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ORIGINAL ARTICLE

Effects of Irrigation-based Potassium Fertilizer on Leaf and Nut Nutrients of Two Pistachio Cultivars

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A R T I C L E I N F O

Keywords:

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A B S T R A C T

The annual consumption of soil potassium by pistachio trees is high. Since the clay soil absorbs potassium, the amount of potassium that can be exchanged or used by the tree in the soil solution is little, and every year this deficiency must be compensated by fertilizers. To evaluate the role of potassium in improving the nutritional elements of the leaves and nuts of two pistachio cultivars (‘Ahmad-Aghaei’ and ‘Kalleh-Qoochi’), four levels of potassium fertilizer were used (0, 200, 300 and 400 mg of potassium per liter of irrigation water) in a factorial experiment in the form of a randomized complete block design with three replications. This experiment was performed on the ON and OFF branches in 2017 and 2018. The results showed that the application of potassium increased the amounts of nutritional elements in the leaf and nut. Specifically, the amounts of nitrogen, phosphorus and potassium were higher in the leaves of OFF branches, whereas the concentrations of calcium and magnesium were higher in the leaves of the ON branches. In both cultivars, the application of potassium increased the amounts of nitrogen and phosphorus in the leaf, as well as the potassium content in the nut. The amounts of calcium and magnesium in the leaf and the amount of calcium in the nuts were higher in the Kalleh-Qoochi cultivar. This treatment can be recommended when plants are in their OFF year, so that potassium can be reserve into the tissues until the ON year.

Introduction

As a cultivated species of the Anacardiaceae family, pistachio (*Pistacia vera* L.) is an established, domesticated nut crop that originated from diverse regions, including Iran (Hosseini *et al.*, 2022). Pistachios are native to western Asia and were brought to European countries by traders (Mandalari *et al*., 2021). Among the many species in the *Pistacia* genus, only *P. vera* has mostly received commercial attention. The word “pistachio” originates from “pistak” in ancient Farsi (Saitta *et al*., 2011). According to archeological data, pistachio consumption has a history of 300,000 years when it

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was first consumed by the Neanderthals (Monnier, 2012). Presently, more than four-fifths of pistachio production in the world occurs in the USA, Iran, and Turkey (FAOstat, 2018). While the majority of the world population does not see pistachio nuts as an inseparable part of the human diet, pistachios are rich in vitamins, proteins, minerals, phenolic compounds, and antioxidants (Tomaino *et al.,* 2010; Akbari *et al.,* 2018; Sheikhi *et al.*, 2019).

Fruit quality and yield in many tree species, including pistachio, are mostly affected by mineral nutrients (Tagliavini and Marangoni, 2002). In a regular plant cycle, and the annual cycle of deciduous trees, one of the main macronutrients is potassium (K) which has multiple functions in tree physiology and cellular metabolism, including protein synthesis and enzyme activation (Pallardy, 2010). K is commonly known for its roles in regulating normal water-use efficiency, maintaining plant growth, and increasing plant tolerance to disease (Ferguson and Haviland, 2016). The nutritional content of leaves is affected by factors that shape plant physiology and adapt to the environmental settings in which the trees are situated. In particular, the presence of K in the leaf is significantly important in healthy forms of leaf physiology (Tränkner *et al*., 2018). The leaves of pistachio trees are regarded as a reservoir of storable K which can be relocated to pistachio nuts during their development and ripening, especially since the kernels are a strong nutritional sink (Elloumi *et al*., 2022). Potassium uptake usually happens at a faster rate at the time of nut-filling because the development of embryos requires an ample supply of potassium. In turn, this can affect leaf K content, depending on the amount of nut yield on the tree (Zeng *et al*., 2001). Deficiencies in K content were reportedly compensated by applications of foliar K treatments on pistachio trees, whereby relationships between K, nitrogen, and other nutrients were regarded as indicators of a correct balance in leaf and fruit nutrients (Hussein, 2008). In an experiment on olive trees, two concentrations of potassium nitrate (2% and 4%) were used as foliar spraying at two stages, immediately after fruit set and pit hardening. The results of this experiment showed that the concentration of 4% at both stages improved the nutritional status, comprising nitrogen, phosphorus, and potassium in the leaves in two years (2009 and 2010) with the use of potassium nitrate (Hegazi *et al.,* 2011). As the soil depth increases, the amount of potassium in the soil decreases. In an experiment, the effects of potash fertilizers were examined on returning potassium to K-deficient soils. In this experiment, potassium sulfate was added to pistachio orchards through the drip-irrigation system for three years. Potassium sulfate was used once a month from May to August at ratios of 0, 1.1, 2.2, and 3.3 pounds for each tree as fertilizer. It was shown that the application of potassium increased the amount of potassium in the soil at a depth of 0 to 30 inches. Therefore, it caused more potassium accumulation in pistachio fruits and leaves (Zeng *et al*., 2000). In a research, the effects of different irrigation regimes and potassium fertilizers were evaluated on olive trees. Each tree received 25 and 35 cubic meters of water each year. Irrigation was done with potassium (0, 200, 300 and 400 mg L^{-1}) in irrigation water in the form of potassium sulfate (48% K_2O). It was found that the 400 mg L^{-1} increased the content of nutrients in the leaves and fruits (N, P, K, Ca and Mg), especially at the irrigation level of 35 cubic meters. Therefore, according to this experiment, the irrigation level of 35 cubic meters and potassium (400 mg L^{-1}) through drip irrigation was recommended for olive trees (Hussein, 2008). Also, spraying potassium nitrate increased the nutritional status of olive leaves (Sarrwy *et al*., 2010). The use of potash fertilizers through irrigation with a drip system increased the absorption of potassium by trees (Neilsen and Neilsen, 2006). In a research, the effects of potassium fertilizers were examined on apple orchards. Through drip-irrigation, seven fertilizer treatments were used, including the control, 15 grams of potassium for each tree with potassium chloride and potassium magnesium sulfate, 30 grams

of potassium for each tree with potassium chloride, potassium magnesium sulfate, potassium sulfate, and potassium thiosulfate. The form of fertilizer did not affect the yield, but after three years, the amount of potassium increased at the depth of 30 cm in the soil, under the drippers. Meanwhile, the potassium concentration increased in the leaves and fruits (Neilsen and Neilsen, 2006). In another experiment, the effects of potassium magnesium sulfate, as fertilizer, were evaluated in apple orchards. Three fertilizer treatments were used annually, including 0, 100 and 200 kg ha^{-1} potassium and magnesium sulfate. After three years of fertilizer application, the contents of potassium and magnesium increased at soil depths of 0 to 10 cm and 10 to 20 cm.
Also, this led to higher levels of potassium in the leaves and fruits (Neilsen and Neilsen, 2011).

After entering plant tissues, K becomes capable of various functions, and the relationship between these functions is usually associated with a normal physiological performance that is measurable. The application of fertilizers in irrigation water is known as an efficient method to increase plant performance (Abd-Elrahman *et al*., 2022). The relationships between K absorption and other macro elements in plants were reportedly studied in a number of plants (Chavan *et al*., 2020; Xu *et al*., 2020). Nonetheless, the effects of potassium treatments, after being added to irrigation water, have not been evaluated in pistachio trees. The major objective of the present study was to determine the effects of K nutrition in irrigation water on specific nutrient contents of pistachio leaves and nuts. A relevant hypothesis was that the potassium treatment in irrigation water can effectively improve the amounts of other macro nutrients in pistachio leaves and nuts. For this purpose, two pistachio cultivars were used, i.e. 'Kalleh-Qoochi' and 'Ahmad-Aghaei', because of their prevalence in Iranian pistachio orchards.

Materials and Methods

This research was carried out on 16-year-old pistachio trees of 'Ahmad-Aghaei' and 'Kalleh-Qoochi' cultivars grafted onto the Badamizarand rootstock in a commercial orchard in 2017 and 2018.

The orchard was located near Sirjan (longitude 55 degrees, 52' and 19" east; latitude 29 degrees, 10' and 53" north). Sirjan is characterized by a semi-arid climate with dry, cold winters and hot, dry summers. The average annual temperature, average annual rainfall and average humidity were 25 °C, 144 mm and 33%, respectively. Twenty-four trees were selected for sample collection. The trees were evenly spaced, at a distance of 1.5 × 6 meters. The chemical and physical properties of the soil were measured (Table 1). Orchard management was carried out equally for all trees. Common types of fertilizers were used in the experiment (Table 2). The experimental layout was factorial in a randomized complete block design. The analysis of data on the nutrients in nuts consisted of three replications and three factors, i.e. two cultivars ('Ahmad-Aghaei' and 'Kalleh-Qoochi'), potassium fertilizer at four levels (0, 200, 300 and 400 mg L^{-1} in irrigation water) and year (2017 and 2018). The analysis of leaf nutrients in the form of compound analysis was carried out for both years (2017 and 2018) with two factors, i.e. cultivar at two levels ('Ahmad-Aghaei' and 'Kalleh-Qoochi') and potassium fertilizer at four levels (0, 200, 300 and 400 mg L^{-1} in irrigation water). Evaluations were done on ON and OFF branches.

The potassium was applied as pure solo-potash fertilizer. Irrigation was done based on the ring basin irrigation system. Irrigation stages in this experiment were carried out according to the irrigation calendar of the garden, which was once a month. Irrigation was done at 8 stages from April to September, once a month, and thereafter once in February and once in March. Each tree approximately received 545 liters of water in each irrigation session.

Table 1. Physical and chemical properties of the soil at a depth of 0-40 and 40-80 cm.

Soil properties	0-40 cm	40-80 cm
Sandy (%)	68.5	66.5
Silt (%)	16	18
Clay (%)	15.5	15.5
Soil texture	Sandy loam	Sandy loam
pH	7.53	7.34
EC (ds m^{-1})	1.1	1.1
AbsorbableK (mg kg^{-1})	438.33	396.122
P (mg kg^{-1})	1.23	2.86
Ca (meq L^{-1})	5	5.2
Mg (meq L^{-1})	3.6	4.2
Na (meq L^{-1})	5.6	5.6
Fe (ppm)	1.43	1.21
Mn (ppm)	6	4
Zn (ppm)	2.1	2.87
Cu (ppm)	0.996	0.16
Organic matter (%)	1.45	1.1

Table 2. Common fertilizers used in the tested garden.

Fertilizers of use	Amount of use	time of use	How to use
solo-potash	100 kg per hectare	In two stages: 1-April to May 2-May to June	Spreading fertilizer on the soil surface
Potassium sulfate	200-250 kg per hectare	Once in three years in winter	Local placement method (Chalkood)
urea	500 kg per hectare	In 3 stages: Stage 1 and 2 with Soluptas and Stage 3 in July	Spreading fertilizer on the soil surface
Calcium nitrate	30-50 kg per hectare	April	Spreading fertilizer on the soil surface
fruit set	2 per thousand	Bud swelling	Foliar spraying
Micro fertilizers (Fe, Zn, Mn)	1-1.5 per thousand	May	Foliar spraying
Calfon	2.5 per thousand	April to May	Foliar spraying
N-P-K	50 kg per hectare	May to June	Spreading fertilizer on the soil surface
Wuxal macromix	1-1.5 per thousand	April to July	Foliar spraying

Measurement of nutrients in the leaves and nuts

In August, the measurable nutrients included nitrogen, phosphorus, calcium, potassium, magnesium and sulfur in the leaves. In September, the measurements were on magnesium, calcium, potassium and sulfur in the nuts. . The samples were taken from the middle part of the ON and OFF branches. To prepare the extract, first, 0.5 g of the sample was dried and ground. Then, it wasplaced in an oven at 550°C for 3 hours, so that the samples turned into ash. Using HCl (2 N), 5 cc of acid was added to each sample, and the volume was finally increased to 50cc by distilled water. This extract was used directly to measure the amounts of magnesium, potassium, and calcium (Estefan *et al*., 2013). To measure phosphorus, using the ammonium molybdate and ammonium vanadate (yellow) methods, first, 5 cc of the previously-made extract was mixed with 10 cc of ammonium molybdate vanadate and, finally, distilled water was added. After being brought to a volume of 50 cc (Olsen *et al*., 1954) and after extraction, it was measured at 470 nm using a spectrophotometer. Calcium and magnesium contents were measured after extraction using an atomic absorption spectrometer, whereas potassium was measured using a flame photometer. To measure sulfur, 5 ml of the extract was mixed with 10 ml of barium chloride (5%) and 1 ml of concentrated HCl which were poured together into a 100 ml flask. Then, its absorption was measured by a spectrophotometer at 420 nm (Estefan *et al*., 2013).

Nitrogen was measured at three stages, i.e. digestion, distillation and titration. First, 0.2 g of dried leaf samples was poured into special tubes to allow digestion, and then one cooper selenium tablet was placed into each tube, followed by the addition of 10 ml pure HCl. Then, the tubes were placed in the system so that digestion could occur, which was completed after three hours. After digestion, the distillation of each sample was done separately by the distillation apparatus. In the tanks of the distillation apparatus, there was a mixture of 4% boric acid, 40% NaOH (0.1 N), and distilled water. Finally, titration was performed for each distilled sample and, after titration, the amount of HCl (0.1 N) consumption was recorded. The percentage of nitrogen was calculated by the following formula (Hosseini, 1994).

$$14 \times 100 \times 1000 \times \text{sample weight} / \text{NaOH} \times \text{amount of HCl consumption} = \text{Nitrogen (\%)}$$

Statistical analyses were performed using SAS software and Duncan’s method (P≤0.05).

Results

Tests for the homogeneity of variance (Bartlett test) were carried out in August each year (2017-2018) on the amount of macronutrients in pistachio leaves (nitrogen, phosphorus, potassium, calcium, magnesium and sulfur) in both pistachio cultivars, ‘Ahmad-Aghaei’ and ‘Kalleh-Qoochi’. According to Table 3, the variance of experimental error was not significant and, thus, homogeneity was estimated in the variance of data for these two years. The results of combined analysis of variance were gathered on the amount of macronutrients in pistachios in August. Specifically, the measurable macronutrients were nitrogen, phosphorus, potassium, calcium, magnesium and sulfur (Table 4).

The analysis of variance showed that regarding the simple effects, fruit bearing (ON) and non-bearing (OFF) treatment groups had a significant effect (P≤0.05) on the amount of all macro elements (except sulfur). The difference in the two cultivars also significantly affected the amount of some macro elements in the leaf, including nitrogen, phosphorus, calcium and magnesium (Table 4). The results of analysis of variance showed that the simple effects of fertilizer were significant on the amount of all nutrient elements in the leaf, except sulfur (Table 4). Among the interactions, the reciprocal interactions between cultivar and fertilizer were significant in the case of nitrogen and phosphorus (Table 4). The reciprocal interactions between year and cultivar, year and fertilizer, year and cultivar and fertilizer were significant in the case of nitrogen, phosphorus and potassium (Table 4).

Table 3. Homogeneity tests on trial errors of ON and OFF, within years (Y_1, Y_2) and between years, regarding pistachio leaf nutrients in August.

Mean Square Error	Leaf nitrogen	Leaf phosphorus	Leaf potassium	Leaf calcium	Leaf magnesium	Leaf sulfur
Within Y_1 ON/OFF	2.01^{ns}	3.14^{ns}	1.88^{ns}	1.54^{ns}	1.45^{ns}	3.62^{ns}
Within Y_2 ON/OFF	2.14^{ns}	2.61^{ns}	2.51^{ns}	2.77^{ns}	1.42^{ns}	3.02^{ns}
betweenY_1 and Y_2	1.59^{ns}	1.21^{ns}	2.20^{ns}	1.04^{ns}	1.03^{ns}	1.20^{ns}

Ns: No significant difference

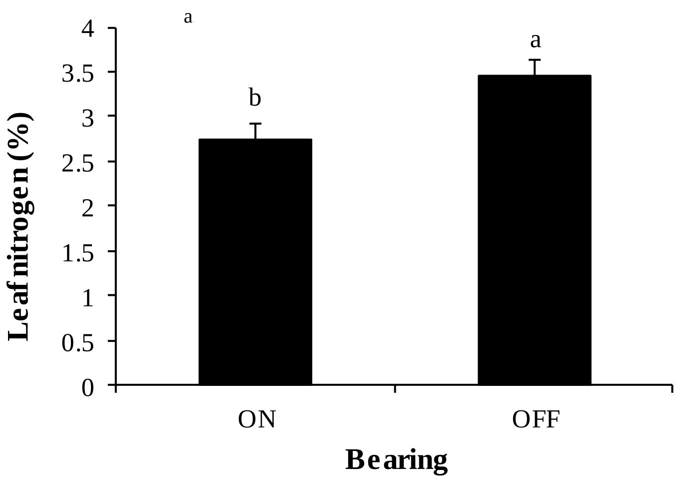
Table 4. Results of combined analysis of variance over two years (y) (2017-2018) and fruit bearing (o) (ON and OFF branches) regarding the two cultivars (v) 'Ahmad-Aghaei' and 'Kalleh-Qoochi' under different levels of potassium fertilizer.

Source	df	Leaf nitrogen	Leaf phosphorus	Leaf potassium	Leaf calcium	Leaf magnesium	Leaf sulfur
y	1	1.6881 **	0.0004550 ns	3.3808 **	0.03046 ns	0.00003 ns	0.00007 ns
o	1	12.1453 **	0.0212713 **	5.7267 **	1.25629**	1.01455 **	0.00120 ns
y*o	1	0.0031 ns	0.0000003 ns	0.0014 ns	0.00005 ns	0.00009 ns	0.00012 ns
r(y*o)	8	0.0082 ns	0.0001697 ns	0.0083 ns	0.01626 ns	0.00317 ns	0.00431 ns
v	1	44.3224 **	0.0029815 **	0.0058 ns	0.08556 *	0.06371 **	0.00005 ns
f	3	22.5626 **	0.0084345 **	1.9645 **	2.18141**	0.20545 **	0.00021 ns
v*f	3	2.1545 **	0.0012286 **	0.0011 ns	0.01539 ns	0.01009 ns	0.00038 ns
o*v	1	0.0021 ns	0.0000013 ns	0.0045 ns	0.00067 ns	0.00461 ns	0.00002 ns
o*f	3	0.0025 ns	0.0000028 ns	0.0009 ns	0.00851 ns	0.00658 ns	0.00004 ns
y*v	1	0.8584 **	0.0043605 **	0.7861 **	0.00003 ns	0.00003 ns	0.00007 ns
y*f	3	5.9465 **	0.0010691 **	0.1215 **	0.00088 ns	0.00005 ns	0.00021 ns
y*o*v	1	0.0021 ns	0.0000046 ns	0.0041 ns	0.00018 ns	0.00007 ns	0.00035 ns
y*o*f	3	0.0024 ns	0.0000032 ns	0.0009 ns	0.00013 ns	0.00006 ns	0.00014 ns
o*v*f	3	0.0028 ns	0.0000053 ns	0.0026 ns	0.00615 ns	0.00224 ns	0.00005 ns
y*v*f	3	0.3809 **	0.0007216 **	0.1035 **	0.00007 ns	0.00005 ns	0.00095 ns
y*o*v*f	3	0.0028 ns	0.0000067 ns	0.0029 ns	0.00023 ns	0.00005 ns	0.00027 ns
Error	56	0.0312	0.0001620	0.0115	0.01208	0.00376	0.00540
CV		5.7	12.7	6.3	6	7.8	15.7

ns, * and **: Non significant, significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Leaf nitrogen

The analysis of variance (Table 4) demonstrated that fruit-bearing, year, cultivar and fertilizer treatments had a significant effect ($P \leq 0.01$) on the amount of leaf nitrogen. A comparison of mean values showed that the leaf nitrogen content in non-bearing OFF branches (3.46%) was significantly higher than in the case of fruit-bearing ON branches (2.74%) (Fig. 1a). An evaluation of the interaction effects between cultivar and fertilizer on the amount of leaf nitrogen in August showed that the highest amount of nitrogen (4.90%) was achieved in the Ahmad-aghaei cultivar by applying fertilizer treatment (400 mg L^{-1} K) in the irrigation water (Fig. 1b). The lowest amount of leaf nitrogen was observed in the control of both cultivars (Fig. 1b). In both cultivars, the nitrogen content was higher in the second year, compared to the first year (Table 5).



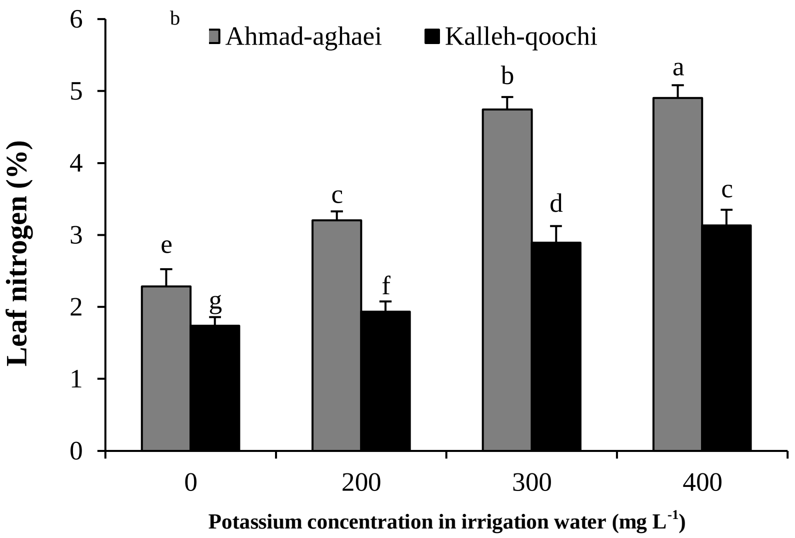
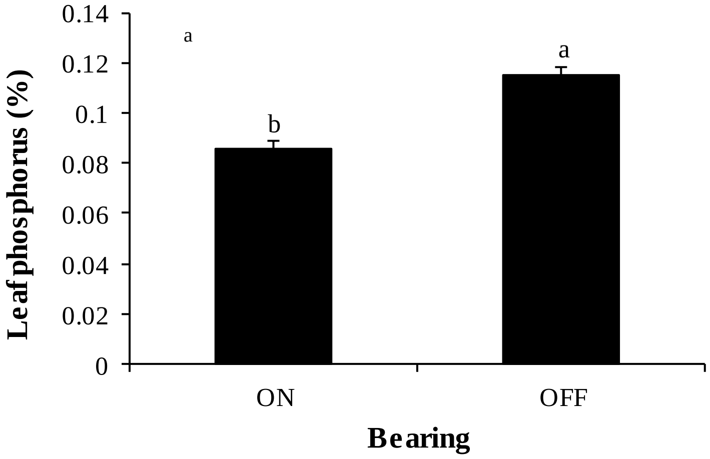


Fig. 1. Comparison of the mean effects of fruit bearing (a) and the interaction between cultivar and different levels of potassium fertilizer (b) on leaf nitrogen content in the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'. ON: fruit-bearing branches and OFF: non-bearing branches. Mean values with at least one common letter in each column are not significantly different (P≤0.05) according to Duncan's test.

Leaf phosphorous

The analysis of variance (Table 4) showed that fruit-bearing, cultivar and fertilizer treatments had significant effects (P≤0.01) on the leaf phosphorus content. A comparison of mean values showed that the leaf phosphorus content in non-bearing OFF branches (0.11%) was significantly higher than in fruit-bearing ON branches (0.08%) (Fig. 2a).

An evaluation of the interaction effects between cultivar and fertilizer on the leaf phosphorus content in August showed that the highest phosphorus content (0.127 and 0.120%) occurred by the application of 400 mg L^{-1} fertilizer on the 'Kalleh-Qoochi' and 'Ahmad-Aghaei' cultivars, respectively (Fig. 2b). The lowest amount of phosphorus in the leaves was observed in the control treatment of both cultivars (Fig. 2b).



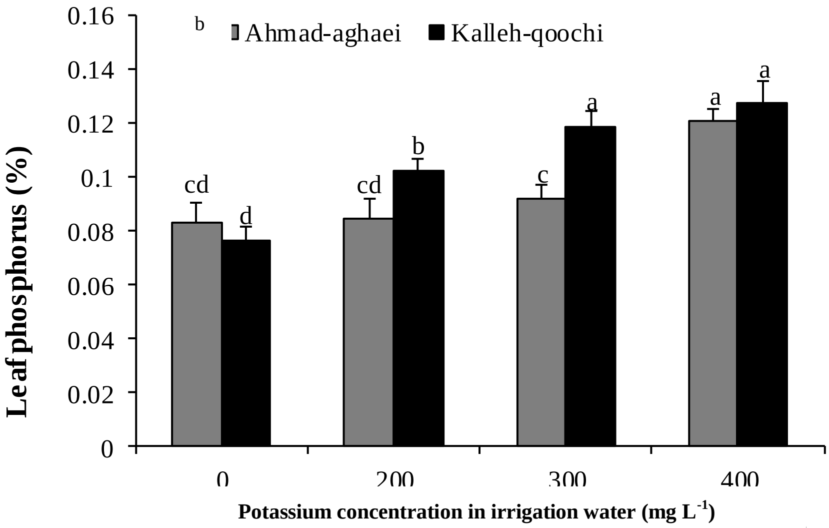
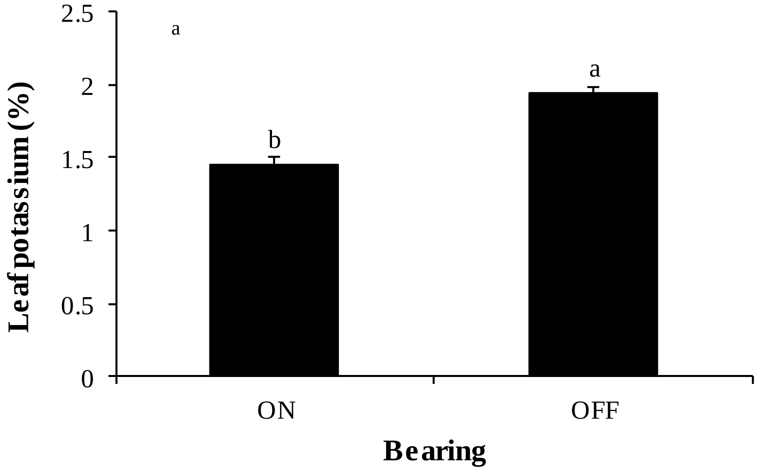


Fig. 2. Comparison of the mean effects of fruit bearing (a) and the interaction between cultivar and different levels of potassium fertilizer (b) on leaf phosphorus content in the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'. ON: fruit-bearing branches and OFF: non-bearing branches. Mean values that share at least one common letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's test.

Leaf potassium

The analysis of variance (Table 4) showed that fruit bearing, year and fertilizer treatments had significant effects ($P \leq 0.05$) on the amount of leaf potassium. The highest amount of potassium (1.93%) occurred in non-bearing OFF branches (Fig. 3a). The fertilizer treatment of 400 mg L^{-1} caused the highest amount of leaf potassium (2.06%) (Fig. 3b). The lowest amount of leaf potassium (1.39%) was measured in the control (Fig. 3b). In both cultivars, higher values of potassium content were observed in the second year (Table 5)



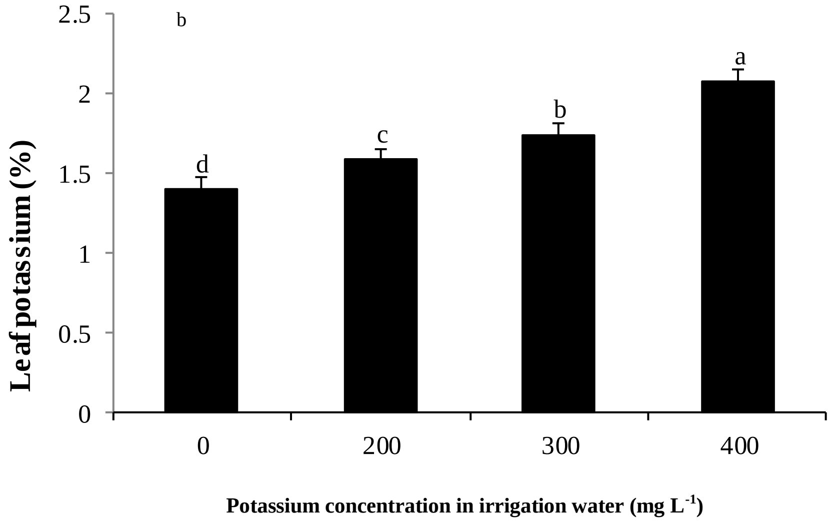


Fig. 3. Comparison of the mean effects of fruit bearing (a) and different levels of potassium fertilizer (b) on leaf potassium. ON: fruit bearing branches and OFF: non-bearing branches. Mean values that share at least one common letter in each column are not significantly different (P≤0.05) according to Duncan's test.

Leaf calcium

The analysis of variance (Table 4) showed that fruit bearing, cultivar and fertilizer treatments had significant effects (P≤0.01) on the amount of leaf calcium (Table 4). A comparison of mean values showed that leaf calcium content in fruit-bearing ON branches (1.94%) was significantly higher than in non-bearing OFF branches (1.72%) (Figure 4a). Leaf calcium content increased in the 'Kalleh-Qoochi' cultivar (1.86%) (Figure 4b). The fertilizer treatment of 400 mg L^{-1} caused the highest amount of leaf calcium (2.21%) (Figure 4c). However, the lowest amount (1.53%) was observed in the control (Figure 4c).

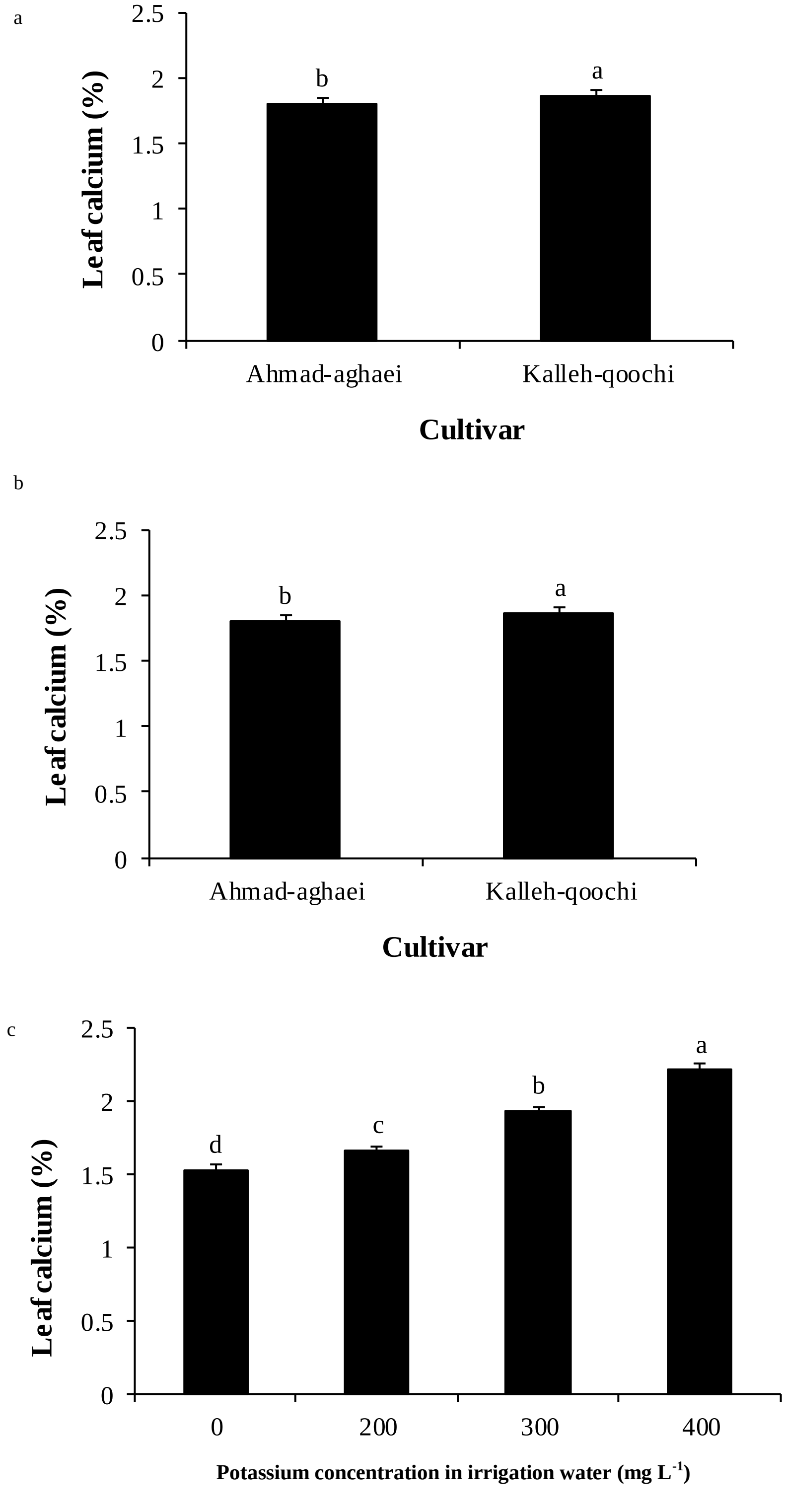


Fig. 4. Comparison of the mean effects of fruit bearing (a), cultivar (b) and different levels of potassium fertilizer (c) on leaf calcium content in the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'. ON: fruit bearing branches and OFF: non-bearing branches. Mean values that share at least one common letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's test.

Leaf magnesium

The analysis of variance (Table 4) showed that fruit bearing, cultivar and fertilizer treatments had significant effects ($P \leq 0.01$) on the amount of leaf magnesium. The comparison of mean values showed that the leaf magnesium content in fruit-bearing ON branches (0.88%) was significantly higher than in non-bearing OFF branches (0.67%) (Figure 5a). The highest amount of magnesium (0.80%) was measured in the 'Kalleh-Qoochi' cultivar and the lowest amount (0.75%) in the 'Ahmad-Aghaei' cultivar (Figure 5b). The fertilizer treatment of 400 mg L^{-1} caused the highest amount of leaf magnesium (0.90%) (Figure 5c), whereas the lowest amount (0.68%) was observed in the control (Figure 5c).

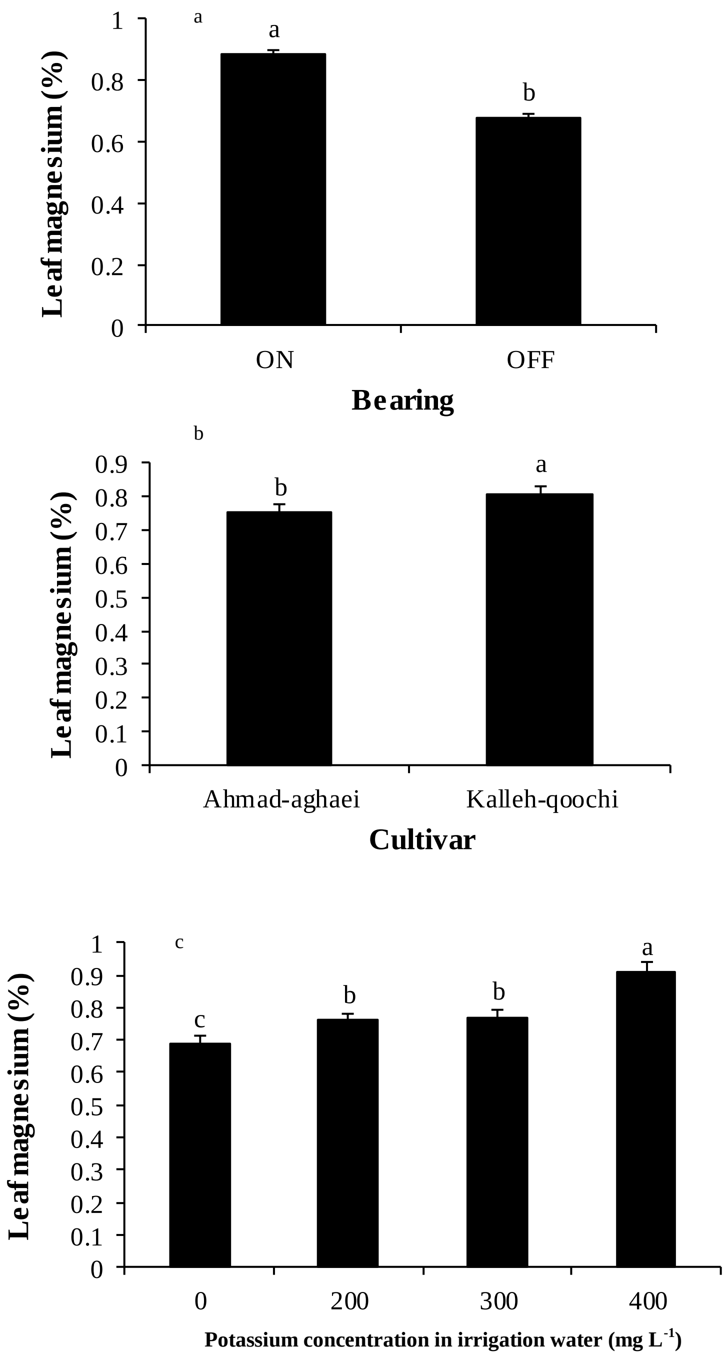


Fig. 5. Comparison of the mean effects of fruit bearing (a), cultivar (b) and different levels of potassium fertilizer (c) on leaf magnesium content in the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'. ON: fruit bearing branches and OFF: non-bearing branches. Mean values that share at least one common letter in each column are not significantly different (P≤0.05) according to Duncan's test.

Table 5. Effect of year (Y1,Y2) on the levels of macro nutrients in pistachio leaves in the two cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi' (V2)

Trait	Leaf Nitrogen (%)		Leaf Phosphorus (%)	
Variety	V1	V2	V1	V2
Y1	3.746	2.198	1.4249	1.5903
Y2	3.822	2.652	1.9812	1.7847
Y2-Y1	0.076 *	0.454 *	0.5563 *	0.1944 *
LSD 5%	0.021		0.0127	

Ns, * and ** non-significant, significant at 0.05 and 0.01 level respectively. *P<0.05, **P<0.01

Nutrient levels in pistachio nuts (September)

Tests for the homogeneity of variance (Bartlett test) were carried out in September each year (2017-2018) on the amounts of macronutrients in the pistachio nuts. Specifically, the measurable macronutrients were potassium, calcium, magnesium and sulfur at harvest (September). The two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi' showed significant differences in this regard (Table 6). The variance in experimental error was not significant and, thus, the variance of data in these two years was considered homogeneous. Accordingly, a combined analysis of variance on the macronutrients (i.e. potassium, calcium, magnesium and sulfur) was carried out in September (Table 7).

The analysis of variance showed that in the simple effect of year, significant differences were observed in the potassium content (P≤0.01) (Table 7). The type of cultivar had a significant effect only on the amounts of potassium and calcium in the kernels (Table 7). The analysis of variance showed that the simple effect of fertilizer was significant on the amounts of all macronutrients, except sulfur (Table 7). Among the interactions, only the reciprocal interactions between cultivar and fertilizer were significant on the potassium content of nuts (Table 7).

Table 6. Tests for the homogeneity of variance (Bartlett test) on experimental errors of macro nutrients in the nuts of the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'.

Mean Square Error	Nut Potassium	Nut Calcium	Nut Magnesium	Nut Sulfur
between Y_1 and Y_2	2.44 ns	1.1 ns	1.5 ns	3.42 ns

Ns: not statistically significant

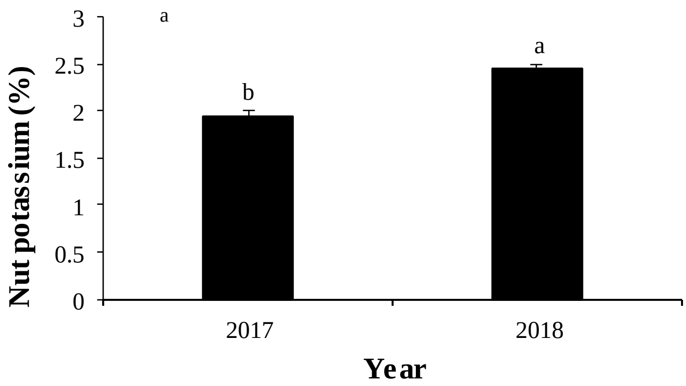
Table 7. Combined analysis of variance in two years (y) (2017-2018) regarding the effects of different levels of potassium (f) (2017-2018) on the macro nutrient levels of nuts in the two pistachio cultivars (v) 'Ahmad-Aghaei' and 'Kalleh-Qoochi'.

Source	df	Nut Potassium	Nut Calcium	Nut Magnesium	Nut Sulfur
y	1	2.920 **	0.0044 ns	0.0039 ns	0.00004 ns
r (y)	4	0.062 **	0.0566 ns	0.0253 ns	0.00032 ns
v	1	0.822 **	0.3414 **	0.0035 ns	0.00009 ns
f	3	0.549 **	0.5212 **	0.1927 **	0.00007 ns
y*v	1	0.023 ns	0.0001 ns	0.0021 ns	0.00039 ns
y*f	3	0.016 ns	0.0106 ns	0.0004 ns	0.00029 ns
v*f	3	0.045 **	0.0074 ns	0.0002 ns	0.00103 ns
y*v*f	3	0.008 ns	0.0029 ns	0.0001 ns	0.00092 ns
Error	28	0.009	0.0390	0.0132	0.00062
CV		4.4	11.1	17.9	24.6

Ns, * and ** non-significant, significant at 0.05 and 0.01 level respectively. *P<0.05, **P<0.01

Nut potassium

The analysis of variance (Table 7) showed that the year factor had a significant effect (P≤0.01) on the amount of potassium content in the pistachio nuts. The amount was significantly affected by the type of cultivar and potassium fertilizer. The comparison of mean values showed that the amount of nut potassium in the second year (2.44%) was significantly higher than the amount in the first year (1.95%) (Figure 6a). The comparison of mean values regarding the interaction between cultivar and different levels of potassium fertilizer showed that the highest potassium content in the nuts was observed by the 400 and 300 mg L^{-1} fertilizer treatments (2.58 and 2.49%, respectively) in the Kalleh-Qoochi cultivar (Figure 6b). The lowest amount of nut potassium content was observed in the control group of the 'Ahmad-Aghaei' cultivar (Figure 6b). Our results showed no significant difference between the two cultivars when affected by the 200 mg fertilizer (Figure 6b).



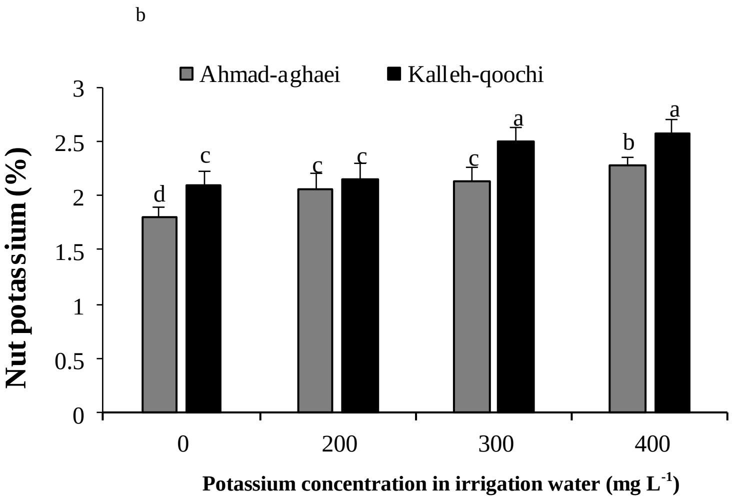


Fig. 6. Comparison of the effects of year (a) and the effects of interaction between cultivar and different levels of potassium fertilizer (b) on the amount of nut potassium content in the two pistachio cultivars 'Ahmad-Aghaei' and 'Kalleh-Qoochi'. Mean values that share at least one common letter in each column are not significantly different (P≤0.05) according to the Duncan's test.

Nut calcium

The analysis of variance showed that the amount of calcium in pistachio nuts was significantly affected by cultivar and fertilizer (Table 7). The comparison of mean values showed that the highest amount of calcium content (1.86%) in the nuts was obtained in the 'Kalleh-Qoochi' cultivar, which was significantly higher than the amount in the 'Ahmad-Aghaei' cultivar (1.69%) (Figure 7a). A comparison of the mean effects of irrigation-based fertilizer showed that the highest amount of nut calcium content (2.04%)

was obtained in response to the 400 mg L^{-1} treatment (Figure 7b). These results also showed that no significant difference was observed between the effects of 300 and 200 mg L^{-1} fertilizer treatments. The lowest amount of nut calcium content (1.53%) was observed in the control group that used no fertilizer treatment (Figure 7b).

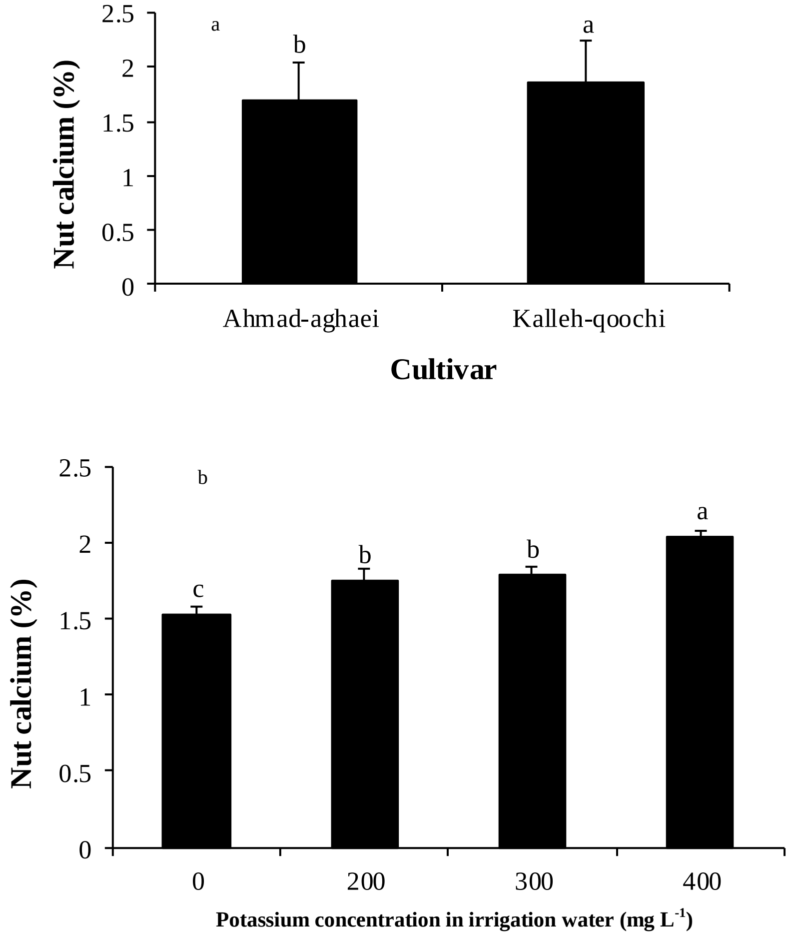


Fig. 7. Comparison of the mean effects of cultivar (a) and different levels of potassium fertilizer (b) on fruit calcium content in the two pistachio cultivars ‘Ahmad-Aghaei’ and ‘Kalleh-Qoochi’. Mean values that share at least one common letter in each column are not significantly different ($P \leq 0.05$) according to the Duncan’s test.

Nut magnesium

The analysis of variance (Table 7) showed that the fertilizer treatments had significant effects ($P \leq 0.01$) on nut magnesium content. The comparison of mean values showed that the highest amount of magnesium in the nuts (0.79%) was obtained in response to the 400 mg L^{-1} fertilizer treatment, whereas the lowest amount (0.49%) was observed in the control (Figure 8). Also, no significant difference was observed between the effects of 200 and 300 mg L^{-1} fertilizer on the magnesium content in the pistachio nuts (Figure 8).

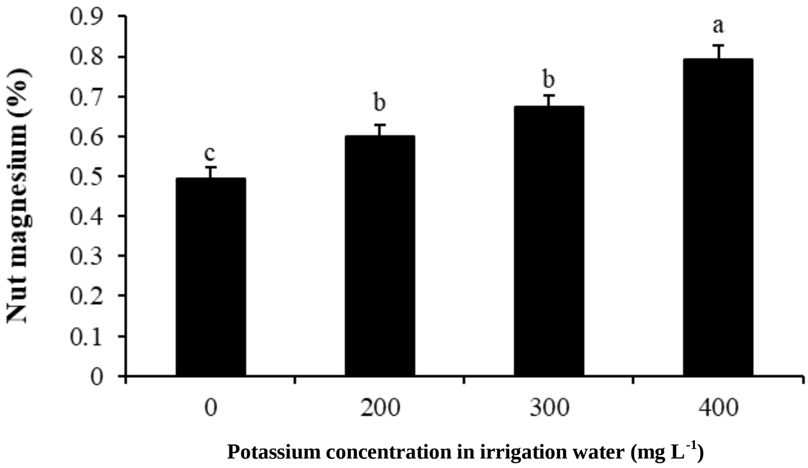


Fig. 8. Comparison of mean effects by different levels of potassium fertilizer on the amount of nut magnesium content. Mean values that share at least one common letter in each column are not significantly different (P≤0.05) according to Duncan's test.

Discussion

While all macronutrients were measured in the leaves, the nitrogen content was the highest and the phosphorus content was the lowest in the measured samples. Nitrogen is a major mineral element in agricultural fertilizers (Torres-Olivar *et al*., 2014). It plays an important role in plant growth and is usually required in more quantities than other minerals. In this regard, previous researchers assessed the physiological function of nitrogen in pistachio nuts (Amiri, 2009; Sarrwy *et al*., 2010). In the current study, we reported that the concentrations of several macro minerals in the leaves, including nitrogen, potassium, and phosphorus, were found in greater amounts in fruit-bearing (ON) branches, compared to non-bearing (OFF) branches. In contrast, the concentrations of other elements such as calcium and magnesium were higher in trees with fruit-bearing (ON) branches. Previous research indicated that nitrogen concentrations in the leaves of OFF trees were higher than in the leaves of ON trees (Baninasab *et al*., 2007). Competition for nitrogen by flower buds and fruit growth may be an important factor that leads to this difference. Similar to previous results by Khezri *et al*. (2020) and Gunes *et al*. (2010), we observed that the presence of fruits on the trees and the growth of fruits reduced the amount of nitrogen in the leaves. Paschalidis *et al*. (2019) reported that low concentrations of nitrogen in plant tissues may reduce the concentration of polyamines in plants. In this regard, Aziz (2003) showed that polyamines can serve as sources of nitrogen or as signal molecules that regulate the ripening of fruits in grapes. Flower buds in non-bearing branches of pistachio trees have higher levels of polyamines, compared to fruit-bearing shoots during growth (Roussos *et al*., 2004). Thus, polyamines can be a major cause of the premature shedding of pistachio flower buds. Polyamines act as anti-aging agents and counteract the activity of abscisic acid and ethylene (Chen *et al*., 2002; Soda, 2015; Diao *et al*., 2017; Chen *et al*., 2019; Lechowska *et al*., 2022). According to a report, nitrogen depletion can occur due to the presence of fruits on branches, which can ultimately lead to seed growth. But nitrogen deficiency can cause the shedding of flower buds (Baninasab *et al*., 2007). In this regard, our results showed that the amount of leaf nitrogen in OFF trees was higher than in those of ON trees, probably because there were fewer fruits on the OFF trees, compared to the ON trees. Consequently, this weakened the effects of sink-source relations. The amount of nitrogen in these trees remained higher, so that the absorption of nitrogen by the fruits corresponded with the growth rate of the fruits. Since the fruits acted as sinks to absorb nitrogen, the demand for nitrogen increased when the embryo began to grow (Chapline and Westwood, 2008; Iqbal *et al*., 2011). Weinbaum and Muraoke (1989) reported that during rapid seed growth, nitrogen was translocated from other parts of the tree to the fruits. Competition between the growing fruits and flower buds usually resulted in the shedding of flower buds (Bihmidin *et al*., 2013; Ling Roan *et al*., 2012).

The results of this study showed that the concentration of phosphorus in the leaves of OFF trees was higher than in ON trees, which was similar to previous results by Gundesli *et al*. (2021) and Gunes *et al*. (2010). Phosphorus plays an important role in flowering and fruit formation in olives (Erel *et al*., 2011; Bustan *et al*., 2013). According to our findings, phosphorus appeared as an essential element for pistachio production, especially during the stages of fruit growth, as evidenced by the decrease in phosphorus concentration in the leaves of ON trees. Accordingly, fruits are the main sinks of phosphorus. Therefore, optimizing the use of phosphorus may increase the efficiency of fertilizer applications on pistachio trees. The occurrence of high phosphorus concentrations in OFF trees, compared to ON trees, was previously reported in the case of other pistachio cultivars (Rosecrance *et al*., 1998; Stevenson and Shackel, 1998; Gunes *et al*., 2010; Khezri *et al*., 2020), olive trees (Erel *et al*., 2011; Bustan *et al*., 2013), pecan trees (Smith, 2009) and apple trees (Cheng and Raba, 2009). Meanwhile, the decrease in phosphorus concentration, a year after the ON stage of trees, was previously reported in pistachios (Rosecrance *et al*., 1998), olives (Erel *et al*., 2011; Bustan *et. al*., 2013), and mandarins (Golomb and Goldschmidt, 1987). Most of these observations could be explained by the presence of a greater level of root growth in the trees when they were at the ON stage.

Pistachio trees usually show seasonal fluctuations in potassium content in the different parts of the tree (Khezri *et al*., 2020; Elloumi *et al*., 2022). The current research showed that the presence of fruits on the branches significantly reduced the potassium content in each leaf. Meanwhile, leaf potassium content in the OFF branches was higher than in the ON branches. In general, potassium uptake reflects the activity of sinks (Zeng *et al*., 2001; Chapline and Westwood, 2008), especially when the pistachio nuts are forming and the nutrients are mainly allocated to embryonic growth in the ON branches. The current research showed that the amount of leaf potassium in the OFF branches was higher than in the ON branches. These results are consistent with previous findings on pistachios (Gundesli *et al*., 2021; Khezri *et al*., 2020), olives (Ulger *et al*., 2004), mandarins (Glomb and Goldschmidt, 1987), and pecans (Smith, 2009) which indicated that leaf potassium content decreased rapidly with the onset of fruit growth. In other words, fruit set caused the amount of potassium in the ON trees to be less than in the OFF trees. In the leaves of ON branches, lower potassium concentrations were observed, especially during the period when the pistachio nuts were developing. This usually takes 100 days from the full-flowering stage and could emanate from a greater potassium requirement by the nuts, compared to other nutrients, along with the possible movement of potassium from the buds and the leaves to the fruits (Baninasab *et al*., 2007). This hypothesis was affirmed in previous results by Elloumi *et al*. (2022) that removing fruits and leaves from the orchard can remove 131 kg of potassium and 18 kg of phosphorus per hectare each year. Rosecrance *et al*. (1998) reported that ON trees accumulated potassium in fruits, whereas OFF trees stored potassium in perennial tissues during the seed filling period. However, in pistachios, 95% of potassium uptake usually occurs during nut filling (Bustan *et al*., 2013; Elloumi *et al*., 2022) and may reflect the role that potassium has in sugar transport. This includes the role of binding to carboxylates and their transport from the phloem to the fruits and roots (Touranie *et al*., 1988). It also has the role of an osmolyte that establishes a gradient of pressure in the phloem to enable the transport and storage of sugars (Pallardy, 2008; Prajapati and Modi, 2012; Marschner, 2012; Behzadi Rad *et al*., 2021). Therefore, an adequate supply of potassium in pistachio trees during the ON year and fruit growth is very important to produce a good yield.

In the current research, it was found that the accumulation of calcium and magnesium in the leaves of the ON branches was higher than in the OFF branches. Similar findings were reported by Baninasab *et al*. (2007) and Gundesli *et al*. (2021). There was a close, positive correlation between the distribution of calcium/magnesium and the transpiration rate of shoots and leaves (Palma-Favaro *et al*., 2007). The reason for the increase in the calcium and magnesium content of the ON branches could be the occurrence of higher transpiration rates in the ON branches, compared to the OFF branches, meaning that the movement of calcium was particularly affected by the movement of water and transpiration. In pistachios, the measurement of stomatal conductance for 2 years showed that ON trees had significantly higher levels of stomatal conductance than OFF trees (Tränkner *et al*., 2018). Therefore, the accumulation of calcium and magnesium in the organs of the ON trees was probably due to their enhanced water uptake (Tränkner *et al*., 2018).

Fertilizer treatments showed that fertigation, at different levels of potassium, increased the amounts of all macro elements in the leaves and nuts, compared to the control. The 400 mg L^{-1} concentration was considered the best treatment that led to the highest increase in macro elements in the leaves and nuts. Research on various plants such as peanuts (Afify *et al*., 2019), mango (Bibi *et al*., 2019), olives (Zivdar *et al*., 2018), and tangerines (Nasir *et al*., 2016) showed that potassium application increased the amounts of potassium in the leaves, buds, and fruits. The results of the current research also indicated that the use of potassium (400 mg L^{-1}) increased the amount of this element in pistachios of ‘Kalleh-Qoochi’ and ‘Ahmad-Aghaei’ cultivars, thereby confirming previous reports on other pistachio cultivars (Zeng and Brown, 1998; Celik *et al*., 2010). The current results demonstrated that the amounts of potassium, calcium, phosphorus, and magnesium in the leaves and nuts of the ‘Kalleh-Qoochi’ cultivar were higher than those of the ‘Ahmad-Aghaei’ cultivar, which probably reflects their genetic differences. Zeng *et al*. (2001) observed a positive relationship between tree yield and leaf potassium content during the process of nut development. Since pistachios have the highest amount of potassium (408 mg per 100 g) among dried nuts (Deeba *et al*., 2013), and because pistachio kernels are a major sink for the absorption of nutrients (Ben-Mimoun *et al*., 2004), potassium acts as an important element in its transport within the tree (Mengel, 2007). Thus, using potassium treatments on pistachio trees can increase leaf potassium and nut potassium contents. The application of potassium increased the amount of this element in pistachio leaves and nuts. Similar to our results, Hamze *et al*. (2012) observed that potassium intake increased the potassium content of fruits. Various other studies also showed that foliar application of potassium nitrate increased the yield of mango fruits (Afiqah *et al*., 2014), oranges of the “Washington” cultivar (Abd El-Rahman *et al*., 2012), mandarins “Clementine” cultivar (Hamza *et al*., 2012), pomegranate (Khayyat *et al*., 2012), and olives (Hegazi *et al*., 2011). The absorption of potassium usually reflects the presence of a sink-source relationship (Ben-Mimoun *et al*., 2004). As the sink becomes more active during nut development, the nutrients are mainly used for the growth of embryos in the nuts of ON trees. Also, they are used for nutrient storage in the perennial organs of OFF trees (Zeng *et al*., 2001). Therefore, it seems that the application of potassium through fertigation is a good way to supply potassium during nut development, thereby increasing fruit weight and adding to the amount of potassium in the nuts (Ben-Mimoun *et al*., 2004). Similar to our results, Norozi *et al*. (2019) reported that using potassium fertilizers in the pistachio cultivar "Cherokee" increased the amount of phosphorus, potassium, and magnesium in the leaves and nuts. In this regard, potassium treatments on other tree species reportedly led to an increase in some macro elements such as potassium, calcium, magnesium, and phosphorus in different parts of the trees (Dinçsoy and Sönmez, 2019). The positive role of potassium fertilizers on pistachio yield is related to their role in the transport of photosynthetic products to the kernel (Marschner, 2012). Meanwhile, the increase in pistachio yield can be achieved also through the vital roles of potassium in protein synthesis, osmotic regulation, enzyme activation, phloem transport, energy transfer, cation-anion balance, and stress tolerance (Marschner, 2012). In previous research, sufficient amounts of potassium have reportedly maintained nitrogen metabolism, while showing that potassium is required for efficient nitrogen utilization. Also, sufficient amounts of potassium should be available for plants so that a maximum response can be taken from phosphorus. A relevant report indicated a positive correlation between these two elements as they are usually absorbed simultaneously by plants (Dinçsoy and Sönmez, 2019). On the other hand, research on different plant species showed that the presence of excess potassium in the soil can adversely affect the absorption of other macronutrients such as NH_4, Ca, and Mg (Dinçsoy and Sönmez, 2019). Decreasing the excess amount of potassium can assist in the absorption of other elements such as magnesium and calcium, but a proper rate of absorption by these elements requires a sufficient amount of potassium that does not exceed a specific limit (Mengel, 2007). According to the findings of this research, using potassium (400 mg L^{-1}) in the irrigation water had beneficial effects on the absorption of other elements and was considered an optimal potassium treatment for pistachio orchards of the 'Kalleh-Qoochi' and 'Ahmad-Aghaei' cultivars.

Conclusions

In August, the amounts of nitrogen, phosphorus, and potassium in the leaves were lower at the time of kernel filling in the ON branches, compared to the OFF branches. The amounts of nitrogen, potassium, and phosphorus were affected by the growth of the kernel on the trees. The levels of these three elements in the leaves decreased during this period, thereby showing that pistachio kernels are valuable sources of nitrogen, phosphorus, and potassium. Since these three elements are crucial for the formation of flower buds, the current findings indicated the importance of these three essential elements (N, P, and K) in pistachio orchards, especially for the ON trees. According to the findings, the application of potassium (400 mg L^{-1}) through fertigation had beneficial effects on the absorption of other elements and could therefore improve yield in pistachio orchards of 'Kalleh-Qoochi' and 'Ahmad-Aghaei' cultivars.

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Conflict of interests

The authors confirm that there are no conflicts of interest.

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