

## The Effect of Salicylic Acid and Potassium on Some Characteristics Nut and Physiological Parameters of Pistachio Trees Cv. Owhadi

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### Abstract

The effect of three salicylic acid (0, 50 and 100 mg l<sup>-1</sup>) and K<sub>2</sub>SO<sub>4</sub> (0, 0.1 and 0.2 %) levels on some characteristics nut and physiological parameters of pistachio trees cv. 'Owhadi' were investigated. Treatments were applied at endospermic growth stage of seed and cotyledons appearance. The results showed that potassium increase yield, splitting percentage; nut fresh mass and kernel dry mass and decrease blank percentage whereas the application of salicylic acid was unaffected on splitting percentage and decrease blank percentage. Simultaneous application of salicylic acid and potassium increase K and Zn concentrations of leaves.

**Key words:** Blank, Bud abscission, Mineral nutrient, *Pistacia vera*, Splitting

### Introduction

Pistachio (*Pistacia vera* L.) a member of Anacardaceae family is an important and exportable nut crop of Iran cultivated since old times. At this time, Iran is the leading producer of this crop followed by USA, Turkey and Syria. The majority of the pistachio production areas are located in arid and semi-arid regions adjacent to desert regions. Low irrigation water quality, soil salinity, nutrient imbalance and heat and drought stress are the most limiting factors in these areas. Under environmental stress condition, reactive oxygen species (ROS) produced in plant. In order to avoid the harmful effects of ROS, plants evolve an effective scavenging system including of non-enzymatic antioxidants and enzymatic antioxidants.

Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development, seed germination, fruit yield, rooting of cuttings and resistance to abiotic stresses (Isfendiyaroglu and Ozeker, 2008). Ion uptake and transport (Harper and Balke 1981) photosynthetic rate, stomatal conductance and transpiration (Khan et al. 2003) could be affected by SA application. Khan et al. (2003) investigated the effects of exogenous application of SA on net photosynthetic rate, stomata conductance and transpiration rate in plants of corn and soybean and reported that SA increased photosynthetic rates in both corn and soybean and leaf area and shoot dry mass in soybean plants. Exogenous SA could regulate the activities of antioxidant enzymes and increase plant tolerance to the abiotic stress (He et al. 2002). Moreover, the effects of SA on reducing plant a biotic stress have reported by numerous reports (Amin et al. 2007; Abdel-Wahed et al. 2006; Eraslan et al. 2007; Wajahtullah et al. 2003; Metwally et al. 2003; Tissa et al. 2000). In research, Amin et al. (2007) studied effects of SA on salt treated *Chlorella vulgaris* seedling and

reported that SA increased total free amino acids content in *Chlorella vulgaris* leaf and alleviated salt stress via osmotic adjustment.

Numerous studies showed that improvement of potassium (K)-nutritional status of plants, can greatly lower the ROS production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport (Cakmak 2005). Potassium deficiency causes severe reduction in photosynthesis, CO<sub>2</sub> fixation and impairment in partitioning and utilization of photosynthates. In addition, soil moisture regime affects potassium release or fixation (Brown, 1995), thus, during summer time, K availability is reduced in the soil of pistachio production areas because of drought and heat stresses. In addition to pistachio trees exhibit some physiological disorders such as abscission of inflorescence buds, fruit abscission, the production of blank, non split, early split and deformed nuts. The foliar K application could alleviate this problem. Ashworth et al. (1987) and Ben-Mimoun (2004) reported that foliar potassium application improved fruit weight and splitting percentage in pistachio. Zeng et al. (2001) studied effects of K fertilization on leaf K concentration and nut yield and quality of pistachio trees and reported that leaf K concentration increased following K fertilization and there were no significant differences among the K sources. They also found a positive correlation between nut yield and leaf K concentration during nut fill and reported that K application at the rate of 110 to 220 Kg/ha significantly increased nut yield and quality, but nut yield tended to decrease when the annual rate exceeded 220 Kg/ha. There are limited data in the literature about SA and K effects on alleviating environmental stress of fruit trees. The objective of this study was to determine the effect of SA and potassium on nut characteristics and physiological parameters of pistachio cv. 'Owhadi'.

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## Material and Methods

### Orchard management

This research was conducted in 2010 (on year) on mature 'Owhadi' pistachio trees in a commercial orchard located in Serjan, Iran. The climate of the area is hot and dry with a desertic characteristics. Absolute annual max and min temperatures are 40 and -8 degree (°C), respectively. Annual rainfall average 150 mm which is mostly concentrated in winter. The soil was sandy loam with a pH and EC of 8.3 and 5 dsm<sup>-1</sup> respectively as measured in 1: 1 ratio soil and water suspension. The 25-year-old trees were grafted on 'Badami-e-Zarand' rootstock, at a spacing of 6×2 m. The orchard had not previously been treated with K fertilizers and was irrigated as follow system every 45 days.

### Treatments

Trees were sprayed with K<sub>2</sub>SO<sub>4</sub> solution at 0, 0.1 and 0.2% or SA at 0, 50 and 100 mg L<sup>-1</sup> single or in combination. Trees were sprayed at endospermic growth stage of seed and cotyledons appearance. Sprays were applied in the morning (7-9 a.m) using a hand pressure sprayer at 10% leaf drop stage. Treatments were nine treatment (Table 1) including distilled water as control (S<sub>1</sub>K<sub>1</sub>), 0.1% K without SA (S<sub>1</sub>K<sub>2</sub>), 0.2% K without SA (S<sub>1</sub>K<sub>3</sub>), 50 mg l<sup>-1</sup> SA without K (S<sub>2</sub>K<sub>1</sub>), 50 mg l<sup>-1</sup> SA plus 0.1% K (S<sub>2</sub>K<sub>2</sub>), 50 mg l<sup>-1</sup> SA plus 0.2% K (S<sub>2</sub>K<sub>3</sub>), 100 mg l<sup>-1</sup> SA without K (S<sub>3</sub>K<sub>1</sub>), 100 mg l<sup>-1</sup> SA plus 0.1% K (S<sub>3</sub>K<sub>2</sub>), 100 mg l<sup>-1</sup> SA plus 0.2% K (S<sub>3</sub>K<sub>3</sub>). The treatments were applied to plots consisting of nine adjacent trees on the same row, and the plots were arranged in a randomized complete-block design with three replications.

### Measurements

#### Inflorescence bud abscission and nut characteristics

Inflorescence bud abscission was recorded during plant growth. To determine these parameters, five branches from each tree were marked and their inflorescence bud was counted monthly from beginning until ending experiment. The pistachio trees were harvested handily in September. Total fresh mass of fruits (including hulls, shells, and kernels) was recorded. Each tree yield was harvested and hulled with hand within 24 h of harvest and was dried at temperature room at 25 °C for 10 days. The hulled nuts each tree were weighed to determine tree yield. The kernel dry mass, nut splitting percentage and blank were determined by counting and weighing 300 hulled nuts randomly collected from each tree (Zeng and Brown 1998).

#### Relative water content of leaf (RWC)

Twenty leaves were randomly sampled from current shoot of around each tree for measuring RWC and chlorophyll and carotenoids of leave after 4 weeks of last treatment. The relative water content was calculated using the following equation.

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where FW and DW represent the fresh and dry weight of leaf discs, respectively and TW represent weight of leaf discs after soaked in distilled water for six hours.

### Extraction chlorophyll and carotenoids

One g of fresh leaves was grinded with 20 ml of 80% acetone and was centrifuged at 5000 rpm for 5 min and the supernatant was transferred to a 100 ml volumetric flask. After extraction, the supernatant was diluted to a final volume of 100 ml by 80% acetone. The absorbance was measured at 663, 652, 645, 480 nm using a spectrophotometer. The chlorophyll a, b, total and carotenoid contents were calculated on an exponential basis, using the equations (Arnon 1949).

$$\text{Chlorophyll a (mg/g FW)} = [12.7(\text{OD}663) - 2.69(\text{OD}645)] \times [V/1000 \times W]$$

$$\text{Chlorophyll b (mg/g FW)} = [22.9(\text{OD}645) - 4.68(\text{OD}663)] \times [V/1000 \times W]$$

$$\text{Total chlorophyll (mg/g FW)} = [(OD652) \times 1000] / [34.5 \times (V/1000 \times W)]$$

$$\text{Carotenoids (mg/g FW)} = [7.6(\text{OD}480) - 1.49(\text{OD}510)] \times [V/1000 \times W]$$

Where V and W represent the volume of used acetone and used weight of leaf sample, respectively and D represent absorbance.

### Nutrients analysis

To examine leaf mineral, twenty leaves of middle current shoot were randomly sampled from branches around each tree in all treatments in last July. It was dried at 75 °C for 48 h in oven, and was ashed in a muffle oven at 550 ± 25° C. The resulting white ash was then dissolved in 10 ml of 2N HCl and adjusted to a volume of 100 ml for determination of elements concentration. The potassium, magnesium and sodium content were determined by flame photometer and Fe, Mn and Zn were measured using a Perkin-Elmer (Model 3110).

### Experimental design and data analysis

The experiment was conducted as factorial in the framework of CRD design with four replications, each including a single tree. Data was analyzed using MSTATC software. The means were separated by Duncan's Multiple Range Test (DMRT) at  $P \leq 0.05$ .

## Results

### Characteristics nut

The results of interaction of SA and K on inflorescence bud abscission indicated that bud abscission was no affected by treatments, however the most bud abscission was observed with S<sub>3</sub>K<sub>3</sub>.

The result of interaction SA and K on yield showed that the highest yield was gained with S<sub>1</sub>K<sub>2</sub> and the lowest yield was found in control and S<sub>3</sub>K<sub>3</sub>, however there were no significant difference between S<sub>1</sub>K<sub>2</sub> and S<sub>2</sub>K<sub>2</sub> and S<sub>3</sub>K<sub>3</sub> (Table 2).

Nut splitting percentage was affected by K, so that the application of 0.02% K<sub>2</sub>SO<sub>4</sub> increased splitting percentage compared to control. Splitting percentage was

unaffected by application of SA. The results of interaction between SA and K on splitting showed that splitting increased with S<sub>1</sub>K<sub>3</sub>, S<sub>2</sub>K<sub>2</sub>, S<sub>2</sub>K<sub>3</sub> and S<sub>3</sub>K<sub>2</sub> treatments. The highest nut splitting was observed with S<sub>1</sub>K<sub>3</sub> and the lowest was found in S<sub>2</sub>K<sub>1</sub> (Table 2).

Kernel dry weight was unaffected by SA, whereas it was increased by application of K<sub>2</sub>SO<sub>4</sub>. The highest and lowest kernel dry weight was observed with 0.2% K<sub>2</sub>SO<sub>4</sub> and control respectively however there were no significant difference in comparison with 0.1% K<sub>2</sub>SO<sub>4</sub>. The interaction of salicylic acid and K showed that the combined application of salicylic acid and K was unaffected kernel dry weight in comparison with control (Table 2).

The results showed that the application of SA and K decreased blank percentage of nut and the lowest was obtained with 0.2% K<sub>2</sub>SO<sub>4</sub> and 100 mg l<sup>-1</sup> SA; however there was no significant difference in comparison with 0.1% K<sub>2</sub>SO<sub>4</sub> and 50 mg l<sup>-1</sup> salicylic acid. The results of interaction of SA and K identified that the combined application of SA and K was decreased blank nut in comparison with control. The lowest blank percentage was obtained with S<sub>1</sub>K<sub>2</sub>, however there was no significant different in comparison with S<sub>2</sub>K<sub>2</sub>, S<sub>2</sub>K<sub>3</sub> and S<sub>3</sub>K<sub>2</sub>. The highest blank percentage was found in control (Table 2).

#### Physiological parameters

The chlorophyll a was increased by SA and K application. The highest chlorophyll a was obtained with S<sub>3</sub> and K<sub>3</sub>. The results of interaction of SA and K on chlorophyll a showed that all treatments increased chlorophyll a in comparison with control (Table 1). The chlorophyll b was affected by SA and K, so that the application of 50 and 100 mg l<sup>-1</sup> SA and 0.2% K<sub>2</sub>SO<sub>4</sub> increase chlorophyll b compared to control. The results of interaction between SA and K on chlorophyll b showed that all treatments increased chlorophyll b compared to control except for S<sub>1</sub>K<sub>2</sub> and S<sub>3</sub>K<sub>3</sub> which were no significant difference in comparison with control (Table 2).

The carotenoid contents of leaf were decreased by application of SA. The results of interaction of SA and K on carotenoid contents of leaf showed that the highest carotenoides was observed with S<sub>3</sub>K<sub>2</sub> (Table 3).

Relative water content of leaves no was affected by K, whereas it was decreased by SA. The lowest RWC was observed with 50 mg l<sup>-1</sup> SA, which had no significant difference with 100 mg l<sup>-1</sup> SA.

#### Leaf mineral nutrient

Potassium concentration of leaf was unaffected by SA treatment, whereas it was affected by K, so that the highest K of leaf was found in 0.2% K<sub>2</sub>SO<sub>4</sub>. Mg leaf was unaffected by application of SA and K treatments. The interaction of SA and K on potassium concentration of leaf showed that the highest K concentration was observed with S<sub>1</sub>K<sub>3</sub> treatment; however there was no significant difference in comparison with S<sub>2</sub>K<sub>1</sub>, S<sub>2</sub>K<sub>3</sub> and S<sub>3</sub>K<sub>1</sub>. The results of interaction between SA and K on Mg concentration showed that the highest Mg concentration of leaf was observed with S<sub>2</sub>K<sub>3</sub>, however there was no significant difference in comparison with other treatments except S<sub>2</sub>K<sub>1</sub> (Table 4). Na concentration of leaf was decreased with K treatment, so that the lowest Na was observed with K<sub>2</sub>. Na concentration of leaf was unaffected by SA and interaction with K.

The results of interaction between SA and K on Fe concentration of leaves showed that there were no significant difference between treatments and control, however Fe concentration was higher with S<sub>2</sub>K<sub>3</sub>.

The results of interaction of SA and K on leaf Zn concentration indicated that the highest Zn concentration of leaf observed with S<sub>3</sub>K<sub>3</sub> which had significant difference compared to control. The results of interaction between SA and K on Mn concentration of leaf showed that SA and K no had significant effects on Mn concentration of leaf compared to control, however it was observed significant difference between S<sub>2</sub>K<sub>3</sub> and S<sub>2</sub>K<sub>1</sub>; S<sub>3</sub>K<sub>2</sub> and S<sub>3</sub>K<sub>1</sub>.

Table 1. Treatments and application rates used for the pistachio field experiment.

Treatments	Application rate		Treatments	Application rate	
	SA (mg l <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> (%)		SA (mg l <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> (%)
control (S <sub>1</sub> K <sub>1</sub> )	0	0	S <sub>2</sub> K <sub>3</sub>	50	0.2
S <sub>1</sub> K <sub>2</sub>	0	0.1	S <sub>3</sub> K <sub>1</sub>	100	0
S <sub>1</sub> K <sub>3</sub>	0	0.2	S <sub>3</sub> K <sub>2</sub>	100	0.1
S <sub>2</sub> K <sub>1</sub>	50	0	S <sub>3</sub> K <sub>3</sub>	100	0.2
S <sub>2</sub> K <sub>2</sub>	50	0.1			

Table 2. Interaction of salicylic acid and K<sub>2</sub>SO<sub>4</sub> on nut yield per tree, 100 kernel mass, splitting percent, blank percent and bud abscission percent.

K <sub>2</sub> SO <sub>4</sub>	Salicylic acid		
	S <sub>1</sub> (control)	S <sub>2</sub> (50 mg/l)	S <sub>3</sub> (100 mg/l)
Yield (kg/tree)			
K <sub>1</sub> (control)	1.65 c *	2.21 b	2.55 ab
K <sub>2</sub> (0.1 %)	3.18 a	2.45 ab	2.85 ab
K <sub>3</sub> (0.2 %)	1.95 bc	2.52 ab	1.70 c
100 kernel mass ( g)			
K <sub>1</sub> (control)	38.68 b-d	35.92 d	39.85 c
K <sub>2</sub> (0.1 %)	40.20 b	35.23 d	38.85 b-d
K <sub>3</sub> (0.2 %)	49.65 a	41.45 b	35.47 d
Splitting (%)			
K <sub>1</sub> (control)	23.50 ef	20.83 f	27.33 d-f
K <sub>2</sub> (0.1 %)	38.50 e	40.33 d	48.83 c
K <sub>3</sub> (0.2 %)	73.33 a	57.67 b	27 d-f
Blank (%)			
K <sub>1</sub> (control)	8.66 a	3.83 b	2.5 bc
K <sub>2</sub> (0.1 %)	1.83 c	3.bc	3.16 bc
K <sub>3</sub> (0.2 %)	2.0 c	2.83 bc	2.50 c
Bud abscission (%)			
K <sub>1</sub> (control)	94.0 ab	93.5 ab	92.5 b
K <sub>2</sub> (0.1 %)	93.5 ab	93.0 b	94.0 ab
K <sub>3</sub> (0.2 %)	93.0 b	93.17 b	95.17 a

\* Different letters in each column and row show significant differences at 5% of probability (Duncan's)

Table 3. Interaction of salicylic acid and K<sub>2</sub>SO<sub>4</sub> on chlorophyll a, chlorophyll b and carotenoids pistachio leaves cv. 'Owhadi'.

K <sub>2</sub> SO <sub>4</sub> (%)	Salicylic acid (mg l <sup>-1</sup> )		
	S <sub>1</sub> (control)	S <sub>2</sub> (50 mg/l)	S <sub>3</sub> (100 mg/l)
Chlorophyll a (mg/g fresh weight)			
K <sub>1</sub> (control)	0.062 b *	0.069 b	0.074 a
K <sub>2</sub> (0.1 %)	0.069 b	0.067 b	0.075 a
K <sub>3</sub> (0.2 %)	0.072 ab	0.069 b	0.076 a
Chlorophyll b (mg/g fresh weight)			
K <sub>1</sub> (control)	0.034 c	0.041 a	0.037 b
K <sub>2</sub> (0.1 %)	0.032 c	0.039 b	0.05 a
K <sub>3</sub> (0.2 %)	0.044 a	0.038 b	0.034 c
Carotenoids (mg/g fresh weight)			
K <sub>1</sub> (control)	0.038 b	0.035 bc	0.033 c
K <sub>2</sub> (0.1 %)	0.038 b	0.035b c	0.048 a
K <sub>3</sub> (0.2 %)	0.037 b	0.036 bc	0.035 bc

\* Different letters in each column and row show significant differences at 5% of probability (Duncan's).

Table 4. Interaction of salicylic acid and K<sub>2</sub>SO<sub>4</sub> on K, Mg, Fe, Zn and Mn of pistachio leaves cv. 'Owhadi'.

K <sub>2</sub> SO <sub>4</sub> (%)	Salicylic acid (mg l <sup>-1</sup> )		
	S <sub>1</sub> (control)	S <sub>2</sub> (50 mg/l)	S <sub>3</sub> (100 mg/l)
		K (%)	
K <sub>1</sub> (control)	1.70 b *	1.95 ab	1.88 ab
K <sub>2</sub> (0.1 %)	1.74 b	1.77 b	1.80 b
K <sub>3</sub> (0.2 %)	2.04 a	1.89 ab	1.80 b
		Mg (%)	
K <sub>1</sub> (control)	0.26 ab	0.20 b	0.24 ab
K <sub>2</sub> (0.1 %)	0.25 ab	0.25 ab	0.23 ab
K <sub>3</sub> (0.2 %)	0.28 a	0.30 a	0.28 a
		Fe (mg/g dry weight)	
K <sub>1</sub> (control)	95.12 ab	76.73 b	93.12 ab
K <sub>2</sub> (0.1 %)	77.07 b	90.10 ab	98.97 ab
K <sub>3</sub> (0.2 %)	95.03 ab	116.80 a	94.35 ab
		Zn (mg/g dry weight)	
K <sub>1</sub> (control)	33.50 b	39.17 ab	32.50 b
K <sub>2</sub> (0.1 %)	42.50 ab	41.0 ab	33.33 b
K <sub>3</sub> (0.2 %)	35.50 ab	42.50 ab	48.50 a
		Mn(mg/g dry weight)	
K <sub>1</sub> (control)	26.52 ab	22.52 b	23.45 b
K <sub>2</sub> (0.1 %)	26.25 ab	26.27 ab	32.63 a
K <sub>3</sub> (0.2 %)	29.38 ab	33.32 a	24.25 b

\* Different letters in each column and row show significant differences at 5% of probability (Duncan's).

### Discussion

Our findings showed that potassium was increased yield; splitting percentage and kernel dry mass. It may be associated with the role of potassium in translocation of carbohydrates to growing fruit, as reported by Zeng et al. (2001), Zeng and Brown (1998), Brown et al. (1995) and Ben-Mimoun et al. (2004). They postulated that the application of potassium was increased yield nut and splitting. The present study indicated that salicylic acid increase splitting percentage at 100 mg l<sup>-1</sup> of SA. The lignin compound hydrolyzes in suture layer during maturation phase and salicylic acid decreased splitting by inhibition of the hydrolysis of lignin compound by affecting polygalacturonase enzyme. Our results indicated that salicylic acid and potassium was decreased blank nut. Carbohydrate pool is the main factor affecting blankness in pistachio. The high yield depletes the carbohydrates and on the other hand potassium and salicylic acid affects photosynthesis and translocation carbohydrates to growing fruits. The interaction of salicylic acid and potassium indicated that inflorescence bud abscission was decreased 1.5% and 1% with S<sub>1</sub>K<sub>1</sub> and S<sub>2</sub>K<sub>2</sub> compare to control respectively. It may be related to alleviating environmental stresses and increasing translocation carbohydrate content resulted to photosynthesis.

marked increases in chlorophyll content and anthocyanins in SA treated *Spirodea* plants. Chandra and Bhatt (1998) found that SA treatment increased or

Our findings showed that potassium increased chlorophyll a, and chlorophyll b. It may be associated with the role of potassium in decreasing oxidative cell damage and production of ROS. Environmental stress damages to cells by production of ROS. There is evidence that exposed environmental stress plants have a large requirement for K (Cakmak and Engels, 1999). It may be to be related to the fact that K is required for maintenance of photosynthetic CO<sub>2</sub> fixation. When plants are exposed stress conditions, ROS production can be additionaly enhanced due to K-deficiency-induced disturbances in stomata opening, water relations and photosynthesis. In addition to chloroplasts loses high amounts of K to further depress photosynthesis and ROS formation. These results strongly support the idea that K can be protected chloroplasts from oxidative damage. In similar results, Sangakkara et al. (2000) reported that K deficiency decreased tolerance legumes plants to drought stress. Our results confirmed Kumar and Kumar (2007) in banana plants. They reported that the foliar spray of 0.5 and 1% potassium increased total chlorophyll in banana plants. The application of K increased K that confirmed by previous reports (Zeng and Brown, 1998; Celik et al. 2010). The present study indicated that salicylic acid increased chlorophyll a, chlorophyll b. In similar study, Rhoads and McIntosh (1992) observed decreased chlorophyll content in cowpea, depending on the genotypes. Our results indicated that interaction of

salicylic acid and K was affected physiological parameters such as chlorophyll a, chlorophyll b and leaf minerals except to Na. The highest chlorophyll, carotenoids, Mn and Zn was observed with S<sub>3</sub>K<sub>2</sub>, S<sub>2</sub>K<sub>3</sub> and S<sub>3</sub>K<sub>3</sub>. It may be related to alleviating environmental stress, increasing photosynthesis and root growth. The effects of SA and K on decreasing of abiotic stress and increasing photosynthesis on crop plants reported by numerous works (Amin et al. 2007; Eraslan et al. 2007;

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