PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-11
https://doi.org/10.14719/pst.6192

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RESEARCH ARTICLE

Preharvest melatonin application on enhancing quality and extending shelf life in papaya

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OPEN ACCESS

ARTICLE HISTORY
Received: 20 November 2024
Accepted: 23 January 2025
Available online
Version 1.0 : 22 March 2025
Version 2.0 : 01 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE
Priyaxee B, Kavitha C, Sivakumar V, Senthil A, Johnson I, Padmadevi K. Preharvest melatonin application on enhancing quality and extending shelf life in papaya. Plant Science Today. 2025; 12(2): 1-11. https://doi.org/10.14719/pst.6192

Abstract

Papaya is an important fruit crop and its cultivation has gaining importance in recent times due to its high economic returns . However, due to its climacteric behaviour and high perishability, the shelf life of papaya after harvest is very limited. A field experiment was conducted to evaluate the effect of preharvest melatonin applications at different concentrations – 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM, along with a control treatment (water spray) on enhancing postharvest quality and prolonging the shelf life of papaya. Among these, the applications of melatonin at 1.5 mM, administered 15 days before harvest, was found to be the most effective in extending the shelf life of papaya by significantly delaying changes in quality parameters. This treatment also resulted in increased antioxidant enzymes activity, improved fruit firmness, delayed ripening enzyme activity and reduced weight loss. Papaya fruits harvested from trees sprayed with 1.5 mM melatonin exhibited a firmness of 6.37 kg/cm^2 under ambient storage and and 6.26 kg/cm^2 under cold storage, compared to 5.54 kg/cm^2 and 5.33 kg/cm^2, respectively, for control fruits. Additionally, total soluble solids (TSS) levels in fruits from the 1.5 mM melatonin treatment were recorded as 12.90 ºB under ambient storage and 13.50 ºB under cold storage, compared to 15.00 ºB and 15.40 ºB, respectively, for control fruits. In conclusion, preharvest melatonin application at 1.5mM effectively delayed postharvest senescence, enhanced fruit quality and reduced postharvest losses in papaya. This approach could significantly improve market access and industrial adaptability, benefiting both producers and consumers.

Keywords

antioxidant capacity; delay senescence; melatonin; papaya; postharvest storage; preharvest spray; quality parameters

Introduction

Papaya (*Carica papaya* L.) is a highly nutritious fruits, and its commercial cultivation is gaining attention due to its high productivity and economic significance. Papaya also known as the "Wonder Fruit of the Tropics," is a potential source of calcium, fibre, folate, riboflavin, vitamin C and vitamin A (2020 IU/100g). It is a vital economic crop in global trade, valued for its high nutritional value, versatile uses (fresh and processed) and demand for papain in industries. Major exporters, including India and Mexico, cater to the growing global demand, supporting livelihoods and economies. Its year-

round production, adaptability and health benefits make it a key commodity in international markets.

In 2022, global papaya production reached 13.8 million mt (1). In India, total papaya production for 2023-24 was recorded at 5.34 million mt, with Tamil Nadu contributing 0.13 million mt (2). However, papaya's climacteric nature and high respiration rate accelerate its ripening process, leading to rapid pulp softening, skin browning and susceptibility to fungal infections, all of which significantly shorten its postharvest shelf life. Despite its high yield potential, approximately 40-60 % of papaya production is lost due to postharvest losses (PHL) (3).

Measures such as field sanitation, postharvest disease management, timely harvesting, suitable packaging and a cold supply chain are employed to minimize PHL. However, papaya remains highly vulnerable to chilling injury and fungal rot when stored at temperature below 9-10 °C. Ripe papaya fruits can be stored at 25 °C and 12 °C for two and three weeks, respectively (4). Most of the techniques used to prevent fruit degradation and infections after harvest in papaya are either very expensive or involve usage of chemicals that are harmful to both the environment and human health. Papaya fruits are generally protected from postharvest anthracnose infestation by application of fungicides like carbendazim and benomyl. However, the "Banning of Insecticides Order, 2020" is set to prohibit the use of carbendazim in India (5).

Melatonin is a naturally occuring indole amine that has recently been utilized to extend the postharvest shelf life of fruits by delaying ripening and reducing oxidative stress (6). It is environmentally friendly, leaving no harmful residues and is endogenously present in both plants and animals, making it safe for human and animal consumption. Melatonin is recognized as an activator of the antioxidant system, a potent free radical scavenger and a protective agent against oxidative damage to proteins and membrane lipids in plants.

According to previous study, melatonin has been identified as a signal molecule involved in multiple physiological process, including senescence, flowering, germination of seed, fruit maturation, abiotic stress responses and ripening of fruit (7). Researchers have recently emphasised on understanding the effects of melatonin application on biochemical pathways, antioxidant shielding systems and its mechanism in delaying senescence in fruit crops. Studies have demonstrated that melatonin application has effectively prolonged the postharvest shelf life of tomatoes, peaches, strawberries, sweet cherries and bananas by delaying ripening and senescence while preserving quality attributes (8-11). A previous study stated that melatonin application also improved the antioxidant defense system, decreased peel browning and postponed low-temperature damage in banana (12).

Hence, melatonin treatment might be a safer and greener choice because of its natural origin, low toxicity and biodegradability than other chemical treatments for maintaining the freshness of fruits during storage. However, there has not been a thorough investigation on the effects of preharvest spray of melatonin and its dosage on papaya. Therefore, the present study has been conducted to investigate the effect of preharvest spray of melatonin to extend shelf life in papaya by lowering postharvest senescence and delaying ripening.

Materials and Methods

The field trial was implemented at Tamil Nadu Agricultural University, Coimbatore, during 2023 - 2024. TNAU Papaya CO 8 trees raised in the college orchard and at full bearing stage, served as the experimental material for the study. CO 8 is a dioecious, red-pulped, high-yielding variety, known for its comparative tolerance to papaya ring spot virus and its suitability for both local and distant markets.

The experiment involved five treatments viz., preharvest sprays of melatonin (dissolved in ethanol, a non-polar solvent) at 0.5, 1.0, 1.5 and 2.0 mM, applied to the entire tree 15 days before harvest, along with a control treatment (water spray). These concentrations were selected based on prior research identifying effective doses for enhancing fruit quality and extending shelf life. The treatments were replicated four times, with five trees per replication and the experiment was arranged in a randomized block design.

Healthy, uniformly matured fruits weighing 800-850 g at the color break stage were harvested 15 days after the preharvest spray. The harvested fruits were sorted, brought o the analytical laboratory and left in the open for 1-2 hrs to remove the field heat. The fruits were then stored under ambient storage (32 ± 2 °C and 55 ± 5% RH) and cold storage (10 ± 2 °C and 90 ± 5% RH). Ambient storage reflects local market conditions, while cold storage simulates controlled environments for export and long-distance transport. Studying both conditions ensures the effectiveness of treatments across the supply chain. Observations were recorded at three days for ambient storage and seven-days intervals for cold storage.

Determination of fruit firmness and physiological loss in weight

A Fruit Hardness Tester (Bestone Industrial Ltd., China) was used to measure the firmness of the fruit. Physiological loss in weight (PLW) was computed by calculating the difference between the initial and final weight of the fruit.

Determination of quality attributes

TSS was measured using a hand refractometer (Erma Inc., Japan) with a range of 0 to 32°Brix and the readings were recorded in °Brix. Titratable acidity was analysed following the procedure given by (13). A 5 g fruit sample was homogenized and distilled water was added to make up 30 mL. After filtration, 5 mL of the filtrate was titrated against 0.1 N sodium hydroxide using two to three drops of phenolphthalein indicator until a pale pink color appeared. The TSS to acid ratio was calculated by dividing TSS by titratable acidity.

Total sugars of the samples were computed using the procedure given by (14). A 0.5 g pulp sample was homogenized with 10 mL of 85 % ethanol, followed by centrifuging three times to extract sugars. The supernatant was pooled, diluted to 100 mL with distilled water and reacted with 4 mL anthrone reagent and 0.5 mL distilled water. After

incubation and cooling, the absorbance at 630 nm was measured using a spectrophotometer.

Total phenol content was determined following a standard procedure (15). 1 g of plant material was macerated with 10 mL of 80 % ethanol, centrifuged and the residue was dissolved in 5 mL distilled water. A 0.2 mL aliquot was diluted to 3 mL, reacted with Folin-Ciocalteu reagent and mixed with sodium carbonate. After boiling and cooling, absorbance at 650 nm was measured. The phenol content was calculated using a catechol standard curve and expressed as mg/100 g of tissue.

Ascorbic acid was analysed using the procedure described in a previous study (16). A 5 g pulp sample was homogenized with 4 % oxalic acid to prepare 50 mL of solution. After filtration, 5 mL of the filtrate was mixed with 10 mL of 4 % oxalic acid and titrated against sodium bicarbonate and 2,6-dichloroindophenol dye until a pink hue (V2) appeared, which persisted for a few minutes. The ascorbic acid content was expressed in mg/100 g.

Determination of ripening and antioxidant enzymes

Enzymes extraction was carried out using the method which was previously outlined (17), with phosphate buffer. The activity of ripening enzymes viz., pectin methyl esterase (PME), polygalacturonase and cellulose, was measured using established protocols.

PME activity was assessed by mixing pectin, sodium chloride and bromothymol blue. Absorbance at 620 nm was recorded after 3 min and the activity was expressed as milliequivalents of COOH/min/g fresh weight (18).

Polygalacturonase activity was determined by incubating sodium acetate buffer, sodium chloride, polygalacturonic acid and enzyme extract for 1 hr. DNSA was then added and absorbance at 540 nm was measured. The activity was expressed in µg/min/g) (19).

Cellulase activity was analysed by incubating carboxymethyl cellulose, sodium acetate buffer and enzyme extract incubated at 37 °C. The reaction was stopped with DNSA and absorbance at 540 nm was measured. The activity was expressed in µg/min/g (20).

Similarly, the activity of antioxidant enzymes viz., peroxidase activity was measured by mixing 3.5 mL phosphate buffer (0.2 M), 0.2 mL enzyme extract, 0.1 mL o-Dianisidine (1 mg/mL) and 0.2 mL hydrogen peroxide (0.1 M). Absorbance at 430 nm was monitored for 3 min and the activity was expressed as ΔA/min/g fresh weight (21).

Catalase activity was determined using 1.5 mL phosphate buffer (0.2 M), 50 µL enzyme extract, 1.5 mL distilled water and 0.5 mL hydrogen peroxide (12.5 mM). Absorbance at 240 nm was recorded for 1 min and the activity was expressed as activity/min/g fresh weight (22).

Superoxide dismutase (SOD) activity was assessed using a reaction mixture containing 0.1 mL enzyme extract, 0.03 mL riboflavin and 1 mL assay mix (27 mL sodium phosphate buffer, 1 mL NBT, 1.5 mL methionine and 0.75 mL Triton X-100). Absorbance at 560 nm measured and the activity was expressed as units/mg protein (23).

Statistical Analysis

The current field experiment was carried out using a randomized block design (RBD). Treatment means were compared using one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) analysis was employed to determine significant differences among treatment. Statistical analysis was performed using R software (version 4.3.1) with the appropriate R Studio packages. The level of significance was set at $p = 0.05$.

Results

Preharvest melatonin on physiological loss in weight and fruit firmness

The weight loss in papaya fruits varied significantly across treatments (Table 1). The control fruits exhibited the highest physiological weight loss (12.46 %), followed by fruits treated with 0.5 mM melatonin (11.88 %), while the lowest PLW (7.97 %) was recorded in fruits sprayed with 1.5 mM melatonin on the ninth day of storage at room temperature.

Regardless of the treatment or storage temperature, fruit firmness declined as storage duration increased; however, melatonin application had a significant impact by, as shown in Table 2. The fruits sprayed with 1.5 mM melatonin registered the highest firmness, measured at 6.37 kg/cm^2 on the 9th day of ambient storage and 6.26 kg/cm^2 on the final day of cold storage. Under both storage conditions, the firmness of these fruits was 1.2-1.4 times higher than that of the control fruits.

Preharvest melatonin on quality attributes

The preharvest application of melatonin had a substantial effect on the TSS of papaya fruits stored under both ambient and cold storage conditions (Fig. 1A). As ripening progressed, control fruits recorded the highest TSS (15.0 ° Brix) on day 9, comparable to the TSS of 15.30 °Brix recorded in fruits treated with 2.0 mM melatonin () on day 28 under cold storage. In contrast, the treatment sprayed with 1.5 mM melatonin recorded the lowest TSS values of 12.90 °Brix in ambient storage and 13.50 °Brix in cold storage.

The reduction in titratable acidity in papaya fruits over the whole storage period was considerably decreased by exogenous melatonin treatment (Fig. 1B). Fruits treated with 1.5 mM melatonin exhibited the highest TA (0.48 %), whereas control fruit registered the lowest TA (0.28 %) under ambient storage. Similarly, in cold storage, control fruits registered the lowest TA (0.18 %), while fruits treated with 1.5 mM melatonin showed a slower decline in TA (0.38 %) on the final sampling day. It was clear that fruits kept in cold storage had higher TA levels than fruits kept in ambient storage.

The TSS: acid ratio of papaya fruits is depicted in Fig. 1C for both ambient and cold storage. Regardless of storage temperatures, the TSS: acid ratio increased from day 0, with no distinct variation, suggesting that melatonin treatment had no significant effect on the ratio of TSS to acid.

The total sugar content of papaya fruits exhibited

an increasing trend from the color break stage to full ripeness (Fig. 1D). Melatonin had a highly significant impact by slowing the rate of sugar accumulation in treated fruits. On day 9 of ambient storage, fruits preharvest-sprayed with 1.5 mM melatonin recorded the lowest total sugar content (41.67 g/100 g), whereas control fruits registered the highest sugar content (51.43 g/100 g). Under cold storage, control fruits maintained higher total sugars (42.55 g/100 g), while fruits treated with 1.5 mM melatonin recorded lower total sugars (31.95 g/100 g) on the final sampling day. The lower sugar levels in cold-stored fruits indicate that ripening was delayed at lower temperatures compared to ambient storage conditions.

The total phenol content of papaya fruits, as shown in Fig. 2A, was significantly impacted by MT treatment under both storage conditions. On day 9 of ambient storage, fruits sprayed with 1.5 mM melatonin showed a total phenol content of 50.05 mg/100g, which was higher than that of control fruits (40.05 mg/100g). Similarly, on the 28th day of cold storage, fruits treated with preharvest spray at 1.5 mM melatonin had a larger peak total phenol content of 42.85 mg/100g, while control fruits recorded 32.75 mg/100g. The lower total phenol content observed under cold storage suggests that peroxidase activity is temperature-dependent.

The changes in ascorbic acid content in papaya fruits stored under various concentrations of preharvest treatments are presented in Fig. 2B for both ambient and cold storage conditions. The decline in ascorbic acid content was considerably mitigated by MT treatment during storage. In contrast to control fruits, which recorded 9.05 mg/100g of ascorbic acid under ambient storage, spray of 1.5 mM melatonin retained 13.09 mg/100g on the ninth day. On the 28th day, T4 retained 10.71 mg/100g of ascorbic acid, whereas control fruits exhibited a reduced level of 8.24 mg/100g. . Notably, fruits stored at lower temperatures retained higher ascorbic acid content compared to those stored at ambient temperature, indicating a slower degradation of vitamin C under cold storage conditions. .

Preharvest melatonin on ripening and antioxidant enzymes

In this study, PME activity increased as storage days prolonged till the ripening stage, after which it declined (Fig. 3A), irrespective of storage conditions. The PME activity in fruits harvested from trees sprayed with 1.5 mM Melatonin as a preharvest spray treatment was significantly decreased in comparison to control fruits in both ambient storage (on day 6th it was 17.62 m.eq. COOH/ min/g) and cold storage (on day 21st it was 17.46 m.eq. COOH/min/g). The peak PME activity was delayed by 3 days under room temperature and 7 days under cold storage.

The maximum activity of polygalacturonase was registered in control fruits on day 6 (60.16 µg/min/g), while the fruits from trees treated with 1.5 mM melatonin exhibited their peak polygalacturonase activity on 9th day (17.83 µg/ min/g) under ambient storage conditions (Fig. 3B). A similar trend was observed cold-stored fruits, where control fruits reached their peak activity on day 21 (42.91 µg/min/g), while melatonin-treated fruits peaked on day 28 (13.00 µg/min/g). The lower peak activity under cold storage indicates that polygalacturonase activity declined as storage temperature decreased.

Cellulase activity followed a similar pattern, with control fruits peaking on day 6 (80.23 µg/min/g) and fruits treated with a 1.5 mM melatonin reaching their peak on 9th day (46.52 µg/min/g) under ambient storage condition. A comparable trend was observed in cold storage on day 21, where control fruits showed a peak of 70.45 µg/min/g, while melatonin-treated fruits peaked at 46.88 µg/min/g. This decline in peak cellulase activity at lower temperatures suggests that cellulase activity decreased as storage temperature was reduced (Fig. 3C).

On the 9th day of ambient storage, fruits from trees sparyed with 1.5 mM melatonin exhibited higher peroxidase activity (0.75 ΔA/min/g) compared to the control fruits (0.62 ΔA/min/g). Similarly, under cold storage, fruits harvested from trees sprayed with 1.5 mM Melatonin had a peak activity of 0.69 ΔA/min/g, whereas control fruits recorded a lower activity (0.47 ΔA/min/g).

Catalase activity was significantly impacted by the preharvest MT spray and catalase activity rose as the ripening process progressed. On the final day of ambient storage, papaya fruits from 1.5 mM Melatonin as a preharvest spray registered higher catalase activity (4.05 ΔA/min/g) than the control (3.14 ΔA/min/g). Fruits that were stored in cold storage also showed a similar pattern.

The activity of superoxide dismutase in fruits from preharvest melatonin sprayed trees was considerably higher than in control fruits, exhibiting a continous increase during both ambient and cold storage. On the final day of storage, fruits from preharvest sprayed (1.5 mM Melatonin) registered the highest SOD activity (0.62 and 0.63 units/mg protein in ambient and cold storage conditions, respectively), while control fruits registered the lowest in both storage conditions).

Discussion

To reduce postharvest losses and extend the shelf life of climacteric fruits viz., papaya, various pre and postharvest techniques have been developed and investigated. However, the impact of preharvest spraying of melatonin on ripening and postharvest life has not yet been thoroughly investigated most particularly in case of tropical and sub tropical fruits. Nonetheless, there are reports of varying results according to the fruit type, quantity administered and application timing.

Melatonin is most effectively administered as a preharvest spray at the mature green stage, prior to the onset of of ripening, to reduce ethylene production and prolong shelf life (24). Melatonin acts as an ethylene synthesis regulator at the appropriate point of administration by inhibiting the expression of genes involved in ethylene synthesis, including the ACS and ACO genes. As ethylene production increases during the colour break stage, melatonin can help to slow down the ripening process. Additionally, melatonin aids in boosting the

antioxidant enzymes in the fruit, which play a crucial role in maintaining fruit quality this stage. Given that the color break stage is associated with significant biochemical and physiological alterations leading to ripening, melatonin application at this stage helps delay these processes by slowing cell wall degradation, preserving fruit firmness and extending shelf life. .

Melatonin is naturally found in the flesh, seeds and skin of grapes, with levels increasing in the flesh and seeds but decreasing in the skin during ripening (25). Melatonin administration significantly increased the size of the berries in grapes during the pre-veraison stage. This increase was linked to an increase in the endogenous melatonin concentration (26). Similarly, in cherry cultivars such as Rainier and Hondeng, melatonin levels peaked during the second stage of fruit development, coinciding with endocarp lignification (27). Furthermore, melatonin foliar application in apricots has been shown to increase fruit yield and weight without significantly influencing on-tree ripening (28).

Elevated respiration and ethylene production are the primary reasons for the reduction in fruit weight during storage (Table 1). Melatonin treatments delays ripening processes, allowing fruits to remain marketable for longer periods. This is particularly advantageous for exporters and retailers aiming to reduce waste and maintain product quality during transportation and storage also by minimizing postharvest water loss, melatonin helps maintain fruit weight, directly impacting profitability since fruits are often sold by weight (5). Similarly, a previous study observed a noteworthy rise in weight loss throughout the papaya's postharvest ripening phase (29). The preharvest melatonin administration considerably reduced the rate of senescence and weight loss during the storage periods as compared to the control. Similar results have been documented, showing that strawberries treated with EMT exhibited much lower rates of senescence and weight loss under cold storage (30). Similarly, a study demonstrated that melatonin administration significantly decreased weight loss and the deterioration of peach quality over time (31). According to recent studies, melatonin treatment significantly reduced weight loss and delayed senescence in sweet cherry fruits (10).

Melatonin-treated fruits may remain firm in both ambient and cold storage conditions and the results indicated that treatments with melatonin postponed the decrease in firmness (Table 2), which was consistent with earlier findings (32). A key feature that decreases with fruit age is fruit flesh hardness, which is caused by the disintegration of various cell wall constituents and a decrease in cell turgor pressure, which determines the fruit's storage potential (33). Furthermore, a study found that peach fruits treated with melatonin exhibited more hardness because they up-regulated numerous genes that are effective in breaking down cell walls during storage (31). In many other fruits, including bananas (34) and guavas (35), prior research documented a softening of fruit firmness as a result of water loss upon harvesting as a result of high respiratory transpiration.

An increase in TSS is associated with the breakdown of starch to sugar, an increase in organic acid content and protein accumulation. While the higher TSS observed in control fruits might be associated with faster ripening, the lower TSS in melatonin-treated fruits suggests delayed ripening (Fig. 1A). These results corroborated with the findings of (36) in melatonin-treated nectarines. In papaya, organic acid content increases as it ripen, with citric and malic acids being the most prevalent organic acids. Citric acid is produced as an intermediate in the Krebs cycle intermediate (TCA) during respiration. The production is regulated by both mitochondrial and glyoxysomal citrate synthases (37). Meanwhile, the glyoxylate cycle, which is initiated by ethylene and regulated by malate synthase, controls the buildup of malic acid in the pulp. Fruits sprayed with 1.5 mM melatonin, registered a decreased

Table 1. Preharvest melatonin application on physiological loss in weight (%) in papaya

Storage period Treatment	Ambient			Cold			
	Day 3	Day 6	Day 9	Day 7	Day 14	Day 21	Day 28
T_1	1.98 ^{a}	7.92 ^{a}	12.46 ^{a}	2.20 ^{a}	6.98 ^{a}	9.59 ^{a}	12.99 ^{a}
T_2	1.92 ^{a}	6.58 ^{b}	11.88 ^{b}	1.79 ^{b}	5.21 ^{b}	8.96 ^{b}	11.89 ^{b}
T_3	1.63 ^{c}	4.88 ^{d}	8.34 ^{d}	1.72 ^{c}	4.27 ^{c}	6.91 ^{c}	10.63 ^{d}
T_4	1.46 ^{d}	4.00 ^{e}	7.97 ^{e}	0.19 ^{d}	3.88 ^{e}	6.33 ^{d}	10.40 ^{d}
T_5	1.84 ^{b}	5.67 ^{c}	9.50 ^{c}	1.67 ^{c}	4.13 ^{d}	7.02 ^{c}	10.97 ^{c}
SE (d)	0.02	0.08	0.15	0.02	0.04	0.15	0.14
CD	0.05	0.17	0.33	0.04	0.08	0.33	0.29

Significant differences at $p \leq 0.05$ are shown by the mean values having different superscript letters. Data is accumulated from the mean of four replications. Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin

Table 2. Preharvest melatonin application on fruit firmness (kg/cm^2) in papaya

Storage period Treatment	Ambient				Cold				
	Day 0	Day 3	Day 6	Day 9	Day 0	Day 7	Day 14	Day 21	Day 28
T_1	7.75 ^{ab}	7.36 ^{b}	6.66 ^{b}	5.54 ^{e}	7.82 ^{a}	7.48 ^{abc}	7.21 ^{a}	6.81 ^{bc}	5.33 ^{c}
T_2	7.73 ^{ab}	7.21 ^{c}	6.41 ^{c}	5.76 ^{d}	7.84 ^{a}	7.32 ^{c}	6.87 ^{b}	6.60 ^{cd}	5.45 ^{c}
T_3	7.78 ^{a}	7.45 ^{a}	6.83 ^{a}	6.21 ^{b}	7.78 ^{a}	7.59 ^{ab}	7.29 ^{a}	6.97 ^{ab}	6.18 ^{a}
T_4	7.83 ^{a}	7.50 ^{a}	6.94 ^{a}	6.37 ^{a}	7.80 ^{a}	7.66 ^{a}	7.37 ^{a}	7.14 ^{a}	6.26 ^{a}
T_5	7.58 ^{b}	7.00 ^{d}	6.57 ^{bc}	6.00 ^{c}	7.72 ^{a}	7.40 ^{bc}	6.77 ^{b}	6.49 ^{d}	5.90 ^{b}
SE (d)	0.09	0.02	0.07	0.07	0.11	0.11	0.07	0.12	0.06
CD	0.19	0.05	0.16	0.15	0.24	0.25	0.17	0.26	0.13

Mean values show significant differences at $p \leq 0.05$, which have different letters. Data were collected from the mean of four replicates. Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin

respiration rate and it might be the reason for slowing the decline in titratable acidity (0.46 % in ambient storage and 0.34 % in cold storage), which in turn delayed senescence (Fig. 1B) and consequently, one of the co-factors contributing to the buildup of organic acids is respiration.

The TSS: acid ratio is a significant determinant of fruit flavor and taste (38). In the current study, the TSS: acid ratio increased until the climacteric peak, followed by a subsequent decline. TSS: acid ratio of the control fruits was lower than fruits treated with melatonin (Fig. 1C), indicating that delayed ripening resulted in a slower rise in titratable acidity.

Simple sugar accumulation increases as a result of starch breakdown during fruit ripening (39). Glucose and fructose make up the majority of total sugars. In addition, galactose, arabinose, mannose and fucose sugars are derived from trace amounts of non-starch polysaccharides. The control fruits in this study showed a greater tendency to accumulate total sugars than the fruits treated with melatonin (Fig. 1D). Reduced sugar accumulation is a key

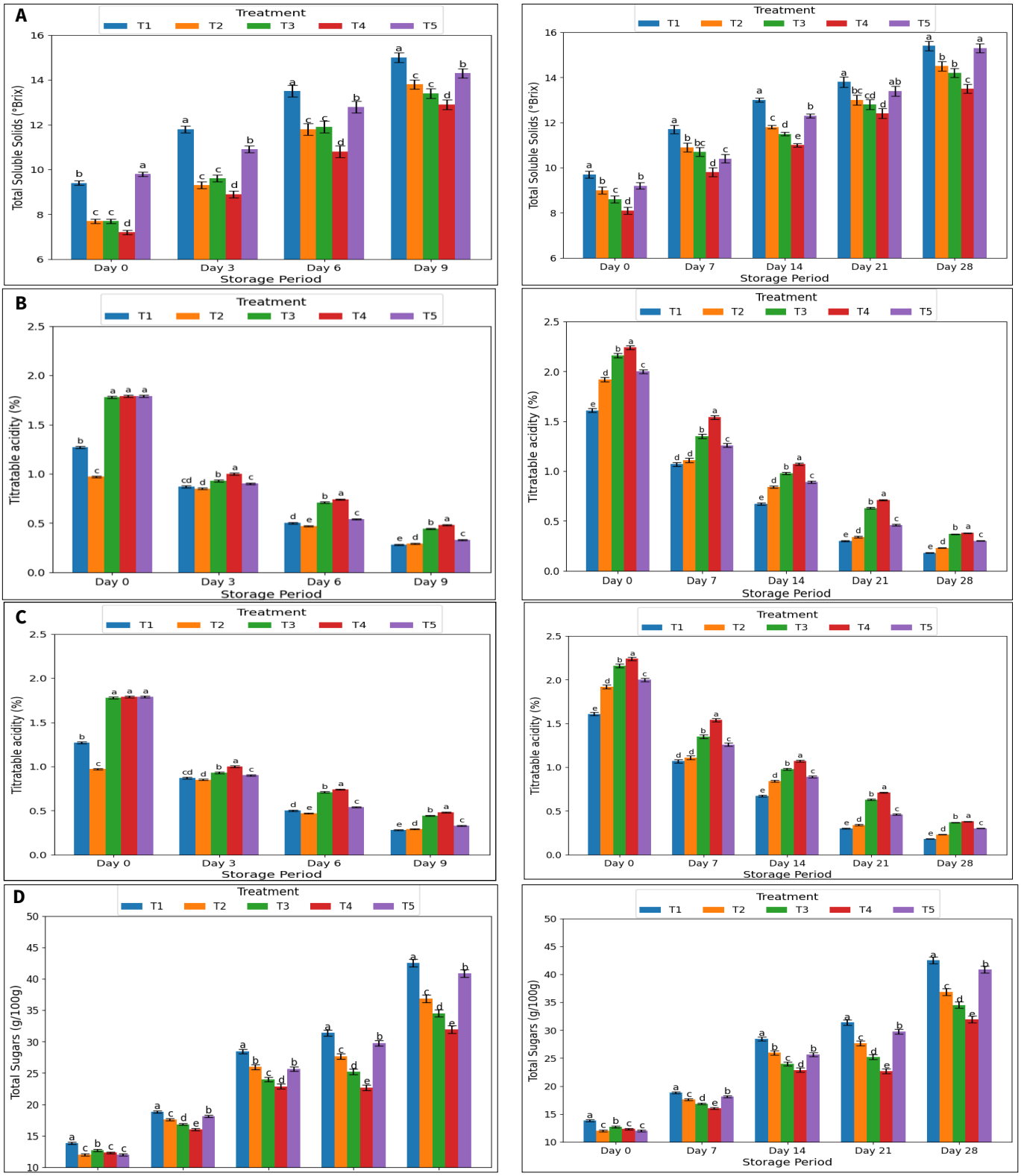

Fig. 1. Preharvest melatonin application on total soluble solids (A) titratable acidity (B) TSS: acid ratio (C) and total sugars (D) in papaya. The standard error of the means ($p < 0.05$) is shown by the bars and the collected data is computed from the mean of four replicates. Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin.

indicator of postharvest ripening and senescence, as it suggests a slower enzymatic activity of amylase and phosphorylase (39). Additionally, consumers' tolerance for sweetness and the flavor of fruit were influenced by sugars, but organic acids increased the amount of sugar in respiratory substrates (9).

One of the critical indicators of fruit nutritional value is total phenolic content, which can be preserved during storage. The findings of this study indicate that exogenous melatonin application led to a steady increase in total phenolic content throughout storage (Fig. 2A), accompanied by a decrease in H_2O_2, O_2- and malondialdehyde (MDA). Recent research suggests that reduced polyphenol oxidase activity and enhanced phenylpropanoid pathway enzyme activity contribute to increased phytochemical accumulation in fresh produce (40). Melatonin has been reported to enhance total phenol content in strawberries (8) and to delay fruit senescence while increasing phenolic compound synthesis in jujube fruits through the upregulation of related genes (41).

Furthermore, melatonin treatment preserved higher ascorbic acid levels throughout storage. In contrast, control fruits exhibited greater ascorbic acid depletion (Fig. 2B). The higher ascorbic acid content in treated fruits suggests slower ripening and reduced consumption of this antioxidant. Ascorbic acid plays a critical role as a co-substrate in ethylene biosynthesis and functions as a co-factor in various metabolic reactions due to its electron-donating properties (42, 43).

The process of fruit softening is closely associated with pectin modification. A specific demethylesterification of homogalacturonan (HGA) is catalyzed by pectin methylesterase, a member of the carbohydrate esterase family, which releases protons and methanol while generating negatively charged carboxyl groups. Following demethylesterification, HGA interacts with Ca^{2+} ions, forming an "egg-box" structure that helps maintain cell wall stiffness and fruit tissue integrity (44). The activity of polygalacturonase, an enzyme involved in pectin breakdown, correlates positively with fruit softening and similar results have been observed in table grape berries (45).

In addition, a group of three cellulase enzymes-endoglucanase, exoglucanase and β-glucosidase-degrades cellulose glycosidic linkages. Endoglucanase randomly breaks down cellulose into oligosaccharides, which are subsequently converted into smaller cello-oligosaccharides by exoglucanase. Finally, β-glucosidase, a rate-limiting enzyme, catalyzes the hydrolysis of cello-oligosaccharides into glucose. Similar enzymatic activities have been previously reported in mangoes (46) and kiwis (47). This study found that melatonin treatment inhibited cell wall-degrading enzymes, such as cellulase and pectin-modifying enzymes, particularly under cold storage conditions (48), providing greater tolerance to chilling stress compared to ambient storage. Control fruits exhibited higher enzymatic activity, with peak enzyme activity occurring earlier than in melatonin-treated fruits (Fig. 3A, B and C). The extended shelf life of melatonin-treated fruits can be attributed to

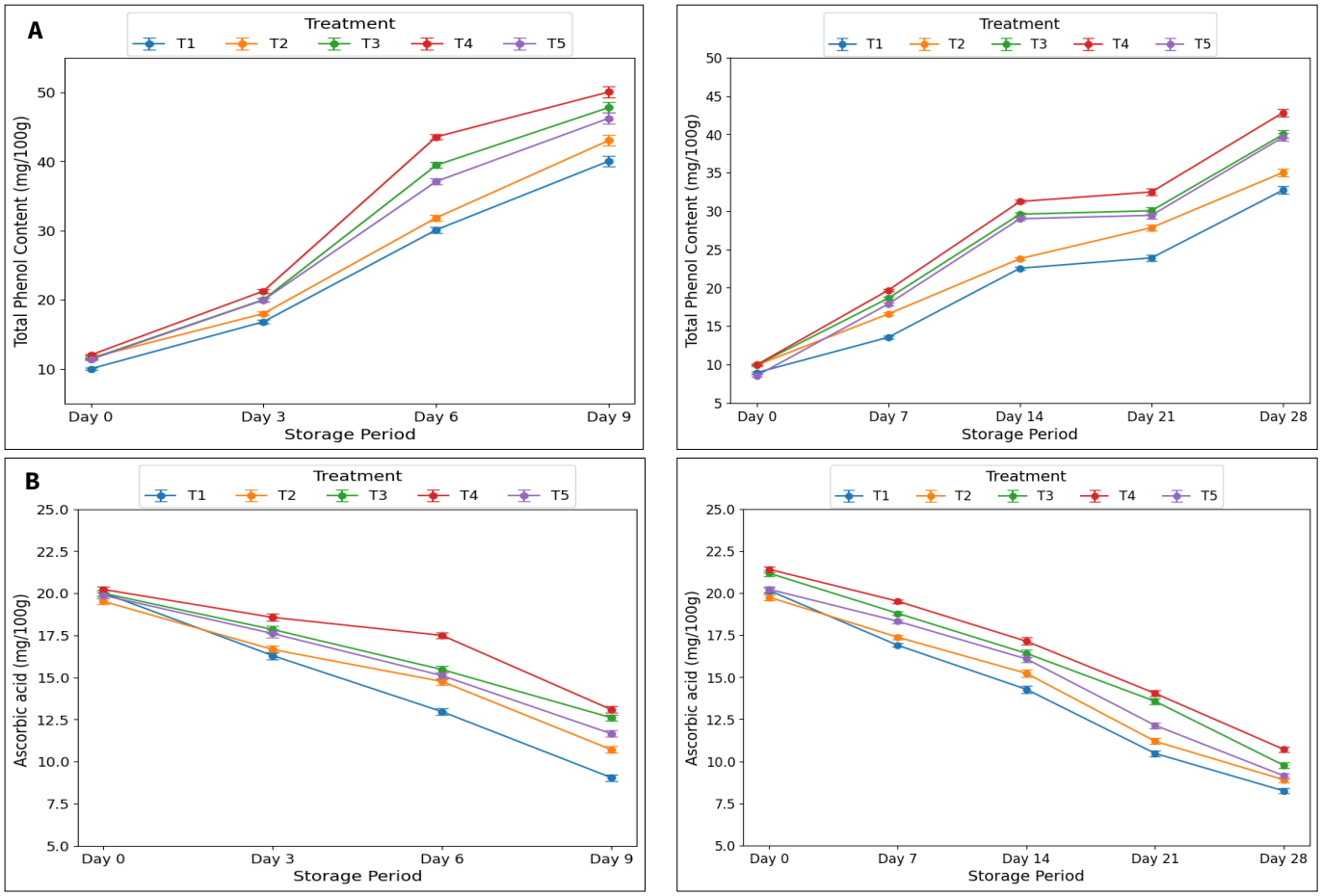

Fig. 2. Effect of EMT treatment on total phenol content (A) and ascorbic acid (B) activity in papaya fruits during ambient and cold storage. Collected data is calculated from the mean of four replicates and the standard error of the means ($p \leq 0.05$) is indicated by the lines. Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin.

reduced enzymatic activity and a delayed peak in enzyme expression. Lipid peroxidation and ROS buildup are regulated by antioxidant enzymes, such as SOD, POD and CAT (6). An increase in antioxidant enzyme activity reduces lipid peroxidation, thereby delaying fruit senescence. The findings (Fig. 4A-C) are consistent with previous studies demonstrating that SOD, POD and CAT activity increased in sweet cherries following exogenous melatonin treatment (EMT) (10). EMT treatment also reduced ROS generation, enhanced antioxidant enzyme activity and inhibited ethylene biosynthesis, which collectively contributed to delayed ripening and protection against chilling injuries under cold storage conditions (49). Similar effects have been reported in pears (32) and bananas (9) during storage.

As a result, following EMT, oxidative stress decreased, which slowed down senescence and prolonged the shelf life of papaya. This study suggests that preharvest application of 1.5 mM melatonin significantly outperformed the control group in maintaining fruit quality. Key parameters such as antioxidant enzyme activity (SOD, CAT, POD), TSS, titratable acidity, TSS: acid ratio, total sugar content, total phenolic content, ascorbic acid levels, physiological weight loss, fruit firmness and ripening enzyme activity (pectin methylesterase, polygalacturonase and cellulase) demonstrated slower degradation under cold storage conditions compared to ambient storage, further supporting the efficacy of melatonin treatment in extending shelf life.

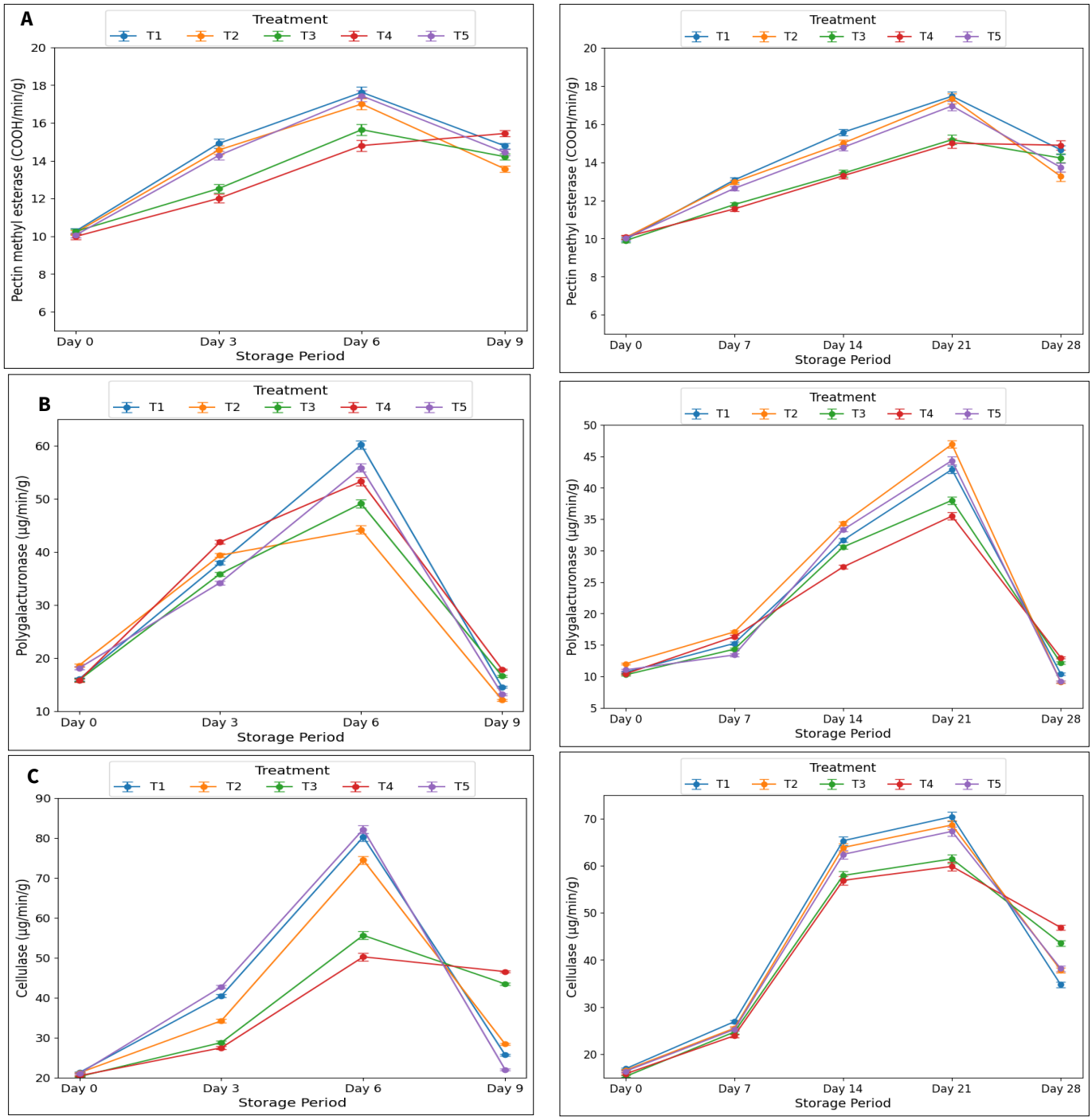

Fig. 3. Preharvest melatonin application on pectin methylesterase (A) polygalacturonase (B) and cellulase (C) activity in papaya.

Data collected is calculated from the mean of four replicates and the standard error of the means ($p \leq 0.05$) is indicated by the lines. Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin.

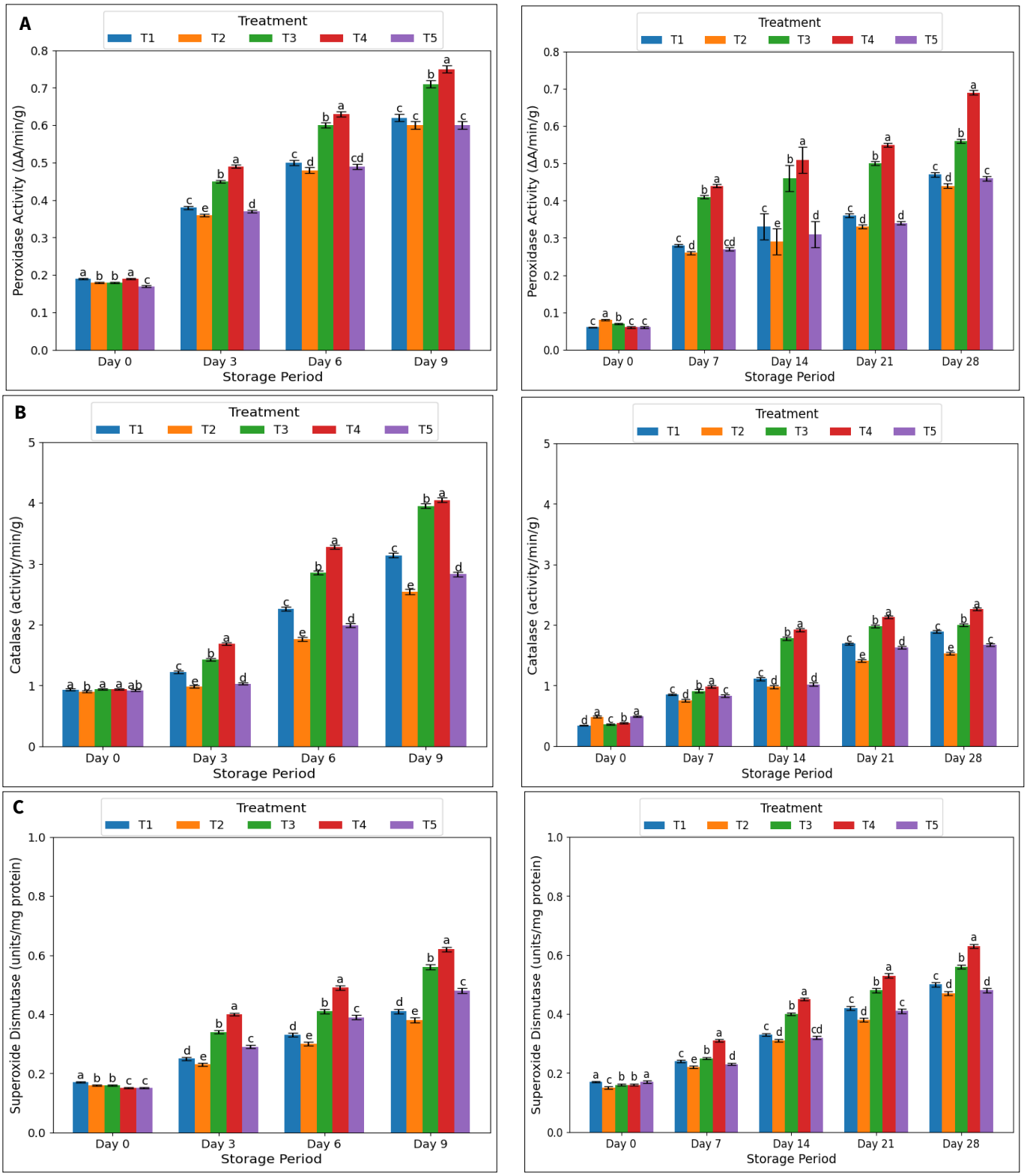

Fig. 4. Preharvest melatonin application on peroxidase (A) catalase (B) and superoxide dismutase (C) activity in papaya.

Four replicates' means are used to calculate the collected data and the bars show the standard error of the means ($p < 0.05$). Treatment details: T_1- Control, T_2- 0.5 mM Melatonin, T_3- 1.0 mM Melatonin, T_4- 1.5 mM Melatonin, T_5- 2.0 mM Melatonin

Conclusion

A preharvest spray of melatonin significantly delayed postharvest senescence, reduced weight loss and enhanced antioxidant enzyme activity under both ambient and cold storage conditions. Additionally, melatonin treatment slowed fruit softening and ripening by regulating cell wall-degrading enzymes and potentially modulating ethylene synthesis in response to CO_2. Fruits from melatonin-treated trees remained fresh for 28 days in cold storage and up to 8 days under ambient conditions, demonstrating the effectiveness of exogenous melatonin treatment (EMT) in extending shelf life, preserving fruit quality and delaying ripening.

Moreover, as an eco-friendly and biodegradable compounds, melatonin aligns with sustainable agricultural practices, providing a safer alternative to synthetic chemicals. Future research could explore various aspects of melatonin application in fruit crops, including its integration into organic farming, its interactions with other biostimulants,

minerals and hormones and the potential for breeding or genetic modification to enhance melatonin levels or responsiveness in crops. Additionally, optimizing administration techniques, such as soil soaking, seed priming and large-scale field applications, as well as assessing commercial viability, could further maximize melatonin's potential in postharvest management.

Acknowledgements

Authors wish to thank to the Tamil Nadu Agricultural University for supporting their research work.

Authors' contributions

PB carried out the formal analysis, investigation, methodology, software, writing original draft. KC conceived of the conceptualization, methodology, resources, funding acquisition, supervision, writing, review and editing. SV looked into the methodology, software, writing review and editing. SA participated in acquiring resources, writing, review and editing. JI participated in writing - review and editing. PK participated in writing, review and editing. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethical Standards

Conflict of interest: The authors declare no conflicts of interest.

Ethical issues: None

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