Biological Forum – An International Journal **7**(2): 554-561(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of humic acid and salinity stress on germination characteristic of savory (*Satureja hortensis* L.) and dragonhead (*Dracocephalum moldavica* L.)

S. Khalesro*, M. Salehi** and B. Mahdavi***

**Assistant Professor, Agronomy and Plant Breeding Department, University of Kurdistan, Sanandaj, Iran.*
***Assistant Professor, Department of Medicinal Plants Production, Nahavand University, Nahavand, Iran.*
****Assistant Professor, Agronomy and Plant Breeding Department,*
Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.

(Corresponding author: S. Khalesro)
(Received 25 July, 2015, Accepted 27 September, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: This study was conducted in a factorial experiment as completely randomized design with three replications in University of Kurdistan in 2014. The aim of this study was investigation of salinity stress and humic acid effects on germination indicators of savory and dragonhead, as, aromatic and medicinal plant. The treatments were humic acid (0, 0.5 and 1%) and salinity (0, 2 and 4 dS/m). The results showed that the maximum length of radicle and plumule and the highest amount of radicle and plumule dry and fresh weight was produced in 1% humic acid. The lowest values of mentioned traits, germination percentage and germination speed were created in 6 dS/m salinity level. Generally the most favorable interaction treatment was the third level of salinity combined with the highest level of humic acid in both plants. Furthermore, humic acid application can be improved germination and plant growth under salinity condition.

Keywords: Lamiaceae, germination, humic acid, Sodium cloride

INTRODUCTION

Consumption of medicinal herbs, the crude source of drugs in indigenous systems of medicine as well as for the pharmaceutical industry, is increasing exponentially. In order to meet the demand, some medicinal plants need to be cultivated commercially. Marginal Lands often comprise toxic soils due to the presence of heavy metals and salinity that pose serious threats to plant production (Qureshi *et al*., 2005). The abiotic stresses especially salinity and drought stress have negative effect on plant growth and development. In fact the effect of salinity on plant growth is a complex response which includes osmotic stress, ion toxicity and mineral deficit (Roy *et al*., 2005). Salinity is one of the most important obstacles in producing crops in arid and semi arid regions. These regions produced nearly one third of the world food (Munns, 2002). Two main characteristics of saline soils are low osmotic potential and high solutes concentration that are toxic for plants potentially. The soil solutes cause decrease in radicle water potential and radicle water absorption then the plant faced to physiological drought. On the other hand the high concentration of solutes in the soil and consequently Na and Ca uptake cause toxicity in plant (Edward and Bison, 1996). Seedling establishment is a sensitive step in plant production. Uniformity and emergence percentage in direct cultivation has a direct effect on yield and production quality.

Humic matter, the major component of soil organic matter, composed 65 up to 70% of soil organic matter, is the subject of study in various areas of agriculture such as soil chemistry, fertility, plant physiology as well as environmental sciences. Since this material plays multiple roles (Cimrin *et al*., 2005; Costa *et al*., 2008) its application is an effective step in order to reach sustainable production. Humic acid is a main component of humus, humates, natural organic matter in humic acid, are included essential mineral matter for plant growth (Senn, 1991). Humic matters are a mixture of different organic matters which are composed of plants and animals remains (Maccarthy , 2001). Humic acid has many benefits for example chelation of different nutrients such as Na, K, Mg, Zn, Ca, Fe, Cu and other nutrients in order to overcome the nutrients deficit. This causes increase in radicle length and weight and initiation of lateral radicles (Aiken *et al*., 1985). *Dracocephalum moldavica* L. is an annual and herbaceous plant that its origin is from the south of Siberia and it grows in Kazakhstan,China and Russia as an anthropophilous plant too. The root of this plant is erect with many branches. The stems are erect and violet because of Antociyanin. The flowers are violet bluish and have nectar. The fruits are achene, their length 2.4 mm up to 2.8 mm. A thousand seed mass is equal to 1.7 g up to 2.1. The secondary metabolites of vegetative organs are tranquillizing and appetizing.

Its essence has antibacterial properties and is used for stomachache and bloat. Its essence is used in cosmetic, soft drink industry and food industry too. In this study, this plant was chosen because of many industrial uses. The young leaves and stems of *Dracocephalum moldavica* L. are aromatic. The essence amount is differed by climatic conditions. The essence is composed of different compounds that their most important are Sitral (40 up to 54%) and Asetat Jeranil (10 up to 51%) (Eman *et al*., 2013). The *Satureja hortenssis* L. belongs to Labiateae family and grows in form of shrub. It has many flowers, their size are 5.1 mm. The leaves are lance shaped and have secretary glands which included essence (Salehi -Arjmand *et al*., 2014). *Satureja hortenssis* L. is an aromatic plant which has many different effects such as treat of muscle aches, nausea and infectious disease (Vesquez *et al*., 2000). It is also used as a flavor in food materials (Mahfouz and, 2007). This plant has been showed antibacterial and antioxidant effects, antispasmodic and soporific (Ratti *et al*., 2001). Since application of aromatic substances in food consumption, drinks preparation and antibacterial and antifungal effects, *Satureja hortenssis* L. is very interesting (Chen, 2006). In this study the use of humic acid in addition to salinity stress was investigated.

MATERIAL AND METHODS

This study was conducted to investigate the effect of humic acid and salinity stress on germination in university of Kordestan. NaCl was used for creating salinity. This study was conducted in a factorial experiment as completely randomized design with 3 replications. The treatments were included three levels of humic acid (0, 0.5 and 1%) and three levels of salinity (0, 2dS/m and 4dS/m). this experiment was done using healthy seed with high viability. For sterilization, the seeds were located in sodium hypochlorite for 3-5 minutes and washed 3 times with water and then were immersed in Chitosan and water with different portions. The sterile Petri with diameters of 9mm was used. Every petri was included 25 seed that was located on Watman paper. Every petri was filled with 10 mm of prepared solutions. Then the petri was located in germinator with 25°C heat and 75% relative humidity. The Petries were covered with parafilm in order to stop evaporation. The indicator of germination was 1mm radicle emergence. The germinated seed was calculated every day. At the final of experiment the length of radicle and plumule and their fresh weight were measured. For determining of dry weight of shoot and radicle, these parts were dried in oven with 70°C for 48 hours (until there was no decrease in weight).

The germination percentage and germination speed were calculated as follows:

Germination percentage= $n/N \times 100$

n: total seed germinated

N: total seed used (25 seeds)

$$\text{Germination speed} = \frac{\sum Dn}{\sum n}$$

n: number of germinated seed on the sightly day

D: days after beginning

The data were analyzed using MSTATC software and the mean comparisons were done following Duncan test significant different at $p \leq 0.05$.

RESULTS

A. Germination percentage

Tables 1 and 2 showed that humic acid had very significant effect ($p \leq 0.01$) on germination percentage in both *Satureja hortenssis* L. and *Dracocephalum moldavica* L.. Mean comparison showed significant different between various levels of humic acid, as the lowest germination percentage was obtained in treatment without humic acid and the highest germination percentage was produced in 1% humic acid (Tables 3 and 4). Using the third level of humic acid increased the germination percentage 32.41% in *Dracocephalum moldavica* L. and 57.38% in *Satureja hortenssis* L. than the first level of it (without using humic acid) (Figures 2 and 4). As well the tables showed that salinity stress had very significant effect on germination percentage in both of studied plants. With considering the mean comparison the highest level of salinity caused the less germination percentage. The third level of salinity decreased the germination percentage in *Dracocephalum moldavica* L. and *Satureja hortenssis* L. 38.12% and 41.18% respectively than first level of salinity (0 dS/m) (Figure 1 and 3). The interaction of humic acid and salinity stress wasn't significant on germination percentage (Tables 1 and 2).

Table 1: Analysis of variance for germination characteristics of *Dracocephalum moldavica* L. in presence of salinity and humic acid levels.

		Mean Squere							
Source of variations	**Free degree**	**Germination percentage**	**Germination speed**	**Radicle length**	**Plumule length**	**Radical fresh weight**	**Plumule fresh weight**	**Radicle dry weigth**	**Plumule dry weigth**
Salinity	2	6741.926**	21.118**	7.023**	2.614**	135.93**	100.25**	14.87**	10.663**
Humic acid	2	613.926**	1.576**	2.154**	1.850**	21.240**	44.834**	2.345**	4.120**
Salinity*humic acid	4	46.815ns	0.212 ns	0.021*	0.021**	0.723**	5.933**	0.093**	0.357**
Error	18	17.185	0.109	0.006	0.006	0.122	0.019	0.017	0.06
CV		6.65	10.26	4.38	6.01	4.82	2.12	5.69	11.21

ns: nonsignificant, *: significant at 0.05 probability levels, **: significant at 0.01 probability levels

Table 2: Analysis of variance for germination characteristics of *Satureja hortenssis* L. in presence of salinity and humic acid levels.

		Mean Squere							
Source of variations	**Free degree**	**Germination percentage**	**Germinati on speed**	**Radicle length**	**Plumule length**	**Radical fresh weight**	**plumule fresh weight**	**Radicle dry weight**	**Plumule dry weight**
Salinity	2	8652.444^{**}	31.660^{**}	8.896^{**}	2.110^{**}	65.049^{**}	47.196^{**}	5.374^{**}	5.708^{**}
Humic acid	2	421.333^{**}	1.737^{**}	2.985^{**}	1.551^{**}	11.345^{**}	12.500^{**}	1.741^{**}	1.434^{**}
Salinity* humic acid	4	31.111^{ns}	0.154^{ns}	0.04	0.019^{**}	0.156^{**}	0.124^{**}	0.036^{*}	0.061^{*}
Error	18	30.322	0.166	0.005	0.004	0.019	0.019	0.012	0.016
CV		9.59	13.21	3.55	5.02	2.57	2.12	6.09	7.56

ns: nonsignificant, *: significant at 0.05 probability levels, **: significant at 0.01 probability levels

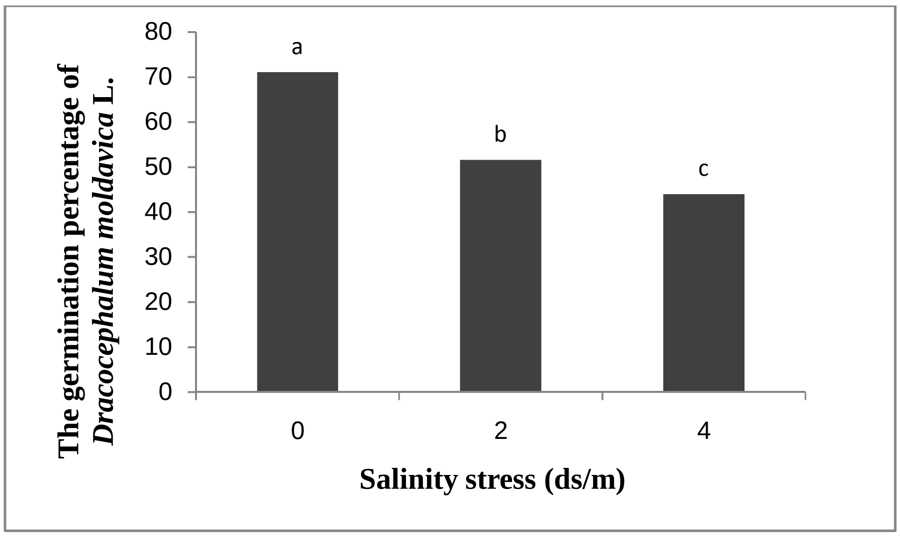

Fig. 1. The effect of salinity levels on germination percentage of *Dracocephalum moldavica* L.

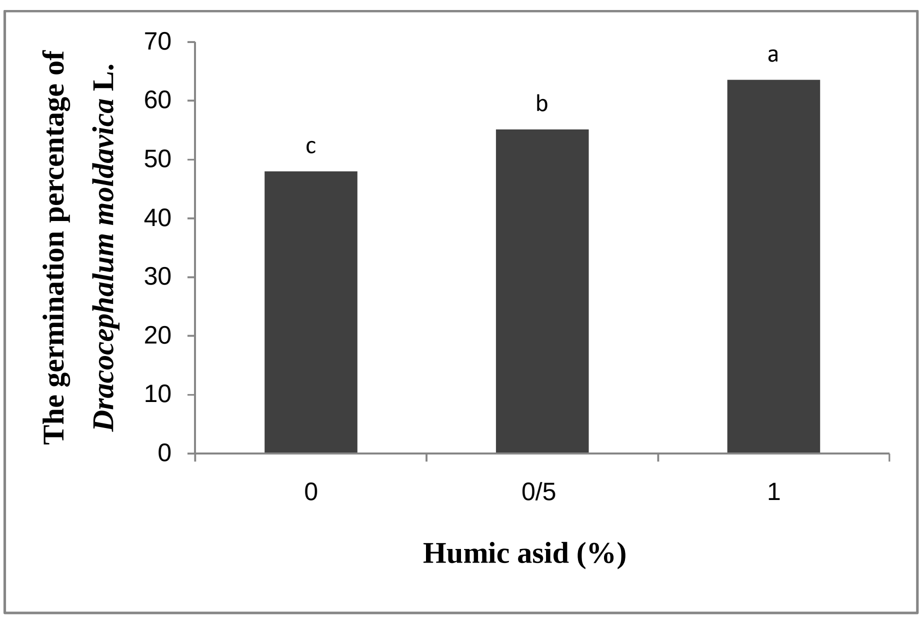

Fig. 2. The effect of humic acid levels on germination percentage of *Dracocephalum moldavica* L.

B. Germination speed

The results showed that humic acid and salinity levels had very significant effect on germination speed. With using 1% humic acid the highest germination speed was produced. Using the third level of humic acid increased the germination speed 32.48% in *Dracocephalum moldavica* L. (figure 2) and 57.32% in *Satureja hortenssis* L. than the first level of it (without using humic acid) (Fig. 4). By Using third level of salinity the lowest germination speed was created. The third level of salinity decreased the germination speed in *Dracocephalum moldavica* L. and *Satureja hortenssis* L. 38.13% and 41.18% respectively than first level of salinity (0 dS/m) (Figures 1 and 3). The interaction of two treatments had no effect on germination percentage (Tables 1 and 2).

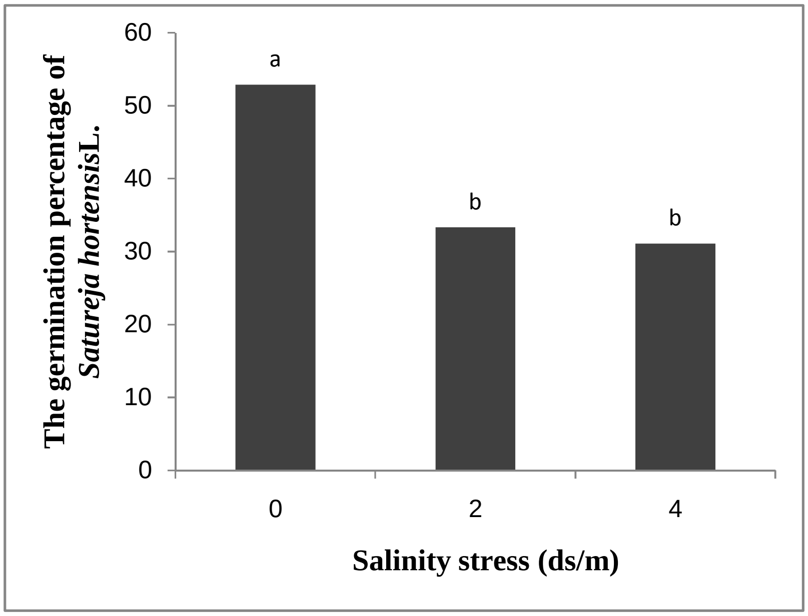

Fig. 3. The effect of salinity levels on germination percentage of *Satureja hortensis* L.

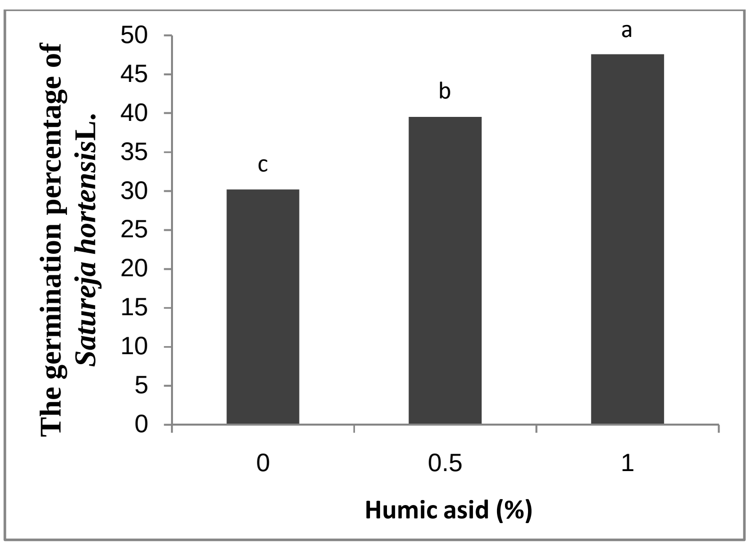

Fig. 4. The effect of humic acid levels on germination percentage of *Satureja hortensis* L.

C. Radicle length

The results showed that the interaction effect of humic acid and salinity had significant effect on radicle length in both of the plants (Tables 1 and 2). The longest radical length was obtained in third level of humic acid without salinity (Tables 3 and 4).

D. Plumule length

The tables 1 and 2 showed that the interaction effect of humic acid and salinity had very significant effect on plumule length in both of the studied plants. The longest plumule length in both plants was created with using third level of humic acid and first level of salinity (*Dracocephalum moldavica* L. 2.3cm and *Satureja hortenssis* L. 2.23cm). The shortest plumule length in both plants was created with using first level of humic acid and third level of salinity.

E. The fresh weight of radicle

The interaction effect of salinity and humic acid had very significant effect on fresh weight of radical (Tables 1 and 2).

Fig. 5. The effect of salinity levels on germination speed of *Dracocephalum moldavica* L**.**

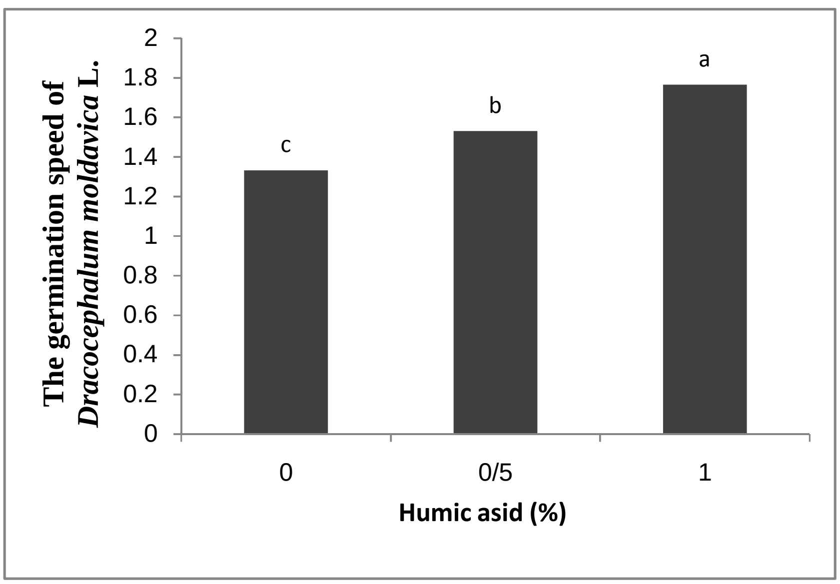

Fig. 6. The effect of humic acid levels on germination speed of *Dracocephalum moldavica* L.

The highest radicle fresh weight in both plants was produce when the third level of humic acid and the first level of salinity were used (*Dracocephalum moldavica* L. and *Satureja hortenssis* L. respectively, 13.63gr and 9.83gr) and the least was created in treatment without using humic acid and third level of salinity (Tables 3 and 4).

F. The fresh weight of plumule

Tables 1 and 2 showed that the interaction effect of salinity and humic acid had very significant effect on fresh weight of plumule. As you see, the highest fresh weight of plumule was created in third level of humic acid and the first level of salinity, and the least was created without humic acid and third level of salinity.

G. The dry weight of radicle

The results showed that the interaction effect of salinity and humic acid had significant effect on Satureja and had very significant effect on *Dracocephalum moldavica* L. The Mean comparison showed different in interaction effect of salinity and humic acid, as you observe the most dry weight of radicle was obtained in third level of humic acid and the first level of salinity and the least of dry weight was in first level of humic acid and third level of salinity (Tables 3 and 4).

H. The dry weight of plumule

The interaction effect of salinity and humic acid had significant effect on dry weight of plumule in two studied plants (Tables 1 and 2). The highest amount of dry weight of plumule was created in third level of humic acid and the first level of salinity and the least of dry weight was produced in first level of humic acid and the third level of salinity (Tables 3 and 4).

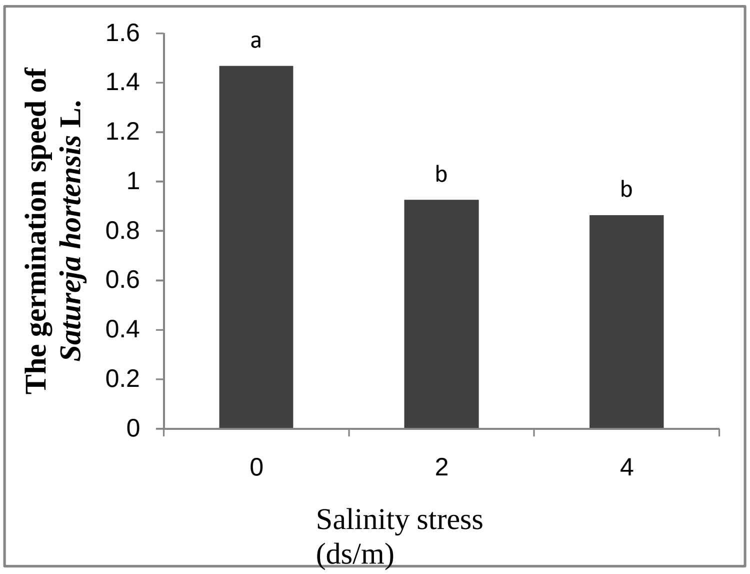

Fig. 7. The effect of salinity levels on germination speed of *Satureja hortensis* L.

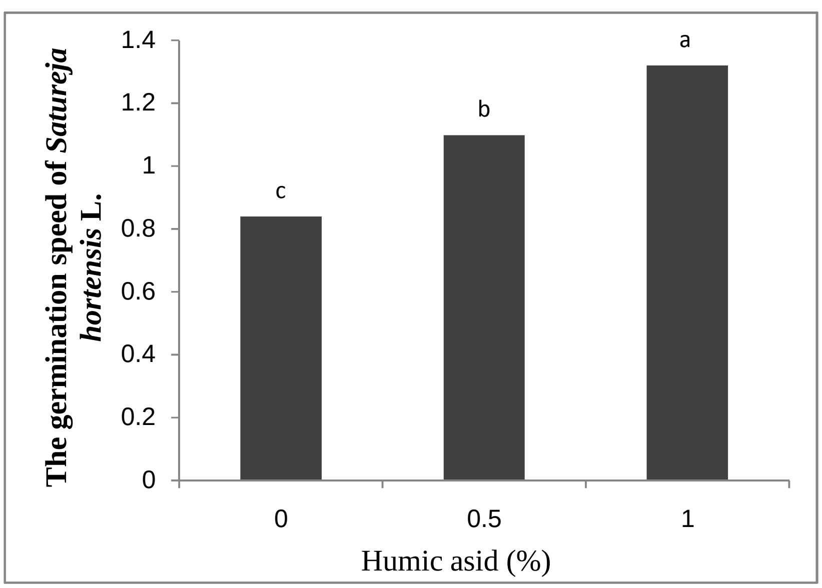

Fig. 8. The effect of humic acid levels on germination speed of *Satureja hortensis* L.

Table 3. The effect of salinity and humic acid on germination characteristics in *Dracocephalum moldavica* L.

Treatment	Radicle length (cm)	Plumule length (cm)	Radical fresh weight (gr)	plumule fresh weight (gr)	Radicle dry weigth (gr)	Plumule dry weigth (gr)
S0H0	2.17c	1.43d	9.67c	7.2c	3.13c	2.5c
S0H1	2.73b	1.73b	10.6b	8.57b	4.43b	2.97b
S0H2	3.33a	2.3a	13.63a	14.2a	4.47a	4.5a
S1H0	1.03f	0.8f	5.8f	4.73e	1.77f	1.6e
S1H1	1.47e	1.17e	6.4e	5.93d	2.07e	2.1cd
S1H2	1.93d	1.87b	8.37d	8.43b	2.63d	2.47c
S2H0	0.63g	0.43h	2.43i	1.93h	0.73i	0.63g
S2H1	1f	0.63g	3.4h	3.67g	1.1h	1.07f
S2H2	1.5e	1.17e	4.83g	4.37f	1.53g	1.77de

Within column means followed by the same letter (a …) are not significantly different at the 0.05 level, according to Duncan test.

Table 4. The effect of salinity and humic acid on germination characteristics in *Satureja hortenssis* L.

Treatment	Radicle length (cm)	Plumule length (cm)	Radical fresh weight (gr)	Plumule fresh weight (gr)	Radicle dry weight (gr)	Plumule dry weigth (gr)
S0H0	2.53c	1.23c	7.13c	6.43c	2.27c	2.03c
S0H1	2.87b	1.67b	8.27b	7.33b	2.53b	2.4b
S0H2	3.83a	2.23a	9.83a	9.17a	3.2a	3.13a
S1H0	1.37f	0.86e	3.67f	4.17e	1.13g	1.27d
S1H1	1.83d	1.1d	4.3e	5.13d	1.43f	1.43d
S1H2	2.53c	1.57b	5.57d	6.4c	1.9d	1.93c
S2H0	0.66h	0.4g	2.4h	2.13g	0.7h	0.7e
S2H1	1.03g	0.66f	2.93g	2.9f	1.3fg	0.86e
S2H2	1.6e	1.17cd	4.43e	4.17e	1.63e	1.27d

Within column means followed by the same letter (a …) are not significantly different at the 0.05 level, according to Duncan test.

DISCUSSION

The results showed the positive effect of humic acid on germination parameters in both of studied plants. Other studies also emphasize this result. Results of a research showed that humic acid can increase length of radicle and stem of corn (Eyheraguibel *et al*., 2008). Another study showed that using humic acid and Ca in tomato seeds increased the seedling growth and seedling nitrogen and Ca content and K and nitrogen content in radical (Tavakoli *et al*., 2008). The researchers studied the effect of two types of humic acid, one type was derived from municipal waste and the other was obtained from organic sources (Pit and Leonadrit), on barley and tobacco, the results showed that humic acid which was derived from municipal waste had more regulatory function on germination speed and reduced the germination time (Ayuso *et al*., 1998). Based on the findings of the current study, salinity stress caused significant decrease on germination parameters in *Dracocephalum moldavica* L. and *Satureja hortenssis* L. Similar finding was reported that salinity stress declined germination speed and radicle length of *Trachyspermum ammi* (Mahdavi and Rahaimi, 2013).The other researchers reported that salinity stress had significant effect on stem and root length of *Oenothera biennis* (Sharma *et al*., 2014). As a result, the application of humic acid with salinity decreased the negative effect of salinity on germination. In order to study the results of this experiment, it is suggested to study the application of humic acid in field experiments.

REFERENCES

Aiken, G. R., McKnight, D. M., Wershaw, R. L. & Mac Carthy, P. (1985). Humic substances in soil, sediment, and water. New York. USA: Wiley Inter Science, pp: 329.

Ayuso, M., Hernandez, T. & Garcia, C. (1996). Effect of humic fractions from urban wastes and other more evolved organic materials on seed germination. *Journal of the Scince of Food and Agriculture*, **72**(4): 461-468.

Chen, J. (2006). The combined use of chemical and organic fertilizer and biofertilizer for crop growth and soil fertility. International Workshop on Sustained Management of Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use. October 6-20. Thailand., pp: 11.

Cimrin, M. K. & Yilmaz, I. (2005). Humic acid application to lettuce do not improve yield but do improve phosphorus availability. *Plant & Soil Science,* **55**(1): 58- 63.

Costa, G., Labrousse, P., Bodin, C., Lhernould, S., Carlue, M. & Krausz, P. (2008). Effect of humic substances on the rooting and development of woody plant cutting. *Acta Horticulture*, **779**: 255-258.

Edward, A. K. & Bison, M. A. (1996). Plasma membrane Na+ transport in salt tolerant charophyte. *Plant Physiology*, **111**: 1191-1197.

Eman, E., Aziz, M., Hussein Hend, S., Wahba, E. & Razin, A. M. (2013). Essential oil constituents of *Dracocephalum moldavica* L. grown under salt stress and different sources of soil amendment. *Middle-East Journal of Scientific Research,* **16**(5): 706-713.

Eyheraguibel, B., Silvestre, J. & Morard, P. (2008). Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresource Technology*, **99**(10): 4206-4212.

Maccarthy, P. 2001. The principles of humic substances. *Soil Science*, **166**: 738-751.

Mahdavi, B., Rahimi, A. (2013). Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum* copticum) under salt stress. *EurAsian Journal of Bio Sciences*, **7**: 69-76.

Mahfouz, S. A. & Sharaf-Eldin, A. (2007). Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). *Agrophysics Journal*, **21**: 361-366.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant Cell Environment, **25**: 239-250.

Qureshi, M. I., Israr, M., Abdin, M. Z. & Iqbal M. (2005). Responses of Artemisia annual to lead and salt-induced oxidative stress. *Environmental and Experimental Botany*, **53**: 185-193.

Ratti, N., Kumar, S., Verma, H. N. & Gautam, S. P. (2001). Improvement in bioavailability of calcium phosphate to *Cymbopogon martini* var. motia by rhizobecteria, AMF and Azospirillum inoculation. *Microbiological Research*, **156**: 145- 149.

Roy, P. K, Niyogi, D., Sengupta, N. & Ghosh, B. (2005). Spermidine treatment to rice seedling recovers salinity stress induced damage of plasma membrane and PM- bound H+ ATPase in salt- tolerant and salt- sensitive rice cultivars. Plant Sciences, **168**: 583- 591.

Salehi-Arjmand, H., Mazaheri, D., Hadian, J., Majnoon Hosseini, N. & Ghorbanpour, M. (2014). Essential oils composition, antioxidant activities and phenolies content of wild and cultivated *Satureja bachtiarica* bunge plants of Yazd origin. *Journal of Medicinal plants.* **15**(31): 6-14.

Senn, T. L. 1991. Humates in Agriculture. Principles and procedures of statistics. 2nd ed. Mc Graw Hill international book company, New York, USA, pp: 203.

Sharma, S., Sunil, P., Arti, J., Sujata, B., Navdeep, D. & Kranti, T. (2014). Effect of salt stress on seedling growth and survival of Oenothera biennisL. *International Research Journal of Environment Sciences* **3**(9): 70-74.

Siam, H. S., Abd El-moez, M. R. and El- Ashry, S. M. (2008). Responce of lettuce followed by sorgum to application of different types of phosphorus, compost and sulfur. *Australian journal of Basic and Applied Sciences*, **2**(3): 447- 457.

Tavakoli Kakhki, H. R., Kazemi, M. & Tavakoli, H. (2008). Analysis of seed size effect on seedling characteristics of different type of wheat (*Triticum aestivum* L.) cultivars. *Asian Journal of Plant Science*, **7**(7): 666-671.

Vesquez, P., Holguin, G., Puente, M. E, Lopez-Cortes, A. & Bashan, Y. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils*, **30**: 460-468.