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RESEARCH ARTICLE

Influence of pre-harvest application of calcium and putrescine on fruit quality of peach during storage

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Abstract

A study was aimed at analyzing the effect of pre-harvest sprays of calcium and putrescine on extending the shelf life and improving the fruit quality attributes of peach cv. Pratap was undertaken at G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Peach fruit is generally characterized by high perishability and short storage potential as fruits ripen quickly, shrivel by losing water and can be destroyed by fruit rotting microorganisms, thus rendering them unmarketable. For this, fifteen-year-old peach trees were sprayed with putrescine and calcium sprays and their combination treatments 10 days before harvesting. Fruit quality assessment such as fruit weight, physiological loss in weight (PLW), fruit shrinkage, fruit firmness, fruit decay, total soluble solids (TSS), titratable acidity (TA), ascorbic acid content and total sugars were analyzed at harvest and after every 6 days up to 30 days of storage at controlled conditions of 4±1˚C and 85± 3% relative humidity (RH). All other pre-harvest foliar sprays showed better fruit quality and storage outcomes than the control. The findings of the present investigation revealed that foliar spray of calcium nitrate at the rate of 1.0% along with the application of putrescine at the rate of 100 ppm followed by calcium nitrate at the rate of 1.0% along with the application of putrescine at the rate of 200 ppm improved the quality and storability of peach cv. Pratap. The combined treatment of putrescine and calcium enhanced firmness, TSS and total sugars and reduced weight loss, shrinkage and fruit decay percentage.

Introduction

Peach fruit is considered one of the most popular temperate fruits around the world because of its delightful flavor and high nutritive value, such as dietary fiber, minerals, proteins, vitamins, phenolic compounds, antioxidants and soluble sugars, which can protect against chronic illnesses like heart disease and certain types of cancer (1). As per the reports published by the Ministry of Agriculture and Farmers Welfare (PIB, 2022) in India, peaches are cultivated at a scale of 19.16 thousand ha, mainly in areas like Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim and Mizoram etc. with an annual production of 121.21 thousand metric tons (MT) (2). Under *Tarai* region of Uttarakhand, India, low-chill peaches are grown successfully because of nutrient-rich soil, high atmospheric humidity and low winter temperature. Like any other climacteric fruit, peaches are characterized by a sudden rise in respiration and ethylene production rates, resulting in loss of firmness and postharvest shelf life of fruits. This short “green” life of fruit at post-harvest is responsible for the huge economic losses to the peach industry (3,4). Due to the overabundance of low-chill peaches during the peak crop period, the price of fruit remarkably goes down; thus, it is essential to extend the shelf life of peaches to obtain maximum profit out of the harvested produce. Also, the consumers' concern over food safety and the potential adverse health effects of chemical residues has prompted research into developing safe replacement chemicals that can assure marketable quality for extended storage periods.

Calcium is an important plant nutrient that helps prevent fruit softening and senescence, thus enhancing shelf life. Exogenously given calcium not only stabilizes but also protects the plant cell wall from enzymes responsible for cell degradation, such as polygalacturonase (PG) and pectin methyl esterase (PME), which are produced during the ripening process (5,6). Similarly, polyamines like putrescine, spermidine, etc. help plants fight out stresses like heat stress, drought stress and salinity stress, assisting in fruit growth and development. Putrescine-treated fruits reduce reactive oxygen species (ROS) by increasing antioxidant enzyme activity, regulating gene expression, stabilizing cellular structures and helping to mitigate oxidative damage, thereby contributing to the overall resilience of plants in challenging environments (7).

However, decreased polyamine concentrations result in smaller amounts of endogenous polyamines, whereas excessive buildup of endogenous polyamines causes plant toxicity (8). As a result of these considerations, the current experiment was carried out to improve the quality and prolong the shelf life of peach fruit by pre-harvest application of calcium and putrescine.

Materials and Methods

The field experiment was conducted on a 15-year-old peach cv. "Pratap" during 2020-21 at Horticultural Research Center, GBPUA&T, Pantnagar, Uttarakhand, India. The 36 uniform vigorous plants, planted at a 6 m × 6 m distance, were selected for the study. During the period of investigation, all the trees were maintained according to cultural practices that were uniform for all the trees (9). The peach trees were sprayed with putrescine and calcium sprays as well as with their combination treatments which consists a total of twelve treatments (control, calcium nitrate at the rate of 0.5%, calcium nitrate at the rate of 1.0%, putrescine at the rate of 100 ppm, putrescine at the rate of 200 ppm, putrescine at the rate of 300 ppm, calcium nitrate at the rate of 0.5% + putrescine at the rate of 100 ppm, calcium nitrate at the rate of 0.5% + putrescine at the rate of 200 ppm, calcium nitrate at the rate of 0.5% + putrescine at the rate of 300 ppm, calcium nitrate at the rate of 1.0% + putrescine at the rate of 100 ppm, calcium nitrate at the rate of 1.0% + putrescine at the rate of 200 ppm and calcium nitrate at the rate of 1.0% + putrescine at the rate of 300 ppm). 10 days before harvesting, 15 liters of respective spray solutions were sprayed using a foot-operated sprayer equipped with a duronist spray nozzle. The fruits were harvested in the last week of April, during commercial ripening and brought to the laboratory. They were separated into different lots according to various treatments and 25 fruits of each of three replications of all treatments were packed in polyethylene bags and were stored at low temperature (4±1^{0}C, 85±3 % RH) for post-harvest evaluation. Physico-chemical analysis was done at 6 days intervals from the date of harvesting till 30 days of storage at low -temperature conditions. As mentioned earlier, an experiment was laid out for the study using a factorial completely randomized design (FCRD) with three replications.

The PLW of fruits was calculated based on the original weight and the weight loss, with the results stated in percentage (%). Decay percentage was calculated by dividing the total number of fruits that displayed fungal mycelia or sporulation by the total number of examined fruits. The result was expressed as a decay percentage. The penetrometer (Model No. BGS-25, Make-Biogen) was employed to measure fruit firmness. Fruit shrinkage was calculated by dividing the fruit's initial diameter and the diameter of the fruit on the date of observation, with the results stated in percent. Total Soluble Solids were determined using an Erma hand refractometer and other quality parameters *viz*. TA (%), total sugars (%) and ascorbic acid (mg/100g) were assessed following standard procedures described by AOAC (10). Analyses of variance (ANOVA) were performed for overall experimentation with Duncan's multiple range tests at $p<0.05$ to find significant differences between the groups. The IBM SPSS Statistics 19 statistical programme was used for all computations and statistical analyses (IBM, NY, USA).

Results and Discussion

Physiological loss in weight

A continuous decline in fruit weight with the advancement of the storage period was observed irrespective of the treatments. However, the maximum and most rapid weight loss rate was noted in control (untreated) fruits after 30 days of storage (Fig. 1a). Out of all the combinations, treatment T_{10} (calcium nitrate (1%) and putrescine (100 ppm) recorded the lowest weight loss percentage (3.89%) after 30 days of storage under low-temperature conditions. The higher PLW in untreated fruits during storage can be ascribed to the rapid upsurge in respiration and transpiration rates compared to the treated fruits and the increase in physiological weight loss with storage advancement could be attributed to uncontrolled respiration rate and transpiration rates, combined with associated metabolic activities. However, the reduction in weight loss in fruits in calcium and putrescine treatments could be due to calcium's role in maintaining membrane functionality and integrity and decreasing enzyme activity responsible for cellular structure disintegration, which reduces gaseous exchange. Due to its anti-senescence effects, putrescine lowered the rate of moisture loss from fruits and thus was able to preserve the membrane stability of peach fruit (6,11,12).

Fruit shrinkage

Shrinkage of fruit is a common phenomenon that ultimately deteriorates fruit appearance. It is evident from the data (Fig. 1b) that as the storage interval increased, there was a gradual increase in fruit shrinkage percentage. The highest percent shrinkage (11.99%) was examined in the control fruits whereas the application of calcium nitrate at the rate of 1% + putrescine at the rate of 100 ppm resulted in the least shrinkage of fruit (3.19%) followed by T_8 (3.94%) treatment (calcium nitrate at the rate of 0.5% + putrescine at the rate of 200 ppm) till the 30th day of low-temperature storage. Shrinkage is mostly due to water loss through transpiration and carbon stocks being depleted due to respiration. It can be inferred that putrescine and calcium-treated fruits help in reducing the respiration rate in fruits, which subsequently helps lower the shrinkage rate of fruits compared to untreated ones. The findings of peach (13) and papaya (14) likewise support the conclusions of the current study.

Fruit decay

No decay of fruits was observed in the T_{10} treatment (calcium nitrate at the rate of 1.0% + putrescine at the rate of 100 ppm) till the 24th day of storage. In contrast, in T_{12} and T_8 treatments the decay of fruits was observed after the 18th day and in T_4 (putrescine at the rate of 100 ppm) and T_{11} (calcium nitrate at the rate of 1.0 % + putrescine at the rate of 200 ppm) treatments after 12th day of storage (Fig. 2a). Minimum fruit decay (6.67%) was recorded in T_{10} treatment, which was *at par* with T_{12} treatment (13.33%). While the highest fruit decay (55%) was observed in the control treatment (T_1). The low decay percentage in fruits in response to the spray application of calcium and putrescine after the 30th day of low-temperature storage can be ascribed to putrescine's better defence mechanism against possible infections or

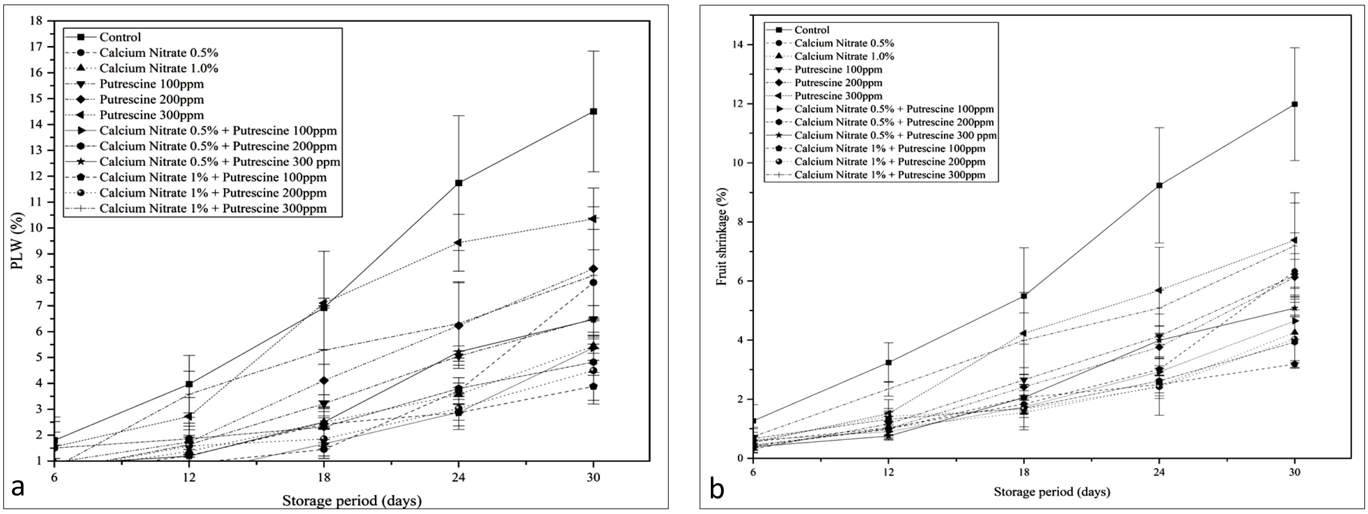

Fig. 1. Effect of pre-harvest sprays of calcium, putrescine and their combinations on (a) weight loss and (b) fruit shrinkage in peaches during low-temperature storage. Vertical bars represent ± SE of the mean.

higher activities of highly coordinated biomolecules (3). The results were in accordance with peach fruits (15), where exogenous putrescine application reduced decay percentage and controlled disease incidence in peach fruits.

Fruit firmness

It as observed that with the increase in storage days, a gradual decrease was observed in fruit firmness irrespective of the treatment, but the rate of decline in firmness was slow in T_{10} treatment (Fig. 2b) compared to others. Fruits treated with a higher $CaCl_2$ concentration had more firmness than untreated fruits, which could be attributable to less fruit softening was also observed in "Desert Red" peaches (16). Earlier workers also suggested a possible delay in softening, with higher fruit firmness and shelf life of both climacteric and non-climacteric fruits with exogenous putrescine application (17). The preservation of firmness of fruits with various pre-harvest treatments is primarily due to the inhibition of the enzymatic activity of two enzymes polygalacturonase (PG) and PME (pectin methyl esterase), which have a capacity of cross-linking pectic compounds which results in cell rigidification.

Total soluble solids and titratable acidity

It is understood that TSS and TA are the two most important quality parameters ascribed to the post-harvest life of any fruit. On the date of harvesting, the maximum TSS (10.40°B) was documented in control fruits (T_1) followed by T_3, T_8, T_9 and T_{10} treatments (Table 1). While the minimum TSS content in fruits was observed in T_6 treatment (putrescine at the rate of 300 ppm). The mean values for storage intervals showed that TSS content generally increased from zero to 24th day of storage after which it is decreased on the 30th day of storage and was 11.14°B. Whereas in control treatment, it was seen that the TSS content of fruit started decreasing from 12th day onwards. In treatments T_2 and T_3, the TSS content of fruit increased up to the 18th day of storage and started decreasing thereafter while, in treatments, T_4, T_5, T_6, T_7, T_8, T_9 and $T_{12,}$ the fruit TSS content started decreasing from the 24th day. However, the TSS of fruits treated with calcium nitrate at the rate of 1.0% + putrescine at the rate of 100 ppm (T_{10}) and calcium nitrate at the rate of 1.0% + putrescine at the rate of 200 ppm (T_{11}) showed increased with storage duration and was found maximum on 30th day of storage. In contrast, the minimum TSS in fruits was recorded in the control (T_1). The delayed increase in TSS of treated fruits with putrescine and calcium might be due to slower ripening in such fruits, which might be due to ethylene biosynthesis inhibition. Similarly, research on the effects of different concentrations of putrescine due treatments on kiwifruit concluded that a 2 mM concentration produced the best results and showed that putrescine slowed the increase of TSS, ultimately contributing to an extended shelf life of the fruit (18). Maximum TA (0.80%) on harvesting

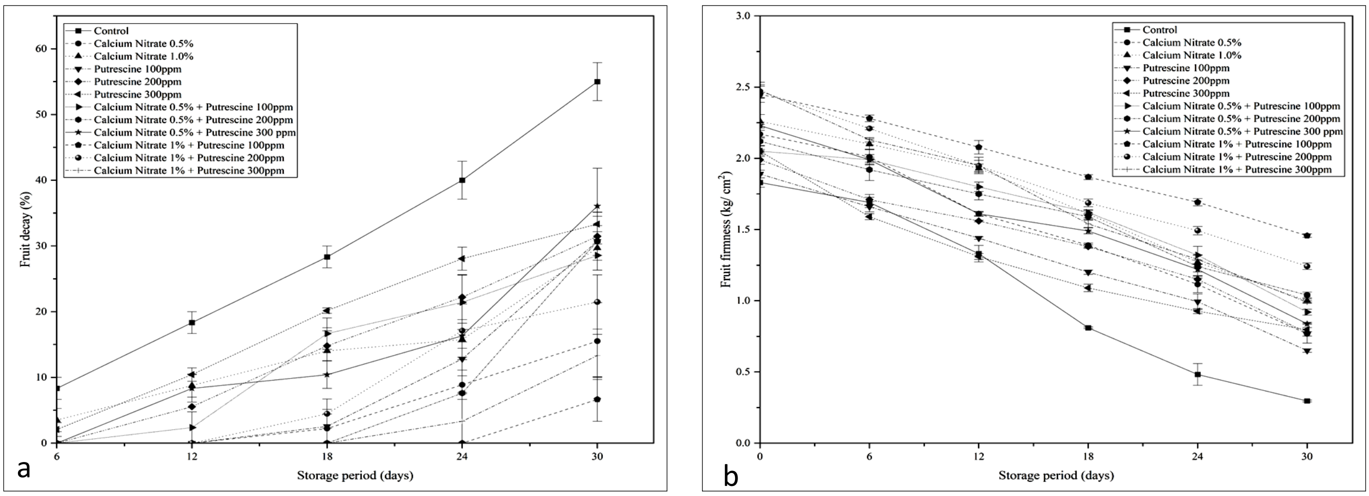

Fig. 2. Effect of pre-harvest sprays of calcium, putrescine and their combinations on (a) fruit decay and (b) fruit firmness in peach during low-temperature storage.

Table 1. Effect of pre-harvest sprays of calcium, putrescine and their combinations on TSS and TA in peach during low-temperature storage

Treatment (T)	TSS (°B)						Titratable Acidity (%)					
	Storage period (P)						Storage period (P)					
	0	6	12	18	24	30	0	6	12	18	24	30
T_1 (Control)	10.40a	11.60a	12.40a	12.00abc	11.50abc	9.80e	0.56f	0.44e	0.35d	0.32e	0.30e	0.29f
T_2 (Calcium Nitrate at the rate of 0.5%)	9.90abcde	11.10abc	11.80b	12.30a	12.00a	11.50ab	0.60ef	0.53d	0.49c	0.47d	0.44d	0.42e
T_3 (Calcium Nitrate at the rate of 1.0%)	10.30ab	11.30ab	11.80b	12.10ab	11.50abc	11.40abc	0.63de	0.58cd	0.54bc	0.50bcd	0.47cd	0.45de
T_4 (Putrescine at the rate of 100ppm)	9.70cde	10.60cdef	10.90cde	11.30def	11.80ab	11.50ab	0.63de	0.58cd	0.53bc	0.49cd	0.47cd	0.46bcde
T_5 (Putrescine at the rate of 200ppm)	9.60de	10.30efg	10.70def	11.10def	11.20bc	10.70d	0.65cde	0.60bcd	0.56b	0.54bc	0.52bc	0.51bc
T_6 (Putrescine at the rate of 300ppm)	9.40e	9.90g	10.30f	10.80f	11.40bc	11.00bcd	0.67cd	0.62bc	0.58b	0.55b	0.53b	0.52b
T_7 (Calcium Nitrate at the rate of 0.5% + Putrescine at the rate of 100ppm)	9.60de	10.20fg	10.60ef	11.30def	12.00a	10.70d	0.65cde	0.60bcd	0.55bc	0.50bcd	0.47bcd	0.45cde
T_8 (Calcium Nitrate at the rate of 0.5+Putrescine at the rate of 200ppm)	10.20abc	10.90bcde	11.20cd	11.40de	11.50abc	10.90cd	0.67cd	0.62bc	0.55bc	0.52bcd	0.47bcd	0.46bcde
T_9 (Calcium nitrate at the rate of 0.5+ Putrescine at the rate of300 ppm)	10.10abcd	10.40defg	10.60ef	10.90ef	11.10c	10.90cd	0.70bc	0.64ab	0.58b	0.54bc	0.52bc	0.50bcd
T_{10} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 100ppm)	10.10abcd	11.00abcd	11.30bc	11.50cd	11.70ab	11.80a	0.74b	0.70a	0.67a	0.67a	0.64a	0.62a
T_{11} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 200ppm)	9.70cde	10.50cdefg	10.73def	10.90ef	11.30bc	11.80a	0.76ab	0.71a	0.67a	0.64a	0.62a	0.61a
T_{12} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 300ppm)	9.80bcde	10.40defg	11.10cde	11.60bcd	11.80ab	11.70a	0.80a	0.67ab	0.59b	0.53bc	0.51bc	0.50bcd
	Significance	Treatment (T)			-S		Significance	Treatment (T)				-S
		Storage period (P)				-S		Storage period (P)				-S
		Treatment (T) x Storage period (P)				-S		Treatment (T) x Storage period (P)				-NS

Means within a column having the same letters are statistically non-significant as per Duncan's multiple-range test (p= 0.05); S-Significant; NS-Not Significant

day was recorded in T_{12} treatments (1% calcium nitrate + 300 ppm putrescine), while minimum TA (0.56%) was recorded for control (T_1). During the storage period, the mean values of TA of peach fruits decreased irrespective of treatments. A minimum decrease in the percentage of TA of fruits over storage intervals was recorded for T_{10} treatment (1% calcium nitrate + 100 ppm putrescine). In contrast, a maximum percentage decrease was observed in case of control. The higher acid in putrescine and calcium-treated fruits could be attributed to reduced organic acid hydrolysis and subsequent organic acid accumulation, which slowly oxidized due to slowed respiration, ethylene synthesis and delayed ripening. Similar findings were also reported in peach (15) and pear (19).

Ascorbic acid

The maximum ascorbic acid content (Table 2) was recorded for T_3 treatment on the harvesting day and was *at par* with T_2 and T_{10} treatments whereas minimum ascorbic acid content was observed for T_9 treatment. However, on day 30th of storage, again, the highest ascorbic acid content was observed in T_3 treatment and was found *at par* with T_7 and T_{11} treatments, whereas the lowest ascorbic acid was observed in the control treatment (T_1). Irrespective of treatment, the ascorbic acid content of fruits decreased throughout the storage period. It has been reported in the control treatment (T1) that oxidizing enzymes such as ascorbic acid oxidase, peroxidase and polyphenol oxidase aid in lowering ascorbic acid levels in fruits during storage (20). This trend was slower in calcium and putrescine-treated fruits, which can be attributed to putrescine's ability to reduce or delay ascorbate oxidase activity, hence retaining ascorbic acid concentration. In apricots, similar findings have been recorded (21).

Total sugar

The total sugar content in all the treatments increased for initial periods and decreased during later periods. On the day of harvesting, the maximum total sugar was recorded in control fruits (T_1) followed by T_3 (9.69%), T_8 (9.44%) and T_{10} (9.91%) treatments. The minimum total sugar content in fruits was observed in T_9 treatment (calcium nitrate at the rate of 0.5% + putrescine at 300ppm). In all other treatments, the total sugar content started decreasing from 24th day of storage except in T_{10} and T_{11} treatments. Fruits treated with calcium nitrate at the rate of 1.0% + putrescine at the rate of 100 ppm (T_{10}) and calcium nitrate at the rate of 1.0% + putrescine at the rate of 200 ppm (T_{11}) showed an increased with storage duration and was found maximum respectively on 30th day of storage as compared to all other treatments. The minimum sugar content of fruit (10.00%) was recorded in the control treatment (T_1) on the 30th day of storage. It can also be inferred from data that treatments T_{10} and T_{11} maintained the sugars in fruits throughout storage, thus positively impacting quality. A sharp increase in total sugar was found in control fruits compared to fruit plants sprayed with calcium and putrescine. The faster increase in total sugar in untreated fruits might be due to the high rate of fruit ripening and senescence process. Our results comply with those observed in peach (18).

The synergistic action of calcium nitrate and putrescine may improve the physiological stability of peaches during storage. Calcium is vital in maintaining cell turgor and firmness, while putrescine can help mitigate stress responses. The interaction between polyamines and calcium ions can modulate the activity of ion channels, affecting cellular homeostasis and signalling (22). Together, they can enhance the overall resilience of the fruit, reducing the likelihood of spoilage and extending its shelf life, similar to the findings observed in pistachio fruit, where the application of calcium nitrate, in conjunction with polyamines like putrescine, leads to a reduction in physiological disorders and malformations. This combination not only promotes better fruit set and development but also enhances the overall quality of the fruit, resulting in improved marketability and extended shelf life (23).

Conclusion

Based on the results summarized above, it was observed that pre-harvest foliar spray of putrescine and calcium nitrate was effective in maintaining the physico-chemical traits and improving the shelf life of peach cv. Pratap as compared to untreated fruits due to slower ripening in fruits, which might be caused by inhibition of ethylene biosynthesis. Further, it was observed that combined foliar sprays of calcium and putrescine showed much more promising results, such as improving the shelf life of fruits compared to their spray. The findings of the present investigation revealed that foliar spray of calcium nitrate at the rate of 1.0% + putrescine at the rate of 100 ppm followed by calcium nitrate at the rate of 1.0% + putrescine at the rate of 200 ppm improved the fruit quality and storability of peach cv. Pratap.

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Authors' contributions

SJ carried out the experiment, took observations, analyzed the data and prepared the manuscript. SU guided the research by formulating the research concept, helped secure research funds and approved the final manuscript. DCD contributed by developing the ideas, reviewing the manuscript and helped in procuring research grants. RK contributed by helped in summarizing and revising the manuscript. RK helped with data interpretation and editing the manuscript. MK and PS edited, summarized and revised the manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

Table 2. Effect of pre-harvest sprays of calcium, putrescine and their combinations on ascorbic acid and total sugars in peach during low-temperature storage

Treatment (T)	Ascorbic Acid (mg/100g)						Total Sugars (%)					
	Storage period (P)						Storage period (P)					
	0	6	12	18	24	30	0	6	12	18	24	30
T_1 (Control)	11.23abc	9.53e	8.02g	7.26g	6.55g	6.05e	9.73a	10.53a	11.13a	10.75abc	11.47a	10.00c
T_2 (Calcium Nitrate at the rate of 0.5%)	11.51ab	10.75bc	10.32bcd	10.02bc	9.44bc	9.00a	9.29abcd	9.84bc	10.49bc	10.90ab	11.12abc	10.15bc
T_3 (Calcium Nitrate at the rate of 1.0%)	11.60a	11.45a	11.04a	10.57a	9.95a	9.27a	9.69ab	10.12ab	10.60b	10.95a	11.17ab	10.52bc
T_4 (Putrescine at the rate of 100ppm)	10.59def	10.34cd	10.07cde	9.56d	9.07c	8.53b	9.44abcd	9.92bc	10.27bcd	10.59abcd	10.88bcd	10.12bc
T_5 (Putrescine at the rate of 200ppm)	10.79cde	10.27d	9.89de	9.44de	9.05c	8.60b	9.19abcd	9.60bcd	9.91d	10.20d	10.92bcd	10.29bc
T_6 (Putrescine at the rate of 300ppm)	10.50def	10.06d	9.67e	9.05e	8.56d	7.91c	9.00cd	9.60bcd	10.05cd	10.57abc	10.81bcd	10.59b
T_7 (Calcium Nitrate at the rate of 0.5% + Putrescine at the rate of 100ppm)	10.98bcd	10.79bc	10.50bc	10.25abc	9.95a	9.26a	9.51abc	9.91bc	10.25bcd	10.50abcd	10.89bcd	10.39bc
T_8 (Calcium Nitrate at the rate of 0.5+Putrescine at the rate of 200ppm)	11.34abc	11.00ab	10.60ab	10.45ab	9.85ab	9.00a	9.20abcd	9.69bcd	10.02d	10.33cd	10.46d	10.33bc
T_9 (Calcium nitrate at the rate of 0.5+ Putrescine at the rate of 300 ppm)	10.34ef	9.58e	9.01f	8.23f	7.70e	6.76d	8.84d	9.39cd	9.89d	10.36cd	10.59cd	10.40bc
T_{10} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 100ppm)	11.44ab	11.25ab	11.07a	9.83cd	9.26c	9.00a	9.41abcd	9.60bcd	9.95d	10.40bcd	10.91bcd	11.27a
T_{11} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 200ppm)	11.23abc	10.78bc	10.40bcd	10.13abc	9.77ab	9.11a	9.10bcd	9.41cd	9.98d	10.25cd	10.77bcd	11.21a
T_{12} (Calcium nitrate at the rate of 1%+Putrescine at the rate of 300ppm)	10.15f	9.55e	9.10f	8.30f	7.25f	6.75d	8.92cd	9.20d	9.81d	10.15d	10.45d	10.35bc
	Significance		Treatment (T)		-S		Significance		Treatment (T)		-S	
			Storage period (P)		-S				Storage period (P)		-S	
			Treatment (T) x Storage period (P)		-S				Treatment (T) x Storage period (P)		-S	

Means within a column having the same letters are statistically non-significant as per Duncan's multiple-range test (p = 0.05)87/, S-Significant; NS-Not Significant

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