Original Article

Effect of Salinity and Humic Acid on Morphological Traits and Essential Oil Content of Thymus Kotschyanus

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Abstract

Thymus Kotschyanus, as a native plant of Iran, is considered to be one of the most important medicinal plants with its unique properties. To evaluation of the effect of salinity and humic acid (HA) on aerial yield essential oil content, a factorial experiment was conducted based on a Completely Randomized Design (CRD) with five replications in 2014 in Qom, Iran. The first factor was five native accessions of *T. kotschyanus*, the second factor was salinity stress like control (0), 50, 100, 150 and 200 mM sodium chloride and the third factor was humic acid with three levels of 0, 1.5 and 3.0 g/l. Data were collected for seedling growth characteristics and essential oil content. The results of the analysis of variance showed significant effects of genotype, salinity on all of the traits. The effect of HA was significant on stem length, root length, aerial dry weight, and dry root weight. There were significant interaction effects for salinity by humic acid (root length, root weight, and essential oil content), for salinity by genotypes (stem length, root/shoot length ratio, essential oil yield) and for HA by genotype (root dry weight and essential oil yield). Results of means comparison between accessions showed that accession Divandarre had higher mean values for all of the traits except stem length and oil content. Results showed that by increasing salinity stress, all of the traits except root dry weight and RS were decreased. The application of HA was effective only on stem and root length. It was concluded that low salinity (50 mM NaCl) coupled 1.5 g/l HA had increased both essential oil content and oil production in all of the accessions. The response of Divandarre to salinity through the application of HA was positively higher than other accessions.

Keywords: essential oil, Humic Acid, Salinity, *Thymus kotschyanus*

Introduction

Thymus kotschyanus is one of the most important Thymus species that has vast applications in the health, pharmaceutical and food industries. The essential oil of Thyme is the world's ten most important essential oils, which have antibacterial, antifungal, antioxidant, and natural preservatives [1]. Due to the severe climatic condition and the possibility of occurrence of saline stress in the germination stages of this species and the lack of information regarding the needs of agriculture and its response to environmental stress, it is necessary to study the response of this plant to saline stress conditions and optimum conditions for its cultivation.

Salinity is one of the most important environmental stress that can affect the growth of plants and severely limit the production of the products. Increasing salinity in agricultural lands in the next 25 years and its global destructive effects are expected, including the loss of 30% of cultivated land [2, 3]. Soil salinity limits the growth of plants in several ways. The water absorbed by the plant is reduced, in other words, in saline soils; plants will wilt sooner because plants cannot absorb water inside the soil due to the soil's salinity [4]. Some of the ions are rich in saline soils, and because of their high absorption by the plant, they cause poisoning, the most important of which are chlorine, sodium, and borate. Also, salinity causes a nutritional imbalance in the plant. In saline soils, it is difficult to feed the plant due to the high levels of some ions. For example, in saline soil, due to the high concentrations of chlorine in the soil solution and its absorption by the plant, the nitrate and sulfate absorption by

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the plant are reduced [4]. Increasing salt concentration in the culture medium causes osmotic stress and ultimately leads to the imbalance of ions in the cell's space. In osmotic stress conditions, osmotic regulators are induced in the cell cytoplasm and lead to the accumulation of obsoletes such as glycine, betaine, and proline [5].

Saline stress in medicinal plants is important at different physiological stages. Researchers have determined that germination of medicinal plants such as *Ocimum basilicum*, *Petroselinum Hortense*, *Origanum vulgare* and *Thymus moroccanus*is sensitive to saline stress [6]. The growth stages of medicinal plants that are negatively affected by salt stress are the seedling growth stage. Belaqziz *et al*. (2009) reported negative effects of saline stress in the growth stage of *Thymus Moroccans* seedlings [7]. Saline stresses the movement of food stored in the plant, causing a delay in the cell division and causing damage to the hypocotyl [8].

The negative effects of salt stress on seedling survival have been reported for thyme [9], *Majorana hortensis* and peppermint [10, 11], *T. Vulgaris*, *T. Moroccans* and *M. pulegium* [7, 12, 13].

Ghavami & Ramin (2008) found that plants, growth parameters such as plant height and leaves the number of *Silybum marianum* was significantly decreased in saline stress of 9 ds/m [14].

Amini Dehchi *et al.* (2009) found that by increasing the sodium chloride concentration, seedling growth parameters and thymol content thyme was decreased [15]. They declared the highest proline content was obtained in severe saline stress (150 mM sodium chloride). Bagheri *et al.* (2012) in a study of the effects of salinity stress on seed germination traits of *Thymus kotschyanus and T. daenensis* found that *T. kotschyanus* was more tolerant to salinity than *T. daenensis*[16]. However, Khoshsokhan *et al.* (2011) in similar species found that the response of both species was similar at the germination stage [17]. Osmotic pressure reduction prevented the germination of the two species of thyme. Zarezadeh *et al.* (2010) in a study of the effects of saline stress on *T.daenensis* reported that saline although the soils may decrease crop yields, could increase its essential oil content [18]. Atti-Santos (2004) found a lower amount (0.25%) of essential oil of Brazilian thyme cultivated in Brazil and suggested cultivation of *Thymus vulgaris* was more appropriate in the spring than other seasons [19].

The *Thymus kotschyanus* is currently harvested in its natural habitat in Iran. This can lead to the destruction of a large part of the germplasm of this valuable plant. Therefore, its domestication and cultivation in saline conditions and assessment of the reduction of harmful effects of salinity through the application of humic acid are not clear. Therefore, the aim of this research was to evaluation of *T. kotschyanus* in saline stress for morphological traits and essential oil yield.

Material and Methods

This experiment was conducted in the greenhouse condition. The soil compositions consisted of 25% clay, 25% decayed animal fertilizer and 50% sandy soil. Plastic pots in size of 20 and 25 cm in diameter were used. Pots had some holes for drainage. A bit of gravel was placed at the bottom of the pots to facilitate the discharge of water. The pots were filled by soil and seeds were disinfected by Mancoseb fungicide with the 1:2000 ratios to prevent the fungal contamination. Seeds sown in the pots in February 2013. Pots were irrigated regularly until the seeds go to germination and seedlings were appeared.

A factorial experiment was conducted based on a completely randomized design (CRD) with five replications in 2014 in Qom, Iran. The first factor was five native accessions of *T. kotschyanus* (Table 1), the second factor was salinity stress levels as control (0), 50, 100, 150 and 200 mM sodium chloride solution with a purity of 98%. Sodium chloride salt in treatments of 50, 100, 150 and 200 mM NaCl were 299.5, 585, 877.5 and 1170 g per 100 liters of water, respectively. The third factor was humic acid with three levels of 0, 1.5 and 3.0 g/l.

Until the onset of stress, irrigation was done routinely. The temperature range of the greenhouse was adjusted between 15 and 25°C. After seed sowing to prevent possible damage to salinity levels on the plants, all pots (except control) were irrigated by 50 mM sodium chloride. Then the soluble irrigations (treatments) were applied to plots The irrigation intervals was based on the plant requirement and the occurrence of thirst symptom. Irrigation intervals decreased with increasing plant growth and increasing air temperature in the late period. Also, to reduce the effect of salinity accumulation, after irrigation of saline water in all treatments under salt stress, three times the irrigation interval was carried out. However, almost all of the different shrubs were dried in saline treatment with 200 mM sodium chloride. Therefore, this treatment was removed. The duration of specific stress application was 50, 100 and 150 mM NaCl and was for 61, 61 and 58 days, respectively.

Data were collected for stem length, root length, root to stem length ratio (RS), fresh and dry weight of stem, root fresh and dry weight, aerial dry matter yield, and essential oil content and essential oil yield.

At the end of the experiments, the variance analysis was carried out and the mean comparisons were made using Duncan method for all traits. SAS and MSTATC software were used for statistical analyses.

Table 1: Information of accessions of *Thymus Kotschyanus* used in the present research

No.	Accessions code	Accessions origin	Altitude m	Longitude N	Latitude E	seed thousand weight	Germination percent
1	12953	Qazvin 1	2000	36 34′ 00″	49 55′ 00″	0.36	98
2	19587	West Azarbaijan	2100	36 12′ 09″	44 57′ 07″	0.43	87
3	17091	Qazvin 2	1500	36 26′ 00″	50 07′ 00″	0.31	96
4	17010	Divandarre	2400	35 55′ 00″	46 40′ 00″	0.53	63
5	18063	Naqade	1389	36 55′ 12″	45 22′ 45″	0.34	87

Results

The results of the analysis of variance showed significant effects of genotype, salinity for all of the traits. The effect of humic acid was significant for stem length, root length, aerial dry weight, and dry root weight. There were significant interaction effects for salinity by humic acid (root length, root fresh and dry weight and an essential oil content and essential oil yield), for genotype by salinity (stem length, RS, essential oil yield) and for genotype by humic acid (root dry weight and essential oil yield). There were significant effects of three-way interaction effects for genotypes by salinity by humic acid for stem length, RS, essential oil yield (Table 2).

Table 2. Results of Analysis of the variance of five accessions of *Thymus kotschyanus* in different levels of Salinity stress and humic acid on their seedling growth characteristics in greenhouse condition

SOV	DF	Stem length	Root Length	Root/shoot Length ratio	Arial fresh weight	Arial dry weight	Root Fresh weight	Root dry weight	Oil %	Oil yield mg/p
Genotype(G)	4	153.4**	30.93*	0.184**	33.56**	6.91**	1.09*	0.610**	0.581*	383.4**
Salinity (S)	3	380.3**	301.53**	0.112*	443.4**	30.38**	2.47**	0.471**	4.347**	7533.1**
Humicacid(H)	2	92.21**	195.64**	0.074	19.71	3.79*	0.92	0.521**	0.026	83.06
S × H	6	8.16	36.65*	0.045	12.35	1.76	1.29*	0.344**	0.610**	669.7**
G × S	12	29.65**	5.81	0.087**	6.37	1.11	0.48	0.113	0.276	283.12**
G × H	8	7.69	10.48	0.031	9.84	1.55	0.69	0.185**	0.124	326.5**
G× S × H	24	13.52*	9.55	0.055*	3.76	1.06	0.20	0.039	0.095	130.5**
Error	240	8.13	14.77	0.03	9.61	1.03	0.42	0.07	0.00	38.27
CV%		14.63	19.38	17.87	27.28	28.80	25.25	34.48	0.00	28.72

** and * = significant differences based on 1 and 5% probability levels

Results of means comparison between accessions showed that accession Divandarre had higher mean values for all of the traits except stem length and oil contents. For stem length, Qazvin 2 with an average value of 21.93 cm had longer stem than other accessions and for oil content, the Azarbijan with 0.71% had higher oil content (Table 3).

In comparison among salinity levels, results showed that by increasing salinity stress, all of the traits except root dry weight and RS were decreased. However, for many of the traits as stem length, root length, root fresh and dry weight, essential oil content and essential oil yield, the higher values were obtained in 50 mM NaCl than that for control. In 50 mM and 100 mM NaCl, the essential oil content was 40% and 30% higher than that for control, respectively. For Root dry weight the high values were obtained in 150 mM NaCl that it was 18% higher than that for control (Table 4).

The application of humic acid was effective only for stem and root length. The higher stem and root length with average values of 20.46 and 20.91 cm were obtained in 3.0 g/l humic acid that they were 10% and 15% higher than that for control (No fertilizer), respectively (Table 5).

The salinity by humic acid interaction effects was significant for root length, root weight, and essential oil content. This means that responses of humic acid on salinity levels were not similar for these traits and in the other word, the positive effect of humic acid on the reduction of the harmful effect of salinity were not similar on different salinity levels. In such a case that interaction effects are significant for some of the traits, the attention has to pay on means comparison of interaction effects than that for main effects. For stem and root length, root fresh weight, the higher mean values of were obtained 50 mM NaCl coupled with the application of 3 g/l humic acid (Fig 1). Similarly, for root dry weight the higher value was obtained in 150 mM NaCl without fertilizer, for essential oil content and yield, the higher values were obtained in 50 mM NaCl coupled with the application of 1.5 g/l humic acid (Fig 1).

The genotype by salinity interaction effects were significant stem length, RS and essential oil yield). For stem length, the higher values of 26.6 and 23.7 cm were obtained in Qazvin2 and Qazvin1, respectively by using 50Mn salinity treatment. This trend was also observed in both genotypes for 100Mn but with lower stem length values (Fig2). For RS, the higher values were contained in Azerbaijan accession in control followed by Naqade with 100 mM NaCl salinity treatment, indicating the relative resistance of the latter genotype to salinity stress than other accessions (Fig2). The genotype by salinity interaction for essential oil yield showed that all of the accessions had higher oil yield in 50 mM NaCl. However, by increasing salinity stress, the higher values were obtained in Azerbijan by 100nM salinity followed by qazvin2 (Fig 2)

The results of the genotype by humic acid interaction effects for root dry weight and essential oil yield are presented in Fig 3. For root dry weight, the higher values were obtained in Divandarre and Naqadeh by application of 1.5 g/l humic acid. By increasing of humic acid concentration, the root dry weight of all of the accessions was significantly decreased (Fig3). For essential oil yield, the higher value was obtained in Divandarre by application of 3 g/l humic acid (Fig3).

Since the mean comparisons of three-way interaction effects for genotypes by salinity by humic acid for stem length, RS, essential oil yield has 24 treatments therefore data not shown. However, the higher stem length with average values of 25 cm was obtained in Qazvin2 accession by low salinity 50 mM NaCl coupled with 3 g/l humic acid. By increasing salinity to (100 mM NaCl) coupled with 1.5 g/l humic acid, the stem length value (22 cm) was decreased and ranked in the second class. The higher value of RS with average values of 1.26 and 1.22 was obtained in Azerbaijan and Divandarre, respectively that were subjected to salinity 100 mM NaCl coupled with 1.5 g/l humic acid.

For essential oil yield, the higher values of 96, 87 mg/p were obtained in Naqade and Divandarre, respectively in low salinity (50 mM NaCl) coupled with 1.5 g/l humic. By increasing salinity to (100 mM NaCl). Qazvin2 produced lower values of 60 mg/p essential oil yield without fertilizer

Table 3. Means comparison of the main effect of five *Thymus kotschyanus* accessions on seedling growth characteristics of in greenhouse condition

Accessions	Stem length cm	Root length cm	Root-shoot Length Ratio	Arial fresh weight g/p	Arial dry Weight g/p	Root Fresh Weight g/p	Root dry weight g/p	Oil %	Oil yield mg/p
Qazvin 1	20.12 b	19.43 a	0.98 b	10.59 b	3.21 c	2.40 b	0.64 b	0.67b	22.45 a
Qazvin 2	21.93 a	21.08 a	0.97 b	10.94 ab	3.31 c	2.56 ab	0.75 ab	0.62b	21.11 a
West Azarbijan	18.28 c	19.73 a	1.09 a	11.10 ab	3.33 c	2.46 ab	0.70 b	0.71 a	23.40 a
Divandarre	19.12 bc	19.60 a	1.02 ab	12.49 a	3.99 a	2.73 a	0.87 a	0.57c	23.41 a
Naqade	17.98 c	19.32 a	1.08 a	11.72 ab	3.77 b	2.65 ab	0.86 a	0.45 d	17.34b

In each column, data with similar alphanumeric characters do not have a significant difference ($p<0.05$).

Table 4. Means comparison of the main effect of salinity effects on seedling growth characteristics of Thymus kotschyanus accessions in greenhouse condition

Salinity levels	Stem length cm	Root length cm	Root-shoot Length Ratio	Arial fresh weight g/p	Arial dry Weight g/p	Root Fresh Weight g/p	Root dry weight g/p	Oil %	Oil yield mg/p
Control	19.60 b	20.42ab	1.07 a	14.54 a	4.17 a	2.44 b	0.73 b	0.51 b	22.38b
50 mM	22.45 a	21.92 a	0.98 b	11.83 b	3.97 a	2.83 a	0.78 ab	0.88 a	33.29 a
100 mM	18.85 b	19.86 b	1.05 ab	10.19 c	3.08 b	2.49 b	0.68 b	0.71 a	21.70b
150 mM	17.04 c	17.12 c	1.01 ab	8.90 c	2.88 b	2.48 b	0.86 a	0.32 c	8.79c

In each column, data with similar alphanumeric characters do not have a significant difference ($p<0.05$).

Table 5. Means comparison of the main effect of humic acid fertilizer on seedling growth characteristics of *Thymus kotschyanus* accessions in greenhouse condition

Humic Acid	Stem length cm	Root length cm	Root-shoot Length Ratio	Arial fresh weight g/p	Arial dry Weight g/p	Root Fresh Weight g/p	Root dry weight g/p	Oil %	Oil yield mg/p
0.0 g/l	18.54 b	18.25 b	1.00 a	11.85 a	3.52 ab	2.46 a	0.77 ab	0.59 a	21.19 a
1.5 g/l	19.46 b	20.33 a	1.05 a	10.98 a	3.72 a	2.65 a	0.83 a	0.62 a	22.58 a
3.0 g/l	20.46 a	20.91 a	1.03 a	11.27 a	3.33 b	2.57 a	0.69 b	0.60 a	20.86 a

In each column, data with similar alphanumeric characters do not have a significant difference ($p<0.05$).

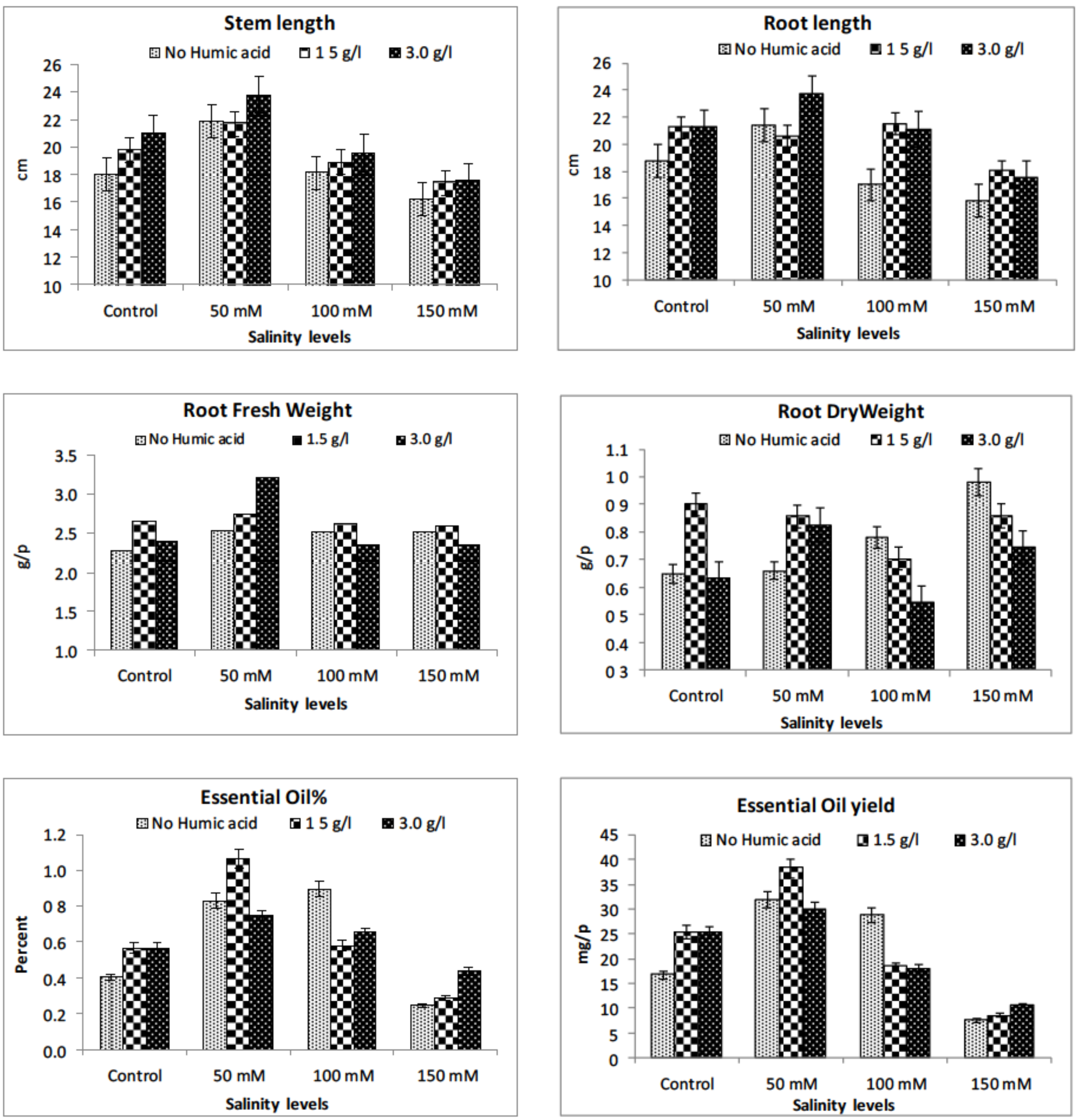

Figure 1: Means of salinity by hemic acid interaction for stem length, root length, root fresh, and dry weight and essential oil content and production

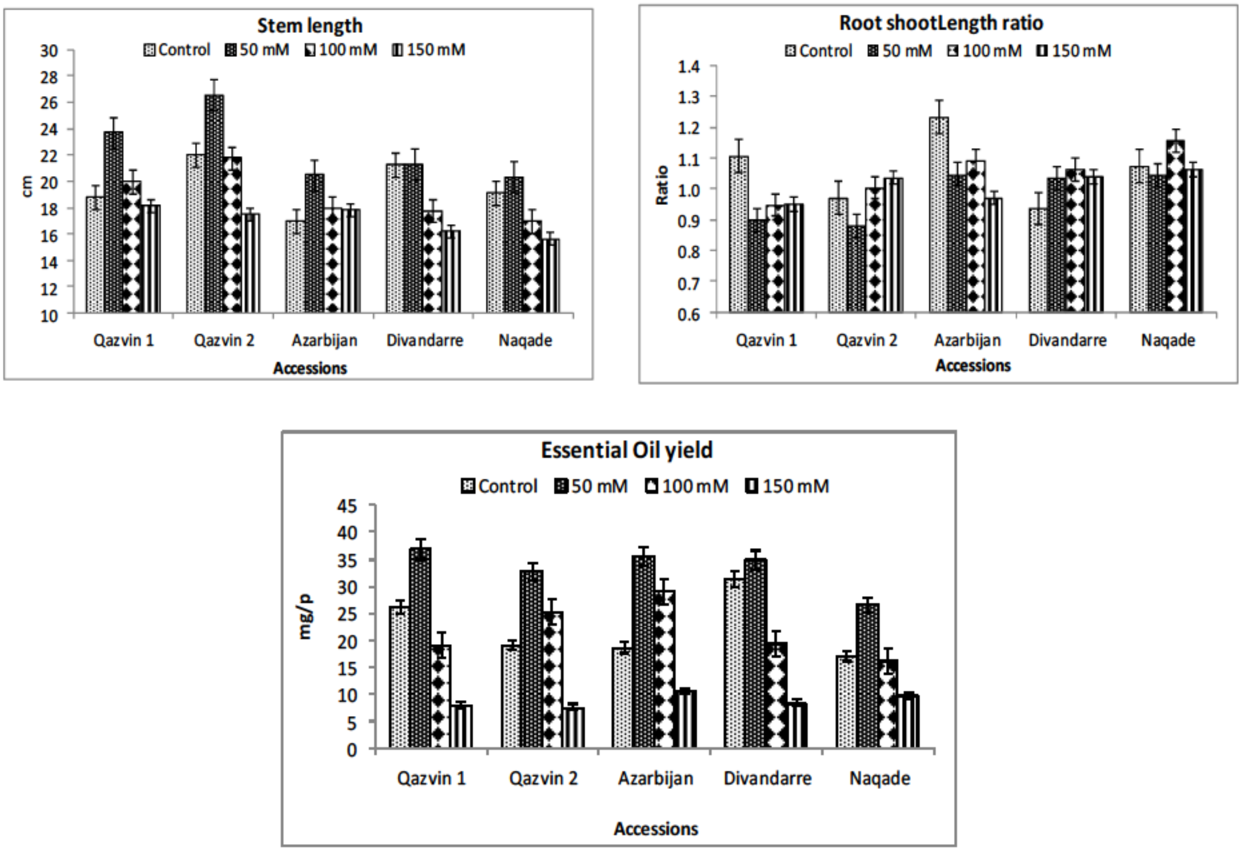

Figure 2: Means of accession by salinity interaction for stem length, root-shoot length ratio, and essential oil content and production

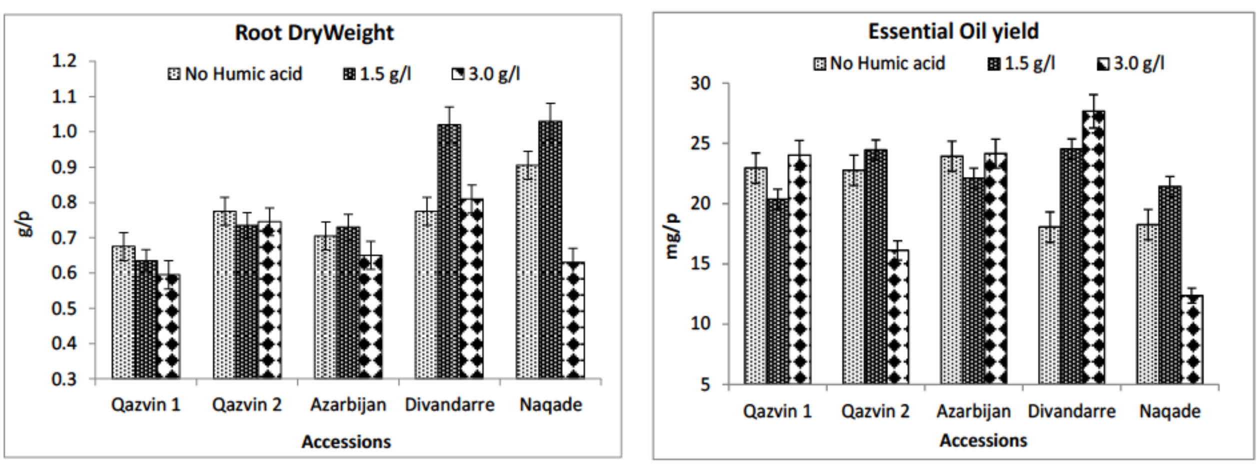

Figure 3: Means of accession by hemic acid interaction for stem length, root-shoot length ratio, root dry weight and essential oil production in

Discussion

The result showed that by increasing salinity stress up to 50 mM NaCl, the length and weight of the stem increased in most accessions and ten reduced by severing salinity. In normal conditions, there was not much variation between the accessions and in most cases they were commonly ranked in class a.

There are various reports on variation in response to saline stress on the amount of essential oil production in salt stress conditions. Similar to present research, the saline stress reduced essential oil yield in *Trachyspermum ammi* [20], in *Mentha piperata* [21], *Thymus maroccanus* [7] and *Chamomilla Recutita* [22] and *Foeniculum vulgare* [23]. In all the accessions, the essential oil percentage in low salinity (50 mM sodium) was higher than control. These results are similar to some reports on the effects of saline stress. Low levels of salinity led to the increase of the essential oil yield in some species, including *Satureja hortensis* [24] and *Salvia officinalis* [25] and *Coriandrum sativum* [26]. The latter authors reported that the essential oil yield is increased only at low and medium levels of saline stress and the oil production under high levels of salt in this plant is greatly reduced [26].

The essential oil content (%) was increased up moderate salinity and then decreased. Using 50 mM and 100 mM NaCl, the essential oil content was increased 40% and 30% higher than that for control, respectively This finding was in agreement on *Matricaria recutita* [27], *Salvia officinalis* [25], *Thymus kotschyanus* [9], *Ocimum basilicum* [28]. In contrast, in some studies on *Origanum majorana* [11] and Savory [29], salinity significantly reduced the essential oil content. Increasing the electrical conductivity due to sodium chloride (1.5 g/ kg soil) on the essential oil percentage and its yield in *Ocimum basilicum* was similar to the control plants [28]. However, by increasing salinity levels to 4.5 g/kg soil, the percentage and yield of oil significantly decreased in *Cymbopogon* [30].

By the application of humic acid, the areal fresh weight was significantly increased higher than the control.

The Divandarre accession showed a good response to Humic acid for areal fresh weight Haghparast *et al.* (2012) did find a significant effect of Humic acid on grain yield of three chickpea varieties [31]. The results also indicate that the usage of Humic acid increases root dry weight. The increase of root size led to resistance to salinity stress. Also, the higher essential oil yield was obtained in Divandarre by application of 3 g/l humic acid. In contrast to our result, Fatemi *et al.* (2011) in field study find no significant effect of humic acid on *Ocimum basilicum* on the essential oil [32]. They concluded that though humic acid has a positive influence on morphological parameters of basil, such as stem length, dry and wet weight, and root length, it did not affect essential oil content and oil yield.

Conclusion

There was good variation among accessions for all of the traits. The accession Divandarre had higher mean values for many traits and its response to salinity through the application of humic acid was higher than other accessions. The Qazvin accession had a longer stem and Azerbaijan had higher oil content (0.71%). From this useful variation could improve breeding new *T. kotschyanus* varieties.

The essential oil content was increased by increasing salinity up to 100 mM NaCl and then decreased so that, in 50 mM and 100 mM NaCl stress, the essential oil content was 40% and 30% higher than that for control average overall of accessions, respectively. It was concluded that low salinity (50 mM NaCl) coupled 1.5 g/l HA had increased both essential oil content and oil production in all of the accessions. The application of 1.5 g/l humic acid had increased both root dry weight and essential oil in all of the accessions. Humic acid had a positive effect on the reduction of a harmful effect of salinity on new *T. kotschyanus*.

References

1. James, T. K., A. Rahman, Douglas, J. A. Control of weeds in five herb crops. In Proceedings of the New Zealand Weed and Pest Control Conference, 1991; 44:116-120.
2. Mahajan, S., Tuteja, N. Cold, salinity and drought stresses: an overview. Archives of biochemistry and biophysics, 2005; 444: 139-158.
3. Wang, W., Vinocur, B., Altman, A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 2003; 218. 1-14.
4. Sela, G. Fertilization and Irrigation: Theory and Best Practices" by Fertilization and Irrigation: Theory and Best Practices. Asesoría Agrícola, Spanish, 2019.
5. Kosova, K., Vitamvas, P., Renaut, J. Plant proteome changes under abiotic stress contribution of proteomics studies to understanding plant stress response. Proteomics, 2011; 74. 1301-1322.
6. Fetri, M., Dargahikhoo, A., Rajabi, M. Effect of drought and salinity stress on germination and seedling growth of Common Yarrow (Achillea mellifolium L.) in laboratory conditions. International Journal of Advanced Biological and Biomedical Research, 2014; 2(2). 383- 391.
7. Belaqziz, R., Romane, A., Abbad, A. Salt stress effects on germination, growth and essential oil content of an endemic thyme species in Morocco (Thymus maroccanus Ball.).Appl. Sci Res. 2009; 5, 858-863.
8. Rahman, M., Soomro, U., Zahoor-Ul-Hag, M., Gul, Sh. Effects of NaCl on wheat (Triticum aestivum L.) cultivars. World J Agri Sci. 2008; 4(3):398-403.
9. Ezz El-Din, A. A., Aziz, E. E., Hendawy, S. F., Omer, E. A. Response of Thymus vulgaris L. to salt stress and alar (B9) in newly reclaimed soil. J Appl Sci Res, 2009; 5(12): 2165-2170.
10. Al-Amier, H., Craker, L. E. In-Vitro selection for stress-tolerant spearmint. Reprinted from: Issues in new crops and new uses. J. Janick and A. Whipkey (Eds.). ASHS Press, Alexandria, VA. 2007; 306-310.
11. Shalan, M. N., Abdel-Latif, T. A. T., Ghadban, E. A. Effect of water salinity and some nutritional compounds of the growth and production of sweet marjoram plants (Majorana hortensis L.). Egypt J Agric Res. 2006; 84(3): 959.
12. Queslati, S., Karray-Bouraoui, N., Attia, H., Rabhi, R., Lachaal, M. Physiological and antioxidantresponses of Mentha pulegium (Pennyroyal) to salt stress. Acta Physiol Plant. 2010; 32(2):289-296.
13. Najafian, S., Khoshkhui, M., Tavallali, V., Saharkhiz, M. J. Effect of salicylic acid and salinity in thyme (Thymus vulgaris L.): investigation on changes in gas exchange, water relations, and membrane stabilization and biomass accumulation. Aust J Basic Appl Sci. 2009; 3(3):2620-2626.
14. Ghavami, A., Ramin, A. Grain yield and active substances of milk thistle as affected by soil salinity. Comm Soil Sci Plant Anal. 2008; 39(17 & 18): 2608-2618.
15. Amini Dahaghi, M., Zawiya, D., Babaei, K., Tabatabaei, R., Jalali Artist, Q. Effect of Drought Stress and Salinity on Adaptive Metabolites, Essences, and Effective Thyme. First National Conference on Plant Physiology in Iran, 2009.
16. Bagheri, M., Yeganeh, H., Bandak, E., Ghasemi Aryan, Y. Effect of salinity stress on seed germination characteristics of medicinal species Thymus kotschyanus and Thymus daenensis. Journal of Rangeland Science. 2012; 2.2. 507-512.
17. Khoshsokhan, F., Babalar, M., Chaghazardi, H. R., Fatahi Moghadam, M. R. Effect of salinity and drought stress on germination indices of two Thymus species. Cercetări Agronomice în Moldova. 2011; 1(149):27-35.
18. Zarezadeh, M., Shahin, M., Tohidi, M. Effect of salinity and drought stress on thyme germination. Journal of Plant Physiology, 2010; 4: 61-70.

19. Atti-Santos, A. C., M. R. Pansera, N. Paroul, L. Atti-Serafini, and P. Moyna. Seasonal variation of essential oil yield and composition of Thymus vulgaris L.(Lamiaceae) from South Brazil. Journal of Essential Oil Research, 2004; 16(4): 294-295.
20. Ashraf, M., Orooj, A., Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (Trachyspermum ammi [L.] Sprague). Journal of Arid Environments, 2006; 64: 209-220.
21. Tabatabaie, S.J. Nazari, J. Influence of nutrient concentrations and NaCl on the growth, photosynthesis, and essential oil content of peppermint and lemon verbena. Turkish Journal of Agriculture and Forestry, 2007; 31: 245-253.
22. Ghanavati, M., Sengul, S. Salinity effect on the germination and some chemical components of Chamomilla recutita L. Asian J Chem, 2010; 22(2): 859-66.
23. Abd El-Wahab, M. A., The efficiency of using saline and freshwater irrigation as alternating methods of irrigation on the productivity of Foeniculum vulgare Mill subsp. vulgare var. vulgare under North Sinai conditions. Res J Agr Biol Sci, 2006; 2(6):571-577.
24. Baher, Z. F., Mirza, M., Ghorbanli, M., Rezaii, M. B. The influence of water stress on plant height, herbal and essential oil yield and composition in Satureja hortensis L. Flav Fragr J. 2002; 17:275-277.
25. Hendawy, S. F., Khalid, K. h. A. Response of sage (Salvia officinalis L.) plants to zinc application under different salinity levels. J Appl Sci Res, 2005; 1:147-55.
26. Neffati, M., Marzouk, B. Roots volatiles and fatty acids of coriander (Coriandrum sativum L.) grown in saline medium. Acta Physiol Plant. 2009; 31(3):455-461.
27. Baghalian, K., Haghiry, A., Naghavi, M. R., and Mohammadi, A. Effect of saline irrigation water on agronomical and phytochemical characters of chamomile (Matricaria recutita L.). Scientia Hort. 2008; 116:437-441.
28. Said-Al Ahl, H. A. H., Mahmoud, A. A. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (Ocimum basilicum L.) under salt stress. Ozean J Appl Sci, 2010; 3(1):97-111.
29. Najafi, F., Khavari-Nejad, R. A., Ali, M. S. The effects of salt stress on certain physiological parameters in summer savory (Satureja hortensis L.) plants. J Stress Physiol Biochem, 2010; 6(1):13-21.
30. Ansari, S. R., Frooqi, A. H. A., Sharma, S. Interspecific variation in sodium and potassium ion accumulation and essential oil metabolism in three Cymbopogon species raised under sodium chloride stress. J Essent Oil Res, 1998; 10:413-418.
31. Haghparast, M., Maleki Farahani, S., Siniqi, J. M., Zerae, Gh. Reduction of negative effects of drought stress in chickpea using humic acid and seaweed extract. Crop production under environmental stress. 2012; 4(1), 59-71. [In Persian with English summary].
32. Fatemi, H., Ameri, A., Aminifard, M. 2011. Investigation of Effect of humic acid Fertilizer on growth and Yield of Ocimum basilicum under Field Conditions. Proceeding of National Conference on Modern Issues in Agriculture, Islamic Azad University, Savah, Branch, Iran (In Persian).