

Salinity effects on growth, chlorophyll content, total phenols, and antioxidant activity in *Salvia lavandulifolia* Vahl.

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Key words: Biomass, electrolyte leakage, total flavonoids, water content.



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Citation:
BAYAT H., SHAFIE F., SHAHRAKI B., 2022 - *Salinity effects on growth, chlorophyll content, total phenols, and antioxidant activity in Salvia lavandulifolia Vahl.* - Adv. Hort. Sci., 36(2): 145-153.

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 4 September 2021
Accepted for publication 2 March 2022

Abstract: Although the effect of salinity stress on some species of *Salvia* has been studied, so far no research has been done on *S. lavandulifolia* species. Therefore, a greenhouse pot experiment was carried out to investigate the impacts of salt stress on vegetative parameters, chlorophyll content, and antioxidants activity in *Salvia lavandulifolia* Vahl. Treatments included different irrigation water salinity levels ($S_0=1.3$, $S_1=3.3$, $S_2=5.3$, $S_3=7.3$, $S_4=9.3$, $S_5=11.3$, and $S_6=13.3$ dS m⁻¹) which were arranged in a completely randomized design. The results showed that salinity treatments significantly affected the plant growth attributes. The lowest plant height, leaf number, leaf length, and shoot dry weight was recorded in the S_6 treated plants with 62%, 41%, 44%, and 82% decrease compared to the control, respectively. Treatment of *S. lavandulifolia* plants with the highest salinity level (S_6) decreased the content of chlorophyll a, chlorophyll b, and total chlorophyll by 57%, 53%, and 54% compared to the control, respectively. Salt stress at all levels increased the total phenolic content, and the highest value was obtained in the S_6 treated plants. Free radical scavenging capacity was significantly increased by all the levels of salinity stress, and the highest (85.14%) value was obtained in the S_6 treated plants. In general, *S. lavandulifolia* can be classified as a species-sensitive plant.

1. Introduction

The genus *Salvia*, belonging to the Lamiaceae family, has about 1000 species worldwide (Walker *et al.*, 2004; Will and Claßen-Bockhoff, 2017). Different species of *Salvia* have various applications in the pharmaceutical and therapeutic industries due to their antibacterial, antifungal, anti-tumor, and antioxidant properties. It is traditionally used to treat bronchitis, colds, sore throats, gastrointestinal disorders, eczema, and tuberculosis (Li *et al.*, 2013; Bahadori *et al.*, 2015). Terpenoids and phenolic compounds are the main secondary metabolites of the genus *Salvia* (Lu and Foo, 2002). *Salvia lavandulifolia* Vahl. is a perennial herbaceous plant native to South France, Spain, and Northwest Africa. This species is well adapted to the semi-arid Mediterranean climate and grows up to 100 cm height and has opposite green or gray-white leaves. Several secondary

metabolites including polyphenolics, flavonoids, triterpenes and monoterpenes have been extracted from the aerial parts *S. lavandulifolia* (Amalia and Kintzios, 2005).

Salinity stress is considered one of the most significant environmental stresses that restrict the growth and yield of plants, especially in arid and semi-arid areas (Deng *et al.*, 2015). In these areas, low rainfall, high evaporation, and poor drainage increase salt concentration in the soil and create salinity stress (Abdel Latef, 2010). Due to the scarcity or low quality (saline waters) of water resources worldwide, the management of crop production in saline conditions is critical. Salinity stress occurs with the accumulation of salts, especially sodium chloride, in the root zone. It causes disturbances in vital plant processes such as nutrient uptake and transport, transpiration, photosynthesis, and biosynthesis of primary and secondary metabolites (Valifard *et al.*, 2014; Ahanger and Agarwal, 2017). Salinity stress impairs plant growth and development by increasing the osmotic potential of the soil solution, disturbing the nutrient balance, and the toxicity caused by the accumulation of sodium (Na^+) and chlorine (Cl^-) ions (Rehman *et al.*, 2019). Salinity stress increases reactive oxygen species (ROS) in the cells that damage nucleic acids, proteins, and membrane lipids (Foyer, 2018). The decrease in growth, dry matter production, and yield were reported in most plants such as *Salvia hispanica*, feverfew (*Tanacetum parthenium* L.), and *Salvia splendens* due to salinity stress (Raimondi *et al.*, 2017; Mallahi *et al.*, 2018; Karimian *et al.*, 2019). Karimian *et al.* (2019) reported that salt stress treatments (0, 20, 40, 60, and 80 mM NaCl) caused the decrease in growth parameters, relative water content, chlorophyll content and increase electrolyte leakage, total phenols and total soluble sugars in *Salvia splendens*. Gengmao *et al.* (2014) demonstrated that salt treatments less than 100 mM NaCl had no effect on growth parameters of *Salvia miltiorrhiza*, but significantly decreased the accumulation of dry matter. In *Salvia officinalis*, the decrease in plant height, chlorophyll content, and essential oil content were reported due to salinity stress (150 mM NaCl) (Es-sbihi *et al.*, 2021).

Plants have developed different physiological, biochemical, and molecular mechanisms to deal with salinity stress (Zhao *et al.*, 2020). Osmotic regulation is one of the mechanisms for maintaining cellular turgidity and membrane stability. In the osmotic regulation process, cellular concentrations of osmotically compatible solutes such as sugars increased (Chakhchar *et al.*, 2015). Moreover, plants to deal with oxidative stresses enhance enzymatic and non-enzymatic antioxidant activities to reduce the deleterious effects of the ROS (Acosta-Motos *et al.*, 2017; Bayat and Moghadam, 2019).

The increasing population of the world coupled with the depletion of freshwater resources and the salinization of agricultural lands necessitates further studies on plants resistant to adverse environmental conditions. Although the effect of salinity stress on some species of *Salvia* has been studied (Valifard *et al.*, 2014; Raimondi *et al.*, 2017; Karimian *et al.*, 2019), so far no research has been done on *S. lavandulifolia* species. Considering the medicinal importance of *Salvia lavandulifolia*, it is necessary to investigate the tolerance to salt stress. Hence, this study was aimed to study the effects of salt stress on vegetative and physiological indices and some secondary metabolites in *Salvia lavandulifolia*.

2. Materials and Methods

Plant materials and experimental design

This study was carried out in Research Greenhouse, Faculty of Agriculture, University of Birjand, Iran. *Salvia lavandulifolia* var. *Lavandulifolia* seeds were purchased from Jelitto Seed Co (Germany) and sown in 105 cell seedling trays in April 2017. Coco peat and peat with a ratio of 1:1 were used for the substrate. Irrigation was done daily during the seedling emergence and growth. After forty days, the seedlings were transplanted into 3-liter plastic pots at the 6-8 leaf stage. The physiochemical characteristics of the soil are given in Table 1 (Sparks, 1996). Organic matter (OM) was determined by the Walkley-Black method, and soil texture was mea-

Table 1 - Some physicochemical characteristics of the experimental soil sample

Texture	pH	EC dS m ⁻¹	Organic matter	Field capacity (FC) (%)	N	K meq lit ⁻¹	Ca meq lit ⁻¹	Na meq lit ⁻¹	Cl meq lit ⁻¹	Mg meq lit ⁻¹	Sodium adsorption ratio (SAR)
Sandy loam	7.9	1.3	0.3	17.8	0.02	8.27	8.61	23.1	25.3	1.49	10.28

sured by the hydrometer method. Soil pH and electrical conductivity (EC) were measured with pH meter (HANNA HI2211-02, USA) and EC meter (Jenway EC meter, Germany), respectively. Phosphorus (P), potassium (K), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) were extracted by the Mehlich 1 extracting solution. Sodium and potassium concentrations were measured by a flame photometer. Phosphorus was determined colorimetrically, and Cu, Zn, Fe, and Mn were measured by atomic absorption spectroscopy. Calcium and Mg were extracted with 1 M potassium chloride and determined by titration with ethylenediaminetetraacetic acid (EDTA). Chlorine was determined by titration method. The SAR was calculated by computing Na^+ , Ca^{2+} and Mg^{2+} concentrations (in meq/L) from the saturation extract. The experiment was conducted under greenhouse conditions at temperatures of 25/20°C and relative humidity of 50-60%.

The salinity stress started four weeks after the transplantation of seedlings into the pots. A completely randomized design with four replications was used to compare seven different irrigation water salinity treatments ($S_0=1.3$, $S_1=3.3$, $S_2=5.3$, $S_3=7.3$, $S_4=9.3$, $S_5=11.3$, and $S_6=13.3$ dS m^{-1}). To prepare solutions S_1 , S_2 , S_3 , S_4 , S_5 , and S_6 , sodium chloride (NaCl) was dissolved in irrigation water in the amounts of 1.14, 2.18, 3.27, 4.43, 5.49, and 6.65 g, respectively. Some physicochemical parameters of control water (S_0) were: EC= 1.3 dS m^{-1} , pH= 7.79, Na= 5.6 meq l^{-1} , Cl= 6.8 meq l^{-1} , and K= 0.35 meq l^{-1} . The pots were irrigated twice a week with saline water based on the field capacity by pot weighting. Salinity treatments were applied for one month, and then the traits were measured.

Growth indices

Plant height, leaf number, leaf length, leaf width, and maximum root length were measured. To determine the dry weight of shoots and roots, the samples were dried in an oven for 48 hours (78°C) (Bayat et al., 2016).

Relative water content (RWC) and electrolyte leakage (EL)

The leaf RWC was measured using the method reported by Gonzalez and Gonzalez-Vilar (2003) and calculated according to the formula:

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

Leaf dry weight was measured after oven-drying of the samples for 48 h (78°C). Turgid weight was

determined after soaking leaves in distilled water in the refrigerator for 6 h.

Electrolyte leakage (EL) of the leaf was measured based on the method reported by Lutts et al. (1996) and calculated according to the formula:

$$\text{EL} = (\text{EC}_1/\text{EC}_2) \times 100$$

where EC1 and EC2 are the primary and secondary electrical conductivities, respectively. Fresh leaves (0.5 g) were dispensed with distilled water (10 ml) in test tubes and then were shaken for 24 hours (24°C). The EC1 was measured by the EC meter. The test tubes were then transferred to an autoclave (121°C) for 15 minutes and EC2 was determined.

Chlorophyll content and total soluble sugars

The pigments of fresh leaves (0.1 g) were extracted by 5 ml of acetone 80%. The amount of chlorophyll a and b were measured by a spectrophotometer (Model Unico 2100, China) at 645 and 663 nm (Arnon, 1949).

The content of leaf total soluble sugars was measured according to the anthrone method (Irigoyen et al., 1992). For this purpose, 0.1 g of dried leaves was extracted with 1 ml of ethanol. Leaf sampling was performed at 10:00 AM.

Total phenols, total flavonoids, and free radical scavenging capacity (FRSC)

Fresh leaves (1 g) were homogenized in methanol for 24 h and then centrifuged at 6000 rpm for 15 min. The Folin-Ciocalteu method was used to measure the total phenolic content (Singleton and Rossi, 1965). Total flavonoids were determined based on the method of Yoo et al. (2008). The FRSC was determined using the method reported by Koleva et al. (2002) and calculated according to the formula:

$$\text{FRSC} = 1 - \text{A Sample (517 nm)} / \text{A Control (517 nm)} \times 100$$

Data analysis

The JMP 13 statistical software (SAS Campus, Cary, NC, USA) was subjected to analysis of variance of the data. The means were separated by the least significant difference (LSD) test at the 5% significance level.

3. Results

Growth attributes

The results demonstrated that the plant growth traits were significantly affected by increasing the

salinity of irrigation water. The lowest plant height, leaf number, leaf length, and leaf width values were recorded in the S6 treated plants by 62%, 41%, 44%, and 46% decrease compared to the control, respectively (Table 2). Salt stress affected the root length of *S. lavandulifolia* plants. Increasing salinity to S3 level had no significant effect on the root length, but its amount decreased with increasing salinity to S6 level (Table 2). Biomass production was significantly influenced by salt treatments. Increasing salt stress to S2 level had no significant effect on the root dry weight. However, with increasing salinity to S6 level, its values significantly decreased (Table 2). All salinity levels significantly reduced the shoot dry weight, and the lowest value was obtained from S6 treated plants with an 82% decrease compared to the control (Table 2). With increasing salt levels, total dry weight decreased significantly. Treatment of *S. lavandulia* plants with S6 decreased total dry weight by 78% compared to the control (Table 2). Salinity stress significantly affected shoot/root dry weight ratio of *S. lavandulifolia* plants. The highest and the lowest values of shoot/root dry weight ratio were obtained from the S4 and S6 treated plants, respectively.

The leaf RWC and EL

Salinity stress decreased the leaf RWC of *S. lavandulifolia* plants. The lowest leaf RWC was achieved in S6 treated plants by a 68% decrease compared to the control (Fig. 1A). The leaf EL significantly increased with increasing salinity stress levels. The lowest (19.34%) and the highest (86.15%) leaf EL values were obtained from the S0 and S6 treated plants, respectively (Fig. 1B).

Chlorophyll content and total soluble sugars

The salinity effect was significant on the content of photosynthetic pigments. Treatment of *S. lavandulifolia* plants with the highest salinity level (S6)

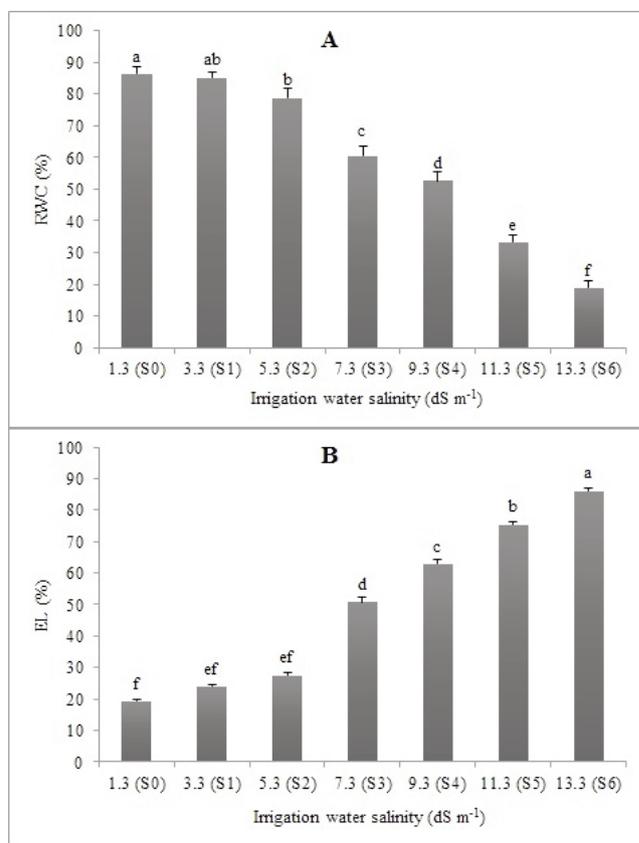


Fig. 1 - Effects of salinity stress on the leaf relative water content (RWC) and electrolyte leakage (EL) in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05. Values are mean ± standard error (SE).

Table 2 - Effects of different levels of irrigation water salinity on the plant height, number of leaves per plant, leaf length, leaf width, root length, root, shoot, and total dry weight, and shoot/root dry weight ratio of *S. lavandulifolia*

Irrigation water salinity (dS m ⁻¹)	Plant height (cm)	Number of leave per plant	Leaf length (cm)	Leaf width (cm)	Root length (mm)	Root dry weight (g. plant ⁻¹)	Shoot dry weight (g. plant ⁻¹)	Total dry weight (g. plant ⁻¹)	Shoot/root dry weight ratio
1.3 (S0)	9.87 ± 0.31 a	52.50 ± 1.44 ab	5.32 ± 0.04 a	2.07 ± 0.04 a	35.51 ± 2.46 a	0.328 ± 0.02 a	0.45 ± 0.01 a	0.78 ± 0.01 a	1.42 ± 0.16 bc
3.3 (S1)	7.25 ± 0.25 b	52.76 ± 2.09 ab	4.92 ± 0.04 b	1.80 ± 0.07 b	33.00 ± 1.35 a	0.291 ± 0.01 a	0.33 ± 0.01 b	0.62 ± 0.01 b	1.16 ± 0.06 bcd
5.3 (S2)	6.87 ± 0.42 b	57.25 ± 1.65 a	4.45 ± 0.18 c	1.77 ± 0.02 b	32.25 ± 0.75 a	0.284 ± 0.01 a	0.29 ± 0.02 c	0.57 ± 0.03 b	1.10 ± 0.05 cd
7.3 (S3)	6.62 ± 0.23 b	48.25 ± 1.10 b	4.27 ± 0.04 c	1.55 ± 0.06 c	31.50 ± 1.19 ab	0.164 ± 0.01 b	0.25 ± 0.01 d	0.41 ± 0.04 c	1.56 ± 0.14 ab
9.3 (S4)	5.37 ± 0.12 c	36.25 ± 1.18 c	3.12 ± 0.04 d	1.21 ± 0.04 d	26.51 ± 2.17 bc	0.113 ± 0.01 cd	0.21 ± 0.008 e	0.32 ± 0.007 d	1.91 ± 0.21 a
11.3 (S5)	4.62 ± 0.31 c	32.75 ± 4.30 c	3.02 ± 0.02 d	1.17 ± 0.04 d	25.01 ± 0.70 c	0.147 ± 0.01 bc	0.17 ± 0.004 f	0.31 ± 0.01 d	1.19 ± 0.11 bcd
13.3 (S6)	3.66 ± 0.16 d	30.50 ± 0.28 c	2.95 ± 0.05 d	1.10 ± 0.05 d	23.78 ± 2.47 c	0.094 ± 0.008 d	0.08 ± 0.004 g	0.17 ± 0.009 e	0.90 ± 0.14 d
Significance	**	**	**	**	**	**	**	**	**

Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05.

** represent significant at 1% level of probability. Values are mean ± standard error (SE).

decreased the amount of chlorophyll a and b, and total chlorophyll by 57%, 53%, and 54% compared to the control, respectively (Table 3). Total soluble sugars were significantly affected by salt stress. By increasing the level of salt stress, the content of total soluble sugars increased upwards. The highest total soluble sugars were achieved by S6 treated plants with a 2.8 times increase compared to the control (Table 3).

Total phenols, total flavonoids, and the FRSC

Irrigation with saline water significantly affected the total phenols and total flavonoids of the leaves. All the levels of salt stress increased the total phenols, and the highest value was obtained in the S6 treated plants (Fig. 2A). The lowest total flavonoid content was obtained in the S1 treated plants (Fig. 2B). The FRSC was significantly increased by all the levels of salinity stress. The lowest (68.16%) and the highest (85.14%) leaf FRSC values were obtained in control and S6 treated plants, respectively (Fig. 3).

4. Discussion and Conclusions

The present results demonstrated that salt stress influenced the vegetative parameters in *S. lavandulifolia*. The negative impacts of salt stress on plant growth have been reported in *Salvia hispanica* (Raimondi et al., 2017), in *Salvia splendens* (Karimian et al., 2019), and *Salvia officinalis* L. (Es-sbihi et al., 2021). The decrease in growth parameters under salinity stress can be related to the reduction of soil water potential and toxicity of Na⁺ and Cl⁻ ions, which leads to a nutritional imbalance (Kasrati et al., 2014; Es-sbihi et al., 2021). Salinity stress reduces cell divi-

sion and elongation, thereby reducing plant growth (Netondo et al., 2004; Kamran et al., 2020). Moreover, the decrease in plant growth under salinity stress can be due to the reduction of photosynthesis and energy reserves. Usually, in saline conditions, the leaf stomata are closed, and the photosynthesis rate decreases due to reduced gas exchange (Chaves

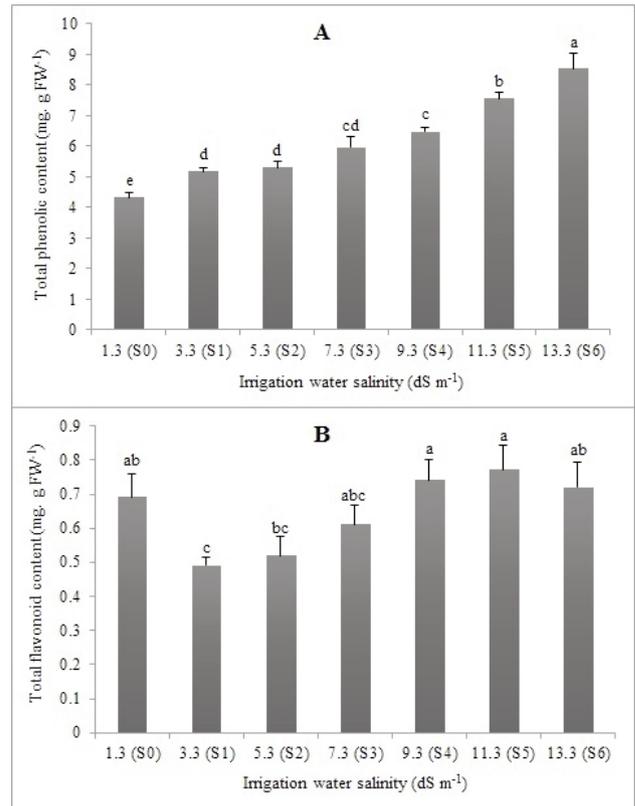


Fig. 2 - Effects of salinity stress on the leaf total phenolic and flavonoid content in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05. Values are mean ± standard error (SE).

Table 3 - Effects of different levels of irrigation water salinity on the chlorophyll a, chlorophyll b, total chlorophyll, and total soluble sugars in *S. lavandulifolia*

Irrigation water salinity (dS m ⁻¹)	Chlorophyll a (mg. g FW ⁻¹)	Chlorophyll b (mg. g FW ⁻¹)	Total chlorophyll (mg. g FW ⁻¹)	Total soluble sugars (mg. g DW ⁻¹)
1.3 (S0)	1.14 ± 0.05 a	0.58 ± 0.04 a	1.71 ± 0.09 a	7.62 ± 0.55 d
3.3 (S1)	0.79 ± 0.04 b	0.41 ± 0.03 b	1.21 ± 0.06 b	8.33 ± 0.91 d
5.3 (S2)	0.70 ± 0.05 bc	0.33 ± 0.01 bc	1.00 ± 0.04 cd	10.39 ± 1.11 cd
7.3 (S3)	0.70 ± 0.02 bc	0.35 ± 0.02 bc	1.05 ± 0.06 bc	11.69 ± 1.37 bc
9.3 (S4)	0.64 ± 0.03 c	0.30 ± 0.05 c	0.94 ± 0.06 cde	12.85 ± 0.48 bc
11.3 (S5)	0.51 ± 0.04 d	0.30 ± 0.03 c	0.79 ± 0.05 de	13.84 ± 0.85 b
13.3 (S6)	0.49 ± 0.01 d	0.27 ± 0.01 c	0.78 ± 0.11 e	21.61 ± 1.39 a
Significance	**	**	**	**

Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05.

**= represent significant at 1% level of probability. Values are mean ± standard error (SE).

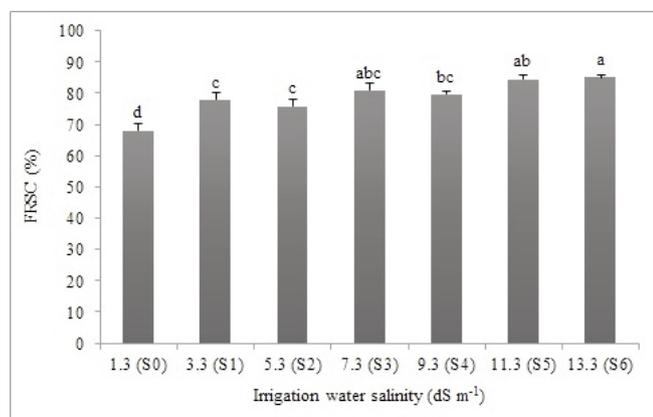


Fig. 3 - Effects of salinity stress on the leaf free radical scavenging capacity (FRSC) in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at $P < 0.05$. Values are mean \pm standard error (SE).

et al., 2009). Salinity can also inhibit root growth, thereby reducing the absorption and transport capacity of water to the shoot (Acosta-Motos *et al.*, 2017).

Irrigation with saline water decreased the RWC of *S. lavandulifolia* leaves (except S1). The leaf RWC is commonly used to estimate the water status of plants under stress conditions (Parida and Das, 2005). Salinity decreases the leaf RWC due to a reduced availability of water from the soil solution as a result of lowered osmotic potential triggered by the toxic effects of the Na^+ and Cl^- ions (Munns, 2005; Álvarez *et al.*, 2012; Bayat *et al.*, 2012).

In this study, the EL increased with increasing salinity levels (except S1). Increased leaf EL under salinity stress has been reported in different crops (Bayat *et al.*, 2013; Hniličková *et al.*, 2019; Karimian *et al.*, 2019). Electrolyte leakage is one of the standard parameters for examining salinity tolerance in plants. Salinity stress causes inefficiency of the leaf cell membrane and consequently increases membrane permeability for ions (Zhao *et al.*, 2020).

In this experiment, the content of chlorophylls decreased with increasing salinity stress levels. Reduced leaf chlorophyll content under salt stress conditions has been reported in various crops (Taïbi *et al.*, 2016; Rahnesan *et al.*, 2018; Es-sbihi *et al.*, 2021). Valifard *et al.* (2019) reported that photosynthetic pigments in *Salvia mirzayanii* leaves were decreased by increasing salinity stress. The decrease in chlorophyll content may be related to the toxicity effects of Na^+ and Cl^- ions, which prevent the formation of pigments (Yang *et al.*, 2011). Decreased pho-

tosynthetic pigments under salinity stress can be mainly due to the destruction of their structure with the ROS and inhibition of biosynthesis of new chlorophylls (Ashraf, 2003; Yang *et al.*, 2020).

In this study, irrigation with saline water enhanced the content of leaf total soluble sugars in *S. lavandulifolia*. Accumulation of leaf soluble sugars under salinity stress has been reported in sunflower (Zheng *et al.*, 2010), in *Salvia miltiorrhiza* L. (Gengmao *et al.*, 2014), and cotton (Peng *et al.*, 2016). Karimian *et al.* (2019) reported that salt stress significantly increased total soluble sugars in the leaves of *Salvia splendens*. Increased the content of soluble sugars is an indicator for osmotic regulation under stress conditions, to maintain cell turgor and continued water influx (Mittal *et al.*, 2012). Soluble sugars were accumulated under salinity stress and protect plants through osmotic regulation, maintenance of turgor pressure, and preservation of membrane and protein stability (Bayat *et al.*, 2013; Nounjan *et al.*, 2018). The increase in concentration of soluble sugars under stress conditions is due to the higher activity of enzymes such as phosphorylase starch and sucrose phosphate synthase (Peng *et al.*, 2016).

Salinity stress significantly affected the total phenols, total flavonoids, and the FRSC of *S. lavandulifolia* leaves. Various studies have reported the increment in total phenols, total flavonoids, and the FRSC in response to salt stress (Karimian *et al.*, 2019; Sirin and Aslım, 2019). Valifard *et al.* (2014) reported the total phenols and antioxidant activity in *Salvia mirzayanii* were increased by salinity stress. Salt stress causes the production of ROS, which damages proteins, lipids, and nucleic acids (Foyer, 2018). Plants use antioxidant defense systems to scavenge and detoxify these compounds from the cell surface, which leads to increased plant antioxidant activity (Rezayian *et al.*, 2018; Bayat and Moghadam, 2019). Phenols and flavonoids are secondary metabolites that act as potent antioxidants against oxidative stress. These non-enzymatic antioxidants protect plants by increasing their osmotic potential and thereby avoiding the dehydration of cells or regulating the redox potential, and depleting the ROS (Bautista *et al.*, 2016; Yan *et al.*, 2017).

Although the effect of salinity stress on some species of *Salvia* has been studied, so far no research has been done on *S. lavandulifolia* species. The results demonstrated that salt stress had adverse effects on the growth parameters, photosynthetic pigments,

and cell membrane stability of the *S. lavandulifolia* plant. However, the total phenolic content and antioxidant activity of the leaves were increased under salinity stress conditions. In general, *S. lavandulifolia* can be classified as a species-sensitive plant. However, further experiments are needed to investigate other mechanisms of salt stress tolerance.

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