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RESEARCH ARTICLE

Effect of preharvest applications of salicylic acid, CPPU, $CaCl_2$ and neemazal on storage behaviour of nectarine cv. Snow Queen

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ABSTRACT

The present study was conducted to assess the effect of preharvest application of growth regulators, nutrients and fungicides on storage behaviour of nectarine fruit. All the preharvest treatments showed a beneficial effect on physico-chemical, antioxidant and sensory parameter of fruits in comparison to control. Among the preharvest treatments, calcium chloride @ 1.5 per cent, Neemazal @0.45 per cent, salicylic acid @3000 ppm and CPPU @15 ppm proved to be most effective in maintaining fruit quality and minimizing deterioration during 28 days storage at 3 ±1 ºC. CPPU @15 ppm, was the most effective treatment in maintaing fruit size, weight and antioxidant compounds *viz*. ascorbic acid and total phenol content in fruits. However, $CaCl_2$ treatment @ 1.5 per cent proved to be the most effective in reducing physiological loss in weight and minimizing deterioration in fruit quality and in retaining maximum firmness during 28 days storage period.

Keywords Nectarine, Snow Queen, preharvest applications, salicylic acid, CPPU, $CaCl_2$, neemazal.

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INTRODUCTION

Nectarine (*Prunus persica* var*. nucipersica* Schneid.) is a smooth-skinned peach of the family Rosaceae. The word nectarine is derived from a Latin word which means 'Persian plum' although they are not plums. The origin of nectarine is a mystery. These has a smooth fruit in contrast to pubescent fruit of peach, was given a species status in the past, but Bailey (1913) considered only a botanical variety (*Prunus persica* var. *nucipersica*). Now-a-days the cultivation of nectarine gained popularity among stone fruit growers in the mid hill areas of Himachal Pradesh. The area under different cultivars of nectarines is expanding rapidly as they have a tendency to fetch better prices due to their earlier arrival in the market as they have a relatively shorter growth period. But, being a climacteric fruit with high ethylene evolution rates, nectarine undergoes rapid changes during ripening and softens immediately after harvest. Nectarines, like other stone fruits, therefore cannot endure long post-harvest handling periods at normal atmospheres. It is a delicate crop, well known for its poor shelf life. In order to avoid product wastage during transportation and marketing, growers tend to harvest their crop at a slightly immature stage but such fruits are hard for consumption and also lack flavour. Preharvest application of growth regulators, nutrients and fungicides can modify the pace and direction of biochemical changes in developing fruit and have the potential to transform its

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quality at harvest (Ghebreslassie, 2017). The quality of fruits at harvest is known to influence their postharvest behaviour. Growth regulators play a vital role in modulating the ripening processes by affecting changes in fruit firmness, brought about by changes in cellular events (Siddiqui, 2018)**.** N-(2-cholor-4-pyridyl)-N-phenyl urea (CPPU), a diphenylurea compound that has a strong cytokinin like activity affects fruit quality through crop load reduction and enhancement of cell elongation and cell division (Greene 2001). The application of plant nutrients like Ca in the form of calcium chloride has also been found to maintain the cellular integrity and firmness of the fruits during storage (Ochie et al. 1993). Fungicides also help in extension of storage life of perishables and fruits by reducing the spore load and hence reducing the incidence of various pathological disorders (Smith and Kiel 1972; Rinaldi et al., 2017), which also ensures an extended storage life of fruit. The present study was therefore carried out to improve fruit quality at harvest and retain it during storage by the application of various pre-harvest treatments. Effect of salicylic acid is first time reported on nectarine in this study.

MATERIALS AND METHODS

Study area

To study the effect of preharvest treatments on postharvest quality of nectarine cultivar Snow Queen was laid out in the experimental orchard of Horticultural Research Station, Kandaghat, India. Well grown uniform trees were selected which were maintained under a standardized schedule of cultural operations throughout the season and subjected to preharvest treatments of various chemicals like CPPU (N-(2-Chloro-4-pyridyl)-N-phenyl urea) (@5, 10 & 15 ppm) and salicylic acid (@ 1000, 2000 & 3000 ppm) applied at pea stage while Neemazal (@ 0.15, 0.30 & 0.45 %) and calcium chloride (@ 0.5, 1.0 & 1.5 %) applied 15 days before the expected date of harvest of fruit.

Physico-chemical analysis

Procedures adopted for evaluating quality by measuring physical parameters such as fruit size, weight, specific gravity as per method prescribed by Ranganna (1986). Physiological Loss in Weight (PLW) was estimated by pre-weighing the fruit samples on a physical balance after each storage interval. The loss in weight at each interval during storage was expressed as per cent of initial weight. While, fruit firmness was measured with a portable Effigi penetrometer (FT-327) which recorded the pressure required to force a plunger of 8 millimeters (mm) diameter into the flesh of the fruit samples. Two readings were recorded on opposite sides of each fruit and their mean was calculated and results expressed in kg/cm^2.

Antioxidant analysis

Changes in antioxidant attributes viz. ascorbic acid content was determined as per AOAC (2000) method using 2, 6-dichlorophenol-indophenol dye and total carotenoids were estimated by macerating a known weight of the sample with acetone and extracting it in petroleum against 3 per cent acetone in petroleum ether as blank at 452 nm (Ranganna 1986).

Total phenols were extracted in 80 per cent ethanol and estimated on the basis of their reaction with an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent under alkaline conditions (Bray and Thorpe, 1954). The developed blue colour was measured at 650 nm on a UV-VIS spectrophotometer (Shimadzu, Japan).

For total carotenoids, a known weight of the sample was macerated with acetone in a pestle and mortar and the extract was decanted into a conical flask. Extraction was continued till the residue became colourless. All extracts were combined and transferred into a separating funnel. 10-15 ml of petroleum ether and 5 ml of 5 per cent sodium sulphate solutions were added to transfer the pigments into petroleum ether. Again petroleum ether was added to transfer all colour into it and then separated out from separating funnel into 50 mL volumetric flask and volume was made up to 25 mL by petroleum ether. The colour

intensity (optical density) was measured on a UV-VIS spectrophotometer (Shimadzu, Japan) at 452 nm using 3 per cent acetone in petroleum ether as blank. The results were expressed in terms of total carotene as mg/100 g of the sample (Ranganna, 1986).

Statistical analysis

Statistical analysis of the data was conducted using the online software OPSTAT and data analysed for Randomized Block Design (RBD) with three replications (Mahony 1985)

Physico-chemical characteristics

Fruit length, diameter, weight and volume of nectarine fruit cv. Snow Queen increased significantly with the pre-harvest application of growth regulators, nutrients and fungicides (Table 1). Fruit length and diameter generally affected to the greatest extent by 15 ppm CPPU (T_3), followed by 10 ppm CPPU (T_2) and 5 ppm CPPU (T_1) treatments, respectively, while treatment with 15 ppm CPPU (T_3) resulted in maximum fruit weight and volume and followed by T_4, T_2 and T_5, respectively. The physiological loss in weight (PLW) of fruits during storage increased gradually under all treatments, although all the preharvest treatments were effective in reducing PLW to varying extents as compared to the control (Figure 1). Calcium chloride @ 1.5 per cent (T_9) proved to be the most effective treatment in reducing PLW where the mean PLW was only 9.35 per cent. Fruit firmness (kg/cm^2) during storage showed a steady decrease with a progressive advancement in storage durations (Figure 2). The firmest fruits at harvest were obtained from trees receiving preharvest application of calcium chloride @ 1.5 per cent (T_9). Beside calcium chloride treatment, Neemazal and salicylic acid also showed better results in improving fruit firmness at harvest and retaining it during storage.

Table 1: Effect of pre-harvest treatments on fruit length (mm), fruit diameter (mm), fruit weight (g) and volume (cc) of Snow Queen nectarine fruit at harvest

Treatment (T)	Fruit length	Fruit diameter	Fruit weight	Fruit Volume
T_1 (CPPU 5 ppm)	49.08	52.12	82.99	78.89
T_2 (CPPU 10 ppm)	49.16	52.54	87.37	83.99
T_3 (CPPU 15 ppm)	49.22	53.00	91.81	88.33
T_4 (Salicylic acid 1000ppm)	48.99	51.07	88.90	78.96
T_5 (Salicylic acid 2000ppm)	48.95	50.82	85.81	75.75
T_6(Salicylic acid 3000ppm)	48.91	50.42	81.15	72.93
T_7 ($CaCl_2$ 0.5%)	48.86	50.14	81.78	87.82
T_8 ($CaCl_2$ 1.0%)	48.81	50.06	78.95	83.98
T_9 ($CaCl_2$ 1.5%)	48.76	49.94	77.03	79.37
T_{10} (Neemazal 0.15%)	49.03	51.01	83.66	82.47
T_{11} (Neemazal 0.30%)	48.99	50.93	81.19	80.09
T_{12} (Neemazal 0.45%)	48.94	50.80	77.97	76.25
T_{13} (Control)	48.74	49.50	70.12	67.39
CD $_{0.05}$	0.04	0.11	0.25	0.16

Table 2. Effect of pre-harvest treatments on ascorbic acid (mg/100g), total carotenoids (mg/100g) and total phenols (mg/100g) content of Snow Queen nectarine fruit during storage at 3±1ºC

Treatment (T)	Ascorbic acid Storage intervals in days						Total carotenoids Storage intervals in days						Total phenols Storage intervals in days					
	0	7	14	21	28	Mean	0	7	14	21	28	Mean	0	7	14	21	28	Mean
T_1 (CPPU 5 ppm)	13.30	11.68	9.14	7.67	5.31	**9.42**	0.85	1.44	2.10	2.99	4.05	**2.28**	33.3	26.6	20.4	16.3	13.6	**22.01**
T_2 (CPPU 10 ppm)	13.33	11.74	9.17	7.69	5.33	**9.45**	0.88	1.47	2.14	3.02	4.08	**2.31**	33.7	27.2	20.9	16.8	14.0	**22.51**
T_3 (CPPU 15 ppm)	13.38	11.76	9.22	7.73	5.37	**9.49**	0.89	1.50	2.18	3.05	4.10	**2.34**	34.4	27.9	21.6	17.4	14.4	**23.13**
T_4 (Salicylic acid 1000ppm)	13.25	11.64	9.08	7.62	5.32	**9.38**	0.80	1.38	2.04	2.96	3.98	**2.23**	33.8	26.9	20.6	16.5	13.7	**22.28**
T_5 (Salicylic acid 2000ppm)	13.31	11.72	9.14	7.69	5.38	**9.45**	0.82	1.42	2.06	2.98	4.01	**2.26**	34.2	27.6	21.3	17.0	14.1	**22.82**
T_6 (Salicylic acid 3000ppm)	13.29	11.68	9.10	7.64	5.35	**9.41**	0.76	1.35	2.00	2.94	3.97	**2.20**	33.6	26.5	20.2	16.1	13.3	**21.91**
T_7 ($CaCl_2$ 0.5%)	13.14	11.70	9.19	7.81	5.53	**9.47**	0.84	1.39	2.02	2.80	3.87	**2.18**	31.9	26.7	21.4	17.0	14.4	**22.27**
T_8 ($CaCl_2$ 1.0%)	13.17	11.72	9.24	7.85	5.56	**9.51**	0.82	1.37	1.98	2.78	3.84	**2.15**	32.5	27.2	22.0	17.5	14.9	**22.79**
T_9 ($CaCl_2$ 1.5%)	13.20	11.75	9.29	7.89	5.60	**9.55**	0.78	1.34	1.96	2.75	3.81	**2.13**	33.1	27.8	22.5	17.9	15.4	**23.31**
T_{10} (Neemazal 0.15%)	13.18	11.51	9.07	7.56	5.23	**9.31**	0.81	1.42	2.08	2.97	4.00	**2.26**	31.0	25.0	19.5	15.2	12.9	**20.72**
T_{11} (Neemazal 0.30%)	13.14	11.49	9.05	7.53	5.22	**9.29**	0.84	1.45	2.10	3.00	4.03	**2.28**	31.6	25.6	19.9	15.7	13.4	**21.21**
T_{12} (Neemazal 0.45%)	13.11	11.45	9.02	7.50	5.19	**9.25**	0.87	1.47	2.13	3.03	4.06	**2.31**	32.1	26.2	20.5	16.1	13.8	**21.71**
T_3 (Control)	12.87	11.33	8.91	7.34	5.04	**9.10**	0.89	1.55	2.19	3.16	4.21	**2.40**	30.4	24.4	19.0	14.3	11.9	**19.98**
Mean	**13.21**	**11.63**	**9.12**	**7.66**	**5.34**		**0.83**	**1.42**	**2.07**	**2.95**	**4.00**		**32.72**	**26.57**	**20.74**	**16.42**	**13.80**	
$CD_{0.05}$																		
						0.02						0.02						0.06
						0.01						0.01						0.10
						0.04						0.05						0.23

Antioxidants

A gradual decrease in the ascorbic acid content of the fruits was observed under all the treatments with advancement of storage period (Table 2). Fruits treated with $CaCl_2$ generally maintained higher ascorbic acid contents, with the mean ascorbic acid content being proportional to the concentration of $CaCl_2$ used. However, the lowest mean ascorbic acid content recorded in control fruits. Carotenoid content of fruits exhibited an increase during storage with the increase being higher in the control fruits which had the maximum mean carotenoid content (2.40 mg/100 g). On the other hand, fruits treated with calcium chloride @ 1.5 per cent had the lowest carotenoid content (2.13 mg/100 g) and was followed by treatments T_8, T_7 and T_6. A gradual decline in phenolic contents of nectarine fruits was observed under all treatments during the entire storage period of 28 days under refrigerated conditions. At harvest, the total phenol contents were considerably enhanced by various preharvest treatments in comparison to control. The maximum mean phenolic contents (23.31 mg/100 g) were recorded in fruits treated with $CaCl_2$ @ 1.5 per cent (T_9) and the control fruits exhibited the minimum mean phenol content (19.98 mg/100 g).

DISCUSSION

Physico-chemical characteristics

The fruit size (diameter) and weight were significantly improved by all the treatments of CPPU, salicylic acid, calcium chloride and Neemazal. Largest fruits were obtained with the application of CPPU which has cytokinin like activity and cytokinins are well known for their ability to cause cell multiplication and therefore may have a direct effect on fruit size. The increment in fruit size and weight under CPPU treatment might be due to the stimulation of cell division and cell elongation, which increased the number and size of cells in the outer and inner pericarp, as well as the cell number in the core as has been reported by Antognozzi et al. (1997). The increment in fruit size and weight under salicylic acid treatment might be due to the fact that salicylic acid has a direct involvement in plant growth by enhancing the level of chlorophyll and photosynthesis, control of stomatal movement, reversion of effects of ABA on leaf abscission and modifying the activity of some important enzymes

(Hayat et al. 2007). The present results concerning the effect of CPPU on the fruit weight and dimensions are in harmony with those obtained by Ninomiya (2001); Cruz- Castillo et al. (2002); Flaishman et al. (2006) and Kim et al. (2006).

Decrease in physiological weight loss due to these treatments in contrast to control might be due to their effect on slowing down the physiological processes responsible for causing weight loss (Sharples and Johnson 1977). Calcium is known to act as an anti-senescent agent as it provides cellular disintegration by maintaining protein and nucleic acid synthesis (Faust and Klein 1973). Singh et al. (1993) reported that application of calcium chloride 20 and 10 days before harvest in Dashehari mango delayed fruit ripening, lowered cumulative physiological loss in weight, reduced respiration rate and improved fruit quality during storage. Similar results were obtained on “Canino” apricot (Farag et al. 2012) and "Anna" apple fruit (Farag and Nagy 2012).

Flesh firmness has a direct influence on fruit quality. With a decrease in fruit firmness the tissue rigidity decreases as a result of hydrolysis of intercellular pectin and also due to a decrease in cell turgor pressure because of an increase in permeability of cell membranes to water. The decrease in both the components of fruit firmness appears to contribute to tissue softening (Pollard 1975). Calcium is an integral part of the cell wall which combines with pectic acid and forms calcium pectate; and thus helps in maintaining the cellular integrity of fruit. The effect of pre-harvest calcium sprays in nectarine trees provided an increase of calcium content in nectarine fruits both in the peel (14–25%) and in the flesh (8–11%) (Manganaris et al. 2006).

Antioxidants

The main contribution of peach/ nectarine and their processed products to nutrition is undoubtedly the supply of vitamin C. The vitamin C content of the peach fruit ranges from 1 – 27 mg/ 100 gm of fresh weight depending on the variety, ripeness stage and location (Joshi and Bhutani 1995). During the present study calcium chloride was found to reduce degradation and preserve the ascorbic acid, which resulted in the maximum mean ascorbic acid content at the end of storage, although it showed a declining trend in all the treatments. Calcium is reported to delay the rapid oxidation of ascorbic acid (Veltman et al. 2000).

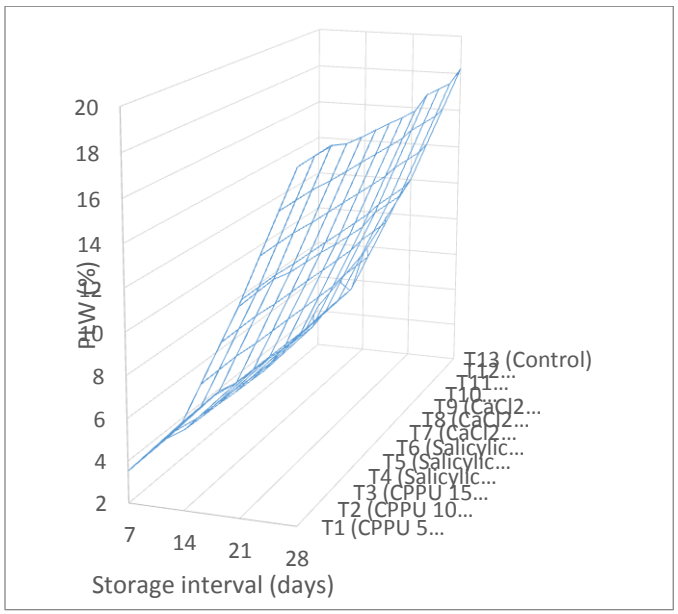

Figure 1. Effect of pre-harvest treatments on physiological loss in weight (%) of Snow Queen nectarine fruit during storage

This might explain the significant retention in fruit ascorbic acid content as a result of calcium application in the present study. Ruoyi et al. (2005) stated that ascorbic acid content of peaches was stable for fifty days storage period with the application of 0.5 per cent $CaCl_2$. Aaddition of calcium improves rigidity of cell wall and prevents enzymes such as polygalacturonase from reaching their active sites (John 1987), thereby, increasing firmness, retarding tissue softening and prolonging harvest season (Marzouk and Kassem 2011). This effect can be explained by the formation of cross links between the carboxyl groups of polyuronide chains found in the middle lamella of the cell wall. It reduces the rate of senescence during commercial and retail storage of fruit, with no detrimental effect on consumer acceptance (Lester and Grusak 2004). Similar increase in ascorbic acid content as a result of calcium application was found by Singh et al. (2007).

Salicylic acid has been shown to interfere with the biosynthesis and/or action of ethylene, abscisic acid and cytokines in plants (Srivastava and Dwivedi 2000). Recently, salicylic acid has been proposed to be a new kind of plant hormone that significantly maintained higher firmness in fruits and lower fruit chilling injury and decay incidences (Rao et al. 2011). Wang et al. (2006) found that salicylic acid alleviated chilling injury by influencing the antioxidant system in order to prevent fruit softening as well as, it affects cell swelling which leads to higher fruits firmness. According to these results, it is expected that salicylic acid treatment may regulate sugars translocation from source to sink. Also, it works as an antioxidant, as it activates ascorbate peroxidase, which increases antioxidant ability and ascorbic acid amount in fruits (Wang et al. 2006). In addition, salicylic acid has been shown to affect the biosynthesis and action of ethylene (Srivastava and Dwivedi 2000), to prevent vitamin C destruction (Wisniewska and Chelcowski 1999) and recently, as anti stress power (Elwan and El-Hamahmy 2009).

In the present studies carotenoid content of fruits increased gradually during storage and the mean carotenoid contents of fruits under various treatments were significantly lower than that recorded in control fruits (6.84 mg/100 g). The direct role of calcium chloride in carotenoid development is not fully understood.

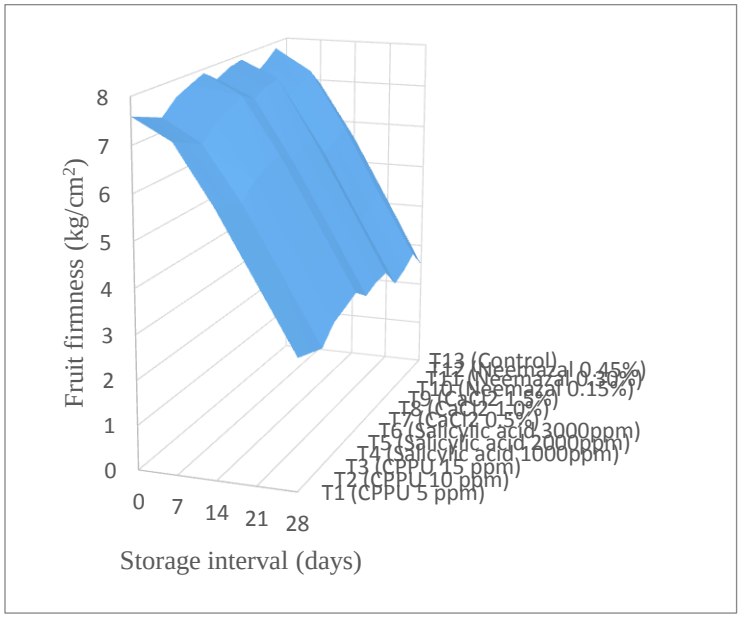

Figure 2. Effect of pre-harvest treatments on fruit firmness (kg/cm^2) of Snow Queen nectarine fruit during storage

Conclusions

The study showed that the fruit length, diameter, weight, volume, TSS, reducing and total sugars of nectarine fruit Cv. Snow Queen were increased significantly with the pre-harvest application of 15 ppm CPPU (T_3). Calcium chloride @ 1.5 per cent (T_9) proved to be the most effective in maintaining the quality during storage of nacterine fruit cv. Snow Queen. In overall

among the pre-harvest treatments calcium chloride @ 1.5 per cent, Neemazal @0.45 per cent, salicylic acid @3000 ppm and CPPU @15 ppm proved to be most effective in maintaining fruit quality and minimizing deterioration during 28 days storage at 3 ±1 ºC. As the storage period increased, the overall acceptability of the fruits, as evaluated on the basis of then appearance, flavour and texture, showed a marked improvement under all treatments upto 21 days before declining during the remaining storage period, with the control fruits being rated as least acceptable.

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