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Effects of the Timing of Foliar Application and Concentrations of Growth Regulators on the Mineral Content of Pistachio Leaves

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A B S T R A C T

Pistachio is one of the economically important horticultural crops in Iran. The main pistachio-producing areas in Iran are located at the edge of the desert and are affected by soil and water salinity. Water and nutrient uptake by the root decreases under saline conditions. In this study, the effects of foliar growth regulators applied at different times on the nutrient uptake and leaf nutrient content of pistachio trees were examined in accordance with a split-plot experimental design based on a randomized complete block design with three replications. The field experiment was conducted with a group of 30-year-old pistachio trees that have been exposed to water and soil salinity. Experimental factors included the timing of foliar application and the applied concentrations of plant growth regulators. The timing of foliar application was the main factor and was split into three levels: application on May 21th, June 21th, and May 21th + June 21th. Different concentrations of plant growth regulators, including sodium nitroprusside, salicylic acid, and jasmonic acid, were split into ten levels and were designated as the sub-factor. Results showed that the timing of application and the applied concentration of plant growth regulators affected root nutrient uptake and leaf nutrient content. The one-time application of plant growth regulators in June and the two-stage application of plant growth regulators in May and June resulted in the highest increase in leaf nutrient content compared with the control treatment. The application of low and moderate concentrations of plant growth regulators increased leaf nutrient content compared with the control treatment. High concentrations of plant growth regulators exerted no significant effects on leaf nutrient content.

Introduction

The pistachio is the most valuable horticultural crop of Iran. According to the Food and Agriculture Organization (FAO), pistachio production in Iran ranks first among that in other countries. In 2013, pistachio exports contributed more than 135 million dollars (FAO, 2005). Iran has more than 420,000 hectares of bearing and non-bearing pistachio orchards. Over 300,000 hectares, or approximately 80% of pistachio orchards, are located in Kerman Province, Iran (Sohrabi *et al*., 2009). The major areas of pistachio cultivation are located at desert margins and are plagued by drought and salinity. High soil and water salinity decrease pistachio growth (FAO, 2005). Nevertheless, the pistachio is recommended as the most appropriate crop

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for the arid areas of Iran given its adaptability to unfavorable conditions, such as water and soil salinity, and relative resistance to drought (Gheibi and Javadi, 2005). Pistachio cultivation is unaffected by soil salinity of 8–12 dS/m. However, once salinity exceeds 18 dS/m, pistachio production drops to zero while pistachio trees remain alive (Heidarinejad, 1996).

Salicylic acid (SA), jasmonic acid (JA), and sodium nitroprusside (SNP) are compounds that affect metabolism, antioxidant synthesis, and other oxidative and biological activities, such as growth, photosynthesis, respiration, and ion uptake and transport. These compounds thus alter the activity of some important enzymes and the structure of chloroplasts (Borsani *et al*., 2001). SA is produced by root cells. SA has an important role in the regulation of physiological processes, such as growth, ion uptake, and photosynthesis, and stimulates seed germination (Raskin, 1992). JA is derived from linoleic acid and is crucial in senescence and leaf abscission (Rubio *et al*., 2009). SNP is a relatively stable radical implicated in various physiological, pathophysiological, and developmental processes, such as germination, stomatal closure, pathogen response, and root development (Duan *et al*., 2007) and (Neil *et al*., 2003). Under salinity stress, the application of SA increases the uptake of calcium, nitrogen, iron, and copper ions while decreasing the uptake of phosphorus, sodium, zinc, and manganese ions. No consensus exists, however, on the impact of SA on ion and nutrient uptake by trees (Momeni *et al*., 2013). Szepesi *et al*. (2005) reported that SA increases the assimilation, photosynthesis rate, and mineral uptake of trees.

Few studies have focused on improving the microelement uptake of pistachio plants through the foliar application of growth regulators, especially JA and SNP, at different times. Therefore, this study aimed to evaluate the effects of various levels and timing of foliarly applied SA, JA, and SNP on nutrient uptake by pistachio trees.

Materials and Methods

In 2014, an experiment was conducted on bearing trees in Rafsanjan Pistachio Research Institute to evaluate the effects of the timing of the foliar application and concentration of SA, JA, and SNP on the leaf nutrient content of pistachio trees (Kalleghouchi cultivar). The study was conducted in accordance with a split-plot design based on a randomized complete block design with three replications. In this experiment, the timing of application was set as the main factor with three levels— late May (D_1), late June (D_2), late May +late June (D_3)—in the main plots. Different concentrations of growth regulators were set as sub-plots with ten levels: 0.5, 1, and 1.5 mM SA, designated as T_1, T_2, and T_3 respectively; 0.5, 1, and 1.5 mM SNP, designated as T_4, T_5, and T_6, respectively; 0.01, 0.02, and 0.03 mM JA, designated as T_7, T_8, and T_9, respectively; and control, designated as T_{10}.

The experiments were conducted with a group of 30-year-old trees, which were planted at intervals of 3 to 6 meters and were exposed to water and soil salinity (Tables 1 and 2). Soil and water sampling and analysis were conducted to determine the required amount of fertilizer (Tables 1 and 2).

Table 1. water experiment result

EC (dSm^{-1})	pH	Carbonate Co_3^{--}	Bicarbonate Co_3H^-	Chlorine Cl^-	Calcium Ca^{++}	Magnesium Mg^{++}	Sodium Na^+	S.A.R.
		Mill equivalents Per Liter						
10.1	6.6	0.0	3.0	82.5	22.5	14.5	54.8	12.7

Table 2. Soil experiment result of experimental garden.

Texture	Sand	Silt	Clay	T.N.V	Cl^-	Mg^{++}	Ca^{++}	Na^+	$P_{(AVA.)}$	$K_{(AVA.)}$	SAR	pH	ECe
loamy	29	53	18	14	36	69.5	58	136	21	208	17	8.1	30.6

ECe * Electric conductivity (dS/m), pH: Acidity of saturated, SAR: Sodium absorption ratio, K (AVA.): absorbable potassium (mg / kg soil), P (AVA.): Absorbable phosphorus (mg / kg soil), Cl, Mgs, Cas, Nas: Sodium, calcium, magnesium and soluble chlorine of soil (Mill equivalents Per Liter), respectively. T.N.V: Percentage of neutralized substance (calcium carbonate equivalent), Clay: Clay percentage, Silt: Silt percentage, Sand: sand percentage, Texture: Soil texture

All fertilizers, including manure and chemical fertilizers were uniformly added at the shading end of trees in grooves. Each plot consisted of three trees. The growth regulators were foliarly applied at the desired concentrations early in the morning (5:00–6:00 Am) in cool air and in accordance with experimental plans. After terminating the experiment in May and June, the pistachio nuts were harvested in accordance with local custom. Leaf samples were collected on July 7 and outsourced to the laboratory of Pistachio Research Institute for the measurement of mineral content. Leaf nutrients were quantified through inductively coupled plasma spectrometry. After data analysis with SAS 9.0 software, means were compared by Duncan's Multiple Range Test at the significance level of 5%.

Results

Effect of experimental treatments on leaf element concentration

Potassium

The timing of growth regulator application, growth regulators, and the interaction of these factors had a significant effect on leaf potassium concentration (Table 3). One-time application in June with 0.5 mM SA and 0.02 mM JA increased leaf potassium by 11% and 27%, respectively, compared with the control treatment. The two-stage application of 1 mM SA and 0.03 mM JA in June and May increased leaf potassium by 61% and decreased leaf potassium by 19%, respectively, compared with the control treatment. In addition, the application of 0.5 mM SNP increased leaf potassium by 29% compared with the control treatment (Table 4).

Sodium

The application of growth regulators and the interaction between the timing of foliar application and application of growth regulator significantly affected leaf sodium content (Tables 3 and 5). The application of 0.03 mM JA and 1 mM SA in late June decreased leaf sodium content by 43% and 21%, respectively, whereas the application of 0.5 mM SNP increased sodium content by 50%. The two-stage application of 0.5 mM SNP decreased leaf sodium content by 38% relative to the control treatment, whereas the application of 0.02 mM JA increased leaf sodium content by 147% (Table 4).

Table 3. Analysis of variance of leaf nutrient contents.

Source of Variations	df	leaf nutrient contents											
		Boron	Copper	Manganese	Zinc	Iron	Magnesium	Calcium	Potassium	Phosphorus	Nitrogen	Sodium	K^+/Na^+
Replication	2	359.54	5.94	615.91	1.39	1988.6	0.131	0.02	0.019	0.00004	0.0007	0.0076	1.083
Spraying time (D)	2	605.21	373.18**	5913.8**	406**	3607**	0.07*	0.55**	0.75*	0.037**	0.13**	0.021^{ns}	2.58^{ns}
Error	4	80.17	8.82	215.39	1.31	75.61	0.018	0.02	0.05	0.0001	0.02	0.0045	5.3
Growth regulators	9	291.41**	452.87*	362.11*	165*	4010**	0.20**	0.21**	0.27**	0.0009**	0.014*	0.33**	27.97**
Growth regulators*spraying time	18	772.29**	523.04**	1721**	93.7**	7791**	0.175*	0.19**	0.2**	0.001**	0.015*	0.037**	40.47**
Error	54	22.73	6.27	94.55	14.22	119.09	0.03	0.07	0.06	0.0001	0.007	0.0017	3.26
Coefficients of variation (%)		3.38	18.36	19.5	19.98	4.91	17.5	13.79	13.56	11.29	4.18	14.3	23.82

ns, *, and ** are respectively non-significant, significant, at the probability of 5% and 1%

Table 4. Means comparison of spraying time and growth regulators on leaf nutrient content of pistachio trees

Treatments	Boron (µg g^{-1})	Copper (µg g^{-1})	Manganese (µg g^{-1})	Zinc (µg g^{-1})	Iron (µg g^{-1})	Magnesium (%)	Calcium (%)	Potassium (٪)	Phosphorus (%)	Nitrogen (%)	Sodium (%)	K^+/Na^+
D_1T_1	153 c-e	5.5 h	34.66 d	12.4 d-f	240 c	0.76 f-g	1.96 b-g	1.60 d-f	0.06 d	1.86 e	0.39 d-f	4.1 h-i
D_1T_2	164 a	9.6 e-g	36 d	13.73 d-f	241 c	0.93 d-g	2.03 b-g	1.76 d-f	0.06 d	1.96 c-f	0.25 i-m	٧ e-g
D_1T_3	141 f-h	5.1 h	27.33 d	14.46 d-f	154 k	0.93 d-g	1.9 d-g	1.63 d-f	0.053 d	1.9 d-f	0.29 h-k	5.66 f-i
D_1T_4	155 b-d	12.8 c-g	22.33 d	13.73 d-e	227 cd	0.76 f-g	1.83 d-g	1.73 d-f	0.053 d	1.83 f	0.19 n-f	8.91 c-f
D_1T_5	130 kl	9.3 f-h	27.66 d	15.63 d-f	195 g-i	1.13 c-e	2.13 b-f	1.19 b-f	0.063 d	1.86 ef	0.29 h-j	6.4 e-j
D_1T_6	153 c-e	16.4 c	36 d	17.5 d-e	286 a	1.53 a-b	2.1 b-g	2.1 b-e	0.07 d	2 b-f	0.4 c-f	5.2 h-j
D_1T_7	136 g-j	15.3 cd	30 d	14.63 d-f	205 f-h	1.20 d-c	1.8 d-g	1.5 f	0.053 d	1.9 d-f	0.19 n-p	7.86 c-g
D_1T_8	126.7 lm	15.3 cd	35.66 d	16.3 d-e	192 g-i	0.66 g	1.56 d-g	1.76 f-d	0.05 d	2.1 a-c	0.19 m-p	9.03 c-f
D_1T_9	118.7 m	11.4 d-g	27.33 d	14.46 d-f	117 l	0.66 g	1.66 d-g	1.6 d-f	0.05 d	2 b-f	0.46 a-d	3.53 j
D_1T_{10}	166 a	27.7 b	35.33 d	16.06 d-f	234 cd	0.96 d-g	1.73 d-g	1.76 d-f	0.063 d	1.9 d-f	0.12 q	14.74 b
D_2T_1	130 lm	8.3 gh	34.33 d	15.6 d-f	183 ij	1.10 c-f	1.93 b-g	2.0 b-f	0.053 d	1.9 d-f	0.25 i-m	7.79 c-g
D_2T_2	163 ab	4.9 h	29.33 d	15.4 d-f	181 ij	0.93 d-g	2.1 b-g	2.03 b-e	0.07 d	1.96 c-f	0.22 j-o	9.22 c-e
D_2T_3	140 f-i	13.9 c-f	34 d	20.3 cd	294 a	0.70 g	1.26 b-d	1.96 b-f	0.066 d	1.9 b-f	0.25 i-m	7.68 c-g
D_2T_4	135.7 g-i	10.5 d-g	34.33 d	15.96 d-f	265 b	0.66 g	1.96 b-g	1.96 b-f	0.09 c	2.0 b-e	0.42 b-d	4.67 h-j
D_2T_5	163 ab	11.2 d-g	36.66 d	28.4 b	218 d-f	0.80 e-g	2.06 b-g	1.63 d-f	0.13 a-b	2.1 a-c	0.27 h-m	6.53 e-g
D_2T_6	119.7 m	12.3 c-g	38.33 d	32.1 b	210 e-g	1.23 b-d	2.03 b-g	1.73 d-f	0.12 a-b	2.0 b-f	0.27 h-m	7.59 d-g
D_2T_7	125 lm	10.6 d-g	23.66 d	18.5 d-e	182 ij	1.10 c-f	1.96 b-g	2.1 b-e	0.123 a-b	2.1 a-c	0.20 m-p	10.73 cd

Table 4. Continued

D_2T_8	144.3 eg	12.3 c-g	36.33 d	16.5 d-f	245 c	1.00 d-g	2.26 b-d	1.83 c-f	0.123 a-b	2 b-f	0.16 o-q	11.26 c
D_2T_9	157.3 a-c	10.7 d-g	93.33 ab	17.83 d-e	289 a	1.60 a	2.43 a-c	2.30 b-c	0.13 ab	2.0 a-d	0.3 h-j	7.57 d-h
D_2T_{10}	148. 3 d-f	12.7 c-g	68.33 c	16.26 d-f	185 h-j	0.76 f-g	1.6 d-g	1.80 c-f	0.12 ab	2.1 a-c	0.38 e-g	4.74 h-j
D_3T_1	154 cd	14.5 c-e	110.33 a	19.23 d-e	296 a	0.96 d-g	2.9 a	2.16 b-d	0.133 ab	2 b-f	0.49 ab	4.36 h-j
D_3T_2	119.6 m	10.5 d-g	100.33 ab	15.73 d-f	196 g-i	1.40 a-c	2.46 a-b	3 a	0.116 b	2 b-f	0.33 f-i	9.00 c-f
D_3T_3	140.6 f-h	12.1 c-g	89.33 b	19.63 d-e	235 d-c	1.10 c-f	2.2 b-e	1.93 b-e	0.123 ab	2.1 a-b	0.20 l-p	9.38 d-e
D_3T_4	147.3 d-e	12.1 c-g	87.33 b	16.56 d-f	296 a	0.93 d-g	1.73 b-g	2.40 b	0.14 a	2.13 ab	0.13 q	19.36 a
D_3T_5	133.3 h-k	77.6 a	67.7 c	27.7 b	283 a	1.00 d-g	1.83 d-g	1.83 d-f	0.12 ab	2.2 a	0.26 h-m	7.05 e-g
D_3T_6	136 g-j	13.7 c-f	91 b	26.46 b-c	195 g-i	1.13 c-e	2.03 b-g	2.4 b	0.116 b	2.0 a-b	0.47 a-c	5.06 h-j
D_3T_7	132.3 h-k	8.3 g-h	95.66 ab	17.66 d-e	225 c-e	1.23 b-d	2.16 b-e	1.60 f- d	0.126 ab	2 b-f	0.28 h-l	5.64 f-i
D_3T_8	141.3 f-h	8.5 g-h	39.33 d	9.46 f	210 e-g	1.10 c-f	1.96 b-e	1.50 f	0.13 ab	2.1 ab	0.52 a	2.92 j
D_3T_9	131.6 i-k	9.4 f-h	36.33 d	26.2 bc	166 jk	0.80 e-g	2.2 b-e	1.80 c-f	0.133 ab	2 b-f	0.33 f-i	5.54 h-j
D_3T_{10}	122.6 m	8.3 h-g	37.66 d	42.16 a	205 f-h	0.90 d-g	1.96 b-g	1.86 c-f	0.13 ab	2.0 ab	0.21 lm	8.76 d-f

Means that are shared at least in one letter have no significant difference in Duncan's multiple range test at the portability level of 5%

D_1: late May , D_2: late June, D_3: late May+late June in main plots and plant growth regulators with different concentrations at ten levels of T_1=SA with concentrations of 0.5 MM, T_2=SA with concentrations of 1 MM, T_3=SA with concentrations of 1.5 MM, T_4=SNP with concentrations of 0.5 MM, T_4=SNP with concentrations of 1 MM, T_5=SNP with concentrations of 1 MM, T_6=SNP with concentrations of 1.5 MM, T_7=JA with concentrations of 0.01 MM, T_7=JA with concentrations of 0.5 MM, T_8=JA with concentrations of 0.02 MM, T_9=JA with concentrations of 0.3 MM, T_{10}=control-(%)=gr/100gr DM, µg g^{-1} =microgram/gr DM.

Table 5. Means comparison of main effects of measured properties.

Treatment	Boron (µg g^{-1})	Copper (µg g^{-1})	Manganese (µg g^{-1})	Zinc (µg g^{-1})	Fe (µg g^{-1})	Magnesium (%)	Calcium (%)	Potassium (%)	Phosphorus (%)	Nitrogen (%)	Sodium (%)	K^+/Na^+
Spraying time (D)												
D_1	146.3 a	12.5 b	31.03 c	14.8 c	209. 8	0.95 b	1.88 b	1.37 b	0.05 c	1.94 b	0.27 a	7.25 a
D_2	142.6 a	10.7 b	42.86 b	19.6 b	255.3 a	0.99 ab	2.06 a	1.93 a	0.1 b	2.03 ab	0.27 a	7.8 a
D_3	135.8 b	17.5 a	75.5 a	22 a	230.9 a	1.5 a	2.14 a	2.05 a	0.12 a	2.07 a	0.32 a	7.70 a
Different concentrations of growth regulators (T)												
T_1	145.6 ab	9.4 de	59.7 a	15.7 b-d	240.1 b-d	0.94 c-e	2.26 a	1.92 b-d	0.082 b	1.93 c	0.38 a	5.42 e
T_2	148.8 a	8.3 e	55.2 ab	14.9 cd	206.1 de	1.08 ab	2.2 ab	2.32 a	0.082 b	1.97 c	0.27 bc	8.41 bc
T_3	140.5 cd	10.5 de	50.2 a-c	18.1 bc	227.7 c	0.91 c-e	2.12 ac	1.84 b-d	0.81 b	2.01 a-c	0.25 b-d	7.63 b-d
T_4	146 ab	11.8 cd	48 bc	15.4 cd	263.1 a	0.78 e	1.8 cd	2.02 ad	0.8 a	2 a-c	0.25 b-d	10.95 a
T_5	142.3 bc	32.7 a	44 cd	23.7 a	232.5 bc	0.97 cd	2.01 a-d	1.78 ad	0.102 a	2.05 ab	0.27 bc	6.67 c-e
T_6	136.2 d	14.1 bc	54.4 a-c	25.3 a	231.7 bc	1.3 a	2.05 b-c	2.07 ad	0.102 a	2.03 ab	0.38 a	5.95 de
T_7	131.3 e	10.4 de	49.7 a-c	16.9 bc	204.3 e	1.17 ab	3 a-c	1.72 d	0.10 b	2 a-c	0.22 d	8.08 bc
T_8	137.1 d	12.0 cd	37.1 d	14.0 d	215.8 d	0.92 c-e	1.93 b-c	1.7 d	0.10 b	3.07 a	0.29 b	7.7 b-d
T_9	135.7 de	10.5 de	52.3 a-c	19.5 b	190.8 f	1.02 b-d	2.1 b-d	1.9 b-d	0.105 a	2.52 a-c	0.36 a	5.54 e
T_{10}	145.6 ab	16.2 b	47.1 bc	24.8 a	208.2 de	0.87 e	1.7 d	1.8 b-d	0.105 a	2.03 ab	0.23 cd	9.41 bc

Means that are shared at least in one letter have no significant difference in Duncan's multiple range test at the portability level of 5%

D_1: late May , D_2: late June, D_3: late May+late June in main plots and plant growth regulators with different concentrations at ten levels of T_1=SA with concentrations of 0.5 MM, T_2=SA with concentrations of 1 MM, T_3=SA with concentrations of 1.5 MM, T_4=SNP with concentrations of 0.5 MM, T_4=SNP with concentrations of 1 MM, T_5=SNP with concentrations of 1 MM, T_6=SNP with concentrations of 1.5 MM, T_7=JA with concentrations of 0.01 MM, T_7=JA with concentrations of 0.5 MM, T_8=JA with concentrations of 0.02 MM, T_9=JA with concentrations of 0.3 MM, T_{10}=control -(%)=gr/100gr DM, µg g^{-1} =microgram/gr DM.

K^+/Na^+ ratio

The application of growth regulators and the interaction effect of the timing of foliar application and growth regulator significantly affected leaf K^+/Na^+ ratio, whereas the timing of foliar application had no significant effect on leaf K^+/Na^+ ratio (Table 3). The one-stage application of 0.01 JA, 0.02 mM JA, and 1 mM SA increased leaf K^+/Na^+ ratio by 67%, 76% and 44%, respectively, compared with the control treatment (Table 3). The two-stage application of 0.5 mM SNP increased K^+/Na^+ ratio by 110% compared with the control treatment, whereas the application of 0.02 mM JA decreased K^+/Na^+ ratio by 66% compared with the control treatment (Table 4).

Calcium

Growth regulators, foliar application, and their interactions significantly affected the calcium content of pistachio leaves (Table 3). The application of 1 mM SA and SNP in May caused calcium content to increase by 17% and 23%, respectively, compared with the control treatment. The one-stage application of 1 mM SA, 1 mM SNP, and 0.02 mM JA in June increased calcium content by 31%, 29%, and 52%, respectively, compared with the control treatment. The two-stage application of 0.5 and 1 mM SA increased leaf calcium compared with the control treatment by 48% and 25%, respectively (Table 4).

Boron

Leaf boron content was significantly affected by the timing of foliar application, the concentration of plant growth regulators, and the interaction between these two factors (Tables 3 and 5). The application of 1 mM SA in June increased leaf boron content by 10% compared with the control treatment, whereas the two-stage application of 0.5 mM SA and SNP increased boron content by 25% and 20%, respectively, compared with the control treatment (Table 4).

Copper

Leaf copper content was significantly affected by the timing of the foliar application of growth regulators, growth regulators, and the interaction between these two factors (Table 3). The two-stage application of 0.5 mM SA, 1 mM SNP, and 1.5 mM SNP increased leaf copper content by 75%, 125%, and 65%, respectively (Table 4).

Manganese

Leaf manganese content was significantly affected by the timing of foliar application and the application of growth regulators (*Table 3*). The two-stage application of the regulators had a significant effect on leaf manganese concentration: treatment with 0.5 mM SA, 0.5 mM SNP, and 0.01 mM JA increased leaf manganese by 192%, 131%, and 154%, respectively, compared with the control treatment. The one-stage application of 0.03 mM JA in June increased leaf manganese content by 36% compared with the control treatment (Table 4).

Zinc

Leaf zinc content was influenced by the timing of foliar application, different growth regulators, and the interaction between these two factors (Table 3). The one-stage application of 1.5 mM SA and SNP in June increased zinc content by 25% and 97%, respectively, compared with the control treatment (Table 5).

Iron

The timing of foliar application, plant growth regulators, and their interaction had significant effects on leaf iron content (Table 3). Treatment with 1.5 mM

SNP in May increased iron content by 22% compared with the control treatment. The one-stage application of 1.5 mM SA and 0.03 mM JA in June increased iron content by 56% compared with the control treatment. The two-step application of 0.5 mM SA and SNP increased iron content by 44% compared with the control treatment (Table 4).

Magnesium

Leaf magnesium concentration was significantly affected by growth regulators, the timing of foliar application, and their interaction (Table 3). The application of 1.0 mM SNP, 1.5 mM SNP and 0.01 JA treatment in May increased leaf magnesium by 18%, 59%, and 25%, respectively, compared with the control treatment. However, the application of 0.5 mM SA, 1.5 mM SNP, and 0.03 mM JA in June increased leaf magnesium concentration by 44%, 62%, and 110%, respectively, compared with the control treatment. In addition, the two-stage application of 1 mM SA increased leaf magnesium content by 55% compared with the control treatment (Table 4.

Phosphorus

Leaf phosphorus concentration was significantly affected by the timing of foliar application, growth regulators, and their interaction (Table 3). The two-stage application of 0.5 mM SNP increased leaf phosphorus by 7.7% compared with the control treatment, whereas the effects of other regulators were not significantly different from that of the control treatment (Table 4).

Nitrogen

Leaf nitrogen concentration was significantly affected by the timing of foliar application, growth regulators, and their interaction (Table 3 and 2). The two-stage application of 1.5 mM SA and 1 mM SNP in May and June increased leaf nitrogen content by 3.4% and 7% respectively, whereas other treatments had no significant effect (Table 4).

Discussion

Results of the current study showed that effect of plant regulators application in different time and stages are significantly different from control treatment without applying any plant regulators. The highest content of copper, manganese, zinc, Fe, magnesium, calcium, potassium, phosphorus, nitrogen, sodium, and K^+/Na^+ recorded for two-stage application of plant regulators, while the lowest content of boron obtained by this treatment. Also, different concentration of plant growth regulators resulted in almost different mean for plant nutrition. Overall results of different concentration showed that, for most of the nutrition, low and moderate concentration had higher effect on nutrition elements than high concentration of regulators. SA, SNP, and JA, respectively showed to increase content of nutritional elements in pistachio leaves.

A previous study reported that SA increases the uptake of potassium, magnesium, and other nutrients (Gunes *et al*., 2007). Potassium ions are necessary to maintain the osmotic potential of cells and to increase the water uptake of the plant. Therefore, improving potassium content will likely improve stomatal function and decrease sodium uptake. Few studies have been conducted on the effects of different concentrations of SA, SNP, and JA on ion uptake. Low concentrations of these hormones considerably decrease sodium uptake. JA acts as a signal molecule that activates the expression of genes involved in defensive mechanisms. For example, JA is implicated in the biosynthetic pathways of phytolectins that attenuate ion toxicity by attaching to ions (Ravnikar *et al*., 1992). Another study, however, stated that JA protects against non-biological stresses by upregulating the expression of genes involved in glutathione metabolism (Zou *et al*., 2001). Certain concentrations of growth regulators increased potassium uptake and decreased sodium uptake by the

root and thus increased K^+/Na^+ ratio. The increase in K^+/Na^+ ratio decreased the destructive effects of sodium and thus improved plant yield (Jeschke *et al*., 1984).

Khan *et al*. (2010) found that the application of SA improves photosynthesis and increases the amounts of nitrogen, phosphorus, potassium, and calcium stored in plant tissue. Low concentrations (0.5 mM) of SA in trees effectively act as plant regulators and improve the growth and nutrition of trees. However, high concentrations of SA do not improve plant growth (Gheibi and Javadi, 2005). Treatment with SA increases the uptake rate of nutrients, such as nitrogen, phosphorus, potassium, calcium, and magnesium. SA produces osmolytes, such as glutamine, proline, and glycine betaine. These osmolytes decrease the osmotic potential of the water inside the plant, thus increasing water and mineral uptake through the root and increasing leaf nutrient concentration (Orabi *et al*., 2010). SNP has a short life and is usually degraded in reactions within a few seconds (Leshem, 1996).

Gunes *et al*. (2007) reported that SA decreases sodium and chloride concentrations and increases potassium, nitrogen, magnesium, iron, manganese, and copper concentrations in trees. The application of 0.1 and 0.5 mM SA increased manganese uptake induces H^+ - ATPase, which is involved in the indirect transport of ions in the plasma membrane (Qinghua and Zhunjan, 2008). SNP improves nutrient uptake, especially zinc uptake, by increasing root dry weight (Magdy *et al*., 2012). Iron is important for chlorophyll production and the activity of many plant enzymes. Iron deficiency is more visible in pistachio plants than other nutrient deficiencies. It can highly affect the growth and productivity of the crop and can weaken and dry out pistachio trees in the long term (Zou *et al*., 2001). Different concentrations of SNP increases iron concentrations because of its oxidative activity on No (Nitric oxide) and Fe^{3+} for conversion into No (Nitric oxide) to $Fe^{+2}No^+$, which increases iron transport in plants (Graziano *et al*., 2002A and 2002B).

Magnesium is one of the highly used nutrients. It activates enzymes involved in growth and energy transport. Adequate magnesium uptake in alkaline soils, especially in the pistachio-producing regions of Iran with high calcium and boron levels, is prevented by competition with the uptake of calcium and other cations on soil colloids (Gheibi *et al*., 2005). JA promotes root growth and thus increases leaf magnesium concentration (Poschenrieder *et al*., 2008). Zhou *et al*. (2001) showed that the application of exogenous nitric oxide increases the activity of H^+ - ATPase in the plasma membrane of root cells of tomato plants under Cu stress. Moreover, the H^+-ATPase of stem cells plays an important role in the uptake of several mineral ions. The application of ≥0.05 mM JA increases the volume and numbers of stem, root, and capillary roots and increases vegetative growth, subsequently increasing root dry matter (Ravanikar *et al*., 1992). These factors facilitate nitrogen uptake and transport to leaves. SNP increases leaf nitrogen concentration given that it increases nitrate uptake (Manai *et al*., 2012).

Results showed that the efficiency of the two-stage foliar application of growth regulators to pistachio trees is better than that of other application methods. Therefore, the highest leaf nutrient content, particularly N, P and K contents, was obtained through the two-stage foliar application of plant growth regulators in late May and late June. In addition, the use of SA and SNP can greatly help increase nutrient uptake by the roots of pistachio trees. Additional experiments are required to elucidate the mechanism that underlies the effects of the timing of applying growth regulators on the nutrient uptake of pistachio trees.

Conflict of interest

No conflict of interest.

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