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Foliar Application of Salicylic Acid, Methyl Jasmonate and Potassium Sulfate on Photosynthetic Characteristics and Fruit Quality of Pomegranate

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ABSTRACT- The present research designed to evaluate the effects of foliar application of methyl jasmonate (MeJA), salicylic acid (SA) and potassium sulfate on improving fruit quality and photosynthesis system. Ten treatments include: SA (0.3, 0.6 and 0.9 mM), MeJA (0.5, 1 and 2 mM), potassium sulfate (0.5, 1 and 1.5%) and distilled water (control) were sprayed on pomegranate trees 2 and 7 weeks after full bloom early in the morning. Results indicated that SA, MeJA and potassium sulfate increased phenolic compound and 1.5% of potassium sulfate and 0.5 mM of MeJA significantly increased the antioxidant activity of pomegranate fruit. Application of SA at 0.6 mM increased significantly hue angle of fruit peel. Foliar application of potassium significantly increased Fv/Fm and Pi parameter. Furthermore, total chlorophyll, b chlorophyll and cartenoids increased by 0.5 % of potassium sulfate, however, the differences were not significant. Besides, 1% and 1.5% of potassium sulfate significantly increased the pH of fruit juice.

Keywords: Antioxidant activity, Fv/Fm, Performance index, Phenolic compound

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family and is one of the oldest known edible fruits. It is sometimes called Chinese apple (23). Pomegranates are mainly grown for fresh consumption of arils (botanic exact term is seed) or juice, although in different countries they are produced for the food and beverage industry as flavouring and coloring agents (10).

The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (24). Pomegranate has been of recent interest for its nutritional, chemical and antioxidant characteristics.

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Very recently, incidence of a physiological disorder called "aril browning" or "aril paleness" (internal breakdown of arils) has threatened the popularity of pomegranate fruit. First time this physiological disorder reported in Ferdows region of Iran in. Affected arils are soft, light creamy-brown to dark blackish-brown, deformed, acidic and possess unacceptable off-flavor and are unsuitable for consumption. The extent of damaged arils could vary from a few to all in a fruit. Fruits having this disorder do not show any external signs and often possess visual appeal. Defective arils are detected only after cutting the fruits open, posing a serious challenge to quality control in export. Sometimes consumers hesitate to buy this fruit because of the hidden brown arils (15).

Shivashankara et al. (36) has been attributed browning of arils in pomegranate to the oxidative damage of membranes leading to higher activities of certain enzymes such as polyphenol oxidase and peroxidase. Similarly, enzymatic browning in pears is attributed to polyphenol oxidase and peroxidase (31).

Potassium is an essential macronutrient in pomegranate and its concentration in peel and aril is higher compared to other macronutrients (24). It is also known as the quality nutrient because of its important effects on quality factors (19). Soares et al. (37) reported that potassium soil application increased antioxidant activity significantly and reduced oxidative damage.

He et al. (12) have shown that exogenous salicylic acid (SA) can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses. Shi et al. (35) also reported that pretreatment by a foliar spray of 1 mM SA may have a signaling function in the induction of heat tolerance in cucumber seedling as indicated by an increase in H_2O_2 concentration. Low concentrations of reactive oxygen species (ROS), especially H_2O_2 are known to act as signal molecules initiating several protective resistance mechanisms against pathogens, chilling and heat stress. SA treatment also caused a decline in total chlorophyll content of wheat and moong. In contrast, an increase in total carotenoid content was observed in both crops (25).

There are some evidences indicating that MeJA can affect the antioxidant system in plant cells (41). The role of MeJA in protecting plants against various stresses has been reported, for example, amelioration of water stress in strawberry (41). It also has been reported that MeJA mitigated the ROS effects in strawberry under water stress and in maize seedlings subjected to paraquat (27). Likewise, MeJA treatment decreased chlorophyll content of *Arabidopsis thaliana* (17).

Many studies have shown a correlation between resistance to environmental stress and the efficiency of the antioxidative systems. The aim of this work was to evaluate the influence of foliar application of salicylic acid, methyl jasmonate and potassium sulfate during fruit growth and maturation on fruit quality of pomegranate.

MATERIALS AND METHODS

Plant Material and Treatment

Experiment was carried out in a commercial orchard of pomegranate cv. Malas Yazdi at Agricultural Research Center of Yazd, with 10 treatments and 4 replications. The treatments including: salicylic acid (0.3, 0.6 and 0.9 mM), methyl jasmonate (0.5, 1 and 2 mM), potassium sulfate (0.5, 1 and 1.5 %) and distilled water (control) applied on the base of factorial with completely randomized block design. Trees were sprayed two times (2^{ed} and 7^{th} week after full bloom). Fruits were harvested at the commercially maturity stage, and were transferred to postharvest laboratory.

Quality Characteristics Assessments

Peel and aril color were determined using a colorimeter (Minolta CR-400) and results were expressed as L*(lightness), a*, b*, hue angle and chroma.

For phenolics measurement, 200 µl of supernatant (5 g. of arils were extracted with 10 ml potassium buffer at pH 7.8 and centrifuged at 5000 rpm for 20 min) was mixed with 300 µl of potassium buffer, 2.5 ml of 0.2 N Folin-Ciocalteu and 2.5 ml of 7.5% sodium bicarbonate. The mixture was allowed to stand for 5 min at 50ºC, before the absorbance was measured at 760nm using a spectrophotometer (Uv/Vis T80). The final result expressed as mg gallic acid /100gfw (34).

The antioxidant activity was measured according to the method described by Serrano et al. (34). In order to determine the antioxidant activity of arils, glycine, hydrogen peroxide and ATBS (2, 2-azinobis (3-ehtylbenzothiazoline-6-sulfonic acid) diamonium salt) were mixed, and after adding proxidase and supernatant (fruit extract) the absorbance was measured at 730 nm by spectrophotometer (Uv/Vis T80). Finally, the antioxidant activity of arils was expressed as mg ascorbic acid equivalent 100 g of aril.

Chlorophylls and carotenoids in leaves were determined according to the Arnon (2). 1 g of leaves was homogenized in 80% acetone. The samples were centrifuged at 3500 rpm for 10 minutes and the absorbance of supernatants were read at 480, 510, 645, 652 and 663 nm by spectrophotometer (Uv/Vis T80).

Chlorophyll fluorescence was measured by chlorophyll fluorometer (Hansatech Instruments LTD Pocket PEA) on leaves after dark adaption for 15 minutes by leaf clips.

TSS was measured from the fruit juice, using a hand refractometer and results were expressed as ºBrix (PAL-1 ATAGO, Japan). Total acidity was determined by titration of 5 ml of juice with 0.2 M of NaOH and the results were calculated as a percentage of citric acid. The pH of juice was measured using a pH meter (Inolab pH 720).

Obtained data were analyzed by SAS software (ANOVA), and mean values were compared at the level of 5% according to Duncan multiple range test.

RESULTS AND DISCUSSION

Total Phenolics and Antioxidant Activity

Although SA, MeJA and potassium sulfate increased the total phenolics, but the differences were not significant compared to control (Table 1). It was reported that total phenolic increased by different treatments including: SA in 'Cara cara' Novel orange (14), potassium foliar application during fruit growth and development in pomegranate (38) and postharvest application of methyl jasmonate in white guava. However, pre-harvest treatment of methyl jasmonate has no effect on total phenolic of white guava (11), that is in agreement with our results. Ghasemnezhad and Javaherdashti (9) were expressed that MeJA could enhance the total phenolics and therefore induce the defense mechanism of raspberry against low temperature stress. SA and MeJA could stimulate the phenylalanine amonia lyase activity, an enzyme involved in the synthesis of phenolics through phenylpropanoid pathway and in consequence increase the amount of phenolic compounds (43).

Table 1. Effect of Salicylic acid, Methyl jasmonate and potassium sulfate on phenolic compound, TSS, total acid and pH of fruit

		Phenolic compound (mg gallic acid $100g^{-1}$ FW)	TSS	Total acidity(%)	pH
Water (control)		47.65†a	13.63a	0.86a	3.79b
Salicylic acid	0.3mM	55.57a	13.60a	0.75a	3.81b
	0.6mM	53.38a	12.69bc	1.03a	3.70b
	0.9mM	56.93a	13.25abc	0.81a	3.83b
Methyl jasmonate	0.5mM	54.71a	12.58c	0.66a	3.82b
	1mM	53.82a	13.50ab	0.96a	3.88ab
	2mM	49.08a	13.20abc	0.81a	3.95ab
Potassium sulfate	0.5%	55.62a	13.20abc	0.84a	3.95ab
	1%	51.80a	13.28abc	0.83a	4.21a
	1.5%	55.49a	13.35abc	0.75a	4.20a
Cv (%)		15.15	4.03	26.48	5.62

† Values with similar letters are not significantly different ($p<0.05$)

The antioxidant activity of arils was influenced by different treatments. 1.5% of potassium sulfate and 0.5 mM MeJA significantly increased the antioxidant activity of pomegranate arils at harvest time, but the increment was not significant in other concentrations and SA treatments (Fig. 1).

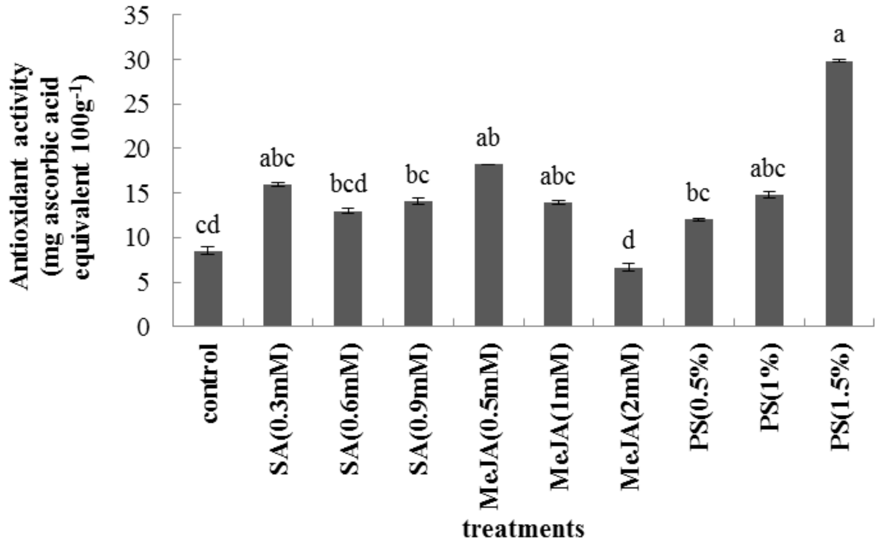

Fig. 1. The effect of Salicylic acid (SA), Methyl jasmonate (MeJA) and potassium sulfate on antioxidant activity of fruit. Data are means ± S.E.

Treatment with signalling molecules like SA and MeJA may induce H_2O_2 production, which in turn may induce the synthesis or activation of various transcription factors and are associated with the induction of different antioxidant enzymes (1). However, SA and MeJA response may vary with organisms, concentration of phytohormones and light intensity (30). It was reported that preharvest application of MeJA increased the antioxidant activity of raspberry fruit (40), and also potassium increased the antioxidant activity in pomegranate fruit (38) and pineapple (37).

It has been shown that potassium would increase growth and photosynthesis of plant and in consequences will increase phenolic compound due to allocation of carbon skeleton to shikimic acid pathway (26). Additionally potassium application was found to be highly correlated with PAL activity (37). On the other hand Wang and Lin (42) found that there is a positive correlation between total phenolic and anthocyanin with antioxidant activity, therefore it may be concluded that potassium has a significant role in antioxidant activity. Similarly, the results has shown a positive correlation (R^2=0.59^{ns}) between phenolic compound and antioxidant activity.

Color Indices

Application of SA at 0.6 mM increased significantly hue angle in fruit peel. Although not significant but the higher value of a* value in fruit peel was observed in 0.3 mM of SA compared to control (Table 2). Contrary to our results, Rudell and Fellman (32) expressed that MeJA treatment could enhance the peel red color and reduced peel hue angle of 'Fuji' apple. Different factors influence the color of fruit such as environmental condition, species and cultivar. In fact it was expressed that fruit response to MeJA treatment depends on the differences of fruit developmental stage (8). There was not any significant differences between all treatments on L* and b* value of arils (data has not shown) and peel color (Table 2) of pomegranate fruit. Also the hue angle and chroma of arils was not influenced by the treatments.

Table 2. Effect of Salicylic acid, Methyl jasmonate and potassium sulfate on peel colors

		L*	a*	b*	hue angle	chroma
Water (control)		**63.12†a**	**19.93abc**	**35.77a**	**60.83b**	**41.00ab**
	0.3mM	**61.27a**	**22.38a**	**35.05a**	**57.36b**	**41.70ab**
Salicylic acid	**0.6mM**	**63.74a**	**18.94bc**	**37.86a**	**70.00a**	**42.48a**
	0.9mM	**61.82a**	**18.32bc**	**34.48a**	**62.02ab**	**39.07b**
	0.5mM	**62.03a**	**17.45bc**	**37.70a**	**65.16ab**	**41.45ab**
Methyl jasmonate	**1mM**	**62.02a**	**20.13ab**	**35.80a**	**60.67b**	**41.09ab**
	2mM	**62.10a**	**20.79ab**	**35.25a**	**59.42b**	**41.09ab**
	0.5%	**61.88a**	**18.72bc**	**34.45a**	**61.46b**	**39.23b**
Potassium sulfate	**1%**	**61.76a**	**16.70c**	**36.28a**	**65.44ab**	**40.02ab**
	1.5%	**62.99a**	**18.22bc**	**35.87a**	**63.04ab**	**40.25ab**
Cv (%)		**2.71**	**10.84**	**6.01**	**8.14**	**4.25**

† Values with similar letters are not significantly different (p<0.05)

Chlorophylls and Carotenoids contents

It can be understood from the results in Table 3 that foliar application of SA at 0.3 and 0.6 mM and MeJA at 0.5 mM significantly decreased total chlorophyll and carotenoid contents. On the other hand, 0.5% of potassium sulfate treatment increased total chlorophyll content, but this was not significantly compared to control (Table 3).

It was reported that foliar spray of 0.5 and 1% of potassium increased total chlorophyll of banana plant as compared to control (18). Canakci and Munzuroglu (5) were shown that SA at 0.2 and 2 mM reduced chlorophyll a+b content of radish, barley and wheat. Beside these results, Cag et al. (3) indicated that SA application increased chlorophyll and carotenoids content of sunflower cotyledons. The decrease caused by high concentrations of SA in chlorophyll content was claimed to have resulted from inhibition of chlorophyll biosynthesis, acceleration of chlorophyll destruction or both (4). Also chlorophyll a and b contents in *Arabidopsis thaliana* decreased by methyl jasmonate treatment (17). Exogenously applied jasmonic acid (JA) and MeJA led to rapid loss of chlorophyll in barley leaves (28). Likewise, Jung

(17) demonstrated that application of methyl jasmonate caused a senescence-like symptom as indicated by a great decline in photosynthesis activity and chlorophylls.

Table 3. Effect of Salicylic acid, Methyl jasmonate and potassium sulfate on leaf pigments

		Chlorophyll a	Chlorophyll b	total Chlorophyll	Cartenoids
Water (control)		1.25†a	0.77a	2.36ab	0.60a
Salicylic acid	0.3mM	1.23ab	0.63bc	2.09cd	0.55b
	0.6mM	1.20b	0.65abc	2.02d	0.54b
	0.9mM	1.24ab	0.72ab	2.26abc	0.59ab
Methyl jasmonate	0.5mM	1.21ab	0.54c	2.00d	0.54b
	1mM	1.23ab	0.66abc	2.17bcd	0.57ab
	2mM	1.25a	0.73ab	2.21a-d	0.57ab
Potassium sulfate	0.5%	1.25a	0.78a	2.42a	0.61a
	1%	1.22ab	0.67abc	2.17bcd	0.57ab
	1.5%	1.25a	0.76a	2.26abc	0.60a
Cv (%)		4.08	24.24	12.58	10.64

† Values with similar letters are not significantly different ($p<0.05$)

Chlorophyll and carotenoid contents of leaves declined 21 weeks after full bloom significantly compared to other sampling dates (Fig. 2), and it may be happen due to the increasing of leaf age. Lin and Ehleringer (20) were shown that metabolic activity increases in the young leaf during growth and development and reaches to a maximum point at the complete leaf expansion and then decreases toward senescence of leaf.

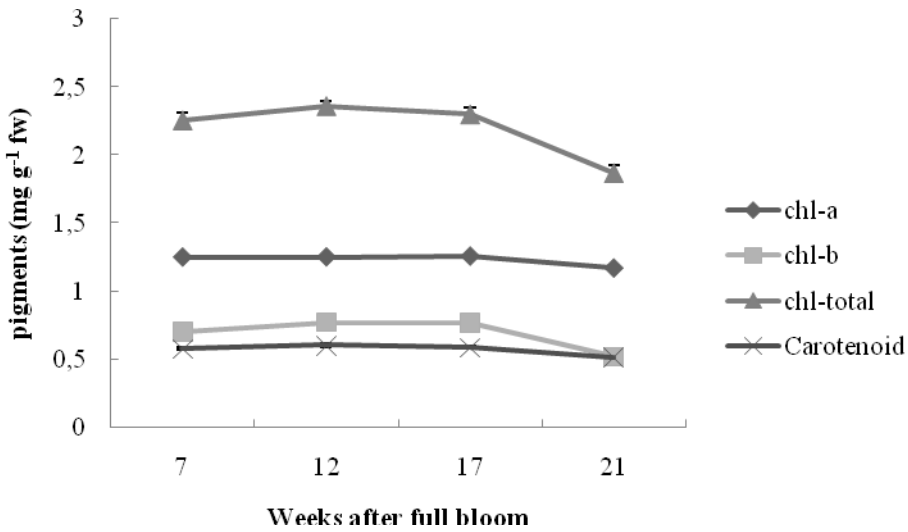

Fig. 2. Leaf pigment content of pomegranate during growth and development

Fv/Fm and Pi

Data presented in Table 5 shows that Fv/Fm and Pi parameters increased significantly by different level of potassium sulfate. The trend of Pi and Fv/Fm during growth and development shown that Pi decreased up to the end of the season (Fig. 3).

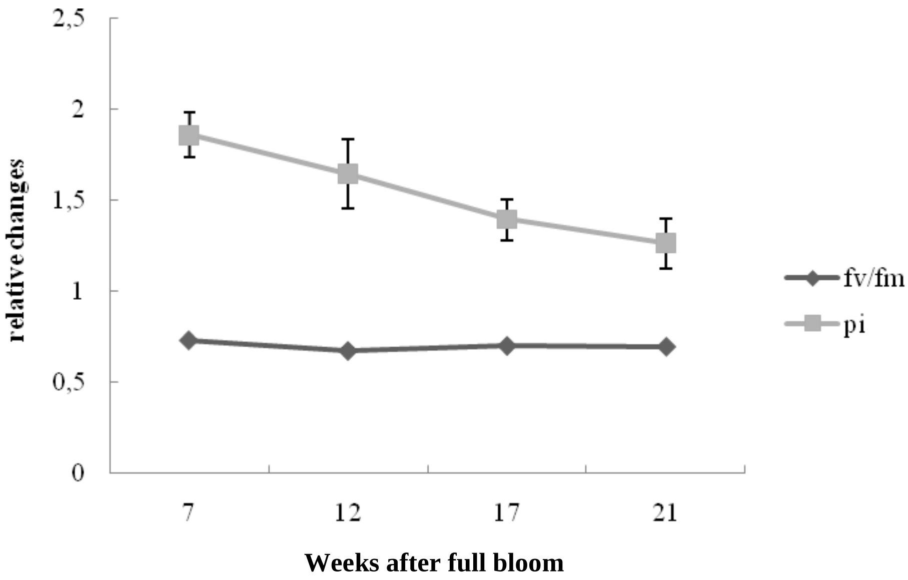

Fig. 3. Fv/Fm and Piof pomegranate during growth and development

As mentioned before, compounds containing potassium could increase leaf pigment and in consequences, higher performance of photosynthetic system was obtained (21). There are positive correlations between Fv/Fm and leaf pigments (Table 4).

Table 4. Correlations between parameters

	chlorophyll b	total chlorophyll	Pi	Fv/Fm	carotenid
chlorophyll a	0.78**	0.9***	0.44ns	0.51ns	0.85**
chlorophyll b		0.85**	0.48ns	0.44ns	0.84**
total chlorophyll			0.45ns	0.49ns	0.96***
Pi				0.96***	0.64*
Fv/Fm					0.65*

ns represents non-significance
***** represents significance at the 0.001 level**
**** represents significance at the 0.01 level**
*** represents significance at the 0.05 level**

A similar relationship was found between Fv/Fm and chlorophyll content by Jamil et al (16) in seedling of radish under salinity stress. These results indicated that difference in chlorophylls and carotenoids contents might be a possible reason of Fv/Fm variation indicating the efficiency of photosynthetic system. The results of the present study indicated that there are positive relationship between Pi with leaf pigments (Table 4), In accordance with noted results, Malceva et al (22) were reported the differences in Pi level were mostly due to changes in the ratio of active chlorophyll molecules in the reaction center of photosystem II.

Table 5. Effect of Salicylic acid, Methyl jasmonateand potassium sulfate on Fv/Fm and Pi

		Fv/Fm	Pi
Water (control)		0.69†dc	1.32bc
Salicylic acid	0.3mM	0.64e	0.84c
	0.6mM	0.67de	1.25bc
	0.9mM	0.66de	1.06bc
Methyl jasmonate	0.5mM	0.68de	1.13bc
	1mM	0.70dc	1.49b
	2mM	0.72bc	1.50b
Potassium sulfate	0.5%	0.74ab	2.07a
	1%	0.74ab	2.15a
	1.5%	0.76a	2.61a
Cv (%)		6.86	25.35

† Values with similar letters are not significantly different ($p<0.05$)

The effect of SA and MeJA on plant function depends on organisms, concentration of phytohormones and light intensity (30). Pretreatment of barley seedlings exposure to paraquat by 500 μM SA or 23 μM MeJA would induce plant to recover from stress condition while it would not happen in the control (29). It could be concluded that SA and MeJA could help the plant to maintain metabolic activity like photosynthesis during stress condition. While Horvath et al. (13) expressed that pretreatment of *Triticum aestivum* cv. Chinese spring plant under drought stress by 0.5 mM of SA could decrease Fv/Fm compared to control significantly.

Moreover, reduction of Fv/Fm ratio also suggested the occurrence of photoinhibition, also known as photo damage (6). At this time, accumulation of reduced electron acceptors may increase the generation of reactive radicals such as ROS, which can induce oxidative injuries (6). These oxidative injuries could enhance chlorophyll degradation or the inhibition of its biosynthesis (6).

TSS, Acidity and pH

The influence of all treatment on total soluble solids (TSS), titratable acidity and pH are shown in table 1. Although the TSS and titratable acidity of fruit juice were not influenced by the most treatments, but 0.6 mM of SA decreased the TSS of fruit juice significantly comparing to control. Also the obtained results showed that 1% and 1.5% of potassium sulfate significantly increased the pH of fruit juice in compare to other treatments. Delgado et al. (7) showed that application of potassium may decrease the amount of tartaric acid in grape and in consequence may increase the pH. Sayyari et al. (33) has shown that the amount of acidity and TSS was not influenced by SA treatment in pomegranate. Beside these results, Wang (39) was found that MeJA application may reduce the glucose, fructose and sucrose levels of radishes.

In general, the results of this experiment shown that the different concentrations of potassium sulfate could maintain and improve the photosynthetic activity in terms of Pi index and Fv/Fm ratio. Moreover this compound especially at concentration of 1.5% causes the highest antioxidant activity of pomegranate arils giving the essential potential of scavenging ROS molecules against stress condition and in consequences preserve fruit quality of pomegranate.

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محلول‌پاشی برگی اسید سالیسیلیک، متیل‌جاسمونات و سولفات پتاسیم بر کیفیت میوه و ویژگی‌های فتوسنتزی انار

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چکیده- پژوهش حاضر به منظور بررسی اثرات اسید سالیسیلیک، متیل‌جاسمونات و سولفات پتاسیم بر بهبود کیفیت میوه و ویژگی‌های فتوسنتزی طراحی شد. ۱۰ تیمار شامل اسید سالیسیلیک (۰/۳، ۰/۶ و ۰/۹ میلی‌مولار)، متیل‌جاسمونات (۰/۵، ۱ و ۲ میلی‌مولار)، سولفات پتاسیم (۰/۵، ۱ و ۱/۵ درصد)و آب مقطر (شاهد) بر روی درختان انار، ۲ و ۷ هفته بعد از مرحله تمام گل محلول‌پاشی شدند. نتایج به‌دست آمده نشان داد که اسید سالیسیلیک، متیل‌جاسمونات و سولفات پتاسیم می‌توانند ترکیبات فنلی را افزایش دهند و سولفات پتاسیم ۱/۵ درصد و متیل‌جاسمونات ۰/۵ میلی‌مولار به طور معنی‌داری فعالیت آنتی اکسیداسیونی میوه را افزایش دادند. از طرف دیگر کاربرد ۰/۶ میلی‌مولار اسید سالیسیلیک به طور معنی‌داری هیوانگل پوست میوه را افزایش داد. کاربرد برگی پتاسیم به طور معنی‌داری نسبت Fv/Fm و شاخص Pi را افزایش داد. همچنین مقدار کلروفیل کل، کلروفیل b و کارتنوئیدها توسط سولفات پتاسیم (۰/۵ درصد غلظت) افزایش یافت، اما تفاوت‌ها معنی‌دار نبود. علاوه بر این نتایج، سولفات پتاسیم ۱ و ۱/۵ درصد به طور معنی‌داری pH آب‌میوه را در مقایسه با دیگر تیمارها افزایش داد.

واژه‌های کلیدی: ترکیبات فنلی، شاخص عملکرد، فعالیت آنتی اکسیداسیونی، Fv/Fm

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