The Importance of Applying Moisture Retaining Compounds in Pistachio Seedling Growth Medium under Drought Stress

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Abstract

Introduction: Water scarcity is one of the most serious threats to the sharp decline of yield in different cultivars of pistachio in Iranian orchards. Therefore, attention should be paid to the maximum productivity of water resources and the use of modern agricultural methods to increase water use efficiency.

Materials and Methods: The purpose of this study was to evaluate the effects of perlite (0 and 10 g kg-1soil), humic acid (0, 7.5 and 15 g kg-1 soil) and irrigation interval (7, 20 and 30 days) on growth parameters of pistachio seedlings. This research was based on a completely randomized factorial design on "Badami Zarand" rootstock under greenhouse conditions. The studied factors included plant height, root length, leaf area, fresh and dry weights of root and shoot and uptake of nutrient elements in leaves. Finally, the statistical analysis of the data was done through a three way factorial ANOVA using the SPSS software, and the means of the data were compared using Duncan's method.

Results: The results of the study indicate that the application of humic acidin the concentration of15 g kg-1 soil and perlite 10 g kg-1 soil have a significant effect on plant growth and nutrient uptake under drought stress, which is associated with increasing effective roots and water abundance.

Conclusion: The results of the interaction effects showed that the application of humic acid and perlite can efficiently reduce the negative effects of drought stress on pistachio seedlings during irrigation intervals of 30 days, and it also causes more tolerance to stress.

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1. Introduction

Climate change has already started, and greenhouse gases have increased in the global scale. The phenology, physiology, distribution and interactions of plants are mostly determined by climate [1, 2]. Irrigation plays an important role in crop production in arid and semi-arid regions. Therefore, the maximum efficiency of water resources and the application of modern agricultural methods should be taken seriously to increase water use efficiency. Pistachio, which belongs to the Anacardiaceae family, has a high level of tolerance to drought and also has economic importance in Iran [3]. Although adult pistachio trees are known to be tolerant of drought, studies have shown that physiological adaptation of pistachio seedlings to drought limits growth [4]. Therefore, the use of methods and technologies that result in maximum efficiency of water should be considered in such circumstances. The application of moisture-retaining compounds is one of these methods [5]. Although micro-irrigation methods reduce water in the wetted surface of the soil and improve irrigation efficiency, there are still significant amounts of irrigation water wasted through soil surface transpiration by weeds [6]. Humic acid as an organic complex affects soil properties and the physiological characteristics of plants due to its carboxyl (COOH–) and phenolic (OH–) groups. Many studies have demonstrated the positive effects of humic acids on nutrient uptake [7], the growth of root length [8], and also the fresh and dry weights of plants [9-10]. Perlite is an amorphous volcanic glass (SiO_2) which has a relatively high water-holding capacity, typically formed by hydration. It can improve water uptake by three to four times of its weight. In fact, perlite is free of mineral nutrients [11]. Mohammadi and Mohammad Abadi investigated the effects of perlite on irrigation intervals of pistachio trees in Rafsanjan pistachio orchards. Their results showed that the highest profitability was related to the use of 3 tons of perlite per hectare with animal manure [12]. Therefore, in this experiment we studied the role of perlite and humic acid to save water and to improve growth of pistachio seedlings under drought stress.

2. Materials and Methods

The greenhouse experiments were carried out to study the effect of moisture retaining compounds (humic acid and perlite) on the morphological indices and nutrient uptake in Pistachio seedlings ("Badami Zarand") during growth seasons in Iran, Rafsanjan (Elevation 1469 m, Latitude East 56, Longitude North 30). The soil properties in this study included sandy loam texture with pH: 8.4 and EC: 1.8 dS m^{-1} and sufficient nutrient elements. The soil sample was air-dried and passed through a 4 mm sieve. About 4 kg of the soil was placed into plastic pots. Before planting, pistachio seeds were sterilized three times with 10% Sodium hypochlorite for 10-12 minutes, washed in each stage and soaked in distilled water for 24 hours [13]. One seedling ("Badami Zarand") was sown in a plastic pot in greenhouse conditions. After germination, one seedling was kept in each pot. The application of treatments were performed on

one-year-old seedlings. The various concentration of humic acid (0, 7.5 and 15 g kg^{-1} soil) and perlite (0 and 10 g kg^{-1}soil) were added to the pots and mixed into soil. This research was conducted in a completely randomized factorial design. Treatments included irrigation intervals (7, 20 and 30 days), humic acid (the substance of coal mine oxide from Tab as with humic purity of 60%) (0, 7.5 and 15 g kg^{-1} soil) and two levels of perlite (coarse) (0 and 10 g kg^{-1}soil) with three replications. After seven months of vegetative growth, the plants were harvested. The studied factors included plant height, root length, leaf area (Leaf Area Meter, AM200, ADC, Bioscientific LTD, UK), fresh weight of root and shoot. Plant samples were dried at 65°C and dry weight of root and shoot was determined. In order to determine uptake of the elements, the plant samples were dry digested using HCl. The samples were analyzed for phosphorus by tri-acid digestion using vanadomolybdo phosphoric acid yellow color method by spectrophotometry and plant potassium by flame photometry through Olsen method [3]. The Fe^{+}, Zn^{+2} and Mn^{+2} values were determined through the inductively coupled plasma mass spectrometry method (ICP-OES) [14]. Finally, the statistical analysis of the data was done through a three way ANOVA using the SPSS software and the means of the data were compared using Duncan's method at 1% and 5% levels.

3. Results

3.1. The effect of humic acid on the growth of pistachio seedlings

In this study, the interaction effect of humic acid, perlite and irrigation interval on height, root length, fresh and dry weight of shoot and root were statistically significant at the 1% level (Table 1). Drought stress decreased the growth of pistachio seedlings. Reductions of height, leaf area, fresh and dry weight of shoot were observed under drought stress (Table 2).

Table 1. The effect of humic acid, perlite and irrigation intervals on the growth parameters of pistachio seedlings: The results of a three way factorial ANOVA.

Means Square							
Source of variance	**DF**	**Height**	**Root length**	**Leaf area (mm^2)**	**Dry weight of root (g)**	**Dry weight of stem (g)**	**Dry weight of leaf (g)**
Humic acid(A)	2	7.755^{ns}	24.125^{*}	80783612.89^{**}	13.467^{ns}	7.443^{ns}	0.04^{**}
Interval irrigation(B)	2	516.96^{**}	86.742^{**}	53676298.55^{**}	20.129^{*}	104.69^{**}	0.099^{**}
Perlite(C)	1	188.907^{**}	28.167^{ns}	39792920.10^{ns}	13.023^{ns}	0.089^{ns}	0.005^{ns}
A* B	4	29.421^{ns}	31.75^{**}	36599151.14^{**}	6.792^{ns}	7.63^{ns}	0.014^{*}
A* C	2	123.06^{**}	22.79^{*}	90968619.19^{**}	2.406^{ns}	10.377^{ns}	0.011^{ns}
B*C	2	61.68^{ns}	6.167^{ns}	3333166.25^{ns}	5.141^{ns}	5.33^{ns}	0.039^{**}
A* B*C	4	71.255^{**}	19.54^{**}	34947379.96^{**}	17.705^{**}	26.83^{**}	0.021^{**}
Error	36	20.505	4.69	5780422.95	4.23	6.0262	0.005
CV%	-	9.16	7.244	14.01	15.13	13.03	12.9

Significant **P< 0.01, *P< 0.05 and Non-Significant (NS)

Table 2. The mean comparisons of humic acid, perlite and irrigation intervals on the growth parameters of pistachio seedlings: The results of Duncan's method

Means Square							
Factors	**Treatments**	**Height**	**Root length**	**Leaf area (mm^2)**	**Dry weight of root (g)**	**Fresh weight of stem (g)**	**Dry weight of leaf (g)**
Humic acid	0	48.66 a	27.41 b	15826 a	5.19 a	7.17 a	0.315 b
	7/5	47.4 a	29.33 a	18161 ab	4.87 ab	8.28 a	0.396 a
	15	47.6 a	30.25 a	20507 a	4.47 ab	8.28 a	0.409 a
irrigation Interval	7	67.66 a	24.66 b	24138 a	4.47 b	8.45 a	0.5417 a
	20	52.66 b	26.39 b	13261 b	6.749 a	6.6 b	0.3342 b
	30	45.11 c	36.39 a	9116.5 c	6.723 a	4.6 c	0.2048 c
Perlite	0	44.76 b	27.27 a	17499 a	4.78 a	7.87 a	0.347 a
	10	49.3 a	28.72 a	16865 a	5.57 a	7.95 a	0.366 a
Interaction effects (irrigation Interval* Perlite* Humic acid)	$I_7P_0H_0$	67 a	24 fg	24138 ab	5 d	8.03 b	0.54 ab
	$I_7P_0H_{7.5}$	56 bc	23 g	26205 a	4.71 de	8.75 ab	0.53 ab
	$I_7P_0H_{15}$	52 cd	30.5 bc	23713 ab	4.05 de	8.08 b	0.54 ab
	$I_7P_{10}H_0$	45 f	25.5 defg	23763ab	4.17 de	9.13 ab	0.52 ab
	$I_7P_{10}H_{7.5}$	56 b	29 bcd	21280 b	4.37 de	9.7 ab	0.53 ab
	$I_7P_{10}H_{15}$	52 cd	32.5 ab	22281 b	5.08 d	9.04 ab	0.52 ab
	$I_{20}P_0H_0$	52 cd	26.5 cdefg	13261 d	6.69 a	6.44 c	0.38 c
	$I_{20}P_0H_{7.5}$	49 d	28 cdef	16204 c	4.24 de	6.52 bc	0.42 bc
	$I_{20}P_0H_{15}$	52 d	28.5 bcde	18701 bc	5.02 d	7.83 b	0.36 b
	$I_{20}P_{10}H_0$	55 bc	29.5 defg	19130 bc	4.87 d	6.83 bc	0.43 bc
	$I_{20}P_{10}H_{7.5}$	53 cd	27.5 cdef	19408 bc	5.08 d	7.9 b	0.42 bc
	$I_{20}P_{10}H_{15}$	54 cd	29.5 bcd	17739 c	5.59 cd	8.24 b	0.36 b
	$I_{30}P_0H_0$	45 f	36 a	9116 e	6.29 b	4.69 d	0.21 e
	$I_{30}P_0H_{7.5}$	51 d	26.5 cdefg	11814 d	4.11 de	6.24 c	0.35 c
	$I_{30}P_0H_{15}$	52 d	29 bcd	11662 d	3.88 de	6.27 c	0.35 c
	$I_{30}P_{10}H_0$	49 de	30.5 bc	13178 d	4.83 d	6.51 c	0.25 d
	$I_{30}P_{10}H_{7.5}$	50 cde	24.5 efg	14056 d	4.65 d	6.58 c	0.32 c
	$I_{30}P_{10}H_{15}$	51 cde	27 cdefg	14346 d	4.54d	6.26 c	0.34 c

Means followed by same letter are not significantly different using Duncan's method

The comparison of means using Duncan's method showed that the root length and dry weight of leaves increased by applying humic acid. Also, the application of perlite significantly increased the height of seedlings (Table 2). In this study, the interaction effect of humic acid, perlite and irrigation interval showed that humic acid significantly increased the seedling height, leaf area and fresh and dry weight of the stem and fresh and dry weight of the leaf compared to control (without humic acid and perlite) in an irrigation interval of 30 days. The results showed that humic acid decreased seedling height in an irrigation interval 7 days, which was related to producing more lateral branches in this irrigation interval. It was also indicated that perlite (10 g kg^{-1} soil), similar to humic acid, was able to reduce the effects of drought stress in 30 days irrigation intervals as compared to control (without humic acid and perlite) (Table 2). The comparison of means using Duncan's method did not show any significant effect on the dry weight of root in perlite and humic acid treatments (Table 1 and 2). The results of the comparison of root length means showed that in irrigation interval of 7 days, the maximum root length was in the treatment of humic acid 15 g kg^{-1} soil plus perlite 10 g kg^{-1} soil. There was no significant difference between treatments in 20 days irrigation intervals. However, in irrigation intervals of 30 days, the highest root length was in the control treatment (without humic acid and perlite) (Table 2).

3.2. The effect of humic acid on nutrient uptake of pistachio seedlings

Drought stress had negative effects on the uptake of the nutrient element in pistachio seedlings. The results from the analysis of variance showed that the uptake of phosphor, calcium and zinc were statistically significant at 1%, and the uptake of potassium, iron and manganese were statistically significant at 5% (Table 3).

Table 3. The effect of humic acid, perlite and irrigation intervals on nutrient uptake of pistachio seedlings: The results of a three way factorial ANOVA.

	Means Square						
Source of variance	**DF**	**P (%)**	**K (%)**	**Ca (%)**	**Fe (g $100g^{-1}$ DM)**	**Zn (g $100g^{-1}$ DM)**	**Mn (g $100g^{-1}$ DM)**
Humic acid (A)	2	0.004 **	0.13*	0.069 *	274395.7 **	119.515 **	198.74*
Interval irrigation (B)	2	0.003**	0.046*	0.312**	243550.55**	458.176**	461.65*
Perlite(C)	1	0.002 ^{ns}	0.108*	0.134 ^{ns}	287700.304 **	1102.8 **	12.76 ^{ns}
A* B	4	0.006 **	0.122 ^{ns}	0.108 ^{ns}	122443 *	891.81 **	1023. 53**
A* C	2	0.001 ^{ns}	0.602**	0.422**	6823.351 **	605.612 **	1369.8 *
B*C	2	0.001 ^{ns}	0.133 ^{ns}	0.252 ^{ns}	28555.076 **	699.51 **	1059.733*
A* B*C	4	0.002**	0.068*	0.362**	129775.59*	263.43**	238.167*
Error	36	0.000	0.108	0.046	29421.52	41.56	257811. 5
CV%	-	9.8	8.9	10.09	8.8	9.5	7.4

Significant **P< 0.01, at *P< 0.05 and Non-Significant (NS)

Reductions of nutrient elements were observed under drought stress (Table 4). The comparison of means by Duncan's method showed that the uptake of potassium, phosphor, calcium, zinc, iron and manganese increased by applying humic acid. Also, it was found that applying perlite significantly increased the uptake of potassium, iron and zinc elements (Table 4). The results of the interaction effect between humic acid, perlite and irrigation interval are shown in the table 4.

Table 4. The mean comparisons of humic acid, perlite and irrigation intervals on nutrient uptake of pistachio seedlings: The results of Duncan's method.

Means Square							
Factors	**Treatments**	**P (%)**	**K (%)**	**Ca (%)**	**Fe (mg l^{-1})**	**Zn (mg l^{-1})**	**Mn (mg l^{-1})**
Humic acid	0	0.1512 b	1.23 b	1.28 b	419.24 b	34.29 c	31.508 b
	7/5	0.1763 a	1.76 a	1.63 a	592.8 b	54.08 b	36.042 a
	15	0.1813 a	1.86 a	1.76 a	653.9 a	75.74 a	38.6 a
irrigation Interval	7	0.074 a	0.6185 a	0.4951 a	195.14 a	10.903 a	16.313 a
	20	0.045 b	0.4284 b	0.4561 b	109.94 b	5.2432 b	11.536 b
	30	0.032 c	0.4004 a	0.3832 b	80.69 c	4.1431 b	8.3979 c
Perlite	0	0.16 a	1.102 b	1.74 a	482.32 b	45.139 b	35.894 a
	10	0.15 a	1.812 a	1.64 a	428.31 a	87.06 a	34.922 a
Interaction effects (irrigation Interval* Perlite* Humic acid)	$I_7P_0H_0$	0.07 cd	0.62 c	0.5 e	195.0139 c	10.90239 d	16.31368 c
	$I_7P_0H_{7.5}$	0.1 ab	0.72 bc	0.56 d	229.4751 b	12.48915 d	21.20445 ab
	$I_7P_0H_{15}$	0.11 a	0.78 bc	0.7 cd	258.5426 a	11.00628 d	19.79985 b
	$I_7P_{10}H_0$	0.07 cd	0.62 c	0.58 d	249.295 a	12.09975 d	22.8855 ab
	$I_7P_{10}H_{7.5}$	0.09 b	0.88 bc	0.58 d	227.3394 b	10.28008 d	21.99028 ab
	$I_7P_{10}H_{15}$	0.09 b	1.06 a	0.7 cd	261.97 a	13.3331 d	26.25565 a
	$I_{20}P_0H_0$	0.05 e	0.43 d	0.46 f	109.949 e	5.2432 f	11.526 d
	$I_{20}P_0H_{7.5}$	0.06 de	0.69 bc	0.84 c	160.9815 cd	19.7094 b	16.1463 c
	$I_{20}P_0H_{15}$	0.06 d	0.79 b	1.22 a	150.4373 cd	30.4911 a	15.74235 c
	$I_{20}P_{10}H_0$	0.05 de	0.58 c	0.43 f	96.063 e	10.593 d	11.429 d
	$I_{20}P_{10}H_{7.5}$	0.06 d	0.67 bc	0.53 de	136.815 d	15.785 c	15.2425 c
	$I_{20}P_{10}H_{15}$	0.06 d	0.6 c	0.87 c	181.824 c	18.8453 bc	20.71563 b
	$I_{30}P_0H_0$	0.03 f	0.43 d	0.38 f	80.69715 f	4.14315 f	8.3979 e
	$I_{30}P_0H_{7.5}$	0.06 d	0.58 c	0.66 d	104.7591 e	10.7646 d	7.7616 e
	$I_{30}P_0H_{15}$	0.06 d	0.6 c	1.03 b	102.467 e	12.60413 d	14.091 cd
	$I_{30}P_{10}H_0$	0.03 f	0.44 d	0.36 f	88.69715 ef	7.00785 e	8.7696 e
	$I_{30}P_{10}H_{7.5}$	0.06 d	0.55 c	0.71 d	104.7591 e	10.28075 d	14.0794 cd
	$I_{30}P_{10}H_{15}$	0.06 d	0.52 c	0.48 de	107.467 e	17.0863 c	13.29983 cd

Means followed by same letter are not significantly different using Duncan's method

The results of interaction effect showed that the application of humic acid significantly increased the nutrient uptake on pistachio seedlings under drought stress as compared with the control treatment (without humic acid and perlite in each irrigation interval).

The highest concentration of potassium uptake in the irrigation interval of 7 days was observed in the treatment with humic acid (15 g kg^{-1} soil) with perlite (10 g kg^{-1} soil). Under drought stress, the lowest uptake of potassium uptake in the irrigation interval of 30 days was observed in the control treatment (without humic acid and perlite) and perlite treatment 10 g kg^{-1} of soil without humic acid (Table 4).

The highest phosphor uptake in the irrigation interval of 7 days was observed in the humic acid treatment 15 g kg^{-1} soil without perlite treatment. The lowest phosphor uptake was observed in the irrigation intervals of 30 days without humic acid treatment in both with and without using perlite conditions. In the irrigation interval of 30 days, application of humic acid treatments (7.5 and 15 g kg^{-1} soil) significantly increased phosphor uptake in the plant in comparison with the treatment without humic acid in both with and without using perlite. But there was no significant difference between them. Calcium uptake decreased under drought stress in this study.

The results of interaction effects showed that humic acid (7.5 and 15 g kg^{-1} soil) had positive effects in irrigation intervals of 20 and 30 days, and the highest uptake was observed in the treatment of humic acid 15 g kg^{-1} soil without perlite. The comparison of means by Duncan's method showed that the maximum uptake of iron was in the humic acid treatment of 15 g / kg soil in irrigation interval of 7 days. The lowest was for the control treatment (without perlite and humic acid).

In irrigation intervals of 20 and 30 days, application of humic acid treatments (7.5 and 15 g kg^{-1} soil) in both with and without using perlite significantly increased iron uptake. The interaction effects of irrigation interval, humic acid and perlite on zinc uptake in pistachio seedlings are shown in table 4. The results of the comparison of means using Duncan test did not show any significant difference in irrigation intervals of 7 days. In the irrigation interval of 20 days, the highest zinc uptake in the treatment of humic acid was 15 g kg^{-1} soil without perlite, and the lowest uptake was observed in the treatment without humic acid and perlite. In the irrigation interval of 30 days, the highest of uptake was observed in the treatment of humic acid (15 g kg^{-1} soil) and perlite (10 g kg^{-1} soil), and the lowest uptake was in the treatment without humic acid and perlite. The interaction effects of irrigation intervals, humic acid and perlite on manganese uptake of pistachio seedlings are shown in table 4. The results of the comparison of means with Duncan test in the irrigation interval of 7 days showed that the maximum uptake was observed in humic acid treatment of 15 g kg^{-1} soil with perlite 10 g kg^{-1} soil. The lowest uptake was for perlite and without humic acid treatment. In the irrigation interval of 30 days, humic acid (7.5 and 15 g kg^{-1} soil) with perlite treatments of 10 g kg^{-1} soil and treatment of humic acid 15 g kg^{-1} soil without perlite significantly increased the manganese uptake in the leaves.

4. Discussion

Drought stress decreased the growth of the pistachio seedlings. Reductions of height, leaf area, fresh and dry weight of shoot were observed in drought stress. Reducing tungsten pressure during drought stress decreases cell growth, development, photosynthesis and, consequently, dry matter production [15], which is in line with the results of the present study. The effects of humic acid on plant growth, including the shoot and the amount of hair roots, were in line with the results obtained by many other researchers [16- 21, 13]. They reported that humic acid significantly increases plant growth. The results showed that perlite (10 g kg^{-1} soil) was able to reduce the effects of drought stress in 30 days irrigation intervals. The results of this study are agreement with the researchers' findings on pepper seedling growth (*Capsicum annuum grossum* var. Demre) and pistachio trees. The results of their studies showed that perlite has a positive effect on increasing the water-holding capacity and improving plant growth under stress [12, 22]. The comparison of means by Duncan's method did not show any significant effect on the dry weight of root in perlite and humic acid treatments. Mahajan and Tuteja reported that root growth under drought stress can also be increased in tolerant plants. This makes it possible for the root system to absorb more water from deeper layers of soil. In pistachio trees, the developmental habit of root is phreatophyte, which allows the root system to penetrate deeply into the soil [23]. Therefore, pistachio trees adapt to survive in long periods of drought [24], which is confirmed by the results of this research. The structural characteristics of the humic acid include a large number of oxygen structural groups (c= o, CO_2H_2, and OH phenols) which reacts with these ionic elements. In some of these interactions, the complex of humic compounds with metal elements affects plant nutrition [25]. The literature has shown that the application of humic acid has a positive effect on plant growth parameters and nutrient uptake under drought tolerance [26, 27], which is in agreement with the results obtained in this study. The most important role of humic acid is in stimulating the growth of the root system. The researchers stated that the humic acid leads to the reconstruction of root morphology, which results in an increase in the level of root uptake [28-29], hence, increasing the uptake of the elements, especially phosphorus, which is in agreement with the results obtained in the present study under drought stress and with reduced transpiration. Therefore, the transfer of the calcium element into the tissues is slow [30]. The reduction of calcium uptake under drought stress conditions was in accordance with the results obtained in this study. The literature has shown that humic compounds can act as ligands and form complexes with metals [31, 32].

5. Conclusions

The results showed that under drought stress, seedling height decreased by 33%. Leaf area and leaf dry weight decreased by 62%. However, the root length growth and root dry weight increased by 47%. The most important effect of humic acid was in increasing the effective hair root 11% and in the leaf dry weight 30%. Increasing the irrigation interval

significantly reduced the uptake of nutrients, especially micronutrients (iron 60%, copper 52%, zinc 60%, and manganese 49%). The results of the comparison of nutrient uptake showed that perlite treatments (iron 30%, potassium 64%, and zinc 2 times) and humic acid (51% potassium, calcium 37%, phosphorus 18%, manganese 24%, iron 55%, and zinc 2 times) improved nutrient uptake significantly, which is associated with increased effective hair roots and water abundance. Overall, the results of the interaction effects showed that the use of humic acid and perlite can effectively reduce the negative effects of drought stress on pistachio seedlings during irrigation intervals of 20 and 30 days and cause more tolerance of stress.

Conflict of interest

The authors declare no conflicts of interest.

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