

## EFFECT OF COMBINED APPLICATION OF FUNGICIDES AND HOT WATER QUARANTINE TREATMENT ON POSTHARVEST DISEASES AND QUALITY OF MANGO FRUIT

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### Abstract

Postharvest diseases and disorders reduce mango fruit quality and cause severe losses, sometime yielding completely unmarketable fruit. Further, the risk of fruit fly presence has made it mandatory to use hot water quarantine treatment (HWQT) as a pre-requisite for market access to countries like China and Iran. In this study, different fungicides and hot water quarantine treatments combinations were evaluated for their effects on mango fruit cv. Samar Bahisht Chaunsa, which were stored for 21 days at ( $13\pm1^{\circ}\text{C}$ ,  $85\pm5\%\text{RH}$ ). Application of Topsin-M @  $1\text{ g L}^{-1}$  as field dip for 1 min (pre-transport) followed by HWQT @  $48^{\circ}\text{C}$  for 60 min., significantly suppressed postharvest diseases. HWQT generally led to increased internal discoloration as compared to control, and hot water injury was higher in fruit subjected to Iran protocol ( $45^{\circ}\text{C}$  for 75 min) compared to China protocol ( $48^{\circ}\text{C}$  for 60 min). NaOCl alone or with HWQT, caused higher internal discoloration of fruit. All physical treatments induced some degree of soft nose but combination of NaOCl with HWQT was found to accelerate the problem compared to control. Fruits subjected to NaOCl @  $2.5\text{ g 10L}^{-1}$  and Topsin-M @  $1\text{ g L}^{-1}$  both followed by + HWQT @  $48^{\circ}\text{C}$  for 60 min showed higher levels of total titratable acidity. However, non-significant effects of the treatments were observed on fruit colour, total soluble solids, total and non-reducing sugar contents and organoleptic acceptability of the fruits. Overall, postharvest, pre-transport application of Topsin-M @  $1\text{ g L}^{-1}$  followed by HWQT ( $48^{\circ}\text{C}$  for 60 min) helped reduce incidence of postharvest diseases, besides fulfilling market access criteria. The higher degree of soft nose development in HWQT fruits; and generally poor post-storage peel colour development warrant further studies.

### Introduction

Mango has become a popular fruit in the world and is praised due to its delicious taste, unique and attractive flavor with high nutritive, diuretic and therapeutic values. Mango fruit contains 10-20% sugars and is a good source of carbohydrates, amino acids, fatty acids, organic acids and minerals. Eating mangoes in the season may provide a store of vitamin A ( $4016\text{ IU }100\text{g}^{-1}$ ) and ascorbic acid ( $28.5\text{ mg }100\text{g}^{-1}$ ) (Meadows, 1998).

For Pakistan, mango is an important foreign exchange earning commodity. Pakistan is one of the leading mango exporting countries ranks 4<sup>th</sup> (Maqbool *et al.*, 2007). For successful shipment of mango fruit to distant markets, mango fruit storage potential and fruit quality consistency needs to be improved (Simmons *et al.*, 1997). As the world is becoming global village, new challenges like WTO, strict quarantine measures and other sanitary and phyto-sanitary protocols are emerging for the export and import of mango. In order to meet these challenges, we have to be competitive both in mango production and export. This can only be possible if producing countries can thoroughly understand the modern production and harvesting techniques as well as postharvest handling and storage requirements of mango (Anwar, 2004).

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Mango postharvest diseases and disorders reduce fruit quality and cause severe losses. In many cases, because the blemished fruit does not meet the cosmetic standards for the A-class fruit, mangoes fetch low price in the international markets. In most mango growing countries, there is less awareness about postharvest diseases and disorders incidence and their control are of crucial importance (Cappellini *et al.*, 1988). Susceptibility of mango fruit to postharvest diseases increases after harvest and prolonged storage as a result of physiological changes in the fruit, favoring pathogen development (Eckert *et al.*, 1996). Major postharvest fruit quality threats include anthracnose, stem end rot and soft nose (Jeffries *et al.*, 1990; Crane & Campbell, 1991). Postharvest management of mango fruits is one of the major challenges faced by mango industry (Amin *et al.*, 2008).

Postharvest hot water dips with fungicides have been proven to be effective in protecting mango against postharvest pathogen infection and in extending storage life of mango fruit during overseas shipments (Swart *et al.*, 2002). There is need to evaluate the effect of these chemicals in commercial mango cultivars of Pakistan. The adverse effects of synthetic chemicals residues on human health (Lichtenberg & Zilberman, 1987) and the environment (Weaver *et al.*, 1990) have led to the intensified worldwide research efforts to develop alternative control strategies. Non-chemical quarantine treatments in mango industry are increasingly becoming important. In recent times, wide international interest in heat treatment technology for quality maintenance and disease control has been observed. Apart from quarantine insect pest, such treatments allow mango shipments out of areas where fruit flies are endemic (Mitchman & McDonald, 1993). Among different heat treatments, use of hot water as a disinfestation treatment, has been widely adopted because of its efficacy (Jacobi *et al.*, 1995). Mango industry in Pakistan is facing problem of fruit fly and various importing countries are imposing restrictions like hot water quarantine treatment (HWQT) for specific duration for disinfestation of this pest. Pakistan has signed mango export protocols (China: 48°C for 60 min; Iran: 45°C for 75 min) for fruit fly disinfestation, before export shipments. Therefore, a detailed study about HWQT effect on disease control and quality attributes was needed. This study intended to explore the potential effects of combined application of HWQT and fungicides on commercial mango cv. Samar Bahisht Chaunsa.

## Materials and Methods

Uniform mature mango fruit of cv. Samar Bahisht Chaunsa were harvested at physiological maturity with a 4-5 cm fruit stalk attached, from a commercial orchard located in district Multan Pakistan, (latitude: 30°12'N; longitude: 71°26'E; altitude: 710 feet above mean sea level). After harvesting, the fruits were desapped and subjected to different treatments as T<sub>1</sub>: Control; T<sub>2</sub>: HWQT @ 45°C for 75 min., (Iran Protocol); T<sub>3</sub>: NaOCl @ 2.5 g 10L<sup>-1</sup> for field dip for 1 min., T<sub>4</sub>: NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min; T<sub>5</sub>: Topsin-M @ 1 g L<sup>-1</sup> in field dip for 1 min., T<sub>6</sub>: Topsin-M @ 1 g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min., T<sub>7</sub>: HWQT @ 48°C for 60 min (China Protocol) and T<sub>8</sub>: HWQT @ 48°C for 60 min + Carbendazim @ 0.4 g L<sup>-1</sup> at 52°C for 5 min. The experiment followed Completely Randomized Design (CRD) along with three replicates.

Fruit of T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> were treated at farm; air dried, packed in cardboard boxes and transported to Postharvest Research Centre at Ayub Agricultural Research Institute (AARI), Faisalabad, where respective HWQT were applied according to treatment plan. In case of

T<sub>8</sub> fruit after HWQT were treated with hot Carbendazim solution (52°C for 5 min). All treated fruits were stored at 13±1°C for 21 days. After storage, fruits were kept at ambient temperature (25±1°C) for ripening. The effects of different treatments on postharvest diseases and disorders and physico-chemical fruit quality were assessed at ripe stage.

Fruit softness (1: hard, 2: sprung, 3: slightly soft, 4: eating soft and 5: over ripe) and skin colour development (1: 100% green and 0% yellow, 2: 75% green and 25% yellow, 3: 50% green and 50% yellow, 4: 25% green and 75% yellow and 5: 0% green and 100% yellow) was rated according to score scale of Miller & McDonald (1991).

Internal discoloration and skin shriveling (1: 25% affected area, 2: 50% affected area, 3: 75% affected area and 4: 100% affected area), hot water damage (1: no lesions on fruit surface; 2: 1 to 3 lesions on fruit surface; 3: 4 to 6 lesions on fruit surface; 4: 7 to 15 lesions on fruit surface; and 5: >30 lesions on fruit surface) and chilling injury (0: no injury, 1: very mild <1 cm<sup>2</sup>, 2: mild 1-2cm<sup>2</sup>, 3: moderate 2-4cm<sup>2</sup>, 4: severe >4cm<sup>2</sup>) were recorded on the basis of self-made rating scales, whereas anthracnose (1: no fruit lesions, 2: 1-3 fruit lesions, 3: 4-6 fruit lesion, 4: 7-15 fruit lesions and 5: >30 fruit surface covered with lesions) and stem-end rot and soft nose (1: none, 3: traces, 5: slight, 7: moderate and 9: severe) were recorded as described by Akhtar & Alam (2002).

Total soluble solids (TSS) were determined using Atago RX 5000 Digital Refractometer (Atago, Japan). Total titratable acidity and sugars were determined by methods given by Hortwitz (1960). Organoleptic evaluation of the fruit for pulp colour, taste, flavor, texture and aroma was done by a panel of ten judges, using the Hedonic scale (Jacobi & Wong, 1991). All judges were asked to score the above mentioned parameters using the 9 point Hedonic scale, 1 being “dislike extremely” and 9 “like extremely”.

The data were subjected to analysis of variance (ANOVA) using Genstat Release 8.2 (Lawes Agricultural trust, Rothmsted Experimental Station, UK). Within the analysis of variance, effects of different treatments and their interactions were assessed. Least significant difference (Fisher's protected LSD) was calculated following significant F test ( $P=0.05$ ).

## Results and Discussion

### a. Postharvest diseases and disorders:

**i. Anthracnose development:** Minimum anthracnose incidence score (0.03) was recorded in fruit subjected to T<sub>6</sub> (Topsin-M @ 1 g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) followed by T<sub>2</sub> (HWQT @ 45°C for 75 min) and T<sub>8</sub> (HWQT @ 48°C for 60 min + Carbendazim @ 0.4 g 10L<sup>-1</sup> at 52°C for 5 min) as compared to control (Table 1). Maximum anthracnose incidence score (1.30) was recorded in fruit of T<sub>5</sub> (Topsin-M @ 1 g L<sup>-1</sup>, field dip for 1 min) followed by T<sub>3</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min) which remained at par with T<sub>1</sub> (Control). The results indicated that Topsin-M or NaOCl treatments alone at field level can not provide required level of protection against anthracnose incidence. The subsequent HWQT @ 48°C for 60 min, after the fungicidal application provides some additional control of disease. HWQT @ 45°C for 75 min, besides disinfecting fruit fly, helped to reduce anthracnose incidence in mango fruit during storage. Anthracnose is an important post-harvest disease of tropical and subtropical fruit. There are many reports about the control of HW fungicidal treatments for its control i.e., Carbendizim, mancozeb and propiconazole (Mortuza *et al.*, 2003), Benomyl and Bavistin etc., (Wasker & Masalkar, 1997). The effectiveness of hot-water dips in the control of anthracnose in mango has also been demonstrated (Spalding & Reeder, 1972; Muirhead, 1976).

**ii. Stem-end rot development:** The results regarding stem end rot (SER) were mixed and without any logical trend. T<sub>2</sub> (HWQT @ 45°C for 75 min), T<sub>3</sub> (NaOCl @ 2.5g 10L<sup>-1</sup> in field dip for 1 min) and T<sub>6</sub> (Topsin-M @ 1g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) had almost none stem-end rot incidence compared with control and other treatments (Table 1). Higher stem-end rot incidence was observed in fruits of T<sub>4</sub>, T<sub>5</sub>, T<sub>7</sub> and T<sub>8</sub> and results of these treatments were statistically at par with each other. HWQT at higher water temperature with low treatment time (China protocol: 48°C for 60 min) alone or with carbendazim or NaOCl did not show better results compared to control. Stem end rot has been described as major threat to mango industry (Johnson *et al.*, 1993). Previously, Esguerra *et al.*, (2004) reported that hot water treatment @ 53°C for 10 min., hot water brush @ 60°C for 20-35 seconds and a brief exposure to hot water dip @ 60°C for 20-35 seconds resulted in retardation of stem-end rot incidence. Furthermore, hot water treatment combined with 0.1% Bavistin was also found effective in controlling the postharvest development of these two fungal diseases (Wasker & Masalkar, 1997). In this study; the two HWQT regimes (Iran: 45°C for 75min; China: 48°C for 60min) showed different results. Further studies are required to establish a clear relationship between stem end rot and HWQT.

**iii. Soft nose development:** Minimum soft nose development (1.80 score) was recorded in fruit of T<sub>1</sub> (Control) followed by T<sub>3</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> for field dip for 1min), while results of T<sub>2</sub>, T<sub>6</sub> and T<sub>7</sub> were statistically at par with each other (Table 1). Maximum soft nose (6.73 score) was recorded in fruit of T<sub>4</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) followed by T<sub>5</sub> (Topsin-M @ 1 mg L<sup>-1</sup> in field dip for 1 min) i.e., 5.10 score. It is evident that all physical treatments induced some degree of soft nose but combination of NaOCl with HWQT was found to accelerate the problem. On the other hand, HWQT alone or in combination with Topsin-M or Carbendazim produced statistically same level of soft nose. Soft nose is a postharvest physiological disorder reported to be caused due to the deficiency of calcium (Gunjate *et al.*, 1979; Burdon *et al.*, 1991; Singh *et al.*, 1993; Hermoso *et al.*, 1997; Chitarra *et al.*, 2001; Torres & Saúco, 2004; Torres *et al.*, 2004) so the fungicidal applications had no influence on control of this disorder during storage. Therefore, nutritional remedies need to be looked at in this regard.

**iv. Hot water damage:** The data regarding HWT damage was recorded to observe the effect of Iran and China protocols. Among the fruit treated with HWQT, minimum hot water damage (0.17 score) was recorded in fruits of T<sub>4</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min). The highest hot water damage was recorded in T<sub>6</sub> (Topsin-M @ 1g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) (Table 1). HWQT @ 48°C for 60 min, induced lower HW damage as compared to prolonged HWQT @ 45°C for 75 min, but higher hot water damage in T<sub>6</sub> may be due to the possible effect of Topsin-M fungicide on osmotic potential of cells. An earlier report indicated that temperatures above 46°C produces fruit damage (Sharp, 1994). The HWT damage constitutes skin scalding, abnormal erratic yellow patches of color development with ripening, damaged lenticels and accelerated respiration rates during pre-climacteric period (Jacobi & Wong, 1991, 1992; Joyce *et al.*, 1993; Paull, 1994; Singh & Chundawat, 1991). Critical limit of hot water treatment temperature for effective disease control in different mango cultivars of Pakistan is yet to be known.

**Table 1. Effect of HWQT, fungicides and their combinations on postharvest diseases and disorders of mango cv. Samar Bahisht Chaunsa.**

Treatment	Anthraco-nose	Soft nose	Stem-end rot	Chilling injury	Hot water damage	Internal discoloration	Skin shriveling
T <sub>1</sub>	0.67b	1.80d	0.30b	0.00	0.00d	0.87de	0.00
T <sub>2</sub>	0.13cd	3.80bc	0.00b	0.07	0.50b	1.33bcd	0.07
T <sub>3</sub>	0.70b	2.40cd	0.00b	0.00	0.00d	1.70ab	0.03
T <sub>4</sub>	0.20bcd	6.73a	1.00a	0.00	0.17cd	1.97a	0.00
T <sub>5</sub>	1.30a	5.10b	1.00a	0.00	0.00d	0.53e	0.00
T <sub>6</sub>	0.03d	3.70bc	0.00b	0.00	0.83a	0.97cde	0.00
T <sub>7</sub>	0.57bc	3.63bc	1.00a	0.00	0.33c	1.20bcd	0.00
T <sub>8</sub>	0.13cd	2.87cd	1.00a	0.03	0.33c	1.53abc	0.00

Means not sharing similar letters are significantly different ( $P \leq 0.05$ )

**v. Internal discoloration:** The results indicated negative response of mango fruit towards NaOCl treatment. It was found that the fruit treated with NaOCl, alone or with HWQT, caused higher internal discoloration in mango fruit during prolonged period of cold storage. On the other hand, HWQT alone (T<sub>2</sub>, T<sub>7</sub>) and in combination with Topsin-M (T<sub>5</sub>) statistically remained at par with control, for internal discoloration. Minimum internal discoloration (0.53 score) was recorded in fruits of T<sub>5</sub> (Topsin-M @ 1 mg L<sup>-1</sup> in field dip for 1 min) followed by T<sub>1</sub> (Control) i.e., 0.87 score. Maximum internal discoloration (1.97 score) was recorded in fruits of T<sub>4</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) followed by T<sub>3</sub> (NaOCl @ 2.5g 10 L<sup>-1</sup> in field dip for 1 min) (Table 1). The relationship between NaOCl and browning/discoloration of the fruit pulp needs more investigations.

**vi. Chilling injury and skin shriveling:** The analysis of variance indicated non significant effects of fungicides and HWQT on chilling injury and skin shriveling in mango fruit (cv. Samar Bahisht Chaunsa) stored at 13±1°C, when tested statistically at 5% level of significance. However, further considerations are necessary to establish the relationship of HWQT with chilling injury and skin shrivelling.

## a. Physico-chemical analysis

**i. Fruit softness and skin colour development:** Fruit softness and skin colour development are basic criterion to examine the fruit ripening rate during cold storage of mango fruit. Optimal HWT conditions have been reported to accelerate the rate of ripening (Jacobi & Wong, 1991, 1992) and promote the uniformity of color development in the mango peel of 'Tommy Atkins' fruit (Jacobi *et al.*, 1998). Non-significant differences were observed among the treatment regarding fruit skin colour development. The fruit of all treatment exhibited poor colour development (i.e. <25%), which appears to be a varietal characteristic, and it leads towards the necessity of developing special protocol for poststorage ripening for cv. Samar Bahisht Chaunsa. Maximum fruit softness (4.07 score) was recorded in fruit of T<sub>3</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min) and T<sub>4</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min) followed T<sub>6</sub> (Topsin-M @ 1g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min), while minimum fruit softness (3.73 score) was observed in fruit of T<sub>1</sub> (Control). NaOCl treated fruit showed higher softness development, while hot water quarantine treatment alone could not perform better (Table 2). In line to the findings of Govender *et al.*, (2005), the treatments did not show any negative effects on the textural softness and peel colour compared to control and the fruits were marketable at ripening, although colour was not optimal.

**Table 2. Effect of HWQT, fungicides and their combinations on physico-chemical attributes of mango cv. Samar Bahisht Chaunsa**

Treatment	Fruit softness	Fruit colour NS	Total soluble solids (°Brix) <sup>NS</sup>	Total titratable acidity (%)	Reducing sugars (%)	Non-reducing sugars (%) <sup>NS</sup>	Total sugars (%) <sup>NS</sup>
T <sub>1</sub>	3.73c	1.67	30.42	0.28c	2.97a	13.49	16.46
T <sub>2</sub>	3.93ab	1.17	24.09	0.57ab	2.09c	13.52	13.71
T <sub>3</sub>	4.07a	1.67	27.32	0.45b	2.71ab	13.36	16.07
T <sub>4</sub>	4.07a	1.23	30.92	0.63a	2.59abc	13.49	16.07
T <sub>5</sub>	3.93ab	1.73	30.69	0.55ab	2.36bc	14.51	16.87
T <sub>6</sub>	4.00a	1.73	30.61	0.65a	2.31bc	12.17	14.48
T <sub>7</sub>	3.83bc	1.87	28.47	0.56ab	2.22bc	12.44	14.66
T <sub>8</sub>	3.97ab	1.70	30.64	0.43b	2.18c	11.63	13.82

Means not sharing similar letters are significantly different ( $P \leq 0.05$ )

**Table 3. Effect of HWQT, fungicides and their combinations on organoleptic characteristics of mango cv. Samar Bahisht Chaunsa**

Treatment	Pulp colour <sup>NS</sup>	Taste <sup>NS</sup>	Flavor <sup>NS</sup>	Texture <sup>NS</sup>	Aroma <sup>NS</sup>
T <sub>1</sub>	6.08	5.17	6.67	6.33	6.17
T <sub>2</sub>	5.33	4.31	5.22	5.56	4.11
T <sub>3</sub>	4.39	4.58	5.19	4.97	4.25
T <sub>4</sub>	5.37	5.79	5.62	5.43	5.18
T <sub>5</sub>	4.32	4.84	4.21	4.27	4.48
T <sub>6</sub>	4.56	5.17	5.17	4.53	4.44
T <sub>7</sub>	5.18	6.50	6.41	6.68	5.77
T <sub>8</sub>	6.00	6.32	6.68	6.41	6.18

NS = Non-significant

**ii. Biochemical attributes:** Effect of different fungicidal treatments and HWQT on total titratable acidity of mango fruit after storage was highly significant. Maximum total titratable acidity contents (0.65%) were recorded in fruit of T<sub>6</sub> (Topsin-M @ 1 g L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min), which was statistically at par with T<sub>4</sub> (NaOCl @ 2.5 g 10L<sup>-1</sup> in field dip for 1 min + HWQT @ 48°C for 60 min), while minimum total titratable acidity (0.28%) was found in fruit of T<sub>1</sub> (Control) followed by T<sub>8</sub> (0.43%) and T<sub>3</sub> (0.45%) (Table 2). The fruit subjected to HWQT alone or treated alongwith with fungicides (Topsin-M or NaOCl) had higher total titratable acidity contents, while control treatments showed least values (0.28%). Among treated fruits, those subjected to HWQT @ 48°C for 60 min + Carbendazim at 52°C for 5 min (T<sub>8</sub>) showed minimum level of total titratable acidity (0.43%) after storage, because this treatment had additional exposure to HW (52 °C for 5 min) for disease control, which might have affected ripening process of the fruit and be the cause of low total titratable acidity contents.

Effect of different combinations of HWQT and fungicides on TSS, total sugars and non-reducing sugars were statistically non-significant, while reducing sugras were significantly affected (Table 2). Maximum reducing sugars (2.97%) were estimated in control treatment, followed by fruit of T<sub>3</sub> (NaOCl @ 2.5g 10 L<sup>-1</sup> in field dip for 1 min) with reducing sugar contents at 2.71%, while minimum reducing sugars (2.01%) were recorded in case of T<sub>2</sub> (HWQT: 45°C for 75 min). Results of T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> for reducing sugars percentage were statistically at par with each other. Previously, Ram *et al.* (1983) reported that hot water treatment did not appreciably affected pH, ascorbic acid and total sugars in treated fruits of cv. ‘Deshehari’.

Effect of different combinations of HWQT and fungicides on organoleptic characteristics (Pulp colour, Taste, Flavor, Texture and Aroma) of mango was statistically non-significant (Table 3).

## Conclusion

The results indicate that after harvest, pre-transport fungicide dip (Topsin-M @ 1 g L<sup>-1</sup>) followed by HWQT @ 48°C for 60 min, for fruit fly disinfestation, helps reduce incidence of postharvest diseases of mango fruit during storage and transit. A comparative analysis of the two HWQT protocols showed that longer exposure time (45°C for 75 min, Iran protocol) although reduced the incidence of diseases, but caused higher degree of hot water damage, compared with those subjected to HWQT for shorter period at higher temperature (48°C for 60 min: China protocol). Overall, HWQT increased fruit soft nose incidence and internal pulp discolouration but did not negatively affect other physico-chemical fruit quality and organoleptic acceptability. Further studies are needed to understand relationship between fruit soft nose and hot water treatments, as well as on post-storage colour development of cv. Samar Bahisht Chaunsa.

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