

ARTICLE

EFFECTS OF SALT STRESS AND SALICYLIC ACID ON VEGETATIVE AND REPRODUCTIVE TRAITS OF POT MARIGOLD

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ABSTRACT

A factorial experiment with two factors using the randomized complete block design with four replications was carried out in the greenhouse of the Shirvan Branch of Islamic Azad University in 2015 to evaluate the effects of salicylic acid sprays on vegetative and reproductive traits of pot marigold under salt stress. The experimental factors were four levels of salt stress (0, 50, 100, and 200 mM), and four levels of salicylic acid sprays (0, 1, 1.5, and 2 mM). Sodium chloride was used to induce the levels of salt stress. Results showed all of the studied traits including number of flowers and flower diameter, flower dry weight, number of buds, plant height, leaf surface area, chlorophyll content, fresh and dry weights of aerial organs, and root dry weight and length were influenced by salicylic acid, salt stress, and by their mutual effects at the 1% level. Comparison of the means indicated that raising the level of salt stress to 200 mM reduced the above-mentioned traits by 89, 73, 94, 52, 90, 46, 78, 67, 83, and 69%, respectively. Moreover, salicylic acid sprays improved vegetative traits, and the dry flower yield per plant of 3.21 grams was achieved by applying 1 mM sprays of salicylic acid without salt stress. The maximum dry plant weight (10.62 grams) was observed in the treatment of applying 1 mM salicylic acid under 50 mM salt stress. In all, salicylic sprays improved plant growth and modified the negative effects of salt stress on plants.

INTRODUCTION

Salt stress is considered one of the limiting factors in the production of agricultural products in many regions of the world, and about 30-50% of irrigated land is affected by salinity. About half of the 9.5 million hectares of land under cultivation in Iran is faced with the salinity problem (Safarnezhad et al., 2007). Salt stress restricts crop plant growth and yield through reducing osmotic potential and by disrupting absorption of some elements. Plants growing in saline soils also face water deficit stress in addition to salt stress due to osmotic properties, and this water deficit stress reduces plant growth rate, which disrupts cell division, causes enlargement of cells, and influences all the metabolic reactions in plants. Moreover, increased levels of sodium and chlorine ions decrease absorption of necessary ions including potassium, calcium, ammonium, and nitrate ions, reduce activities of enzymes, and disrupt membrane structure (Demir Kaya et al., 2006; Netondo et al., 2004).

At present, medicinal plants are among important economic plants utilized raw or in processed forms in traditional or modern industrial medicine (Akbarinia, 2010). Pot marigold is an annual or perennial plant of the Asteraceae family, its flower, in addition to edible uses (as a flavoring and coloring agent of food), contains active ingredients and compounds that have applications in industry (manufacturing dyes and industrial nylons) and in pharmacy. Its seeds contain 15-20 percent oil, 45-60 percent of which consists of calendic acid (Martin and Deo, 2000). The active ingredients of this plant are produced and stored in its flowers, and the most important of them are water-soluble flavonoids, carotenoids, essential oil, and mucilaginous compounds (Edla, 3000; Baco et al. 2002).

Salicylic acid (SA) is an antioxidant, a plant hormone, and an important signaling molecule in plant reactions to environmental stresses, is produced by root cells, and plays a pivotal role in regulating various physiological processes such as growth, plant evolution, ion absorption, photosynthesis, and germination. It has been discovered that salicylates accumulate in many plant species (Hayat and Ahmad, 2007).

Salinity had negative effects on growth, protein content, and nutrient absorption in strawberry, but plants treated with SA often had greater stem weight, higher dry weight of stems, more fresh and dry root weights, and greater quantities of chlorophyll under saline conditions (Karlidag et al., 2009).

Salinity treatment decreased growth parameters, but application of SA increased protein content and changed leaf and root fresh and dry weights in artichoke (Bagherifard et al., 2013).

Nasiri and Izan (2014) studied the effects of SA and salt stress on yield of the medicinal plant dragonhead, and showed the mutual effects of salinity and SA on plant height and yield and plant chlorophyll content were significant.

Therefore, this research intended to study the effects of various salinity levels on vegetative and reproductive traits of pot marigold.

KEY WORDS

Pot marigold, salt stress, vegetative and reproductive traits

Published: 10 October 2016

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MATERIALS & METHODS

A factorial experiment was conducted in this research with two factors using the randomized complete design with four replications in the research greenhouse of the Shirvan Branch of Islamic Azad University in 2015. The first factor was four levels of SA spray (0, 1, 1.5, and 2 mM), and the second factor four levels of NaCl salinity (0, 50, 100, and 200 mM). Pot marigold seeds were bought from the Pakan Seed Company in Isfahan.

The seeds were planted in 64 plastic pots at an identical depth with 10 seeds per pot in soil of known composition and were irrigated. The plants grew in the open air at $20\pm 5^\circ\text{C}$, and, when established and at four-leaf stage, were thinned to have three plants per pot. Salt stress was applied 45 days after thinning using various concentrations of NaCl solutions. Once every 10 days, normal water was used in irrigating the pots to prevent salt shock. The experiment continued for 100 days (until the end of the flowering stage). Taking samples started at 50% flowering and every 20 days (after the flowers opened) flower samples were taken. The studied traits included number of flowers, flower diameter, flower dry weight, plant height, leaf surface area, chlorophyll content, fresh- and dry-weights of aerial organs, root length, and root dry weight. MSTATC was employed to analyze the data, EXCEL to draw the diagrams, and the LSD test at 1% probability to compare the means.

RESULTS

Results of ANOVA showed that the effects of SA concentration, of the various levels of salinity, and their mutual effects on all the measured traits were significant at the 1% probability level.

Number of flowers

Comparison of the means indicated the maximum number of flowers per plant (9.83) belonged to the treatment of spraying mM SA with no salt stress, and the minimum (1.08) to the treatment of 2 mM SA and 200 mM salt stress, with a difference of 80% between the two treatments. In general, number of flowers per plant declined with increases in the level of salt stress [Fig. 1].

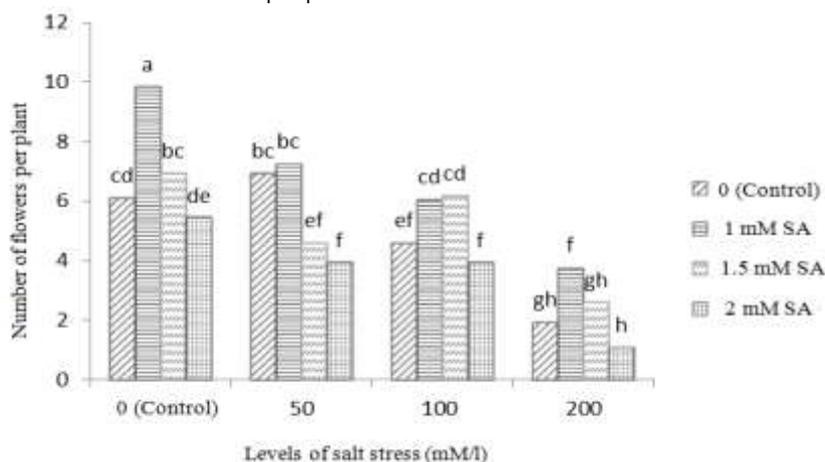


Fig. 1: Comparison of the means of number of flowers per plant at various levels of SA concentrations and salt stress

Flower diameter

The largest flower diameter (2.97 cm) was that of 1 mM SA spray with no salt stress, and the minimum (0.8 cm) that of 2 mM SA spray and 200 mM salt stress, with a difference of 73 percent between the 2 treatments. Flower diameter decreased under the influence of salt stress, and low and moderate concentrations of SA sprays were able to somewhat increase flower diameter at high levels of salt stress [Fig. 2].

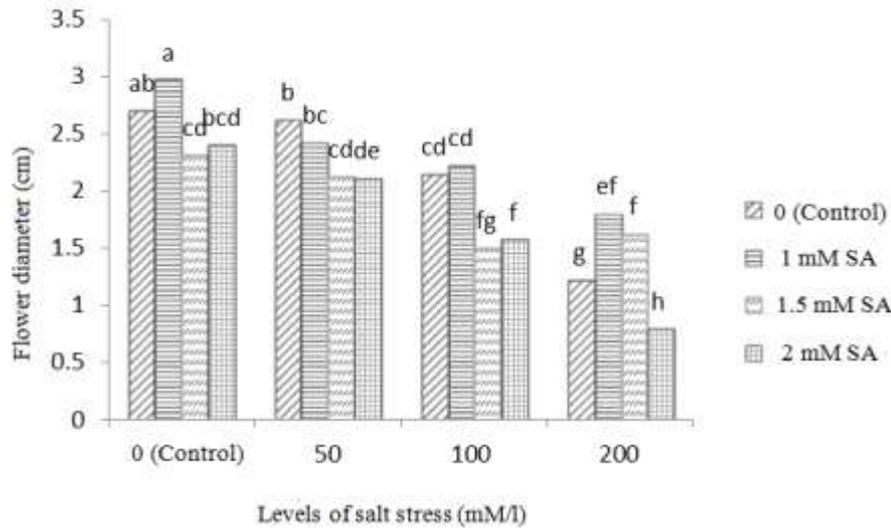


Fig. 2: Comparison of the means of flower diameter at various levels of SA concentration and salt stress

Dry flower weight

The maximum dry flower weight (3.21 g/plant) was achieved in the treatment os spraying 1 mM SA and no salt stress, and the minimum (0.19 g/plant) in the treatment of 2 mM SA and 200 nM salt stress, with a difference of 94% between the two treatments [Fig. 3]

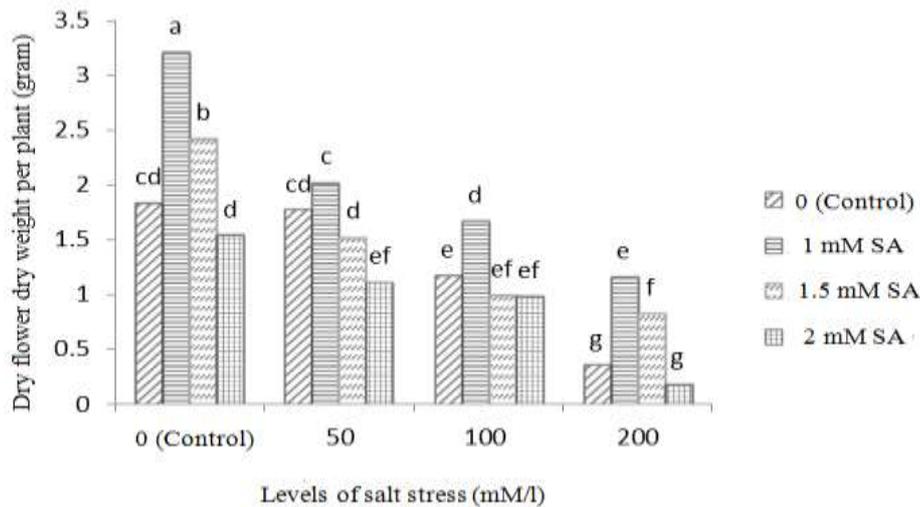


Fig. 3: Comparison of the means of dry flower weight at various levels of SA concentrations and salt stress

Plant height

Results revealed the tallest plants (54.00 cm) were observed in the treatment of spraying 1 mM SA with no salt stress, and the shortest (25.50) in the treatment of not spraying SA acid under 200 mM salt stress conditions, with a difference of 52% between the two treatments. In general, plant height declined under the influence of salt stress. Spraying SA at low concentrations under low levels of salt stress increased plant height, and at higher levels of salt stress higher concentrations of SA increased plant height the most [Fig. 3].

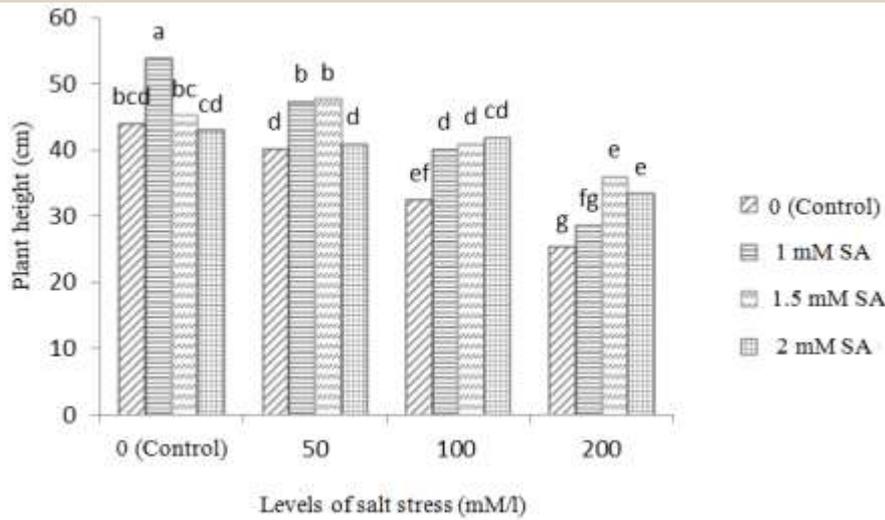


Fig. 4: Comparison of the means of plant height at various levels of SA concentration and salt stress

Leaf surface area

Comparison of the means showed that the largest leaf surface area (411.64 cm²) belonged to the treatment of spraying 1.5 mM SA and no salt stress, and the smallest (38.45 cm²) to the treatment of not spraying SA and 200 mM salt stress, with a 90% difference between the two treatments [Fig. 5].

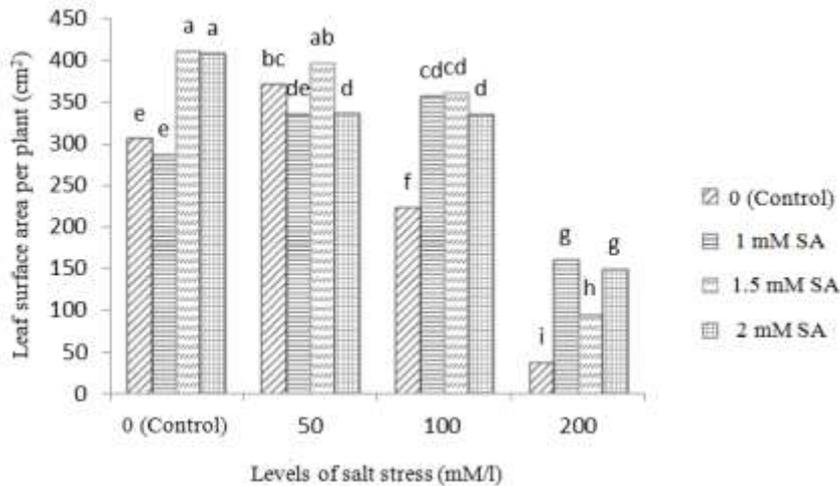


Fig. 5: Comparison of the means of leaf surface area at various levels of SA concentration and salt stress

Chlorophyll content

The highest chlorophyll content (SPAD 46.00) was achieved in the treatment of spraying 1 mM SA with no salt stress, and the lowest (SPAD 24.42) in the treatment of spraying 1.5 mM SA and 200 mM salt stress, with a difference of 46% between the two treatments [Fig. 6].

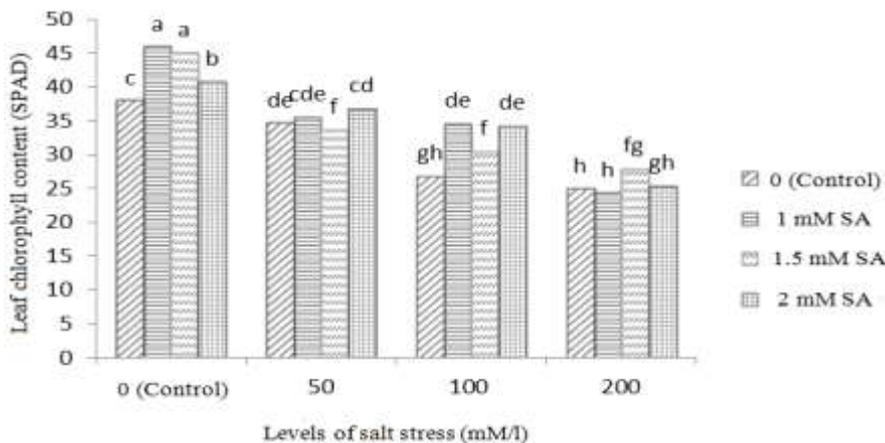


Fig. 6: Comparison of the means of chlorophyll content at various levels of SA concentration and salt stress

Fresh weight of aerial organs

The largest fresh weight of aerial organs (62.97 g/plant) was obtained in the treatment of spraying 1.5 mM SA with no salt stress, and the smallest (13.52 g/plant) in the treatment of not spraying SA and 200 mM salt stress, with a difference of 78% between the two treatments [Fig. 7].

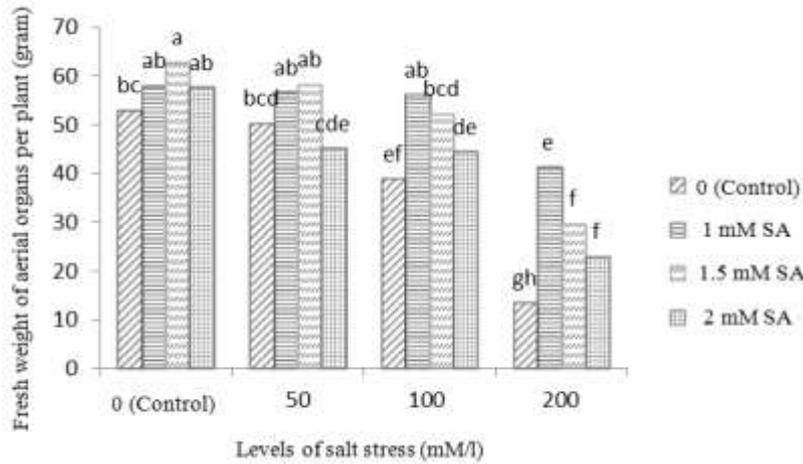


Fig. 7: Comparison of the means of fresh weight of aerial organs at various levels of SA concentration and salt stress

Dry weight of aerial organs

The maximum dry weight of aerial organs (10.62 g/plant) was observed in the treatment of spraying 1 mM SA and 50 mM salt stress, and the minimum (3.41 g/plant) in the treatment of not applying SA and 200 mM salt stress, with a difference of 67% between the two treatments [Fig. 8].

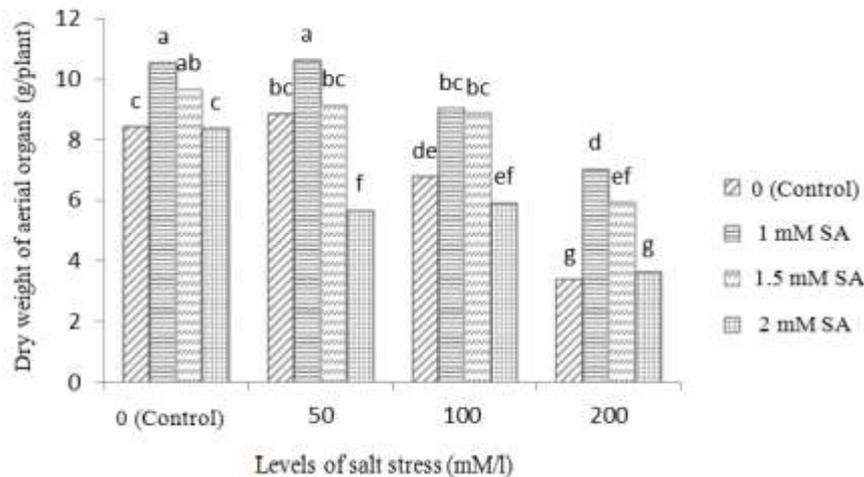


Fig. 8: Comparison of the means of dry weight of aerial organs at various levels of SA concentration and salt stress

Root dry weight

The largest root dry weight (6.30 grams) belonged to the treatment of spraying 1.5 mM SA with no salt stress, and the minimum (1.02 grams) to the treatment of not spraying SA and 200 mM salt stress, with a difference of 83% between the two treatments [Fig. 9].

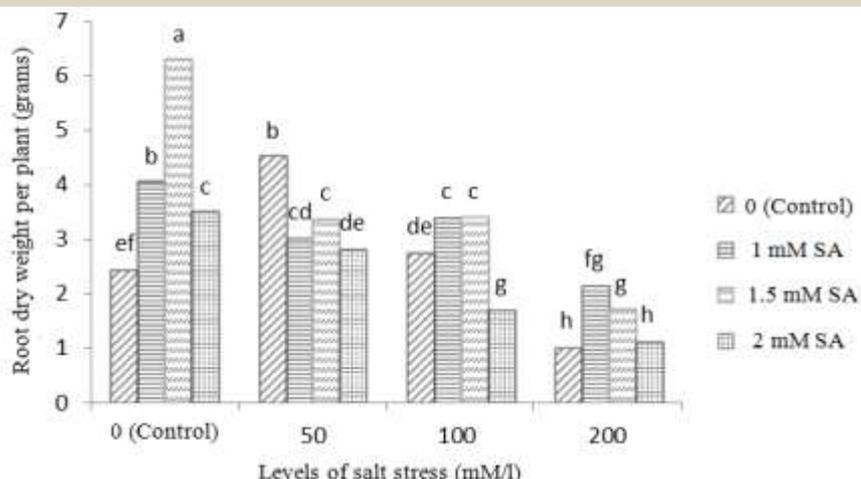


Fig. 9: Comparison of the means of root dry weight at various levels of SA concentration and salt stress

Root length

The longest roots (31.12 cm) were observed in the treatment of spraying 1.5 mM SA and 100 mM salt stress, and the shortest (9.50 cm) in the treatment of not spraying SA and 200 mM salt stress, with a difference of 69% between the two treatments [Fig. 10].

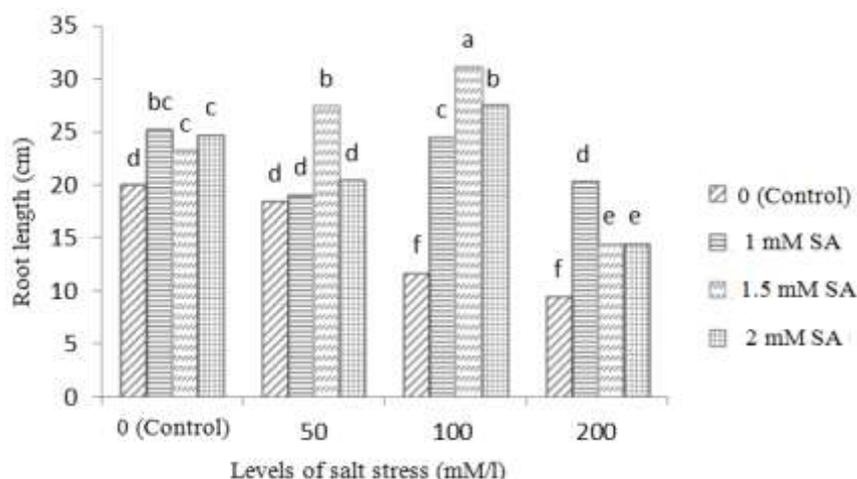


Fig. 10: Comparison of the means of root length at the various levels of SA concentration and salt stress

DICUSSION

Considering the above results, spraying 1 mM SA alone increased number of flowers per plant. Moreover, the minimum number of flowers per plant was produced under conditions of 200 mM salt stress. Plants normally produce fewer flowers under saline conditions to maintain assimilation and food reserves (Aldosivi et al., 1998). In relation to the effects of salicylic acid, Glass and Dunlop (1974) also reported SA treatment increased number of flowers in African violets. Furthermore, Kamali et al. (2012) noticed 300 mM sodium chloride reduced number of flowers by 66.4% compared to the control. In addition, 200 ppm was the most suitable concentration of SA spray in the experiment and resulted in the largest number of flowers. Salicylic acid stimulates flowering in some plants. In tobacco tissue culture, use of SA caused flowering in tobacco plants. Moreover, SA together with kinetin and IAA stimulate bud formation. Since other photosynthetic materials can also cause these effects, researchers believe this effect of SA is not a direct one but rather SA stimulates flowering through stimulating some other reactions.

In the present research, SA at 1 mM increased height of plants that were not under salt stress and, under conditions of salt stress, 1.5, and 2 mM SA sprays significantly improved plant height. In all, studies carried out by other researchers also proved the positive effect of SA in increasing internode distance. Hegazi and El-Sherif (2007) showed that application of 1 mM SA on beans increased plant height by 58% compared to the control. Furthermore, research by Martin Max et al. (2005) indicated SA treatment on African violets increased length of petioles. In another study conducted by Amin et al. (2007), foliar sprays of edible onion plants improved most growth parameters including plant height and length of petioles.

Results of this research showed that leaf surface area decreased under salt stress conditions, increased in the treatment of spraying 2 mM SA, and this increase was significant compared to the control when 1.5 mM SA was sprayed. The reason for this increase is the positive effect of SA on improving growth characteristics. Research conducted by Amin et al. (2007) revealed that foliar sprays of SA on edible onions increased leaf surface area by 8% compared to the control. Furthermore, the reduction in leaf surface area under salt stress that they observed was in agreement with results Rodriguez et al. (2005) found regarding reduction in leaf surface area of strawberry under salt stress conditions.

In this study, leaf chlorophyll content was influenced in the treatment of spraying SA and under salt stress conditions at the statistical level of 1%: SA sprays increased chlorophyll content but salt stress decreased it compared to the control. Misra et al. (2000) reported salt stress caused destruction of chloroplasts and reduced chlorophyll content. It seems chlorophyll content decreases under salt stress conditions because it is not synthesized and ethylene concentration increases (Khan, 2003). Moreover, chlorophyllase becomes more active under salt stress conditions (Singh and Jane, 1981). Therefore, chlorophyll content decreases under salt stress conditions both because chlorophyll is not synthesized and since its degradation and destruction increases (Singh and Jane, 1981). One of the most important reasons for the reduction in chlorophyll content is its destruction by active oxygen species. Moreover, competition of glutamyl kinase with glutamate ligase (the first enzyme in the route for chlorophyll biosynthesis) and its greater content under salt stress conditions cause the precursor of glutamate to be used more for the production of amino acids, especially proline. Consequently, chlorophyll synthesis will face restrictions (Games et al., 2000). Research carried out by Gharib (2006) on two medicinal plants (marjoram and basil) proved that spraying them with SA could increase their fresh weights. In another study conducted by Kanaki (2008) on radish seedlings, it was proved SA treatment increased fresh weight of aerial organs.

CONFLICT OF INTEREST

None

ACKNOWLEDGEMENTS

None

FINANCIAL DISCLOSURE

None

REFERENCES

- [1] Adela P, Constantin B, Sanda A, Carmen S. [2003] HPLC analysis of carotenoids in four varieties of (*Calendula officinalis* L.) Flowers. *Acta Biologica*. 47: 37-40.
- [2] Akbari-Nia A. [2010] Effect of salt stress on germination and seedling growth of *Nepeta Pogonosperma* Jamzad and Assadi. *The proc.11 Iran.Crop.Sci Cong. 1: Crop Production*. Shahid Beheshti University, Tehran, Iran. 24-26.
- [3] Aldesuquy HS, Mankarios AT, Awad HA. [1998] Effect of some antitranspirants on growth, metabolism and productivity of salinetreated wheat plants. Induction of stomatal closure, inhibition of transpiration and improvement of leaf turgidity. *Acta Botanica Hungarica*, 41:1-10.
- [4] Amin AA, M Rashad HM H El-Abagy. [2007] Physiological effect of Indol-3-butric acid and salicylic acid on growth, yield and chemical constituents of onion plants. *J. Appl. Sci. Res.* 3(11): 1554- 1563.
- [5] Bagheri-Fard G, Bagheri Fard A, Amarlou A. [2013] The effect of salicylic acid and salinity on some morphological characteristics artichoke plant (*Cynara scolymu* L), the first regional conference on medicinal plants north of the country, Gorgan, Golestan Research Center for Agriculture and Natural Resources.
- [6] Bako E, Deli J, Toth G. [2002] HPLC study on the carotenoid composition of *Calendula* Products. *Journal of biochemical and biophysical methods*.53:241-250.
- [7] Canakci S. [2008] Effect of salicylic acid on fresh weight changes, chlorophyll and protein amounts of radish seedling. *J. Biol. Sci.* 8 (2): 431-435.
- [8] Demir Kaya M, Gamze Okc U, Atak M, Yakup C. [2006] Seed treatment to overcome salt and 77 drought stress during germination in sunflower (*Helianthus annuus* L.). *Europe. J. Agronomy* 24:291-295.
- [9] Gemes K, Poor Z, Sulyok A, Szepesi M, Szabo P, Tari I. [2008] Role of salicylic acid pre-treatment on the photosynthetic performance of tomato plants (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego) under salt stress. *Acta Biol. Szeged*. 52: 161-162.
- [10] Gharib EL. [2006] Effect of salicylic acid on growth, metabolic activities and oil content of basil and marjoram. *Int. J. Agric. Biol.* 4: 485- 492.
- [11] Glass ADM, Dunlop J. [1974] Influence of phenolic acids on ion uptake. IV Depolarization of membrane potentials. *Plant Physiology*, 54:855-858.
- [12] Hayat S, Ali B, Ahmad A. [2007] Springer, Salicylic cide: Biosynthesis, V\ Metabolism and physiological Role in plant, Salicylic Acid. A Plant Hormone.M chapter. 1: 1-14.
- [13] Hegazi AM, El-shraiy. [2007] Impact of salicylic acid and paclobutrazol exogenous application on the growth, yield and nodule formation of common bean. *Aus. J. Basic Appl. Sci.* 1(4): 834- 840.
- [14] Kamal M, Kharazi SM, Selahvarz Y, Tehranfar A. [2012] Effect of Salicylic Acid on the Growth and some morphological characteristics of flower bud in saline conditions. *Journal of Horticultural Science (Agricultural Science and Technology)*. 26 (1): 112-102.
- [15] Karlidag H, E Yildirim, M Turan. [2009] Salicylic acid ameliorates the adverse effect of salt stress on strawberry. *Sci. Agric.*66(2): 180-187.
- [16] Khan MA. [2003] An ecological overview of halophytes. From Pakistan. In: H. Lieth and M. Moschenco.(Eds), cash crop halophytes.
- [17] Martin F. [2005] A growers manual for *calendula officinalis* L. ADAS bridget research centre.
- [18] Martin RJ, B Deo. [2000] Effect of plant population on *calendula officinalis* flower production. *New Zealand Journal of crop and Hortic. Sci* 28(1): 37-44.
- [19] Misra A, Sricastatva NK. [2000] Influence of water stress on Japanese mint. *Journal of Herbs, Spices & Medicinal Plants*, 7:51-58.
- [20] Nasiri Y, Ayzan T. [2014] The effect of salicylic acid on performance *Dracocephalum moldavicaby* under salt stress, *Crop Science Conference of Iran and Iran's Third Conference on Science and Technology Seed, Crop Science Society of Iran*.
- [21] Netondo GW, JC Onyango, Beck E. [2004] Sorghum and salinity: I. Response of growth, water relation and ion

- accumulation to NaCl salinity. *Crop Science*. 44: 797-805.
- [22] Noreen Z, Ashraf M, Akram, NA. [2010] Salt-induced regulation of some key antioxidant enzymes and physiobiochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). *Journal of Agronomy and Crop Science* 196: 273-285.
- [23] Rodriguez PA, Torrecillas MA, Morales MF, Ortuno MJ, Sanchez- Blanco. [2005] Effect of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* L. plants. *Environ. Exp. Bot.* 53: 113-123.
- [24] Safarnejhad A, Sadr SV, Hamidi H. [2007] Effect of salinity stress on morphological characters of *Nigella sativa*. *Journal of Rangeland and Forests plant Breedings and Genetic Research*, 15(27): 75-84.
- [25] Singh G, Jain S. [1981] Effect of some growth regulators on certain biochemical parameters during seed development in chickpea under salinity. *Indian Journal of Plant Physiology*, 20:167-179.
- [26] Sobhani Gh, Golchin A, F Shekari. [2010] Effects of salicylic acid on the growth and yield of tomato plants under salt stress, the first National Congress of science and new technologies of agriculture, Zanjan, Zanjan University.