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Abstract. Recently, a pomegranate physiological disorder called 'aril browning' or 'aril paleness' in which a part or all of the arils show discoloration, affect the quality of fruit and such fruits are not suitable for consumption. The present research design to evaluate the effects of jasmonates, salicilates and potassium nutrient on reducing this disorders and improving fruit quality. Ten 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), were sprayed on pomegranate tree 10 and 45 days after full bloom and after harvest. Different parameters related to quality of fruit were measured. The obtained results indicated that salicylic acid could improve the total acidity, TSS, chroma and hue angel of skin and b* index of arils. Although the phenolic content of arils increased in all treatment compare to control, the differences were not significant. The highest antioxidant activity was observed in the 1.5% potassium sulfate treatment and the differences with the control were significant.

Keywords. Aril and skin color – Internal breakdown – Physiological disorder – Antioxidants.

I – 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 (Mars, 1994). Pomegranates are mainly grown for fresh consumption of arils (botanic exact term is seed) or juice, although in various countries they are produced for the food and beverage industry as flavouring and coloring agents (Gil *et al.*, 2000).

The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). Pomegranate has been of recent interest for its nutritional, chemical and antioxidant characteristics. Very recently, the incidence of a physiological disorder called 'aril browning' or 'aril blackening' (internal breakdown of arils) has threatened the popularity of pomegranate fruit. This physiological disorder was reported for the first time in Ferdows region of Iran in the year of 2001. Affected arils are soft, light creamy-brown to dark blackish-brown, deformed, acidic and possess unacceptable off-flavour 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 good eye 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 (Jalikop,*et al.*, 2010).

Shivashankara *et al*. (2004) attributed browning of arils in pomegranate to the oxidative damage of membranes leading to higher activities of certain enzymes like polyphenol oxidase and peroxidase. Similarly, enzymatic browning in litchi, pears and apples is ascribed to polyphenol oxidase and peroxidase (Murata *et al*., 2000).

Potassium is an essential macronutrient in pomegranate and its concentration in peel and aril of pomegranate fruits was the highest compared to other macronutrients (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). It is also known as the quality nutrient because of its important effects on quality factors (Lester *et al*., 2006). Soares, *et al*. (2005) reported that potassium soil application increased significantly antioxidant activity and reduced oxidative damage.

He, *et al.* (2002) have shown that exogenous salicylic acid (SA) can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress (He *et al*., 2002). Shi, *et al*. (2006) 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.

There are some evidences indicating that methyl jasmonate (MejA) can affect the antioxidant system in plant cells (Wang, 1999). The role of MeJA in protecting plants from various stresses has been reported, for example, amelioration of chilling injury and water stress in rice (Lee *et al*., 1996), tomato (Ding *et al*., 2001) and strawberry (Wang, 1999). It also has been reported that MeJA mitigated the ROS effects in strawberry under water stress and in maize seedlings subjected to paraquat (Norastehnia and Nojavan-Asghari, 2006).

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 the fruit growth and maturation on fruit quality of pomegranate.

II – Material and methods

Plant material and treatments. 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 included: 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 mM) and distilled water (control) applied on the base of factorial with completely randomized block design. Trees were sprayed two times at 2010/4/29 and 2010/6/3 dates ($2^{éd}$ and 7^{th} week after full bloom respectively). Fruits were harvested on 2010/9/12 date at the commercially maturity stage, and were transferred to postharvest labratory in Vali-e-Asr university of Rafsanjan.

Quality characteristics assessments. Total soluble solids (TSS) was measured from the fruit juice, using a hand refractometer and results were expressed as ºBrix (PAL-1 ATAGO, Japan). Total acidity (TA) 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). Peel and aril color were determined using a colorimeter (Minolta CR400) and results were expressed as L*(lightness), a*, b*, hue angle and chroma.

For phenolics measurement, 200 µl of supernatant (after 5 gr of arils were extracted with 10 ml potassium buffer at pH 8.7) was mixed with 300 µl of potassium buffer, 2.5 ml of 0.2 N Folin-Ciacalteu 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 galic acid /100 gfw. The antioxidant activity was measured based on ATBS (diamonium salt), described by Serrano *et al*. (2005) and the absorbance was measured at 730nm.

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

III – Results and discution

1. Total phenolics and antioxidant activity

Although SA, MejA and potassium sulfate increased the total phenolics, this was not significant compared to control (Fig. 1). It was reported that total phenolic increased by different treatment include: SA in 'Caracara Novel' orange (Huang *et al.*, 2008), potassium foliar application during fruit growth and development in pomegranate (Tehranifar and Mahmoodi Tabar, 2009) and grape (Delgado *et al.*, 2004) 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 (Gonzalez-Aguilar 2004), that is in agreement with our results. Ghasemnezhad and Javaherdashti (2008) expressed that MejA could enhance the total phenolics and therefore induce the defense mechanism of raspberry. SA and MejA could stimulate the phenylalanine ammonia lyase activity, an enzyme involved in the synthesis of phenolics and flavonoids through the phenylpropanoid pathway, (Chen *et al.*, 2006), and in consequence increase the amount of phenolic compounds (Yao and Tian, 2005).

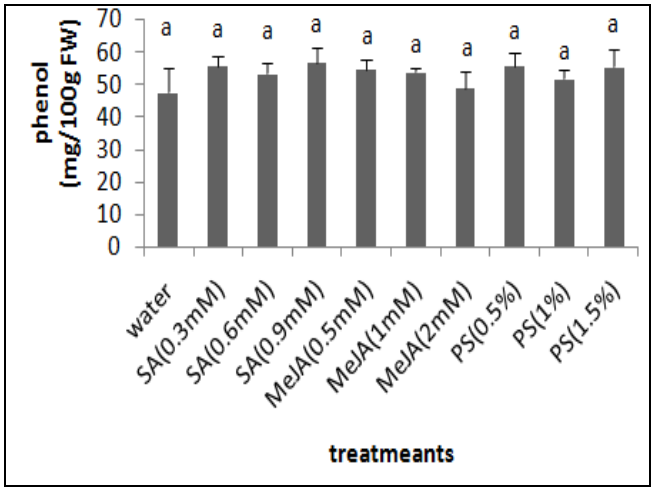

Fig. 1. The effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on phenolics of fruit.

The antioxidant activity of arils was influenced by the treatment. 1.5% of potassium sulfate and 0.5 mM MejA significantly increased the antioxidant activity of pomegranate arils at harvest, but the increment was not significant in other concentration and SA treatments (Fig. 2).

Treatment with signalling molecules like SA or MejA may induce H_2O_2 production, which in turn may induce the synthesis or activate various transcription factors and are associated with the induction of different antioxidant enzymes (Agarwal *et al*., 2005). However, SA and MejA response may vary with organisms, concentration of phytohormones and light intensity (Raman and Ravi, 2011). It was reported that preharvest application of MejA increase the antioxidant activity of raspberry fruit (Wang and Zheng, 2005), and also potassium increased the antioxidant activity in pomegranate fruit (Tehranifar amd Mahmoodi Tabar, 2009) and pineapple (Soares *et al.*, 2005).

Wang and Lin (2000) found that there is a positive correlation between total phenolic and

anthocyanin with antioxidant activity. Likewise in our experiment a correlation (0.59) was found between antioxidant activity and total phenolics.

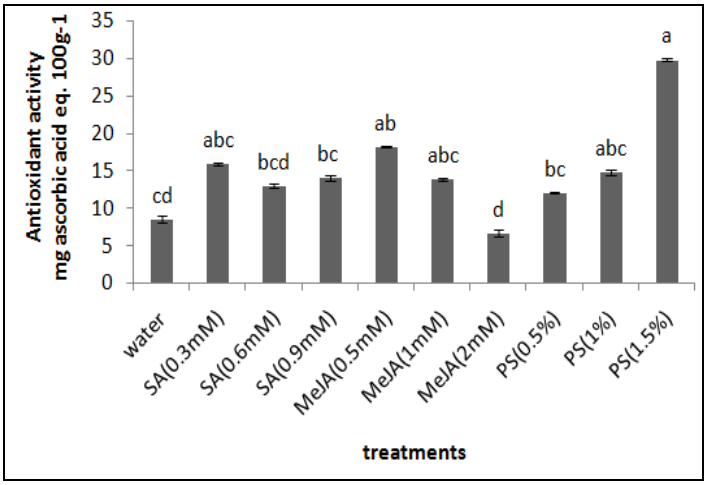

Fig. 2. The effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on antioxidant activity of fruit.

2. Total soluble solids, acidity and pH

The influence of treatments on TSS, titrable acidity and pH are shown in Table 1. Although the TSS and titrable acidity of fruit juice were not influenced by most of the treatments, 0.3 and 0.9 mM of SA decreased the TSS of fruit juice significantly comparing to control. 1% and 1.5% potassium sulfate significantly increased the pH of fruit juice compared to other treatments. Delgado *et al*. (2006) showed that application of potassium may decrease the amount of tartaric acid in grape and in consequence may increase the pH. Sayyari *et al*. (2009) and Ding *et al*. (2007) showed that the amount of acidity and TSS were not influenced by SA treatment in pomegranate and mango respectively. Besides these results Wang (1998) found that MejA application may reduce the glucose, fructose, and sucrose levels of radishes.

3. Color indices

There was not any significant differences between all treatment on L* and b* value of arils and peel color of pomegranate fruit (Tables 2 and 3). Also the hue angle and chroma of arils was not influenced by all the treatments. A higher value of a* and hue angle in fruit peel was observed in 0.3 SA compared to other treatments, although the differences were not significant. Contrary to our results, Rudell and Fellman (2005) expressed that MejA treatment could enhance the peel red color and reduce peel hue angle of 'Fuji' apple. Different factors influence the color of fruit such as environmental condition, species and cultivar, etc. In fact it has been observed that fruit response to MejA treatment could be ascribed to differences in the fruit developmental stage (Fan *et al.*, 1997).

Table 1. Effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on TSS, total acidity and pH of fruit juice

		TSS	Total acidity	pH
Water (control)		13.625a	0.8615a	3.785b
Salicylic acid	0.3 mM	13.6a	0.7477a	3.81b
	0.6 mM	12.685bc	1.0256a	3.7025b
	0.9 mM	13.25abc	0.8123a	3.83b
	0.5 mM	12.575c	0.6585a	3.82b
Methyl jasmonate	1 mM	13.5ab	0.9631a	3.8825ab
	2 mM	13.2abc	0.8123a	3.95ab
	0.5%	13.2abc	0.84a	3.9475ab
Potassium sulfate	1%	13.275abc	0.8287a	4.2124a
	1.5%	13.35abc	0.7477a	4.2025a
CV		4.03	26.48	5.62

Table 2. Effect of treatments SA (0.3, 0.6 and 0.9mM), MejA (0.5, 1 and 2mM) and potassium sulfate (0.5, 1 and 1.5 %) on aril colors

		L*	a*	b*	Hue angle	Chroma
Water (control)		29.93a	1.475a	13.775ab	6.07a	13.864a
Salicylic acid	0.3 mM	34.053a	2.14a	15.8152a	7.78a	15.968a
	0.6 mM	31.7a	4.438a	14.37ab	15.324a	15.587a
	0.9 mM	34.108a	1.735a	15.415ab	6.524a	15.517a
	0.5 mM	35.093a	1.745a	14.5425ab	6.724a	14.674a
Methyl jasmonate	1 mM	30.495a	1.855a	14.2725ab	7.406a	14.394a
	2 mM	34.825a	2.933a	13.895ab	11.996a	14.404a
	0.5%	33.313a	1.74a	13.6475b	7.24a	13.763a
Potassium sulfate	1%	33.485a	1.668a	14.8475ab	6.47a	14.197a
	1.5%	29.288a	1.948a	14.3425ab	7.909a	14.49a
CV		16.66	12.65	8.62	10.2	11.53

Table 3. Effect of treatments SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on peel colors

		a*	L*	b*	Hue angle	Chroma
Water (control)		19.933abc	63.123a	35.765a	29.168abc	41.004ab
Salicylic acid	0.3 mM	22.38a	61.273a	35.053a	32.641a	41.695ab
	0.6 mM	18.935bc	63.743a	37.855a	26.663bc	42.475a
	0.9 mM	18.318bc	61.82a	34.475a	27.978abc	39.074b
	0.5 mM	17.448bc	62.03a	37.695a	24.841c	41.45ab
Methyl jasmonate	1 mM	20.135ab	62.02a	35.795a	29.328abc	41.089ab
	2 mM	20.785ab	62.103a	35.253a	30.582ab	41.085ab
	0.5%	18.718bc	61.883a	34.45a	28.549abc	39.226b
Potassium sulfate	1%	16.703c	61.763a	36.275a	24.667c	40.016ab
	1.5%	18.22bc	62.988a	35.87a	26.969bc	40.25ab
CV		10.84	2.71	6.01	12.14	4.25

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