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Effects of pre-harvest spray of salicylic (SA) and methyl jasmonate (MeJA) on the phytochemicals and physiological changes during the storage of grapefruit Cv. ray ruby

Waseem Ahmed*, Saeed Ahmed, Liaquat Ali, Imatiaz Hussan

Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan

Key words: Grape fruit, ray ruby, total phenolic compound, phytochemicals, chilling injury.

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Abstract

Grapefruit is an important fruit crop in the world as well as in Pakistan. The signalling molecules like salicylic acid (SA) and methyl jasmonate (MeJA) are endogenous plant growth substances which can play a key role in plant growth and development, and responses to environmental stresses. Phytochemicals in grapefruit are considered very important for human body due to their medicinal properties. Therefore it is important that Grapefruit should be stored proper temperature and consumed when it has maximum quantity of these chemicals. Phytochemical and postharvest losses are crucial issues for citriculture industry. Therefore it is also important to establish techniques or to adopt some measures that can reduce these losses. This study was carried out to investigate the effect Pre-harvest spray of SA and Me JA on the shelf life and quality of Ray Ruby. Pre-harvest sprays of SA @ 12mM and MeJA @ 5 mM showed higher biochemical parameters such as higher total phenolic compounds (166.29 and 165.76 mgGAE/100g), total antioxidants (72.63 and 71.37 %), Total carotenoids (16.40 and 16.32,18.09 and 18.03 mg/100g), total flavonoids contents (55.74 and 53.43 mgCEQ/100g) and total limonin contents (11.95 and 12.04 µg/mL) with minimum chilling injuries (1.57 and 1.42%) and fruit rots (4.23 and 3.90%) were also recorded in same fruits. On the basis of the result of this study, Pre-harvest sprays of SA @ 12mM and MeJA @ 5Mm and storage temperature of 8°C was found the best for maintaining phytonutrient quality of grapefruit after 90 day storage.

* **Corresponding Author:** Waseem Ahmed ✉ waseemuaf12@yahoo.com

Introduction

Citrus fruits are grown in more than 64 countries of the world (Chaudhry *et al.*, 2004) with a total production among the fruit crops of more than 105.4 million tonnes annually (PHDEB, 2006). Citrus ranks first with respect to area and production in Pakistan (Anonymous, 2013). Pakistan stands at 12th position among the citrus producing counties of the world with a total area of 2150,000 acres and total annual production of 490.27 (tonnes) (FAO, 2012). Grapefruit and lemon limes species. Grapefruit is ancestor of pummelo and was separated from pummelo in 1830 (Webber ,1943). Name is due to bearing habit just like a cluster of grapes. It contributes important nutrients to the diet, existing vitamins A vitamin C, folic acid, potassium, and dietary fibre (Mukherjee, 1997). Grapefruit is considered as preventive against cancer as it is a rich source of flavonoids and limonoids contents (Tanaka *et al.,* 2000). They are also rich sources of naringin, narirutin, and poncerin. Pharmacological properties of these flavonoids are linked to the ability of these compounds to promote differentiation function as antioxidants (Kuo,1996). Moreover flavonoids have a quality to act as anti-inflammatory, reduces the cholesterol level and modulates the immune system (Kuo 1996). Pre storage techniques such as application of salicylic acid and methyl Jasomonate (Me JA), fungicide, hot water dipping (HWD), wax application and postharvest technologies including modified and controlled atmosphere (MA and CA) storage, intermittent warming (IW) and temperature conditioning (TC) treatments are helpful to prolong the high quality storage life of Grapefruit.

The signalling molecules like salicylic acid (SA) and methyl jasmonate (MeJA) are endogenous plant growth substances which can play a key role in plant growth and development, and responses to environmental stresses. Salicylic acid is cosmically spread in plant kingdom (Raskin *et al.,* 1990) and is included in plant hormones group (Raskin, 1992).

Jasmonates are also play significant role in numerous physiological fruit processes (Hartmond, 2000), specifically in stress, senescence and leaf abscission (Gross & Parthier, 1994). It is also well known that methyl jasmonate (MeJA) is a natural compound of plants which play an important role in growth, development and response to different stresses. Its pre or post-harvest treatment reduce the brown rot in sweet cherry (Yao & Tan ,2005), suppress the gray mold in rose flowers (Darras *et al.,* 2005) and reduces the decay in papaya fruits (Gonzalez-Aguilar *et al.,* 2003). It also induces the promotion of senescence which can be characterized by the chlorophyll degradation, inhibition of lycopene accumulation (Sanieswky & Czapsky,1985). It is also known that salicylic acid plays an important role against fungi and diseases (Li *et al.,* 1999). Treatment of salicylic acid suppress the postharvest anthracnose diseases caused by *Collectotrichum gloeosporioides* in mango fruit (Zainuri *et al.,* 2001), Pear fruit (Cao *et al.,* 2006) and reduce the fungal decay in sweet cherry through defense resistance (Chan & Jiang, 2006). Zhang & Zheng (2004) reported that MeJA treatments inhibit the fruit decay in strawberries. Moreover, the total phenolic contents increased more rapidly and stayed at significantly higher levels in fruits treated with MeJA than the fruits of control. Higher antioxidants activities were also observed in the SA pre-harvest treated fruits when compare with the control (Renhua *et al.,* 2008). Hongjie *et al.,* (2004) also studied the effect of foliar application of SA and MeJA on sweet cherry. They found that SA @ 2mM and MeJA @ 0.2mM showed best results to inhibit the spore germination on fruits.

Pre-harvest spray of different chemicals reduces the Cl symptoms and enhances antioxidants in fruits. There are some references in the literature those indicate that different groups of growth regulators such as salicylic acid (SA) , methyl Jasmonate (Me JA), abscisic acid (ABA), and jasmonic acid (JA) enhance the mechanism that protects fruits from CI (González *et al.,* 2003). SA is signaling molecule, mediates defense for many pathogens and also play an essential role in thermogenesis during storage. JA and Me JA are called growth regulators. Wound of these compounds release an 18 systemin, amino acid

polypeptide that activates cell membrane lipase enzyme. These compounds are volatile in nature as they provide quick signals to neighboring cells and promote them to produce some chemicals before any injury occurred. It is reported that papaya and strawberries were treated with MeJA (5-10 mM) and stored in MA packaging at 10°C and found inhibition in fungal decay and in CI (González-Aguilar *et al.,* 2003). Jasmonates are also play significant role in numerous physiological fruit processes (Hartmond, 2000), specifically in stress, senescence and leaf abscission (Gross and Parthier, 1994). It is also well known that methyl jasmonate (MeJA) is a natural compound of plants which play an important role in growth, development and response to different stresses. Its pre or post-harvest treatment reduce the brown rot in sweet cherry (Yao and Tan, 2005), suppress the gray mold in rose flowers (Darras *et al.,* 2005) and reduces the decay in papaya fruits (Gonzalez-Aguilar *et al.,* 2003). It also induces the promotion of senescence which can be characterized by the chlorophyll degradation, inhibition of lycopene accumulation (Sanieswky & Czapsky, 1985). Methyl jasmonate also plays an important role to improve the color by stimulating the anthocyanin biosynthesis (Prez *al et.,* 1997). It is also known that salicylic acid plays an important role against fungi and diseases Li *et al.,* 1999). Treatment of salicylic acid suppress the postharvest anthracnose diseases caused by *Collectotrichum gloeosporioides* in mango fruit (Zainuri *et al.,* 2001), Pear fruit (Zhang & Zheng ,2004 reported that MeJA treatments inhibit the fruit decay in strawberries. Moreover, the total phenolic contents increased more rapidly and stayed at significantly higher levels in fruits treated with MeJA than the fruits of control. Higher antioxidants activities were also observed in the SA pre-harvest treated fruits when compare with the control (Renhua *et al.,* 2008). Hongjie *et al.* (2004) also studied the effect of foliar application of SA and MeJA on sweet cherry. They found that SA @ 2mM and MeJA @ 0.2mM showed best results to inhibit the spore germination on fruits

The aim of this study to investigate the pre harvest application of MeJA and SA on the phytochemical and fruit quality changes during and after storage maximum quantities of these phytochemicals present in grape fruit juice after storage.

Materials and methods

Experimental Details and Treatments

The studied factors included treatment and storage period. Thirty uniform and healthy grape fruit trees grafted on lough lemon rootstock were selected at orange Research Institute Sargodha (latitude 32° 03' N and longitude 72° 40' E) Punjab, Pakistan. Fruits were randomly harvested from selected trees with fruit clipper and brought to the Pomology Lab Institute of Horticultural Sciences, University of Agriculture Faisalabad Pakistan. Some analytical work was conducted in the laboratories of Post-harvest Research Centre, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, Biological and Bioassay Laboratory of Chemistry and Biochemistry Department, University of Agriculture Faisalabad, Pakistan and Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

Treatments

T_0 = Distilled water applied
T1 = Salicylic acid (SA) @ 6.0 mM
T2 = Salicylic acid (SA) @ 8.0 mM
T3 = Salicylic acid (SA) @ 12.0 mM
T4 = Methyl Jasmnate (MeJA) @ 3 mM
T5 = Methyl Jasmnate (MeJA) @ 4 mM
T6 = Methyl Jasmnate (MeJA) @ 5 mM

Preparation of salicylic acid solution

Salicylic acid (SA) solution was prepared by dissolving SA powdered in ethanol solution than gently heat was applied so that its dissolved completely it was @ 6, 8 and 12 mM and then applied on trees of grapefruits foliar application before harvesting at 20 days intervals.

Preparation of methyl jasmonate solution

Different concentrations of MeJA (3, 4 and 5 mM) were used. MeJA solutions were prepared by dissolving powder in ethanol solution and stirred. The

solution of 3, 4 & 5 mM MeJA solution was used for spraying.

Harvesting and storage

Fruits were harvested on 10 October at the commercially mature stage, sorted to eliminate damaged or shrivelled fruit, further selected for uniform size and color. Fruits were stored at 8˚C under normal air at 80-90% relatively humidity.

Washing and cleaning of fruits

Fruit were surface-sterilized with 2% (v/v) sodium hypochlorite for 3 min dipping than washed with tap water and air-dried. Each treatment contained three replications (10 fruits) and the entire experiment was performed twice.

Phytochemical parameters

Total phenolic contents

Total phenolic contents (TPC) were calculated by using Folin-Ciocalteu reagent method as reported by Ainsworth and Gillespie, (2007). The FC-reagent (10 mL) was dissolved in distilled water to make the solution 100 mL. In each sample (100 mL), FC-reagent (200 µL) was added and vortex thoroughly. The 700 mM Na_2CO_3 (800 µL) was added into each sample and incubated at room temperature for 2 h. Sample (200 µL) was transferred to a clear 96-well plate and absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as Gallic acid equivalent.

Total antioxidants

Total antioxidants activities of the grapefruit juice was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals as described by Amira *et al.,* (2012). The absorbance was read against a blank at 517 nm using micro-plate ELISA reader (BioTek, USA). Inhibition of free radical by DPPH in percent (%) was calculated by following formula:

I % = (Ablank -Asample /Ablank) × 100

Where Ablank is the absorbance of the control reaction mixture excluding fruit sample, and Asample is the absorbance of the test compounds. IC50 values, which represented the concentration of date fruit extracts that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentrations.

Total flavonoids contents

Flavonoids were determined by the method of (Kim *et al.,* 2003). Distilled water (4 ml) was added to 1 ml of fruit juice. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1M sodium hydroxide were added to the mixture and then the volume of reaction mixture was made up to 10 ml with distilled water. The mixture was thoroughly vortex and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents. All the measurements were taken in triplicate and the mean values were calculated.

Limonin contents

Total Limonin and limonin glycoside contents were isolated and evaluated for purity (Breksa *et al*., 2004) were used to prepare 500 µg/ mL stock solutions in acetonitrile were stored at 20°C. Juice samples were clarified by centrifugation (16000g, 5 min,10 C), and the supernatant was collected and filtered through filter paper (Whatman #1, Whatman Inc., Clifton, NJ) for the estimation of limonin contents.

Using these values, the limonin equivalence (µg/mL) of the sample was calculated using the equation.

Total carotenoids contents

Total carotenoids contents were estimated according to the method of (Lichtenthaler and Buschmann, 2001). Frozen grapefruit juice (5ml) was extracted with 1mL of pure acetone and then mixture was homogenized for 1 min and incubated at 4oC in darkness until the cap turned white. The homogenate was centrifuged at 16,000×g for 15min and 200µL of supernatant from each tube were placed in 96-well plates. The absorbance was read at 470 nm in a

micro-plate reader (Power Wave HT, Bio. Tek). The concentration of total carotenoids was calculated as follows:

TC (µg/mL) = (1000×A470)/214, and expressed as mg/100 g fresh weight.

Weight loss

Ten fruits (n=10) were randomly selected from each treatment unit. These fruits were weighted as fresh and at 30 days internal during the storage period and weight was calculated using the following formula (Takur, 2002).

$$WL(\%) = \frac{\text{Original Fruit weight- final fruit weight after storage}}{\text{Average fruit weight}} \times 100$$

Chilling injury

Chilling injury during the storage was calculated by using the following formula.

$$\text{Chilling injury}(\%) = \frac{\text{Number of affected fruits per treatment}}{\text{Total number of fruits per treatment}} \times 100$$

Fruit rot

Fruit rot during the storage was estimated by using the following formula.

$$\text{Fruit rot}(\%) \frac{\text{Number of affected fruits per treatment}}{\text{Total number of fruits per treatment}} \times 100$$

Statistical analysis

Collected data were statistically analyzed using computer software MSTAT-C. Analysis of variance was used to test the significance of variance. While difference among treatment means were compared using LSD test (P=0.05) (Steel *et al.,* 1996). Standard errors (SE) were computed by MS-Excel and data were presented graphically using the same program.

Results

Texture score

Statistically significant differences were found at P≤0.05 regarding the effects of treatments (SA and MeJA) and storage periods while interaction between them showed non-significant results for texture score (Figure 1). The fruits those were sprayed with salicylic acid @ 12 mM (T_3), methyl jasmonate @ 5 mM (T_6) and salicylic acid @ 8 mM (T_2) showed higher color scores of 7.88, 7.66 and 7.33 were marked by the panellists, respectively and these were at par with each other. While minimum texture score of 3.88 was liked by the panellists in the fruits those were untreated (T_0). The fruits those were analysed 30 days after storage showed higher texture score of 7.28 ranked by the panellists as compared to the fruits those were analysed 60 and 90 days after storage where texture scores were 6.57 and 5.61, respectively.

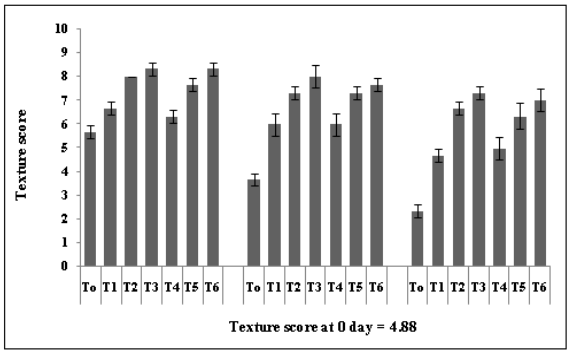

Fig. 1. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on texture score during storage (8°C) in the fruits of Ray Ruby.

T_0=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Chilling injury

Statistically significant differences (P≤0.05) were found regarding the effects of treatments (SA and MeJA) and storage periods while their interaction was found non-significant (Figure 2). Minimum chilling injury indexes of 0.11, 0.11, 0.22 and 0.22% were recorded in the fruits of T_6 (methyl jasmonate @ 5 mM), T_3 (salicylic acid @ 12 mM), T_5 (methyl jasmonate @ 4 mM) and T_2 (salicylic acid @ 8 mM), respectively and these were at par with each other. While fruits those were untreated (T_0) showed higher index (3.66%) of chilling injury in the fruits. The fruits those were analysed 90 days after storage showed higher index of chilling injury (1.57%) than the fruits those were analysed 60 and 30 days after storage where chilling injury indexes were 0.90 and 0.66%, respectively and these were at par with each other.

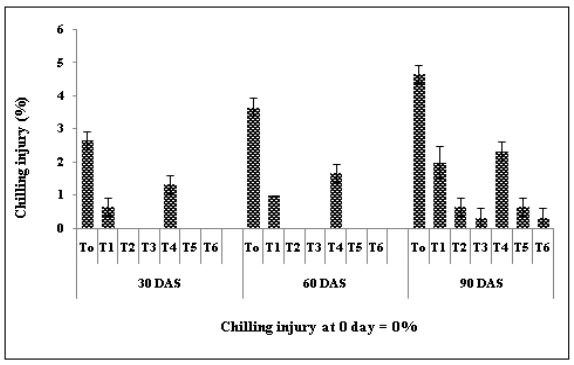

Fig. 2 . Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on chilling injury (%) during storage (8°C) in the fruits of Ray Ruby.

T_0=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

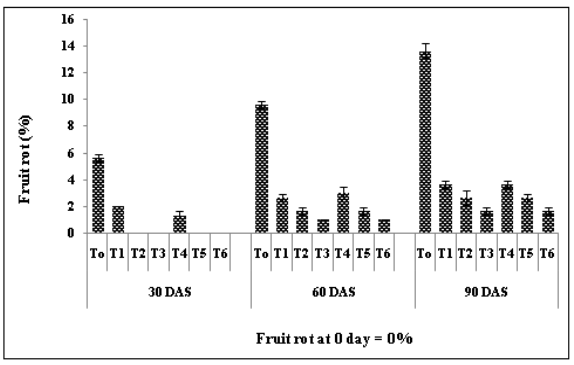

Fig. 3. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate i(MeJA) on fruit rot (%) during storage (8°C) in the fruits of Ray Ruby.

T_0=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Fruit rot

The effects of treatments (SA and MeJA), storage periods and interaction between them showed statistically significant differences ($P \leq 0.05$) regarding the fruit rot (Figure 3). Lower indexes of fruit rot (0.88, 0.88, 1.44 and 1.44%) were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T_3), methyl jasmonate @ 5 mM (T_6), methyl jasmonate @ 4 mM (T_5) and salicylic acid @ 8 mM (T_2), respectively and these were at par with each other. While higher index of fruit rot (9.66%) was noted in the fruits those were untreated (T_0). The fruits those were analysed 90 days after storage showed higher index of fruit rot (4.23%) than the fruits those were analysed 60 and 30 days after storage where fruit rot indexes were 2.95 and 1.28%, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that fruits those were sprayed with methyl jasmonate @ 5 mM (T_6), methyl jasmonate @ 4 mM (T_5), salicylic acid @ 12 mM (T_3) and salicylic acid @ 8 mM (T_2) showed zero index of fruit rot when analysed 30 days after storage, respectively and these were at par with each other. Whereas, higher index of fruit rot (13.66%) was noted in the control fruits (T_0) when analysed 90 days after storage.

Fruit weight loss

The analysed data presented in Figure 4 showed significant differences at $P \leq 0.05$ regarding the effects of treatments (SA and MeJA), storage periods and their interaction. Fruits those were sprayed with salicylic acid @ 12 mM (T_3), methyl jasmonate @ 5 mM (T_6) and salicylic acid @ 8 mM (T_2) showed lower losses in weights of 2.22, 2.66 and 2.77%, respectively and these were at par with each other. While higher loss in weight (11.00%) was noted in the fruits those were untreated (T_0). The fruits those were analysed 90 days after storage showed higher loss in weight of 6.28% than the fruits those were analysed 60 and 30 days after storage where losses in weights were 4.33 and 2.80%, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that lower losses in weights (1.33, 1.66, 1.66, 2.00 and 2.00%) were recorded in the fruits of T_3 (salicylic acid @ 12 mM), T6 (methyl jasmonate @ 5 mM), T_2 (salicylic acid @ 8 mM), T_3 (salicylic acid @ 12 mM) and T_5 (methyl jasmonate @ 4 mM) when analysed 30, 30, 30, 60 and 30 days after storage, respectively and these were at par with each other. Whereas, fruits those were untreated (T_0) showed higher loss in weight of 15.33% when analysed 90 days after storage.

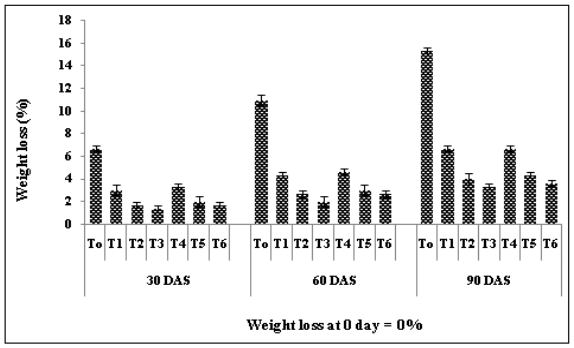

Fig. 4. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on weight loss (%) during storage (8°C) in the fruits of Ray Ruby.

T_o=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Total phenolic contents

Total phenolic contents (TPC) showed significant differences P≤0.05) regarding the effects of treatments (SA and MeJA) and storage periods while their interaction was found non-significant (Figure 5). The fruits those were sprayed with salicylic acid @ 12 mM (T_3) showed higher total phenolic contents of 166.29 mg GAE/100 g as compared to the fruits of all other treatments. While lower total phenolic contents of 138.76 mg GAE/100 g were recorded in the control fruits (T_o). The fruits those were analysed 30 days after storage showed higher total phenolic contents (161.51 mg GAE/100 g) than the fruits those were analysed 60 and 90 days after storage where total phenolic contents were 153.00 and 147.25 mg GAE/100 g, respectively.

Total antioxidants activities

Statistically significant differences (P≤0.05) were found regarding the effects of treatments (SA and MeJA), storage periods and their interaction on total antioxidants activities in the fruits of Ray Ruby (Figure 6). Higher total antioxidants activities (72.63 and 71.37%) were recorded in the fruits of T_3 (salicylic acid @ 12 mM) and T_6 (methyl jasmonate @ 5 mM), respectively and these were at par with each other. While lower antioxidants activities of 52.02% were noted in the fruits those were untreated (T_o). The fruits those were analysed 30 days after storage showed higher total antioxidants activities (71.21%) as compared to the fruits those were analysed 60 and 90 days after storage where total antioxidants activities were 63.96 and 57.36%, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that higher antioxidants activities of 78.33 and 77.39% were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T_3) and methyl jasmonate @ 5 mM (T_6) when analysed 30 days after storage, respectively and these were at par with each other. Whereas, lower antioxidants activities of 39.69% were noted in the control fruits (T_o) when analysed 90 days after storage.

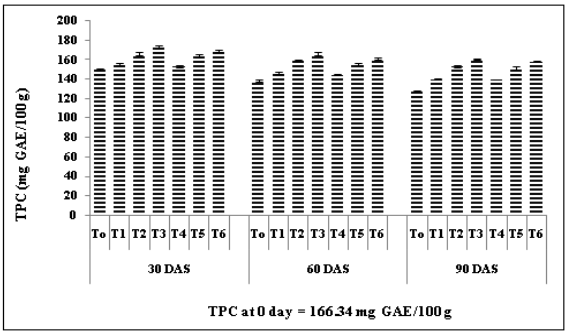

Fig. 5. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on total phenolic contents (mg GAE/100 g) during storage (8°C) in the fruits of Ray Ruby.

T_o=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Total flavonoids contents

The analysed data presented in Figure 7 showed statistically significant differences (P≤0.05) regarding the effects of treatments (SA and MeJA), storage periods and their interaction on total flavonoids contents (TFC) in the fruits. Higher amounts of TFC (55.74 and 53.43 mg CEQ/100 g) were recorded in the fruits of T_3 (salicylic acid @ 12 mM) and T_6 (methyl jasmonate @ 5 mM), respectively as compared to the

fruits of other treatments. While lower TFC of 38.68 mg CEQ/100 g were noted in the fruits those were untreated (T_0). The fruits those were analysed 30 days after storage showed higher total TFC (54.81 mg CEQ/100 g) than the fruits those were analysed 60 and 90 days after storage where total TFC values were 48.28 and 42.23 mg CEQ/100 g, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that higher amounts of TFC (60.55 and 58.50 mg CEQ/100 g) were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T_3) and methyl jasmonate @ 5 mM (T_6) when analysed 30 days after storage, respectively. While, lower amounts of TFC (29.39 mg CEQ/100 g) were noted in the control fruits (T_0) when analysed 90 days after storage.

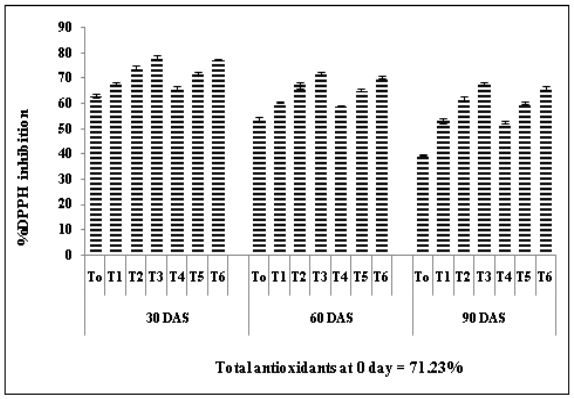

Fig. 6. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on total antioxidants (%DPPH inhibition) during storage (8°C) in the fruits of Ray Ruby.

T_0=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Total carotenoids contents

Statistically significant differences ($P \leq 0.05$) were found regarding the effects of treatments (SA and MeJA), storage periods and their interaction on total carotenoids contents in the fruits of Ray Ruby (Figure 8). Higher amounts of total carotenoids contents of 16.40% and 16.32 mg/100 g were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T_3) and methyl jasmonate @ 5 mM (T_6), respectively and these were at par with the fruits of T_2 (salicylic acid @ 8 mM) and T5 (methyl jasmonate @ 4 mM). While lower amounts of total carotenoids (12.14 mg/100 g) were noted in the fruits those were untreated (T_0). The fruits those were analysed 30 days after storage showed higher total carotenoids contents (16.40 mg/100 g) as compared to the fruits those were analysed 60 and 90 days after storage where total carotenoids contents were 15.48 and 14.63 mg/100, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that higher total carotenoids contents of 16.75 and 16.68 mg/100 g were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T3) and methyl jasmonate @ 5 mM (T_6) when analysed 30 days after storage, respectively and these were at par with the fruits of T_2 (salicylic acid @ 8 mM), T_5 (methyl jasmonate @ 4 mM) and T_3 (salicylic acid @ 12 mM) when analysed 30 and 60 days after storage, respectively. Whereas, lower total carotenoids contents (8.77 mg/100 g) were noted in the control fruits (T_0) when analysed 90 days after storage.

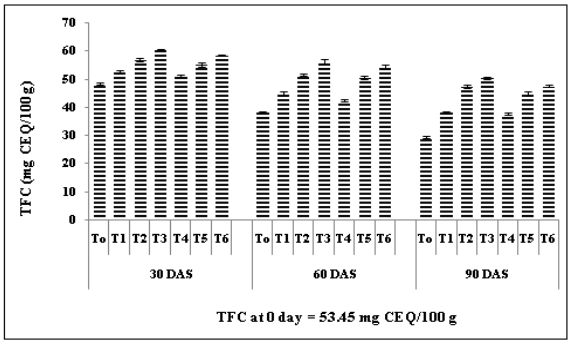

Fig. 7. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on total flavonoids contents (mg CEQ/100 g) during storage (8°C) in the fruits of Ray Ruby.

T_0=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Total limonin contents

Total limonin contents showed statistically significant

differences (P≤0.05) regarding the effects of treatments (SA and MeJA), storage periods and their interaction in the fruits of Ray Ruby (Figure 9). Lower amounts of total limonin contents (11.95 and 12.04 µg/mL) were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T_3) and methyl jasmonate @ 5 mM (T6), respectively. While higher amounts of total limonin contents (14.34 µg/mL) were noted in the fruits those were untreated (T_o). The fruits those were analysed 30 days after storage showed higher amounts total limonin contents (14.11 µg/mL) as compared to the fruits those were analysed 60 and 90 days after storage where total limonin contents were 12.80 and 11.48 µg/mL, respectively. The interaction between treatments (SA and MeJA) and storage periods showed that lower total limonin contents of 10.07 and 10.15 µg/mL were recorded in the fruits those were sprayed with salicylic acid @ 12 mM (T3) and methyl jasmonate @ 5 mM (T_6) when analysed 90 days after storage, respectively and these were at par with each other. Whereas, higher amounts of total limonin contents (14.51 and 14.38 µg/mL) were noted in the fruits of T_o (control) and T_4 (methyl jasmonate @ 3 mM) and when analysed 30 days after storage, respectively and these were at par with each other (Figure 4.182a).

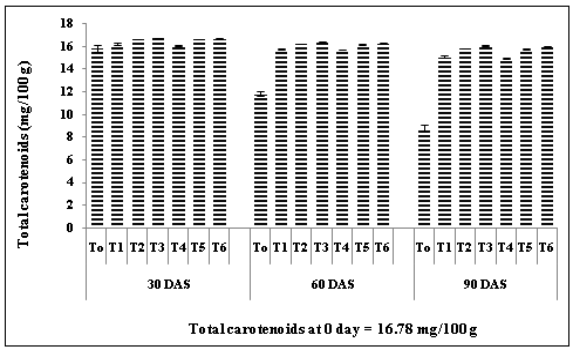

Fig. 8. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on total carotenoids contents (mg/100 g) during storage (8°C) in the fruits of Ray Ruby.

T_o=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

Discussion

The signalling molecules like salicylic acid (SA) and methyl jasmonate (MeJA) are endogenous plant growth substances which can play a key role in plant growth and development, and responses to environmental stresses. Salicylic acid is cosmically spread in plant kingdom (Raskin *et al.* 1990) and is included in plant hormones group (Raskin, 1992). Fruit texture is mainly attributed to cell wall integrity and stored carbohydrates such as pectin, starch etc. SA and MeJA maintained fruit textural score after 90 days of storage which may be due to decreased breakdown of insoluble pectin substances. Weichmann,(1987) similarly stated that the activity of pectin enzymes (esterase and polygalacturonidase) is involved in the breakdown of insoluble pectin forms to soluble. Untreated fruit showed more reduction of textural properties due to decrease in fruits texture depicts hydrolytic changes in the fruits resulting in exhaustion of sugar compounds and decrease in firmness (Malundo *et al.,* 2001; Abbasi *et al.,* 2010). Phenolics being secondary plant metabolites are synthesized by all plants. These are responsible for the flavor and color of fruit products (Jeong *et al.,* 1993). Robert *et al.,* (2003) stated that phenolics are involved in several functions such as nutrient absorption in plants, protein synthesis, enzymatic activities and photosynthesis. Many phenolic compounds act as antioxidants, but in higher quantity they become browning substrates. PPO and reactive oxygen species function as main oxidants during phenolics function, as substrates and antioxidants (Robarts *et al.,* 2011). They exist generally as flavonols in fruit peel (Hamauzu, 2006). The fruits treated with SA @ 12 mM and MeJA @ 5mM showed minimum reduction in phenolic compound during the storage (maintained) as compared to the fruits those were treated with lower concentrations and without treatments (control). Untreated fruit showed more reduction of TPC which might be due to reduction of different enzymes and reducing electronic transfer- based antioxidant which might have reduced many TPC during storage. The loss of phenolic compounds during storage can be associated

with several enzymatic and non-enzymatic reactions, ethylene production being superior (McDonald,1998). Similar findings have also been described by Huang *et al.,* (2008) who reported that that SA treated 'Cara cara' navel oranges showed increased total phenolic content, higher concentration of SA having more profound effect in this respect. There is also evident that exogenously applied SA with suitable dose enhanced the efficiency of antioxidant system in plants (Hayat *et al.,* 2007). Zeng *et al.,* (2008) also reported that salicylic acid treatment significantly enhanced phenylalanine (PAL, peroxidase POD and -1,3-glucanase activity in grape berries which may help them to protect themselves against chilling stress during storage. Antioxidants are compounds capable of quenching ROS without undergoing conversion, themselves, to destructive radicals (Hodges, 2003). To ascertain dietary importance of fruits and vegetables it is also important to estimate their antioxidant activity. Higher concentration of both chemicals maintained TAC during storage of both cultivators which might be due to counteracted balancing between increased free radicals and increased FRSA. Both chemicals being TAC, regulates some of fruit defense systems and nutrition components biosynthesis including antioxidant activity (Huang *et al.,* 2008). Untreated fruit reduced TAC which might be due to imbalance electronics structure and more leakage of cell membrane. Results of present study are supported by the previous findings of various scientists (Lu, 2002). The other reason for lower reduction in TC and TF contents during storage by the application of higher doses of both chemicals might be due to decreased respiration which prevents fruit senescence during storage. It seems that these compounds prevent enzymatic activities which have a role in anthocyanin synthesis by slowing down ripening process. It is well documented that MeJA affects many physiological events including coloration in fruit (Rohwer & Erwin, 2008). Higher doses SA @ 12 mM and MeJA @ 5 mM showed better results to maintain phytochemical properties in compounds after storage. Maximum TL were found with application of higher doses of SA@ 12mM and MeJA @ 5 mM which may be attributed to fruit bitterness, because under low pH conditions, the A-ring lactone (LARL) can be converted to limonin. It previous research it is reported that higher doses maintain this convection (Rolle & Chism, 1987). Untreated fruits showed increased emzyamtic changes as compare to those fruit that were treated with SA and MeJA. This is temporary because, at a more advanced stage of oxidation, the molecules gradually lose this property, and there is a drastic reduction in TL. These findings are in accordance with the findings of some previous researchers (Goodner *et al.,* 2001; Fan *et al.,* 2005).

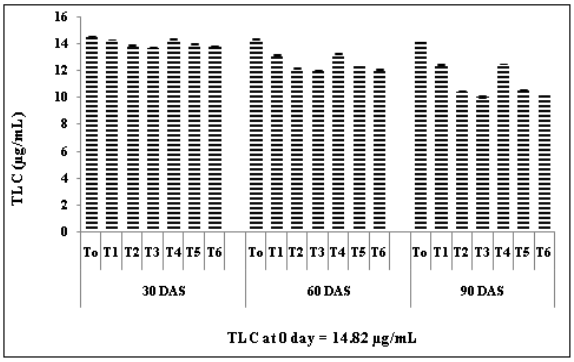

Fig. 9. Effects of pre-harvest spray of salicylic acid (SA) and methyl jasmonate (MeJA) on total limonin contents (µg/mL) during storage (8°C) in the fruits of Ray Ruby.

To=control, T_1=salicylic acid @ 6 mM, T_2=salicylic acid @ 8 mM, T_3=salicylic acid @ 12 mM, T_4=methyl jasmonate @ 3 mM, T_5=methyl jasmonate @ 4 mM, T_6=methyl jasmonate @ 5 mM (DAS=days after storage).

Each vertical bar represents mean of three replicates ± S.E.

The fruits treatments with SA @ of 12 mM and MeJA @ of 5 mM effectively controlled the chilling injury development in grapefruit, as estimated by CI symptoms percentage after 30, 60 and 90 days storage, but very little symptoms (0.33 and 0.33%) were noted after 90 days. While, controlled fruits showed 2.66 and 2.33% chilling injury after 90 days storage. It can be concluded that treated fruits might be build up some defensive system due to less loss of antioxidants against the chilling injury therefore these remained safe from the chilling injury. It is because oxidative stress caused by the accumulation of reactive oxygen species (ROS) together with a

reduction in the anti-oxidant system are involved in CI development in fruits during storage Similar results were also reported by Wang *et al.,* (2006) and Cao *et al.,* 2009 of those worked on peaches and pomegranates respectively and found that higher concentrations of SA in the range from 0.35 to 12.0 mM were more effective to control the chilling injury than lower ones. The effect of SA and Me JA against CI in grapefruit was attributed to its ability to develop antioxidant systems and heat shock protein (HSPs) that minimized the Cl symptom during storage (Wang *et al.*, 2006). It is also reported that higher doses develop expression of a set of defense genes that protect the fruit against the CI (Fung *et al.*, 2004; Cao *et al.*, 2009). More chilling injury symptoms in untreated fruits could also be due to the leakage of cell membrane and loss of integrity of membrane with imbalance of electron due to more ethylene production during storage. Many researchers also reported the similar findings (Martine *et al.* 2004; Zhou *et al.,* 2002; Lurie & Crisosto, 2005). No fungal decay was visually observed on treated fruits after 30 to 60 days of storage but minimum decay was noted after 90 days storage on fruits those were treated with lowers doses. However, SA @ 12 mM and MeJA @ 5 mM concentrations proved to be the most effective to reduce fungal decay at the end of storage time which may be due to better developed defense system (Jiankanget *et al.,* 2006) or working of these chemicals as anti-senescent effects (Asghari & Aghdam, 2010). In earlier studies, SA treatment prevented the decay in peaches (Wang *et al.*, 2006), strawberry (Babalar *et al.,* 2007) and grape (Ranjbaran *et al.,* 2011). Moreover it is also reported that SA treatment strengthens defense system through enhancing activities of antioxidant enzymes that improve resistant in treated fruit against the fungal attack (Xu & Tian ,2008).

Fruit weight losses are known as most significant physiological disorder during postharvest life. Low fruit losses during storage in treated fruit also singled the superiority of SA MeJA by reducing the respiration, transpiration and metabolic activities of fruit and these three activities are directly related with fruit weight loss during storage.Treatment of SA and MeJA cause the hindrance in respiration by generating free radicals (Wolucka *et al.,* 2005) by closing stomata (Manthe *et al.,* 1992; Zheng & Zhang, 2004) and slowing down respiration which may have ultimately reduced the weight loss of fruit. The finding of Shafiee *et al.,* (2010) also shown that strawberry fruits showed less fruit weight loss than control when salicylic acid was supplied accomplished with nutrients.

Conclusions

In conclusion, our study showed that Grapefruit juice is rich source of different phytochemical which play a vital role for health improvement substance. In this study it proves that before harvesting MeJA and SA application significantly maintained these compounds during storage. SA (@12 mM), MeJA (@4 mM) are best dozes to control the Fruit loss, chilling injury, fruit rot and to improve the phytochemicals under storage. Grower should used pre harvest spray of SA (12mM) and MeJA (4mM) to improve the shelf life and quality of grape fruit

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