

# Effect of pre- and postharvest application of salicylic acid on quality attributes and decay of pomegranate fruit (cv. Shishe-Kab)

Tahereh Boshadi<sup>1</sup>, Farid Moradinezhad<sup>1\*</sup> and Mehdi Jahani<sup>2</sup>

<sup>1</sup>Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Iran. <sup>2</sup>Department of Plant Protection, Faculty of Agriculture, University of Birjand, Iran. \*E-mail: [fmoradinezhad@birjand.ac.ir](mailto:fmoradinezhad@birjand.ac.ir)

## Abstract

This study was aimed to assess the effect of salicylic acid (SA) on quality and storage life of pomegranate (*Punica granatum* L.) and performed in two experiments including foliar application and postharvest dipping of fruits in salicylic acid solutions. In the first experiment, pomegranate trees were sprayed at concentrations of 0, 1, 3 or 9 mM L<sup>-1</sup>. After two months of storage at 5 °C, ionic leakage, total soluble solids, and decay of pomegranate fruit was examined. The results showed that ionic leakage and fungal decay decreased and total soluble solids increased in treated fruits compared to the control. In the second experiment, fruits were dipped in SA at concentrations of 0, 1 or 3 mM L<sup>-1</sup> and stored at 5 °C for two months to investigate the effects of salicylic acid on chilling injury (CI), decay, and chemical attributes of stored samples. The results revealed that postharvest application of SA significantly increased total antioxidants and decreased chilling injury and decay of treated fruits. However, it did not significantly affect total phenol, total soluble solids, total anthocyanin and colour of fruits compared to the control.

**Key words:** Salicylic acid, pomegranate, fungal decay, chilling injury, anthocyanin, fruit color.

## Introduction

The pomegranate (*Punica granatum* L.) belongs to the Punicaceae family. The tree is grown in many subtropical countries especially in the Mediterranean region, India, Pakistan, Afghanistan, Iran, Saudi Arabia and in the subtropical areas of South America (Elyatem *et al.*, 1984).

Currently, interest in a number of fruits which are high in polyphenolic compounds has raised due to their reported chemopreventive and/or chemotherapeutic properties (Turrini, 2015). Pomegranate has been shown to exert anticancer activity, which is generally attributed to its high content of polyphenols including ellagitannins, ellagic acid, flavonoids (quercetin and luteolin glycosides) (Seeram *et al.*, 2005) and other polyphenols, such as anthocyanins (3-glucosides, cyanidin, and pelargonidin) (Benzie *et al.*, 2011). However, tropical and subtropical horticultural crops including pomegranate fruits are susceptible to chilling injury when stored at low temperatures after harvest (Wang, 1993). It is therefore very important to optimize the postharvest care of fruits to obtain satisfactory quality (Ozer *et al.*, 1997). Moradinezhad and Khayyat (2014) also reported that pomegranate fruit cv. Shishe-kab is susceptible to chilling injury, when stored at 5 °C for long-term.

Recently, there is an increasing interest in the use of natural compounds for maintenance of fruit quality and extension of shelf life. Salicylic acid (SA), a widely distributed compound in plants, belong to a group of phenolic compounds (Luo *et al.*, 2011) which is known as an endogenous signal molecule modulating both biotic and abiotic stresses (Asghari *et al.*, 2010), and is accepted as a safe natural chemical compound for the pre and postharvest application (Supapvanich and Promyou, 2013). Thus, salicylic

acid has remarkable ability to maintain the quality of the fruit during storage life of fruits (Tareen *et al.*, 2012). Babalar *et al.* (2007) reported that pre and postharvest SA treatments resulted fruit quality maintenance in strawberry. In grape fruit, Champa *et al.* (2015) reported that preharvest treatment of SA improved quality and postharvest life of table grapes. Pre-harvest SA enhanced shelf life of mango fruit during cold storage (Barman and Asrey, 2014). In addition, postharvest application of SA prolonged storage life and reduced fruit softening rate of kiwifruit (Zhang *et al.*, 2003), and delayed discoloration with the inhibition of browning on grapes (Ranjbaran *et al.*, 2011). In previous reports, SA application was effective in reducing CI and rate of the decline in ascorbic acid (vitamin C) losses in pomegranates (Sayyari *et al.*, 2009). Moradinezhad and Khayyat (2014) also reported that postharvest application of SA improve quality and postharvest life of pomegranate fruit cv. 'Shishe-Kab' during cold storage. However, little information is available about preharvest application of SA on cold storage of pomegranate cv. Shishe-kab and more research is needed in this regard.

The aim of the present study therefore was to evaluate the effects of pre and postharvest application of SA on quality, chilling injury and decay of pomegranate fruit cv. Shishe-kab.

## Materials and methods

**Preparing the plant material and storage conditions:** This study was performed in two individual experiments.

**Experiment I:** In the first experiment, pomegranate trees (cv. Shishe-kab) in the Shaukatabad Research Orchard of University of Birjand were sprayed one month before harvest, by different concentrations (1, 3 and 9 mM L<sup>-1</sup>) of salicylic acid. Control

trees were sprayed by distilled water. Fruits harvested early in November at full maturity stage and stored at 5 °C with 85-90 % RH. After 2 months, fruits transferred to the laboratory and electrolyte leakage and total soluble solid were measured. Also, a group of fruits were separated and inoculated with the fungus (*Botrytis*, *Penicillium*, *Aspergillus*) and then, the diameter of the lesion and the percentage of infected aril was measured.

**Experiment II:** In the second experiment, pomegranate fruits cv 'Shishe-Kab' were harvested at maturity stage, from trees that were grown at the uniform conditions, in November. Diseased, sunburn, bruised and injured fruits were discarded and uniform-sized fruits were selected. Thereafter, the fruits were immersed in salicylic acid solution (1 or 3 mM L<sup>-1</sup>) for 5 min. Control fruits were dipped in distilled water. Fruits were allowed to completely air-dried at ambient temperature and stored at 5 °C and 85-90 % RH for two months. Fruits transferred to the Physiology Laboratory at the University of Birjand and physicochemical parameters, decay and chilling injury were evaluated.

#### Physicochemical assessment

**Antioxidant activity:** DPPH method was used to measure the antioxidant activities of pomegranate juices based on the evaluation of the free radical scavenging capacities of the juices (Blois, 1958). Briefly, 1 mL of juice was mixed with 2 mL of 0.1 mM DPPH in methanol. The absorbance was measured at 517 nm using spectrophotometer (Unico 2100, China). Antioxidant activity was expressed as the percentage decline in absorbance relative to the control, corresponding to the percentage of DPPH scavenged (% DPPH), which was calculated as follows

$$\text{DPPH (\%)} = \left(1 - \frac{A(\text{sample})}{A(\text{control})}\right) \times 100$$

**Anthocyanin:** Total anthocyanin was studied in two groups of fruits, fruits that have been sprayed before harvest and fruits that had been dipped in salicylic acid after harvest. The pH-differential method was used to quantify the total anthocyanins (Giusti and Wrolstad, 2001). Extracts were diluted with 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) in 4 mL plastic cuvettes. Absorbance was measured at 510 and 700 nm and anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyanidin 3-glucoside) and absorbance (formula 2). Results were expressed as mg cyanidin 3-glucoside equivalents/100 g fresh weight.

$$\text{Total anthocyanin (mg L}^{-1}\text{)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times d}$$

$$A = (A_{\text{MAX}} - A_{700\text{nm}}) \text{pH}_1 - (A_{\text{MAX}} - A_{700\text{nm}}) \text{pH}_{4.5}$$

Where, A=absorbance; MW= molecular weight of cyanidin-3-glucoside; DF=the degree of dilution;  $\epsilon$ = molar absorptive coefficient.

**Decay and chilling injury:** In the first experiment, at first, fruits were treated with a disinfectant (bleach 10 %) to eliminate any possible contamination. Then, the skin of fruit was scratched and inoculated with a suspension of fungi to determine the influence of salicylic acid to prevent the growth of common fungi of pomegranate (*Aspergillus*, *Penicillium*, *Botrytis*). For this purpose, 12 fruit were selected from each treatment, randomly and were divided into 3 groups of 4, and each group was inoculated by one of the fungi and finally were stored at 5 °C. Immediately

after viewing the signs of mold, factors such as the diameter of mold on the surface of fruit (using digital callipers) and the percentage of infected arils were measured in each sample and compared with each other:

$$\text{Aril infection (\%)} = \frac{\text{Number of decayed arils in the fruit}}{\text{Number of intact arils in the fruit}} \times 100$$

In the second experiment, fruits transferred to the laboratory from cold storage, then decayed and chilling fruits counted and percentage of chilling and decay was determined. To calculate the percentage of decay, fruits containing moldy spots in diameter of more than 1 cm were considered decayed fruits and the decay percentage was calculated based on the method prescribed by El-Mougy *et al.* (2012)

Also, to calculate the percentage of chilling injury, fruits with chilling spots, in diameter of more than 1 cm, were considered as chilling affected fruits (Zhu *et al.*, 2004)

**Total soluble solids (TSS):** Total soluble solids were measured in pre and postharvest treated fruits. Fruit samples from each treatment were pooled and juiced to determine total soluble solids in the extracted juice by a hand-held refractometer (Extech Co., Model RF 10, °Brix, 0–32 %, USA) at 20 °C.

**Ionic leakage:** Ionic liquid, examined in pre-harvest treated fruits. To calculate the ionic liquids, discs with a diameter of 2.5 cm from the skin of the fruit were prepared and placed in a tank containing 20 mL of distilled water. After 24 hours, the initial electrical conductivity (EC1) was read. The samples were placed in water bath at 100 °C for 1 hour. After cooling, the second electrical conductivity (EC2) was calculated. Finally, the ion leakage was calculated using the formula suggested by Beckerson and Hofstra (1980)

**Phenol:** Pomegranate fruit juice was used to determine total phenolics content with Folin–Ciocalteu reagent by the method of Chuah *et al.* (2008) and expressed as mg gallic acid equiv. Plant sample (0.5 mL) was transferred to a test tube. After 5 minutes, 0.5 mL Folin was added. Then, 2 mL sodium bicarbonate (200 grams per liter) was added to it and shaken. The solution was left for 15 minutes at room temperature and 10 mL of deionized water was added to it and centrifuged for 5 minutes and the absorbance of the sample was measured with a spectrophotometer at a wavelength of 725 nm, and finally, results calculated in milligrams gallic acid per 100 g dry weight.

**Fruit skin colour:** Aril colour of pomegranate fruits was measured by using colorimeter (TES-135A, Taiwan) and recorded as L\* (lightness), a\* (-greenness to +redness) and b\* (-blueness to +yellowness). The different colour indexes (hue, chroma and Browning Index) were calculated according to Nunes *et al.* (1995).

**Statistical analysis:** The data were statistically analyzed in a completely randomized design experiment with four replications in the second experiment. Data were analyzed using GLM (generalized linear model) procedure of SAS program version 6.1. The difference between mean values of parameters was investigated by using LSD's test to examine if differences were significant at  $P \leq 0.05$ .

## Results and discussion

### Experiment I: Pre-harvest SA foliar application

**Total soluble solids:** Foliar application of salicylic acid had a significant effect ( $P < 0.05$ ) on TSS of pomegranate fruits compared to the control. The results indicated that all the treatments increased the total soluble solids compared with the control. However, there was no statistically significant difference among different concentrations of SA (Table 1). In some non-climacteric fruits such as pomegranate (Sayyari *et al.*, 2009) and grape (Ranjbaran *et al.*, 2011) non-significant effect of SA on TSS in fruits during cold storage has been reported. However, Khalili *et al.* (2014) reported that TSS in berries was not significantly affected by SA treatments. The increase in the TSS content during storage was probably due to the concentrated juice content as a result of dehydration and hydrolysis of polysaccharides.

**Ionic leakage:** Ionic leakage was significantly lower at 3 (40.73) and 9 (40.89) mM L<sup>-1</sup> concentrations than in the control (100.60) (Table 1). The highest electrical conductivity (ionic leakage percent) was observed in the control treatment and the lowest observed at concentration of 3 mM L<sup>-1</sup>. Also salicylic acid at concentration of 9 mM L<sup>-1</sup>, lead to a significant reduction in the ionic leakage ( $P < 0.05$ ). But the leakage of ions in 1 (70.76) mM L<sup>-1</sup> salicylic acid treatment was not significantly different compared to the control (Table 1). Similarly, salicylic acid decreased ion leakage in grape (Wang *et al.*, 2006). Sayyari *et al.* (2009) reported that in pomegranate fruits pre-storage treatment with salicylic acid, reduced ionic liquids, which is consistent with the results of our research. Ion leakage measurement of tissues under cold stress is an acceptable criterion to assess resistance to low temperatures (Wang *et al.*, 2006). Previous studies reported, when the plant tissue was damaged by the cold temperature, free radicals accumulated and damage lipids and fatty acids of the membrane (Han *et al.*, 2004). This leads to further damage the cell membrane and with drawal of water from cells and consequently ionic liquids increase (Azzarello *et al.*, 2009).

**Total anthocyanin:** There was no significant differences in anthocyanin values among the different treatments of SA used (0, 1, 3 and 9 mM L<sup>-1</sup>) (Table 1). The results of this study corresponded to the findings of Huang *et al.* (2008) in orange. Obinata *et al.* (2003) also reported that SA could markedly increase the production of procyanidin in grape. Our results agree with findings of Obinata *et al.* (2003) and Sayyari *et al.* (2011). Total anthocyanin was increased with storage period in both control and treated pomegranate (Sayyari *et al.*, 2011). Salicylic acid increases the synthesis of plant hormones, enzymes and photosynthesis, and thus accumulates pigments such as carotenoids and anthocyanins (Capitani *et al.*, 2005). Anthocyanins degrade by polyphenol oxidase during post-harvest, and this might be the main reason behind the reduction of anthocyanin compounds. The role of SA on anthocyanin production is unknown. One may hypothesize that SA could activate the key enzyme (Chalcone synthase) in the anthocyanin biosynthetic pathway (Godoy- Hernandez and Loyola-Vargas, 1997).

**Colour:** Pre-harvest SA treatments had no significant effect on the colour attributes of aril and peel of pomegranate fruits as compared to the control (Table 2). SA treatments were not

Table 1. Effect of pre-harvest salicylic acid (SA) foliar application on total soluble solids, ionic leakage and total anthocyanin of pomegranate fruits cv. 'Shishe-Kab' after two months of storage at 5 °C

SA Concentration (mM L <sup>-1</sup> )	Total soluble solid (%)	Ionic leakage	Anthocyanin (mg L <sup>-1</sup> )
0 (control)	16.54b	100.60a	148.69a
1	17.12a	70.76a	115.98a
3	18.37a	40.73b	150.22a
9	17.12a	40.89b	129.66a

effective on chroma in comparison with the control. Also, the application of SA did not change fruit hue angle and BI (Table 2). Similarly, SA had no effect on the colour properties of papaya fruits during storage (Promyou and Supapvanich, 2014). Also in apple, SA application had no effect on fruit colour (Supapvanich *et al.*, 2017). However, Tareen *et al.* (2012) showed that salicylic acid treatments, significantly affected skin colour of peach. SA treatments increased sensory quality parameters of apricot fruits including flesh color, taste, and texture (Ezzat *et al.*, 2017).

Table 2. Effect of pre-harvest salicylic acid (SA) foliar application on the colour of aril and peel of pomegranate fruits cv. 'Shishe-Kab' after two months of storage at 5 °C

SA (mM L <sup>-1</sup> )	Aril Color			Skin Color		
	Chroma	Hue	BI	Chroma	Hue	BI
0 (control)	60.81a	16.54a	123.54a	139.68a	26.32a	129.62a
1	66.07a	16.39a	93.25a	142.66a	20.75a	146.72a
3	82.62a	26.22a	1130.69a	128.27a	18.51a	132.27a
9	77.55a	21.83a	125.66a	134.69a	20.83a	137.78a

### Fungal decay

**Aspergillus:** Results showed that salicylic acid treatments had a significant effect in controlling the growth of *Aspergillus* spores. It was shown that 9 mM L<sup>-1</sup> salicylic acid treatment (19.94) was the most effective treatment to prevent the growth of fungus spores. In addition, fruits treated with 3 mM L<sup>-1</sup> salicylic acid (44.06) showed greater mold diameter compared to 1 mM L<sup>-1</sup> treatment (35.99). Also, the control treatment (51.66) showed the maximum diameter of mold (Fig 1, A and Fig 2, A). *Aspergillus* is known as pathogen that create secondary infections through small wounds and fissures in the pomegranate that has been stored at low temperatures (Adaskaveg, 1995). Several species of the genus *Aspergillus* have been observed to cause postharvest decay in pomegranates, particularly *A. niger*, *A. flavus* L., *A. niveus* Blochwitz, *A. versicolor* (Vuill.) Tirab, *A. nidulans* (Eidam) G. Winter, and *A. clavatus* Desm (Kanwar *et al.*, 1972). *Aspergillus* spp. were also found on moldy stamens of pomegranates stored at ambient temperatures (Labuda *et al.*, 2004). SA has been shown to inhibit the mycelial growth and mycotoxin production of *A. flavus* in pistachio (*Pistaica vera* L.) fruits (Panahirad *et al.*, 2014).

**Botrytis:** The results did not show significant differences between 1 mM L<sup>-1</sup> (48.36) and 3 mM L<sup>-1</sup> (49.78) concentration of SA, and the control treatment (48.45) in controlling the growth of spores. Thus, 1 and 3 mM L<sup>-1</sup> concentration of SA had no impact on decreasing the symptoms of *Botrytis* growth. But SA application at 9 mM L<sup>-1</sup> (41.25) significantly reduced the diameter of mold caused by the fungal inoculum (Fig 1, B and Fig 2, B). *Botrytis* fruit rot, also known as gray mold, caused by *Botrytis cinerea*, is the most serious disease of strawberry and is widespread in the environment. Microbial spoilage caused by *Botrytis* is the most important disease that causes economic losses in the pomegranate

(Tedford *et al.*, 2005). Some pathogens, such as *B. cinerea*, are able to infect stored pomegranates by mycelia spread from infected fruit to adjacent healthy fruit, causing ‘nests’ of decay (Tedford *et al.*, 2005). SA potential to control grey mould disease caused by *Botrytis cinerea* in tomato has been reported by Li and Zou (2017).

**Penicillium:** The most effective concentration of SA in controlling the *Penicillium* fungus was 3 mM L<sup>-1</sup> (25.97). However, the 1 (35.96) and 9 mM L<sup>-1</sup> (28.86) treatments significantly reduced mold diameter compared to the control (48.39) (Fig 1, C and Fig 2, C). Three *Penicillium* species, namely *P. crustosum*, *P. expansum* and *P. solitum*, are known to be destructive pathogens on pome fruits (Frisvad *et al.*, 2000). From the infrageneric point of view, they belong to subgenus *Penicillium* with penicilli of predominantly terminal terverticillate structures (Pitt, 1979). The *Penicillium* species is specific and obvious species in the pomegranate fruit. Rocha Neto *et al.* (2016) reported that in apple fruit, SA may be an alternative to the commercial fungicides currently used against *P. expansum*.

#### Experiment II: Postharvest SA dipping treatment

**Chilling injury:** Postharvest SA dipping significantly decreased chilling injury of fruits treated with 1 (13.5 %) and 3 mM L<sup>-1</sup> (7.75 %) concentrations of SA, after 2 months of cold storage (5 °C) compared to control (23 %) (Fig 3). These results indicate a role for salicylic acid in reducing chilling injury, as has been reported for loquat (Cai *et al.*, 2006) and pomegranate fruits (Sayyari, 2011). Similar results have also been reported in peach that SA concentration of 1 mM L<sup>-1</sup> reduced chilling injury (Wang *et al.*, 2006). SA application was effective in reducing chilling injury in pomegranates by decreasing electrolyte leakage and PAL activity (Sayyari *et al.*, 2009). In mango, treatment with SA was effective for delaying the increase in CI symptoms and lowering the CI index to about 70-80 % compared with the control fruits (Junmatong *et al.*, 2015). Generally, CI affects primarily the cell

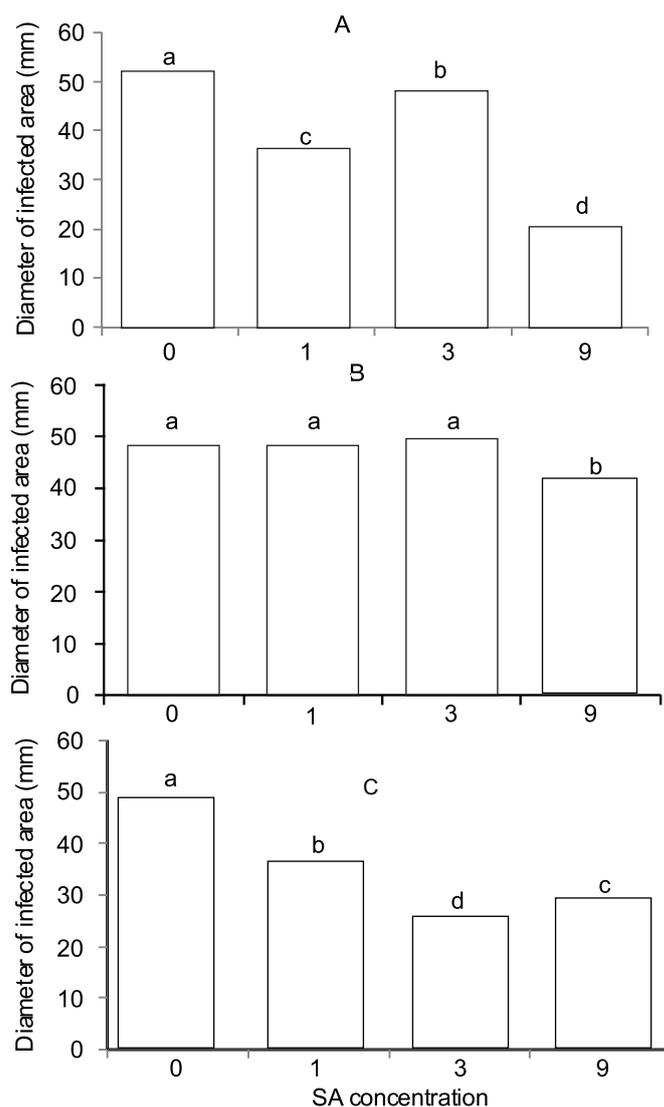


Fig. 1. The diameter of infection area (mm) created on the pomegranate fruit by inoculation of *Aspergillus* (A), *Botrytis* (B) and *Penicillium* (C).

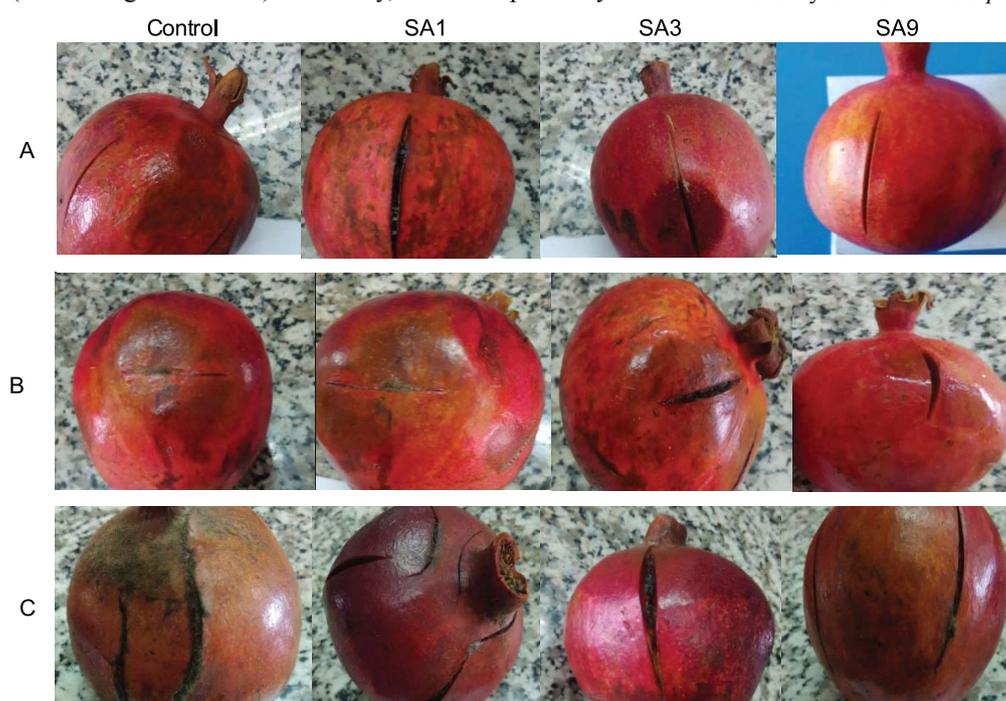


Fig. 2. Rot lesions caused by *Aspergillus* (A), *Botrytis* (B) and *Penicillium* (C).

membrane with changes in the fatty acid composition of phospholipids, and then the membrane damages initiate a cascade of secondary events leading to disruption of cell structure (Lurie *et al.*, 1987).

**Decay:** The effect of SA on fruits fungal decay is shown in Fig. 4. Fruits treated with different concentrations of SA significantly lowered decay incidence than control (11.75 %) after two months of storage. However, SA at 3 mM L<sup>-1</sup> concentration was more effective in reducing fungal decay at the end of storage time. Although, 1 (2.25 %) and 3 (1.5 %) mM L<sup>-1</sup> concentration of SA showed no significant difference (Fig. 4). SA does not have direct fungicidal effects, nevertheless, different studies show that it affects and decreases fungus development (Amborabe *et*

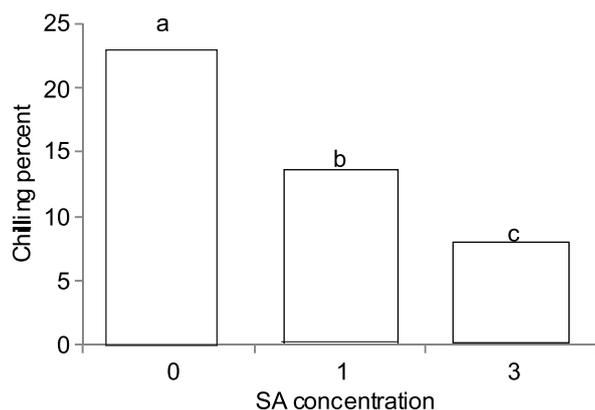


Fig. 3. Effect of postharvest salicylic acid dipping on chilling injury of pomegranate fruit *cv.* Shishe-Kab after two months of storage at 5 °C.

*al.*, 2002). Salicylic acid, enhanced expression of genes that control the production of phenolic compounds by activating the phenylalanine ammonialyase enzyme, increases resistance to infection (Eraslan *et al.*, 2007). Dipping of pear fruit in the SA solution, effectively controlled fruit decay during 5 months of cold storage (Asghari *et al.*, 2007). SA application had a positive effect in reducing berry decay (Samra, 2015).

**Total phenol:** There was no significant difference in the content of total phenolics among different concentrations of applied SA (Table 3). Phenolic compounds play an important role in increasing antioxidant capacity and fruit quality, and fruits with high phenolic compounds usually have the high antioxidant capacity (Fang *et al.*, 2009). In a previous study, pomegranate arils treated with salicylic acid exhibited higher total phenolic contents during storage (Dokhanieh *et al.*, 2016). The results of this study are consistent with the findings of the Sayyari *et al.* (2011) that postharvest salicylic acid treatments had no significant effect on total phenol of fruit, during storage. Ghasemnezhad *et al.* (2010) mentioned that the decrease of total phenolic levels might be due to the breakdown of cell structure in order to senescence phenomena during the storage period. The effect of salicylic acid treatments on maintenance of total phenolics content may be attributed to delay in senescence process.

**Total soluble solids:** Postharvest treatment of SA application did not change fruit total soluble solids (Table 3), which is in agreement with the findings of Garcia *et al.* (1995), Hernandez-Munoz *et al.* (2006) on the strawberry and on some non-climacteric fruits such as pomegranate (Sayyari *et al.*, 2009). However, no significant effect of SA on SSC in fruits during cold storage has been reported, as it has been observed in the present study. Also, a number of researchers reported that SA treatment had no effect on TSS of some fruits such as grape (Ranjbaran *et al.*, 2011) and persimmon (Ahmadi *et al.*, 2012).

**Antioxidant activity:** The effect of postharvest SA application on antioxidant activity of pomegranate fruits is shown in Table 3. Significant differences were observed in antioxidant activity among treated fruits and control (33.99). The highest antioxidant activity was observed in treated fruit with SA at 3 mM L<sup>-1</sup> (57.14). The total antioxidant activity was relatively low in the untreated fruits in comparison to SA-treated fruits, which might be due to retention of lower ascorbic content in the untreated fruits towards the end of storage period. In previous studies, the positive

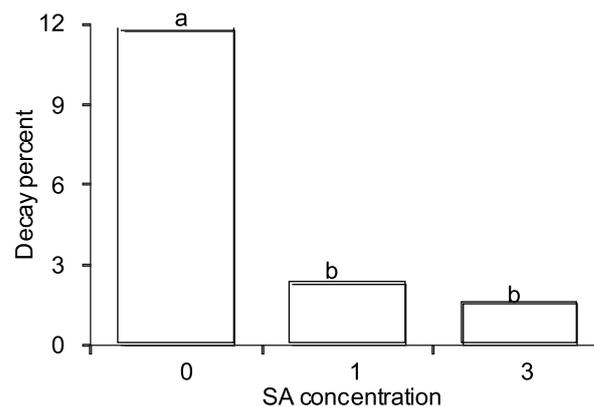


Fig. 4. Effect of postharvest salicylic acid dipping on decay of pomegranate fruit *cv.* Shishe-Kab after two months of storage at 5 °C. correlation between antioxidant activity and total phenolics has been reported (Diaz-Mula *et al.*, 2009; Ghasemnezhad *et al.*, 2010). The treatments of salicylic acid maintained antioxidant activity of the fruit significantly during storage. This effect of salicylic acid treatment was probably due to maintaining total phenolics and ascorbic acid levels during storage (Davarynejad *et al.*, 2015).

Table 3. Effect of postharvest salicylic acid (SA) dipping on total phenol, total soluble solids (TSS) and antioxidants of pomegranate fruits *cv.* 'Shishe-Kab' after two months of storage at 5 °C

SA (mM L <sup>-1</sup> )	Phenol (mg 100g <sup>-1</sup> )	Total soluble solids (%)	Antioxidants (%)
0 (control)	0.80a	17.77a	33.99c
1	0.86a	17.87a	39.31b
3	0.84a	18.09a	57.14a

**Color:** Postharvest salicylic acid treatments had not effect on the skin colour of pomegranate fruits after 2 months of cold storage (Table 4). The same results were obtained from postharvest SA application on strawberry that SA treatments were not effective on a\* value in comparison with control (Shafiee *et al.*, 2010). However, Babalar *et al.* (2007) reported that SA significantly affected a\* value of strawberry. The a\* value is a useful index of maturation and the degree of ripening in tomato (Artes *et al.*, 1999) and the external colour is a key factor indicating the quality of tomato (Supapvanich, 2015). The salicylic acid treatment causes a decrease in respiration and a delay in the appearance of the climacteric peak, which is concentration-dependent (Srivastava and Dwivedi, 2000). Shafiee *et al.* (2010) reported that the effect of SA treatments might be due to the reduction of respiration, and it prevents the increase in a\* value, so it could have an advantage in delaying the senescence. The SA application did not affect b\* value in SA-treated fruit, which showed higher level than control set.

Foliar application of salicylic acid (1, 3 and 9 mM L<sup>-1</sup>) had a significant effect on ionic leakage, the growth of fungi and Table 4. Effect of postharvest salicylic acid (SA) dipping on the skin colour properties of pomegranate fruits *cv.* 'Shishe-Kab' after two months of storage at 5 °C

SA (mM L <sup>-1</sup> )	Color properties					
	a*	b*	L*	Hue	BI	Chroma
0 (control)	44.95a	9.05a	34.9a	12.54a	112.50a	97.90a
1	48.61a	9.71a	34.8a	11.99a	119.57a	102.94a
3	48.84a	10.77a	36.3a	13.66a	122.07a	109.07a

total soluble solids while it had no significant effect on total anthocyanin and colour of fruit. In addition, the results of present study conclusively showed that postharvest dipping of pomegranate fruit at 1 and 3 mM L<sup>-1</sup> salicylic acid had a significant effect on the chilling injury, decay, antioxidants and total anthocyanin of pomegranate fruits cv. 'Shishe-Kab' after two months of cold storage. Nevertheless, SA had no significant effect on total phenol, total soluble solids, colour and performed nearly to that of control fruits.

Thus salicylic acid, a natural and safe phenolic compound, exhibits a high potential in controlling postharvest losses of pomegranate fruits. The result indicates that SA maintained the fruit quality and reduced the fruit decay of pomegranate in the both pre and post-harvest treatments, without any additional need for use of chemicals. However, further studies are needed to evaluate the response of different pomegranate cultivars.

## References

- Adaskaveg, J.E. 1995. Postharvest sanitation to reduce decay of perishable commodities. *Perishables Handling Newslett.*, 82: 21-25.
- Ahmadi, A., S. Kalantari, Y. Mostofi, Z. Zamani and O. Khademi, 2012. Extending storability of persimmon fruit cv. Karaj by postharvest application of salicylic acid. *J. Agr. Sci. Technol.*, 14: 1067-1074.
- Amborabe, B.E., P.F. Lessard, J.F. Chollet and G. Roblin, 2002. Antifungal effect of salicylic acid and other benzoic acid derivatives towards eutypalata: structure-activityrelationship. *Plant Physiol. Biochem.*, 40: 1051-1060.
- Artes, F., M.A. Conesa, S. Harnandez and M.I. Gil, 1999. Keeping quality of fresh-cut tomato. *Postharvest Biol. Technol.*, 17: 153-162.
- Asghari, M. and M.S. Aghdam, 2010. Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends Food Sci. Technol.*, 21: 502-509.
- Asghari, M., H. Shirzad and R. Hajitagilo, 2007. Postharvest treatment of salicylic acid effectively controls pear fruit diseases and disorders during cold storage. Novel approaches for the control of postharvest diseases and disorders. *Proceedings of the international congress, bologna, Italy, 3-5 may*, Crioif, University of Bologna.
- Azzarello, E., S. Mugnai, C. Pandolfi, E. Marone and S. Mancuso, 2009. Comparing image (fractal analysis) and electrochemical (impedance spectroscopy and electrolyte leakage) techniques for the assessment of the freezing tolerance in olive. *Trees*, 23:159-167.
- Babalar, M., M. Asghari, A. Talai and A. Khosroshahi, 2007. Effect of pre-and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit. *Food Chem.*, 105: 449-453.
- Barman, K., and R. Asrey, 2014. Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. *J. Sci. Ind. Res.*, 73: 713-718.
- Beckerson, D. and G. Hofstra, 1980. Effects of sulphur dioxide and ozone, singly or in combination, on membrane permeability. *Canadian J. Botany*, 58: 451-457.
- Benzie, I.F.F. and S. Wachtel-Galor. 2011. *Herbal Medicine*. Second Edition. Biomolecular and Clinical Aspects, CRC Press, Boca Raton, Fla, USA.
- Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nat.*, 181: 1199-120
- Cai, C., X. Li and K.S. Chen, 2006. Acetylsalicylic acid alleviates chilling injury of postharvest loquat (*Eriobotrya japonica* Lindl.) fruit. *Eur. Food Res. Technol.*, 223: 533-539.
- Capitani, F., S. Biondi, G. Falasca, V. Ziosi, A. Balestrazzi, D. Carbonera, P. Torrigiani and M.M. Altamura, 2005. Methyl jasmonate disrupts shoot formation in tobacco thin cell layers by over-inducing mitotic activity and cell expansion. *Planta*, 220: 507-519.
- Champa, W.A.H., M.I.S. Gill and B.V.C. Mahajan, 2015. Preharvest salicylic acid treatments to improve quality and postharvest life of table grapes (*Vitis vinifera* L.) cv. Flame Seedless. *J. Food Sci. Technol.*, 52: 3607-3616.
- Chuah, A.M., Y-Chi. Lee, T. Yamaguchi, H. Takamura, L.J. Yin and T. Matoba, 2008. Effect of cooking on the antioxidant properties of coloured peppers. *Food Chem.*, 111: 20-28.
- Davarynejad, G.H., M. Zarei, M.E. Nasrabadi and E. Ardakani, 2015. Effects of salicylic acid and putrescine on storability, quality attributes and antioxidant activity of plum cv. Santa Rosa. *J. Food Sci. Technol.*, 52: 2053-2062.
- Diaz-Mula, H.M., P.J. Zapata, F. Guillen, D. Martinez-Romero, S. Castillo, M. Serrano and D. Valero, 2009. Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars. *Postharvest Biol. Technol.*, 51: 354-363.
- Dokhanieh, A.Y., M.S. Aghdam and M.A. Askari Sarcheshmeh, 2016. Impact of postharvest hot salicylic acid treatment on aril browning and nutritional quality in fresh-cut pomegranate. *Hort. Environ. Biotechnol.*, 57: 378-384.
- El-Mougy, S., M. Abdel-Kader and H. Aly, 2012. Effect of a new chemical formula on postharvest decay incidence in citrus fruit. *J. Plant Prot. Res.*, 52: 156-164.
- Elyatem, S., M. and A.A. Kader, 1984. Post-harvest physiology and storage behaviour of pomegranate fruits. *Sci. Hort.*, 24: 287-298.
- Eraslan, F., A. Inal, A. Gunes and M. Alpaslan, 2007. Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Sci. Hort.*, 113: 120-128.
- Ezzat, A., A. Ammar, Z. Szabo and J. Nyeki, 2017. Postharvest treatments with methyl jasmonate and salicylic acid for maintaining physico-chemical characteristics and sensory quality properties of apricot fruit during cold storage and shelf-life. *Pol. J. Nutr. Sci.*, 67: 159-166.
- Fang, Z., Y. Zhang, Y. Lu, G. Ma, J. Chen, D. Liu and X. Ye, 2009. Phenolic compounds and antioxidant capacities of bayberry juices. *Food Chem.*, 113: 884-888.
- Frisvad, J.C., O. Filtenborg, F. Lund, R.A. Samson, 2000. The homogenous species and series in subgenus *Penicillium* are related to mammal nutrition and excretion. In: *Integration of Modern Taxonomic Method for Penicillium and Aspergillus Classification*. Samson RA, Pitt JI, eds. Amsterdam Overseas Publishers Association, 265-284.
- Garcia, J.M., A. Cayetano and A.A. Miguel, 1995. Postharvest heat treatment on Spanish strawberry (*Fragaria x ananassa* cv. Tudla). *J. Agric. Food Chem.*, 43: 1489-1492.
- Ghasemnezhad, M., M.A. Shiri and M. Sanavi, 2010. Effect of chitosan coatings on some quality indices of apricot (*Prunus armeniaca* L.) during cold storage. *Caspian J. Env. Sci.*, 8: 25-33.
- Godoy-Hernandez, G. and V.M. Loyola-Vargas, 1997. Effect of acetylsalicylic acid on secondary metabolism of *catharanthus roseus* tumor suspension cultures. *Plant Cell Rep.*, 16: 287-290.
- Giusti, M.M. and R.E. Wrolstad, 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current Protocols in Food Analytical Chemistry*. F1.2.1-F1.2.13.
- Han, B. and J.C. Bischof, 2004. Direct cell injury associated with eutectic crystallization during freezing. *Cryobiol.*, 48: 8-21.
- Hernandez-Munoz, P., E. Almenar, M. Jose and R. Gavara, 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*). *Postharvest Biol. Technol.*, 39: 247-253.
- Huang, R., R. Xia, Y. Lu, L. Hu and Y. Xu, 2008. Effect of preharvest salicylic acid spray treatment on postharvest antioxidant in the pulp and peel of Cara caranavel orange (*Citrus sinensis* L. Osbeck). *J. Sci. Food Agric.*, 88: 229-236.
- Junmatong, C., B. Faiyue, S. Rotaryanont, J. Uthaibutra, D. Boonyakiat and K. Saengnil, 2015. Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit. *Science Area*, 41: 12-21.

- Kanwar, Z.S. and D.P. Thakur, 1972. Post-harvest fungal diseases of pomegranate in Haryana. *Indian J. Mycol. Plant Pathol.*, 2: 189-191.
- Khalil, H.A., 2014. Effects of pre-and postharvest salicylic acid application on quality and shelf life of flame seedless grapes. *Eur. J. Hort. Sci.*, 79: 8-15.
- Labuda R., K. Hudec, E. Pieckova, J. Mezey, R. Bohovic, S. Mataova and S.S. Lukac, 2004. *Penicillium implicatum* causes a destructive rot of pomegranate fruit. *Mycopathol.*, 157: 217-223.
- Li, L. and Y. Zou, 2017. Induction of disease resistance by salicylic acid and calcium ion against *Botrytis cinerea* in tomato (*Lycopersicon esculentum*). *Emirates J. Food Agric.*, 29: 78.
- Luo, Z., C. Chen and J. Xie, 2011. Effect of salicylic acid treatment on alleviating postharvest chilling injury of Qingnai plum fruit. *Postharvest Biol. Technol.*, 62: 115-120.
- Lurie, S., L. Sonego and R. Ben-Arie, 1987. Permeability, microviscosity and chemical changes in the plasma membrane during storage of apple fruit. *Sci. Hort.*, 32: 73-83.
- Moradinezhad, F. and M. Khayat, 2014. Effects of intermittent warming and prestorage treatments (hot water, salicylic acid, calcium chloride) on postharvest life of pomegranate fruit cv. 'Shishe-Kab' during long-term cold storage. *Int. J. Hort. Sci. Technol.*, 1: 43-51.
- Nunes, M.C.N., J.K. Brecht, A.M.M.B. Morais and S.A. Sargent, 1995. Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. *Postharvest Biol. Technol.*, 6: 17-28.
- Obinata, N., T. Yamakawa, M. Takamiya, N. Tanaka, K. Ishimaru and T. Kodama, 2003. Effects of salicylic acid on the production of procyanidin and anthocyanin in cultured grape cells. *Plant Biotechnol.*, 20: 105-111.
- Ozer, M., H. Eris, R. Turk and N. Sivritepe, 1997. Biochemical changes and quality losses on normal, modified and controlled atmosphere stored of kiwifruits. In: *The Storage of Horticultural Products and Marketing Symposium*. K. Kaynas (Editor). Atatürk Central Horticultural Research Institute, Yalova, Turkey, Oct 21-24, pp. 125-133.
- Panahirad, S., F. Zaare-Nahandi, N. Mohammadi, S. Alizadeh-Salteh and N. Safaie, 2014. Effects of salicylic acid on *Aspergillus flavus* infection and aflatoxin B<sub>1</sub> accumulation in pistachio (*Pistacia vera* L.) fruit. *J. Sci. Food Agric.*, 94: 1858-1763.
- Pitt, J.I. 1979. *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*. Academic Press, 634 p.
- Promyou, S. and S. Supapvanich, 2014. Effects of salicylic acid immersion on physicochemical quality of Thai papaya fruit 'Kaek Dam' during storage. XXIX International horticultural congress on horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): IV 1111.
- Ranjbaran, E., H. Sarikhani, A. Wakana and D. Bakhshi, 2011. Effect of salicylic acid on storage life and postharvest quality of grape (*Vitis Vinifera* L. cv. 'Bidaneh Sefid'). *J. Fac. Agr.*, 56: 263-269.
- Rocha Neto, A.C., C. Luiz, M. Maraschin and R.M. Di Piero, 2016. Efficacy of salicylic acid to reduce *Penicillium expansum* inoculum and preserve apple fruits. *Int. J. Food Microbiol.*, 221: 54-60.
- Samra, B.N. 2015. Impact of postharvest salicylic acid and jasmonic acid treatments on quality of Crimson Seedless grapes during cold storage and shelf life. *Int. J. Adv. Res.*, 3: 483-490.
- Sayyari, M., M. Babalar, S. Kalantari, M. Serrano and D. Valero, 2009. Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates. *Postharvest Biol. Technol.*, 53: 152-154.
- Sayyari, M., S. Castillo, D. Valero and M. Serrano, 2011. Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds and antioxidant activity during postharvest storage of pomegranates. *Postharvest Biol. Technol.*, 60: 136-142.
- Seeram, N.P., L.S. Adams, S.M. Henning, Y. Niu, Y. Zhang, M.G. Nair and D. Heber, 2005. *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J. Nutr. Biochem.*, 16: 360-367.
- Shafiee, M., T.S. Taghavi and M. Babalar, 2010. Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. *Sci. Hort.*, 124: 40-45.
- Srivastava, M.K. and U.N. Dwivedi, 2000. Delayed ripening of banana fruit by salicylic acid. *Plant Sci.*, 158: 87-96.
- Supapvanich, S. and S. Promyou, 2013. Efficiency of salicylic acid application on postharvest perishable crops. *Springer.*, 339-355.
- Supapvanich, S. and S. Promyou, 2013. Efficiency of salicylic acid application on postharvest perishable crops. In: *Salicylic Acid: Plant Growth and Development*. S. Hayat, A. Ahmad and M.N. Alyemini (eds). New York, Springer. Pp. 339-355.
- Supapvanich, S. 2015. Effects of salicylic acid incorporated with lukewarm water dips on the quality and bioactive compounds of rambutan fruit (*Nephelium lappaceum* L.). *CMU J. Nat. Sci.*, 14.1: 23-27.
- Supapvanich, S., P. Mitsang, and P. Youryon, 2017. Preharvest salicylic acid application maintains physicochemical quality of 'Taaptimjaan' wax apple fruit (*Syzygium samarangense*) during short-term storage. *Sci. Hort.*, 215: 178-183.
- Tareen, M.J., N.A. Abbasi and I. Ahmad Hafiz, 2012. Postharvest application of salicylic acid enhanced antioxidant enzyme activity and maintained quality of peach cv. Flordaking fruit during storage. *Sci. Hort.*, 142: 221-228.
- Tedford, E.C., J.E. Adaskaveg and A.J. Ott, 2005. Impact of Scholar (a new post-harvest fungicide) on the California pomegranate industry. *Plant Health Progress*, 10: 94.
- Turrini, E., L. Ferruzzi and C. Fimognari, 2015. Potential effects of pomegranate polyphenols in cancer prevention and therapy. *Oxidative medicine and cellular longevity*. doi.org/10.1155/2015/938475.
- Wang, C.Y. 1993. Approaches to reduce chilling injury of fruits and vegetables. *Hort. Rev.*, 15: 63-95.
- Wang, L.J. and S.H. Li, 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca<sup>2+</sup> homeostasis and antioxidant systems in young grape plants. *Plant Sci.*, 170: 685-694.
- Wang, L., S. Chen, W. Kong, S. Li, and D.D. Archbold, 2006. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. *Postharvest Biol. Technol.*, 41: 244-251.
- Zhang, Y., K. Chen, S. Zhang and L. Freguson, 2003. The role of salicylic acid in postharvest ripening of kiwi fruit. *Postharvest Biol. Technol.*, 28: 67-74.
- Zhu, Z., G. Wei, J. Li, Q. Qian and J. Yu, 2004. Silicon alleviates salt stress and increases antioxidants enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.*, 167: 527-53.

Received: November, 2017; Revised: November, 2017; Accepted: December, 2017