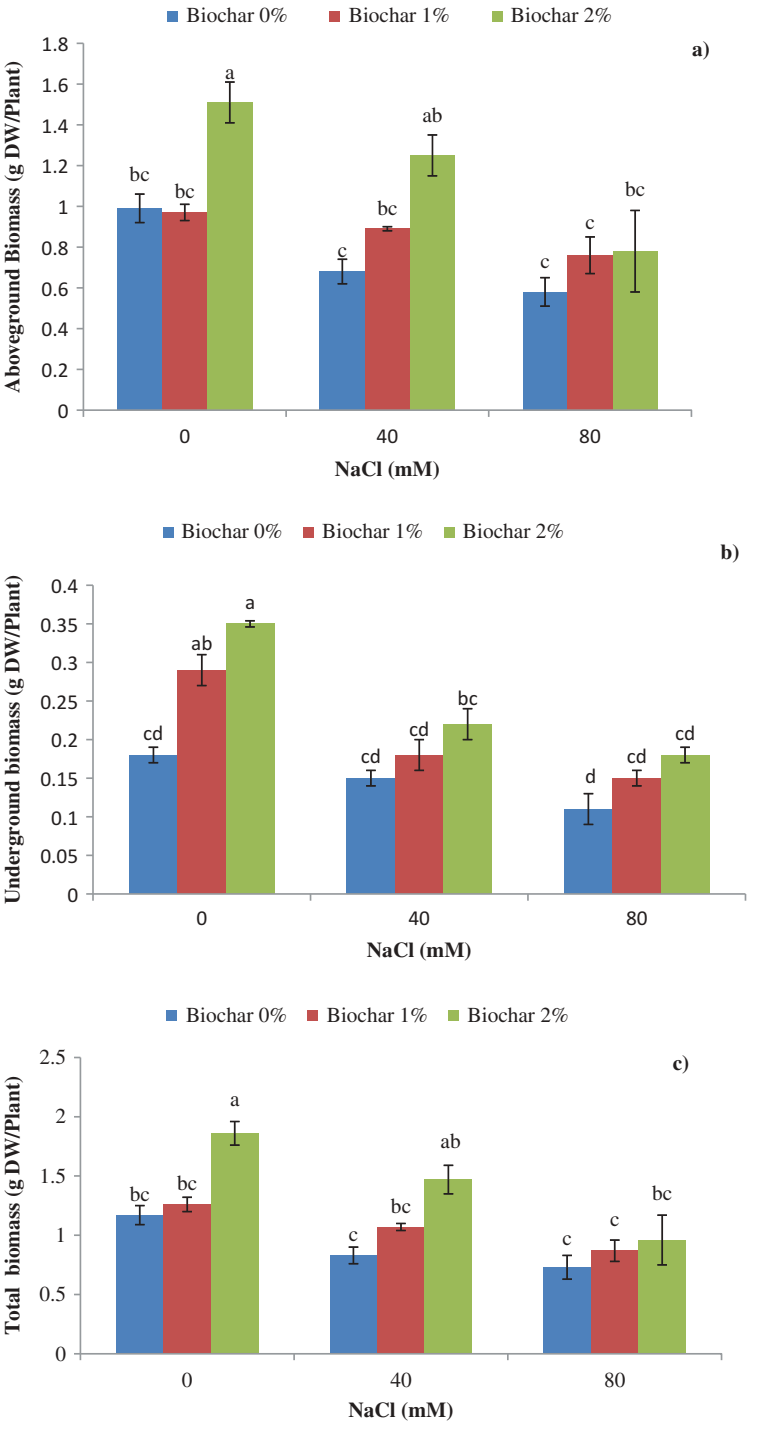

Figure 1. Effect of NaCl concentration and BC application on (a) above ground biomass (b) underground biomass (c) total biomass of summer savory at flowering stage. Bars with the same letter are not significantly different at ($P < 0.05$) according to Bonferroni test.

Photosynthetic pigments

Chlorophyll a content

According to the findings of this study the chlorophyll a content of salt stressed plants at vegetative stage decreased by increasing NaCl concentration. In this growth stage, the lowest chlorophyll a content (0.42 mg g^{-1} FW) was observed under 120 mM salinity without using BC, however there was no significant difference between 80 and 120 mM NaCl treatments. Application of BC increased chlorophyll a content especially at 2% BC treatment (Table 1). At flowering stage, the lowest chlorophyll content (0.66 mg g^{-1} FW) was observed at 80 mM NaCl without using BC (Table 1) and chlorophyll a content increased significantly at 2% w/w BC under 80 mM NaCl.

Chlorophyll b content

The lowest chlorophyll b content (0.72 mg g^{-1} FW) was obtained under 120 mM NaCl without using BC at vegetative growth stage. The highest amount of chlorophyll b (6.7 ± 0.16 mg g^{-1} FW) was observed at 2% BC treatment (Table 1). At flowering stage, the lowest chlorophyll b content (0.64 mg g^{-1} FW) of plants was observed at 80 mM NaCl without using BC (Table 1). The highest amount of chlorophyll b content (2.87 ± 0.17 mg g^{-1} FW) was seen at 2% w/w of BC with a significant difference compared to other treatments.

Total chlorophyll content

The lowest total chlorophyll content (2.99 mg g^{-1} FW) was obtained under 80 mM NaCl without using BC at vegetative growth stage. The highest amount of total chlorophyll content (10.85 ± 0.14 mg g^{-1} FW) was observed at 2% w/w of BC with a significant difference compared to other treatments (Table 1). At flowering stage, the lowest total chlorophyll content

Table 1. Effect of NaCl concentration and BC application on photosynthetic pigments content of summer savory at vegetative and flowering stages.

Growth stage	NaCl concentration (mM)	BC (% w/w of soil)	Chlorophyll a (mg g^{-1}FW)	Chlorophyll b (mg g^{-1}FW)	Total chlorophyll (mg g^{-1}FW)	Carotenoid (mg g^{-1}FW)
Vegetative stage	0	0	1.6 ± 0.05 bc*	3.62 ± 0.26 cd	6.75 ± 0.3 cd	1.26 ± 0.06 b
	40	0	0.71 ± 0.2 d	2.89 ± 0.31 de	4.31 ± 0.35 e	1.06 ± 0.08 bc
	80	0	0.58 ± 0.03 d	2.07 ± 0.26 ef	2.99 ± 0.19 fg	0.34 ± 0.05 ef
	120	0	0.42 ± 0.23 d	0.72 ± 0.09 g	4.04 ± 0.35 e	0.41 ± 0.08 d-f
	0	1	2.4 ± 0.14 ab	4.2 ± 0.23 c	7.84 ± 0.11c	1.26 ± 0.07 b
	40	1	2.54 ± 0.11 a	3.65 ± 0.15 cd	6.63 ± 0.17 cd	1.31 ± 0.03 ab
	80	1	1.72 ± 0.11 bc	3.3 ± 0.09 cd	5.89 ± 0.18 d	0.31 ± 0.14 f
	120	1	1.62 ± 0.14 c	1.72 ± 0.27 fg	7.65 ± 0.29 c	1.08 ± 0.04 bc
	0	2	2.62 ± 0.2 a	6.7 ± 0.16 a	10.85 ± 0.14 a	1.32 ± 0.06 ab
	40	2	2.59 ± 0.14 a	5.86 ± 0.3 ab	9.7 ± 0.26 ab	1.7 ± 0.06 a
	80	2	2.72 ± 0.22 a	5.75 ± 0.12 ab	9.27 ± 0.21 b	1.24 ± 0.12 b
	120	2	2.45 ± 0.12 ab	5.36 ± 0.22 b	2.4 ± 0.17 g	1.19 ± 0.01 b
Flowering stage	0	0	1.81 ± 0.06 d	1.93 ± 0.09 ab	6.19 ± 0.36 b-d	1.79 ± 0.17 a
	40	0	1.22 ± 0.15 e	1.97 ± 0.09 ab	5.14 ± 0.29 d	0.71 ± 0.15 c
	80	0	0.66 ± 0.01 f	0.64 ± 0.17 c	2.16 ± 0.06 e	0.87 ± 0.12 b
	0	1	4.11 ± 0.09 b	1.99 ± 0.19 ab	7.55 ± 0.21 ab	1.82 ± 0.19 a
	40	1	2.77 ± 0.03 c	2.1 ± 0.32 ab	6.25 ± 0.19 b-d	1.55 ± 0.16 ab
	80	1	2.69 ± 0.17 c	1.67 ± 0.38 bc	5.85 ± 0.4 cd	1.29 ± 0.18 ab
	0	2	3.73 ± 0.06 b	2.87 ± 0.17 a	8.53 ± 0.3 a	1.81 ± 0.19 a
	40	2	4.65 ± 0.004 a	2.68 ± 0.21 ab	7.24 ± 0.2 a-c	1.59 ± 0.11 ab
	80	2	4.67 ± 0.15 a	2.02 ± 0.21 ab	7.61 ± 0.4 ab	2.03 ± 0.13 a

*Data are mean ± SE. Means with different letter in a column at each growth stage are statistically significant at 5% level probability according to Bonferroni test.

(2.16 mg g^{-1} FW) of plants was seen at 80 mM NaCl without using BC (Table 1). The highest amount of total chlorophyll content (8.53 ± 0.3 mg g^{-1} FW) was obtained at 2% w/w of BC with a significant difference compared to other treatments.

Carotenoid content

The lowest carotenoid content (0.34 mg g^{-1} FW) was observed under 80 mM salinity without using BC at vegetative growth stage. By using BC carotenoid content increased especially at 2% BC treatment (Table 1). At flowering stage, the lowest carotenoid content (0.71 mg g^{-1} FW) of plants was observed at 40 mM NaCl without using BC (Table 1). The use of BC especially at 2% w/w BC, significantly increased carotenoid content at this growth stage under 80 mM NaCl.

Total soluble sugar

Due to the findings of this study, there was no significant difference between NaCl levels in relation to soluble sugar content at vegetative growth stage, however this trait increased (0.253 mg g^{-1} FW) partially at the highest NaCl concentration (Figure 2(a)). Moreover, by using BC the soluble sugar content increased. At flowering stage, soluble sugar content decreased in comparison with vegetative stage, but by increasing NaCl concentration, it increased and application of BC lead to increase soluble sugar content (Figure 2(a)). The highest soluble sugar content (0.162 mg g^{-1} FW) was observed at 80 mM NaCl by using 2% w/w BC in soil.

Total phenolic content

Due to the findings of this experiment, the total phenolic content in the leaves increased by raising NaCl concentration at vegetative and flowering stages (Figure 2(b)). At vegetative stage the highest phenolic content (10.26 mg g^{-1} FW) was observed at 120 mM NaCl without using BC application, although there were no significant differences between this concentration and the other NaCl concentrations (40 and 80 mM) without using BC. On the other hand, using BC caused to reduce total phenolic content. So, the lowest phenolic content (3.8 mg g^{-1} FW) was observed at 120 mM NaCl with using BC 2% w/w (Figure 2(b)).

At flowering stage, the total phenolic content was higher than vegetative growth stage significantly. The highest total phenolic content (18.16 mg g^{-1} FW) was observed at 80 mM NaCl without using BC. The use of BC especially at 2% w/w, significantly decreased total phenolic content at this growth stage in all treatments particularly at 80 mM NaCl (Figure 2(b)). According to the results, leaf extracts at flowering stage exhibited an important amount of phenolic content (18.16 mg g^{-1} FW) at 80 mM NaCl, which was about 1.77 times higher than the total phenolic content at vegetative growth stage (10.26 mg g^{-1} FW).

Antioxidant activity

Due to the findings of this study, antioxidant activity at vegetative stage increased by raising NaCl concentration. The highest antioxidant activity (92.91%) was observed at 120 mM NaCl, and there was no significant difference between the NaCl concentrations (0, 40, 80 and 120 mM) without using BC (Figure 3(a)). Antioxidant activity decreased in each concentration of NaCl by applying BC.

At flowering stage, antioxidant activity increased by raising salinity concentration and enhanced partially (about 1 times) more than vegetative stage. Therefore, the highest antioxidant activity (93.83%) was observed at 80 mM NaCl (Figure 3(a)). In salinity treatments, by using BC the antioxidant activity decreased. The lowest antioxidant activity (59.29%) was observed by using 2% w/w BC without salt stress (Figure 3(a)).

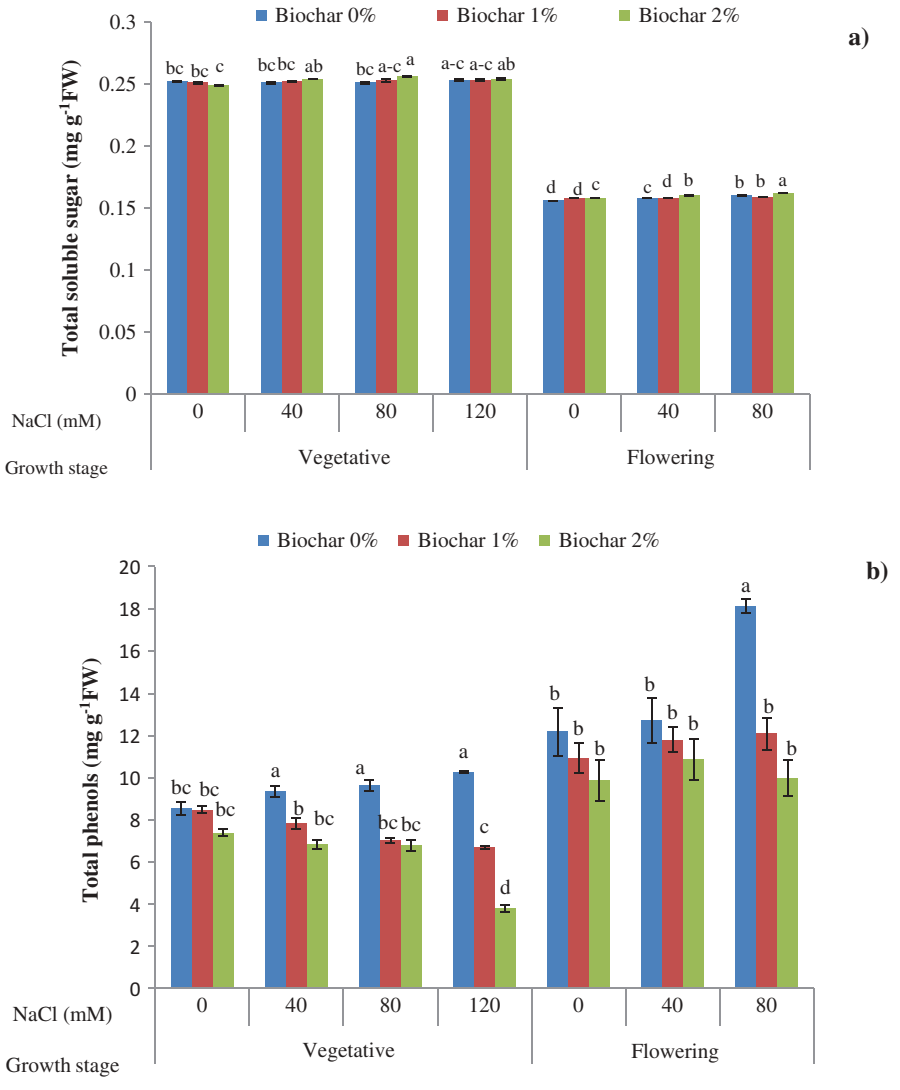

Figure 2. Effect of NaCl concentration and BC application on (a) total soluble sugar and (b) total phenolic content of summer savory at vegetative and flowering stages. Bars with the same letter are not significantly different at ($P < 0.05$) according to Bonferroni test.

Polyphenol oxidase (PPO)

According to the results of this study, (PPO) activity increased by salinity until 40 mM of NaCl at vegetative stage and then decreased by increasing NaCl concentration. The highest PPO activity (2.03 unit mg^{-1} protein) was observed at 40 mM NaCl without using BC (Figure 3(b)). Application of BC reduced PPO activity, but at the highest NaCl concentration (120 mM), the usage of BC increased PPO activity. At flowering stage, PPO activity decreased by increasing NaCl concentration and BC usage increased PPO activity (Figure 3(b)). The highest PPO activity (1.69 unit mg^{-1} protein) was obtained in the absence of salinity and BC. Moreover, the lowest amount of PPO activity (0.67 unit mg^{-1} protein) was observed under 80 mM NaCl without using BC and there was no significant difference between 0 and 40 mM NaCl treatments without using BC (Figure 3(b)).

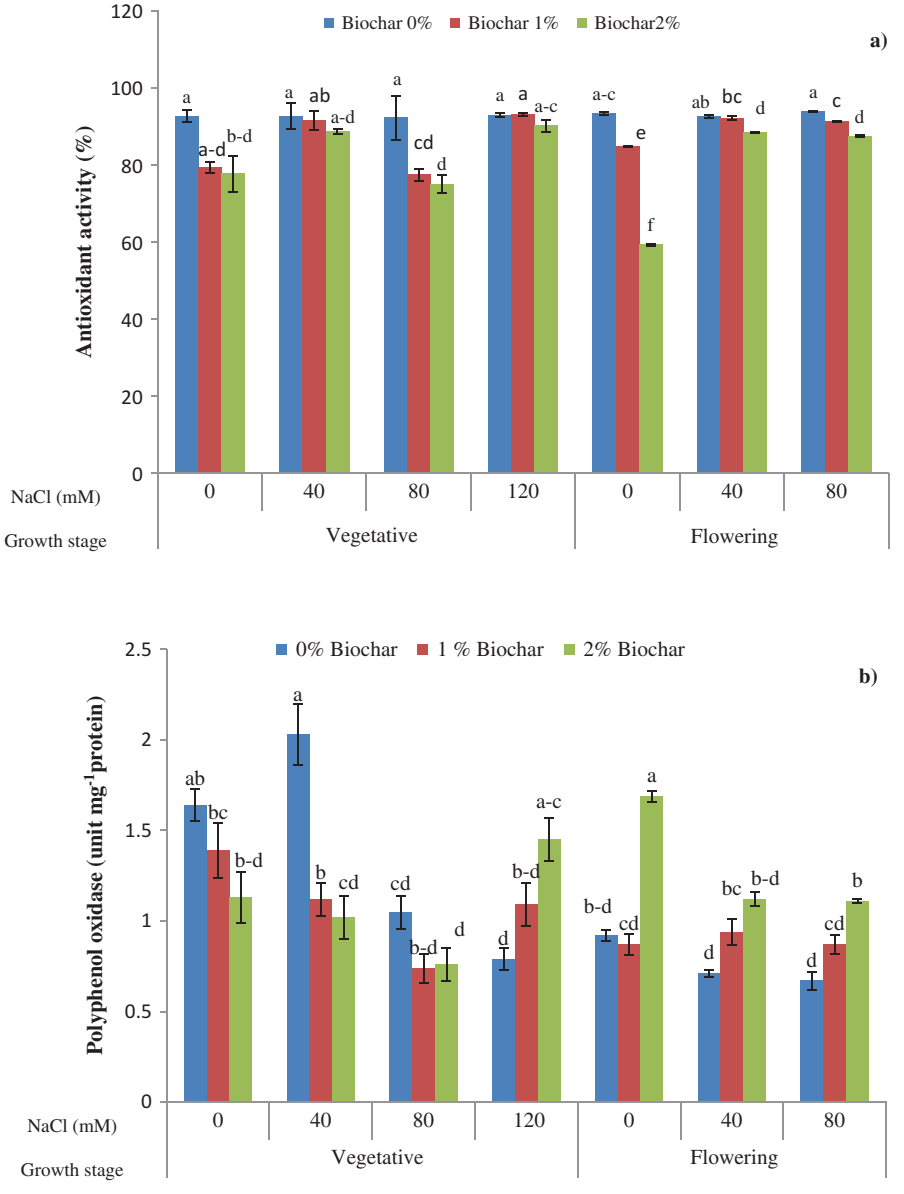

Figure 3. Effect of NaCl concentration and BC application on (a) antioxidant activity and (b) PPO activity of summer savory at vegetative and flowering stages. Bars with the same letter are not significantly different at ($P < 0.05$) according to Bonferroni test.

Discussion

Among environmental limitations, salinity as one of the major adverse environmental factors can restrict growth of plants and their production, reduce water content and biomass of plants by ionic and osmotic affects (Haddadi et al. 2016). In some plant species from Lamiaceae family, the growth and biomass of some of them such as *Mentha pulegium* (Oueslati et al. 2010) and *Salvia officinalis* (Taarit et al. 2011), decreased under saline condition. This reduction may be due to the toxicity of salt ions, high osmotic capability, restriction of the formation of plants growth promoters such as cytokinine and increasing the production of the inhibitors (Gupta and Huang 2014). High salt stress

can influence multiple physiological and biochemical characteristics on the cellular and the complete levels of plants (Aghaleh et al. 2009).

Soil moisture content decrease under salinity stress and plants are exposed to osmotic stress by the limitation of water absorption from soil (Gupta and Huang 2014), however osmotic potential increase remarkably. Therefore, water uptake reduces and the leaves dehydrate because of excessive accumulation of solutes. Contrary effects of salt stress on plants on water storage potential of plants was reported in previous researches and BC amendment addition to soil can improve soil-water storage capability and play its role in overcoming the harmful influence of salt stress on leaf water potential (Novak et al. 2012; Akhtar et al. 2015). In previous research, soil amended with BC increased biomass of plants in comparison with control soil (Thomas et al. 2013). BC enhanced plant biomass in our study, as documented in other literature (Graber et al. 2010). Furthermore, using BC may be increase the growth and biomass of plant under salt stress (Akhtar et al. 2015), although its mechanism is unclear. It probably connect with increasing in stomatal conductance and improvement the water usage in herbaceous plants (Thomas et al. 2013; Akhtar et al. 2015). The largest growth responses of plants to BC application are probably related to poor soil quality and nutrient conditions, high soil acidity or low water holding capacity (Atkinson et al. 2010).

Under salinity stress, usually the physiological reactions are observable through leaf necrosis, content and composition of pigments such as chlorophyll loss and discoloration of plants which are quality characteristics that noticeably related to visual appearance (Heidari 2012). In conformity with our results, chlorophylls content decreased by increasing salt concentration in basil (Heidari 2012) that connected with the photo inhibition or creation of ROS. The decrease in chlorophyll content under salinity stress possibly because of increasing in chlorophyllase enzyme activity, instability of protein complexity of pigments (Heidari 2012). Furthermore, based on the previous research, BC application resulted in increasing chlorophyll content in wheat in all treatments (Kanwal et al. 2017) that it is in accordance with our findings in this study. BC application raise photosynthesis rate which is a symptom of increasing chlorophyll content. Moreover, enhanced level of chlorophyll is due to the increased level of N in leaves by using BC (Akhtar et al. 2015). Negative impacts of salinity on different physiological procedure of plants were recorded before (Heidari 2012; Gupta and Huang 2014). According to the findings of this study, in stressed plants at the higher salinity levels, the negative influences of salinity and osmotic abilities were observed.

In the cell, a common way to grow under stress conditions is the formation and accumulation of soluble sugars, as important compatible solute that resulted in osmotic adjustment of the plants. Salinity increases the soluble sugar. In accordance with our findings, in wheat soluble sugar enhanced in saline condition (Tiwari et al. 2011). As a matter of fact, under salinity stress, the carbohydrate synthesis of plants diverts to produce secondary metabolites. The reason behind the enhanced levels of soluble sugar under stress condition are division of larger carbohydrate molecules that preserve turgidity of the cell (Aghaleh et al. 2009). Their increased levels under stress conditions save different membranes and other cellular structure from negative effects of stress (Gupta and Huang 2014).

In addition, salinity stress can increase production of some secondary metabolites like phenols (Khan et al. 2011). Phenols comprise a part of cellular solutes and its reduction in environmental stress was reported (Sonar et al. 2011). Therefore, improving synthesis of phenols under stressful conditions protect the cellular structure against oxidative damage (Khan et al. 2011). In some plant species, phenols concentration increase under salinity stress (Ksouri et al. 2007; Sonar et al. 2011) that in accordance with our results in summer savory. Evidently, the variation in the capacity to collect phenols is related to variation in salt tolerance of plants (Ksouri et al. 2007). Usually polyphenolic compounds are discovered in a wide range of plants with antioxidant activity (Ben Abdallah et al. 2013). Phenolic content varied between two growth stages may be due to the duration of salinity. According to the previous studies, the correlation between antioxidant properties and polyphenols in plant species were reported (Ksouri et al. 2007; Sonar et al. 2011). Therefore, the findings indicated that the plants rich in phenolic compounds

or using the treatments to increase polyphenol levels of plants could be of considerable attention to the different industries.

Furthermore, phenolic compounds have important function in absorbing and neutralizing free radicals, relieving singlet oxygen and disintegrating peroxide (Ksouri et al. 2007). In this experiment, total phenolic content under saline conditions was significantly higher at flowering stage. Polyphenol distribution within plant may express various necessity for counteracting abiotic stresses at different growth stages (Ben Abdallah et al. 2013). In this experiment, the enhancement in phenolic compounds content was more pronounced in flowering stage. In conclusion, the normal saline endurance pathway was induced by salinity through the content of phenolic compounds. The significant increase of total phenols accumulation in response to salinity suggest that the phenolic compounds are perhaps stress-induced in *S. hortensis*.

Antioxidant molecules have important role in detoxification of free radicals. Therefore, determining the antioxidant potential of plants for using in various industries like food and pharmaceutical have growing interest to produce natural products (Ben Abdallah et al. 2013). Antioxidants can prevent the initiation or proliferation of oxidizing chain responses, and as a result postpone or inhibit the oxidation of proteins, lipids and other vital molecules in cells (Krishnaiah et al. 2010). Various abiotic stresses cause to the excessive production of ROS in plants that are reactive and toxic highly and damage proteins, carbohydrates and DNA. To avoid this issue, plants improve active oxygen scavenging systems (Sonar et al. 2011). Salinity stress can also cause to oxidative stress by enhancing in ROS, which are greatly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Noctor et al. 2014). In plant tissues, the antioxidant system may control the levels of ROS (Gupta and Huang 2014). In this case, we can explain the increase of *S. hortensis* phenolic content at flowering stage as a protective strategy because they are potential relievers of ROS. There is increasing interest in evaluating the ability of medicinal plants in decreasing ROS-induced tissue injury (Krishnaiah et al. 2010). To avoid oxidative damage caused by salt stress, various adaptive mechanisms have developed via the biosynthesis of antioxidants. In fact, polyphenolic compounds involve in the protection versus ROS, which are produced when photosynthetic metabolism is damaged due to environmental stresses (Ksouri et al. 2007). Moreover, the antioxidant effect of plant production is related to different phenolic constituents include phenolic diterpenes, phenolic acids and tannins (Lee et al. 2004). NaCl usage, even at low concentrations, stimulate the activities of antioxidant enzymes in some plants species, which suggest a role of salt stress in ROS formation (Wang et al. 2005). Under stress conditions, for maintaining cellular metabolic functions and minimizing oxidative damage, the balance between production and degradation of ROS is needed. Phenolic compounds dominate antioxidant activity. In many plant species, highly positive relationship between total phenols and antioxidant activity have been reported (Rainha et al. 2011) that in conformity with our results. In addition, the antioxidant activity of plants depends on the nature, the concentration and the interactions among the antioxidants in the mixture of phenolic compounds because of the synergistic interactions between them (Ben Taârit et al. 2012). Therefore, the high antioxidant potential would be explained by the high content of phenolic compounds. Also, diversity in salt tolerance is connected with differences in antioxidant activities. Furthermore, under stress conditions the antioxidant capacity of defense system is often improved (Ben Taârit et al. 2012) which may be connected to salt tolerance (Abogadallah 2010). In several salt-tolerant plant species, antioxidant activity increases to demonstrate that antioxidants play an important role in the salt stress response (Abd Elgawad et al. 2016). Previous report indicated that in the hard environmental conditions, the amount of antioxidant activities increase in the plant tissues (Maisuthisakul et al. 2007) that in accordance with our findings in this research that the highest phenolic contents was observed at highest NaCl concentration (80 mM). Under salinity condition, the major factor of total phenolic oxidation in plants may be in consequence of increasing in (PPO) activity (Tarchoune et al. 2012). PPO is an enzyme that

oxidizes some phenols to chinone. Wounding and enzymatic browning in several plants are connected to PPO (Demir and Kocaliskan 2001).

Synthesis of polyphenolic compounds and their accumulation in plants are usually influenced by different kinds of stresses (Ksouri et al. 2007), and PPO is the main responsible enzyme for phenolic compounds oxidation. During flowering stage, *S. hortensis* could be considered more tolerant than vegetative stage against salinity stress that showing higher phenolic content and antioxidant activity with lower damage in former stage. In contrast with our findings, PPO activity increased in *Mentha pulegium* under salt stress (Abogadallah 2010). But in conformity with our results, salt stress significantly decreased PPO activity in *Mentha aquatic* (Haddadi et al. 2016). In this study, PPO activity decreased significantly under salinity in *S. hortensis* leaves. It may indicate that PPO did not have a function in modification of the phenol content and protection of *S. hortensis* plant under salt stress. Moreover, it indicated that PPO was unable to oxidize and degrade the toxic substance such as phenolic compounds which are generally accumulated during salinity stress in *S. hortensis*.

Physiological responses of plants to BC application have been investigated in few studies, but it is important to explain the mechanism of BC is related to increase growth and other effects. In most cases BC acts to mitigate the negative effects of plant stress due to its sorptive properties, either by reducing exposure of plants to stress agents or by ameliorating the stress responses of plants (Beesley et al. 2010, 2011). The negative influences of salts on plants occur through osmotic effects, ionic toxicity and decreasing water availability is expected to relieve both of them (Gupta and Huang 2014). Thus, BC's capacity to enhance water availability could be explained the improvement of the salt effect that observed in this experiment. BC adsorb meaningful amounts of added salt in the soil. Therefore, BC may immobilizes salt ions in saline soils or it may produce non-saline microsites that improve nutrient uptake (Thomas et al. 2013). From the other point of view, BC characteristics such as the porosity and specific surface area which are affected by the raw material type and pyrolysis conditions greatly influence the effects of BC amendment to improve the soil-plant water connection and improve the salt-stressed soil (Case et al. 2012). Consequently, based on these characteristics of BC, the main mechanism for alleviation the stress is sorption of NaCl that cause to reduce the plant reaction to salt stress.

Conclusion

The results of this study indicated that photosynthetic pigments, total soluble sugar, total phenolic content and antioxidant activity of summer savory were influenced by salinity. As we demonstrated in the results, summer savory growth stage showed different reactions to salinity and BC application to soil, particularly at 2% w/w of soil, mitigated negative effects of salt stress on osmotic adjustments and increased photosynthetic pigments at both growth stages significantly. Based on our findings, the plants have shown complex mechanisms for adaptation to osmotic and ionic stresses caused by high salt concentrations. There is little information in the literature about the effect of the BC amendment in saline soil on biochemical characteristics. In this experiment, we found that BC can absorb NaCl and reduce negative effects of salt stress. An intensive effort needs to understand the effects of salinity on plants and find new strategies for reducing harmful effects of salt stress on plants. Furthermore, based on the BC properties as an environmental friendly soil amendment, we recommend more investigation about the usage of BC different levels under saline condition and examine the influence of BC on different medicinal and edible plants under salinity stress.

Disclosure statement

No potential conflict of interest was reported by the authors.

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