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Effect of Pre-harvest Application of Fungicides on Fruit Quality of Kinnow Mandarin Stored Under Ambient Conditions

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ABSTRACT

The experiment was conducted at farmer's field of district Sirsa, Haryana during the years 2015-16 and 2016-17 to enhance the shelf life of fruits by using pre-harvest application of fungicides. The pre-harvest treatments comprising six applications of fungicides were laid out in a randomized block design with four replications. Spray schedules comprising Copper oxychloride (0.3%) and Carbendazim (0.1%) and Copper oxychloride (0.3%) and Thiophanate methyl (0.1%) proved significantly superior to rest of the fungicides treatments and control in controlling the post-harvest fruit decay. The microbial counts were found significantly lowest in Copper oxychloride (0.3%) and Carbendazim (0.1%) followed by Copper oxychloride (0.3%) and Mancozeb (0.2%) as compared to control. The quality parameters viz., total soluble solids (TSS), acidity, fruit firmness and ascorbic acid were found non-significant with different fungicides treatments as compared to control. Pre-harvest or prophylactic fungicidal umbrella must be provided to overcome latent infection before the pathogen establishes in the host tissues particularly perishables intended to be kept under storage.

Key words: *Fungicides, Shelf life, Decay loss, Ascorbic acid, Ambient storage.*

INTRODUCTION

Citrus occupies an important place in horticultural wealth of India by covering approximately 1055 thousand hectare area with an annual production of about 12746 thousand MT. Mandarin occupies 429 thousand hectare area and annual production is 4754 thousand MT. Total area under citrus fruits in Haryana is 11.52 thousand hectares and annual production is 225.05 thousand MT[19]. Aspects like precocity and prolificacy in bearing, attractive fruit color, higher juice content, absence of granulation disorder and heavy returns have attracted the growers in North India. Citrus fruits are non-climacteric, having low rate of respiration and they are comparatively poorer in post-harvest life as compared to climacteric fruits. Due to its short shelf life, it cannot be carried from production area to faraway places, results in surplus in local market and farmers are constrained to dispose off their produce at throwaway price. Kinnow is a hybrid between King and Willow leaf mandarin developed by Dr. H. B. Frost at California during the year 1935.

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In India, it was introduced during mid sixties at Regional Fruit Research Station, Abohar (Punjab). Kinnow constantly performed well in semi-arid and sub-mountainous tract of India. Kinnow fruits display changes in fruit texture, colour, aroma and biochemical characteristics beside fruit quality parameters *viz.*, fruit weight, fruit colour, taste, total soluble solids, acidity and sugars, which are immensely influenced with storage, resulting of poor fruit quality and post-harvest losses. Various factors have been reported to be associated with post-harvest losses of Kinnow mandarin. Post-harvest losses caused by fungal diseases and physiological activities (respiration, ethylene liberation and enzyme) are the major factors responsible for limiting the shelf life of Kinnow[22]. It is important to store the Kinnow fruits for an ample period to avoid a surplus in the market during harvesting season. The fruits may be contaminated in orchards right from the setting to harvesting stage leading to pre-mature fruit drop, and incipient pre-harvest infections causes subsequent post-harvest decaying during storage and transit under cordial conditions of temperature and moisture[16]. Approximately 24 per cent post-harvest fruit losses have also been reported due to pre-harvest quiescent infection[3]. Latent or well established pre-harvest infection at the time of harvest is difficult to be abolished by mere post-harvest application of fungicides. Government regulations allow only pre-harvest employment of particular chemicals for decay control but not for the post-harvest treatment. The best approach to control the post-harvest fruit decaying is pre-harvest application of fungicides[20]. Post-harvest impairment caused by green mould rot, blue mould rot, stem end rot and core rot are the most economically significant post-harvest diseases of Kinnow. Presently, these diseases are mainly handled by the application of synthetic fungicides. To maintain shelf-life and fruit quality in the market, management of post-harvest diseases is crucial, as transport and marketing from producers to consumers takes long time.

MATERIAL AND METHODS

The experiment was conducted at farmer's field of district Sirsa, Haryana during the years 2015-16 and 2016-17 with the objective to enhance the shelf life of Kinnow fruits using fungicides. The experiment was laid out in Completely Randomized Design (CRD) comprising seven treatments, *i.e.*, Control (water spray), Copper oxychloride (0.3%), Copper oxychloride (0.3%) and Carbendazim (0.1%), Copper oxychloride (0.3%) and Thiophanate methyl (0.1%), Copper oxychloride (0.3%) and Mancozeb (0.2%), Copper oxychloride (0.3%) and Streptocyclin (500 ppm), Copper oxychloride (0.3%) and Aureofungin (500 ppm) with four replications. The Copper oxychloride was applied in 1st week of July and 1st week of August and other fungicides were applied in the 3rd week of September. Uniform cultural practices and plant protection measures were followed for these trees throughout the study period as per package of practice[4]. The healthy Kinnow trees in orchard were selected. Mature Kinnow fruits of uniform size, bruise and disease free were harvested from all four sides of the Kinnow tree canopy to get homogenous fruit samples during third week of December. Fruits were harvested with the help of secateurs by clipping the fruit stalk just above the point of attachment to the fruit. The Kinnow fruits were graded to obtain uniform size fruits for further handling. After grading the fruits were collected in plastic crates and shifted to post-harvest laboratory for different treatments in Department of Horticulture, CCSHAU, Hisar. The treated fruits were packed in Corrugated Fibre Board (CFB) boxes with newspaper lining. The experiment was laid out with four replications and each replication comprised of four kilogram fruits. The boxes were kept at an ambient temperature (7-20°C, 80-85% RH) and relative humidity in the post-harvest laboratory. The physico-chemical changes in the fruit were recorded at harvest and at five days interval during storage. The quality parameters *viz.*, total soluble solids (TSS), acidity, fruit firmness, ascorbic acid and physicl parameters *viz.*, Decay loss and Microbial load were recorded during storage period. Decay loss of fruits was calculated by weight of decayed fruits divided by initial weight of fruits and then converted into percentage value. The haemocytometer is used for counting the fungal spores in liquid suspension. It is a special microscope slide with a counting chamber 0.1 mm deep so that volume of liquid over a one square mm is 0.1 cubic mm. The counting chamber has a total of nine squares, each of 1mm x 1 mm engraved over it but only one square per field is visible under 100x microscope magnification. Each one mm square is divided into 25 medium sized squares, each one of which is further sub divided into 16 small squares, thus a total of 400 squares in one mm. One ml of a cell suspension is put into the counting chamber, number of cells is counted and the total cell number is determined mathematically (Spores/ml = Number of spores counted on the middle square of the grid x 10^4). Fruit firmness was measured by using a stand penetrometer of 0-20 kg scale (Deccan Techno Corporation). A plunger of 6mm diameter was used for the determination of rupture force and the readings were expressed as kg/cm^2. The total soluble solids of fruits were determined by using hand Refractometer having a range of 0 to 32, by placing a drop of juice and taking the readings. The Refractrometer was calibrated with distilled water with every use. For recording the acid content, 2 ml of juice was titrated against 0.1 N sodium hydroxide solution using phenolphthalein as an indicator[1]. The acid content was expressed as % of citric acid. Ascorbic acid content of the juice was estimated using the detective dye 2, 6- dichlorophenol indophenols visual titration method[1]. Two millilitres of fruit juice was mixed with 2 millilitres of 3% metaphosphoric acid as buffer. It was titrated against 2, 6-dichlorophenol indophenol dye. The end point was appearance of light pink persistent for five seconds. The results have been expressed as milligrams of ascorbic acid per 100 ml of juice.

RESULTS AND DISCUSSION

The pooled mean of both year 2015-16 and 2016-17 is presented in results.

Decay loss (%): The data presented in the Table 1 reveals that decay loss was significantly affected by the treatments and storage duration. Decay loss was observed minimum (4.66 and 4.76%) in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%) and Copper oxychloride (0.3%) and Thiophanate methyl (0.1%), respectively in an ascending order and both the treatments are at par, followed by Copper oxychloride (0.3%) and Mancozeb (0.2%) (4.80%), where as maximum decay loss was recorded in control (5.54%). There was a continuous increase in decay loss with increase in storage interval. The decay loss increased significantly from the minimum (2.31%) at 15th day of storage to the maximum (7.94%) at 25th day of storage period. The interactive effect of treatments and duration of storage found significant. At 15th day of storage, the minimum (2.11 and 2.21%) decay loss was observed in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%) and Copper oxychloride (0.3%) and Thiophanate methyl (0.1%), respectively and maximum decay loss (2.58%) was observed in control. Similar trend was observed at 20th and 25th day of storage.

Table 1: Effect of pre-harvest spray of fungicides on decay loss (%) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments	**Days after storage**			**Mean**
	15	**20**	**25**	
Control	2.58	5.44	8.61	**5.54**
Copper oxychloride (0.3%)	2.46	5.34	8.43	**5.41**
Copper oxychloride (0.3%) and Carbendazim (0.1%)	2.11	4.54	7.32	**4.66**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)	2.21	4.64	7.42	**4.76**
Copper oxychloride (0.3%) and Mancozeb (0.2%)	2.24	4.67	7.50	**4.80**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)	2.34	5.27	8.24	**5.28**
Copper oxychloride (0.3%) and Aureofungin (500ppm)	2.24	5.17	8.08	**5.16**
Mean	**2.31**	**5.01**	**7.94**	
Initial value	0.00			
CD at 5% level of significance	**Treatment**	**Storage**	**Interaction**	
	0.12	0.08	0.20	

Up to 10 days of storage, there was no decay loss observed in the treatments and control. Copper oxychloride is contact fungicide and other fungicides are systematic in nature and combination of Copper oxychloride with Carbendazim and Thiophanate methyl proves best to minimize the decay loss during the storage period as compared to control.The minimum decay loss was observed in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%) followed by Copper oxychloride (0.3%) and Thiophanate methyl (0.1%), at 15th day of storage and maximum decay loss was observed in control. Similar trend was observed at 20th and 25th day of storage. Pre-harvest sprays of fungicides checks the growth of pathogenic fungi, restricts latent infection of fungi and resulted in change in biochemical constituents of the fruits. This induces resistance to the fruits against the penetration and growth of fungus resulted in less decaying of fruits[18]. During storage, the fruit rot incidence increased might be due to breakdown of fungicides and weakening of defence system of the fruits against the microbial attack due to reduce in pectin substances and growth of already existing pathogen during storage[8]. Prochloraz came out to be most effective fungicide in checking the post-harvest fruit decaying up to 30 days followed by Carbendazim provide the protection to the fruits up to 15 days during storage[21]. Pre-harvest application of fungicides has been used to lesser pre-harvest inoculums load and subsequent post-harvest decay in various citrus fruits[6]. Chadha[7] conducted a study on the efficacy of foliar sprays of Carbendazim before harvest in controlling the post harvest decaying of citrus fruits. Pre-harvest sprays of Carbendazim @ 0.1% and Propineb @ 0.2% exhibited average decay of 4.81% and 5.74% respectively and found significantly superior than other spray treatments in controlling post-harvest fruit decay under cold storage to the extent of 77.99 and 73.73 per cent, respectively over control[11].

Microbial load (cfu/ml): The data (Table 2) reveals that microbial load was significantly affected by the treatments and storage duration. The above data indicates that the microbial load was significantly minimum (16.07 x 10^4 cfu/ml) in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%), followed by Copper oxychloride (0.3%) and Mancozeb (0.2%) (17.19 x 10^4 cfu/ ml) and Copper oxychloride (0.3%) and Thiphanate methyl at 0.1% (17.51 x 10^4 cfu/ml), respectively in an ascending order. The maximum microbial load was recorded in control (26.31 x 10^4 cfu/ ml) There was a continuous increase in microbial load with increase in storage interval. The microbial load increased significantly from the minimum (7.04 x 10^4 cfu/ ml) at 5th day of storage to the maximum (37.22 x 10^4 cfu/ml) at 25th day of storage period. The combined influence of treatments and duration of storage had a significant reflection on microbial load. At 5th day of storage, the minimum microbial load (5.83 x 10^4 cfu/ ml) was observed in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%) and maximum microbial load (9.27 x 10^4cfu/ml) was observed in control. Similar trend was observed during the storage interval and at 25th day of storage, the minimum microbial load (30.84 x 10^4 cfu/ml) was observed in fruits treated with Copper oxychloride (0.3%) and Carbendazim (0.1%) and maximum microbial load (49.75 x 10^4 cfu/ml) was observed in control.

Table 2: Effect of pre-harvest spray of fungicides on microbial load (cfu/ml) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments	Days after storage					Mean
	5	10	15	20	25	
Control	9.27	15.17	23.64	33.71	49.75	**26.31**
Copper oxychloride (0.3%)	7.81	11.59	15.90	27.80	39.08	**20.44**
Copper oxychloride (0.3%) and Carbendazim (0.1%)	5.83	9.27	14.04	20.35	30.84	**16.07**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)	6.56	10.38	15.17	22.46	32.97	**17.51**
Copper oxychloride (0.3%) and Mancozeb (0.2%)	6.00	9.88	14.42	22.96	32.70	**17.19**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)	7.07	10.71	15.28	25.09	37.80	**19.19**
Copper oxychloride (0.3%) and Aureofungin (500ppm)	6.76	10.31	15.01	24.77	37.42	**18.85**
Mean	**7.04**	**11.04**	**16.21**	**25.30**	**37.22**	
Initial value	2.33					
CD at 5% level of significance	**Treatments**		**Storage**	**Interaction**		
	0.15		0.13	0.34		

Copper oxychloride is contact fungicide and other fungicides are systematic in nature and combination of Copper oxychloride with Carbendazim and Thiophanate methyl proves best to minimize the microbial load during the storage period as compared to control. The treatment of Satsuma fruits with the fungicide mixture, followed by cold storage at 2°C and 93% relative humidity, extended the storage period at least 40 days and has least microbial counts. The absence of antifungal treatment and the increase of RH levels caused a greater rise in colony forming units (cfu) than the increase of the storage period or temperature[10]. Pre-harvest applications of Thiophanate methyl control post-harvest green mould effectively. Green mould among de-greened orange fruit was 16% when Thiophanate methyl was applied 1 week before harvest, whereas, among the not treated fruit, the incidence was 89.5%[24].

Fruit firmness (Kg/cm^2): The data (Table 3) reveals that the pre-harvest sprays of Copper oxychloride in combination with different fungicides and Streptocyclin did not affect the fruit firmness of Kinnow during storage. The firmness of the fruits followed a declining trend commensurate with the advancement in storage period. The fruit firmness decreased significantly from the maximum (1.39 kg/cm^2) at 5th day of storage to the minimum (1.12 kg/cm^2) at 15th day of storage period. The combined influence of treatments and duration of storage had a non- significant effect on fruit firmness.

Table 3: Effect of pre-harvest spray of fungicides on fruit firmness (Kg/cm^2) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments	Days after storage			Mean
	5	**10**	**15**	
Control	1.37	1.25	1.09	**1.24**
Copper oxychloride (0.3%)	1.38	1.26	1.12	**1.25**
Copper oxychloride (0.3%) and Carbendazim (0.1%)	1.39	1.27	1.13	**1.26**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)	1.39	1.27	1.13	**1.26**
Copper oxychloride (0.3%) and Mancozeb (0.2%)	1.40	1.28	1.14	**1.27**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)	1.38	1.26	1.11	**1.25**
Copper oxychloride (0.3%) and Aureofungin (500ppm)	1.41	1.28	1.15	**1.28**
Mean	**1.39**	**1.27**	**1.12**	
Initial value	1.48			
CD at 5% level of significance	**Treatment**	**Storage**	**Interaction**	
	NS	0.02	NS	

The firmness of the fruits followed declining trends commensurate with the advancement in storage period. Pre-harvest sprays of Copper oxychloride in combination with different fungicides and Streptocyclin did not affect the fruit firmness of Kinnow during storage. The decline in the firmness might be due to moisture loss from the fruit cells. Softening of fruits is caused either by the breakdown of insoluble proto pectins into soluble pectin or by hydrolysis of starch[14]. Fruit softening is attributed to the degradation of cell wall components, mainly pectin, due to action of specific enzymes such as polygalacturonase. Fruit firmness diminishes as the degree of ripening increases due to the action of pectolytic enzymes[15]. The loss of pectic substances in the middle lamella of the cell wall is perhaps the key steps in the ripening process that lead to the loss of cell wall integrity thus cause loss of firmness and softening[25]. Firmness declines in all the treatments and difference became insignificant in treated and non-treated fruits. Under refrigerated storage condition the fruit firmness was found non-significant among treated and non-treated fruits[26].

Total soluble solids (%): The data (Table 4) indicates the influence of different pre-harvest treatments on total soluble solids and non-significant results were found during storage period. Total soluble solids increased progressively with the advancement of storage period irrespective of the pre-harvest treatment. The total soluble solids increased significantly from the minimum (9.81%) at 5th day of storage to the maximum (10.40%) at 15th day of storage. The combined influence of treatments and duration of storage had a non-significant effect on total soluble solids.

Table 4: Effect of pre-harvest spray of fungicides on total soluble solids (%) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments		**Days after storage**			**Mean**
		5	**10**	**15**	
Control		9.80	10.10	10.50	**10.13**
Copper oxychloride (0.3%)		9.85	10.15	10.40	**10.13**
Copper oxychloride (0.3%) and Carbendazim (0.1%)		9.80	10.00	10.30	**10.03**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)		9.80	10.15	10.40	**10.12**
Copper oxychloride (0.3%) and Mancozeb (0.2%)		9.85	10.15	10.45	**10.15**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)		9.80	10.10	10.40	**10.10**
Copper oxychloride (0.3%) and Aureofungin (500ppm)		9.80	10.05	10.35	**10.07**
Mean		**9.81**	**10.10**	**10.40**	
Initial value		9.55			
CD at 5% level of significance	**Treatment**	**Storage**		**Interaction**	
	NS	0.13		NS	

The initial rise in total soluble solids of the fruit and it declines later was observed under storage period regardless of the pre-harvest treatments.During initial days of storage the total soluble solids increases due to loss in moisture from fruit surface and breakdown of complex organic metabolites into simple molecules or due to hydrolysis of starch into sugars[27]. The decrease in total soluble solids in the later stage of storage might be due to increased metabolic and respiratory activity of fruits. Total soluble solids were increased during storage and not affected by the treatments during ambient and cold storage of fruits[26]. No significant effect on total soluble solids content of Kinnow fruits up to 45 days of storage in cold storage conditions and up to 30 days under ambient conditions were observed under different treatments[13].

Acidity (%): The perusal of the pooled data (Table 5) reveals that the acidity was non-significantly affected by the treatments. The results show that with an increase in storage duration, the acidity in fruit juice decreased significantly. The acidity decreased significantly from the maximum (0.54%) at 5th day of storage to the minimum (0.48%) at 15th day of storage period. The interactive effect of treatments and duration of storage had a non-significant reflection on acidity.

Table 5: Effect of pre-harvest spray of fungicides on acidity (%) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments		**Days after storage**			**Mean**
		5	**10**	**15**	
Control		0.52	0.50	0.46	**0.49**
Copper oxychloride (0.3%)		0.54	0.51	0.48	**0.51**
Copper oxychloride (0.3%) and Carbendazim (0.1%)		0.55	0.52	0.50	**0.52**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)		0.55	0.52	0.49	**0.52**
Copper oxychloride (0.3%) and Mancozeb (0.2%)		0.55	0.53	0.50	**0.53**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)		0.53	0.50	0.47	**0.50**
Copper oxychloride (0.3%) and Aureofungin (500ppm)		0.52	0.50	0.47	**0.50**
Mean		**0.54**	**0.51**	**0.48**	
Initial value		0.56			
CD at 5% level of significance	**Treatment**	**Storage**		**Interaction**	
	NS	0.01		NS	

The acidity in fruit juice decreased significantly in all the treatments with respect to the advancement of storage period. The decreasing trend in the fruit acidity with the increasing storage period might be due to the oxidation of organic acid and its further utilization in metabolic processes[17]. The decrease in titratable acids during storage may be attributed to utilization of organic acid in pyruvate decarboxylation reaction occurring during the ripening process of fruits[9]. Change in acidity was inconsistent and non-significant among the treatments during ambient and cold storage of fruits[26]. The acidity in fruit juice among the treatments was found to vary non-significantly in both ambient and cold storage conditions[13].

Ascorbic acid (mg/100ml): The perusal of the pooled data (Table 6) reveals that the ascorbic acid was non-significantly affected by the treatments. The data reveals that the ascorbic acid content decreased progressively with the advancement of storage period irrespective of the pre-harvest treatment. The maximum ascorbic acid (22.30 mg/100ml) was found in Copper oxychloride (0.3%) and Carbendazim (0.1%) as compared to control (22.17 mg/100ml). The ascorbic acid decreased significantly from the maximum (23.72 mg/100ml) at 5th day of storage to the minimum (20.48 mg/100ml) at 15th day of storage period. The interaction between treatments and duration of storage had a non-significant reflection on ascorbic acid.

Table 6: Effect of pre-harvest spray of fungicides on ascorbic acid (mg/100ml) in Kinnow [Pooled mean (2015-16 and 2016-17)] stored under ambient conditions

Treatments	Days after storage			Mean
	5	10	15	
Control	23.67	22.43	20.42	**22.17**
Copper oxychloride (0.3%)	23.69	22.42	20.45	**22.19**
Copper oxychloride (0.3%) and Carbendazim (0.1%)	23.82	22.52	20.56	**22.30**
Copper oxychloride (0.3%) and Thiophanate methyl (0.1%)	23.77	22.47	20.49	**22.24**
Copper oxychloride (0.3%) and Mancozeb (0.2%)	23.77	22.48	20.51	**22.25**
Copper oxychloride (0.3%) and Streptocyclin (500ppm)	23.65	22.42	20.45	**22.17**
Copper oxychloride (0.3%) and Aureofungin (500ppm)	23.69	22.41	20.47	**22.19**
Mean	**23.72**	**22.45**	**20.48**	
Initial value	24.29			
CD at 5% level of significance	**Treatment**	**Storage**	**Interaction**	
	NS	0.16	NS	

The ascorbic acid content decreased progressively with the advancement of storage period irrespective of the pre-harvest treatment. Ascorbic acid is highly sensitive to oxygen and is susceptible to oxidative deterioration as well as mild oxidation resulting in the formation of de-hydro ascorbic acid. Presence of oxygen accelerates oxidation process in fruits[2]. Ascorbic acid is less stable compound[12] and normally decreases with increase in storage period[5]. Ascorbic acid was found non-significant among different treatments as compared to control after 60 days of cold storage of Kinnow fruits[23].

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