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ALLEVIATING THE SALT STRESS EFFECTS IN *Salvia splendens* BY HUMIC ACID APPLICATION

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ABSTRACT

Salinity stress is a serious problem in urban landscape of arid and semi arid regions. To overcome the adverse impact of salinity, the application of organic matter and plant nutrients in the growth media for improving the plant growth is essential. An experiment was conducted in order to determine the response of *Salvia splendens* to salinity levels and also the role of humic acid in the salt stress alleviation. In the current experiment, five salinity levels (0, 20, 40, 60, and 80 mM NaCl) and three humic acid (0, 100, 500 and 1000 mg/l) treatments were prepared. The effects of these treatments were investigated on some growth parameters, physiological characteristics and also biochemical compounds. The results indicated that the growth parameters decreased in saline-treated than control plants. Different salinity levels significantly affected relative water content, evaporation rate and also electrolyte leakage. Salinity caused the increase in proline, malondialdehyde, sugar content, DPPH, total phenol content and decrease in chlorophyll, compare to the control plants. Application of humic acid on *Salvia splendens* decreased the leaf area and plant height compared to the control plants. Thus, regarding the growth parameters, it is probable that the effect of humic acid on the biochemical compounds is similar to salinity effect. The findings suggest that sage is an ornamental plant sensitive to salinity and humic acid (in the studied levels) could not alleviate the negative effects of salt stress on this plant.

Key words: humic acid, organic matter, salinity, seasonal flower

INTRODUCTION

In the arid and semi-arid regions, limitation in water quantity and quality is an important challenge in the urban landscape. One of the major indicators of a decline in water quality is salinity [Cabrera 2014]. Salt stress negatively affects plant growth and development, germination and yield due to specific ions effects, nutritional imbalance, low osmotic potential of soil solution and combinations of these factors [Ashraf and Harris 2004].

To decrease the negative effects of salt stress, the addition of supplemental organic matter and plant nutrients to growth media as an improver agent could be necessary [Aydin et al. 2011]. In salt-affected areas, different properties of soil (the physical, chemical and biological characteristics) are improved by the application of organic matters, leading to enhanced plant growth and development. Therefore, the application of organic matter for soil remediation is important for sustainable land use and crop productivity [Wong et al. 2009].

Humic acid as a new organic matter, according to soil scientists is defined as humus materials that are soluble in aqueous alka-line solutions, but precipitate when the pH is adjusted to 1–2. Humic acid is made

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of associations of predominantly hydrophobic compounds (polymethylenic chains, fatty acids, steroids compounds) which are stabilized at neutral pH by hydrophobic dispersive forces [Canellas et al. 2015].

Some studies showed an increase in rooting, growth and early flowering in ornamental plants by humic acid application [Baldotto et al. 2010, 2012]. One of potential applications for humic acid in horticulture results from its effects on abiotic stress alleviation. Humic acid can induce shifts in plant primary and secondary metabolism related to abiotic stress tolerance, which collectively modulate plant growth as well as promoting fitness [Canellas et al. 2015].

Cimrin et al. [2010] reported that the application of humic acid improved the growth of pepper under salt stress. Other study showed that humic acid was highly effective for soil conditioners in vegetable growth, to improve crop tolerance and growth saline conditions [Aydin et al. 2011]. According to Ouni et al. [2014] the effect of humic acid on salt stress was divided into indirect and direct plant effects. The indirect effects of HS are linked to improvements in the physical, chemical and microbiological properties of the soils. The direct actions on the plant are due to their effects on germination, plant growth and hormone-like activity. Paksoy et al. [2010] found that application of humic acid positively affected the yield parameters of plants under salinity condition. According to Khalesro et al. [2015] humic acid can be improved germination and plant growth in *Satureja hortensis* and *Dracocephalum moldavica* under salt stress.

Landscape plants in particular, seasonal plants, however, are judged by their aesthetic value rather than growth rate or production, the salt concentration in water must be acceptable and or by providing the suitable conditions they can tolerate salinity and grow with acceptable quality [Wu and Dodge 2005]. In spite of ornamental plants in their degree of salinity tolerance from sensitive to tolerant, they usually are damaged due to low or moderate salinity on the irrigation water [Ma and Yamaji 2006].

Salvia splendens is considered one of the most commonly observed ornamental plants in the landscape. Some researchers have reported it as a moderately sensitive plant to salt stress [Villarino and Mattson 2011]. In this work, we characterized the response during greenhouse production of *Salvia splendens* exposed to different levels of NaCl salinity in the irrigation water and different levels of humic acid as foliar spray. We aim to find the mitigating effect of humic acid on *Salvia splendens* under salt stress.

METHODS AND MATERIAL

A greenhouse experiment was conducted at Ferdowsi University of Mashhad, Iran from 15 Feb. to 5 Apr. 2016. A total of 100 plants ((5 × 4 treatments) × 5 replicates) were transplanted into pots (20 cm diameter and 20 cm depth) filled with a soil that its texture was sand clay. Plants were watered daily to pot capacity (determinated by gravimetric method) at regular intervals prior to the initiation of the treatments. The five salts (0, 20, 40, 60, and 80 mM NaCl) and three humic acid (0, 100, 500 and 1000 mg l^{-1}) treatments were prepared. Salinity and humic acid treatment were imposed after plant establishment (10 cm height). Salinity treatment was planned using field capacity (FC) method, so that the plants were irrigated with saline water to field capacity. In order to prevent the accumulation of salt in soil, leaching was done by applying sufficient water (20% more than FC) per irrigation. Humic acid was applied to pot plants as a foliar spray, once every two weeks. Plants received the treatments for 8 weeks.

Growth parameter

Growth parameters such as leaf area, plant height, inflorescence height were measured during the experiment.

Relative water content (RWC). A fully expanded leaf of a similar age was sampled and immediately weighed fresh weight (FW). Then, they placed in distilled water at 4°C in the dark for 24 h to rehydrate and the turgid weight (TW) determined. The leaves were dried in an oven at 75°C for 48 h and the dry weight (DW) determined. The RWC was calculated using the following formula [Silveira et al. 2003]:

$$(1): RWC = (FW - DW)/(TW - DW) \times 100.$$

Evaporation rate (ER) and electrolyte leakage (EL). Evaporation rate was measured by a portable photosynthesis system (LCA-4) using the youngest fully expanded leaves. Electrolyte leakage (EL) was

determined by method of Ben Hamed et al. [2007]. The leaf samples were placed in 15 mL of distill water and then were incubated in a water bath at 25°C for 3 h. Initial electrical conductivity (EC) was measured by an EC meter. Then, for releasing all the electrolytes, the leaf samples were heated for 20 min at 96°C. After, EC of cool samples at 25°C, was measured. Electrolyte leakage was calculated by the following formula:

$$(2): EL = (EC_{initial}/EC_{final}) \times 100.$$

Biochemical analyses

Proline. Samples of fresh leaves were weighed (0.1 g) and homogenized in liquid nitrogen into microtube by electrical homogenizer, after that one ml of 3% sulphossalicilic acid as solvent was added to sample. Than the extract was centrifuged at 3500 g for 10 min and the supernatant was preserved 4° celsius for the proline determination. An aliquot of this supernatant was taken and after adding reactive ninhydrin acid reagent (ninhydrin, phosphoric acid 6 M, glacial acetic acid) and glacial acetic acid at 99%, was placed in a water bath at 100° celsius for 30 min. Soon after removal from the water bath, the microtubes were cooled in ice bath. The absorbance was read in a spectrophotometer at 520 nm, as indicated by Bates [1973]. Proline content in fresh tissue was calculated by comparing the sample absorbencies with the standard proline curve in a concentration range of 0 to 25 mg l^{-1}. The result of proline concentration was expressed as μg g^{-1} f.w.

Sugar content. Soluble sugars were determined based on the modified method of phenolsulforic [Dubois et al. 1956]. Samples of fresh leaves (0.1 g) homogenized in liquid nitrogen into microtube by electrical homogenizer, after that 500 μl of ethanol as solvent was added to sample. The extraction was centrifuged at 3500 g for ten min and the supernatant was preserved 4°C. Than 500 μl of 70% ethanol was added to primary sample. The extraction was centrifuged at 3500 g for 10 min after 4 h and this supernatant added to primary supernatant. Finally 400 μl of supernatant, 400 μl chloroform and 200 μl distilled water mixed together. 200 μl of light phase, phenol and sulfuric acid mixed and after 20 min in boiling water, their absorbance are read in 480 nm. Soluble sugar concentration was calculated using glucose solution as a standard curve.

Chlorophyll. Chlorophyll pigments were determined based on the modified method of Dere et al. [1998]. 0.1 g of fresh leaves homogenized in liquid nitrogen into microtube by electrical homogenizer, after that one ml of methanol as solvent was added to sample. This sample preserve in dark and cold situation for 24 h. Than sample in microtubes were mixed by vortex for 5 min. Than the extract was centrifuged at 2500 g for 10 min and the supernatant was preserved 4°C for the chlorophyll determination. Supernatant diluted 10 times with methanol. The absorbance was read in a spectrophotometer at 470, 653 and 666 nm. Finally chlorophyll a, b and total carotenoid were calculated from this equation.

(3): Ca = 15.65 A666 – 7.340 A653

(4): Cb = 27.05 A653 – 11.21 A666

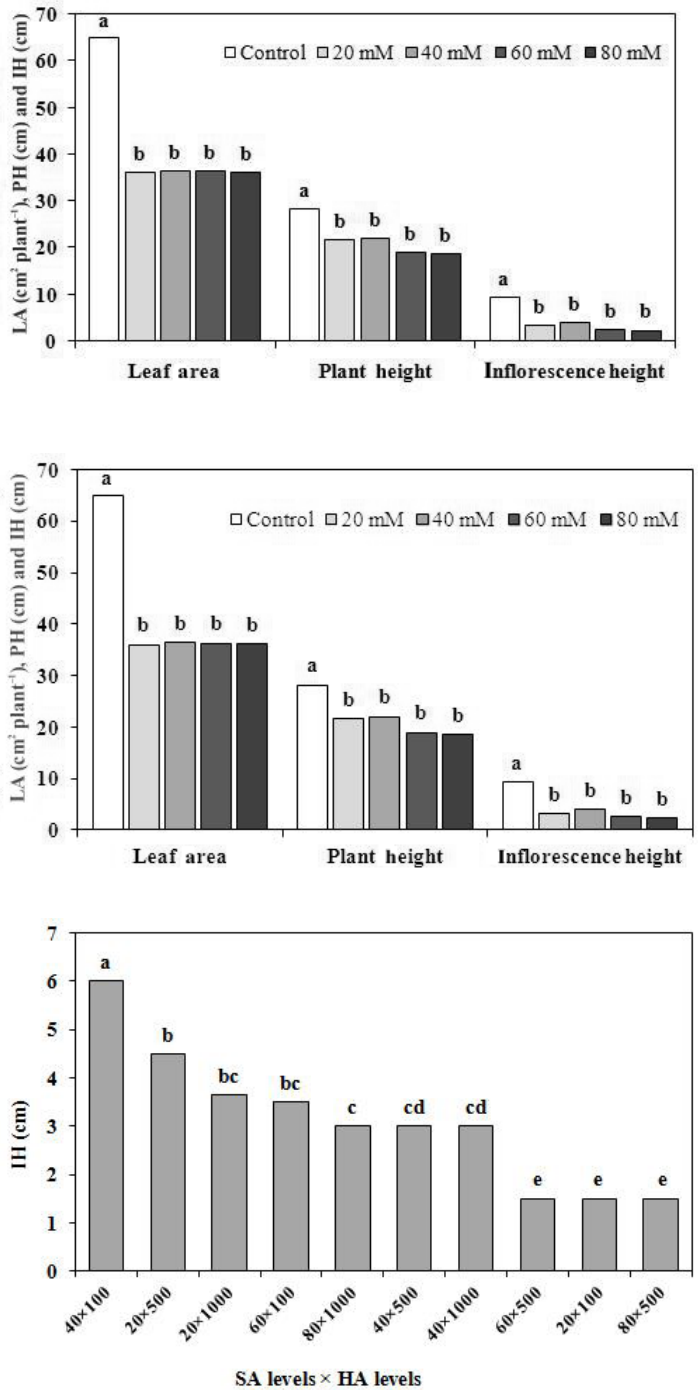
(5): Cx + c = 1000 A470 – 2.860 Ca – 129.2 Cb/245

(6): Ca = Chlorophyll a, Cb = Chlorophyll b, Cx + c = Total carotene, A666 = Absorbance in 666nm, A653 = Absorbance in 653nm and A470 = Absorbance in 470nm

Malondialdehyde (MDA). Malondialdehyde (MDA) was measured following the method described by Stewart and Bewley [1980]. 500 mg of leaf tissue were homogenized in 5ml of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid solution was added and the sample incubated at 95°C for 30 min. Then the samples centrifuged at 10000 × g for 30 min. Absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm read and 51 subtracted from this value. The level of lipid peroxidation was expressed as μM of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1}.

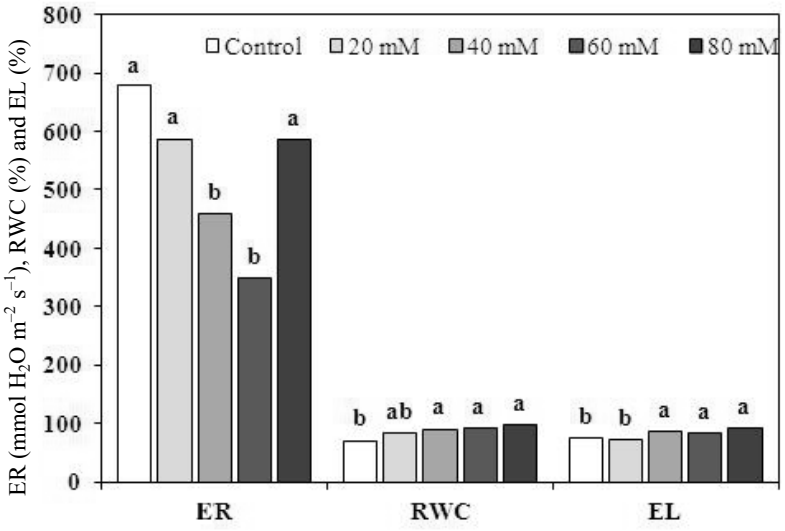
1,1-Diphenyl-2-picryl-hydrazyl (DPPH). For determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity we used Abe et al. [1998] method. Four ml of a 0.004 % solution of DPPH in methanol 80% was mixed with 1ml of concentrations (0.02–0.1 mg ml^{-1}) of methanolic extract. The reaction mixture was thoroughly vortex and left in the dark for 30 min. The absorbance was measured at 517 nm a spectrophotometer. Acorbic acid was applied for constructing the standard curve.

Total phenol (TPh) content. The total phenol content was measured using method of Singleton et al. [1999], with some modifications. 0.5 ml of methano-



Means followed by the some letters did not differ significantly at $p \leq 0.05$, n = 5

Fig. 1. The effect of salinity levels (0, 20, 40, 60, and 80 mM NaCl), humic acid levels (0, 100, 500 and 1000 mg l^{-1}) and salinity × humic acid levels on the leaf area (LA), plant height (PH) and inflorescence height (IH) in *Salvia splendens*



Means followed by the some letters did not differ significantly at $p \leq 0.05$, n = 5

Fig. 2. The effect of salinity levels (0, 20, 40, 60, and 80 mM NaCl) on the relative water content (RWC), evaporation rate (ER) and electrolyte leakage (EL) in *Salvia splendens*

lic extract (1 mg ml^{-1}) was mixed with 1.5 ml (1 : 10 v/v diluted with distilled water) Folin Ciocalteau's reagent. Then it was stand for 22°C for 5 min. 2 ml of sodium carbonate (Na_2CO_3, 7.5%, w/v) was added and then the mixture were allowed stand for 90 min. The absorbance of the blue colour was measured at 725 nm using spectrophotometer. Gallic acid was applied for constructing the standard curve The total phenol concentration was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE g^{-1}) of extract.

The experiment was conducted in completely randomized factorial design with five replications. The data obtained was statistically analyzed by General Linear Model (GLM) using Minitab 17. Mean values were grouped with Tukey multiple range test ($P \leq 0.05$).

RESULTS

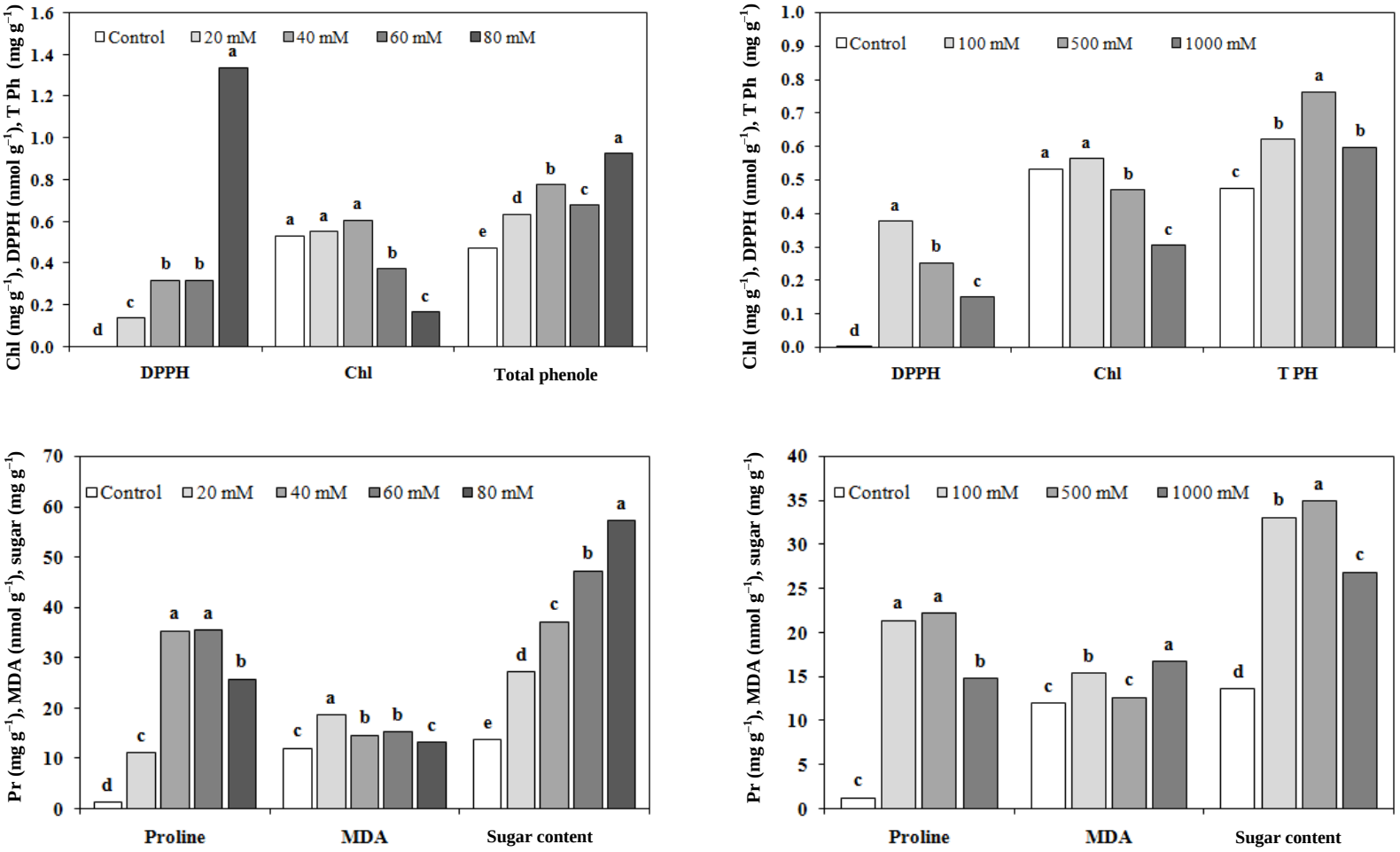
Leaf area, plant height and inflorescence height

Results showed that different salinity and humic acid levels and also some their interactions, significantly affected leaf area, plant height and inflorescence height (Tab. 1). Salinity levels, significantly decreased leaf area, plant height and inflorescence height compared to control plants, but no significant differences were found among salinity levels (Fig. 1).

In plants that were treated with 500 and 1000 mM humic acid, leaf area was decreased significantly. Plant height in all humic acid tratments reduced than control plants. Some interactions indicated different growth responses (inflorescence height) to salt stress among humic acid levels (Fig. 1).

Relative water content (RWC), evaporation rate (ER) and electrolyte leakage (EL). As be shown in table 1, different salinity levels significantly affected relative water content (RWC), evaporation rate (ER) and electrolyte leakage (EL). Raising the salinity levels from 40 to 80 mM significantly increased RWC than control plants (Fig. 1). Salt water irrigation under 40 and 60 mM salinity levels, decreased significantly ER and EL in comparison with control plants and also 20 mM salt stress (Fig. 2).

Biochemical compounds. At the end of the experiment, the effects of salinity and humic acid levels on proline (Pr), chlorophyll (Chl), malondialdehyde (MDA), sugar content (Sugar), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and total phenol (TPh) were significant (Tab. 1). Proline content was increased by



Means followed by the some letters did not differ significantly at $p \leq 0.05$, n = 5

Fig. 3. The effect of salinity 0, 20, 40, 60, and 80 mM NaCl and humic acid levels (0, 100, 500 and 1000 mg l^{-1}) on proline (Pr), malondialdehyde (MDA), sugar content (sugar), chlorophyll chlorophyll (Chl), DPPH and total phenol (T Ph) in *Salvia splendens*

Table 1. Analysis of variance (Mean Square) the effects of different levels of salinity (0, 20, 40, 60, and 80 mM NaCl) and humic acid (0, 100, 500 and 1000 mg l^{-1}) on leaf area, plant height, inflorescence height, leaf relative water content, evaporation rate, electrolyte leakage, proline, chlorophyll, malondialdehyde, sugar content, DPPH and total phenol in *Salvia splendens*

Measured taits	Salinity levels (SL)	Humic acid levels (HL)	SL × HL
Leaf area (LA)	237833.8*	12.55*	18.45 ns
Plant height (PH)	225.62*	23.10*	4.90 ns
Inflorescence height (IH)	63.18*	0.44ns	2.88*
Evaporation rate (ER)	0.24*	0.15 ns	0.11 ns
Electrolyte leakage (EL)	644.91*	37.40 ns	33.82 ns
Relative water content (RWC)	0.17*	0.04 ns	0.002 ns
Proline (Pr)	3020.71*	21.83*	308.72 ns
Chlorophyll (Chl)	0.031*	0.013*	0.054 ns
Malondialdehyde (MDA)	102.65*	62.7*	89.39 ns
Sugar	2443.99*	212.65*	123.86 ns
1,1-Diphenyl-2-picryl-hydrazyl (DPPH)	1.68*	0.045*	0.062 ns
Total phenol (TPh)	0.318*	0.168*	0.144 ns

* Significant in 5% probability, ns: non significant, n = 5

all salinity and humic acid levels compared to control plants. The highest proline content was observed in 40 and 60 mM salinity and 100 and 500 g l^{-1} humic acid levels (Fig. 3).

MDA increased in 20, 40 and 60 mM salinity levels than treated plants with 80 mM salinity and also control plants. Significant differences were found between 100 and 1000 than 500 mM humic acid treated and control plants (Fig. 3). Increasing the salinity level from 20 to 80 mM significantly increased sugar content compared to control plants. Sugar content increased under all humic acid levels in comparison with control plants (Fig. 3).

In the plants subjected to different salinity and humic acid levels, except 20 mM salinity, there were no significant differences in Chl than control plants (Fig. 3). Increasing the salinity level (from 20 to 80 mM) and decreasing humic acid level (from 100 to 1000 mM) significantly increased and decreased DPPH in comparison with control plants, respectively. A remarkable increase in DPPH was observed in 80 mM and 100 mM salinity and humic acid, respectively (Fig. 3). Significant differences were found in total phenol among different salinity and humic acid levels compared to control plants. The highest total phenol was obtained at 80 mM and 500 mM salinity and humic acid level, respectively (Fig. 3).

DISCUSSION

Leaf area, plant height and inflorescence height. The results of this study indicate that the plant species of *Salvia splendens* have several morphological adaptations to imposed salinity, including the reductions of leaf area, plant height and inflorescence height, which is in line with previous studies on Rose [Safi et al. 2005], Ornamental bulbous plants [Shillo et al. 2002], fourteen floriculture species [Villarino and Mattson 2011] and halophytes [Rodríguez et al. 2005, Ventura et al. 2014]. A reduction in leaf area may be a response to water deficit caused by salinity, due to a reduction

in the transpiration surface [Bañón et al. 2006, Burnett and van Iersel 2008]. Salinity reduces elongation and cell division as blade and foliar area decrease [Munns and Tester 2008]. Studies show one of the good indicators to salt tolerance is plant size. Negative effects of salinity on growth could be due to a decrease the rate of photosynthesis and/or disruption of their intake of ions [Niu and Cabrera 2010, Navarro et al. 2007].

Relative water content (RWC), evaporation rate (ER) and electrolyte leakage (EL). Evaporation rate decreased with increasing salt concentrations (except 80 mM). It has been suggested that plants in response to salinity, as an important component of salt tolerance, close their stomata that the reaction lead to decreases in transpiration [Vysotskaya et al. 2010].

Although many studies reported that, increased levels of salinity, reduces the RWC, but the results of the present study indicate that the plants grown under salt stress have a higher RWC. RWC increment could be occurring due to gradual adaptation of leaf cells against salt stress [Sabet Teimouri et al. 2007]. Also one of another technique to survive the plants under salinity conditions is maintenance of high RWC [Ahmad and Malik 2002].

Electrolyte leakage was enhanced in salinity levels (except 20 Mm) compared to the control plants. Electrolyte leakage is a constituent part of the plant's response to stress. The studies indicated that the electrolyte leakage could be due to membrane damage that lead to cell death [Demidchik et al. 2014]. This phenomenon was already observed by several authors in different crops [Ghoulam et al. 2002].

Biochemical compounds. Generally, different salinity levels caused the increase in proline, MDA, sugar content, DPPH, total phenol and decrease in Chl, compare to control plants. In many plant species, proline accumulates as a response to metabolic salt stress, thus it plays a key role in the osmotic adjustment in plants under stress [Mademba et al. 2003] and serves as an osmoprotectant [Wanichananan et al. 2003]. The MDA (as the product of lipid peroxidation) concentration, also in plant cells increases under stress conditions. Lipid peroxidation is an important index to determine the stress degree in the plants exposed to salt stress, by measuring cell membrane stability [Parvanova et al. 2004. Due to osmotic adjustment under condition of environmental stress, the plants accumulate sugar in their tissues [Dhanapackiam and Ilyas 2010]. Increasing of sugar content under salt stress in such plants as tomato [Amini and Ehsanpour 2005] and barley [Bagheri and Sadeghipour 2009] was reported. The phenomenon is recognized as a result of starch degradation, sugar synthesis by non-photosynthesis pathways, non-converting of these components to other productions and decreasing of transporting from leaves [Premachandre et al. 1991]. Many researches have suggested high salinity can induce the oxidative stress which caused the biological damage and production of free radical. DPPH assay is a reliable technique to determine the ability of antioxidants to scavenge the free radicals [AbdElgawad et al. 2016].

Some studies have reported increases in total phenolic and falvonoid compounds in response to salt stress [Hanen et al. 2008, Rezazadeh et al. 2012]. Another effect of salt stress is decreasing photosynthetic rate, which caused to decreasing chlorophylls [Francois and Mass 1999] that it was confirmed by the study.

In the present study application of humic acid on Sage, decrease leaf area and plant height compared to control plants. Thus maybe it can be said the effect of humic acid on the growth parameter was similar to salinity effect. According to many researchers, humic substances may enhance the uptake of some nutrients, reduce the uptake of toxic elements, and improve the plant response to salinity. Some other researches indicated that the application of very high doses of humic acids is less effective. According to some researches, the results change due to the levels of treatment, growing media, and origin of humic substances [Arancon et al. 2006].

CONCLUSIONS

The data obtained from the present study suggest that *Salvia splendens* is a sensitive ornamental plant to the salinity. It can be interpreted from the morphological and biochemical reactions of the plants in confront with salt stress. Based on the findings, humic acid could not alleviate the negative effects of salinity on the Sage and even the data suggest that in some characteristics, plant responses to the humic acid was similar to stress conditions. Therefore, for the future studies, we recommend the assessment of the lower humic acid levels on Sage under salt stress.

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REFERENCES

Abdelgawad, H., Zinta, G., Hegab, M.M., Pandey, R., Asard, H., Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front. Plant Sci., 7(276), 1–11.

Abe, N., Murata, T., Hirota. A. (1998). Novel 1,1-diphen yl-2-picrylhydrazyl-radical scavengers, bisorbicillin and demethyltrichodermol, from a fungus. Biosci. Biotechnol. Biochem., 62, 661–662.

Ahmad, R., Malik, K.A. (eds.). (2002). Prospects for saline agriculture. International Seminar of Prospects For Saline Agriculture, Islamabad, Pakistan. Kluwer Academic Publisher, Dordecht, p. 244.

Amini, F., Ehsanpour, A.A. (2005). Soluble proteins, proline, carbohydrates, and Na^+/K^+ changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under *in vitro* salt stress. Am. J. Biochem. Biotechnol., 1(4), 212–216.

Arancon, N.Q., Edward, S.L., Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. Eur. J. Soil Biol., 42, 65–69.

Ashraf, M., Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166, 3–16.

Aydin, A., Kant, C., Turan, M. (2011). Humic acid application alleviate salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane Leakage. Afr. J. Agric. Res., 7(7), 1073–1086.

Bagheri, A., Sadeghipour, O. (2009). Effects of salt stress on yield, yield components and carbohydrates content in four Hull less Barley (*Hordeum vulgar* L.) cultivars. J. Biol. Sci., 9(8), 909–912.

Baldotto, L.E.B., Baldotto, M.A., Canellas, L.P., Smith, R.E.B., Olivares, F.L. (2010). Growth promotion of pineapple 'Vitória' by humic acids and *Burkholderia* spp. during acclimatization. Braz. J. Soil Sci., 34(5), 1593–1600.

Baldotto, L.E.B., Baldotto, M.A., Soares, R.R, Martinez, H.E.P., Venegas, V.H.A. (2012). Adventitious rooting in cuttings of croton and hibiscus in response toindolbutyric acid and humic acid. Rev. Ceres, 59, 476–483.

Bates, L., Waldren, R., Teare, I. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39(1), 205–207.

Ben Hamed, K., Castagna, A., Salem, E., Ranieri, A., Abdelly, C. (2007). Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. Plant Growth Regul., 53(3), 185–194.

Cabrera, RI. (2014). Alternative water sources for urban landscape irrigation in arid regions. J. Arid Land Stud., 24(1), 89–92.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. Sci. Hortic., 193, 15–27.

Cimrin, K.M., Onder, T., Turan, M., Burcu, T. (2010). Phosphorus and humic acid application alleviate salinity stress of pepper seedling. Afr. J. Biotechnol., 9, 5845–5851.

Demidchik, V., Straltsova, D., Medvedev, S, Pozhvanov, G., Sokolik, A., Yurin, V. (2014). Stress-induced electrolyte leakage: the role of K^+ permeable channels and involvement in programmed cell death and metabolic adjustment. J. Exp. Bot., 65(5), 1259–1270.

Dere, S., Gunes, T., Sivaci, R. (1998). Spectrophotometric determination of chlorophyll A, B and total carotenoid contents of some algae species using different solvents. Turk. J. Bot., 22, 13–17.

Dhanapackiam, S., Ilyas, M. (2010). Effect of salinity on chlorophyll and carbohydrate contents of *Sesbania grandiflora* seedlings. Ind. J. Sci. Technol., 3(1), 64–66.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28, 350–356.

Francois, L.E., Maas, E.V. (1999). Crop response and management on salt-affected soils. In: Handbook of plant and crop stress, Pessarakli, M. (ed). Marcel Dekker, New York, 169–201.

Ghoulam, C., Foursy, A., Fares, K. (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot., 47, 39–50.

Khalesro, S., Salehi, M., Mahdavi, B. (2015). Effect of humic acid and salinity stress on germination characteristic of savory (*Satureja hortensis* L.) and dragonhead (*Dracocephalum moldavica* L.). Biol. Forum Int. J., 7(2), 554–561.

Ma, J.F., Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. Trends Plant Sci., 11, 329–397.

Mademba, F., Boucherea, U.A., Larher, F.R. (2003). Proline accumulation in cultivated citrus and its relationship with salt tolerance. J. Hortic. Sci. Biotechnol., 78(5), 617–623.

Ouni, Y., Ghnayaa, T., Montemurro, F., Abdellya, C., Lakhdara, A. (2014). The role of humic substances in mitigating the harmful effects of soil salinity and improveplant productivity. Int. Plant Prod., 8, 353–374.

Paksoy, M., Türkmen, Ö., Dursun, A. (2010). Effects of potassium and humic acid onemergence, growth and nutrient contents of okra (*Abelmoschus esculentus* L.) seedling under saline soil conditions. Afr. J. Biotechnol., 9, 5343–5346.

Parvanova, D., Ivanov, S., Konstantinova, T., Karanov E., Atanassov, A., Tsvetkov, T., Alexieva, V., Djilianov, D. (2004). Transgenic tobacco plants accumulating osmolytes show reduced oxidative damage under freezing stress. Plant Physiol. Biochem., 42, 57–63.

Premachandre, G.S., Saneoka, H., Fujta, K. (1991). Osmotic adjustment and stomata response to water deficit in maize. J. Exp. Bot., 43, 1451–1456.

Rezazadeh, A., Ghasemnezhad, A., Barani, M., Telmadarrehei, T. (2012). Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara scolymus* L.) leaves. Res. J. Med. Plants, 6(3), 245–252.

Rodrıguez, P., Torrecilla, A., Morales, M.A., Ortuño, M.F., Sánchez-Blanco, M.J. (2005). Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. Environ. Exp. Bot., 53, 113–123.

Sabet Teimouri, S.T., Khazaie, H., Nezami, A., Nasiri Mahallati, M. (2007). Investigation of different levels of salinity on physiological characteristics and leaf anti oxidant enzyme rate of sesame (*Sesamum indicum* L.). J. Agric. Res., 7, 171–190.

Safi, M., Fardous, A., Muddaber, M., El-Zuraiqi, S., Al-Haddidi, L., Bashabsheh, I. (2005). Effect of treated saline water on flower yield and quality of roses *Rosa hybrida* and carnation *Dianthus caryophyllus*. ScienceAsia, 31, 335–339.

Silveira, J.A.G., Viegas, R.A., Rocha, I.M.A., Moreira, A.C.D.M., Moreira, R.A., Oliveira, J.T.A. (2003). Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. J. Plant Physiol., 160, 115–123.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299, 152–178.

Stewart, R.R.C., Bewley, J.D. (1980). Lipid peroxidation associated aging of soybean axes. Plant Physiol., 65, 245–248.

Ventura, Y., Myrzabayeva, M., Alikulov, Z., Omarov, R., Khozin-Goldberg, I., Sagi, M. (2014). Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. AoB Plants, 6, 1–11.

Villarino, G.H., Mattson, N.S. (2011). Assessing tolerance to sodium chloride salinity in fourteen floriculture species. HortTechnology, 21(5), 539–545.

Vysotskaya, L., Hedley, P.E., Sharipova, G., Veselov, D., Kudoyarova, G., Morris, J., Jones, H.G. (2010). Effect of salinity on water relations of wild barley plants differing in salt tolerance. AoB Plants, 1–8.

Wanichananan, P., Kirdmanee, C., Vutiyano, C. (2003). Effect of salinity on biochemical and physiological characteristic in correlation to selection on salt-tolerance in aromatic rice (*Oryza sativa* L.). ScienceAsia, 29(4), 333–339.

Wong, V.N.L., Dalal, R.C., Greene, R.S.B. (2009). Carbon dynamics of sodic and saline soil following gypsum and organic material additions − a laboratory incubation. Appl. Soil Ecol., 41, 29–40.

Wu, L., Dodge, L. (2005). Landscape plant salt tolerance selection guide for recycled water irrigation. A special report for the Elvenia J. Slosson Endowment Fund. Available: http://slosson.ucdavis.edu/files/215300.pdf