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ORIGINAL ARTICLE **OPEN ACCESS**

The Effect of Salicylic Acid on Some Morphological Features And Yield of *Dracocephalum Moldavica* In Saline Conditions

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ABSTRACT
Salicylic acid (2-hydroxybenzoic acid) plays an important role in regulating physiological processes like plant growth, ion uptake, photosynthesis, and germination. In order to study the effects of salicylic acid on growth features of Dracocephalum moldavica, a factorial experiment with a completely random design was conducted at the research station of Natural Resources and Irrigation Office, Piranshahr in 2013-2014. A pot experiment consisting of four levels of salicylic acid (0, 0.5, 1, and 1.5 mM) and three levels of chloride acid (0, 2.5, and 5 mM) with three replications was utilized. Salicylic acid was foliar sprayed once in two weeks. Sodium chloride was given to the plants through the earth and irrigation once in three days. Measured features included chlorophyll level, stem diameter, number of leaves, length of flowering branches, number of flowering stems, number of lateral branches, and height of bushes. The results of the study indicated that salinity stress of 5 MS decreases number of flowering stems, stem diameter, and number of leaves by 28, 37, and 25% in the experimental group compared to the control groups. Utilizing salicylic acid with a concentration of 1.5 mM in the experimental group increases the number of flowering stems by 26.35% compared to the control group (without spraying salicylic acid solution). The effect of salinity stress and salicylic acid on features like chlorophyll, length of flowering branches, number of lateral branches, and height of bushes was meaningful. According to the results of the study, utilizing salicylic acid can improve growth features of Dracocephalum moldavica under saline conditions.
Keywords: *Dracocephalum moldavica; salicylic acid; salinity; yield*

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INTRODUCTION

Due to excessive agricultural activities, saline lands in the world and Iran are constantly developing [1]. Therefore, potential production of agriculture is impossible in these conditions. To tackle this problem, it is necessary to identify and select resistant cultivars [2]. Salinity stress does not influence only one phase of plant growth but it can affect different phases depending on the stress intensity, stress type, level of plant resistance, different phases of growth, and type of plant texture and stem (evolution procedure) [3]. Negative effects of salinity on plant growth are due to low osmotic potential of soil solution (osmotic stress), specific effects of ion (salinity stress), imbalance of nutrient elements, or a combination of these factors [4]. When a plant grows in saline conditions, its photosynthetic activity decreases; therefore, its growth, leaf surface, and chlorophyll content drop and chlorophyll fluorescence rises [5]. Sodium is the most dominant cation existing in soil and water solution of saline areas [6]. A large number of crops have a remarkable sensitivity to saline conditions due to accumulation of sodium ion in cells and its effect on disturbance in ion balance, osmotic regulation, the activities of most enzymes, and cell metabolism and creation of inhibitory toxicity [6,7]. In general, low concentration of cytosolic sodium and imbalanced proportion of potassium to sodium ions (K^+/Na^+) are known as important aspects of salinity resistance. Salinity resistant cultivars of crop species indicate a high proportion of potassium to sodium [8]. Salicylic acid is a phenolic compound that is considered as phytohormones and among phenolic compounds of benzoic derivatives and cinnamic acids, it has some effects on metabolism, biosynthesis, and oxidative and biological activities like growth, photosynthesis, breathing, uptake and transfer of ions, changing the activities of some important enzymes, and chloroplast structure [9-11]. Many studies have proved that salicylic acid as an important messenger molecule has a role in plant responses to various biotic and antibiotic stresses [13, 14]. In agriculture and horticulture, induction of tolerance to different types of stress in plants is possible through treatment by salicylic acid and its derivatives [15]. It has also been reported that salicylic acid is effective in plant responses to biological stresses like ultraviolet, ozone, and drought stress [16]. Salicylic acid enhances plant resistance against biological and antibiotic stresses [17]. It is confirmed that salicylic acid reduces accumulation and leakage of toxic ions in plants [18, 19] and decreases the effects of environmental stresses by increasing the number of growth regulating hormones such as auxins and cytokines [20].

Dracocephalum moldavica is a perennial herbaceous plant that is a kind of lamiaceae, native to central Asia, and has been domesticated in central and East Europe. All parts of the plant contain essence and its amount varies in different parts of the plant. The flower and vegetative organs (young leaves and stems) contain the highest percentage of essence. *Dracocephalum moldavica* essence has healing and anti-microbial and -bacterial properties. It is also widely used in pharmaceuticals, cosmetics and personal care, food industry, and perfumery. Moreover, it is utilized to alleviate headache, abdominal pain, flatulence, flu, general body weakness, nerve pain and spasms in the gastrointestinal and renal pain, toothache, and mouth rinse. It can be used as poultice in rheumatic pains. This plant also has anti-tumor properties [21,22]. Since salinity stress is one of the most important causes of the plant yield reduction and using growth regulating hormones is one of the plant strategies to reduce the effects of stress, the present study was conducted in order to examine and evaluate the effects of utilizing salicylic acid on the yield and quality of *Dracocephalum moldavica* in saline conditions.

METHOD AND MATERIALS

The present study was conducted in crop year of 2013 in the form of pot experiment. It was conducted at the research farm of Piranshahr's Natural Resources and Irrigation Office, with longitude of 36' and 51^{o}, latitude of 57' and 40 ^{o}, and altitude of 1420 m. Piranshahr is located in the south of West Azerbaijan Province, Iran and has a semi-humid climate. Average annual rainfall is 700 mm, the maximum and minimum temperatures are respectively 39^{o}C and 11^{o}C and sometimes temperature reaches below 0. It has an average humidity of 73%. The experiment was a factorial one with a completely random design and consisted of three levels of salinity stress (0, 2.5, and 5 MS) and four levels of salicylic acid spray (0, 0.5, 1, and 1.5 mM) with three replications. Plastic pots with height of 32 cm and diameter of 30 cm were provided, labeled, and arranged in a random order. Seeds were planted in four surface points of the pots using a pile method on 9 April, 2013. After the seeds had emerged, thinning was conducted at 2-leaf stage and only one plant was kept at every planting point as a result there left only four plants en each pot. During stem growth period, treatment was conducted by spraying salicylic acid once in two weeks. Spraying salicylic acid was done in the morning and before sunrise. Afterwards, all of the pots were watered. Salinity stress treatment was applied a week after the first salicylic acid spray. In flowering stage, harvesting was completely done. Afterwards, every sample was measured and related data were collected for further variance analysis. Bush height and reproductive organ length were measured using a ruler and stem diameter was measured using calipers. In every bush, the number of lateral stems, flowering stems, and leaves was counted and the means scores were registered for further analysis. Collected data were analyzed using MSTAT-C software. Duncan's multiple range method at a significant level of 5% was utilized in order to compare the means of each feature.

RESULTS

1. Bush height

The results of the present study indicated that the interaction between irrigation (salinity stress) and salicylic acid in terms of bush height as significant at a level of 1% (See Table 1). The highest level of this feature was at 0 level of salinity irrigation and spray of 0, .05, and 1 mM of salicylic acid without any significant difference at these levels. And its lowest level was related to the treatment with 5 MS salinity and without salicylic acid spray (See Table 2 & Figure1).

Table 1. Variance analysis of salicylic acid spray and salinity stress on yield of *Dracocephalum moldavica*

S.O.V.	Mean Squares							
	df	Bush Height	N. of Lateral stems in the Bush	N. of Flowering stems	Length of Reproductive Organ	Main Stem Diameter	N. of Leaves in the Bush	Chlorophyll Amount
Salinity A	2	220.083**	20.028**	15.750**	60.778**	2.581**	1401.444**	100.750**
Salicylic Acid B	3	15.741**	2.444**	7.731**	11.296**	0.011^{ns}	1.963^{ns}	6.556*
A x B	6	5.269**	1.806**	1.009^{ns}	3.963*	0.026^{ns}	2.852^{ns}	7.750**
Experimental Error	24	1.000	0.306	0.417	1.528	0.017	4.611	1.889
Coefficient of Variation (%)		3.64	5.47	9.33	7.70	6.65	2.93	5.32

2. Number of leaves in each pot

The effect of salinity on the number of leaves was significant at a level of 1%. (See Table 1). The maximum number of leaves was related to the control treatment (83.5) and the minimum to salinity treatment of 5 MS (62). There was not a significant statistical difference between different treatments with salicylic acid spray and interactive effect of spray and salinity (See Table 2 & Figure 2).

Table 2. Comparison of means of salinity stress effect on some morphological features of Dracocephalum moldavica			
Salinity level	N. of Flowering Stems	Main Stem Diameter	N. of Leaves
0	7.91 a	2.4500a	83.50a
2.5 MS	7.16b	1.8333b	74.66a
5 MS	5.66c	1.5417c	62b
Different letters in each column indicate significant difference at a level of 5%.			

3. Number of lateral branches

In regard with the number of lateral branches, the results of variance analysis (See Table 1) indicated that the interaction of studies factors was significant at a level of 5%. The highest level of this feature was related to the treatment with salinity-free irrigation and spray of different amounts of salicylic acid without significant difference. And its lowest level was related to treatment with 5 MS salinity without salicylic acid spray (See Table 4 & Figure 3).

4. Number of flowering stems

The results of variance analysis table showed that an increase in salinity has a significant effect on the number of flowering stems (p=0.01) (See Table 1). The maximum number of flowering stems was related to the control treatment (7.91) and the minimum to the treatment with 5 MS salinity (5.66) (See Table 2 & Figure 4). The number of flowering stems under the effect of spraying salicylic acid also showed a significant difference at a significance level of 1%. Comparing the means of this feature showed that the maximum number of flowering stems was related to the treatments with 1 and 1.5 mM salicylic acid and the minimum to the control treatment (without salicylic acid spray) (See Table 3 & Figure 5).

Table 3. Comparison of means of salicylic acid effect on N. of Flowering Stems of *Dracocephalum moldavica*	
Salicylic Acid	N. of Flowering Stems
0	5.56c
0.5 mM	7.11b
1 mM	7.44ab
1.5 mM	7.55a
Different letters in each column indicate significant difference at a level of 5%.	

5. The length of reproductive organ

In regard with the length of the reproductive organs, the results of variance analysis table (See Table 1) indicated that the interaction of the studied factors was significant at a level of 4%. The highest level of this feature was related to the treatment with salinity-free irrigation and spray of different amounts of salicylic acid without significant difference. And its lowest level was related to treatments with 2.5 and 5 MS salinity without salicylic acid spray and 5 MS salinity with .05 mM salicylic acid spray (See Table 4 & Figure 6).

6. Chlorophyll amount

The interaction between irrigation treatment (salinity stress) and salicylic acid spray was significant at a level of 1% (See Table 1). The maximum amount of chlorophyll was related to the interaction of treatments salinity-free irrigation, 2.5 MS, and spraying different amounts of salicylic acid without significant difference and the minimum was related to treatment with 5 MS without salicylic acid spray (See Table 4 & Figure 7).

Table 4. Comparing the mean effect of factors A and B				
	Bush Height	N. of Lateral Stems	Length of Reproductive Organ	Chlorophyll Amount
A1B1	31.67ab	11.67a	18.67a	28a
A1B2	31.33ab	11.67a	17.67a	27.67a
A1B3	32a	11.67a	18.67a	27a
A1B4	30bc	11.33a	18.33a	28.33a
A2B1	26.67de	9.33bc	13.67cd	27a
A2B2	28.33cd	9c	15bc	28a
A2B3	30bc	9.66bc	16.67ab	27.33a

A2B4	28.67c	10.33b	18.67a	26.67a
A3B1	19.67g	7.33d	12.33d	18.67c
A3B2	22.67f	9.33bc	13.33cd	23.33b
A3B3	25.67e	9.66bc	15bc	24.33b
A3B4	23.33f	10.33b	14.67bc	23.67b
Different letters in each column indicate significant difference at a level of 5%.				

7. Main stem diameter

The results of variance analysis table indicated that an increase in salinity has a significant effect on main stem diameter (See Table 1). The maximum stem diameter was related the control treatment (2.45) and the minimum to the treatment with 5 MS salinity (1.54), which shows a decrease of 37% compared to the control group (See Table 2 & Figure 8). There was no significant difference between the surfaces of salicylic acid and the interaction effect of salicylic spray and salinity.

DISCUSSION

The results of the present experiment showed that bush height, stem diameter, number of lateral stems, length of flowering branches, and yield of *Dracocephalum moldavica* can meaningfully be affected by salinity stress and an increase in salinity their amount reduces remarkably. In most areas of the world, salinity stress is the most dominant environmental stress which reduces crop yield and growth by decreasing osmotic potential and disturbing uptake of some nutrient materials. Due to osmotic properties, plants that grow in saline soil face with not only salinity stress but also draught stress, which reduces the pace of plant growth. This disturbs cell division and growth and influences all metabolic reactions of the plant. Moreover, an increase in sodium and chloride ions reduces the uptake of necessary ions like potassium, calcium, ammonium, nitrate, and enzyme activity and disturbs membrane structure [23, 24]. Reduction in vegetative growth and dry weight due to a decrease in cell turgidity results from osmotic processes [25]. Another reason for growth and yield reduction in plants as a result of salinity is due to an increase in plant energy consumption to send out aggressive sodium ions that exist abundantly in the environment; therefore, a lot of cell energy is consumed to fight against salinity stress, as a result growth and yield of the plant reduces [26]. Ghorbanali *et al* [27] have also reported that salinity stress causes significant differences in height of aerial organs, dry and wet weight of aerial organs, dry and wet weight of root, life of total mass, and number of nods in Nigella Sativa and reduces these factors. When *Dracocephalum moldavica* was treated with salicylic acid, its height is remarkably increased. Compared to the control plant, the salicylic acid treated one removes oxygen free radicals and cell loss decreases [20]. It has been reported that salicylic acid significantly enhances the average growth speed of tomato stems in 200 Mm salinity stress condition [28], which is in line with the results of the present study. In regard with the number of leaves, it was observed that 5 MS salinity stress decreases this feature by 25.74% compared to the control group. Salinity stress disturbs osmotic balance and as a result water exits from the leaves and cell turgidity will be destroyed [29]. After the plant is exposed to saline conditions, expansion of leaves decreases and the reduction continues for a while. When decay rate of leaves is higher that their expansion, the amount of stored carbohydrate materials in the plant decreases relatively to leaf surface. However, the amount of carbohydrate needed for the plant growth is very likely to increase especially as the root grows compared to the stem and finally the plant will not be able to provide carbohydrate needed for continuation of plant growth; therefore, expansion of leaf surface stops and plant stem weakens gradually. This emerges as the plant becomes one day old, which is generally associated with yellow leaves. Reduction in the number of leaves is one of the reasons for reduction in leaf surface in plants in saline conditions [30]. Reduction in leaf surface as a result of salinity and number of leaves due to the amount of photosynthesis is because of turgor pressure drop. As a result of reduction in leaf surface, light absorption and plant's total photosynthesis capacity or canopy cover decrease which causes a reduction in necessary photosynthesis products for plant growth [31]. According to the available reports, salinity reduces weight of dry stem, root and leaf, number of leaves, leaf surface, and stem length in wheat, corn, barley, rice, sorghum, and sesame [32-35]. The amount of chlorophyll in *Dracocephalum moldavica* was affected by salinity stress. An increase in salt uptake and ionic toxicity results in cell dysfunction and damage to physiological processes like photosynthesis and breath. By making harmful changes in ion balance, water status, nutrient materials, stomata function, and photosynthesis efficiency, salinity reduces plant's growth processes like germination and seedling growth and finally the amount of product in the plant [40]. Salinity stress leads to formation of active types of oxygen like superoxide and hydrogen peroxide. Active types of oxygen can extremely disturb the natural metabolism of the plant through oxidative damage to lipids, nucleic acids, and proteins. Therefore, the salicylic acid treated plant can remove oxygen free radicals more effectively and there will be less cell damage (Shakirova, 2003). When a plant grows in saline conditions, its photosynthesis activity decreases; therefore, growth rate, leaf surface, and chlorophyll content decrease. El-Tayeb *et al* [14] have proved that salicylic acid treatment increases the amount of chlorophyll and carotenoid in plants and causes photosynthesis to speed up in saline conditions. The results of variance analysis presented in Table 1 indicated that the number of flowering stems decreases in saline conditions such that the maximum number of flowering stems was related to the control treatment (7.91) and the minimum to the treatment with 5 MS salinity (5.66). Moreover, the number of flowering stems can be affected by salicylic acid spray and the difference was significant at a level of 1%. It has been reported that salicylic acid increases flowering of Lemna paucicostata [36], accelerates initiation of flowering in bean plant [39] and increases the plant size in campanula rotundifolia [37]. Martin Mex *et al* [38] have reported that salicylic acid spray in saintpaulia leads to an increase in the number of flowers, which is in agreement with the results of the present study. Salicylic acid leads to an increase in the number of flower buds by increasing protein synthesis and emerging new bands of isozymes [36].

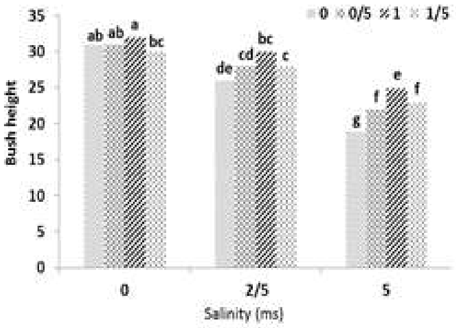

Figure 1. Salinity effect on N. of Flowering stems of *Dracocephalum moldavica* in the levels of Salicylic Acid

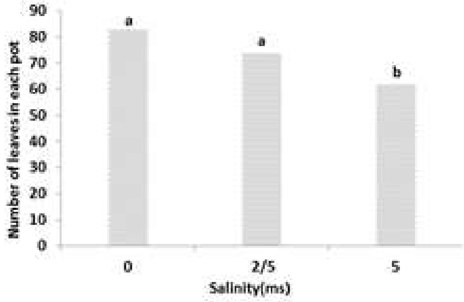

Figure 2. Salinity effect on number of leaves in each pot of *Dracocephalum moldavica*

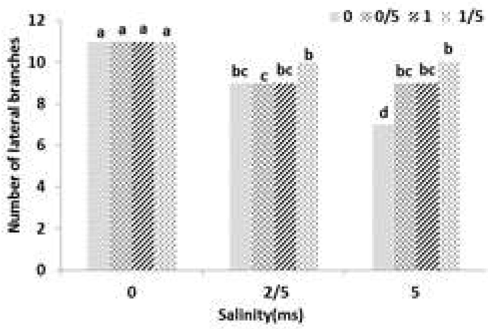

Figure 3. Salinity effect on N. of Flowering stems of *Dracocephalum moldavica* in the levels of Salicylic Acid

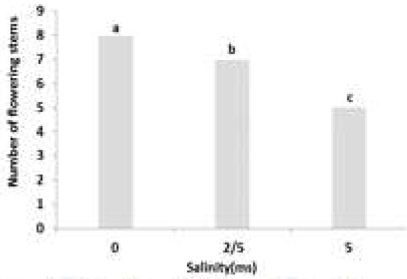

Figure 4. Salinity effect on N. of Flowering Stems of *Dracocephalum moldavica*

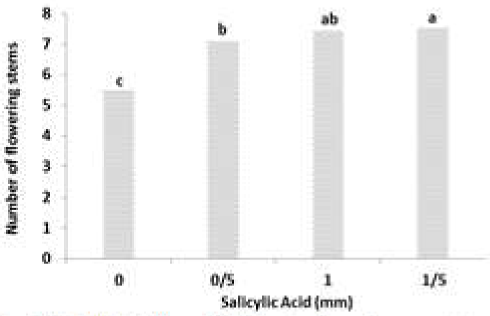

Figure5. Salicylic Acid effect on N. of Flowering Stems of *Dracocephalum moldavica*

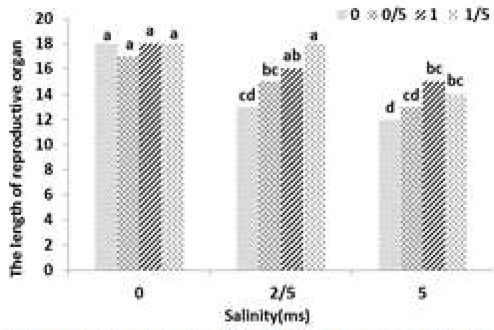

Figure 6. Salinity effect on Length of Reproductive Organ of *Dracocephalum moldavica* in the levels of Salicylic Acid

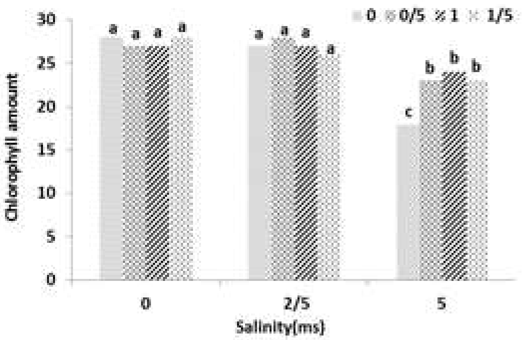

Figure 7. Salinity effect on Chlorophyll Amount of *Dracocephalum moldavica* in the levels of Salicylic Acid

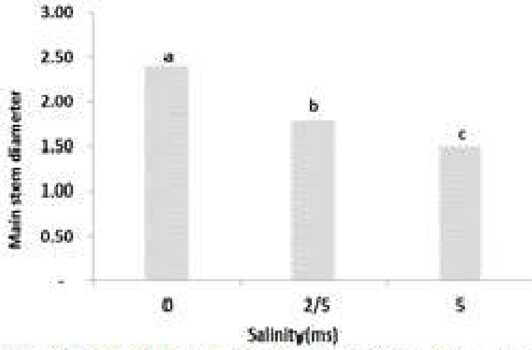

Figure 8. Salinity effect on Main Stem Diameter of *Dracocephalum moldavica*

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