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Pre-harvest methyl jasmonate spray maintains postharvest quality of Kinnow mandarin (*Citrus reticulata*) fruits

K S DHAMI[1], RAM ASREY[1]*, R R SHARMA[1], V R SAGAR[1], SUKANTA DASH[2] and A K DUBEY[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

The study was carried out at the experimental orchard of Indian Agricultural Research Institute, New Delhi (2019–20) to evaluate the pre-harvest effect of methyl jasmonate (MeJA) on postharvest quality of Kinnow mandarin (*Citrus reticulata* Blanco) under low temperature storage. Fruits were sprayed on tree with four concentrations of MeJA (0.1 mM, 0.3 mM, 0.5 mM, 0.7 mM) and control (distilled water) at 40 days and 20 days before commercial harvesting. The harvested fruits were stored consecutively at 2ºC for 20 days followed by at 6ºC for 20 days with 85–95% relative humidity. All the treatments showed significant results than control, but fruits treated with 0.5 mM MeJA retained higher moisture content (least water loss) and firmness with increase in TSS, acidity, total phenols, ascorbic acid, antioxidant and peroxidase enzyme activity during the storage period of 40 days. Therefore, pre-harvest spray @ 0.5mM MeJA could be an effective alternative to synthetic chemical use in maintaining the quality of Kinnow mandarin fruits during low temperature storage.

Keywords: Low temperature storage, Methyl jasmonate, Pre-harvest spray, Quality

The characteristic nature of Kinnow mandarin (*Citrus reticulata* Blanco) such as deep golden color, tight skin, delicious taste and distinct aroma with higher juice percent and dietary fiber have made it one of most popular fruit among citrus group (Asrey and Barman 2020). Also, it is an important source of natural antioxidants and phytochemicals, particularly flavonoids, phenolic and ascorbic acid with essential minerals**.** It is now commercially grown in Punjab, Rajasthan, Haryana, Himachal Pradesh, Uttarakhand, Jammu and some parts of Uttar Pradesh. Mandarin fruits can be stored for long period up to 5-7 weeks under low temperature condition (Luengwilai *et al.* 2007) to reduce market glut and for extended marketing period. However, the antioxidant and nutrients content of the crop deteriorates during storage mainly due to different biotic and abiotic stresses such as pathogens and chilling injury causing loss of nutritional value and quality. Suitable postharvest technologies are required to reduce the adverse effects of biotic and abiotic stresses in order to maintain nutritional quality and storage life of kinnow. Use of synthetic chemicals in postharvest management of horticultural crops is being discouraged now because of the food safety issues and environmental concerns (Asghari and Hasanlooe 2015). Inducing defense mechanism and enhancing natural resistance in fruit itself for extending the storage life of harvested produce has now been considered as an emerging alternate strategy to chemicals (Terry and Joyce 2004).

Therefore, the application of MeJA can be very effective approach as it is a signaling molecule which mediates diverse developmental processes and shows defense responses against biotic and abiotic stresses (Cheong and Choi 2003). It responds actively to stress conditions modulating various plant defense systems including antioxidant system (Wasternack *et al.* 2013). Since, the postharvest stage has remained the main research focus of MeJA applications on fruits however, fruits are more receptive to agrochemical applications at pre-harvest stage (Reyes-Díaz *et al.* 2016), which have been little studied in case of Kinnow mandarin. Therefore, the aim of this study was to ascertain the pre-harvest spray effects of MeJA on post harvest quality of Kinnow mandarin under different low temperature storage conditions.

MATERIALS AND METHODS

Healthy Kinnow mandarin trees were selected in the experimental orchard of Indian Agricultural Research Institute, New Delhi during 2019–20. The fruits were sprayed with the different concentrations of MeJA; T_1: MeJA(0.1 mM) T_2: MeJA(0.3 mM) T_3: MeJA(0.5 mM), T_4: MeJA(0.7 mM) and T_5: control (distilled water spray) at two different time intervals, i.e. last week of December (1st spray) or 40 days before commercial harvesting and 2nd

Present address: [1]ICAR-Indian Agricultural Research Institute, New Delhi; [2]ICAR-Indian Agricultural Statistics Research Institute, New Delhi. *Corresponding author e-mail: ramu_211@yahoo.com.

week of January (2nd spray) or 20 days before commercial harvesting. Tween 20 @ 0.5% was used as a surfactant. The treated fruits were then harvested at commercial maturity (TSS ≈12º brix). The harvested fruits were sorted for uniform shape and free from physical injuries and then stored consecutively at two different temperatures of 2 and 6ºC with 85–95% relative humidity for 40 days. Fruits were retained at 2ºC for 20 days followed by storage exposure at 6ºC for another 20 days. The stored fruits were then finally analysed for following parameters at every 10 days interval period upto 40 days. There were 40 fruits in each treatment and replicated thrice.

Physiological loss in weight (PLW) was determined by weighing the fruits at different intervals which were calculated as the difference between the initial weight and the final weight at the time of measurement and expressed as the percentage of initial fruit weight. Fruit firmness was determined by texture analyzer (model: TA+Di, Stable Micro Systems, UK) using 49 N load cell, 36mm diameter cylinder probe with test speed-2mm/s, and distance-10 mm. Hardness was defined as maximum force during the compression, which was expressed in Newton (N). Total soluble solid was analyzed by putting few drops of juice over the prism of the hand refractometer (Fisher 0-50, Japan) and the reading was recorded in ºbrix. Titratable acidity was determined by titrating the juice with 0.1 N NaOH using a few drops of 1% phenolphthalein solution as indicator and finally acidity was expressed in percentage (%). The total phenol content was determined using Folin-Ciocalteu reagent (Singleton *et al.* 1999) with the help of spectrophotometer. The 0.1 ml sample extract (in 80% ethanol) was mixed with 0.5 ml Folin-Ciocalteu reagent, 2.0 ml Na_2CO_3 solution and 2.9 ml distilled water. Finally, the absorbance of the mixture was taken at 760 nm after 90 min where the mixture without sample was taken as blank. The total phenolics content was expressed in gallic acid equivalent (µgGAE g/FW).

Antioxidant activity of fruit sample was determined on the basis of its radical scavenging effect on the DPPH free radical which was measured at 517 nm wavelength using a UV-Vis spectrophotometer. Inhibition of free radical by DPPH in percent (%) was calculated by following formula:

Inhibition ratio (%) = {(Ac – As)/Ac} × 100; where, Ac = OD of DPPH, As = OD of sample

$$\text{DPPH (µmolTrolox/g)} = \frac{\text{\% Inhibition} \times \text{Volume made up} \times \text{Dilution factor}}{0.061 \times 1000 \times \text{Weight of sample (g)}}$$

Ascorbic acid content was estimated by the standard procedure (Ranganna 1999) using % metaphosphoric acid (HPO_3), ascorbic acid standard and standardized dye of the sodium salt of 2, 6-dichlorophenol-indophenol. Calculation of ascorbic acid content in the sample was done as;

$$\text{mg of ascorbic acid/ 100g} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Vol. made up} \times 100}{\text{Aliquot of extract} \times \text{Volume of sample taken}}$$

Peroxidase (POD) enzyme activity was measured following the method described by Zhang *et al.* (2018) using guaiacol as the substrate with some modifications. The reaction mixture contained 220 µL 0.3% (v/v) guaiacol, 60 µL 0.3% (v/v) H_2O_2 and 20 µL crude enzyme extract. The reaction was initiated immediately by adding H_2O_2, incubated in a water bath at 30 ºC, and the reaction allowed to proceed for 5 min while A_{470} was measured at every 30s. One unit (U) of POD activity was defined as the amount of enzyme extract producing an increase of A_{470} by 0.01 in 1 min and expressed as U/min/ g of fresh weight.

All the statistical analysis has been done through repeated measurement analysis in a factorial set up using SAS 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

MeJA works by increasing the antioxidant activity and related compounds within the fruits in order to minimize deteriorative process in the fruits. Also, it has been found that MeJA induces the enzymes activity responsible for cell wall softening in fruits leading to improved firmness, thus preventing from mechanical damage and microbial attack indirectly (Bari and Jones 2008). Pre-harvest application of MeJA significantly affected the postharvest quality parameters of the Kinnow mandarin fruits during cold storage. However, better response was found with 0.5mM concentration of MeJA which could be selected for practical application where higher concentrations may counteract the positive effect on fruit quality by promoting ripening and senescence of the fruit as reported in peaches by Jin *et al.* (2009).

Physiological loss in weight (PLW): All the fruits treated with MeJA retained significantly higher moisture with concentration of 0.5 mM having lowest (6.75%) moisture loss than control (8.40%) on the 40th day of storage. Higher moisture retention in the treated fruits may be due to higher firmness and lower metabolic activities. Also, Wolucka *et al.* (2005) reported that MeJA causes hindrance in respiration by generating free radicals leading to closed stomata thus slowing down respiration ultimately leading to reduced weight loss of fruit.

Fruit firmness: The MeJA treatments significantly affected the fruit quality during the 40 days of storage period by maintaining higher firmness (Table 1). Higher firmness in MeJA treated fruits may be due ripening-retarding effect of MeJA and higher moisture retention of fruits together. The similar effect of MeJA was reported by Soto *et al.* (2010) in peaches and Saracoglu *et al.* (2017) in sweet cherry.

Ascorbic acid content: All the treatments showed significant increase in ascorbic acid content in the fruits where as control fruits had exhibited decrease amount of ascorbic acid during storage period (Table 1). The 0.5 mM concentration had higher level of average ascorbic acid (27.04 mg/100 g) over control fruits (19.44 mg/100 g) at the last day of storage. Reports through experiments on plant cell mentioned that MeJA can enhance the transcription of

Table 1 PLW, firmness and ascorbic acid of kinnow fruits as affected with pre-harvest spray of MeJA and stored at 2°C followed by 6°C for 40 days

Avg. values treatment	Storage days														
	PLW (%)					Firmness (N)					Ascorbic acid (mg/100g)				
	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
0.1mM	0	1.63	3.30	5.53	7.63	6.85	6.77	6.62	6.49	6.17	21.54	23.22	24.30	25.18	25.55
0.3mM	0	1.48	3.20	5.30	7.40	6.90	6.81	6.69	6.60	6.31	21.59	23.36	24.56	25.36	26.23
0.5mM	0	1.28	2.80	4.95	6.75	7.03	6.93	6.84	6.75	6.56	21.67	23.58	25.05	25.87	27.04
0.7mM	0	1.38	3.00	5.15	7.05	6.95	6.84	6.74	6.64	6.45	21.66	23.48	24.92	25.71	26.53
Control	0	1.80	3.95	6.30	8.40	6.68	6.57	6.42	5.73	5.30	20.88	20.33	20.09	19.95	19.44
LSD (P=0.05),	T = <.0001,					T = <.0001,					T = <.0001,				
T=treatment	D = <.0001,					D = <.0001,					D = <.0001,				
D= day	T × D = <.0001					T × D = <.0001					T × D = <.0001				

genes involved in the *de novo* biosynthesis of ascorbic acid (Wolucka *et al.* 2005) in fruits. This finding is consistent with previous studies on loquat fruit (Cai *et al.* 2011), mango (Muengkaew *et al.* 2016) and blueberry (Wang *et al.* 2019).

TSS, acidity and total phenols: MeJA application at 0.5mM concentration showed higher TSS (13.20 °B), acidity (1.69%) and total phenols (391 mg/100 g) in the fruits as compared to untreated (control) fruits during the storage period of 40 days (Table 2). Higher TSS and acidity in the treated fruits may be due to lower metabolic activities and progression of simultaneous senescence process in the fruits. Increase in total phenols in treated fruits may be due to activation of secondary metabolic pathways leading to accumulation of phenolic compounds in the fruits (De Geyter *et al.* 2012). Also, application of MeJA has been found to increase phenolic content in strawberries (De la Pena *et al.* 2010) and in grapes (Gema and Ruiz del Castillo 2014) during storage.

Antioxidant activity: The increasing trend of antioxidant activity was found in the treated fruits, whereas decreasing trend was seen in the control fruits. MeJA at 0.5 mM had higher antioxidant activity in the fruits compared to all other treatments (Fig 1). Application of MeJA enhances antioxidant activity by acting on the different parts of antioxidant system in a different manner (Asghari and Hasanlooe 2015). Similar results for increase in antioxidant activity through MeJA application were also reported in peaches (Jin *et al.* 2009), loquat (Cao *et al.* 2008) and plum fruits (Zapata *et al.* 2014).

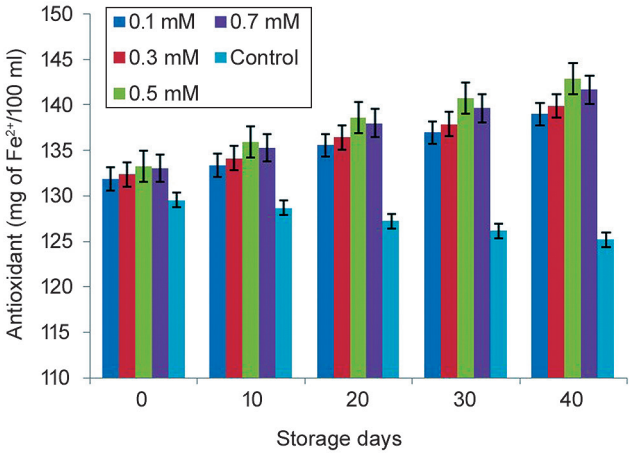

Fig 1 Antioxidant activity (mg of Fe^{2+}/100 ml) of kinnow fruits pre-harvest sprayed with MeJA and stored at 2°C followed by 6°C for 40 days (standard error= ±0.33).

Table 2 TSS, acidity and total phenols of kinnow fruits as affected with pre-harvest spray of MeJA and stored at 2 °C followed by 6°C for 40 days

Avg. values treatment	Storage days														
	TSS (°B)					Acidity (%)					Total phenols (µgGAE g/FW)				
	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
0.1mM	11.20	11.60	12.10	12.30	12.55	1.23	1.35	1.40	1.45	1.48	304.0	314.75	326.75	333.00	344.00
0.3mM	11.25	11.90	12.25	12.43	12.80	1.25	1.40	1.44	1.50	1.52	312.0	326.75	338.75	347.25	360.50
0.5mM	11.50	12.33	12.68	12.95	13.20	1.43	1.54	1.61	1.65	1.69	326.0	346.25	363.25	374.25	391.00
0.7mM	11.30	12.13	12.45	12.75	12.90	1.38	1.48	1.56	1.62	1.65	319.0	339.50	354.75	364.25	379.25
Control	11.00	11.55	11.83	11.60	10.88	1.10	1.21	1.31	1.39	1.41	288.0	289.50	280.00	269.50	256.50
LSD (P=0.05),	T= <.0001,					T= <.0001,					T= <.0001,				
T=treatment	D= <.0001,					D= 0.0001,					D= <.0001,				
D= day	T × D= <.0001					T × D= 0.89					T × D= <.0001				

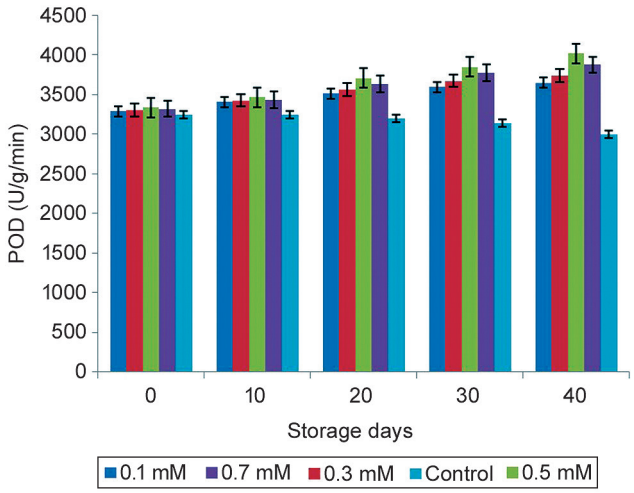

Fig 2 Peroxidase enzyme activity (U/g/min) of kinnow fruits pre-harvest sprayed with MeJA and stored at 2ºC followed by 6ºC for 40 days (standard error= ±0.52).

Peroxidase enzyme activity: POD is important antioxidant and defense enzymes that work together with other enzymes to scavenge reactive oxygen species (Passardi *et al.* 2004) reducing oxidative stress in the fruits (Fig 2). All MeJA treated fruits showed significant increase in POD activity, having highest (4017.5 U/g /min) in 0.5 mM, whereas control fruits showed a decrease (3000 U/g/min) in POD activity during the storage period. The increased POD activity is associated to enhancement of antioxidant capacity and disease resistance in the fruits (Tareen *et al.* 2012). Similar results were obtained in mandarin (Guo *et al.* 2014) and strawberry (Zuniga *et al.* 2020).

The present study indicated that pre-harvest application of MeJA could be a useful option to increase antioxidant and nutritional contents of Kinnow mandarin fruit during postharvest storage. MeJA at 0.5 mM concentration was most effective in maintaining higher moisture content, fruit firmness with increased ascorbic acid, total phenols and peroxidase enzyme activity, thus enhancing the quality and storability of the fruits. Therefore, pre-harvest spray of MeJA could be effective strategy for enhancing postharvest quality of kinnow mandarin fruits leading to extended storage life of the fruits under cold storage conditions.

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