



Effect of Humic Acid on Germination, Growth, and Photosynthetic Pigments of *Medicago sativa* L. under Salt Stress

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Authors

Sofi A.¹ MSc,
Ebrahimi M.* PhD,
Shirmohammadi E.² MSc

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*Department of Range and Watershed Management, Faculty of Water and Soil, University of Zabol, Zabol, Iran

¹Department of Range and Watershed Management, Faculty of Water and Soil, University of Zabol, Zabol, Iran

²Department of Soil Engineering, Faculty of Water and Soil, University of Zabol, Zabol, Iran

Correspondence

Address: Department of Range and Watershed Management, Faculty of Water and Soil, University of Zabol, Zabol, Iran

Phone: +98 (54) 32232600

Fax: +98 (54) 32232600

maebrahimi2007@uoz.ac.ir

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ABSTRACT

Aims Salt stress is one of the most important environmental stresses that cause to reduce biological function in plants. In this context, appropriate biotech is needed that would not only improve the productivity of the product but also improve the soil. Regarding the problem, the use of humic acid in addition to the positive effects on soil properties, it is useful in terms of economic, environmental, and social aspects and can be an appropriate alternative to chemical fertilizers.

Materials & Methods The present research was conducted as a factorial experiment in a completely randomized design. The first factor was humic acid (0, 0.009 mg Li⁻¹). The second factor was salt stress, which was applied at three levels of 2, 6, and 12 dS m⁻¹. In each treatment, seed germination, seed vigor index, growth traits (allometric coefficient, radicle and pedicle length, total dry, and fresh weight), and photosynthetic contents of *Medicago sativa* L. were measured.

Findings The findings showed that under salt stress, humic acid increased the seed germination of *M. sativa* L. Humic acid was effective in increasing the plant growth. In connection with the photosynthetic contents, the humic acid showed positive effects, especially in terms of 2 and 12 dS m⁻¹ salinity levels, respectively.

Conclusion In general, humic acid had high impact on reducing the negative impacts of salt stress. Due to the fact that majority of the world's rangelands are in arid and semi-arid areas, and salt stress is one of the most important factors in reduced plant growth, more comprehensive and accurate survey in the field is recommended.

Keywords Environmental Stress; Humic Acid; Plant Growth; Seed Vigor Index

CITATION LINKS

[1] Breeding for abiotic stresses for ... [2] Impact of salinity stress on photochemical efficiency of photosystem ii, chlorophyll ... [3] Interactive effects of salinity and water stress ... [4] The Impact of water stress and salinity on water ... [5] Mechanisms of salinity ... [6] Growth responses of Secale ... [7] Influence of sodium chloride on seed ... [8] Biosaline agriculture and salinity tolerance ... [9] Morphological analysis of salt stress response ... [10] Effect of humic acid on seed germination and ... [11] Physiological response of ... [12] Effects of humic substances on plant growth ... [13] Effect of foliar-applied humic acid to dry weight and mineral nutrient uptake of maize ... [14] Effect of humic acid on root and shoot growth of two ... [15] Alfalfa ... [16] Factors influencing seed germination of ... [17] Investigation and compare the allelopathic effects for different ... [18] Solid matrix priming improves seedling ... [19] Effects evaluation of salt stress on germination ... [20] PGPR-induced defense responses in the tea plant against ... [21] Copperenzymes in isolated chloroplasts ... [22] Effect of light, salinity, and temperature ... [23] Changes in fruit yield and quality in response to ... [24] Study of the effects of salinity on growth and development ... [25] The effect of humic acid fertilizer on quantitative characteristics ... [26] Assessment of germination indicas for *Vicia monantha* under ... [27] Physiological effects of humic substances on ... [28] Lignite-derived humic acid effect on growth of wheat plants ... [29] Humic acid effect on catalase activity and the generation of reactive ... [30] Investigation effect of humic acid ... [31] Action of humic acid on promotion of cucumber shoot ... [32] Preliminary study on the effects of foliar ... [33] Nutritional value, functional properties and ... [34] Physiological responses of three maize ... [35] Influence of plant-growth-promoting bacteria ... [36] Effect of foliar application of N and ... [37] Mitigating activity of humic substances: Direct influence on biota. In: Perminova IV, Hatfield K, Hertkorn N, editors. Use of humic substances to remediate polluted environments: From theory ...

Introduction

Plants are exposed to a combination of environmental stresses including water shortages, high water, temperature changes, salinity, and lack of nutrients.^[1,2]

Environmental stresses are usually divided into two categories: Biological and abiotic stresses. Among physicochemical stress, salinity and temperature have been more extensive in the world, and therefore, they have been considerably studied.^[3]

Salinity is one of the main limitations affecting plant production in all over the world particularly in arid and semi-arid regions.^[4,2] Salinity may suppress plant growth, germination, plant quality, available water, and cause toxicity to the most plants effects include reducing the available water for the plants, poisoning by certain toxic ions, nutritional malformations, growth retardation, and product quality.^[1,5,6]

Studies have showed that seeds germination may decrease as the salinity increases.^[7,6]

Growth and development of plants starts from seed germination, and for their survival, the buds of the plant must comply with environmental conditions and grow in soil. Furthermore, germination is the most sensitive stage of the plant life, especially for the seedling growth. If the plants can successfully undergo these steps, it can survive.^[8,9] Since the salt stress causes the nutritional imbalance in the plant using the fertilizer could improve plant growth. Organic fertilizers can be considered as one of the new methods to increase plant performance.^[10] Due to the organic acids benefits to both plant and soil, it has been increasingly used in arable and natural lands. Organic acids could improve plant quality and productivity as well as soil physical, chemical, and biological properties. They are also efficient in increasing production and improving the quality of the plants due to their quasi-hormonal compounds. Using the natural or synthetic fertilizers with the organic basis is a new method for reducing the environmental stress associated with increased plant performance.^[11]

In this context, humic acid is one of this compounds that derived from humus and

other natural resources, exhibits no harmful environmental effect on the improvement of the performance can notably and effectively be used in changing the environment.^[12,13]

This acid can have positive effects on plant growth and increases the absorption of nitrogen, potassium, calcium, magnesium, and phosphorus by the plant.^[14]

In this context, one of the most important palatable forage plants for livestock is *Medicago sativa* L. It is rich in protein and mineral materials. Studies have shown that *M. sativa* L. is moderately salt tolerant, so that salinity more than 2 dS m⁻¹ decreases its growth and performance.^[15] We contrast humic acid and salt stress treatments to address the following question: How does humic acid affect the *M. sativa* L. properties under salt stress?

The objective of this study included: The study of the effect of humic acid on the germination, growth, and photosynthetic pigments of *M. sativa* L. under salt stress.

Materials & Methods

This research was conducted as a factorial experiment in a completely randomized design with four replications. Before the experiment, seeds were disinfected using the 3% sodium hypochlorite solution and then rinsed several times with distilled water. Then, the seeds were disinfected with 2% benomyl fungicide solution and again were rinsed with distilled water.^[10] This was done to prevent the attack of fungi. The seeds were prepared from Isfahan Pakan Bazr Co., Iran. All Petri dishes were disinfected with alcohol and were placed in an autoclave.

In each Petri dish, 10 numbers of seeds were set. The treatments included in this study were humic acid fertilizer with concentrations of 0 or 0.009 mg Li⁻¹ and salinity at three levels of 2, 6, or 12 dS m⁻¹, which were used in four replications. The humic acid was prepared from Green Co., Iran. Whatman filter paper was first placed in each Petri dish (diameter of 7.5 cm) and seeds were transferred to Petri dishes. In each Petri dish, the 8 ml solution was added. Petri dishes were closed with parafilm to

prevent evaporation, and then they were transferred to the germinator devices (temperature 25°C, humidity 70%, 16 h of light, and 8 h of darkness).

The germinated seeds counted 24 h after transferring them into the Petri dishes.^[16] Germination seeds were daily counted and recorded,^[17] until the germination was completed. On the 6th day, the radicle and pedicle length, and the dry and fresh weights of the seedlings were measured. When the number of the germinated seeds was fixed in the 14th day, some of the germination properties such as germination rate, percentage, and seed vigor index were measured according to equations (1) to (3):

$$SG = \sum Ni / Di \quad (1)$$

In the equations, SG, Ni, and Di are germination rate, number of germinated seeds in each day, and counted day, respectively.^[18]

$$GP = (n/N) 100 \quad (2)$$

Where, GP is germination percentage, n is total number of the germinated seeds, and N is total number of the germinated seeds in each Petri dish.^[19]

$$SVI = (\text{mean of initial stem length} + \text{the mean of initial root length}) \times \text{viability} \quad (3)$$

The seedling vigor index was determined at the end of growing trial after calculating the pedicle and radicle lengths.^[20] In this respect, viability is the final germination percentage. Allometric coefficient was obtained through calculating the radicle length to pedicle length ratio based on equation (4):

$$\text{Allometric coefficient} = \frac{\text{radicle length}}{\text{pedicle length}} \quad (4)$$

In each Petri dish, the radicle and pedicle lengths of plant were calculated using a caliper. The samples were washed with distilled water to determine the dry weight. The radicles and pedicles were placed in the oven (model: Dena-Iran) in 70°C for 48 h. Then, the dry weights of radicle and pedicle were determined.

To measure chlorophyll a, b, total chlorophyll contents, and carotenoids, 100 mg of fresh tissue was pulverized inside a porcelain mortar with 5 ml of 80% acetone and then centrifuged. The solution was transferred to centrifuge tubes, and the remnant in the

mortar was washed twice with 5 ml of 80% acetone, the solution of which was added to the tubes. After that, the tubes were centrifuged (10 min and 6000 rpm) and the solution was set to a 250 ml flask. The volume of solution was adjusted to 25 ml with 80% acetone. Chlorophyll contents were measured at wavelengths of 470, 663, and 645 nm using spectrophotometer (WPA-S2000).^[21] The contents of chlorophyll a, b and carotenoids were measured according to the equations (5) to (7). Total chlorophyll was calculated by sum of chlorophyll a and b.^[21]

$$\text{Chlorophyll a} = \frac{(19.3 \times A_{663} - 0.86 \times A_{645})}{V/100 W} \quad (5)$$

$$\text{Chlorophyll b} = \frac{(19.3 \times A_{645} - 3.6 \times A_{663})}{V/100 W} \quad (6)$$

$$\text{Carotenoids} = \frac{1000 (A_{470} - 3.27 (\text{mgchl.a})^{-1.04} (\text{mgchl.b})/227)}{V} \quad (7)$$

V = Volume of filtrated solution (upper solution of centrifuges)

A = Absorption of light at wavelengths of 663, 645, and 470 nm

W = Wet weight of sample (g)

The data were statistically analyzed using the SPSS version 18. The statistical processing was mainly conducted by analysis of variance (ANOVA), and the normality of data was tested using Kolmogorov–Smirnov. Equality of variance among treatments was tested using Levene's test for homogeneity of variance. Duncan test *post hoc* analysis was performed to define which specific mean pairs were significantly different.

Findings

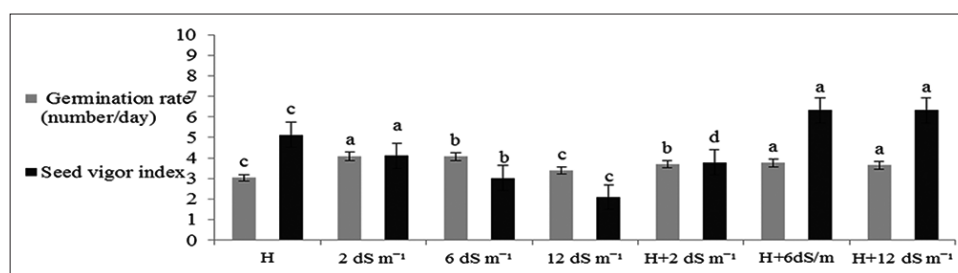
Effect on germination indices

The results of ANOVA of data [Table 1] showed that the main effects of the humic acid, salt stress and interaction effects of humic acid and salt stress on the germination rate and seed vigor index were significant ($P < 0.01$). Although main effect of salt stress and humic acid had no significant effect on the germination percentage, the results of interaction effects of humic acid and salt stress [Figure 1] showed that humic acid had a significant effect on the germination rate under salt stress conditions ($P < 0.01$). The

Table 1: ANOVA of seed germination and seed vigor index of *M. sativa* L. under different salt stress and humic acid treatments

Source of variation	Df	Mean square		
		Germination rate	Germination percentage	Seed vigor index
Humic acid	3	0.45**	26.56n.s	6.26**
Salt stress	3	1.59**	143.22n.s	7.34**
Humic acid × salt stress	9	0.34**	140.45*	5.93**
Error	48	0.09	54.68	1.47
CV (%)		7.73	7.80	10.80

** $P < 0.01$, * $P < 0.05$, n.s $P > 0.05$. ANOVA: Analysis of variance, *M. sativa*: *Medicago sativa*

**Figure 1:** Effects of humic acid and salt stress on seed germination rate and vigor index of *Medicago sativa* L. Error bars represent standard error of the mean

highest germination rate was measured in the treatment of H+6 dS m⁻¹ and the lowest value was calculated in the H treatment. The same result was observed for the germination percentage [Figure 2]. The highest and lowest germination percentage was measured in the treatment of H+6 dS m⁻¹ and the H treatments, respectively [Figure 2]. Germination percentage in the treatments of H+6 and H+12 dS m⁻¹ was the same [Figure 2]. Application of humic acid increased seed germination indices of *M. sativa* L. under salt stress. The results of interaction effects of humic acid and salt stress on seed vigor index of the plant showed that humic acid resulted in a significant increase in plant seed vigor under salt stress [Figure 1]. The highest seed vigor index was measured in the treatments of H+6 and H+12 dS m⁻¹, respectively, and the lowest value related to H+2 dS m⁻¹ treatment [Figure 1].

Effect on growth traits

The results of the ANOVA of data [Table 2] showed that the main effect of the use of humic acid, different levels of salt stress and the interaction effect of humic acid and

salt stress compared with the H treatment had significant effects on the growth of *M. sativa* L. ($P < 0.01$). The results of interaction effects of humic acid and salt stress showed that humic acid increased radicle length of the plant under salt stress, so that maximum radicle length was measured in the treatments of H+12 dS m⁻¹ and the lowest value was measured in the H treatment [Figure 3]. The highest pedicle length was observed in the treatments of H+6 and H+12 dS m⁻¹, and the lowest value was observed in the H treatment [Figure 3]. The highest and lowest allometric coefficient was obtained in the H+6 and H+2 dS m⁻¹ treatments, respectively [Figure 4]. Similar results were observed for the fresh and dry weight of the plants. The highest and lowest fresh and dry weights were observed in the treatments of H+12 and H+2 dS m⁻¹, respectively [Figure 4].

Effect on photosynthetic pigments

The results of the ANOVA of data [Table 3] showed that the main effect of the use of humic acid, different levels of salt stress and the interaction effect of humic acid and salt stress had significant effects on the photosynthetic contents of *M. sativa* L.

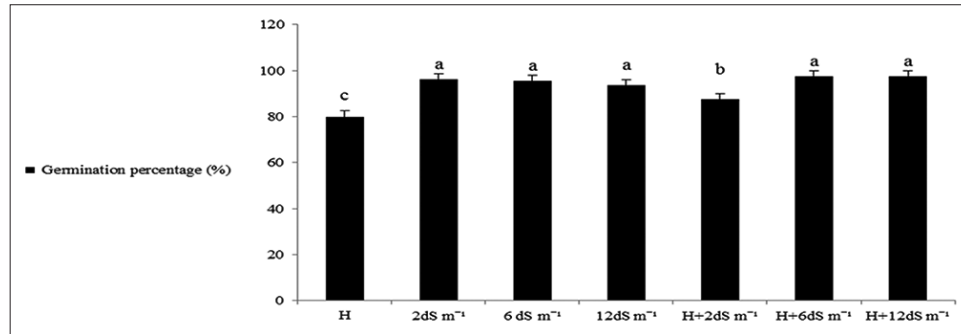


Figure 2: Effects of humic acid and salt stress on seed germination percentage of *Medicago sativa* L. Error bars represent standard error of the mean. H=humic acid

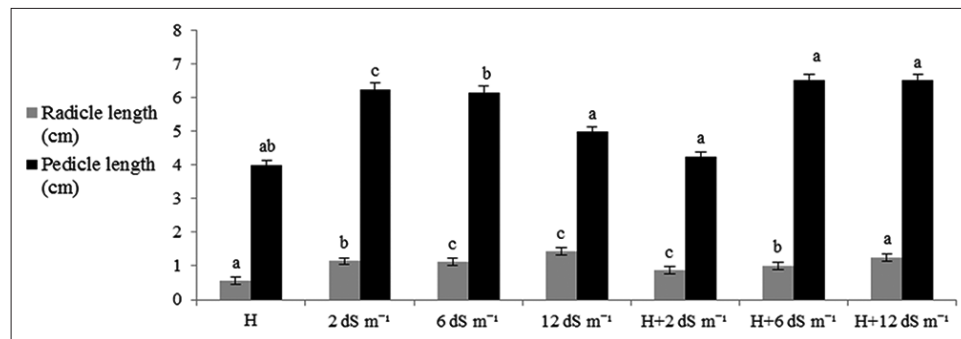


Figure 3: Effects of humic acid and salt stress on the radicle and pedicle lengths of *M. sativa* L. Error bars represent standard error of the mean. H=humic acid

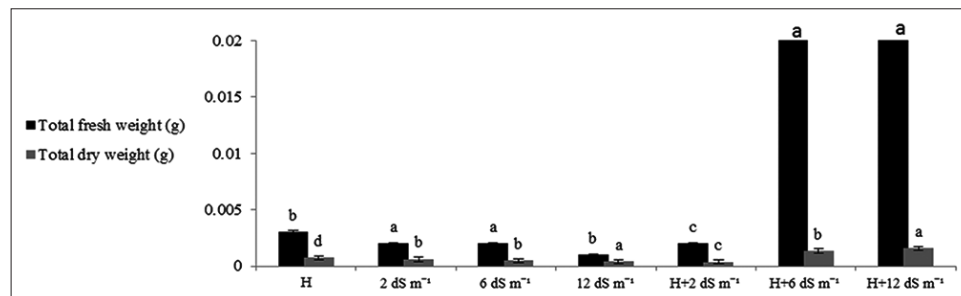


Figure 4: Effects of humic acid and salt stress on the total fresh and dry weights of *Medicago sativa* L. Error bars represent standard error of the mean

($P < 0.01$). The results of interaction effects of humic acid and salt stress showed that the maximum effect of humic acid in chlorophyll a was in the H+2 dS m⁻¹ treatment [Table 4]. While the highest amount of chlorophyll b was observed in the treatment of H+12 dS m⁻¹. The lowest chlorophyll b content was measured in the treatments of H+2 and H+6 dS m⁻¹, respectively. The maximum values of carotenoid and total chlorophyll were measured in the H+6 dS m⁻¹ treatment, and lowest amounts related to

the H and H+2 dS m⁻¹ treatments [Table 4]. The results of this study showed that humic acid under salt stress had different effects on the photosynthetic pigments of *M. sativa* L. [Figure 5].

Discussion

In general, the results of present study showed that salt stress reduced severely seed germination and plant growth while under stress condition humic acid increased seed germination. In this context, Zia and Khan

Table 2: ANOVA of some growth traits of *M. sativa* L. under different salt stress and humic acid treatments

Source of variation	Df	Mean square				
		Radicle length	Pedicle length	Allometric coefficient	Total fresh weight	Total dry weight
Humic acid	3	0.35**	6.64**	40.71**	0.00074**	0.00001**
Salt stress	3	2.73**	7.08**	73.97**	0.00063**	0.00001**
Humic acid × Salt stress	9	0.80**	4.77**	24.53**	0.00058**	0.00001**
Error	48	0.05	1.43	5.5	0.00001	0.00001
CV (%)		2.72	10	10.8	0.31	2.5

** $P < 0.01$. ANOVA: Analysis of variance, *M. sativa*: *Medicago sativa*

Table 3: ANOVA of the photosynthetic pigments of *M. sativa* L. under different salt stress and humic acid treatments

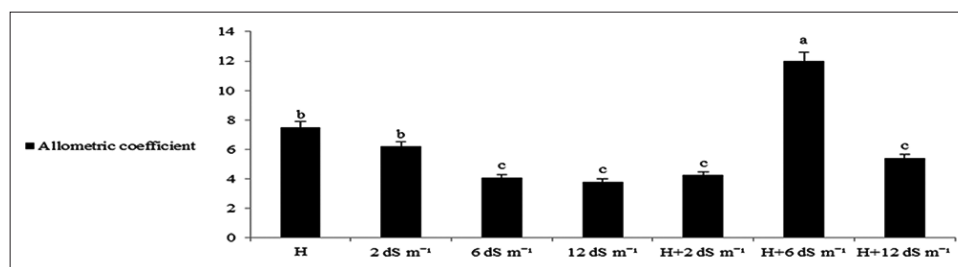
Source of variation	Df	Mean square			
		Chlorophyll a	Chlorophyll b	Carotenoid	Total chlorophyll
Humic acid	3	0.18**	0.15**	0.001**	0.20**
Salt stress	3	0.86**	0.22**	0.001**	1.43**
Humic acid × Salt stress	9	0.97**	0.5**	0.002**	1.20**
Error	48	0.003	0.0004	0.0002	0.002
CV (%)		2.72	11	11.78	8.39

** $P < 0.01$. ANOVA: Analysis of variance, *M. sativa*: *Medicago sativa*

Table 4: Effects of humic acid and salt stress on the photosynthetic pigments of *M. sativa* L.

Characteristic (mg g ⁻¹ fresh weight)	H	2 dS m ⁻¹	6 dS m ⁻¹	12 dS m ⁻¹	H + 2 dS m ⁻¹	H + 6 dS m ⁻¹	H + 12 dS m ⁻¹
Chlorophyll a	0.07 ± 0.03a	0.05 ± 0.00b	0.04 ± 0.00bc	0.03 ± 0.00c	0.29 ± 0.03a	0.10 ± 0.03b	0.12 ± 0.03b
Chlorophyll b	0.12 ± 0.01a	0.04 ± 0.00b	0.01 ± 0.00c	0.01 ± 0.00c	0.08 ± 0.01c	0.08 ± 0.01c	0.32 ± 0.01a
Carotenoid	0.03 ± 0.003b	0.01 ± 0.003b	0.01 ± 0.003b	0.01 ± 0.003b	0.03 ± 0.003b	0.05 ± 0.003a	0.04 ± 0.003a
Total chlorophyll	0.03 ± 0.003b	0.02 ± 0.003b	0.01 ± 0.003b	0.01 ± 0.003b	0.03 ± 0.003b	0.05 ± 0.003a	0.04 ± 0.003ab

Values within a row followed by the same letters are not significantly different ($P < 0.05$, means ± SE). H = humic acid. ANOVA: Analysis of variance, *M. sativa*: *Medicago sativa*

**Figure 5:** Effects of humic acid and salt stress on the allometric coefficient of *Medicago sativa* L. Error bars represent standard error of the mean. H=humic acid

studied the effect of salinity on germination of *Limonium stocksii*.^[22] They reported with increasing salinity, seed germination

decreased. Chachar *et al.* studied the effect of NaCl on germination and seedling growth of cotton and showed that an increase in salinity

reduced seed germination and seedling growth.^[7] Humic substances are produced through decomposition of organic matter and as fertilizer, improve soil structure and microorganisms in the soil.^[23] In fact, by increasing the osmotic pressure resulting from increasing salinity in the environment, on the one hand, water absorbing stage of the seed is disturbed and, on the other hand, the presence of high concentrations of anions and cations (notably sodium and chloride) result in toxic environment and prevent seed germination.^[24] Humic acid due to having a low molecular weight is quickly absorbed by the seed and increases the absorption of nutrients such as nitrogen and phosphorus and thus stimulate germination of the plants.^[10]

The results of the present study showed that under 6 dS m⁻¹ salinity stress, humic acid increased the germination indices of *M. sativa* L., while the H treatment reduced the plant germination. Chachar *et al.* found that humic acid reduced the negative effects of salinity on seed germination and resulted in the early growth of cotton and use of this organic material can increase seedling establishment and final yield of the plant.^[7]

In the present study, it was observed that humic acid enhanced the seed vigor index of the plant under salt stress. Sofi *et al.* reported a similar reduction of seed vigor index of *Trifolium alexandrinum* under salinity stress, while humic acid increased the seed vigor index of the plant.^[25] Seed vigor index is a function of the seed germination percentage and is the average length of seedling.^[25] Gholami *et al.* showed that salt stress decreased seed vigor index of *Vicia monantha*.^[26] The mechanism of action of humic substances on stimulation of germination of different plants is not clearly known, but some studies showed two effects including direct effect on production and performance of plant hormones notably Gibberlic acid^[27] and indirect effect on better absorption of the nutrients,^[12] and therefore, plant growth. Humic acid increases seed vigor by increasing the nitrogen and calcium content of seedlings, nitrogen, and potassium

of the radicle.^[10]

In this study, the use of humic acid increased length of the radicle and pedicle, fresh and dry weight of *M. sativa* L. In this regard, Tahir *et al.* showed that humic acid has hormone-like properties and increased root volume, and thus, increased the nutrient uptake of the plants.^[28] On the other hand, due to the high cations exchange capacity, humic acids may provide useful elements and dispose toxic elements and heavy metals in the root of plants. Cordeiro *et al.* investigated the effect of humic acid on root growth of *Zea mays* and found humic acid with 3 mmol concentration in the presence of low and high concentrations of nitrate can cause root development of *Z. mays* and increase 1`fresh and dry weight of the plant.^[29] The reason for the increase of fresh and dry weight of the plant in the presence of humic acid is that humic acid increases chlorophyll contents and consequently, increases photosynthesis and dry matter produced in the plant. In other words, it can be said that humic acid with hormone-like effects increases the root growth and dry root weight.^[10] Ebrahimi and Miri reported that the humic acid increased the yield of *Plantago ovata* due to enhancing stress resistance and increasing availability of primary nutrients to the plant.^[30] The used humic acid in the present study includes the nutrients such as nitrogen, potassium, phosphorous, and iron which have effective impacts in the activity of ATPase in the plant. The results of the experiments carried out by Veronica *et al.* showed that the use of humic acid causes a significant increase in growth of the cucumber and this was associated with increase in activity of H⁺-ATPase.^[31] Furthermore, an increase in stalk nitrate concentrations and its decrease in the roots were observed. These changes occurred with a significant increase in the concentration of cytokines and polyamines in the cucumber stem and reduction in the root. The researchers showed that the positive effect of humic substances on the development of stem of plant could be directly related to the effect of nitrate on the concentration of cytokines and polyamines in the stem.

The results showed that humic acid increased the photosynthetic contents of *M. sativa* L. in salt stress conditions compared with the H treatment. The most important biological effects of humic acid on the plants include stimulating the seed germination and plant growth,^[28] increase of plant biomass, nitrogen accumulation, and the absorption of nutrients.^[13] By activating the physiological processes, chlorophyllization increases and consequently, photosynthesis improves and plant growth increases. Sofi *et al.* reported that humic acid increased growth, photosynthetic pigments, and performance of *T. alexandrinum*.^[25] Perhaps increase in chlorophyll contents of the plant occurs due to the cytokinin like properties of the material that reduce the damage of the chloroplasts^[27] and increases root growth and the amount of chlorophyll and photosynthesis pigments in leaves.^[32] Carotenoids act as antioxidants and protect the chlorophylls in the plants.^[33] Carotenoids are plant secondary metabolites that protect the plants from the oxidative stress by eliminating free radicals.^[34,35] Delfine *et al.* reported that the humic acid by providing the plant with higher water and nutrients increase the producing the chlorophyll a, b and carotenoid and to make the photosynthetic materials easier.^[36] According to Nardi *et al.* humic acid through positive physiological effects, including effects on the metabolism of plant cells and increase in concentrations of chlorophyll increases the life of the photosynthesis tissues and improves plant performance.^[27] In other words, humic substances can show anti-stress effects.^[37] Humic substances may increase nutrient uptake and reduce toxicity of some absorbed elements. Hence, it can be said that using the humic substances can be improve plant growth under salt stress.^[24]

Conclusion

The results of present study showed that humic acid affects the germination, growth, and photosynthetic pigments of *M. sativa* L. under salt stress. Among the different concentrations of salinity, humic acid had the highest effect on the salt stress of 6

and 12 dS m⁻¹. Application of humic acid can improve quantity and quality of plants, reduces the effects of salt stress and also decreases the use of chemical fertilizers and environmental pollution. Moreover, since these types of fertilizers used in the low amounts, they can be economical. In general, the results of this study showed that the effects of humic acid on *M. sativa* L. are positive in terms of it being used in the restoration and improvement of rangelands. However, the question as to what extents can the humic acid promotes the plant's resistance to salt stress effects needs further research. Therefore, a more comprehensive and accurate survey and study in the field is recommended. Furthermore, it is suggested different concentrations of humic acid on other salt-sensitive plants are studied.

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References

- 1- Witcombe JR, Hollington PA, Howarth CJ, Reader S, Steele KA. Breeding for abiotic stresses for sustainable agriculture. *Philos Trans R Soc Lond B Biol Sci.* 2008;363(1492):703-16.
- 2- Ranjbar Fordoei A, Dehghani Bidgholi R. Impact of salinity stress on photochemical efficiency of photosystem ii, chlorophyll content and nutrient elements of nitere bush (*Nitraria schoberi* L.) Plants. *J Rangel Sci.* 2016;6(1):1-9.

- 3- Omami EN, Hammes PS. Interactive effects of salinity and water stress on growth, leaf water relations, and gas exchange in amaranth (*Amaranthus* spp.). *N.Z J Crop Hortic.* 2006;34(1):33-44.
- 4- Salarian M, Alizadeh A, Davary K, Ansari H. The Impact of water stress and salinity on water requirement and crop coefficient of greenhouse bell pepper. *Adv Environ Biol.* 2014;8(19):88-99.
- 5- Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev Plant Biol.* 2008;59:651-81.
- 6- Araghi Shahri SM, Dianati Tilaki GhA, Behtari B, Alizadeh MA. Growth responses of *Secale cereale* and *S. ceremont* to priming treatments under salinity stress. *J Rangel Sci.* 2015;5(3):202-11.
- 7- Chachar QI, Solangi AG, Verhoef A. Influence of sodium chloride on seed germination and seedling root growth of cotton (*Gossypium Hirsutum* L.). *Pak J Bot.* 2008;40(1):183-97.
- 8- Öztürk M, Waisel Y, Khan MA, Görk G. Biosaline agriculture and salinity tolerance in plants. Basel: Birkhäuser Basel; 2009.
- 9- Memon SA, Hou X, Wang LJ. Morphological analysis of salt stress response of pak Choi. *Environ Agric Food Chem.* 2010;9(1):248-54.
- 10- Ebrahimi M, Miri E. Effect of humic acid on seed germination and seedling growth of *Borago officinalis* and *Cichorium intybus*. *ECOPERSIA.* 2016;4(1):1239-49.
- 11- Rezaei MA, Khavari Nejad R, Fahimi H. Physiological response of cotton (*Gossypium hirsutum* L.) plants to soil salinity. *Pajouhesh and Sazandegi.* 2004;62(4):81-9. [Persian]
- 12- Bulentasik B, Turan A, Celik H, Vahap Katkat A. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihi) under conditions of salinity. *Asian J Crop Sci.* 2009;1(2):87-95.
- 13- Çelik H, Katkat AV, Bülent Aşık B, Turan MA. Effect of foliar-applied humic acid to dry weight and mineral nutrient uptake of maize under calcareous soil conditions. *Commun Soil Sci Plant Anal.* 2010;42(1):29-38.
- 14- Sabzevari S, Khazaie HR, Kafi M. Effect of humic acid on root and shoot growth of two wheat cultivars (*Triticum aestivum* L.). *J Water Soil.* 2009;23(2):87-94. [Persian]
- 15- Fu C, Hernandez T, Zhou C, Wang ZY. Alfalfa (*Medicago sativa* L.). *Methods Mol Biol.* 2015;1223:213-21.
- 16- Gorai M, Gasmi H, Neffati M. Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae). *Saudi J Biol Sci.* 2011;18(3):255-60.
- 17- Farajollahi A, Tavili A, Gholinejad B, Darini J, Pouzesh H. Investigation and compare the allelopathic effects for different tissues of *Peganum harmala* in different amounts on the *Bromus tectorum* germination and growth characteristics. *ECOPERSIA.* 2013;1(1):53-62.
- 18- Mereddy R, Hallgren S, Wu L, Wu Y, Conway KE. Solid matrix priming improves seedling vigor Okra seeds. *Proc Okla Acad Sci.* 2000;80:33-7.
- 19- Behbodian B, Lahouti M, Nezami A. Effects evaluation of salt stress on germination of chickpea varieties. *J Agric.* 2005;28(2):127-37. [Persian]
- 20- Saravanakumar D, Vijayakumar Ch, Kumar N, Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. *Crop Prot.* 2007;26(4):556-65.
- 21- Arnon DI. Copperenzymes in isolated chloroplasts, Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 1949;24(1):1-5.
- 22- Zia S, Khan MA. Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*. *Can J Bot.* 2004;82(2):151-7.
- 23- Ozdamar Unlu H, Unlu H, Karakurt Y, Padem H. Changes in fruit yield and quality in response to foliar and soil humic acid application in cucumber. *Sci Res Essays.* 2011;6(13):2800-3.
- 24- Khaleghi E, Ramin AA. Study of the effects of salinity on growth and development of *Lawans* (*Lolium perenne* L.), *Festuca arundinaceae* and *Cynodon dactylon*. *J Water Soil Sci.* 2005;9(3):57-67. [Persian]
- 25- Sofi A, Ebrahimi M, Shirmohammadi E. The effect of humic acid fertilizer on quantitative characteristics of clover. *Proceedings of 2nd International Conference on Sustainable Development, Strategies and Challenges with Focus on Agriculture, Natural Resources, Environment and Tourism.* Tabriz: Permanent Secretariat of the International Conference on Sustainable Development, Solutions and Challenges; 2016. pp. 1-7. [Persian]
- 26- Gholami P, Ghorbani J, Ghaderi Sh, Salarian F, Karimzadeh A. Assessment of germination indicas for *Vicia monantha* under salinity and drought stresses. *Rangeland.* 2010;4(1):1-11. [Persian]
- 27- Nardi S, Pizzeghello D, Muscolo A, Vianello A. Physiological effects of humic substances on higher plants. *Soil Biol Biochem.* 2002;34(11):1527-36.
- 28- Tahir MM, Khurshid M, Khan MZ, Abbasi MK, Kazmi HM. Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere.* 2011;21(1):124-31.
- 29- Cordeiro FC, Catarina CS, Silveira V, De Souza SR. Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (*Zea mays*). *Biosci Biotechnol Biochem.* 2011;75(1):70-4.
- 30- Ebrahimi M, Miri Karbasak E. Investigation effect of humic acid on germination, seedling growth and photosynthesis pigments of medicinal plant *Isabgol* (*Plantago ovata* Forssk). *Iran J Seed Sci Res.* 2016;3(3):35-46. [Persian]
- 31- Mora V, Bacaicoa E, Zamarreño AM, Aguirre E, Garnica M, Fuentes M, et al. Action of humic

- acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to shoot distribution of cytokinins, polyamines and mineral nutrients. *J Plant Physiol.* 2010;167(8):633-42.
- 32- Ferrara G, Pacifico A, Simeone P, Ferrara E. Preliminary study on the effects of foliar applications of humic acids on 'Italia' table grape. *Journal International des Sciences de la Vigne et du Vin.* 2008;42(2):79-87.
- 33- Ramadan MF. Nutritional value, functional properties and nutraceuticals applications of black cumin (*Nigella sativa* L.): An overview. *Food Sci Technol.* 2007;42(10):1208-18.
- 34- Efeoglu B, Ekmekci Y, Cicek N. Physiological responses of three maize cultivars to drought stress and recovery. *S Afr J Bot.* 2009;75(1):34-42.
- 35- Delshadi S, Ebrahimi M, Shirmohammadi E. Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of *Onobrychis sativa* L. under drought stress. *J Plant Interact.* 2017;12(1):200-8.
- 36- Delfine S, Tognetti R, Desiderio E, Alvino A. Effect of foliar application of N and humic acids on growth and yield of durum wheat. *Agron Sustain Dev.* 2005;25(2):183-91.
- 37- Kulikova NA, Stepanova EV, Koroleva OV. Mitigating activity of humic substances: Direct influence on biota. In: Perminova IV, Hatfield K, Hertkorn N, editors. Use of humic substances to remediate polluted environments: From theory to practice: Proceedings of the NATO advanced research workshop on use of humates to remediate polluted environments: From Theory to Practice, held in Zvenigorod. Berlin: Springer Science & Business Media; 2005.

تأثیر اسید هیومیک بر جوانه‌زنی، رشد و رنگیزه‌های فتوسنتزی در *Medicago sativa* L. تحت تنش شوری

اعظم صوفی MSc

گروه مرتع و آب‌خیزداری، دانشکده آب و خاک، دانشگاه زابل، زابل، ایران

مهدیه ابراهیمی PhD*

گروه مرتع و آب‌خیزداری، دانشکده آب و خاک، دانشگاه زابل، زابل، ایران

ابراهیم شیرمحمدی MSc

گروه مهندسی خاک، دانشکده آب و خاک، دانشگاه زابل، زابل، ایران

چکیده

اهداف: تنش شوری یکی از مهم‌ترین تنش‌های محیطی است که باعث کاهش عملکرد زیستی گیاهان می‌شود. برای جلوگیری از این مساله، نیاز به روش زیستی مناسب وجود دارد تا نه‌تنها حاصلخیزی محصول افزایش یابد، بلکه خاک نیز اصلاح شود. با توجه به این مشکل، استفاده از اسید هیومیک علاوه بر تاثیرات مثبت بر خصوصیات خاک، از جنبه‌های اقتصادی، محیط زیستی و اجتماعی نیز مفید است و می‌تواند جایگزین مناسبی برای کودهای شیمیایی باشد.

مواد و روش‌ها: تحقیق حاضر به صورت آزمایش فاکتوریل در قالب طرح کاملاً تصادفی انجام شد. فاکتور اول اسید هیومیک (صفر و ۰/۰۰۹ و میلی‌گرم در لیتر) بود. فاکتور دوم تنش شوری بود که در سه سطح ۲، ۶ و ۱۲ دسی‌زیمنس بر متر اعمال شد. در هر تیمار، جوانه‌زنی بذر، شاخص بنیه بذر، خصوصیات رشد (ضریب آلومتری، طول ریشه‌چه و ساقه‌چه، وزن خشک و تر کل) و محتویات فتوسنتزی *M. sativa* L. اندازه‌گیری شد.

یافته‌ها: تحت تنش شوری، اسید هیومیک، جوانه‌زنی بذر *M. sativa* L. افزایش یافت. اسید هیومیک در افزایش رشد گیاه موثر بود. در ارتباط با محتویات فتوسنتزی، اسید هیومیک به ویژه در سطوح ۲ و ۱۲ دسی‌زیمنس بر متر تاثیر مثبتی داشت.

نتیجه‌گیری: اسید هیومیک تاثیر زیادی در کاهش اثرات منفی تنش شوری دارد. به دلیل این که عمده مراتع دنیا در مناطق خشک و نیمه‌خشک هستند و تنش شوری یکی از فاکتورهای مهم در کاهش رشد گیاه است، ارزیابی جامع و دقیقی در این زمینه پیشنهاد می‌شود.

کلیدواژه‌ها

تنش محیطی؛

رشد گیاه؛

شاخص بنیه بذر؛

اسید هیومیک

* نویسنده مسئول

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آدرس پستی: گروه مرتع و آب‌خیزداری، دانشکده آب و خاک، دانشگاه زابل، زابل، ایران

maebrahimi2007@uoz.ac.ir