

## Physiological and Biochemical Response of Olive (*Olea europaea* L.) Cultivars to Foliar Potassium Application

Sh. Zivdar<sup>1</sup>, K. Arzani<sup>1\*</sup>, M. K. Souri<sup>1</sup>, N. Moallemi<sup>2</sup>, and S. M. Seyyednejad<sup>3</sup>

### ABSTRACT

This study was conducted under Ahvaz, Iran environmental condition during 2012 and 2013 growing seasons in order to investigate the influence of foliar application of potassium on some physiological and biochemical characteristics of three olive cultivars including 'Mission', 'Koroneiki' and 'Dezfuli'. Potassium sulfate in different concentrations of 0, 1, and 2 g l<sup>-1</sup> was sprayed twice on selected mature 10 year old trees; at 50 and 65 days after full bloom. Potassium sprays increased leaf chlorophyll content of all cultivars, leaf potassium content in 'Mission' and 'Koroneiki' cvs., fruit anthocyanin concentration of 'Dezfuli' in two crop seasons. However, K treatments had no significant effect on leaf N and P content, fruit K and P content in all studied cultivars. Fruit soluble carbohydrates increased by foliar application of potassium in 'Koroneiki' and 'Dezfuli' cultivars in the second year of study. Stem water potential and leaf stomatal conductance were significantly affected by cultivar and K treatments during the two years. Our findings showed that foliar application of potassium resulted in the decrease of stomatal conductance of 'Koroneiki' and 'Dezfuli' in two crop seasons. Based on the results, it can be concluded that the studied cultivars differently respond to K treatments during the two crop seasons and foliar potassium application could have a positive impact on leaf chlorophyll, leaf potassium, fruit soluble carbohydrates and fruit anthocyanins in olive cultivars.

**Keywords:** Foliar application, Olive, Potassium, Soluble carbohydrates, Stomatal conductance.

### INTRODUCTION

Olive (*Olea europaea* L.) tree is an evergreen native to the Mediterranean region. In recent years, due to high olive oil demand as well as to the olive oil and fruit nutritional value, the cultivation of olive has been expanded in various regions in Iran. However, the cultivation of olive tree is limited because of harsh environmental conditions and water scarcity in most of the new olive plantation areas (Arji and Arzani, 2008). The limitation of water as well as long hot summers in the regions lead to poor

fruit and oil quality (Khaleghi *et al.*, 2015; Saadati *et al.*, 2013). In addition, low precipitation (200 mm) in the studied area has caused problems to supplying fertilizers during most of the year. To achieve optimal growing conditions in this region new strategies toward orchard management are required (Arzani *et al.*, 2009). For this purpose applying an efficient nutritional management such as foliar nutrient application is one of the important practices. Since foliar feeding provides the stems, leaves or fruits directly with nutrients, it is a suitable method to reduce the environmental risks arising from the use of the soil-applied

<sup>1</sup>Department of Horticultural Science, Tarbiat Modares University (TMU), P. O. Box: 14115-336, Tehran, Islamic Republic Iran.

\* Corresponding author; e-mail: arzani\_k@modares.ac.ir

<sup>2</sup> Department of Horticultural Science, Shahid Chamran University, Ahvaz, Islamic Republic Iran.

<sup>3</sup> Department of Plant Physiology, Shahid Chamran University, Ahvaz, Islamic Republic Iran.



chemical fertilizers (Inglese *et al.*, 2002). Meanwhile, potassium (K) is one of the most important and highly consumed nutrients in plants, which plays important roles in their growth, metabolism and survival. This element participates in the activity of enzymes, protein synthesis, photosynthesis, osmotic adjustment, stomatal movements, carbohydrate synthesis and translocation and stress tolerance (Marschner, 2012). Large amounts of plant potassium is removed through the harvested fruits (Fernandez-Escobar, 2004), while potassium plays an important role in regulating the water status in olive tree (Arquero *et al.*, 2006). Potassium is especially compatible with foliar application method because it has been shown that after being sprayed on the leaves it is rapidly absorbed and translocated throughout the tree (Mengel, 2002). Thus, foliar application is a useful way of applying potassium to fruit trees, especially in arid and semi-arid regions, because in these areas, water scarcity due to low rainfall in summer, remarkably reduces the absorption of nutrients especially potassium from the soil, when trees are in higher potassium demand (Toscano *et al.*, 2002). Therefore, the objective of the present study was to explore the physiological and biochemical response of three commercial olive cultivars to foliar application of potassium under Ahvaz, Iran environmental conditions.

## MATERIALS AND METHODS

### Experimental Site

This experiment was carried out during 2012–2013 growing seasons in the research orchard, department of Horticultural Science, Shahid Chamran University, located at Ahvaz, Khuzestan province, Iran (Longitude: 48° 40' E, Latitude: 31° 20' N, Altitude: 22.5 m). According to the orchard soil analysis results, the soil texture was clay loam with 0.45% organic matter. The electrical conductivity of the soil was 4.05

dS m<sup>-1</sup> with 7.9 soil pH. Note that, the highest amount of N (0.48%) and P (13.31 mg kg<sup>-1</sup>) was observed in the top 30 cm of the soil. Although, data from soil analysis showed that the amount of K in the 30 cm top region of the soil was the lowest (132 mg kg<sup>-1</sup>) compared to the deeper layer of 60 to 90 cm of soil depth (250 mg kg<sup>-1</sup>). The average monthly climatic conditions of 2012 and 2013 growing seasons are presented in Table 1.

### Plant Material and Treatments

This study was performed on 10-year-old mature olive trees of 'Mission', 'Koroneiki' and 'Dezfuli' cultivars. The experimental design was split plot, arranged based on Randomized Complete Block Design (RCBD) with three replications. Treatments were applied at 50 and 65 Days After Full Bloom (DAFB). Full Bloom (FB) occurred at 28<sup>th</sup>, 31<sup>st</sup> March and 4<sup>th</sup> of April for 'Koroneiki', 'Mission' and 'Dezfuli' respectively in the first year (2012) and at 1<sup>st</sup>, 5<sup>th</sup> and 7<sup>th</sup> of April for 'Koroneiki', 'Mission' and 'Dezfuli' respectively in the second year (2013), so FB was adopted on 1<sup>st</sup> of April in both growing seasons. All orchard agricultural practices and nutrition management were similar for all experimental trees. Annual application of fertilizers in the soil was 500 g N (urea), 250 g P (superphosphate) and 500 g K (potassium sulfate) per tree. The annual P and K fertilizers were applied in November and N fertilizer was applied three times in February, May and July. Foliar application of potassium was applied on trees with similar size and vigor. In addition, in order to avoid fast evaporation, trees were sprayed by a small automated sprayer in late afternoon one hour before sunset. Nutrient treatments included control (distilled water), K<sub>2</sub>SO<sub>4</sub> (1 g l<sup>-1</sup>) and K<sub>2</sub>SO<sub>4</sub> (2 g l<sup>-1</sup>). About 20 liters per tree of K<sub>2</sub>SO<sub>4</sub> solutions were applied, note that the wetting agent of Tween-20 (0.5%) was added into the solution before spray as a surfactant. The

**Table 1.** Climatic parameters of 2012 and 2013.

Months	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Relative Humidity (% RH)	Rainfall (mm)	Evaporation (mm)
Jan	7.6	17.1	12.3	72	61.4	46.3
Feb	9.7	20.6	15.2	68	23.5	78.6
Mar	13.6	26.1	19.9	54	18.3	142.5
Apr	17.2	31.6	24.4	43	4.0	248.3
May	22.3	36.6	29.5	40	23.2	336.7
Jun	27.4	44.0	35.3	26	0.0	505.2
Jul	29.9	43.3	38.1	29	0.0	487.7
Aug	30.1	46.4	38.3	28	0.0	471.8
Sep	26.6	43.8	35.2	35	0.0	376.8
Oct	20.2	38.0	29.1	39	0.0	486.2
Nov	16.9	29.2	23.1	58	41.7	126.3
Dec	11.2	20.3	15.8	73	51.8	63.4
Mean	19.4	33.1	26.4	47.1		
Total					223.9	3369.8

nutrient concentration for applied treatments was determined in the experimental site based on the series of preliminary application of different concentrations (1 to 4 g l<sup>-1</sup>) with visual observation of possible toxicity and damage of the leaves.

### Plant Measurements

#### Determination of Nutrient Concentrations, Chlorophyll, Anthocyanin and Soluble Carbohydrate in Plant Tissues

Leaves from middle canopy position of one year old shoots and fruit from middle canopy zone were harvested in July and October in both years of study, respectively. All samples were washed with distilled water, dried at 70°C for 48 hours, and then were ground into powder. N content was determined by Kjeldahl (1883) method. P and K contents were determined using colorimetry and flame photometer, respectively (Chapman and Pratt, 1961). The concentration of chlorophylls a and b in the leaves and fruit samples were determined by spectrophotometer (UV-1201, Shimadzu) at 645 and 663 nm (Lichtenthaler, 1987). The

total chlorophyll contents are expressed in mg kg<sup>-1</sup> fresh weight of leaf and fruit. Fruit anthocyanin content was determined by the pH differential method (Lee *et al.*, 2005) and expressed as mg g<sup>-1</sup> cyanidin-3-glucoside equivalents. The soluble carbohydrates content was determined using the phenol sulphuric acid method (Hellebust and Graigie, 1978). The fruit samples were extracted with 80% ethanol, and then the absorbance of carbohydrate fraction was determined by spectrophotometer (UV-1201, Shimadzu) at 485 nm.

#### Measurement of Water Potential and Stomatal Conductance

The changes in Stem Water Potential (SWP) and stomatal conductance (g<sub>s</sub>) were measured monthly from June to November in both years. Stem Water Potential (SWP) was measured by using a pressure chamber (Soil Moisture Equipment Corp, Model 3000). For this purpose, SWP was recorded immediately at midday on the detached one-year-old branches including leaves. Leaf stomatal conductance was measured at midday using a porometer (AP4, DELTA-T).



## Statistical Analysis

The data were analyzed using the Analysis Of Variance (ANOVA) and Duncan's multiple range test ( $P \leq 0.05$ ) to evaluate the significance of differences between treatment means. A repeated measure ANOVA was used where the same measurement was performed at a different time.

## RESULTS AND DISCUSSION

### Nutrient Concentrations

There was no significant effect of K treatments on N and P content of leaves of the studied cultivars in two consequent years of this research (Table 2). These results are

in agreement with the results of other researchers (Veberic *et al.*, 2002) who reported a limited increase of leaf nutrient concentrations in response to foliar application of macronutrients and potassium (Weinbaum, 1988). They suggested that it is necessary to spray several times per crop season to achieve better results. In the case of leaf potassium, the effect of K treatments was significant in 'Mission' in both years. Foliar application of  $1 \text{ g l}^{-1}$  K had significantly increased leaf K content compared to control in 'Mission' cv. (Table 2). The K effect was also significant on fruit N content in 'Koroneiki' but not in 'Mission' and 'Dezfuli' cultivars. Insignificant differences were recorded among potassium treatments and olive cultivars concerning fruit P and K content, with the exception of 'Koroneiki' in the first

**Table 2.** Nitrogen, P and K concentrations (% ddry weight) in the leaves and fruit of the olive cultivars 'Mission', 'Koroneiki' and 'Dezfuli' at three rates of Foliar Application of Potassium (FAP) during 2012 and 2013 growing seasons.<sup>a</sup>

Treatments		2012 Growing season					
		Leaf			Fruit		
Cultivars	FAP <sup>b</sup>	N	P	K	N	P	K
Mission	T <sub>1</sub>	1.45a	0.10ab	0.53d	1.06abc	0.16a	4.50ab
	T <sub>2</sub>	1.44a	0.09ab	0.81c	1.22a	0.15a	4.71a
	T <sub>3</sub>	1.56a	0.08b	0.61d	1.16ab	0.16a	4.93a
Koroneiki	T <sub>1</sub>	1.31a	0.11a	0.93c	0.83e	0.16a	3.87b
	T <sub>2</sub>	1.37a	0.11a	1.15b	1.11ab	0.15a	4.24ab
	T <sub>3</sub>	1.63a	0.09ab	1.22ab	1.02bcd	0.18a	4.94a
Dezfuli	T <sub>1</sub>	1.36a	0.09ab	1.25ab	0.91cde	0.17a	3.94b
	T <sub>2</sub>	1.39a	0.10ab	1.28ab	0.88de	0.17a	4.50ab
	T <sub>3</sub>	1.59a	0.09ab	1.34a	0.90cde	0.17a	4.51ab
		2013 Growing season					
		Leaf			Fruit		
		N	P	K	N	P	K
Mission	T <sub>1</sub>	1.33d	0.09ab	0.53d	1.09ab	0.19bc	4.83a
	T <sub>2</sub>	1.37cd	0.09ab	0.83bc	1.26a	0.25a	4.74a
	T <sub>3</sub>	1.33d	0.08b	0.69cd	1.23a	0.21ab	4.80a
Koroneiki	T <sub>1</sub>	1.64abc	0.13a	1.07ab	0.93bc	0.14d	4.83a
	T <sub>2</sub>	1.76a	0.11ab	1.09ab	1.18a	0.15cd	4.54a
	T <sub>3</sub>	1.69ab	0.10ab	1.05ab	1.09ab	0.17bcd	4.64a
Dezfuli	T <sub>1</sub>	1.49abcd	0.10ab	1.29a	0.94bc	0.15cd	4.08b
	T <sub>2</sub>	1.42bcd	0.12ab	1.14a	0.91c	0.16bcd	3.80b
	T <sub>3</sub>	1.43bcd	0.09ab	1.21a	0.90c	0.15cd	3.84b

<sup>a</sup> The different letters in the same column symbolize statistically significant differences among three treatments and olive cultivars, according to the Duncan's multiple range test ( $P \leq 0.05$ ). <sup>b</sup> T<sub>1</sub> =  $0 \text{ g l}^{-1}$  K<sub>2</sub>SO<sub>4</sub> (control); T<sub>2</sub> =  $1 \text{ g l}^{-1}$  K<sub>2</sub>SO<sub>4</sub>, T<sub>3</sub> =  $2 \text{ g l}^{-1}$  K<sub>2</sub>SO<sub>4</sub>.

year, where significantly greater fruit K content (4.94 %) was recorded for the T<sub>3</sub> treatment (2 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>) than control treatment (T<sub>1</sub>). These results are similar to other researches which showed that the application of potassium resulted in an increase in leaf potassium content in olive (Restrepo-Diaz *et al.*, 2008; Saykhul *et al.*, 2013), pecan (Diver *et al.*, 1985) and prune (Robbins *et al.*, 1982). However, no significant increase in the leaf potassium concentration of plants in response to K treatments has been reported (Smith *et al.*, 1987). It is well known that leaf uptake is affected by several factors such as nutrient concentration, application rate, air temperature and internal factors such as leaf age, metabolic activity of plant, membrane and cuticle characteristics (Weinbaum,

1988). It has also been reported that cultivars may differently respond to nutrients (Chatzistathis *et al.*, 2009; Loupassaki *et al.*, 2002). In addition, it has been reported that in olive trees the tissue nutrient concentrations in the different plant parts is different. In this context, Rodrigues *et al.* (2012) showed different nutrient accumulation in different tree parts. Their results revealed that the higher K pool was in fruits followed by the leaves. They stated at harvest, 40.0% of total K in a tree appeared in the fruit. Leaves contained only 25.4% of total potassium.

### Chlorophyll, Anthocyanin and Soluble Carbohydrates

**Table 3.** Leaf Chlorophyll (LCh), Fruit Chlorophyll (FCh), Fruit Soluble Carbohydrates (FSC) and Fruit Anthocyanin (FA) (based on fresh matter weight) of the olive cultivars ‘Mission’, ‘Koroneiki’ and ‘Dezfuli’ at three rates of foliar Application of Potassium Sulfate (FAP) during 2012-2013 crop seasons.<sup>a</sup>

Treatments		2012			
Cultivars	FAP <sup>b</sup>	LCh (mg kg <sup>-1</sup> )	FCh (mg kg <sup>-1</sup> )	FSC (mg g <sup>-1</sup> )	FA (mg g <sup>-1</sup> )
Mission	T <sub>1</sub>	6.02c	1.45f	25.87a	0.016c
	T <sub>2</sub>	9.79a	3.60ab	20.16ab	0.018c
	T <sub>3</sub>	7.47bc	2.97cd	22.62ab	0.019c
Koroneiki	T <sub>1</sub>	6.60c	3.39abc	14.66bc	0.025bc
	T <sub>2</sub>	8.44ab	3.83a	8.19c	0.034ab
	T <sub>3</sub>	9.25a	3.16bcd	16.64bc	0.034ab
Dezfuli	T <sub>1</sub>	5.99c	2.85de	23.54ab	0.033b
	T <sub>2</sub>	6.59c	2.39e	20.68ab	0.034ab
	T <sub>3</sub>	8.63ab	2.95cd	28.43a	0.046a
		2013			
		LCh (mg kg <sup>-1</sup> )	FCh (mg kg <sup>-1</sup> )	FSC (mg g <sup>-1</sup> )	FA (mg g <sup>-1</sup> )
Mission	T <sub>1</sub>	3.72e	1.25cde	17.51cd	0.020b
	T <sub>2</sub>	5.19bcd	1.46bc	16.62cd	0.024ab
	T <sub>3</sub>	4.32d	1.32bcd	12.51de	0.020b
Koroneiki	T <sub>1</sub>	6.17b	1.32bcd	8.66e	0.018b
	T <sub>2</sub>	5.47bcd	1.09de	17.92cd	0.021b
	T <sub>3</sub>	8.10a	1.01e	13.44d	0.023ab
Dezfuli	T <sub>1</sub>	4.88cd	1.55bc	21.29c	0.021b
	T <sub>2</sub>	5.64bc	1.94a	29.00b	0.022ab
	T <sub>3</sub>	5.20bcd	1.58b	43.37a	0.030a

<sup>a</sup> The different letters in the same column symbolize statistically significant differences among three treatments and olive cultivars, according to the Duncan's multiple range test ( $P \leq 0.05$ ). <sup>b</sup> T<sub>1</sub>= 0 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> (control); T<sub>2</sub>= 1 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>, T<sub>3</sub> = 2 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>.



The results showed in most cases, foliar application of potassium has led to a significant increase in leaf chlorophyll content of all cultivars compared to control treatments (Table 3). The highest concentrations of leaf chlorophyll was recorded in 'Mission' cultivar under  $1 \text{ g l}^{-1} \text{ K}_2\text{SO}_4$  ( $9.25 \text{ mg kg}^{-1}$ ) and 'Koroneiki' cultivar under  $2 \text{ g l}^{-1} \text{ K}_2\text{SO}_4$  ( $9.79 \text{ mg kg}^{-1}$ ) during 2012. Insignificant differences in fruit chlorophyll were recorded among K treatments and olive cultivars, with the exception of 'Mission' in the first year and 'Dezfuli' in the second year of this research where fruit chlorophyll was significantly increased by applying  $1 \text{ g l}^{-1}$  potassium sulfate ( $T_2$ ). The maximum concentration of fruit chlorophyll was found in 'Koroneiki' ( $3.83 \text{ mg kg}^{-1}$ ) and 'Mission' ( $3.60 \text{ mg kg}^{-1}$ ) treated with  $1 \text{ g l}^{-1}$  potassium sulfate during 2012. In the first crop season, fruit chlorophyll concentration of 'Mission' cultivar was significantly increased by  $1 \text{ g l}^{-1}$  potassium sulfate compared to control; however in other olive cultivars, the similar effect was not observed (Table 3). It is well known that cultivars differently respond to nutrient application (Chatzistathis *et al.*, 2009; Loupassaki *et al.*, 2002). The increase in chlorophyll concentration might be due to the role of potassium as a precursor in the biosynthesis of photosynthetic pigments. Also, potassium plays an important role in preventing decomposition of newly formed Chlorophyll (Tanaka and Tsuji, 1980). In other studies also foliar spray of potassium sulfate increased the chlorophyll content in sunflower (Akram *et al.*, 2009) and banana plants (Kumar and Kumar, 2008).

There was no significant effect of K treatments on Fruit Soluble Carbohydrates (FSC) in the studied olive cultivars in the first year of experiment (2012). However it was found that FSC increased by foliar application of potassium in 'Koroneiki' and 'Dezfuli' cultivars in the second year of research (Table 3). No significant statistical differences were detected for FSC of 'Koroneiki' trees treated with  $1 \text{ g l}^{-1}$  or  $2 \text{ g l}^{-1}$  potassium sulfate in 2013, but differences

with untreated trees were significant. The maximum level of FSC ( $43.37 \text{ mg g}^{-1}$ ) was recorded in 'Dezfuli' trees treated with  $2 \text{ g l}^{-1}$  potassium sulfate. The higher carbohydrates content in fruit of 'Dezfuli' cultivar might be partly related to the fact that this is a native cultivar and therefore more adapted to environmental conditions of the experimental site. Therefore this cultivar was more efficient in using potassium and showed relatively higher photosynthetic activity. Other studies also showed that application of potassium increased total carbohydrates content in fruit of grape (Arshad *et al.*, 2006) and prune trees (Southwick and Olson, 1996). It was reported that carbohydrate biosynthesis and translocation greatly depend on plant K content and the enzyme responsible for synthesis of carbohydrates is activated by potassium. Lower potassium levels result in accumulation of carbohydrates in plants and adequate levels of potassium is necessary for carbohydrate translocation in the sink organs (Amtmann *et al.*, 2008; Amtmann and Armengaud, 2009; Geiger and Conti, 1983).

In this research, anthocyanin content of 'Dezfuli' cultivar was significantly increased by  $2 \text{ g l}^{-1} \text{ K}$  in the first and second year of research (Table 3). The effect of potassium treatments on fruit anthocyanin was insignificant in 'Koroneiki' and 'Mission' cultivars in both years of experimentation (Table 3). The increase in the production of total anthocyanins with increasing potassium levels might be due to enhancement of Total Non-structural Carbohydrate (TNC). Present findings were also in agreement with the results of other researchers (Ghasemzadeh *et al.*, 2010; Norhaiza *et al.*, 2009; Wei *et al.*, 1990), as they showed that total flavonoids such as anthocyanins production is increased under higher K levels. Potassium has distinct effects on photosynthesis and carbohydrate translocation in plants. This indirectly enhances the biosynthesis of total flavonoids under potassium application.

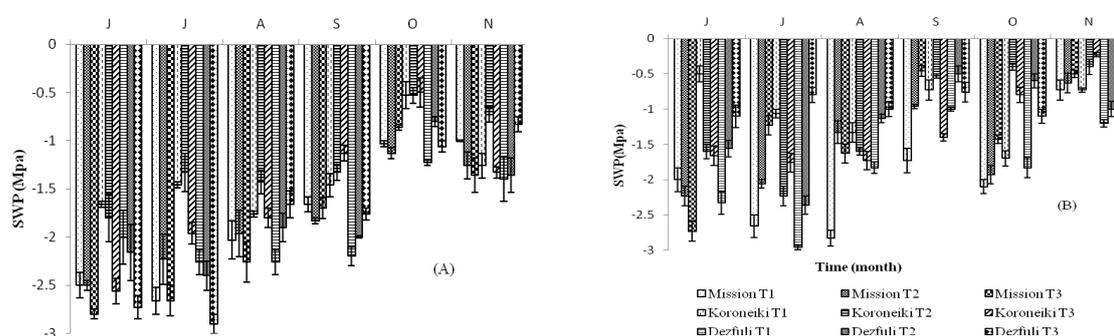
### Water Potential and Stomatal Conductance

Stem Water Potential (SWP) was significantly affected by cultivar in both years, and by K treatments in the second year of this research (Figures 1-A and -B). It was found that the highest stem water potential (-0.5 MPa) in the first year (2012) was recorded in 'Koroneiki' (in October), while the lowest one (-2.9 MPa) was recorded in 'Dezfuli' (in July) (Figure 1-A).

Stem Water Potential (SWP) in all treatments increased from June to November and reached maximum level in October (Figure 1-A). Subsequently, there was a decrease of stem water potential in November which was higher than the SWP values in summer. It was found that stem water potential of 'Koroneiki' was greater than that of 'Mission' and 'Dezfuli' during the summer. Similar results were obtained by other researchers, who showed that the water potential values were different among olive cultivars and during different times of season (Bacelar *et al.*, 2009) and it is normally higher in late summer (Burma, 2011; Giorio *et al.*, 1999).

As shown in Figure 1-B, the highest stem water potential was observed in the T<sub>3</sub> treatment of 'Koroneiki' (-0.23 MPa) in November, and the lowest was observed in the T<sub>1</sub> treatment of 'Dezfuli' (-2.96 MPa) in July. It was observed that in the

measurements from July to November, stem water potential levels of 'Mission' cultivar were higher in treated trees (T<sub>2</sub> and T<sub>3</sub>) compared to control (T<sub>1</sub>). In fact, the SWP of 'Mission' cultivar increased with foliar potassium applications. The maximum level of SWP in 'Mission' was observed in T<sub>3</sub> (-0.46 MPa) in September and its minimum in T<sub>1</sub> (-2.83 MPa) in August. During the summer (from July to September), stem water potential decreased in 'Koroneiki' in the T<sub>2</sub> and T<sub>3</sub> treatments compared to T<sub>1</sub> (control), while during October and November, stem water potential increased in 'Koroneiki' *cv.* in the T<sub>2</sub> and T<sub>3</sub> treatments compared to control. Stem water potential of 'Dezfuli' cultivar increased by applying K treatment (T<sub>2</sub> and T<sub>3</sub>) compared to control during the experiment. The maximum values of stem water potential in 'Dezfuli' cultivar were observed in T<sub>2</sub> treatment in September (-0.5 MPa) and October (-0.6 MPa) and in T<sub>3</sub> treatment in November (-0.6 MPa). It has been suggested that potassium is one of the osmolytes and its accumulation in plant cell might facilitate the occurrence of osmotic adjustment or activate the enzymes involved (Chartzoulakis *et al.*, 2006). In the present research such mechanism might have happened due to reduction of water potential in the treated 'Koroneiki' cultivar during the hot summer period. Our finding of stem water potential in untreated 'Mission' and 'Dezfuli' trees was in agreement with a previously reported research in which stated



**Figure 1.** Effect of foliar application of potassium sulfate on stem water potential (SWP) of three olive cultivars in 2012 (A) and 2013 (B). T<sub>1</sub>= 0 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> (control); Months from June (J) to November (N). T<sub>2</sub>= 1 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>; T<sub>3</sub>= 2 g l<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>. Data are means±SE, n= 3 Replicate plants.

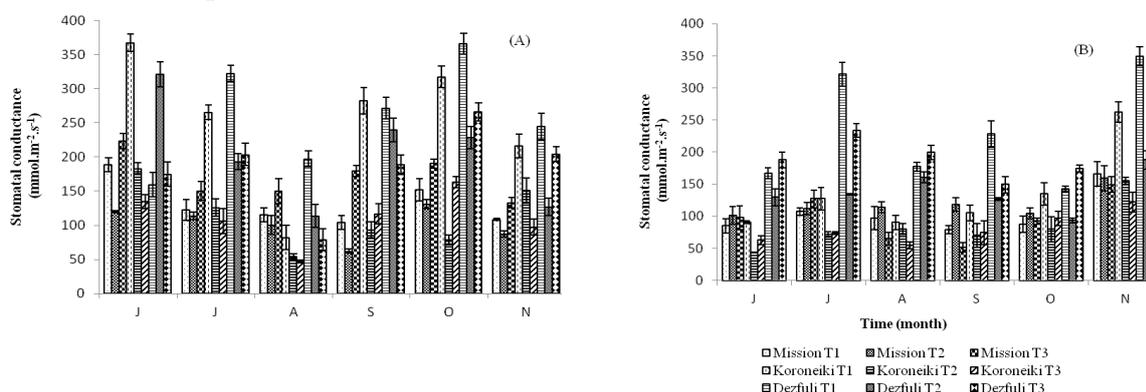


that olive trees are able to reduce the water potential of their tissue and thus create a higher potential difference between their leaves and roots, therefore being able to increase the amount of water absorption from the soil under hot and dry weather conditions (Xiloyannis *et al.*, 1999).

Stomatal conductance ( $g_s$ ) was significantly affected by cultivar and K treatments in both crop seasons (Figures 2-A and -B). 'Koroneiki' trees were more affected than the other two cultivars by the foliar application of potassium in the two studied growing seasons as the level of  $g_s$  was remarkably reduced by application of potassium. In fact, a similar response in the stomatal conductance of 'Koroneiki' and 'Dezfuli' cultivars was found. During the first crop season, the maximum levels of  $g_s$  were 367.78  $\text{mmol m}^{-2} \text{s}^{-1}$  in untreated 'Koroneiki' trees in June and 366.39  $\text{mmol m}^{-2} \text{s}^{-1}$  in untreated 'Dezfuli' trees in October. The minimum levels of  $g_s$  were observed in  $T_2$  (54.07  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and  $T_3$  (47.21  $\text{mmol m}^{-2} \text{s}^{-1}$ ) of 'Koroneiki' cultivar in August (Figure 2-A). During the second crop season, the maximum levels of  $g_s$  were observed in untreated 'Dezfuli' trees recording 321.2  $\text{mmol m}^{-2} \text{s}^{-1}$  in July and 349.53  $\text{mmol m}^{-2} \text{s}^{-1}$  in November (Figure 2-B). 'Mission' cultivar showed different response to foliar application of potassium between the two years of the study. In the first year of experiment,  $g_s$  of 'Mission'

cultivar decreased by applying  $T_2$  (1  $\text{g l}^{-1}$  potassium sulfate) and increased with  $T_3$  (2  $\text{g l}^{-1}$  potassium sulfate) compared to  $T_1$  (control). In the second year of experiment,  $g_s$  in the  $T_2$  treatment of 'Mission' increased during the season until the end of October compared with control treatment. Treated 'Mission' trees ( $T_3$ ) showed an increase in  $g_s$  in June to July, and then  $g_s$  decreased from August to September. Stomatal conductance increased during the next measurements from October until the end of November in all treatments.

Stomatal regulation is an important mechanism in balancing plants water relations (Athar and Ashraf, 2005; Dubey, 2005) and it depends on the distribution of potassium into epidermal cells, guard cells and leaf apoplast (Shabala *et al.*, 2002). In apple it was shown that foliar application of potassium reduced stomatal conductance (Swietlik *et al.*, 1982). In another study (Arquero *et al.*, 2006) on olive trees, higher potassium nutrition led to less stomatal conductance during the whole day compared to low-fed potassium plants. These results are in agreement with those of the present research. However, Akram *et al.* (2009) found that stomatal conductance in sunflower increased significantly with the application of potassium sulfate. In the present study different amounts of potassium sulfate reduced stomatal conductance in 'Koroneiki' and 'Dezfuli' cultivars. This



**Figure 2.** Effect of foliar application of potassium sulfate on stomatal conductance ( $g_s$ ) of three olive cultivars in 2012 (A) and 2013 (B).  $T_1=0 \text{ g l}^{-1} \text{ K}_2\text{SO}_4$  (control);  $T_2=1 \text{ g l}^{-1} \text{ K}_2\text{SO}_4$ ,  $T_3=2 \text{ g l}^{-1} \text{ K}_2\text{SO}_4$ . Data are means $\pm$ SE,  $n=3$  Replicate plants. Months from June (J) to November (N).

unexpected result of K nutrition on stomatal regulation of plants was also described by Benlloch-Gonzalez *et al.* (2008). Since potassium is important in the stomatal opening, the application of potassium is expected to increase stomatal conductivity. It has been suggested that the interactions between abscisic acid and ethylene could possibly explain this unexpected response (Benlloch-Gonzalez *et al.*, 2010a, b).

Moreover, stomatal conductance decreased during the summer (Figures 2-A and -B). In August, in most cases (Figure 2-A),  $g_s$  in all treatments reached to lower values than at the start of the experiment (June). Stomatal conductance increased from August to early autumn (October and November), but in the first crop season, reduction in  $g_s$  was observed in November. It seems that in mid-summer, the increase in air temperature and evaporation (Table 1) is associated to low stomatal conductance, then in late summer the decreased evaporation and air temperature resulted in higher  $g_s$  than the mid-summer. These findings are similar to those obtained by Burma (2011), who stated that  $g_s$  in olive trees grown in Iran, decreased during the summer and increased in the late summer. These results were not similar to those of Giorio *et al.* (1999) in southern Italy which showed that in late summer low evaporative atmospheric demand resulted from low air temperature, VPD (Vapor Pressure Deficit) and radiation was associated to low stomatal conductance in olive trees of 'Kalamon' cultivar.

Our data indicated that the pattern of water potential and stomatal conductance were not exactly similar between the two crop seasons. The differences are probably due to changes in environmental conditions and internal plant conditions. Other researchers have also expressed that physiological and environmental variables as well as other plant internal factors can affect the stomatal conductance and water status parameters (Moriani *et al.*, 2002). Factors such as time of the day (Mencuccini *et al.*, 2000) and the amount of crop load (Palmer *et al.*, 1997) seem to play an

important role in regulating water status and the chemical signals to control stomatal conductance. In general it can be stated that although the technique of foliar application of potassium has been widely accepted (Swietlik and Faust, 1984; Tagliavini and Marangoni, 2002), however little is known about its effectiveness in various specific environmental conditions. Researchers have examined the effect of foliar application of potassium on different species, but the reported results were different. Such variation in the results is probably due to the fact that this technique is affected by many factors such as plant species, leaf age, type and concentration of the nutrients, number of foliar application, weather condition and plant nutrition status (Swietlik and Faust, 1984).

In the present study, although the differences among the cultivars in their physiological and biochemical responses to the application of potassium should not be ignored, it is important to note that the environmental conditions during the year might have a strong influence on olive trees response. In this research it was inferred that foliar application of potassium on olive cultivars affects the studied physiological and biochemical parameters in different ways and possibly several mechanisms such as gene activation or expression, may be involved in these responses. If such mechanisms exist, more precise studies such as molecular and genetic experiments are required.

#### ACKNOWLEDGEMENTS

We thank Tarbiat Modares University (TMU) and Shahid Chamran University (SCU) for providing facilities and financial support.

#### REFERENCES

1. Akram, M. S., Ashraf, M. and Akram, N. A. 2009. Effectiveness of Potassium Sulfate in



- Mitigating Salt-Induced Adverse Effects on Different Physio-biochemical Attributes in Sunflower (*Helianthus annuus* L.). *Flora.*, **204**: 471-483.
2. Amtmann, A. and Armengaud, P. 2009. Effects of N, P, K and S on Metabolism: New Knowledge Gained from Multilevel Analysis. *Curr. Opin. Plant. Biol.*, **12**: 275-283.
  3. Amtmann, A., Troufflard, S. and Armengaud, P. 2008. The Effect of Potassium Nutrition on Pest and Disease Resistance in Plants. *J. Plant Physiol.*, **133**: 682-689.
  4. Arji, I. and Arzani, K. 2008. Effect of Water Stress on Some Biochemical Changes in Leaf of Five Olive (*Olea europaea* L.) Cultivars. *Acta Hortic.*, **791**: 523-526.
  5. Arquero, O., Barranco, D. and Benlloch, M. 2006. Potassium Starvation Increases Stomatal Conductance in Olive Trees. *HortSci.*, **41**: 433-436.
  6. Arshad, M., Gerigourian, V., Nazemieh, A., Mostoufi, Y. and Khalighi, A. 2006. Investigations on Effects of Nitrogen and Potassium Spray on Qualitative Characteristics and Some of Effective Physiological Factors in Bearing of *Vitis vinifera* L. 'Soltani'. *IJHST.*, **7(3)**: 135-146.
  7. Arzani, K., Arji, I. and Javadi, T. 2009. Pruning and Training Systems for Modern Olive Growing. (Eds.): Cantini, C. and Gucci, R. (Translated Book), Pub. By Agricultural Education Publisher, Ministry of Agriculture, Karaj, Iran, 232 PP.
  8. Athar, H. and Ashraf, M. 2005. Photosynthesis under Drought Stress. In: "Handbook of Photosynthesis", (Ed.): Pessaraki, M. Second Edition, CRC Press, New York, PP. 795-810.
  9. Bacelar, E. A., Moutinho-Pereira, J. M., Goncalves, B. C., Lopes, J. I. and Correia, C. M. 2009. Physiological Responses of Different Olive Genotypes to Drought Conditions. *Acta Physiol. Plant.*, **31**: 611-621.
  10. Benlloch-Gonzalez, M., Arquero, O., Fournier, J., Barranco, D. and Benlloch, M. 2008. K Starvation Inhibits Water-stress-induced Stomatal Closure. *J. Plant Physiol.*, **165**: 623-630.
  11. Benlloch-Gonzalez, M., Fournier, J. M. and Benlloch, M. 2010a. K<sup>+</sup> Deprivation Induces Xylem Water and K<sup>+</sup> Transport in Sunflower: Evidence for a Co-ordinated Control. *J. Exp. Bot.*, **61**: 157-164.
  12. Benlloch-Gonzalez, M., Romera, J., Cristescu, S., Harren, F., Fournier, J. M. and Benlloch, M. 2010b. K<sup>+</sup> Starvation Inhibits Water-stress-induced Stomatal Closure Via Ethylene Synthesis in Sunflower Plants. *J. Exp. Bot.*, **61**: 1139-1145.
  13. Burma, L. 2011. Effects of Foliar Application of Kaolin on Some Qualitative and Quantitative Features of Four Varieties of Olive Fruit. MSc. Thesis, University of Shahid Chamran, Iran.
  14. Chapman, H. D. and Pratt P. F. 1961. *Method of Analysis for Soils, Plants and Waters*. Division of Agricultural Sciences, University of California, Riverside, USA.
  15. Chartzoulakis, K., Psarras, G., Vemmos, S., Loupassaki, M. and Bertaki, M. 2006. Response of Two Olive Cultivars to Salt Stress and Potassium Supplement. *J. Plant Nutr.*, **29(11)**: 2063-2078.
  16. Chatzistathis, T., Therios, I. and Alifragis, D. 2009. Differential Uptake, Distribution Within Tissues, and Use Efficiency of Mn, Fe and Zn by Olive Cultivars 'Kothreiki' and 'Koroneiki'. *HortSci.*, **44**: 1994-1999.
  17. Diver, S. G., Smith, M. W. and McNew, R. W. 1985. Foliar Application of Potassium Sulfate, Potassium Nitrate, Urea and Ammonium Nitrate on Pecan Seedlings. *Hortic. Sci.*, **20**: 422-425.
  18. Dubey, R. S. 2005. Photosynthesis in Plants under Stressful Conditions. Handbook of Photosynthesis. Second Edition, CRC Press, New York, PP. 717-718.
  19. Fernandez-Escobar, R. 2004. Fertilizacion. In: "El Cultivo del Olivo", (Eds.): Barranco, D., Fernandez-Escobar, R. and Rallo, L. Mundi-Prensa, Madrid, PP. 287-320.
  20. Geiger, D. R. and Conti, T. R. 1983. Relation of Increased Potassium Nutrition to Photosynthesis and Translocation of Carbon. *Plant Physiol.*, **71**: 141-144.
  21. Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., Wahab, P. E. M. and Halim, M. R. A. 2010. Effect of Different Light Intensities on Total Phenolics and Flavonoids Synthesis and Antioxidant Activities in Young Ginger Varieties (*Zingiber officinale*). *Int. J. Mol. Sci.*, **11**: 3885-3897.
  22. Giorio, P., Sorrentino, G. and Andria, R. G. 1999. Stomatal Behavior, Leaf Water Status and Photosynthetic Response in Field-Grown Olive Trees under Water Deficit. *Environ. Exp. Bot.*, **42**: 95-104.

23. Hellebust, J. A. and Graigie, J. S. 1978. *Handbook of Physiological Methods*. Cambridge University Press, New York, PP. 95-97.
24. Inglese, P., Gullo, G. and Pace, L. S. 2002. Fruit Growth and Olive Quality in Relation to Foliar Nutrition and Time of Application. *Acta Hort.*, **586**: 507-509.
25. Khaleghi, E., Arzani, K., Moallemi, N. and Barzegar, M. 2015. The Efficacy of Kaolin Particle Film on Oil Quality Indices of Olive Trees (*Olea europaea* L.) cv. 'Zard' Grown under Warm and Semi-Arid Region of Iran. *Food Chem.*, **166**: 35-41.
26. Kjeldahl, J. G. C. 1883. A New Method for the Determination of Nitrogen in Organic Matter. *Fresenius. J. Anal. Chem.*, **22**: 366-372.
27. Kumar, A. R. and Kumar, N. 2008. Studies on the Efficacy of Sulphate Of Potash (SOP) on the Physiological, Yield and Quality Parameters of Banana cv. Robusta (Cavendish-AAA). *Eurasia J. Biosci.*, **2(12)**: 102-109.
28. Lee, J., Durst, R. W. and Wrolstad, R. E. 2005. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.*, **88**: 1269-1278.
29. Lichtenthaler, H. K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Method. Enzymol.*, **148**: 350-382.
30. Loupassaki, H. M., Chartzoulakis, S. K., Digalaki, B. N. and Androulakis, I. 2002. Effects of Salt Stress on Concentration of Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sodium in Leaves, Shoots, and Roots of Six Olive Cultivars. *J. Plant Nutr.*, **25**: 2457-2482.
31. Marschner, P. 2012. *Marschner's Mineral Nutrition of Higher Plants*. 3<sup>rd</sup> Edition, Academic Press: London, UK, PP. 178-189.
32. Mencuccini, M., Mambelli, S. and Comstock, J. 2000. Stomatal Responsiveness to Leaf Water Status in Common Bean (*Phaseolus vulgaris* L.) Is a Function of Time of Day. *Plant Cell Environ.*, **23**: 1109-1118.
33. Mengel, K. 2002. Alternative or Complementary Role of Foliar Supply in Mineral Nutrition. *Acta Hort.*, **594**: 33-47.
34. Moriana, A., Villalobos, F. J. and Fereres, E. 2002. Stomatal and Photosynthetic Responses of Olive (*Olea europaea* L.) Leaves to Water Deficits. *Plant Cell Environ.*, **25**: 395-405.
35. Norhaiza, M., Maziah, M. and Hakiman, M. 2009. Antioxidative Properties of Leaf Extracts of Popular Malaysian Herb, *Labisia pumila*. *J. Med. Plant. Res.*, **3**: 217-223.
36. Palmer, J. W., Giuliani, R. and Adams, H. M. 1997. Effect of Crop Load on Fruiting and Leaf Photosynthesis of 'Braeburn'/M26 Apple Trees. *Tree Physiol.*, **17**: 741-746.
37. Restrepo-Diaz, H., Benloch, M., Navarro, C. and Fernandez-Escobar, R. 2008. Potassium Fertilization of Rainfed Olive Orchards. *Sci. Hort.*, **116**: 399-403.
38. Robbins, S., Chaplin, M. H. and Dixon, A. R. 1982. The Effect of Potassium Soil Amendment, Trenching and Foliar Sprays on the Mineral Content, Growth, Yield and Fruit Quality of Sweet Cherry and Prune. *Commun. Soil Sci. Plant Anal.*, **13**: 545-560.
39. Rodrigues, M. A., Ferreira, I. Q., Claro, A. M. and Arrobas, M. 2012. Fertilizer Recommendations for Olive Based upon Nutrients Removed in Crop and Pruning. *Sci. Hort.*, **142**: 205-211.
40. Saadati, S., Moallemi, N., Mortazavi, S. M. H. and Seyyednejad, S. M. 2013. Effects of Zinc and Boron Foliar Application on Soluble Carbohydrate and Oil Contents of Three Olive Cultivars during Fruit Ripening. *Sci. Hort.*, **164**: 30-34.
41. Saykhul, A., Chatzistathis, T., Chatzissavvidis, C., Koundouras, S., Therios, I. and Dimassi, K. 2013. Potassium Utilization Efficiency of Three Olive Cultivars Grown in a Hydroponic System. *Sci. Hort.*, **162**: 55-62.
42. Shabala, S., Schimanski, L. J. and Koutoulis, A. 2002. Heterogeneity in Bean Leaf Mesophyll Tissue and Ion Flux Profiles: Leaf Electrophysiological Characteristics Correlate with the Anatomical Structure. *Ann. Bot.*, **89**: 221-226.
43. Smith, M. W., Cotten, B. C. and Agen, P. L. 1987. Foliar Potassium Sprays on Adult Pecan Trees. *HortScience.*, **22**: 82-84.
44. Southwick, S. M. and Olson, W. 1996. Optimum Timing of Potassium Nitrate Spray Application to "French" Prune Trees. *J. Am. Soc. Hort. Sci.*, **121**: 326-333.
45. Swietlik, D., Korcak, R. F. and Faust, M. 1982. Effect of Mineral Nutrient Sprays on Photosynthesis and Stomatal Opening of Water-stressed and Unstressed Apple



- Seedlings II. Potassium Sulphate Sprays. *J. Am. Soc. Hortic. Sci.*, **107**: 568-572.
46. Swietlik, D. and Faust, M. 1984. Foliar Nutrition of Fruit Crops. *Hortic. Rev.*, **6**: 287-355.
47. Tagliavini, M. and Marangoni, B. 2002. Major Nutritional Issues in Deciduous Fruit Orchards of North Italy. *Hort. Technol.*, **12**: 26-31.
48. Tanaka, A. and Tsuji, H. 1980. Effect of Calcium on Chlorophyll Synthesis and Stability in the Early Phase of Greening in Cucumber Cotyledons. *Plant Physiol.*, **65**: 1211-1215.
49. Toscano, P., Godino, G., Belfiore, T. and Bricolli-Bati, C. 2002. Foliar Fertilization: A Valid Alternative for Olive Cultivar. *Acta Hortic.*, **594**: 191-195.
50. Veberic, R., Stampar, F. and Vodnik, D. 2002. Influence of the Foliar Application of Phosphorus and Potassium on the Photosynthetic Intensity in Apple Trees (*Malus domestica* Borkh.). *Acta Hortic.*, **594**: 165-170.
51. Wei, H., Tye, L., Bresnick, E. and Birt, D. F. 1990. Inhibitory Effect of Epigenin, a Plant Flavonoid, on Epidermal Ornithine Decarboxylase and Skin Tumor Promotion in Mice. *Cancer Res.*, **50**: 499-502.
52. Weinbaum, S. A. 1988. Foliar Nutrition of Fruit Trees. In: "Plant Growth and Leaf Applied Chemicals". CRC Press, Inc. Boca Raton, Florida, USA, pp. 81-100.
53. Xiloyannis, C., Dichio, B., Nuzzo, V. and Celano, G. 1999. Defense Strategies of Olive against Water Stress. *Acta Hortic.*, **474**: 423-426.

### پاسخ های فیزیولوژیکی و بیوشیمیایی ارقام زیتون به کاربرد برگی پتاسیم

ش. زیودار، ک. ارزانی، م. ک. سوری، ن. معلمی، و س. م. سید نژاد

#### چکیده

این پژوهش در سال های ۱۳۹۱ و ۱۳۹۲، در شرایط آب و هوایی اهواز به منظور بررسی اثرات محلولپاشی پتاسیم بر برخی صفات فیزیولوژیکی و بیوشیمیایی سه رقم زیتون (میشن، کروناکی و دزفولی) انجام شد. سولفات پتاسیم در غلظت های متفاوت ( صفر، ۱ و ۲ گرم در لیتر) طی دو مرحله ۵۰ و ۶۵ روز پس از تمام گل، بر روی درختان ۱۰ ساله و بارور زیتون به کار برده شد. محلولپاشی پتاسیم در هر دو سال سبب افزایش کلروفیل برگ در همه ارقام، میزان پتاسیم برگ در ارقام میشن و کروناکی و آنتوسیانین میوه در رقم دزفولی شد. تیمارهای پتاسیم اثر معناداری بر نیتروژن و فسفر برگ، پتاسیم و فسفر میوه نداشتند. کاربرد برگی پتاسیم سبب افزایش کربوهیدرات محلول میوه در ارقام کروناکی و دزفولی در سال دوم آزمایش شد. پتانسیل آب شاخه و هدایت روزنه ای برگ به طور معناداری در هر دو سال تحت تأثیر رقم و تیمارهای پتاسیم قرار گرفتند. نتایج نشان داد که کاربرد برگی پتاسیم سبب کاهش هدایت روزنه ای برگ در ارقام کروناکی و دزفولی در هر دو سال گردید. بر اساس نتایج می توان بیان کرد که ارقام زیتون به طور متفاوت به تیمارهای پتاسیم طی دو فصل رشد پاسخ دادند و کاربرد برگی پتاسیم می تواند اثر مثبت بر کلروفیل برگ، پتاسیم برگ، کربوهیدرات محلول میوه و آنتوسیانین میوه در برخی ارقام زیتون داشته باشد.