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The Effect of Humic Acid on the Growth and Physiological Indices of Pistachio Seedling (*Pistacia vera*) under Drought Stress

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A R T I C L E I N F O

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Drought stress;
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A B S T R A C T

This study was carried out to evaluate the effect of soil application of humic acid on growth and physiological indices of pistachio seedlings ("Badami Zarand" rootstock) under drought stress. This experiment was arranged as a completely randomized design with three replications in greenhouse conditions. The experimental treatments consisted of humic acid (0, 30 and 60 gr in 4 kg^{-1} soil in pot) and irrigation intervals (7, 20 and 30 days). The studied growth parameters included of stem height, leaf number, leaf area, stem diameter, biomass of root and shoot and physiological indices in leaves such as relative water content (RWC), relative water loss (RWL), leaf water content (LWC), excised leaf water retention (ELWR), excised leaf water loss (ELWL) and relative water protection (RWP). In this experiment, the results showed that relative water content reduced and relative water loss increased in leaf in irrigation interval of 30 days. The results indicated that the application of humic acid increased the vegetative growth of pistachio seedling in comparison with the control under high irrigation interval ($p<0.05$). The results also showed that relative water content and relative water protection significantly increased with the application of humic acid ($p<0.05$). These results indicated that the pistachio seedlings were sensitive to water stress and the application of humic acid under drought stress could be an appropriate management strategy to improve the growth of seedlings in pistachio orchards in arid and semi-arid areas of Iran.

Introduction

About 90 percent of Iran is categorized as arid and semi-arid areas. Iran especially Kerman, as the main origin of pistachio, has always the largest cultivation area (450000 ha) with a high genetic diversity of pistachio orchards in the world (Mirzavand *et al.*, 2017). Pistachio (*Pistacia vera* L.) a member of the Anacardiaceae family, is very important in the economic aspect of agricultural production in Iran. Drought tolerance of pistachio species could be related to high water conservation ability by deep taproot, leaf characteristics, stomatal adjustment, stomatal features, leaf shedding (Fardooei, 2001). Although pistachio has a high level of tolerance to drought, studies showed that there must be adequate soil moisture during late winter, spring and early summer for commercial crop production (Picchioni and Myamota, 1990, Ferguson *et al.*, 2002). Irrigation plays an important role in yield, but particularly in pistachio, it also improves the nut quality and regulates the normal alternate bearing pattern (Sedaghati and Hokmabadi, 2015). In recent years, droughts with increasing severity and frequency have been experienced around the world due to climate change effects, especially in Iran. High irrigation intervals are one of the most important problems of pistachio orchards in arid and semi-arid regions. In the

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water crisis, the growth of trees only happens to survive without yield. Under such circumstances, water reservation plays an important role in drought mitigation. Therefore the development of methods of reserving in drought periods could help to decrease the drought impacts by reducing the expected water shortage. Natural and technological methods have been studied in recent years to improve water use efficiency in agricultural products (Walker and Bernal, 2008). Until now, the beneficial effects of humic substances have been mentioned (Abootalebi Jahromi and Hassanzadeh Khankahdani, 2016; Barzegar et al., 2016; Fallahi et al., 2016; Abdipour et al., 2019). Recent literature showed that humic substances as the major component of soil organic matter could be used as a growth regulator to regulate hormone levels, improve plant growth, micronutrient uptake, biochemical processes in plants (respiration, photosynthesis and chlorophyll content) and enhance stress tolerance (Çimrin *et al*., 2010; Khaled and Fawy, 2011). Also Accumulation of low molecular weight organic solutes compounds, like osmoregulations, are an important tolerance mechanism, which lets the retention of cellular turgor and favors the absorption of water (Chaves *et al*., 2003) under drought stress. Although, the favorable effect of these products depends on the origin of the material, extraction method and concentration and composition of the humic extract (Burdon, 2001). Humic substances are heterogeneous, flexible, polyelectrolytes and relatively large stable organic complexes. They improve soil structure, porosity, water holding capacity, cation, and anion exchange, and are involved in the chelation of mineral elements (Davies *et al*., 2001; Cavani, 2003). Therefore, the present investigation was conducted to evaluate the effects of soil application of humic acid on the growth and physiological parameters in pistachio seedlings ("Badami Zarand" rootstock) under drought stress.

Materials and Methods

Plant material and experiment design

This experiment was conducted in the Pistachio Research Center (PRC) in Rafsanjan, Iran during 2018 and 2019. The climate in this region is classified as arid and semi-arid with a mean annual rainfall of 100 mm and the annual temperature range is between 17°C to 42°C (Hasheminasab *et al*., 2012). The present study has investigated the effect of different levels of humic acid applied in soil on the growth and physiological indices of pistachio seedling ("Badami Zarand" rootstock) under drought stress. This experiment was carried out in a factorial arrangement of 3 × 3 × 3 in a randomized complete block design with 3 replications. The experimental treatments consisted of humic acid (0, 30 and 60 gr in 4 kg^{-1} soil in pot) and irrigation intervals (7, 20 and 30 days). The application of treatments was performed on one-year-old seedlings. The soil used for this study was collected from 0–30 cm depth of the pistachio orchards located in Rafsanjan. The soil properties in this study included sandy loam texture with pH: 8.4 and EC: 1.8 dS m^{-1} and the sufficient nutrient elements. The soil was air-dried and passed through 4 mm sieve and four kg were used for each pot. Before planting, pistachio seeds were sterilized with 10% sodium hypochlorite three times for 10-12 minutes, washed in each stage and were soaked in distilled water for 24 hours. Prepared seeds of Pistachio were sown in pots. After germination, one seedling was kept in each pot. Various concentrations of humic acid (the substance of coal mine oxide from Tabas with humic purity of 60%) (0, 30 and 60gr) (determined based on the product label) were added in the pots and mixed into the soil. Drought stress due to irrigation intervals in 3 levels included irrigation intervals of 7 days (control), 20 and 30 days based on the soil weight moisture content at field capacity (FC). Therefore, the weight of pot in each treatment was measurement every 2 days and in the irrigation time (7, 20 and 30 days) was balanced to be at soil weight moisture in FC. After six months of vegetative growth, the plants were harvested.

Measured parameters

Growth parameter

The height of seedlings (by a ruler with 2mm scale), leaf number, and stem diameter (by the digital caliper) were recorded in two periods during six months of the growing season.

Leaf area (LA)

Leaf area was measured by leaf area meter (Leaf Area Meter England Company). To measure the leaf area, the mean of 10 leaves without petiole was calculated

Dry weight of root and shoot

At the end of the experiment, plants were harvested early in the morning. The plant samples were placed in plastic bags, labeled carefully and brought to the laboratory. Each plant was rinsed, catted, and subdivided into shoot and root. Each part was dried in the oven at 70°C for 72 hours and fresh and dry weight was recorded.

Physiological traits

Samples of 10 leaves were taken randomly and weighed for the assessment of physiological traits. Fresh weight (WF) measured and after 2, 4, 6 hours was repeated. Then, samples were placed in distilled water for 24h and reweighed to obtain turgid weight (WT). Then, samples were placed in distilled water for 24h and reweighed to obtain turgid weight (WT). Leaf samples were dried in oven and weight recorded at 70°C for 72h (WD). Physiological traits were calculated using the following formula:

Relative Water Content (RWC) = $\frac{WF-WD}{WT-WD}$
(Clark and McCaig, 1982)

Relative Water Loss (RWL)
$= \frac{(WF-W1)+(W1-W2)+(W2-W3)}{3WD\ (T2-T1)}$

(Clark and McCaig, 1982)

Leaf Water Content (LWC) = $\frac{WF-WD}{WF}$
(Clark and McCaig, 1982)

Excised Leaf Water Retention (ELWR) = 1- ($\frac{WF-W3}{WF}$)
(Clark and McCaig, 1982)

Excised Leaf Water Loss (ELWL) = $\frac{WF-W3}{WF-WD}$
(Mint *et al*., 1988)

Relative Water Protection (RWP) = $\frac{W3-WD}{WF-WD}$
(Hasheminasab *et al*., 2012)

In the above formula, WF, WD, WT, W1, W2 and W3 are fresh leaf weight (at the time of sampling), dry weight (by placing leaves in oven at 70 °C for 24 hours), turgid weight (by placing Leaves in distilled water about 18-20 h), leaf weight after two hours (at 25 °C), after four hours and after six hours, respectively.

Data analyses

The experiment was conducted in a factorial design in RCBD with three replications. Data were analyzed using SPSS software. Mean comparisons were made using Duncan's multiple range test.

Results

The interaction effect of humic acid and irrigation interval on the growth of pistachio seedlings

In this study, high irrigation intervals had negative effects on the growth indicates. The results showed that the interaction effect of humic acid and irrigation interval on height, leaf number, leaf area, and stem diameter were statistically significant at 5% level

(Table1). The comparison of means by Duncan's method showed that the seedling height significantly decreased in high irrigation intervals. The application of humic acid significantly increased height under drought stress, and the maximum was in 30gr in irrigation interval 30 days. However, the different concentrations of humic acid treatments didn't show any significant differences. The interaction effect of humic acid and irrigation interval on height for two periods (three months) showed in Fig. 1. The effect of humic acid treatments on leaf number was significant under drought stress (Fig.2). The application of humic acid significantly increased leaf number compare to control (without humic acid in the same irrigation interval) under drought stress. However, the maximum leaf number was observed in the irrigation interval 7 days (Fig.2). The application of humic acid significantly increased stem diameter, especially under drought stress. The maximum diameter was in 60 gr in irrigation interval 30 days (Fig.3). The comparison of means by Duncan's method showed that humic acid improved the leaf area under drought stress. However, humic acid treatments didn't show any significant differences in irrigation interval 20 days (Fig. 4). Also, the results showed that the dry weight of shoot significantly increased by increasing the humic acid under drought stress (Fig. 5 and 6). Humic acid treatments of 30 and 60 gr significantly increased the dry weight of shoot compared to control (without humic acid in the same irrigation interval) in irrigation interval 30 days (Fig. 5). But there wasn't any significant effect on the dry weight of root in irrigation interval 20 days (Fig. 6). Treatment without humic acid in irrigation interval 30 days significantly increased the dry weight of root compared to all treatments (Fig. 6).

Table 1. Analysis of variance for the effect of humic acid on growth parameters of pistachio seedlings

		Means Square					
Source of variance	df	Height of seedling	Stem diameter	Leaf number	Leaf area (mm2)	Root biomass (g)	Shoot biomass (g)
Irrigation interval (A)	2	200.7 *	0.398*	17.5**	53676298.55**	20.129*	104.69**
Humic acid(B)	2	51.06*	14.108**	4.36*	80783612.89 **	13.467 ns	7.443 ns
(A×B)	4	95. 6**	1.272*	23.26*	3699151.15**	6.79 *	7.66 *
Error	9	57.25	1.581	10.9	5780424.95	4.3	6.2
CV%	-	9.16	11.39	15.01	14.01	13.43	12.38

Significant F-test at **P < 0.01, at *P < 0.05 and non-significant (NS)

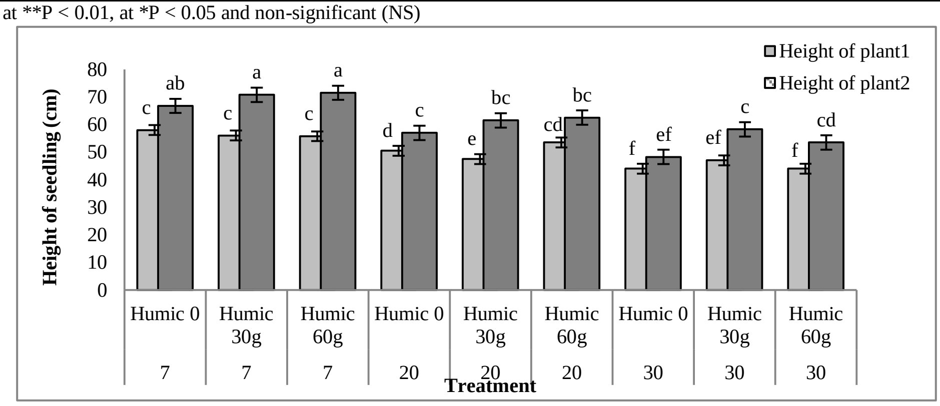

Fig. 1. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on height of pistachio seedlings in cm (height of plants in 3 and 6 months after treatment showed by number 1 and 2, respectively)
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

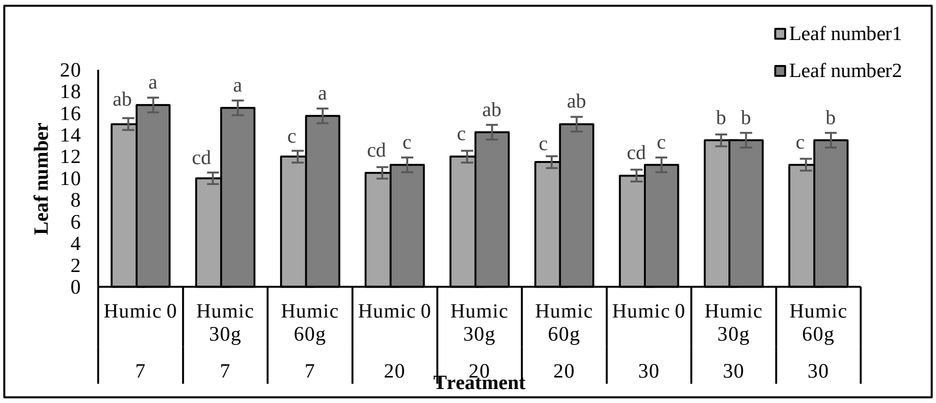

Fig. 2. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on leaf number in pistachio seedlings (leaf number in 3 and 6 months after treatment showed by number 1 and 2 , respectively)(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

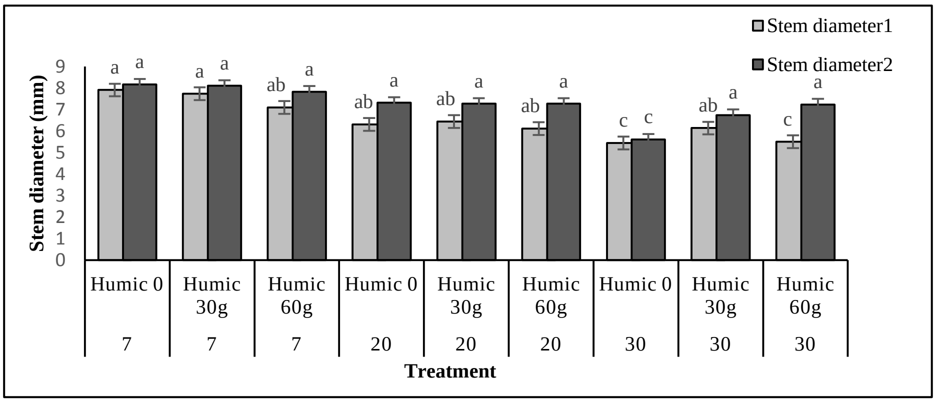

Fig. 3. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on stem diameter (mm) in pistachio seedling (stem diameter in 3 and 6 months after treatment showed by number 1 and 2, respectively)(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

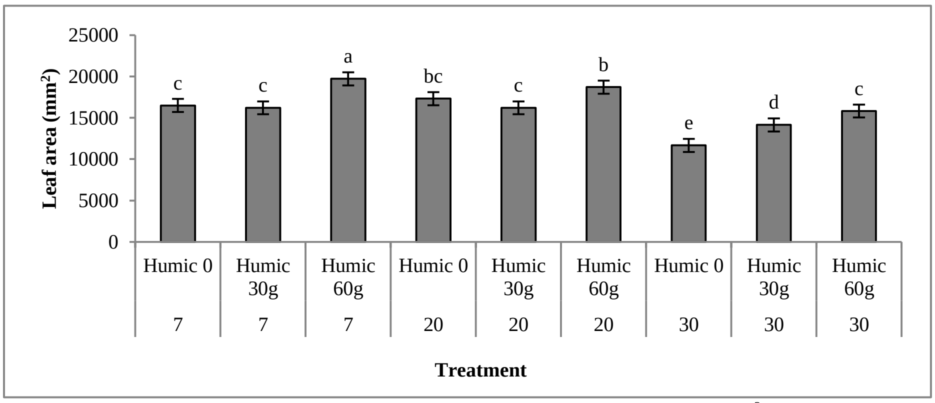

Fig. 4. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on leaf area (mm^2) in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

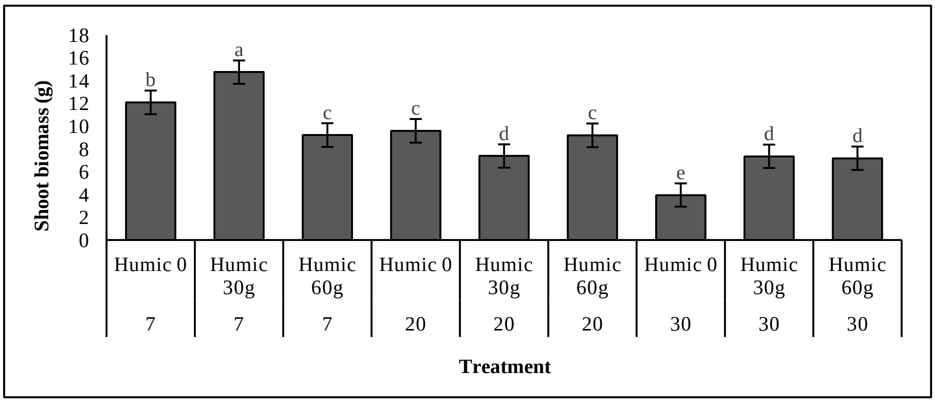

Fig. 5. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on shoot biomass (g) in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

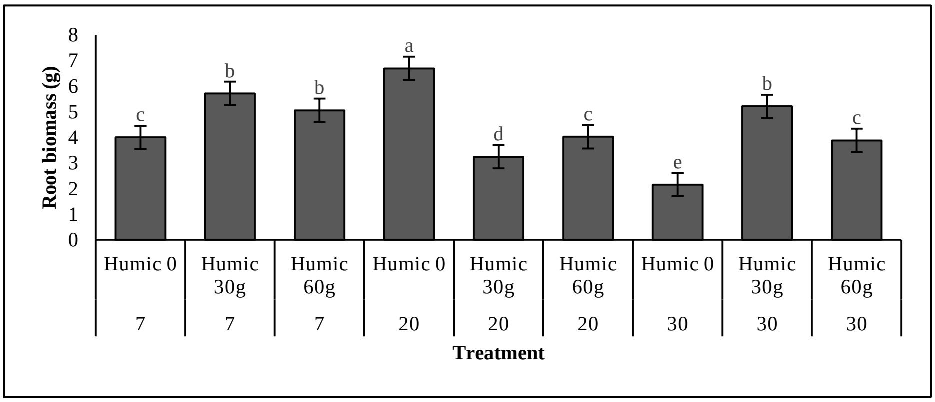

Fig. 6. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on root biomass (g) in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The effect of humic acid and irrigation interval on physiological traits of pistachio seedlings

The results from the analysis of variance showed that the interaction effect of humic acid and irrigation interval treatments on physiological traits were statistically significant at 5% (Table 2). The comparison of means by Duncan's method showed that with increasing the irrigation interval, the relative water content in leaves significantly increased. The maximum relative water content was observed in irrigation intervals 30, 20 and 7 days, respectively. Humic acid treatments had a significant increase in relative water content in irrigation interval 30 days compared to irrigation interval 7 days. But between different concentrations of humic acid didn’t show any significant effects on the relative water content in irrigation interval 30 days (Fig. 7). The interaction effect of humic acid and irrigation interval in leaf water content were statistically significant at 5% (Table 2). The comparison of means by Duncan's method showed that the maximum of relative water content was observed in irrigation interval 20 days. In irrigation interval 30 days, relative water content increased in humic acid treatments compared to control (without humic acid in the same irrigation interval) (Fig. 8). The maximum relative water content in irrigation interval 7 days was observed in humic acid 60, 30 and 0 g, respectively (Fig. 8). The interaction effect of humic acid and irrigation interval in excised leaf water retention showed that in irrigation interval 7 days, the maximum was observed in control (without humic acid in the same irrigation interval) and in irrigation interval 20 and 30 days was observed in humic acid treatments. There wasn’t any significant difference between the concentrations of humic acid (Fig. 9). The interaction effect of humic acid and irrigation interval in excised leaf water loss showed that in irrigation interval 7 days, the maximum was observed in humic acid treatments and irrigation interval 20 and 30 days was observed in control (without humic acid in same irrigation interval) (Fig. 10). The comparison of means by Duncan's method showed that leaf water protection increased in humic acid treatments in high irrigation intervals. In irrigation interval 7 days, the maximum was observed in control (without humic acid in the same irrigation interval). This trend was the same as the excised leaf water retention parameter (Fig.11).

Table 2. Analysis of variance for the effect of humic acid and irrigation interval on physiological parameters of pistachio seedlings

		Means Square				
Source of variance	df	RWC	LWC	ELWR	ELWL	RWP
irrigation Interval (A)	2	0.055 **	0.042**	0.009 **	0.022**	0.030**
Humic acid(B)	2	0.006**	0.004**	0.007**	0.018**	0.017**
(A*B)	4	0.003*	0.005*	0.046*	0.085*	0.090*
Error	9	0.0011	0.0010	0.0009	0.0019	0.0009
CV%	-	3.783	5.658	4.550	3.246	5.774

Significant F-test at **P < 0.01, at *P < 0.05 and non-significant (NS)

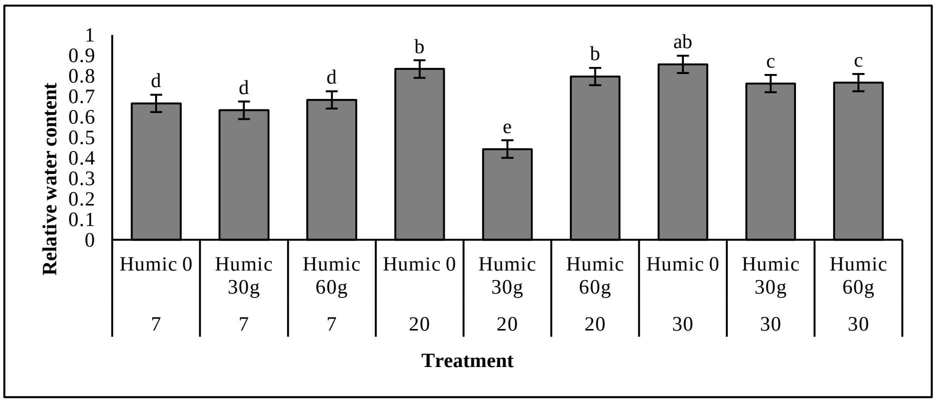

Fig 7. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on relative water content in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

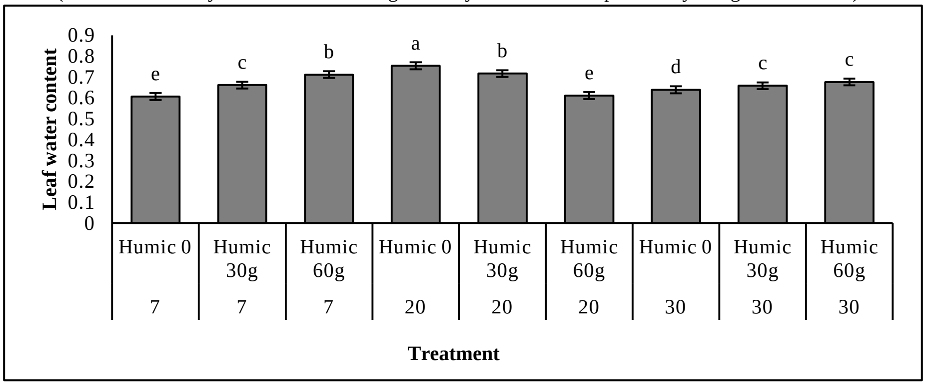

Fig. 8. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on leaf water content in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

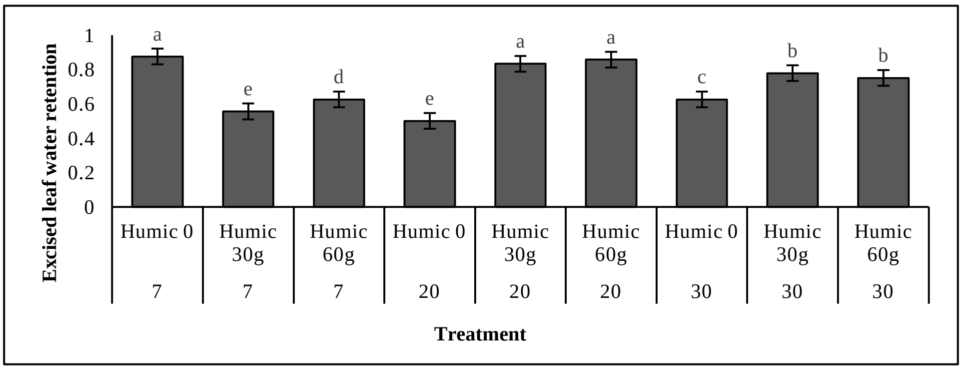

Fig. 9. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on excised leaf water retention in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

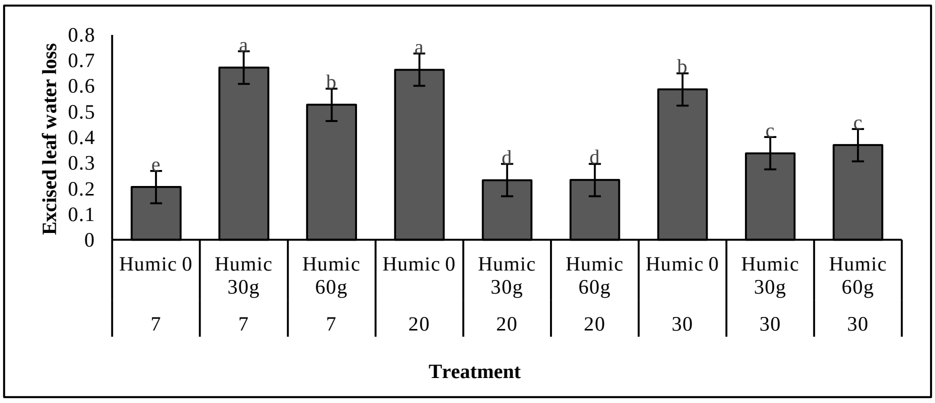

Fig. 10. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on excised leaf water loss in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

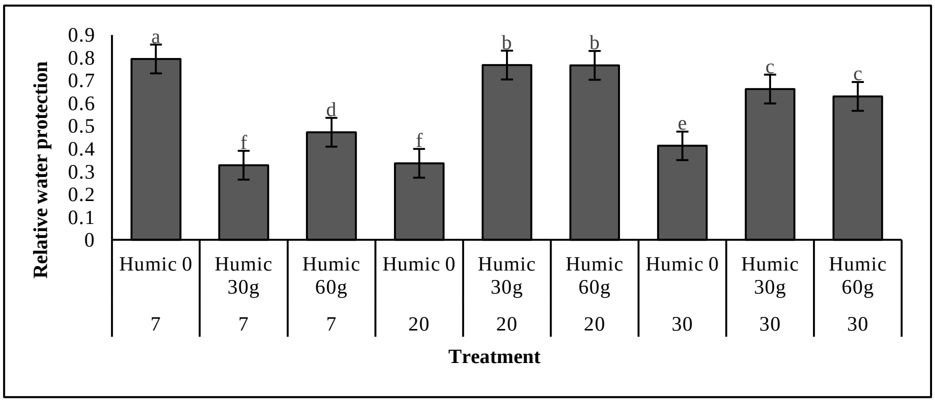

Fig. 11. The interaction effect of humic acid and irrigation interval (7, 20, 30 days) on relative water protection in pistachio seedling (Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Discussion

The responses of plants to drought stress have been observed in many physiological and biochemical parameters include, leaf wilting, reduction in leaf area, stimulation of root growth, changes in relative water content and membrane structure, excessive generation of ROS, and antioxidants and osmolytes, and transcriptional activation of drought-responsive genes (Miller *et al*., 2010; Kang *et al*., 2011; Lata and Prasad, 2011). In these circumstances, some plants effectively tolerance drought stress by reducing transcriptional water loss, which conserves an adequate water status to sustain critical physiological and biochemical processes in the plant (Yoo *et al*., 2010). In this research, the comparison of means by Duncan's method showed that the plant height decreased in control (without humic acid in different irrigation interval) under drought stress. Humic acid treatments significantly increased seedling height compared to control (without humic acid in the same irrigation interval) (Fig. 1). Similar observations were also reported in different plants (Eyheraguibel *et al.,* 2008; Mora *et al.,* 2010; Tahir *et al.,* 2011). The literature showed that humic acid improves plant growth, micronutrient uptake, hormone levels, biochemical processes in plants (respiration, photosynthesis and chlorophyll content) and enhance stress tolerance (Wang *et al.,* 2001; Pena-Mendez *et al.,* 2005; Çimrin *et al.,* 2010; Khaled and Fawy, 2011). The comparison of means by Duncan's method showed that humic acid improved the growth indicates under drought stress (Figs. 1, 2, 3 and 4). Similar observations were also reported zucchini (Mora *et al.,* 2010), wheat (Tahir *et al.,* 2011), corn (Eyheraguibel *et al.,* 2008) and pepper (Çimrin *et al.,* 2010). Also, the results showed that dry weight of root and shoot significantly increased by increasing the humic acid compared to control (without humic acid in the same irrigation interval) under irrigation interval 30 days (Figs. 5 and 6). Also, there was a significant effect on the dry weight of root and shoot in irrigation interval 20 days in control compared to humic acid treatment and significantly increased (Fig. 5 and 6). The literature showed that the root developmental habit is phreatophyte in pistachio trees, which allows the root system to penetrate deeply into the soil (Javanshah and afrousheh, 2018). Therefore, the results of this research on increasing root growth in irrigation interval 20 days related to the tolerant one-year-old seedlings due to absorbing more water from lower parts of soil. In general, the reaction of plants to humic acid has different. In some plants, the humic acid treatments increase root length, while others are increasing root density. Root growth significantly related to the hydrophobic humic acids and these features, especially for plant adaptation to various conditions, including salinity of the soil is important (Römheld and Neumann, 2006). In the same research by Ghorbani *et al.,* (2010) observed humic acid (3500 and 4500g ha^{-1}) increased durability, leaf area and economic performance on the corn. Also, Albayrak and Camas (2005) reported that treatment with 1200 mgl^{-1} increased the leaf area. The deficiency of water was found to decrease the relative water content (RWC) in plant leaves, and also reported that this cause block stomata and reduce photosynthesis rate (Cornic, 2000). Water stress is directly related to the amount of water available in the soil and indirectly saving water in the plant. Some studies report the measurement of water status in the plant could help to understand physiological changes in the plant during drought stress (Blum, 2005). Water retention in plants play an important role in drought stress, so the application of methods for reserving in drought period could help to increase the drought impacts. Relative water content has been the most commonly used method in the plant (Alizade, 2002). Therefore, relative water content and excised leaf water loss have been suggested as important indicators of water status (ElTayeb, 2006, Hasheminasab *et al*., 2014). The results of this study showed that the relative water content (RWC) increased during high irrigation intervals that were considered drought tolerant in pistachio seedlings (Fig. 7). Hasheminasab *et al*. (2014) were reported that Badami was a tolerant cultivar with the highest RWP. A similar study showed that the relative water content (RWC) is high in the tolerant plant (Liu *et al*., 2002). This index is related to the water content imported into the leaves. It reflects the balance between the water content of the leaf tissue and the transpiration rate (Lugojan and Ciulca, 2011). So, a decrease in the relative water content (RWC) of leaves is one of the best-documented symptoms of drought stress. In this research, the results also showed that the relative water content decreased in humic treatments in irrigation interval 30 days compared to control (without humic acid in the same irrigation interval) (Fig. 7). Consequently, it could be concluded that the relative water content decreased in humic acid treatments under drought stress due to changes in cell membrane such as penetrability and the openness of the stomata (Blokhina *et al*., 2003). In this experiment, water deficiency in irrigation interval 30 days was found to reduce relative water content (RWC) and increase relative water loss (RWL) in pistachio seedlings (Fig. 8). Similar observation reported drought-tolerant variety exhibited lower relative water loss and higher LWC than drought-sensitive variety under drought stress (Shi *et al*., 2012a). Low excised-leaf water loss (ELWL) and high excised leaf water retention have been commonly associated with improved growth under drought stress (Chandra and Islam, 2003; David, 2010). The results of this study showed similar observation in high irrigation intervals 20 and 30 days in experimental treatments in the same irrigation interval (Fig. 9 and 10). The results of this study indicated that humic acid treatments increased relative water protection (RWP) in high irrigation intervals in pistachio seedlings and reduce the adverse effects of drought stress (Fig. 11). Relative water protection (RWP) is an important indicator of water status in plants separated susceptible cultivars from tolerant and intermediate, but can't able separated tolerance from intermediate

(Hasheminasab *et al*., 2014). Application of humic acid had a significant effect on the plant growth parameters, including shoot dry weight, height and diameter, number leaf and leaf area and cause them to increase. Humic acid significantly reduced excised leaf water loss and showed a significant effect of absorbed water content in the plant. In this experiment, there were no significant differences between humic acid treatment 30 and 60 g. In Iran, decreased the availability of underground water resources and prolonged drought periods during the last two decades are the major problem for the pistachio producers (Bagheri *et al*., 2012). Thus, improvement of the growth under drought stress can increase tolerance in pistachio seedlings base on the results of this research. Therefore, it is recommended that the application of humic acid looking at the appropriate method in the improvement of plant conditions under drought stress.

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References

Abdipour, M., Hosseinifarahi, M. and Najafian, S., 2019. Effects of humic acid and cow manure biochar (CMB) in culture medium on growth and mineral concentrations of basil plant. International Journal of Horticultural Science and Technology, 6(1), pp.27-38.

Abootalebi Jahromi, A. and Hassanzadeh Khankahdani, H., 2016. Effect of humic acid on some vegetative traits and ion concentrations of Mexican Lime (*Citrus aurantifolia* Swingle) seedlings under salt stress. International Journal of Horticultural Science and Technology, 3(2), pp.255-264.

Albayrak S, Camas N (2005) Effect of different levels and application times of humic acid on root and leaf yield and yield component of forage turpin. Journal of Agronomy. 42, 130-133.

Alizade A (2002) Soil, Water and Plants Relationship. 3rd Edn., Emam Reza University Press, Mashhad, Iran, ISBN: 964-6582-21-4. pp. 737.

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora. 199, 361-376.

Ashraf M, Foolad MA (2007) Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. Environmental Journal of Experiment Botany. 59, 206-216.

Ashraf M, Ozturk M, Athar HR (2008) Salinity and Water Stress (Eds). Series: tasks forvegetation science series 44. Improving Crop Efficiency. Springer-Verlag, Netherland. pp.246.

Bagheri V, Shamshiri MH, Shirani H, Roosta HR (2012) Nutrient uptake and distribution in mycorrhizal pistachio seedlings under drought stress. Journal of Agricultural Science and Technology. 14, 1591–1604.

Barzegar, T., Moradi, P., Nikbakht, J. and Ghahremani, Z., 2016. Physiological response of Okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress. International Journal of Horticultural Science and Technology, 3(2), pp.187-197.

Blokhina O, Virolainen E, Fagerstedt KV (2003) Anti-oxidative damage and oxygen deprivation stress. Annals of Botany. 91, 179-194.

Blum A (2005) Drought resistance, water-use efficiency, and yield potential are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research. 56, 1159–1168.

Burdon J (2001) Are the traditional concepts of the structures of humic substances realistic? Soil Science. 6,752-769.

Cavani L, Ciavatta C, Gessa C (2003) Identification of organic matter from peat, leonardite and lignite fertilisers using humification parameters and electro focussing. Bioresource Technology. 86, 45−52.

Chandra D, Islam MA (2003) Genetic variation and heritability of excised-leaf water loss and its relationship with yield and yield components of F5 bulks in five wheat crosses. Journal of Biological Sciences. 3, 1032−1039.

Chaves MM, Maroco JP, Pereira JS (2003) Understanding Plant Responses to Drought from Genes to the Whole Plant. Functional Plant Biology. 30, 239-264.

Çimrin KM, Türkmen O, Turan M, Tuncer B (2010) Phosphorus and humic acid application alleviate salinity stress of pepper seedling. African Journal of Biotechnology. 9(36), 5845-5851.

Cornic G (2000) Drought stress inhibits Photosynthesis By decreasing stomatal aperture- Not by affecting ATP synthesis. Trends Plant Science. 5, 187-198.

Davies G, Ghabbour EA, Steelink C (2001) Humic acids: Marvelous products of soil chemistry. Journal of Chemical Education. 78, 1609-1614.

El-Tayeb N (2006) Differential response of two Vicia faba cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agronomica Academiae Scientiarum Hungaricae. 54, 25−37.

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, 4206–4212.

Fallahi, H.R., Ghorbany, M., Samadzadeh, A., Aghhavani-Shajari, M. and Asadian, A.H., 2016. Influence of arbuscular mycorrhizal inoculation and humic acid application on growth and yield of Roselle (*Hibiscus sabdariffa* L.) and its mycorrhizal colonization index under deficit irrigation. International Journal of Horticultural Science and Technology, 3(2), pp.113-128.

Fardooei AR (2001) Evaluation of salt and drought resistance of two pistachio species (*Pistacia khinjuk* and *P. mutica*) in terms of ecophysiological and growth characteristics, Doctoral dissertation, Ghent university, 187.

Ferguson L, Poss JA, Grattan SR, Grieve CM, Wang D, Wilson C, Donovan Chao CT (2002) Pistachio rootstocks influence scion growth and ion relations under salinity and boron stress. American Journal of Society Science. 127, 194-199.

Ghorbani S, khazraei H, Kafi M, banayan M (2010) The effect of humic acid in water irrigation on yield and component yields of *Zea mays* L. Agricultural Journal. 2(1), 123-131.

Hasheminasab H, Aliakbari A, Aliakbari A, Baniasadi R (2014) Optimizing the relative water protection (RWP) as novel approach for monitoring drought tolerance in Iranian pistachio cultivars using graphical analysis. International Journal of Biosciences. 4(1), 194-204.

Javanshah A, Afrousheh M (2018) The importance of applying moisture retaining compounds in pistachio seedling growth medium under drought stress. Pistachio and Health Journal. 1(4), 38-47

Kang Y, Han Y, Torres-Jerez I, Wang M, Tang Y, Monteros M, Udvardi M (2011) System responses to long-term drought and re-watering of two contrasting alfalfa varieties. Journal of Plant. 68, 871–889.

Khaled H, Fawy HA (2011) Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity. Soil and Water Resource. 6(1), 21–29.

Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany. 62, 4731–4748.

Liu Y, Fiskum G, Schubert D (2002) Generation of reactive oxygen species by mitochondrial electron transport chain. Journal of Neurochemistry. 80, 780-787.

Lugojan C, Ciulca S (2011) Evaluation of relative water content in winter wheat. Journal of Horticulture, Forestry and Biotechnology. 15, 173–177.

Miller G, Suzuki N, Ciftci-Yilmaz S, Miller R (2010) Reactive oxygen species homeostasis and signaling during drought and salinity stresses. Journal of Plant, Cell and Environment. 33, 453–467.

Mirzavand S, Sorkheh K, Siahpoosh MR (2017) Peroxidase gene-based estimation of genetic relationships and population structure among wild pistachio species populations. Biochemical Genetics. 55, 1-14.

Mora V, Bacaicoa E, Zamarreño AM, Aguirre E, Garnica M, Fuentes M, García-Mina JM (2010) 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. Journal of Plant Physiology. 167, 633–642.

Pena-Mendez EM, Havel J, Patocka J (2005) Humic substances, compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine Journal of Applied Biomedicine. 3, 13-24.

Picchioni GA, Miyamota S (1990) Salt effects on growth and ion uptake of pistachio rootstock seedlings. Journal of the American Society for Horticultural Science. 115, 647–653.

Römheld V, Neumann G (2006) The Rhizosphere: Contributions of the soil-Root Interface to Sustainable Soil Systems. In: Uphoff, N. Ball A, Fernandes E, Herren H, Husson O, Laing M, Palm C, Pretty JN, Sánchez PA, Sanginga N, Thies J. (ed.) Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton, FL. pp. 91-107.

Sedaghati N, Hokmabadi H (2015) Optimizing Pistachio Irrigation Management Using the Relationship between Echo-physiological Characteristics and Water Stress. Journal of Agricultural Science and Technology. 17, 189-200.

Shi H, Wang Y, Cheng Z, Ye T, Chan Z (2012a) Analysis of natural variation in bermudagrass (*Cynodon dactylon*) reveals physiological responses underlying drought tolerance. PLOS ONE. 7(12), e53422.

Tahir MM, Khurshid M, Khan MZ, Abbasi MK, Hazmi MH (2011) Lignite-derived humic acid effect on growth of wheat plants in different soils. Pedosphere. 2, 124–131.

Walker DJ, Bernal MP (2008) The effects of olive mill waste compost and poultry manure on the availability and plant uptake of nutrients in a highly saline soil. Bioresource and Technology. 99, 396-403.

Wang Y, Combe C, Clark MM (2001) The effects of pH and calcium on the diffusion coefficient of humic acid. Journal of Membrane Science. 183, 49-60.

Yoo CY, Pence HE, Jin JB, Miura K, Gosney MJ, Hasegawa PM, Mickelbart MV (2010) The *Arabidopsis* GTL1 transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via trans repression of SDD1. Journal of Plant Cell. 22, 4128–4141.