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Evaluation of the growth and status of some nutrients in pistachio seedlings treated with phosphorus under different levels of irrigation water salinity

Farhad Azarmi-Atajan[1*], Mohammad Hassan Sayyari-Zohan[1] and Abdollah Mirzaei[2]

[1]Department of Soil Science, Faculty of Agriculture, University of Birjand, Birjand, Iran
[2]Department of Agronomy, Faculty of Agriculture, University of Birjand, Birjand, Iran

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***Corresponding author:**
Department of Soil Science, Faculty of Agriculture, University of Birjand, Birjand, Iran.
Email: farhadazarmi@yahoo.com; farhadazarmi@birjand.ac.ir

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ABSTRACT

Purpose: Irrigation with saline water and poor quality and fertility of the soil are the most important factors limiting the growth, establishment, and yield of pistachio trees in many pistachio farming areas of Iran. In addition, phosphorus plays an important role in plant growth, especially under environmental stress conditions. Thus, the purpose of this experiment is to investigate the role of P use in improving the growth of pistachio seedlings at different levels of irrigation water salinity. **Research method:** A greenhouse study was conducted as a factorial combination based on a completely randomized design with three replications. The treatments include two levels of P [Control (P_0) and 30 mg kg^{-1} soil (P_1) as triple superphosphate] and three levels of irrigation water salinity (0, 5, and 10 dS m^{-1}). **Findings:** Irrigation with saline water (10 $dS.m^{-1}$) significantly decreased the shoot dry weight (94%), root dry weight (64%), leaf area (62%), plant height (35%), shoot and root P content (41% and 52%), shoot K content (40%) and shoot and root K/Na (85% and 28%) of pistachio seedlings. However, P application increased the growth parameters and the concentration of P and K elements in the pistachio seedlings shoot and root under water salinity stress. **Research limitations:** No limitations were encountered. **Originality/Value:** According to the results of this experiment, phosphorus application increased the growth of pistachio seedlings in saline condition. Therefore, according to soil and water salinity in pistachio farming areas of Iran, optimal nutrition with nutrients such as P can increase the tolerance of pistachio seedlings to salinity stress and their establishment.

INTRODUCTION

Soil salinity has become one of the most critical environmental and socio-economic problems in the world, and climate change has intensified the process of soil salinization (Hassani et al., 2021). According to estimates, about 20 percent or 62 million hectares of irrigated agricultural lands in the world are facing different degrees of salinity, and their extent is continuously increasing due to climate changes and human activities (Arora, 2019). With the current trend, about 50% of agricultural land will be affected by salinity by 2050 (Hasanuzzaman et al., 2014). Fertilization, irrigation with saline water, salt build-up soils, and scarcity of rainfall are the main reasons for increasing the salinity of agricultural soils (Kumar et al., 2022). Under the saline conditions, due to high osmotic stress and changes in the availability of nutrients in the soil or the physiological destruction of some plant organs involved in the absorption of nutrients, the uptake and transport of nutrients in different parts of the plant are disturbed and nutritional imbalance occurs in the plant (Farooq et al., 2015). Despite the destruction of chlorophyll, the reduction of the rate of photosynthesis, the stomatal closure, and the increase of oxidative damage in plants exposed to salinity, plants deal with it in various ways, such as the production of osmoprotectants and phytohormones and the increase of antioxidant activities (Arif et al., 2020). Soil and water salinity is increasing significantly in irrigated agricultural lands of arid and semi-arid regions (Hu & Schmidhalter, 2005). It has been proven that the decrease of P availability in the soil is intensified by salinity. Due to high ratios of Na^+/K^+, Na^+/Ca^{2+} and Cl^-/NO_3^- in the solution of saline soils, the activity of nutrient ions such as P is low (Bidalia et al., 2019). Plants grown in saline soils usually face phosphorus (P) deficiency, the main reason of which is the decrease in the availability of P in the soil due to precipitation with other cations such as Ca^{2+}, Mg^{2+} and Zn^{2+} depending on the soil pH (Evelin et al., 2019; Azarmi-Atajan & Sayyari-Zohan, 2022). Therefore, it is necessary to maintain and improve the fertility and status of nutrients, including P, in agricultural soils affected by salinity for the growth and optimal performance of agricultural products.

Phosphorus is one of the essential macronutrients for the optimal growth of plants, and after nitrogen, it is the second nutrient limiting the growth and yield of plants. This nutrient plays a vital role in different cellular processes, including high-energy molecules formation, membrane structures maintenance, cell division, biomolecules synthesis and activation/inactivation of enzymes (Razaq et al., 2017). Phosphorus is an essential and fundamental element for all stages of plant growth and stimulates seed germination, root development, stem strength, flower and fruit formation, and increases crop quantity and quality (Malhotra et al., 2018). Despite the high amount of total P in agricultural soils, the amount of soluble P in the soil is meager and the plants grown in these soils often show symptoms of P deficiency. Generally, water-soluble P in soil is often less than 1 mg kg^{-1} (Rodriguez & Fraga, 1999). Abbas et al. (2018) reported that P deficiency (10 µM) had a more negative effect on the growth of the shoot and root of two wheat cultivars compared to moderate salt stress (100mM NaCl) under soil culture conditions. It was also described in the study that the application of P improved the growth and content of some nutrients in pistachio seedlings under saline conditions (Shahriaripour et al., 2011).

Pistachio (*Pistachia vera* L.) is one of the most important agricultural products of Iran and most pistachio orchards in Iran are located in arid and semi-arid areas with salty soils. Despite the relatively high tolerance of pistachio trees to salinity stress, the increase in soil and water salinity in pistachio farming areas of Iran - especially in recent years - has reduced their growth and yield (Azarmi et al., 2016). In addition to salinity stress, other factors such as high pH value and poor fertility of soil have also affected the growth and yield of pistachio trees. Considering soil and water salinity in pistachio farming areas of Iran and the lack of available P in most soils of pistachio orchards in Iran, the purpose of this research is to investigate the application of P on the growth and amount of nutrients of pistachio seedlings at different levels of irrigation water salinity.

MATERIALS AND METHODS

The experiment was carried out as a factorial combination based on a completely randomized design with three replications under greenhouse conditions with the relative humidity of 45% and temperature of 28±2/19±2 ºC on day/ night. The treatments include two levels of P [Control (P_0), and 30 mg kg^{-1} soil (P_1) as triple superphosphate] and three levels of irrigation water salinity (0, 5 and 10 $dS.m^{-1}$). A non-saline soil sample was collected from the agricultural fields of South Khorasan province, and after air drying; it was crushed, passed through a 4-mm sieve to remove gravels and mixed thoroughly. Selected physicochemical properties of the soil used in this study were measured based on standard methods (Sparks, 1996), and are shown in Table 1. After adding nutrients (N, K, Fe, and Zn) to the soil based on the soil analysis and mixing them thoroughly and uniformly with the soil, the plastic pots were filled with 2 kg of homogeneous soil. Phosphorus was also added to the pots before planting based on experimental treatments. For planting, first, 5 germinated pistachio seeds (*P. vera* L. cv. Badami) were planted in each pot and irrigated with non-saline water (EC=1.2 $dS.m^{-1}$) for 4 weeks. Then the number of seedlings in each pot was thinned to 2 and irrigation was done based on experimental treatments with prepared saline water to keep the soil at 80% of field capacity until the end of the growth period (twenty weeks).

Measurements

At the end of the experiment, the pistachio seedlings were collected and measurements were done. The height of the seedlings was measured from the soil surface using a ruler. After cutting the seedlings from the soil surface, the roots were carefully separated from the soil and washed to remove the soil particles the adhered to them. To determine the dry weight of the shoot and root, the plant samples were placed in an oven at a temperature of 70 ºC for 48 hours, weighed, and then ground into powder for chemical analysis. The powdered plant samples were dry-ashed at 500 °C and digested with 2N hydrochloric acid and made to volume with hot distilled water. Total P concentration in the shoot and root of pistachio seedlings were determined the method described by Chapman and Pratt (1961). Also, the content of K and Na in the samples was recorded by flame photometer.

Statistical analysis

The experimental data were used in analysis of variance (ANOVA) by SAS software, and the difference between the mean values was evaluated using the least squares difference (LSD) test at the 5% level of probability.

Table 1. Some characteristics of the soil used in the experiment.

Texture	pH	ECe	OM	SP	P_{av}	K_{av}	Na_s	Ca_s	Mg_s	SAR
		($dS\ m^{-1}$)	%	%	($mg\ kg^{-1}$)		($mEq\ L^{-1}$)			($MEq\ L^{-1})^{0.5}$
Sandy Loam	7.50	1.40	0.57	30	7.12	179	7.30	5.60	7.58	2.85

ECe: Electrical Conductivity, OM: Organic Matter, SP: Saturation Percentage, av: Available forms of element in the soil, s: Soluble forms of element in the saturated soil solution, SAR: Sodium Adsorption Ratio.

RESULTS AND DISCUSSION

Shoot and root dry weight

The analysis of variance revealed that the main and interaction effect of P and irrigation water salinity had a significant effect (p≤0.05) on the shoot and root dry weight of pistachio seedlings (Table 2).

According to the result, the increase of irrigation water salinity to 5 and 10 dS.m^{-1} reduced shoot dry weight by 20% and 94%, and root dry weight by 19% and 64% in comparison with the control, respectively (Table 3). Salinity stresses primarily affect the roots. Murshed et al. (2015) reported that as the salinity level increases, the diameter, length and number of roots decrease. The decrease in the dry weight of seedlings with increasing salinity can be related to the decrease in the number of leaves, smaller leaf surface area and also the decrease in their height. However, treatment with P had a positive effect on the shoot and root dry weight of pistachio seedlings in both saline and non-saline conditions. Application of 30 mg P kg^{-1} at salinity levels of 0, 5 and 10 dS.m^{-1} increased shoot dry weight by 43%, 60% and 61% and root dry weight by 60%, 48% and 96%, respectively, at the same salinity levels (Table 3). Phosphorus plays an essential role in processes such as respiration, photosynthesis, membrane stability and energy production (Marschner, 1995). The increase in dry weight of pistachio seedlings with the application of mineral P has also been reported by Shahriaripour et al. (2011) and Fekri et al. (2015).

Table 2. Analysis of variance (mean of square) for measured traits.

SOV	df	ShDW	RDW	Leaf Area	Plant Height	Shoot P	Root P
Phosphorus (P)	1	0.811**	0.692**	1014**	125**	0.049**	0.009**
Salinity (S)	2	0.492**	0.704**	1489**	43.1**	0.014**	0.010**
P × S	2	0.009*	0.021*	13.9*	1.52ns	0.0007*	0.0004*
Error	12	0.002	0.004	5.16	1.51	0.0002	0.0001
CV		4.75	7.78	4.59	9.92	7.64	5.95

SOV: Source of Variation; ShDW: Shoot Dry Weight; RDW: Root Dry Weight.
**, * and ns: significant at p≤0.01, significant at p≤ 0.05 and non-significant, respectively.

Table 2. (Continued**).**

SOV	df	Shoot K	Root K	Shoot Na	Root Na	Shoot K/Na	Root K/Na
Phosphorus (P)	1	0.084**	0.072**	0.027**	0.039**	3.26*	0.15**
Salinity (S)	2	0.53**	0.074**	0.30**	0.086**	196**	0.70**
P × S	2	0.027*	0.004*	0.004*	0.002*	0.668ns	0.11*
Error	12	0.006	0.001	0.001	0.0005	0.630	0.020
CV		5.73	4.86	8.25	6.24	12.5	6.92

SOV: Source of Variation; ShDW: Shoot Dry Weight; RDW: Root Dry Weight.
**, * and ns: significant at p≤0.01, significant at p≤ 0.05 and non-significant, respectively.

Table 3. The interaction effect of phosphorus × salinity of irrigation water on the dry weight (DW) and phosphorus (P) content of shoot and root of pistachio seedlings.

Phosphorus (mg kg^{-1})	Salinity of irrigation water (dS m^{-1}) 0	5	10	Salinity of irrigation water (dS m^{-1}) 0	5	10
	Shoot DW (g plant^{-1})			Root DW (g plant^{-1})		
0	1.05 ± 0.03 c	0.82 ± 0.04 d	0.54 ± 0.03 e	0.89 ± 0.04 c	0.72 ± 0.02 d	0.32 ± 0.02 e
30	1.50 ± 0.02 a	1.31 ± 0.02 b	0.87 ± 0.03 d	1.42 ± 0.05 a	1.07 ± 0.04 b	0.63 ± 0.03 d
	Shoot P Concentration (%)			Root P Concentration (%)		
0	0.12 ± 0.009 d	0.15 ± 0.007 c	0.07 ± 0.005 e	0.19 ± 0.006 b	0.14 ± 0.005 c	0.09 ± 0.007 d
30	0.23 ± 0.008 b	0.26 ± 0.009 a	0.15 ± 0.006 c	0.22 ± 0.003 a	0.19 ± 0.006 b	0.15 ± 0.005 c

For each parameter, values followed by the same letter are not significantly different (p≤0.05) according to LSD test.

Leaf area and plant height

The leaf area of seedlings was significantly influenced ($p \leq 0.05$) by the P, salinity and P× salinity interaction. Also, only the main effects of P and salinity on the height of pistachio seedlings were significant (Table 2).

The results indicated that the leaf area of seedlings showed 19% and 62% decrease at the 5 and 10 dS.m^{-1} salinity levels relative to the control. Salinity stress in the initial stages causes the stomata to close and the partial pressure of CO_2 between the cells decreases, and as a result, the growth of the leaves is limited by reducing the rate of photosynthesis. Also, salinity causes the premature senescence of leaves and a severe reduction in plant growth (Arif et al., 2020). Although the application of P in both saline and non-saline conditions increased the leaf area of seedlings, but its effect was more significant in high salinity (10 dS.m^{-1}) than other salinity levels. Treatment with P at salinity levels of 10 dS.m^{-1} increased this parameter by 75% in comparison with the same salinity level (Fig. 1). Use of 30 mg P kg^{-1} increased plant height by 54% compared to the control (Fig. 2a). Considering the low amount of P in the studied soil, the application of P has improved various growth indicators of pistachio seedlings such as leaf area and height. The response of plants to the supply of P is different depending on the concentration and plant species (Ben hamed et al., 2019). On the other hands, when the salinity of irrigation water increased from 0 to 5 and 10 dS.m^{-1}, the seedling height decreased by 21% and 35%, respectively (Fig. 2b). One of the most apparent effects of salinity on the plant is to reduce its growth and development. The decrease in plant growth under salinity stress is mainly due to the increase in osmotic pressure, the decrease in water availability in the root environment, the accumulation and toxicity of sodium and chlorine ions, and the loss of nutritional balance in the plant (Munns, 2002).

Shoot and root P content

The analysis of variance showed that the main and interaction effect of P and irrigation water salinity had a significant effect ($p \leq 0.05$) on the shoot and root concentration of P in pistachio seedlings (Table 2).

Based on the results, irrigation with water with low salinity level (5 dS.m^{-1}) increased (25%), but with high salinity level (10 dS.m^{-1}) decreased (41%) the P content in the shoot of pistachio seedlings. But both salinity levels caused a significant decrease in P concentration in the root of seedlings. Application of P at salinity levels of 0, 5 and 10 dS.m^{-1} increased shoot P content by 91%, 73% and 114%, and root P content by 15%, 35% and 61%, respectively, relative to the same salinity levels (Table 3). In general, the relationship between salinity and the content of P in plants is complex, and the concentration of plant P varies depending on the plant species, the growth stage of the plant, and the amount of P in the culture medium (Loupassaki et al., 2002). The reduction of P uptake in leaves and roots of pistachio seedlings under saline conditions was reported by Eskandari and Mozafari (2014).

Table 4. The interaction effect of phosphorus × salinity of irrigation water on the shoot and root potassium (K) and sodium (Na) content of pistachio seedlings.

Phosphorus (mg kg^{-1})	Salinity of irrigation water (dS m^{-1}) 0	5	10	Salinity of irrigation water (dS m^{-1}) 0	5	10
	Shoot K Concentration (%)			Root K Concentration (%)		
0	1.78 ± 0.06 a	1.42 ± 0.04 bc	1.06 ± 0.03 d	0.62 ± 0.02 c	0.68 ± 0.02 b	0.87 ± 0.03 a
30	1.49 ± 0.06 b	1.35 ± 0.04 c	1.02 ± 0.03 d	0.51 ± 0.02 d	0.60 ± 0.01 c	0.69 ± 0.02 b
	Shoot Na Concentration (%)			Root Na Concentration (%)		
0	0.15 ± 0.01 e	0.34 ± 0.02 c	0.64 ± 0.03 a	0.27 ± 0.007 d	0.36 ± 0.01 c	0.54 ± 0.02 a
30	0.11 ± 0.01 e	0.28 ± 0.01 d	0.51 ± 0.02 b	0.22 ± 0.006 e	0.25 ± 0.01 de	0.42 ± 0.01 b

For each parameter, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test.

Shoot and root K and Na content

Based on the results, the content of K and Na in the shoot and root of pistachio seedlings was significantly influenced ($p \leq 0.05$) by the P, salinity and P × salinity interaction (Table 2).

The results revealed that with the increase of irrigation water salinity level, the content of K reduced in the shoot but increased in the root. In comparison with the control, the shoot K concentration decreased by 20% and 39% at the salinity levels of 5 and 10 $dS.m^{-1}$, respectively. On the other hands, at these salinity levels, the content of root K increased by 10% and 40% compared to the control, respectively (Table 4). Despite the relatively high amount of K in the soils of arid and semi-arid regions, the availability of this element for the plant can decrease in different conditions, including salinity. The most important factor limiting the availability of K for the plant in saline conditions is the high concentration of ions that cause salinity, including Na, and also the reduction of root growth. Shahriaripour et al. (2010) showed that the increase of NaCl decreased the concentration of K in the shoot and increased its concentration in the roots of pistachio seedlings. Their results are consistent with the results of this research. The increase in K concentration in the roots with the application of salt water is related to the decrease in plant growth and the dilution effect. However, an increase in K concentration in the leaves of pistachio seedlings with increasing salinity has also been reported (Hojjatnooghi et al., 2014). The use of P only in non-saline conditions was effective on the content of shoot K, which caused it to decrease by 16% compared to the control at same salinity levels. Furthermore, the application of P at salinity levels of 0, 5 and 10 $dS.m^{-1}$ reduced the content of root K by 17%, 11% and 21% relative to the control, respectively (Table 4). Phosphorus plays a role in the process of cell division and thus causes the development of the growth of plant shoot and roots and increases the availability of nutrients in the soil (Razaq et al., 2017).

According to the results, an increase in the irrigation water salinity levels caused an increase in the concentration of Na in the shoot and root of the pistachio seedlings, although the accumulation of this element was higher in the shoot than in the root. For example, with application of high water salinity level (10 $dS.m^{-1}$), the content of Na in the shoot and root increased by 4.2 times and 2.0 times compared to the control, respectively. One of the main effects of salinity stress on plants is the accumulation of toxic ions in their various tissues, especially leaves. Studies have shown that one of the mechanisms of plants resistance to salinity stress is keeping Na concentration low in leaves and reducing its transfer from roots to aerial parts (Chen et al., 2010). Although the resistance of the pistachio plant to salinity stress is relatively high, but with the increase of Na concentration in the root growth environment, the ability of the root to control and accumulate Na is reduced and as a result, its transfer to aerial organs increases. In general, due to the accumulation of toxic elements such as Na and Cl in the shoot of plants, leaves are more vulnerable to these elements than root. Based on the studies of Behzadi Rad et al. (2021), salinity increased the concentration of Na in the shoots and roots of three cultivars of pistachio seedlings. However, the accumulation of Na in the shoot of all three cultivars was more than in the root. The application of P (30 $mg.kg^{-1}$) caused a decrease in the accumulation of Na in the shoot and root of seedlings, especially in saline conditions (Table 4). Considering the role of P in rooting and increasing the root growth of plants, the decrease in Na concentration in pistachio seedlings with the application of P can be attributed to the increase in root growth and, as a result, the increase in the absorption of water and nutrients such as Ca and K. Optimum application of P in saline conditions can improve various soil properties, increase K use efficiency, increase tolerance to salinity stress, and as a result, increase plant yield (Sadji-Ait Kaci et al., 2022).

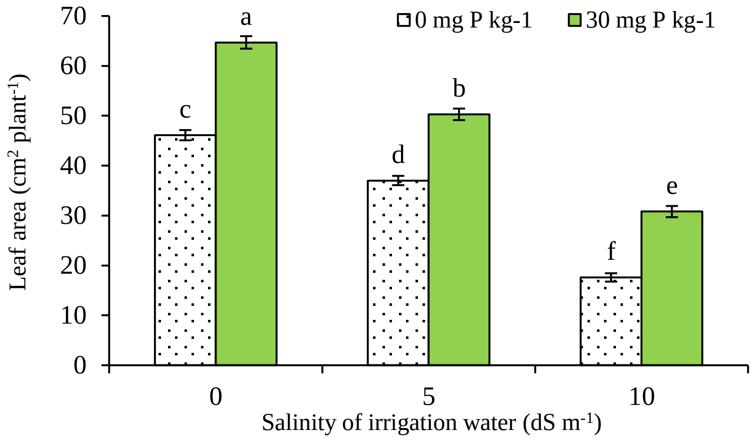

Fig. 1. The effect of phosphorus × salinity of irrigation water on the leaf area of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test. The error bars in the graphs are standard errors.

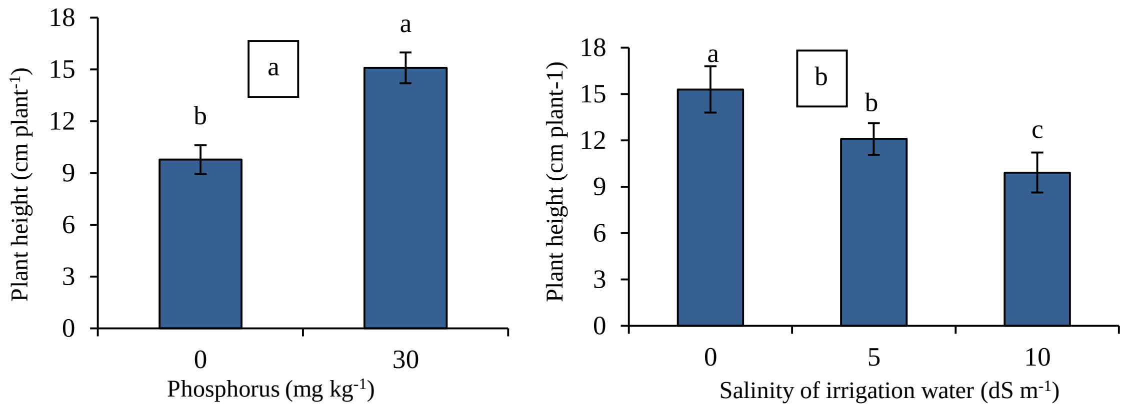

Fig. 2. Main effect of phosphorus (a) and irrigation water salinity (b) on the height of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test. The error bars in the graphs are standard errors.

Shoot and root K/Na

The analysis of variance showed that only the main effects of P and salinity on the shoot K/Na of pistachio seedlings were significant. Also, the root K/Na of seedlings was significantly influenced ($p \leq 0.05$) by the P, salinity and P× salinity interaction (Table 2).

The results indicated that use of P significantly increased shoot K/Na by 14% in comparison with the control (Fig. 3a). Also, the ratio of shoot K/Na showed 64% and 85% decrease at water salinity levels of 5 and 10 $dS.m^{-1}$ compared to the control (Fig. 3b). According to the results, the root K/Na significantly reduced with rising irrigation water salinity. Salinity levels of 5 and 10 $dS.m^{-1}$ decreased root K/Na by 16% and 28% relative to the control. In the plant under salt stress, the outflow of K from the plant increases and as a result the K/Na decreases (Qi & Spalding, 2004). Also, the competition of Na with other nutrients such as K causes nutritional imbalances (Mohamed & Gomaa, 2012). The application of P was effective only at low salinity level (5 $dS.m^{-1}$) on the ratio of root K/Na, which caused it to increase by 26% compared to the control (Fig. 4). The increase in K/Na with the application of P can be related to the improvement of root growth, the plant's access to water and nutrients, and the increase in uptake of nutrients.

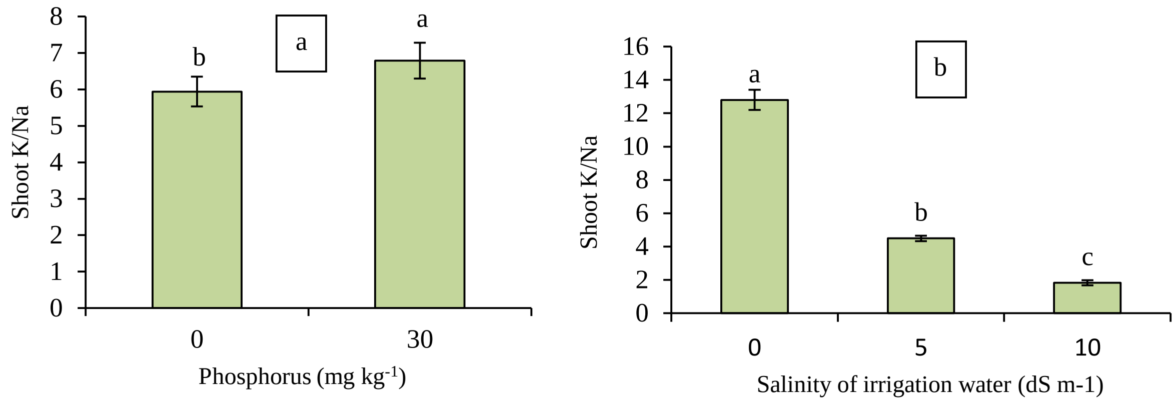

Fig. 3. Main effect of phosphorus (a) and irrigation water salinity (b) on the shoot K/Na of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

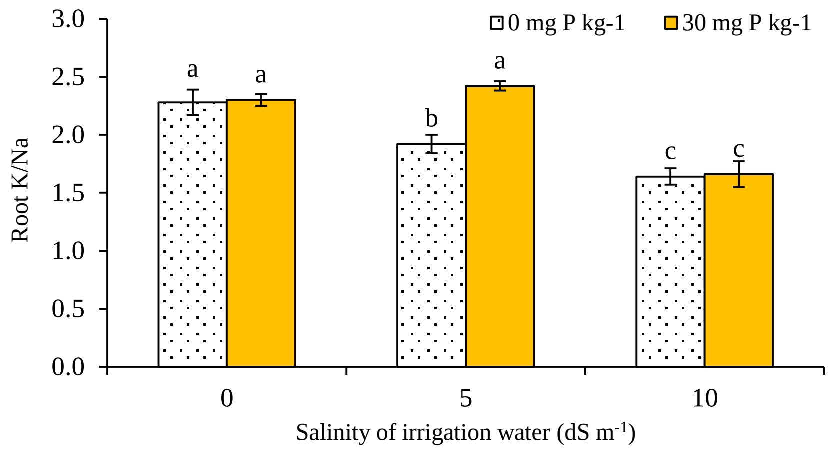

Fig. 4. The effect of phosphorus × salinity of irrigation water on the root K/Na of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

CONCLUSION

Irrigation with saline water is one of the most important factors that reduce the quality and fertility of the soil and as a result the growth, establishment and yield of pistachio trees in a large part of pistachio orchards in Iran. According to the results of the present experiment, irrigation with saline water (especially salinity level 10 $dS.m^{-1}$) decreased the shoot and root dry weight, leaf area, plant height, shoot and root P content, shoot K content and shoot and root K/Na of pistachio seedlings. On the other hand, with increasing salinity of irrigation water the concentration of shoot and root Na and root K in seedlings was increased. However, the application of P significantly increased the growth parameters such as dry weight and nutrient concentration in pistachio seedlings under various salinity conditions. Also, the use of P decreased the content of Na in the shoot and root of pistachio seedlings. Considering the lack of available P in the soil and the role of this element in many plant processes including root growth and development, the application of P was able to increase the growth of the roots and, as a result, increase the access to water and nutrients, and the growth of pistachio seedlings at different levels of water salinity. Therefore, the use of P can help to develop the root system and ultimately increase the establishment of pistachio seedlings in regions with saline soil and water.

Conflict of interest

The authors declare no conflict of interest.

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