ISSN: 2538-2152; ECOPERSIA, 2021; 9(4):287-297.

Influence of humic acid on germination, morphological characteristics and photosynthesis pigments of *Trifolium alexandrium* L. under salinity stress

ARTICLEINFO

Article Type
Original Research

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How to cite this article
Sofi A., Ebrahimi M., Shirmohammadi E. Comparing dormancy breaking treatments in *Parkinsonia aculeata* seeds. ECOPERSIA 2021; 9(4): 287-297.

DOR:
20.1001.1.23222700.2021.9.4.7.9

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Article History
Received: April 30, 2021
Accepted: July 2, 2021
Published: August 20, 2021

ABSTRACT

Aim: The study was done to determine the effect of humic acid on germination, morphological properties, and photosynthesis pigments of *Trifolium alexandrium* L. under salt stress.

Methods: This study was conducted in a as a completely randomized design was humic acid (0,0.009mg Li−1). The second factor was salt stress (0,0.75,1.5,3dSm^{-1}). Seed germination, vigor index, allometric coefficient, radicle and pedicle length, totally dry and fresh weight, and photosynthetic contents were measured.

Findings: The minimum and maximum germination rate (4.00 and 4.90seed/day) and vigor index (1.79 and 6.33) were related to 3dSm^{-1} and H+3dSm^{-1} treatments, respectively. The germination percentage did not show a significant difference ($p<0.05$). The radicle and pedicle lengths, allometric coefficient, germination rate, and vigor index in the zero treatment were more than the H treatment, but they decreased with increasing salinity. The minimum and maximum radicle (0.79 and 2.30cm), pedicle lengths (0.79 and 2.55cm) and dry weight (0.0014 and 0.0026g) were related to the 3dSm^{-1} and H+3dSm^{-1} treatments, respectively. The highest chlorophyll a (0.31 mg g-1 fresh weight), carotenoid (0.07 mg. g^{-1} fresh weight) and total chlorophyll (0.48mg. g^{-1} fresh weight) were related to H+1.5dS. m^{-1}. The highest amount of chlorophyll b (0.34mg. g^{-1} fresh weight) was observed in H+3dS. m^{-1}.

Conclusion: Humic acid had different but positive effects on the plant's quantitative properties and germination characteristics in salinity stress. The use of this fertilizer in salinity stress for salinity-sensitive plants can be helpful, but a more comprehensive survey in the field is recommended.

Keywords: Abiotic stress, seedling vigor index, humic substances, total chlorophyll.

CITATION LINKS

[1] Saberali SF, Moradi MI. Effect of salinity on ... [2] Sofi A, Ebrahimi M, Shirmohammadi E. Effect of humic ... [3] Khaleda L, Park HJ, Yun DJ, Jeon JR, Kim MG, Cha JY, Kim WY. Humic acid ... [4] Sai-Kachout S, Ben Mansoura A, Jaffel A, Leclerc J.J, Rejeb MN, Ouerghi Z. The effect of ... [5] Jie Tang J, Bassham DC. Autophagy in crop ... [6] Zaremanesh H, Akbari N, Eisvand HR, Ismaili A, Feizian M. The Effect of ... [7] Bulentasik B, Turan A, Celik H, VahapKatkat A. Effects of .... [8] Ebrahimi M, Miri E. Effect of humic acid ... [9] Delshadi S, Ebrahimi M, Shirmohammadi E. Influence of ... [10] Azevedo IG, Olivares FLO, Ramos ACR, Bertolazi AA, Canellas ... [11] Canellas LP, Olivares FL, Canellas NOA, Mazzei PL, Piccolo A. Humic acids ... [12] Kaya C, Akram NS, Ashraf M, Sonmez O. Exogenous application of humic acid ... [13] Benazzouk S, Djazouli Z.E, Lutts S. Assessment of the preventive effect of ... [14] Shrestha A, Hesterman OB, Squire JM, Fisk JW, Sheaffer CC. Annual ... [15] Taylor NL. Clovers around the world, In Clover ... [16] Deepak K, Deepika S, Bhatnagar SK. Salinity .... [17] Gorai M, Gasmi H, Neffati M. Factors influencing seed ... [18] Farajollahi A, Tavili A, Gholinejad B, Darini J, Pouzesh H. Investigation ... [19] Mereddy R, Hallgren S, Wu L, Wu Y, Conway KE. Solid ... [20] Behbodian B, Lahouti M, Nezami A. Effects ... [21] Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced .... [22] Munns R, Tester M. Mechanisms of ... [23] Arnon DI. Copper enzymes in isolated chloroplasts, Polyphenol ... [24] Canellas LP, Canellas NOA, Irineu LESS, Olivares ... [25] Aguiar NO, Olivares FL, Novotny EH, Canellas LP. Changes in ... [26] Khaleghi E, Ramin AA. Study of the effects of salinity on growth ... [27] Chachar QI, Solangi AG, Verhoef A. Influence of ... [28] Ozdamar UH, Unlu H, Karakurt Y, Padem H. Changes in ... [29] Canellas LP, Canellas NOA, Soares TS, Olivares FL. Humic ... [30] Nardi S, Pizzeghello D, Muscolo A, Vianello A. Physiological effects .... [31] Araghi Shahri SM, Dianati Tilaki GA, Behtari B, Alizadeh MA. Growth ... [32] Cordeiro FC, Catarina CS, Silveira V, De Souza SR. Humic acid effect ... [33] Rubio V, Bustos R, Irigoyen ML, Cardona-Lopez X, Rojas-Triana M, Paz-Ares J. Plant hormones ... [34] Wahid A. Analysis of toxic and osmotic effects of ... [35] Kaymakanova M. Effect of Salinity on germination ... [36] Matuszak-Slamani R, Bejger R, Cieśla J, Bieganowski A, Koczańska M, Gawlik A, Gołębiowska D. Influence of ... [37] Akladious SA, Mohamed HI. Ameliorative effects of ... [38] Jing-Min Z, Shang-jun X, Mao- Peng S, Bing-Yao M, Xiu-mei C, Chunsheng L. Effect of humic acid ... [39] Samadimatin A, Hani A. Effect of ethanol and humic acid foliar spraying on ...

Introduction

Soil salinity is one factor limiting plant productivity and growth in Iran's rangelands [1,2]. The effects of salinity on plant growth depend on the plant species. A certain salinity level may lead to a decrease in the yield of a plant species, while it is not restrictive for other plants because different plants have different salinity tolerances [3]. To quantify the effect of salinity on the plant, a limit should be defined from which the plant yield decreases. The yield reduction threshold is the amount of salinity after which the yield reduction begins [4,5].

Salinity stress harms soil microbial population, soil characteristics, and nutritional balance in plants, so organic acids can reduce the adverse effects of salinity and improve the growth situation for plants [6-9]. Due to environmental considerations, using various organic acids to improve the quantitative and qualitative of plant products has become widespread. Studies have shown that tiny amounts of organic acids significantly affect the soil's physical, chemical, and biological properties and, consequently, increase plant production [10].

One of these organic acids is humic acid, which causes stable insoluble complexes with trace elements [11]. This compound can directly positively affect plant growth and increase nitrogen, potassium, calcium, and phosphorus by the plant [12, 13].

Trifolium alexandrium L. (Leguminoceae) is one of the valuable forage plants due to its high forage production potential (700-1000 kg ha^{-1}) and ability to improve soil's physical qualities and fertility (such as nitrogen fixation) [14-15]. In recent decades, berseem clover cultivation has been highly welcomed due to its rapid growth and high production of fresh forage with remarkable quality and quantity. This plant has low resistance to salinity, and its relative resistance threshold to salinity is 1.5 $dS.m^{-1}$ [16]. Due to the importance of this plant, the use of organic fertilizers can be important in reducing salinity stress. We contrast humic acid and salt stress treatments to address the following question: Does humic acid reduce the effects of salt stress on germination properties of *T. alexandrium* L. (Leguminoceae) The objective of this study included: The study of the effect of humic acid on the germination, growth, and photosynthetic pigments of *T. alexandrium* L. under salt stress. We hypothesized that the use of humic acid reduces salinity stress in *T. alexandrium* L.

Materials & Methods

Germination medium

This research was conducted as a factorial experiment in a completely randomized design (four replications). The seeds were prepared from Isfahan Pakan Bazr Co., Iran. First off, seeds were disinfected using a 3% sodium hypochlorite solution. Then, the seeds were disinfected with 2% Benomyl fungicide solution and again were rinsed with distilled water to prevent the attack of fungi [8]. All Petri dishes were disinfected with alcohol and were placed in an autoclave. In each petri dish, 10 numbers of seeds were set on Whatman filter paper (7.5 cm). The treatments were humic acid with concentrations of 0 and 0.009 $mg.l^{-1}$ and salinity at levels of 0, 0.75, 1.5, and 3 $dS.m^{-1}$. In each petri dish, an 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%, 16h light, and 8h darkness).

Calculation of plant properties

The germinated seeds counted 24 h after transferring them into the Petri dishes. Germination seeds were daily counted and recorded [17, 18] until the germination was completed. On the 6^{th} day, the radicle and pedicle length (using a caliper), the dry

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and fresh weights (using a digital balance with 0.0001 g precision) of the seedlings were measured. When the number of the germinated seeds was fixed on the 14th day, some of the germination properties were measured according to Eqs (1) to (3):

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

In the equations, SG, Ni, and Di are germination rate, number of germinated seeds each day, and counted day, respectively (19).

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

where GP is germination percentage, n is the total number of the germinated seeds, and N is the total number of the germinated seeds in each petri dish [20].

SVI = mean of initial stem length + the mean of initial root length) × viability (3)

The seedling vigor index (SVI) was determined at the end of the growing period after calculating the pedicle and radicle lengths [21]. In this respect, viability is the final germination percentage. The allometric coefficient was calculated through Eq 4 [22]:

Allometric coefficient = radicle length/pedicle length (4)

The radicles and pedicles were placed in the oven (70°C for 48h). Then the dry weights of radicle and pedicel were determined. To measure chlorophyll contents and carotenoids, 100 mg of fresh tissue was pulverized inside a porcelain mortar with 5ml of 80% acetone and centrifuged. The solution was transferred to centrifuge tubes, and the remnant in the mortar was washed twice with 5ml of 80% acetone, the solution of which was added to the tubes. After that, the tubes were centrifuged (10 min, 6000 rpm), and the solution was set to a 250 mm flask. The volume of the solution was adjusted to 25 ml with 80% acetone. Chlorophyll contents were measured at wavelengths of 470, 663, 645 nm, using a spectrophotometer (WPA-S2000) (23). The contents of chlorophyll were measured according to the Eqs. (5) to (7). Total chlorophyll was calculated by the sum of chlorophyll a and b [23].

Chlorophyll a =19.3 × A663-0.86 ×A645) V/100 W (5)

Chlorophyll b = (19.3×A645-3.6 ×A663) V/100W (6)

Carotenoides= 100(A470)-3.27 (mgchl.a)-104 (mgchl.b)/227 (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 the sample (g)

Statistical analysis

The statistical processing was mainly conducted by analyzing variance (one-way ANOVA), using SPSS version 20. The normality of data was tested using Kolmogorov–Smirnov. Homogeneity of variance among treatments was tested using Levene's test. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different.

Findings and Discussion

Effect on germination indices

The results of ANOVA (Table 1) showed that the main effects of salinity ($p<0.01$) and humic acid ($p<0.05$) and the interaction effects of salinity and humic acid ($p<0.01$) on germination rate and seed vigor index were significant. However, salinity and humic

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Table 1) Analysis of variance of seed germination and seed vigor index

Source of variation	df	Mean square		
		Germination Rate	Germination percentage	Seed vigor index
Humic acid	1	0.059*	1.56$^{n.s}$	0.22*
Salt stress	3	0.46**	1.56$^{n.s}$	3.02**
Humic acid× Salt stress	3	0.01**	1.56$^{n.s}$	0.59**
Error	24	0.02	1.56	0.06
CV (%)		3.32	1.25	14.30

** p<0.01, * p<0.05, n.s p>0.05

acid's direct and interaction effects were not significantly influential on the germination percentage (p≥0.05).

The results showed that interaction effects of humic acid and salt stress (Figure 1) had a significant effect on the germination rate under salt stress (p<0.01). The germination rate in treatment without salinity and humic acid (zero) was 4.3 Seed.day^{-1}. With the addition of humic acid, the germination rate of the plant increased (4.4 Seed.day^{-1}), but the difference in the germination rate was found to be non-significant between zero and H treatments (p>0.05). With increasing salinity up to 1.5 dS.m^{-1}, the germination rate did not decrease, which could be related to the tolerance threshold of the plant to the salinity stress (≤1.5 dS.m^{-1}), but in the 3 dS.m^{-1} treatment, the germination rate of the plant decreased significantly (p<0.01). The minimum value of seed germination rate was related to treatment 3dS.m^{-1}, and the highest (4.9 seed. day^{-1}) germination rate value was observed in H+3dS.m^{-1} treatments.

The seed vigor index in the zero treatment was 2.55, and in the H treatment, the index was 5.12, which had a significant increase (p<0.01). A significant decrease in the seed vigor index (up to 68.18%) was observed with the increasing salinity concentration from 0.75 to 3 dS.m^{-1}. However, there was no significant difference in the seed vigor index between H+3 dS.m^{-1} and H+1.5 dS.m^{-1} treatments (p>0.05). The minimum seed vigor index was measured in the treatment of 3dS.m^{-1} (1.79). The seed vigor index was maximum in the H+3dS.m^{-1} (6.33) followed by H+1.5 dS.m^{-1}(6.32) treatment. The lowest germination percentage (97.5%) of *T. alexandrium* L. was measured in the treatment of H+1.5 dS.m^{-1}. However, the germination percentage did not show significant differences among the humic acid and salt stress treatments (Figure 2).

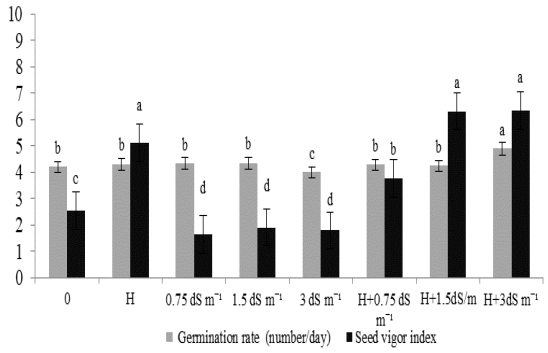

Figure 1) Effects of humic acid and salt stress on seed germination rate and vigor index. Error bars represent the standard error of the mean. H=humic acid

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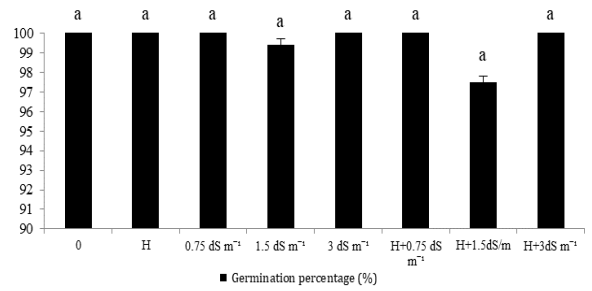

Figure 2) Effects of humic acid and salt stress on seed germination percentage. Error bars represent the standard error of the mean. H=humic acid

Plant growth decreases with increasing salinity in the environment due to physical and chemical effects or due to toxic and osmotic effects of salts in saline solution. In fact, with increasing osmotic pressure due to increased salinity in the environment, the dewatering stage of the seed is disrupted, and the osmotic pressure increases. As a result, the dewatering stage of the seed is disturbed, but the high concentration of anions and cations (especially sodium and chloride) in the environment causes seed poisoning and prevents seed germination.

The study of Canellas et al. [24] showed that germination is directly related to the amount of water absorbed by seed, and salt concentration of the medium delays germination. Salinity prevents seed germination by reducing water availability or interfering with plant growth regulators [24-26]. Chachar et al. [27] reported that increasing salinity (0, 50, 100, 150, and 200 mM) reduced cottonseed germination. In 200 mM NaCl, the germination percent was the lowest (78.7% compared to 86.8% on average for the other salinity treatments). Also, seedling growth, root length, root fresh, and dry weights were severely affected.

In the present study, the use of humic acid caused a significant increase in the germination rate of plant seeds in salinity stress conditions. Due to its low molecular weight, Humic acid is rapidly absorbed by plant seeds and increases the absorption of nutrients such as Nitrogen and Phosphorus. As a result, it stimulates the germination of plants [28]. Canellas et al. [29] showed that humic acid could reduce the adverse effects of salinity on germination and initial growth of Cotton through a decrease in the osmotic potential and increased availability of nutrients. The researchers reported that this organic matter could increase seedling establishment and ultimately increase the final yield of the plant.

The results showed a significant effect of humic acid on plant vigor under salinity stress. The mechanism of humic substances in stimulating the seed germination of plants is not known precisely, but researchers [7,30] have shown two effects: 1. Direct effect, including the production and action of plant hormones [30] and 2. The indirect effect on nutrient uptake and plant growth [7]. Humic acid increases the seed vigor index of seedlings by increasing the Nitrogen and Calcium content of seedlings [8].

Effect on growth properties

The results of ANOVA (Table 2) showed that the main effect of humic acid, salt stress, and the interaction effect of humic acid and salt stress had significant effects on the growth of *T. alexandrium* L. ($p<0.01$).

The results showed that the radicle length of the plant was 0.52 cm in the zero treatment and showed a significant increase ($p<0.01$) in the H treatment (without salinity stress). Also, a comparison of 0.75 dS m^{-1} and zero treatments showed that the radicle length in the zero treatment was less than the 0.75 $dS.m^{-1}$, which could indicate the plant's tolerance to salinity (≤1.5 $dS.m^{-1}$).

The radicle length decreased significantly with increasing salinity so that the minimum value (0.79 cm) was related to the treatment of 3 $dS.m^{-1}$ (Figure 3). In salinity stress conditions, the addition of humic acid

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Table 2) Analysis of variance of some growth properties

Source of variation	df	Mean square				
		Radicle Length	Pedicle length	Allometric coefficient	Total fresh weight	Total dry weight
Humic acid	1	5.26**	0.22**	6.34**	0.00042**	0.00001**
Salt stress	3	6.68**	3.05**	11.69**	0.00032**	0.00001**
Humic acid×Salt stress	3	2.82**	0.57**	5.01**	0.00036**	0.00001**
Error	24	0.03	0.06	0.91	0.00001	0.00001
CV (%)		2.72	10.00	10.80	0.31	2.50

** p<0.01

increased the plant's radicle length, and the maximum radicle length (2.30 cm) was related to H+3dS.m^{-1} treatment. The increasing radicle length indicates that humic acid reduces the effect of salt stress on the radicle length. (Figure 3). A similar trend was observed for the pedicle length (Figure 3). The highest pedicle length was observed in H^{+3} dS.m^{-1} (2.55 cm) which followed by zero (2.50 cm) and H (2.50 cm) treatments. The lowest value (0.79 cm) was observed in the salinity level of 3 dS.m^{-1} (Figure 3).

The allometric coefficient in treatment H (4.80) was higher than treatment zero (4.66), but there was no significant difference between the two treatments (p<0.05). With increasing salinity stress (from 0.75 to 3 dS.m^{-1}), the allometric coefficient decreased significantly so that the minimum value (0.73) was measured in the treatments of 3dS.m^{-1} (Figure 4).

We founded a similar result for the fresh and dry weights of the plant. The amount of fresh (0.039 g) and dry (0.0022 g) weights in the zero treatment was less than the H treatment, but the differences between the two treatments were not statistically significant (p<0.05). Fresh (0.0223 g) and dry (0.0014g) weights of the plant decreased gradually with the increase of salinity dosage and were minimum for the 3 dS.m^{-1} treatment (Figure 5).

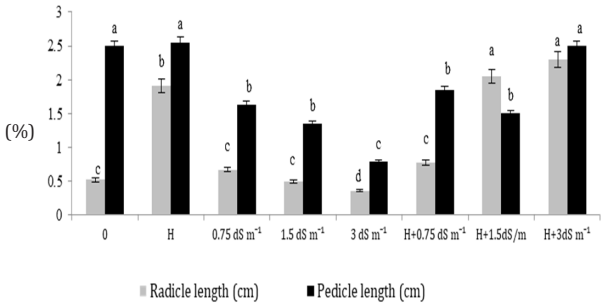

Figure 3) Effects of humic acid and salt stress on the radicle and pedicle lengths. Error bars represent the standard error of the mean. H=humic acid

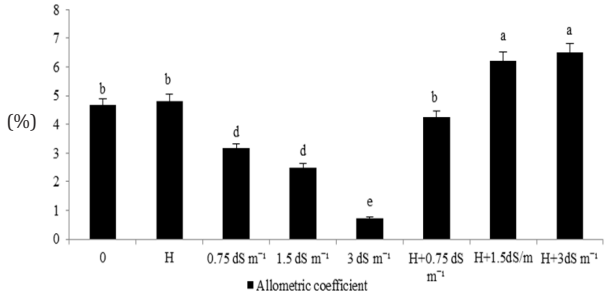

Figure 4) Effects of humic acid and salt stress on the allometric coefficient. Error bars represent the standard error of the mean. H=humic acid

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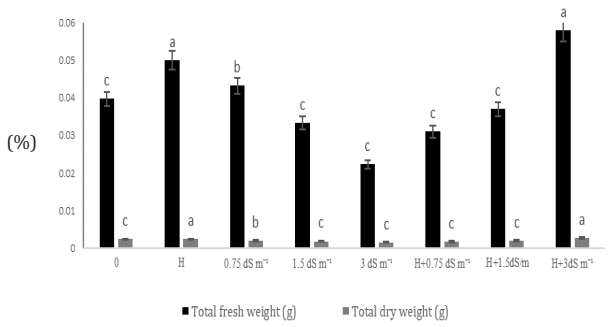

Figure 5) Effects of humic acid and salt stress on the fresh and dry weights. Error bars represent the standard error of the mean. H=humic acid

Radicle and pedicle growth reduces by salinity due to the inhibitory effects of salt on the cell division and enlargement in the growing point [29]. With increasing salinity, a reduction in the radicle and pedicle lengths was observed. With increasing salinity stress, ion toxicity resulting from increasing Na and Cl ions disrupts plants' biological and metabolic activities, ultimately reducing the growth and destruction of the plant organs [3]. Salinity stress by high concentrations of Na and Cl destroys the osmotic balance in the plant cells, and as a result, water leaves the plant tissues. Under salinity stress, excessive energy is consumed to produce organic matters. These organic substances are used to create osmotic balance, and therefore, the shoot and root weight and consequently the plant biomass is reduced [1,3]. Plant dry weight is affected both by reduced vegetative growth and reduced photosynthesis efficiency. In this regard, the root is the first organ directly stressed due to the direct absorption of elements [29].

Humic acid has hormonal-like properties, increases root volume, and thus absorbs more nutrients [31, 32]. Cordeiro et al. [32] investigated the effect of humic acid on the root growth of *Zea mays*. They showed that 3 mm humic acid in the presence of nitrate caused the development of roots and subsequently increased the fresh and dry weight of roots. Humic acid, due to increased activity of hormones involved in cell division, photosynthesis, protein synthesis, and various enzymatic reactions, and increased enzyme activity such as phosphorylase and phosphatase, increase plant yield. Also, the accelerating effect of humic substances on plant growth, primarily stem growth, may be due to root H^+-ATPase activity and root and stem nitrate distribution, which in turn leads to changes in the distribution of cytokinins and polyamines. Therefore, it affects the growth of the plant stem [33]. One of the leading causes of cellular damage during seed exposure to salt stress is reactive oxygen. Reactive oxygen accumulation in plant cells injuries cells membranes. To avoid reactive oxygen injury in cells, anti-oxidative enzymes like peroxidases, catalase, and ascorbate peroxidase are produced. All of these enzymes are triggered by humic substances under salt stress conditions (32,33).

Effect on photosynthetic pigments

The results of ANOVA (Table 3) proved that the main effect of humic acid, salt stress, and the interaction effect of humic acid and salt stress had significant effects on the photosynthetic contents ($p<0.01$).

In treatment H, the amount of chlorophyll a was 0.09 $mg.g^{-1}$ fresh weight, which significantly increased compared to the zero treatment ($p<0.01$). The amount of chlorophyll a in the treatments of zero and 0.75 $dS.m^{-1}$ was 0.07 mg. g^{-1} fresh weight, indicating that the plant's photosynthesis does not decrease at a salinity level below the tolerance threshold (Table 4). However, with increasing salinity stress, chlorophyll a showed a significant decrease ($p<0.01$). Interaction effects of humic acid and salt stress showed that the maximum effect of humic acid in chlorophyll a (0.31 mg. g^{-1} fresh weight) was in the H+1.5 $dS.m^{-1}$ treatment (Table 4). Similarly, the amounts of chlorophyll

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Table 3) Analysis of variance of the photosynthetic pigments

		Mean square			
Source of variation	**df**	**Chlorophyll a**	**Chlorophyll b**	**Carotenoid**	**Total chlorophyll**
Humic acid	1	0.29**	0.25**	0.06**	0.17**
Salt stress	3	5.35**	1.27**	0.04**	11.27**
Humic acid×salt stress	3	0.10**	0.63**	0.02**	0.81**
Error	24	0.007	0.00	0.00007	0.00
CV (%)		8.74	6.51	4.78	5.57

** p<0.01

b and carotenoid in the H treatment were more than the zero treatment. Salinity stress significantly reduced chlorophyll b and carotenoid compared to the H and zero treatments (p<0.01).

The highest amount of chlorophyll b (0.34 $mg.g^{-1}$ fresh weight) was observed in the treatment of H^{+3} $dS.m^{-1}$. The lowest chlorophyll b content (0.02 $mg.g^{-1}$ fresh weight) was measured in the treatments of 3 $dS.m^{-1}$. The maximum values of carotenoid (0.07 $mg.g^{-1}$ fresh weight) and total chlorophyll (0.48 mg g^{-1} fresh weight) were measured in the H+1.5 $dS.m^{-1}$, and the lowest amounts related to the 1.5 $dS.m^{-1}$ treatment (Table 4).

Increasing salinity reduces the plant's potential for photosynthesis. Accumulation of sodium and chloride ions in the leaves reduces photosynthesis in the plant by closing the stomata and reducing chlorophyll. The amount of chlorophyll and photosynthetic pigments are the essential factors in the photosynthetic capacity of the plants because they directly affect the speed and amount of photosynthesis and, ultimately, the production of biomass [24,34]. It is also an essential point that water availability is one of the main factors limiting the photosynthesis and growth of plants. Salinity prevents the absorption of water by the seed [6]. Therefore, it affects the seedling growth of plants by less mobilization of reserve nutrients, preventing cell division, injuring hypocotyls, and limiting photosynthesis [13]. In the present study, the highest chlorophyll a (the primary pigment of photosynthesis), carotenoid, and total chlorophyll was related to H+1.5 $dS.m^{-1}$ treatment. In other words, the maximum efficiency of humic acid in this regard was related to a salinity level of 1.5 $dS.m^{-1}$. While, the highest values of germination rate, allometric coefficient, radicle and pedicle lengths, fresh and dry weights of the plant were measured in H^{+3} $dS.m^{-1}$. However, there is no positive relationship between the plant tolerance to salinity and germination phase and the following stages of growth, such as photosynthesis, the greater tolerance to salinity during germination is associated with lower photosynthesis rates [35].

Our results showed that humic acid had different effects on photosynthetic pigments under salinity stress. Humic acid increases nutrient uptake by essential chelating elements and increases fertility and production in plants [36, 37]. Canellas et al. [24] reported that humic acid increased yield in maize due to increased photosynthesis and nutrient uptake. Jing-Min

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Table 4) Effects of humic acid and salt stress on the chlorophyll contents

Characteristic (mg.g^{-1} fresh weight)	0	H	0.75 dS.m^{-1}	1.5 dS.m^{-1}	3 dS.m^{-1}	H+0.75dS.m^{-1}	H+1.5 dS.m^{-1}	H+3 dS.m^{-1}
Chlorophyll a	0.07±0.00^{d}	0.09±0.00^{c}	0.07±0.01^{d}	0.06±0.01^{de}	0.05±0.02^{e}	0.30±0.02^{a}	0.31±0.03^{a}	0.10±0.01^{b}
Chlorophyll b	0.09±0.02^{c}	0.14±0.02^{b}	0.06±0.01^{d}	0.03±0.01^{e}	0.02±0.02^{e}	0.10±0.02^{b}	0.18±0.03^{b}	0.34 ±0.02^{a}
Carotenoid	0.04±0.00^{b}	0.05±0.00^{b}	0.03±0.01^{c}	0.02±0.02^{d}	0.03±0.00^{c}	0.05±0.02^{b}	0.07±0.01^{a}	0.06±0.01^{a}
Total chlorophyll	0.05±0.00^{e}	0.23±0.00^{c}	0.13±0.02^{c}	0.08±0.01^{d}	0.08±0.00^{d}	0.41±0.02^{b}	0.48±0.01^{a}	0.44±0.01^{a}

Values within a row followed by the same letters are not significantly different ($p<0.05$, means± SE). H=humic acid

et al. [38] showed that with increasing water and use of humic acid, the amount of root activity, chlorophyll contents of *Populus* spp increased. Perhaps an increase in chlorophyll contents of the plant occurs due to the cytokinin-like properties of the material that reduce the damage of the chloroplasts and increases root growth and the number of photosynthesis pigments in leaves [24].

Humic acid, through its positive physiological effects, including the effect on plant cell metabolism and increasing leaf chlorophyll concentration, causes tissue persistence [30]. Humic acid has a vital role in increasing the absorption of nutrients in plants through chelating properties. The mechanism of action of humic acid is mainly the formation of a complex between humic acid and mineral ions, the effect of humic acid on respiration and photosynthesis, and stimulation of nucleic acid metabolism. Increasing the salinity level from 0.75 to 1.5 dS.m^{-1} caused a 28% decrease in chlorophyll a content and a 50% decrease in chlorophyll b content. At the same time, the highest chlorophyll content of the plant was related to the combination of salinity and humic acid treatments. Perhaps an increase in chlorophyll contents of the plant occurs due to the cytokinin-like properties of the material that reduce the damage of the chloroplasts and increases root growth and the amount of chlorophyll and photosynthesis pigments in leaves [38].

Conclusion

Generally, this study showed that humic acid had a practical effect on seed germination of *T. alexandrium* L. under salt stress. It was revealed that treatment humic acid+3 dS.m^{-1} had the highest effect in the study. A high level of salt stress (3 dS.m^{-1} dosage) had a more negative effect on the attributes, such as the plant's radicle and pedicle lengths. Regarding the increase of seed germination indices, plant attributes, and chlorophyll contents by applying humic acid in high salt stress conditions, probably higher concentrations of humic acid would provide better results. In the present study, we examined the application of humic acid in lab conditions; further studies could be done to study the effect of humic acid in soil conditions. Due to the unsuitable management of rangelands in Iran, one of the most critical problems is the lack of information about the use of humic acid to decrease salinity in the soil. Therefore, more studies are necessary about the role of humic acid to mitigate the effects of salinity in the soil and promote the plant's resistance to salt stress.

[DOR: 20.1001.1.23222700.2021.9.4.7.9]

Acknowledgments

The authors express their thanks to the Department of Range and Watershed Management, University of Zabol, for providing the necessary facilities to undertake this study.

Ethical Permissions: The case was not found by the authors.

Conflict of Interest: The author declares that there are no conflicts of interest regarding the publication of this manuscript.

Authors' Contributions: Azam Sofi (First author), Introduction author/Original researcher (35%); Mahdieh Ebrahimi (Second author), Methodologist (35%); Ebrahim Shirmohammadi (Third author), Assistant/ Statistical analyst (30%).

Funding/Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

References

1. Saberali S.F., Moradi M.I. Effect of salinity on germination and seedling growth of *Trigonella foenum-graecum, Dracocephalum moldavica, Satureja hortensis*, and *Anethum graveolens*. J. Saudi Soc. Agr. Sci. 2019; 18(3):316-323.
2. Sofi A., Ebrahimi M., Shirmohammadi E. Effect of humic acid on germination, growth, and photosynthetic pigments of *Medicago sativa* L. under salt stress. ECOPERSIA. 2018; 6(1):21-30.
3. Khaleda L., Park H.J., Yun D.J., Jeon J.R., Kim M.G., Cha J.Y., Kim W.Y. Humic acid confers high-affinity K+ transporter 1-mediated salinity stress tolerance in Arabidopsis. Mol. Cells. 2017; 40(12):966-975.
4. Sai-Kachout S., Ben Mansoura A., Jaffel A., Leclerc J.J., Rejeb M.N., Ouerghi Z. The effect of salinity on the growth of the halophyte *Atriplex hortensis*. Appl. Ecol. Env. Res. 2009; 7(4):319-323.
5. Jie Tang J., Bassham D.C. Autophagy in crop plants: what's new beyond Arabidopsis? Open Biol. 2018; 1-13.
6. Zaremanesh H., Akbari N., Eisvand H.R., Ismaili A., Feizian M. The Effect of humic acid on soil physicochemical and biological properties under salinity stress conditions in pot culture of Satureja khuzistanica Jamzad. ECOPERSIA. 2020; 8(3):147-154.
7. Bulentasik B., Turan A., Celik H., VahapKatkat A. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci. 2009; 2(1):87-95.
8. Ebrahimi M., Miri E. Effect of humic acid on seed germination and seedling growth of *Borago officinalis* and *Cichorium intybus*. ECOPERSIA 2016; 4(1):1239-1249.
9. 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):200-208.
10. Azevedo I.G., Olivares F.L.O., Ramos A.C.R., Bertolazi A.A., Canellas L.P. Humic acids and Herbaspirillum seropedicae change the extracellular H+ flux and gene expression in maize roots seedlings. Chem. Biol. Technol. Agr. 2019; 6(1):1-10.
11. Canellas L.P., Olivares F.L., Canellas N.O.A., Mazzei P.L., Piccolo A. Humic acids increase the maize seedlings exudation yield, Chem. Biol. Technol. Agr. 2019; 6(3):1-10.
12. Kaya C., Akram N.S., Ashraf M., Sonmez O. Exogenous application of humic acid mitigates salinity stress in maize (Zea mays L.) plants by improving some key physico-biochemical attributes. Cereal Res. Commun. 2018; 46(1):67-78.
13. Benazzouk S., Djazouli Z.E., Lutts S. Assessment of the preventive effect of vermicompost on salinity resistance in tomato (*Solanum lycopersicum* cv. Ailsa Craig). Acta Physiol. Plant. 2018; 40(6):121-129.
14. Shrestha A., Hesterman O.B., Squire J.M., Fisk J.W., Sheaffer C.C. Annual medics and berseem clover as emergency forage. Agron. J. 1998; 90(2):197-201.
15. Taylor N.L. Clovers around the world, In Clover Sci. and Technology. Taylor NL (Ed.). America Society of Agronomy, Madison, Wisconsin. 1958; pp:1-6.
16. Deepak K., Deepika S., Bhatnagar S.K. Salinity caused sodium and potassium ions uptake in berseem (*Trifolium alexandrinum* L.). Vegetos. 2009; 22(2):109-115.
17. Gorai M., Gasmi H., Neffati M. Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae). Saudi. J. Biol Sci. 2011; 18(3):255-260.
18. 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.
19. Mereddy R., Hallgren S., Wu L., Wu Y., Conway K.E. Solid matrix priming improves seedling vigor Okra seeds. Proc. Okla Acad. Sci. 2000; 80(2):33-37.
20. Behbodian B., Lahouti M., Nezami A. Effects evaluation of salt stress on germination of chickpea varieties. J. Agr. 2005; 28(2):127-137.
21. Saravanakumar D., Vijayakumar C., Kumar N.,

[DOR: 20.1001.1.23222700.2021.9.4.7.9]

Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. Crop Prot. 2007; 26(4):556-565.
22. Munns R., Tester M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008; 59(1):651-681.
23. Arnon D.I. Copper enzymes in isolated chloroplasts, Polyphenol oxidase in *Beta volgaris*. Plant Physiol. 1949; 24(1):1-5.
24. Canellas L.P., Canellas N.O.A., Irineu L.E.S.S., Olivares F.L., Piccolo A. Plant chemical priming by humic acids. Chem. Biol. Technol. Agr. 2020; 12(7):1-17.
25. Aguiar N.O., Olivares F.L., Novotny E.H., Canellas L.P. Changes in metabolic profiling of sugarcane leaves induced by endophytic diazotrophic bacteria and humic acids. Peer J. 2018; 6(1):1-28.
26. Khaleghi E., Ramin A.A. Study of the effects of salinity on growth and development of Lawans (*Lolium perenne* L.), *Festuca arundinaceae*, and *Cynodon dactylon*. JWSS. 2005; 9(3):57-67.
27. Chachar Q.I., Solangi A.G., Verhoef A. Influence of sodium chloride on seed germination and seedling root growth of cotton (*Gossypium hirsutum* L.). Pak. J. Bot. 2008; 40(1):183-197.
28. Ozdamar U.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; 13(6):2800-2803.
29. Canellas L.P., Canellas N.O.A., Soares T.S., Olivares F.L. Humic acids interfere with nutrient sensing in plants owing to the differential expression of TOR. J. Plant Growth Regul. 2019; 38(1):216-224.
30. Nardi S., Pizzeghello D., Muscolo A., Vianello A. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 2002; 34(11):1527-1536.
31. Araghi Shahri S.M., Dianati Tilaki G.A., Behtari B., Alizadeh M.A. Growth responses of Secale cereale and S. ceremony to priming treatments under salinity stress. J. Rangeland Sci. 2015; 5(3):202-211.
32. Cordeiro F.C., Catarina C.S., Silveira V., De Souza S.R. Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (*Zea mays*). Biosci. Biotechnol. Biochem. 2011; 75(1):70–74.
33. Rubio V., Bustos R., Irigoyen M.L., Cardona-Lopez X., Rojas-Triana M., Paz-Ares J. Plant hormones and nutrient signaling. Plant Mol. Biol. 2009; 69(4):361–373.
34. Wahid A. Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane. Faisalabad- Pakistan. Bot. Bull. Acad. Sinica. 2004; 45(2):133-141.
35. Kaymakanova M. Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.). Biotechnol. Biotechnol. Equip. 2009; 23(1):326-329.
36. Matuszak-Slamani R., Bejger R., Cieśla J., Bieganowski A., Koczańska M., Gawlik A., Gołębiowska D. Influence of humic acid molecular fractions on growth and development of soybean seedlings under salt stress. Plant Growth Regul. 2017; 83(3):465-477.
37. Akladious S.A., Mohamed H.I. Ameliorative effects of calcium nitrate and humic acid on the growth, yield component, and biochemical attribute of pepper (*Capsicum annuum*) plants grown under salt stress. Sci. Hortic. 2018; 236(1):244-250.
38. Jing-Min Z., Shang-jun X., Mao- Peng S., Bing-Yao M., Xiu-mei C., Chunsheng L. Effect of humic acid on poplar physiology and biochemistry properties and growth under different water levels. J. Soil Water Conserv. 2010; 6(2):1-42.
39. Samadimatin A., Hani A. Effect of ethanol and humic acid foliar spraying on morphological traits, photosynthetic pigments, and quality and quantity of essential oil content of *Dracocephalum moldavica* L. Iran. J. Plant Physiol. 2017; 8(1):2299-2306.

[DOR: 20.1001.1.23222700.2021.9.4.7.9]