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Melatonin application extends banana shelf life by delayed ripening

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Abstract

Bananas are among the worlds' most economically significant fruit crops, valued for their distinctive flavour and aroma, despite being highly susceptible to rapid postharvest physiological deterioration. Melatonin, a non-toxic bioactive compound, regulates several plant physiological processes and is widely studied in postharvest management. With this background, the study investigated the effect of different concentrations of melatonin on three banana varieties, namely Grand Naine

'(AAA), Poovan (AAB) and Ney Poovan (AB). The fruits were stored under ambient (AS) (27 ± 2°C) and cold storage (CS) (17°C) with 90-95% relative humidity. Melatonin treatment (1000 µM) effectively reduced weight loss, retained firmness and total soluble solids, reduced ethylene production, enhanced chlorophyll retention and delayed starch degradation. Grand Naine showed the most pronounced effects, followed by Ney Poovan and Poovan. Overall, 1000 µM melatonin was the most effective treatment for extending banana shelf life and maintaining quality under ambient and cold storage conditions.

Keywords

banana; melatonin; ripening; shelf life; storage conditions

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring indoleamine with diverse roles in plant physiological processes (1). Melatonin synthesis starts from the amino acid tryptophan and is produced naturally in various fruit tissues such as peel, pulp and seeds (2, 3). Melatonin is a non-toxic compound involved in multiple physiological processes, including seed and root development, plant growth and flowering (4, 5). It also influences fruit development, ripening, senescence and responses to biotic and abiotic stresses (6-9). Moreover, melatonin is widely studied in postharvest applications as it has efficacy in prolonging storage life and enhancing the quality of fruits and vegetables (10).

Notably, the exogenous application of melatonin delays ethylene production during the ripening process by inhibiting the expression of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzyme activities (11). Melatonin helps maintain several fruit quality attributes such as weight loss, respiration rate, titrable acidity, fruit firmness, soluble sugars, organic acids, carotenoid content and aromatic compounds (12, 13). Studies have shown that exogenous melatonin significantly delays fruit ripening in apples, sweet berries, peaches, pears and mangoes (14-18). It also reduces decay in fruits like kiwifruit(18), litchi(19) and strawberry(20).

Bananas are among tropical and subtropical regions' most widely cultivated and economically essential fruits (21). Bananas are a natural and instant energy source due to their carbohydrate content and important nutrients like vitamin C, B_6, potassium, iron, magnesium and dietary fibre, making them a healthy addition to the diet (22). In India, banana cultivation covers an area of 994.14 thousand hectares ('000 ha) with a production of 366.14 lakh tonnes and a productivity of 36.83 MT ha^{-1} (23). The climacteric nature of bananas causes them to ripen rapidly after harvest, which shortens their postharvest shelf life. Postharvest handling and marketing chain involves significant challenges under various storage conditions, accelerating maturations' physiological and metabolic processes and producing fruit spoilage. Postharvest losses of perishable commodities like fruits and vegetables can reach 20-40%, occurring at various stages such as harvesting, transportation, storage and marketing (24). Notably, delaying the ripening before consumption is a significant challenge for supply chain management in perishable fruits, including bananas (25, 26). During the climacteric phase, bananas change chlorophyll breakdown, cell wall softening and conversion of starch into sugars. These changes affect the fruits' colour, firmness, aroma and shelf life (27).

Optimizing postharvest ripening is crucial for improving banana quality and shelf life, especially for market distribution. During postharvest ripening, there is a notable rise in ethylene production and respiration rate, which coincides with the fruit climacteric phase. Ethylene is an essential gaseous phytohormone linked to the ripening process. It orchestrates the timely expression of genes that govern various ripening and senescence events, including increased respiration, autocatalytic ethylene production and alterations in fruit colour, texture, aroma and flavour. Enzymes such as ACS and ACO play a key role in ethylene biosynthesis, which is triggered during postharvest ripening (21, 28, 29, 30). Ethylene production in climacteric fruits regulates ripening and quality-related processes, while ethylene inhibitors markedly postpone the ripening process (11). However, various postharvest management techniques aim to extend the shelf life of perishable fruits that effectively control ethylene production, maintaining the sensory and nutritional quality of the fruits (24).

Postharvest treatments vary according to regional agricultural practices, local regulations and market demands (25). Fresh fruits are highly perishable and deteriorate rapidly during storage, resulting in reduced taste, nutritional quality, poor marketability and acceptance and leading to economic loss (19). Optimizing quality preservation and extending the postharvest shelf life of fruit represent significant challenges in the fruit industry. Researchers are focused on various postharvest treatments to improve the shelf life of fruits and enhance their quality. Several postharvest technologies have been developed to maintain and extend the shelf life of fresh fruit by targeting ethylene oxidation, inhibiting ethylene action and modulating the ripening process (26).

Melatonin is gaining attention as a promising approach for improving fruit production and prolonging shelf life. Several studies reported that exogenous melatonin application delayed postharvest ripening in various horticultural produce such as apples, pears, peaches, guava and pomegranate (14-16, 27, 28). Melatonin application has shown the potential to enhance disease resistance and reduce deterioration rates in several fruits during storage, including litchi, strawberry and peach (15, 20, 29). However, the use of melatonin in the postharvest management of bananas is limited. Hence, this study aims to elucidate the effect of postharvest application of melatonin on three banana varieties under different storage conditions (ambient and cold). The study aimed to optimize melatonin concentration to extend the shelf life of bananas by modifying morphological, physio-chemical and quality traits.

Materials and Methods

Source of banana and melatonin

Freshly harvested bunches of three banana varieties, Grand Naine (AAA) (V_1), Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3) were sourced from a farmers' field at Thadagam road, Coimbatore (11˚.54'N, 76˚.56'E). The varieties above are widely cultivated and well-known for their high yield and uniform fruit quality. Moreover, the ripening behaviour of these varieties differs slightly, making it valuable for assessing the broad applicability of melatonin treatments. Uniformly sized, healthy banana fruits at the mature green stage (approximately 75 % maturity) were selected for the study. Melatonin (99 % purity) was purchased from Sigma-Aldrich Pvt. Ltd and used for the experiment.

Treatment details

Banana hands were washed with running tap water, immersed in 100 ppm of sodium hypochloride (NaClO) for 2 min and air dried at room temperature. Sodium hypochlorite acts as a disinfectant in the postharvest storage of bananas, preventing microbial decay during storage. Banana hands were tagged and dipped for 3 min in sterile water (control) and different melatonin concentrations with a non-ionic surfactant of 0.05% Tween 20 to reduce the surface tension. The treatment, T_1 - Control (untreated), T_2 - Melatonin at 200 µM, T_3 - Melatonin at 400 µM, T_4 - Melatonin at 600 µM, T_5 - Melatonin at 800 µM and T_6 - Melatonin at 1000 µM. The control and treated fruits were kept in plastic crates with a size of 54 × 35 × 28 cm and stored under ambient (27 ± 2°C) and cold (17°C) storage conditions, one set at each. Morph-physiological, biochemical and quality characteristics were measured at 2-day intervals during storage. The ripening behaviour of the banana was assessed daily and also take the observations on alternate days till the 15th day after treatment. Each replication involved two hands of banana. One hand of banana was used to measure the hand weight and shelf-life longevity, while the other was used to analyze other morpho-physiological, biochemical and quality parameters.

Fruit morphological characteristics

At first, the banana hand, *i.e.,* a cluster of bananas from the same bunch, was selected for each treatment. The hands were separated from the bunches, weighed individually using an electronic balance and expressed in kg hand^{-1}. Fruit firmness was assessed using a penetrometer (TA-XT2 Texture Analyzer, Stable Micro System, UK). The test was conducted on whole bananas at three points: proximal, distal and mid-portion. The maximum force recorded at these points measured firmness, with values expressed in Newton (N).

Fruit physio-chemical and quality characteristics

Ethylene production was measured using a portable analyzer (Bioconservacion, Spain). Bananas were sealed in containers and the ethylene concentration in parts per million (ppm) was determined directly. The rate of ethylene production was expressed as µL kg^{-1} h^{-1}.

Total soluble solids (TSS) were determined from the clear juice filtrate using a handheld refractometer, with results expressed in °Brix. Chlorophyll content was quantified using the DMSO (Dimethyl sulfoxide) extraction method described by Hiscox and Israelstam (30). About 0.5 g of banana peel was subjected to DMSO extraction by incubating it in darkness for 12 h. Then, the volume was made up to 10 mL with DMSO. Total chlorophyll content was determined by measuring absorbance at 652 nm using a UV-1900i spectrophotometer (Shimadzu, UK) and expressed as µg g^{-1} fresh tissue and calculated using the following Equation 1.

$$\text{Total chlorophyll} = \frac{\text{O.D. Value at 652}}{34.5} \times \frac{V}{1000} \qquad \text{(Eqn. 1)}$$

Where, V=Volume of the extract, W=Fresh weight in grams

The starch-iodine staining method was determined to assess starch conversion to sugars during the ripening process. A staining solution containing 1 % potassium iodide and 0.25 % iodine was prepared. Bananas were sliced transversely at the midpoint into 2-3 cm thick sections, including the peel. The cut surfaces of the banana sections were immersed in the starch-iodine solution for 5 seconds. The resulting starch patterns were analyzed by comparing them to a starch-iodine staining chart specific to bananas (31, 32).

Postharvest shelf life of fruit

The shelf life of banana fruit was determined by measuring the interval from the first day of storage to the end of the fruits' edible life, as observed visually after the applied treatment. Maturity indices for each fruit were assessed by comparing the peel colour to a standard colour chart and expressed in days (33).

Statistical analysis

The experimental design was under a Factorial Completely Randomized Design (FCRD). Data are expressed as mean ± standard error of the mean. Mean comparisons were performed using the least significant difference (LSD) method and analysis of variance (ANOVA) with a substantial level of 0.05 was calculated using R software (version 4.3.1) for data analysis and bar diagrams were visualized using Microsoft Excel 2010.

Results and Discussion

Effect of postharvest melatonin treatment on hand weight

Significant differences ($p \leq 0.05$) in hand weight were observed between control and treated fruits under two different storage conditions. The results showed a gradual reduction in hand weight during storage. Under ambient conditions, the maximum weight loss was observed in the control (T_1), with 23.07%, 32.71% and 21.05% recorded for V_1, V_2 and V_3 respectively. In contrast, bananas treated with 1000 µM of melatonin (T_6) had the lowest weight loss with 21.07%, 22.62% and 15.74% for V_1, V_2 and V_3 respectively (Table 1). Under cold storage, fruits treated with melatonin at 1000 µM (T_6) depicted a minimum reduction of hand weight of 8.73, 15.78 and 7.34 % in V_1, V_2 and V_3 compared to control (T_1) (12.25 V_1, 22.46 V_2 and 8.52 % V_3) on 15th day of observation (Table 2). Postharvest application of melatonin at 1000 and 1500 µM significantly diminished weight loss across different banana varieties. In contrast, untreated fruits experienced more significant weight loss, likely due to elevated respiration and transpiration rates. Melatonin treatment postpones the climacteric peak, thus attenuating metabolic activity and reducing weight loss during ripening (34). Similar findings have shown that melatonin prevents weight loss in apple, orange, blueberry and nectarine compared to untreated fruits (14, 35-37).

Effect of postharvest melatonin treatment on firmness and total soluble solids

Statistically significant differences ($p \leq 0.05$) in fruit firmness were observed among the treatments, varieties and their interactions. During the ripening phase, fruit firmness progressively decreased under both storage conditions. Grand Naine treated with melatonin at 1000 µM maintained fruit firmness of 13.85 Newton (V_1T_6) than the untreated fruits of 5.65 Newton (V_1T_1) on the 9th day of ambient storage condition (Fig. 1). Under cold storage conditions, fruits treated with 1000 µM melatonin (T_6) showed a minor reduction in firmness compared to the control. By the 15th day, firmness values were 20.85 to 4.30 N for Grand Naine, 20.15 to 1.62 N for Poovan and 19.10 to 1.73 N for Ney Poovan (Fig. 2). Similar result was reported by Hu *et al.* (11), who found that melatonin-treated fruits showed retention of fruit firmness in bananas, it might be due to repression of ethylene production during postharvest banana ripening. Exogenous melatonin application lowers ethylene production and respiration rates, reducing CO_2 levels. This prevents the build-up of acetaldehyde and ethanol, thereby preserving fruit firmness and strengthening the fruit skin, enhancing postharvest quality (38, 39).

Total soluble solids (TSS) are crucial for assessing fruit maturity and nutrient richness, serving as a key indicator of fruit quality and influencing economic value in the fruit trade. Bananas treated with 1000 µM melatonin (T_6) showed lower TSS values (19.3, 24.8 and 24.7 °Brix for Grand

Table 1. Effect of melatonin on hand weight (kg) in different banana varieties under ambient storage conditions

Treatment details	Ambient storage (27±2°C)					
	1st day	3rd day	5th day	7th day	9th day	Mean
V_1T_1	3.172 ± 0.05^{a}	2.796 ± 0.04^{a}	2.666 ± 0.04^{a}	2.508 ± 0.04^{a}	2.440 ± 0.04^{a}	2.716
V_1T_2	2.155 ± 0.03^{d}	1.903 ± 0.03^{e}	1.818 ± 0.03^{e}	1.719 ± 0.03^{e}	1.667 ± 0.03^{d}	1.834
V_1T_3	2.442 ± 0.03^{c}	2.294 ± 0.03^{c}	2.200 ± 0.02^{c}	2.098 ± 0.02^{c}	2.044 ± 0.02^{c}	2.216
V_1T_4	2.776 ± 0.04^{b}	2.456 ± 0.04^{b}	2.356 ± 0.03^{b}	2.236 ± 0.03^{b}	2.164 ± 0.03^{b}	2.398
V_1T_5	2.416 ± 0.04^{c}	2.288 ± 0.03^{c}	2.192 ± 0.03^{c}	2.090 ± 0.03^{c}	2.006 ± 0.03^{c}	2.198
V_1T_6	1.784 ± 0.03^{ef}	1.566 ± 0.03^{h}	1.504 ± 0.03^{f}	1.426 ± 0.03^{f}	1.408 ± 0.03^{ef}	1.538
V_2T_1	2.152 ± 0.03^{d}	2.068 ± 0.03^{d}	1.938 ± 0.03^{d}	1.786 ± 0.03^{d}	1.448 ± 0.02^{e}	1.878
V_2T_2	1.735 ± 0.03^{f}	1.610 ± 0.03^{gh}	1.260 ± 0.02^{g}	1.170 ± 0.02^{g}	1.052 ± 0.02^{h}	1.352
V_2T_3	1.804 ± 0.02^{ef}	1.702 ± 0.02^{f}	1.564 ± 0.02^{f}	1.412 ± 0.02^{f}	1.182 ± 0.01^{g}	1.533
V_2T_4	1.628 ± 0.02^{g}	1.432 ± 0.02^{i}	1.326 ± 0.02^{g}	1.210 ± 0.02^{g}	1.174 ± 0.02^{g}	1.354
V_2T_5	1.830 ± 0.03^{e}	1.678 ± 0.03^{fg}	1.548 ± 0.02^{f}	1.432 ± 0.02^{f}	1.370 ± 0.02^{f}	1.572
V_2T_6	1.786 ± 0.03^{ef}	1.666 ± 0.01^{fg}	1.550 ± 0.01^{f}	1.436 ± 0.03^{f}	1.382 ± 0.03^{f}	1.564
V_3T_1	0.912 ± 0.01^{hi}	0.840 ± 0.01^{k}	0.798 ± 0.01^{i}	0.750 ± 0.01^{i}	0.720 ± 0.01^{j}	0.804
V_3T_2	0.850 ± 0.01^{ij}	0.798 ± 0.01^{kl}	0.768 ± 0.01^{i}	0.687 ± 0.01^{jk}	0.661 ± 0.01^{kl}	0.745
V_3T_3	0.966 ± 0.01^{h}	0.918 ± 0.01^{j}	0.888 ± 0.01^{h}	0.858 ± 0.01^{h}	0.828 ± 0.01^{i}	0.892
V_3T_4	0.714 ± 0.01^{k}	0.662 ± 0.01^{n}	0.660 ± 0.01^{j}	0.630 ± 0.01^{kl}	0.584 ± 0.01^{m}	0.650
V_3T_5	0.804 ± 0.01^{j}	0.738 ± 0.01^{lm}	0.734 ± 0.01^{i}	0.704 ± 0.01^{ij}	0.682 ± 0.01^{jk}	0.732
V_3T_6	0.724 ± 0.01^{k}	0.676 ± 0.01^{mn}	0.648 ± 0.01^{j}	0.622 ± 0.01^{l}	0.610 ± 0.01^{lm}	0.656
Mean	1.703	1.561	1.468	1.376	1.301	
SE.d	0.039	0.035	0.033	0.031	0.030	
CD (0.05)	0.078	0.071	0.067	0.062	0.059	

V_1 - Grand Naine (AAA); V_2 - Poovan (AAB); V_3 - Ney Poovan (AB); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 -Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM. Treatment groups are scaled from 1 to 10 on different storage conditions. The data shown was one of the four replicates.

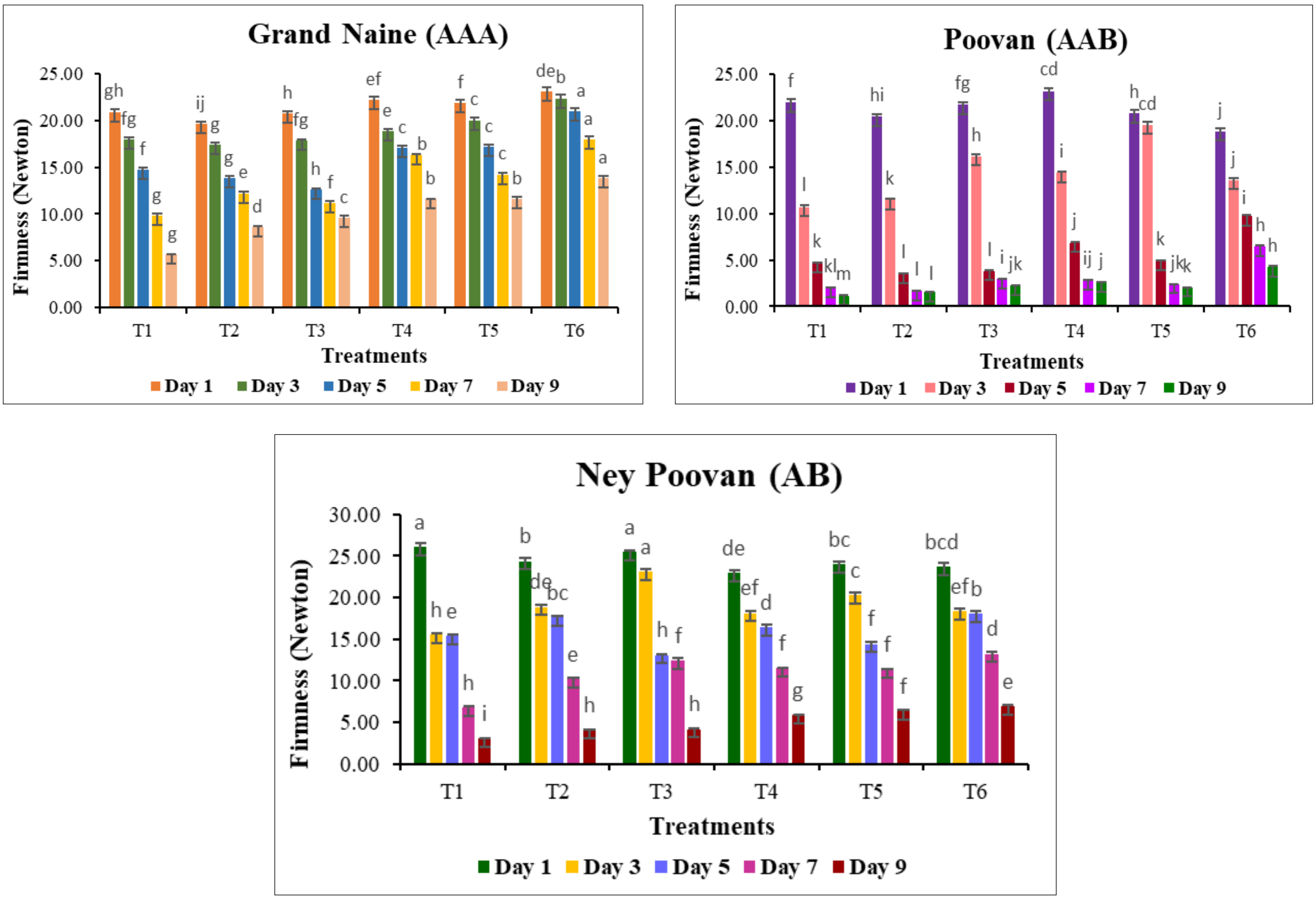

Fig 1. Effect of postharvest melatonin treatment on fruit firmness (Newton) at ambient storage (27±2°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1- Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

Table 2. Effect of melatonin on hand weight (kg) in different banana varieties under cold storage conditions

Treatment details	Cold storage (17 °C)								
	1st day	3rd day	5th day	7th day	9th day	11th day	13th day	15th day	Mean
V_1T_1	2.954 ± 0.04^{a}	2.768 ± 0.04^{b}	2.744 ± 0.04^{b}	2.712 ± 0.04^{b}	2.682 ± 0.04^{b}	2.654 ± 0.04^{b}	2.628 ± 0.04^{b}	2.592 ± 0.02^{b}	2.717
V_1T_2	2.668 ± 0.04^{c}	2.490 ± 0.04^{c}	2.470 ± 0.04^{c}	2.444 ± 0.04^{c}	2.414 ± 0.04^{c}	2.396 ± 0.04^{c}	2.374 ± 0.04^{c}	2.342 ± 0.02^{c}	2.450
V_1T_3	3.096 ± 0.03^{a}	3.060 ± 0.03^{a}	3.034 ± 0.03^{a}	3.006 ± 0.03^{a}	2.970 ± 0.03^{a}	2.940 ± 0.03^{a}	2.906 ± 0.03^{a}	2.867 ± 0.02^{a}	2.985
V_1T_4	2.272 ± 0.03^{e}	2.238 ± 0.03^{de}	2.218 ± 0.03^{de}	2.192 ± 0.03$_{d}$	2.160 ± 0.03^{d}	2.136 ± 0.03^{d}	2.108 ± 0.03^{d}	2.076 ± 0.02^{d}	2.175
V_1T_5	2.482 ± 0.04^{d}	2.448 ± 0.04^{c}	2.424 ± 0.04^{c}	2.396 ± 0.04^{c}	2.362 ± 0.03^{c}	2.338 ± 0.03^{c}	2.312 ± 0.03^{c}	2.278 ± 0.02^{c}	2.380
V_1T_6	1.968 ± 0.04^{f}	1.940 ± 0.04^{f}	1.920 ± 0.04^{f}	1.898 ± 0.04^{e}	1.868 ± 0.04^{f}	1.848 ± 0.03^{f}	1.822 ± 0.03^{f}	1.796 ± 0.02^{e}	1.883
V_2T_1	2.288 ± 0.03^{e}	2.250 ± 0.03^{d}	2.230 ± 0.03^{d}	2.198 ± 0.03^{d}	2.170 ± 0.03^{d}	2.140 ± 0.03^{d}	2.106 ± 0.03^{d}	1.774 ± 0.02^{e}	2.145
V_2T_2	1.934 ± 0.03^{f}	1.788 ± 0.03^{g}	1.776 ± 0.03^{g}	1.654 ± 0.02^{h}	1.510 ± 0.02^{h}	1.494 ± 0.02^{h}	1.476 ± 0.02^{i}	1.458 ± 0.01^{g}	1.636
V_2T_3	1.888 ± 0.02^{f}	1.626 ± 0.02^{h}	1.610 ± 0.02^{h}	1.582 ± 0.02^{h}	1.556 ± 0.02^{h}	1.534 ± 0.02^{h}	1.520 ± 0.02^{i}	1.426 ± 0.01^{gh}	1.593
V_2T_4	2.292 ± 0.03^{e}	2.168 ± 0.03^{e}	2.150 ± 0.03^{e}	2.122 ± 0.03^{d}	2.076 ± 0.03^{e}	2.054 ± 0.03^{e}	2.030 ± 0.03^{e}	1.842 ± 0.02^{e}	2.092
V_2T_5	1.802 ± 0.03^{g}	1.784 ± 0.03^{g}	1.770 ± 0.03^{g}	1.742 ± 0.03^{g}	1.702 ± 0.03^{g}	1.674 ± 0.02^{g}	1.606 ± 0.02^{h}	1.363 ± 0.01^{h}	1.680
V_2T_6	1.900 ± 0.04^{f}	1.870 ± 0.04^{f}	1.838 ± 0.03^{g}	1.820 ± 0.03^{f}	1.794 ± 0.03^{f}	1.774 ± 0.03^{f}	1.720 ± 0.03^{g}	1.600 ± 0.02^{f}	1.790
V_3T_1	1.002 ± 0.01^{h}	0.990 ± 0.01^{i}	0.982 ± 0.01^{i}	0.970 ± 0.01^{i}	0.952 ± 0.01^{i}	0.944 ± 0.01^{i}	0.934 ± 0.01^{j}	0.916 ± 0.01^{i}	0.961
V_3T_2	0.806 ± 0.01^{ij}	0.798 ± 0.01^{jk}	0.788 ± 0.01^{jk}	0.780 ± 0.01^{jk}	0.764 ± 0.01^{k}	0.754 ± 0.01^{k}	0.742 ± 0.01^{l}	0.730 ± 0.01^{k}	0.770
V_3T_3	0.872 ± 0.01^{i}	0.866 ± 0.01^{j}	0.862 ± 0.01^{j}	0.856 ± 0.01^{j}	0.844 ± 0.01^{j}	0.836 ± 0.01^{j}	0.824 ± 0.01^{k}	0.808 ± 0.00^{j}	0.846
V_3T_4	0.678 ± 0.01^{k}	0.670 ± 0.01^{l}	0.668 ± 0.01^{l}	0.660 ± 0.01^{l}	0.654 ± 0.01^{l}	0.646 ± 0.01^{l}	0.634 ± 0.01^{m}	0.618 ± 0.00^{l}	0.654
V_3T_5	0.712 ± 0.01^{k}	0.706 ± 0.01^{l}	0.700 ± 0.01^{l}	0.698 ± 0.01^{l}	0.690 ± 0.01^{kl}	0.682 ± 0.01^{kl}	0.670 ± 0.01^{lm}	0.658 ± 0.01^{l}	0.690
V_3T_6	0.736 ± 0.01^{jk}	0.728 ± 0.01^{kl}	0.724 ± 0.01^{kl}	0.716 ± 0.01^{kl}	0.708 ± 0.01^{kl}	0.704 ± 0.01^{kl}	0.696 ± 0.01^{lm}	0.682 ± 0.01^{kl}	0.712
Mean	1.797	1.733	1.717	1.691	1.660	1.642	1.617	1.546	
SE.d	0.039	0.039	0.039	0.038	0.037	0.038	0.036	0.035	
CD (0.05)	0.077	0.077	0.078	0.076	0.075	0.074	0.073	0.070	

V_1 - Grand Naine (AAB); V_2 - Poovan (AAB); V_3 - Ney Poovan (AB); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM. Treatment groups are scaled from 1 to 10 on different storage conditions. The data shown was one of the four replicates.

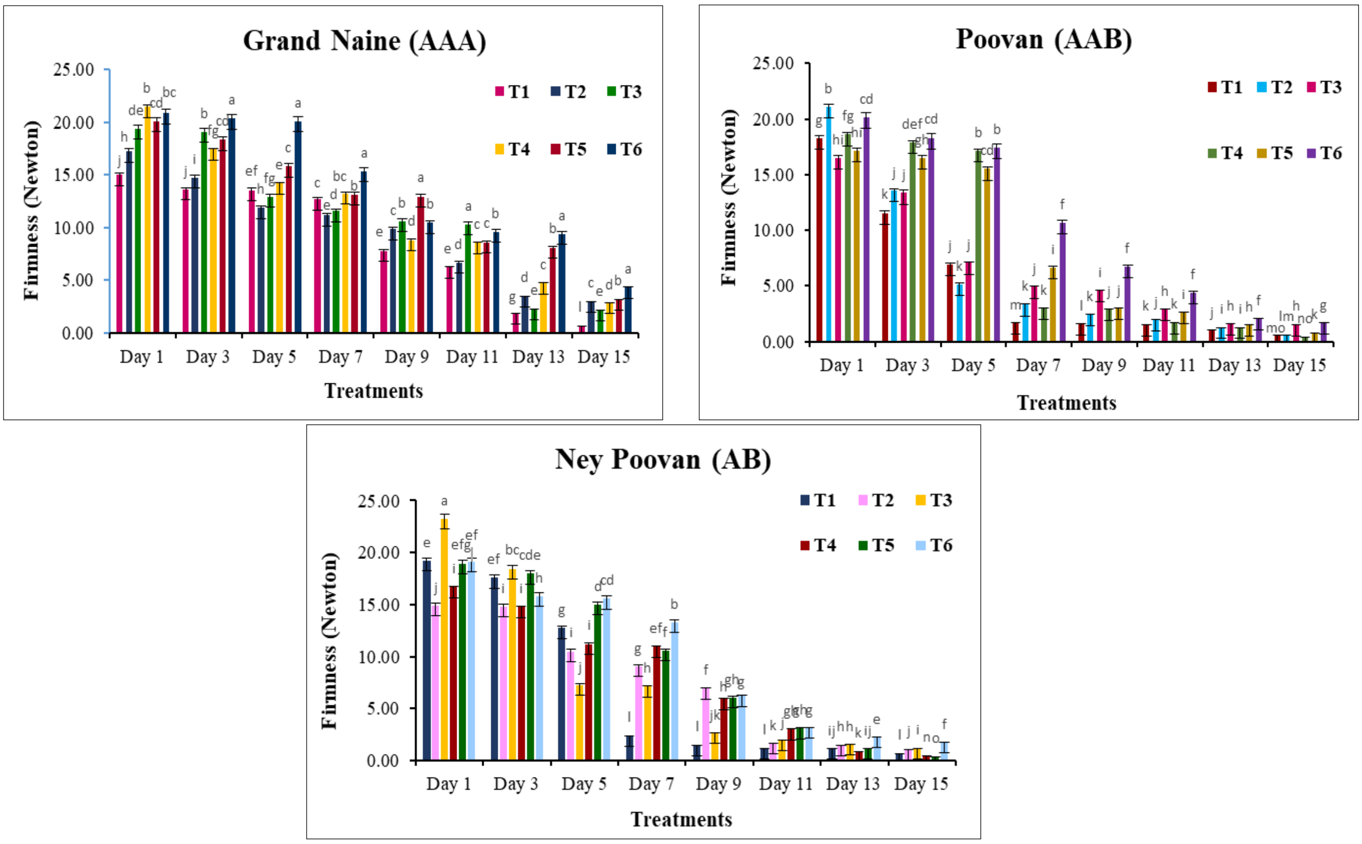

Fig 2. Effect of postharvest melatonin treatment on fruit firmness (Newton) at cold storage (17°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1- Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

Naine, Poovan and Ney Poovan, respectively) compared to the control (T_1: 24.8, 28.5 and 28.8 ˚Brix) on the 9th day under ambient storage conditions (Fig. 3). At 15th day of cold storage, Grand Naine treated with 1000 µM of melatonin (V_1T_6) showed lower TSS values (4.2 to 22.0 ˚Brix) compared to the control (V_1T_1: 7.4 to 24.2 ˚Brix), followed by Ney Poovan and Poovan (Fig. 4). Consistent with the current findings, Bal (34) revealed that melatonin treated at 1000 µM L^{-1} concentration decreased the TSS content in plum. As a climacteric fruit, accelerated ripening and increased respiration convert complex carbohydrates into simple sugars, primarily glucose and fructose, which contribute to an increase in peachs' total soluble solids (TSS) (40).

Effect of postharvest melatonin treatment on ethylene evolution

The data showed that ethylene production was significantly increased over the storage period across all the treatments. The climacteric peak of ethylene production was observed on day 7 and day 11 of ambient and cold storage conditions, consistent across all varieties. Among the three varieties, Grand Naine (V_1) displayed lower production of ethylene (0.78 µL kg^{-1} h^{-1}) throughout the storage period compared to Poovan (V_2: 1.04 µL kg^{-1} h^{-1}) and Ney Poovan (V_3: 0.89 µL kg^{-1} h^{-1}). Melatonin treatment at 1000 µM significantly reduced the ethylene production peak in all three varieties: V_1T_6 (0.59 µL kg^{-1} h^{-1}), V_2T_6 (0.80 µL kg^{-1} h^{-1}) and V_3T_6 (0.62 µL kg^{-1} h^{-1}) compared to the control (Fig. 5). Fruits stored under cold condition exhibited a gradual increase in ethylene production throughout the observation period. Dipping fruits in melatonin at 1000 µM resulted in the lowest ethylene production in Grand Naine (V_1T_6: 0.47 µL kg^{-1} h^{-1}), Poovan (V_2T_6: 0.87 µL kg^{-1} h^{-1}) and Ney Poovan (V_3T_6: 0.68 µL kg^{-1} h^{-1}) compared to the control (V_1T_1: 1.08; V_2T_1: 1.17 and V_3T_1: 1.12 µL kg^{-1} h^{-1}) at the end of cold storage condition (Fig. 6). Melatonin treatment has been shown to delay and suppress climacteric ethylene production and ACC accumulation, which aligns with melatonin mediated reductions in the activities of ACS and ACO in mango (17). Exogenous melatonin treatment has been reported to reduce ethylene production and inhibit the expression of ACS and ACO in banana and pear ripened under ambient storage conditions (11, 41).

Effect of postharvest melatonin treatment on chlorophyll

Chlorophyll content was decreased progressively during the ripening period under both storage conditions. Ambient-stored fruits showed a more rapid decline in chlorophyll than cold conditions across all three varieties. On the 9th day of observation, melatonin-treated fruits (T_6-1000 µM) retained higher total chlorophyll content in Grand Naine 18.52 µg g^{-1} followed by Ney Poovan (11.30 µg g^{-1}) and Poovan (10.43 µg g^{-1}) compared to untreated. Compared to control, melatonin-treated fruits recorded a lower reduction of total chlorophyll content among all three varieties (Fig. 7).

Grand Naine retained chlorophyll content for a more extended period in melatonin-treated fruits (V_1T_6: 9.41 µg g^{-1}) of total chlorophyll followed by Ney Poovan (V_3T_6: 4.64 µg g^{-1}) and Poovan (V_2T_6: 4.43 µg g^{-1}) than control on 15th day of cold condition (Fig. 8). Dipping lettuce in melatonin at concentrations of 100 and 1000 µmol L^{-1} effectively delayed the decrease in total chlorophyll content compared to the control (42). Similarly, Broccoli treated with 100 µmol L^{-1} melatonin maintained 24.15 %

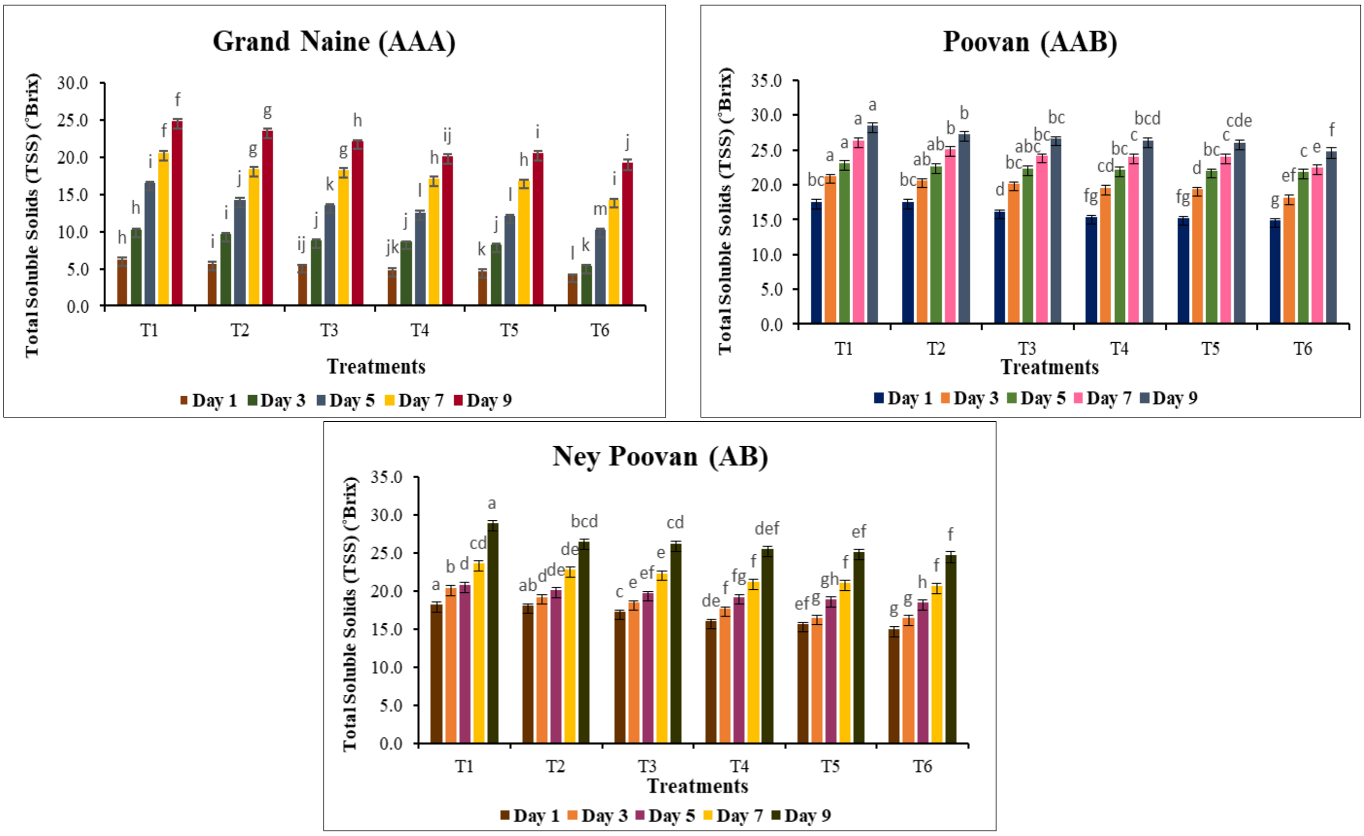

Fig 3. Effect of postharvest melatonin treatment on total soluble solids (˚Brix) at ambient storage (27±2°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1- Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

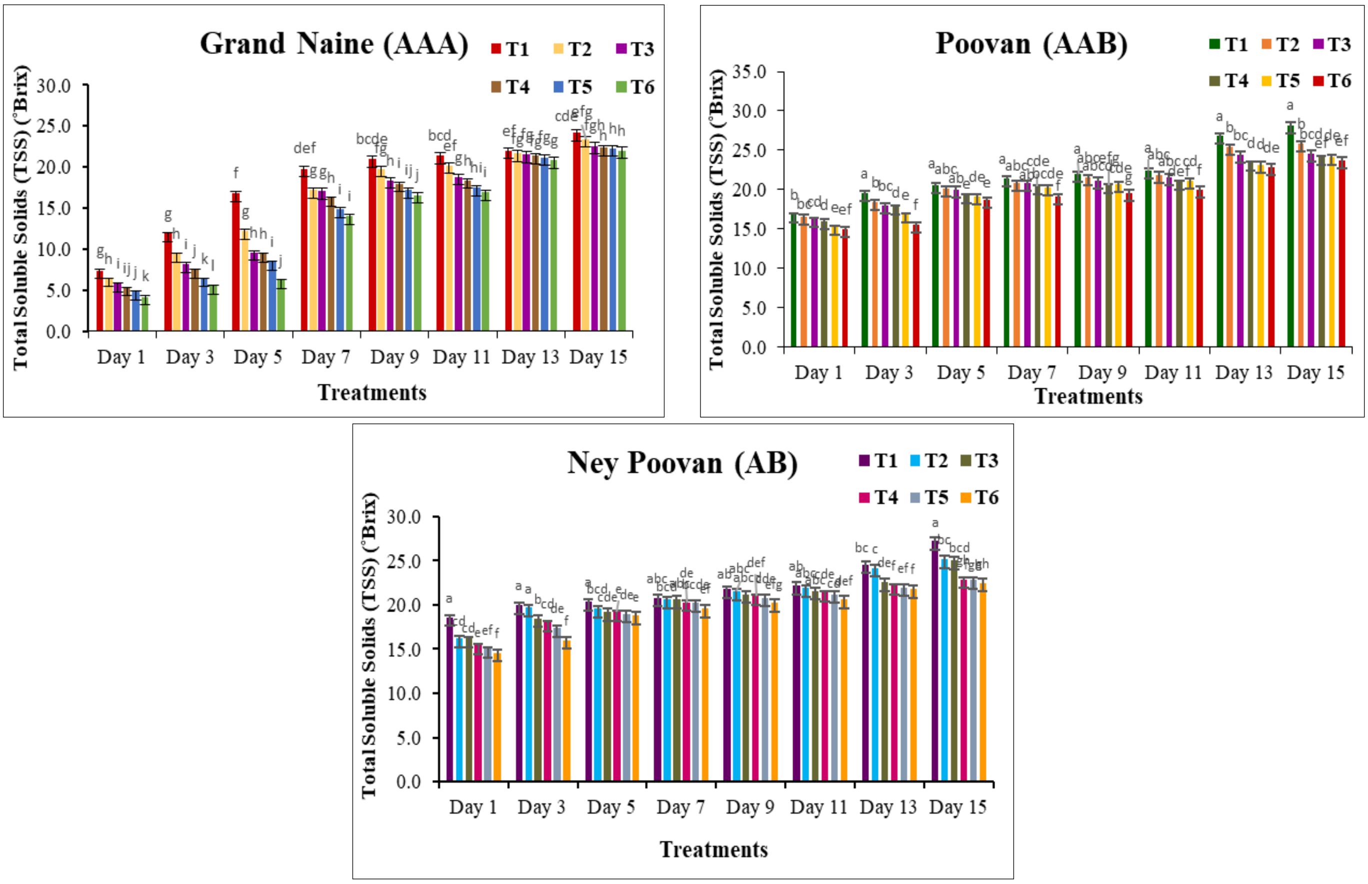

Fig. 4. Effect of postharvest melatonin treatment on total soluble solids (˚Brix) at cold storage (17°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

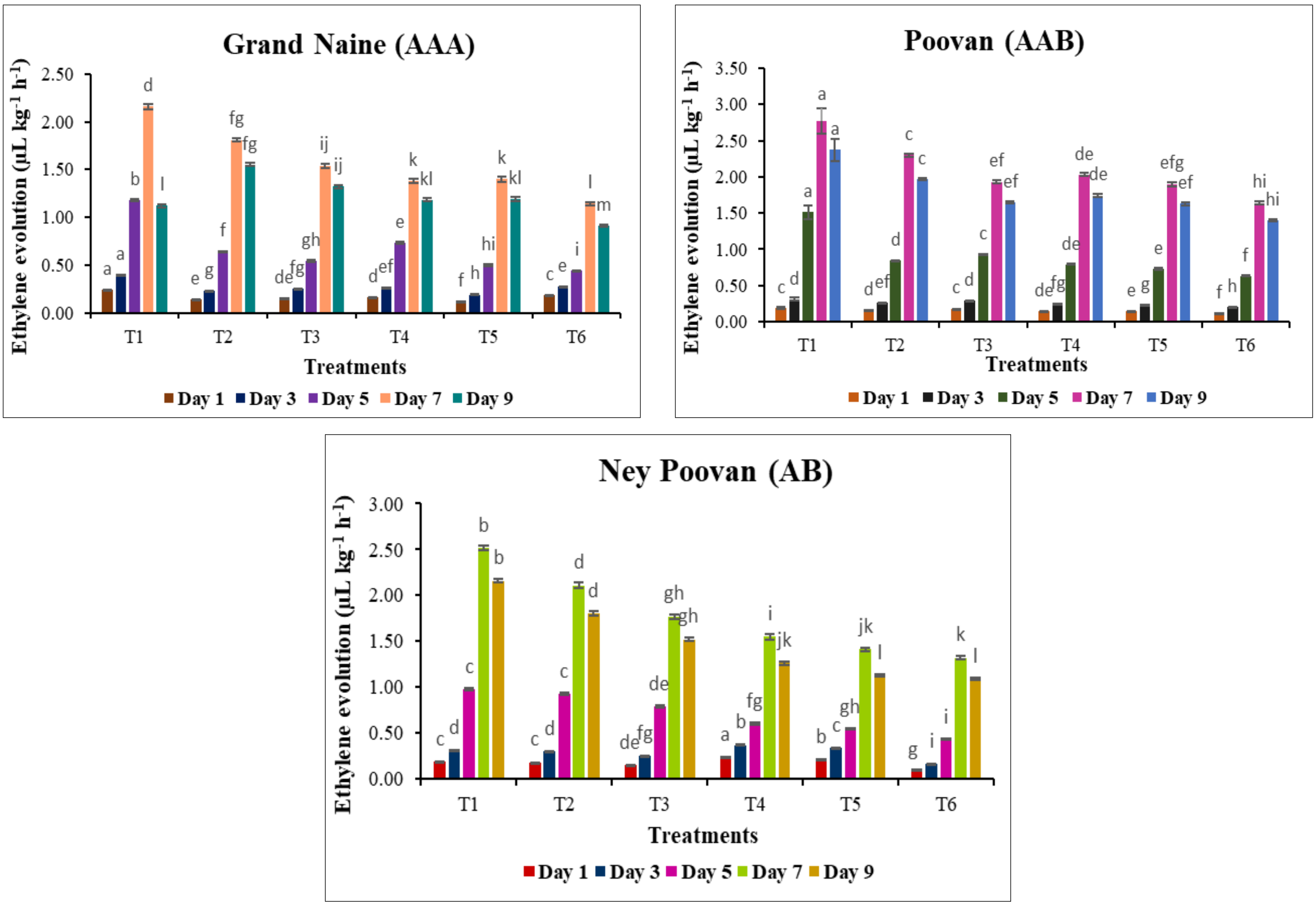

Fig. 5. Effect of postharvest melatonin treatment on ethylene evolution (µL kg^{-1} h^{-1}) at ambient storage (27±2°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

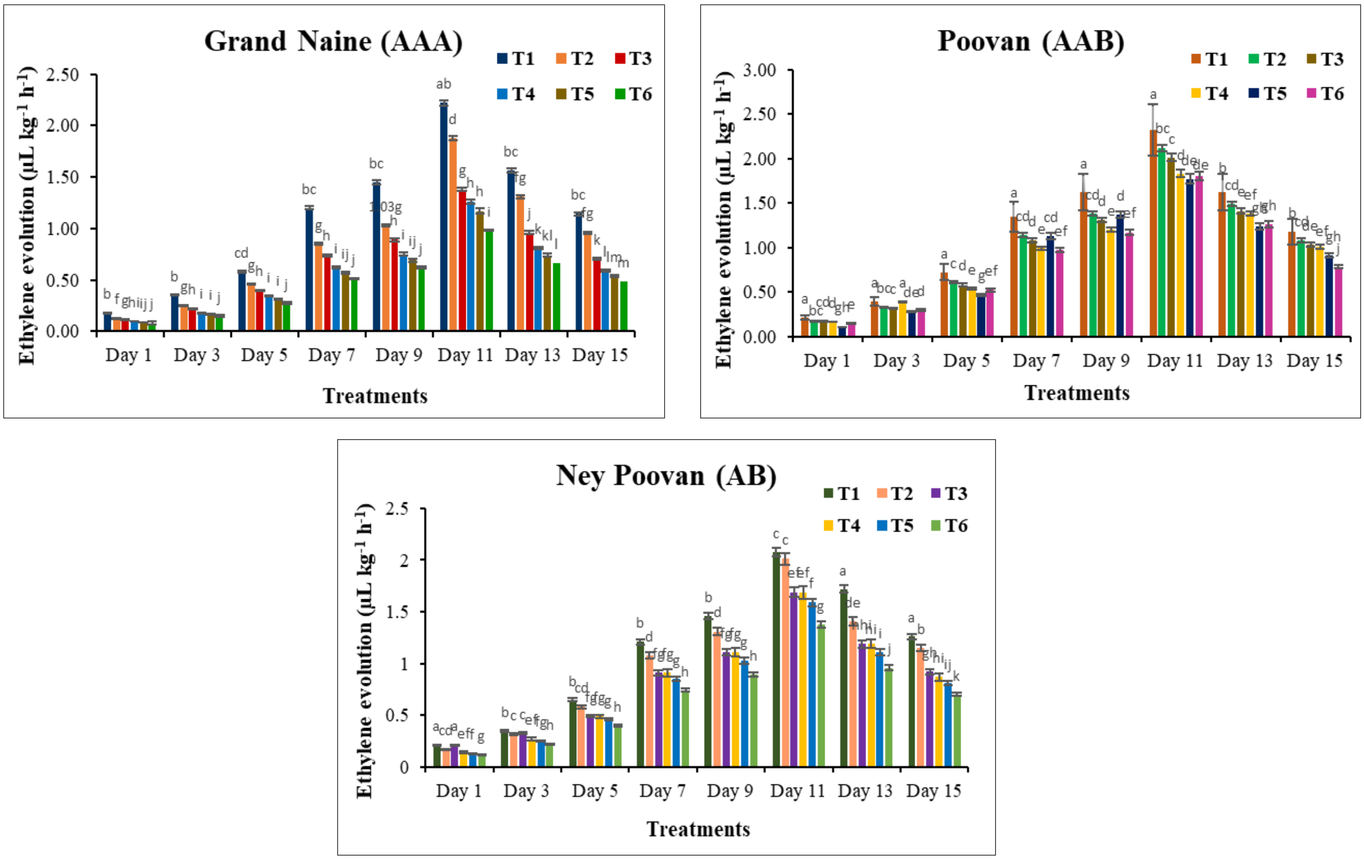

Fig. 6. Effect of postharvest melatonin treatment on ethylene evolution (µL kg^{-1} h^{-1}) at cold storage (17°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

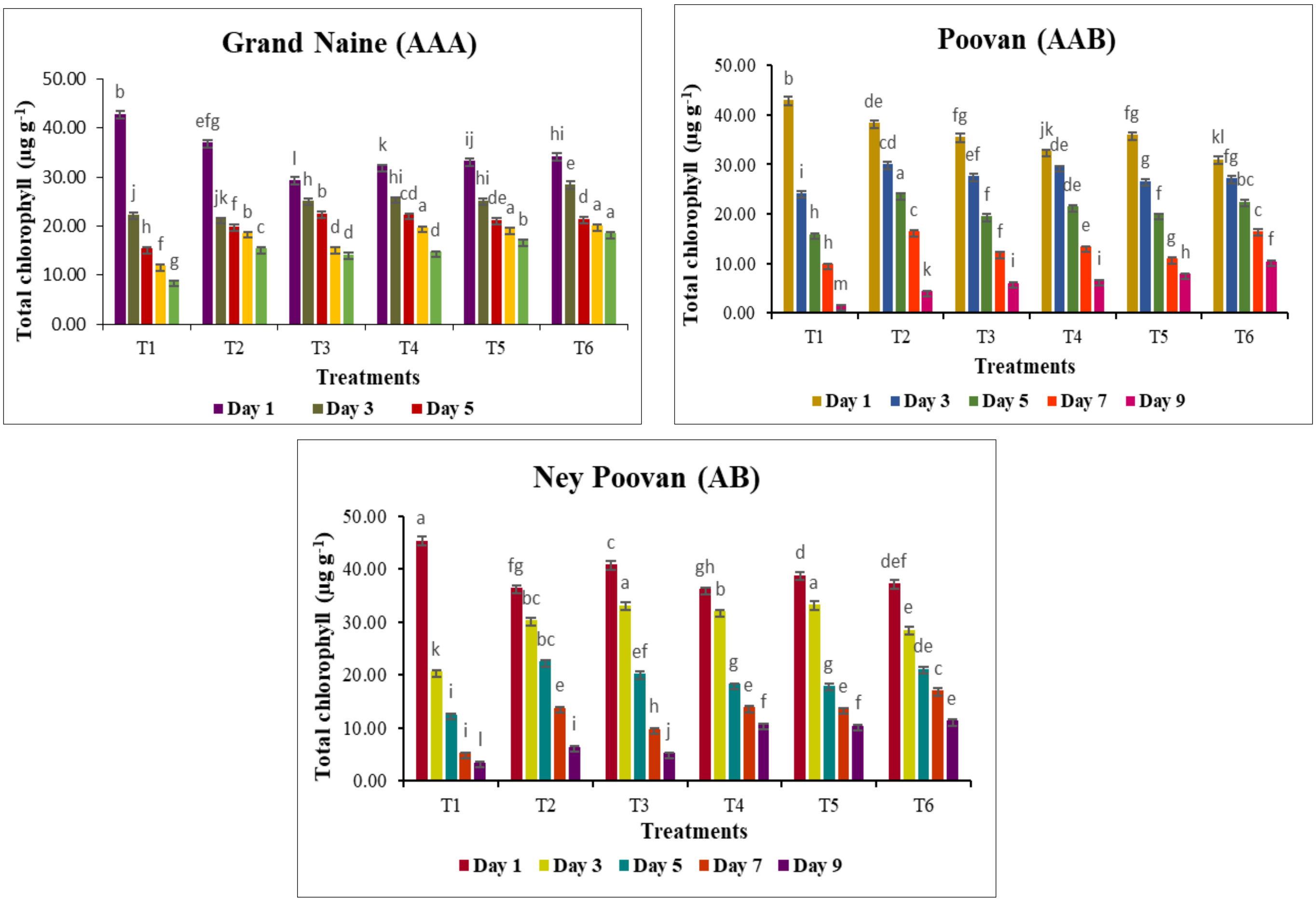

Fig. 7. Effect of postharvest melatonin treatment on total chlorophyll (µg g^{-1}) at ambient storage (27±2°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

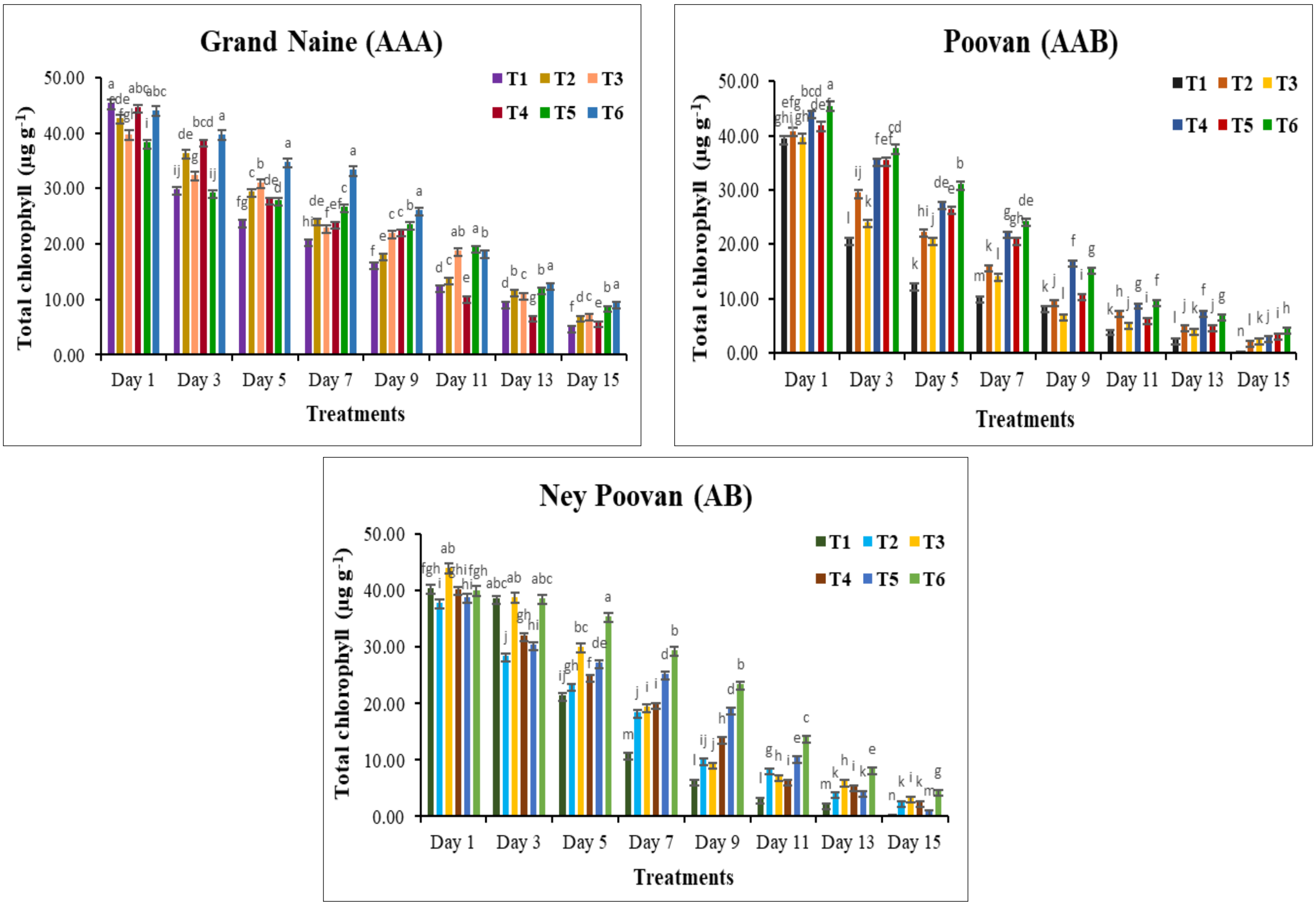

Fig. 8. Effect of postharvest melatonin treatment on total chlorophyll (µg g^{-1}) at cold storage (17°C) of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

higher chlorophyll content than the control on the 6th day of storage (43). Chlorophyll degradation during postharvest storage is primarily caused by reduced chlorophyll-binding proteins and increased chlorophyllase enzyme activity, which melatonin may counteract by reducing reactive oxygen species (ROS) and lipid radicals and thus potentially delaying chlorophyll degradation (44).

Effect of postharvest melatonin treatment on starch degradation

Starch degradation began in the core of the pulp, the placenta and the endocarp, while the mesocarp retained starch for the most extended period during maturation (45). Fig. 9-10 illustrates the starch breakdown in cross-sections of three banana varieties taken from the centre position of the fruit during the ripening period. Starch exhibits a blue-black colouration, whereas regions with depleted starch appear white. During ripening, starch depletion progresses from the centre of the banana towards the periphery. Differences were noticed in the changes in starch degradation pattern on banana pulp between the treatments until the 9th and 15th day of ambient and cold storage conditions, respectively. Bananas treated with 1000 µM melatonin exhibited the most prolonged ripening duration and extended shelf life compared to the control under both storage conditions. The starch pattern of each fruit was compared using the starch pattern of each fruit with the starch iodine staining chart for bananas developed by Blankenship and Ellsworth (31). Melatonin-treated fruits showed about 5% of starch, while the untreated (control) fruits had >65 % on the 9th and 15th day of ambient and cold storage conditions (Table 3-4). Starch-to-sugar conversion is influenced by the levels of O_2 and CO_2, which can inhibit the enzyme activities involved in the starch hydrolysis process (46, 47). Immersing bananas in melatonin from 0.05 to 0.5 mM suppressed ethylene production and starch breakdown, delaying the ripening process during postharvest storage.

Effect of postharvest melatonin treatment on shelf life

Fruit shelf life is a critical factor significantly affecting the quality of horticultural commodities, especially bananas, during the postharvest storage period. Melatonin treatments offer a potential solution for extending shelf life. It is determined through continuous assessment of the fruits' physical appearance and quality analysis tests' results throughout the storage period (48). Maximum shelf life was recorded in T_6 (treated with melatonin at 1000 µM) of Grand Naine (10.25 days), Poovan (9.00 days) and Ney Poovan (9.45 days) compared with control (T_1) fruits at ambient storage conditions. The shelf life of fruits was prolonged when stored under cold conditions. Among the varieties, Grand Naine had the most extended shelf life of 17.58 days, followed by Poovan (16.98 days) and Ney Poovan (16.28 days) in fruits treated with 1000 µM of melatonin, compared to the control (T_1). The results are presented in Fig. 11. However, fruits dipped in 1000 µM of melatonin were considered the best treatment among the three banana varieties, *viz.,* Grand Naine, Poovan and Ney Poovan, under two different storage conditions to increase the fruit shelf life during the postharvest storage period.

1^{st} day of observation 9^{tht} day of observation

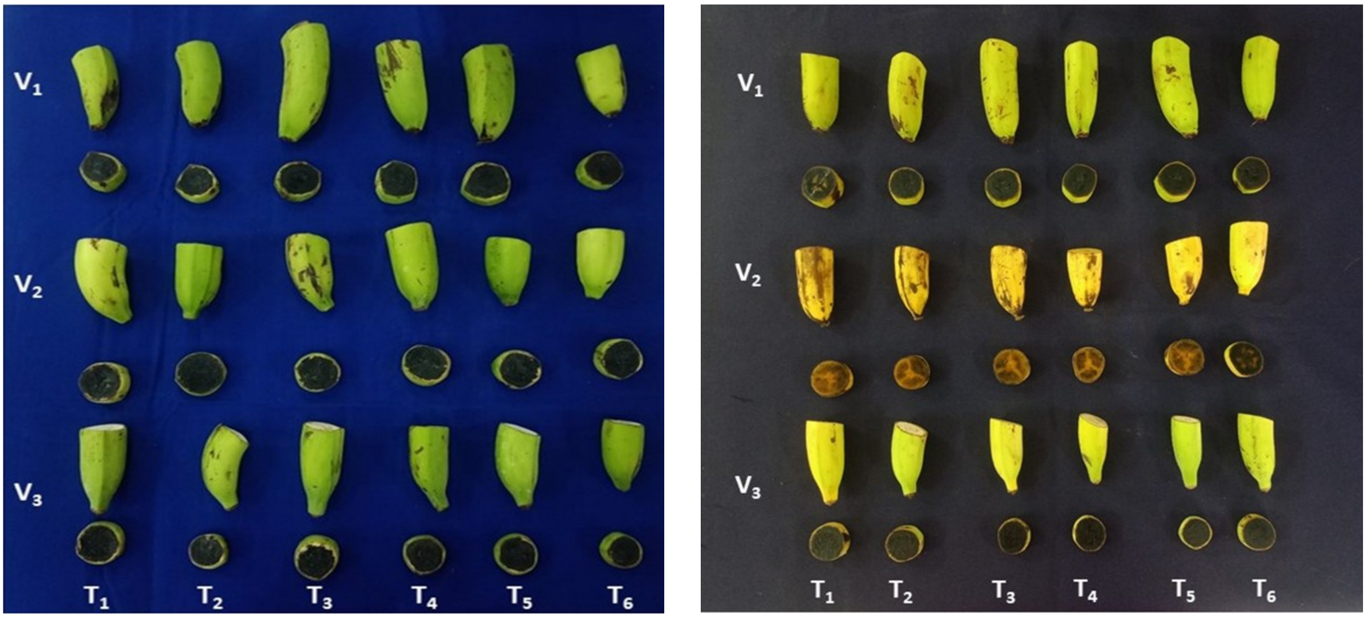

Fig. 9. Effect of postharvest melatonin treatment on starch degradation at 1^{st} and 9^{th} day of ambient storage condition of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

1^{st} day of observation 15^{tht} day of observation

Fig 10. Effect of postharvest melatonin treatment on starch degradation at 1^{st} and 15^{th} day of cold storage condition of Grand Naine (AAA) (V_1); Poovan (AAB) (V_2) and Ney Poovan (AB) (V_3); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM.

Table 3. Effect of postharvest melatonin treatment on starch conversion analysis by starch-iodine test on 1^{st} and 9^{th} of ambient storage condition

Treatments	Scale (1-10) based on starch content					
	Ambient storage (27 ± 2 °C)					
	Grand Naine (AAA)	Poovan (AAB)	Ney Poovan (AB)	Grand Naine (AAA)	Poovan (AAB)	Ney Poovan (AB)
T_1	1	1	1	4	10	4
T_2	1	1	1	2	9	2
T_3	1	1	1	2	9	1
T_4	1	1	1	1	8	1
T_5	1	1	1	1	7	1
T_6	1	1	1	1	4	1

V_1 - Grand Naine (AAA); V_2 - Poovan (AAB); V_3 - Ney Poovan (AB); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM. Treatment groups are scaled from 1 to 10 on different storage conditions. The data shown was one of the four replicates.

Table 4. Effect of postharvest melatonin treatment on starch conversion analysis by starch-iodine test on 1st and end 15th day of cold storage condition

Treatments	Scale (1-10) based on starch content					
	Cold storage (17 °C)					
	1st day of observation			15th day of observation		
	Grand Naine (AAA)	Poovan (AAB)	Ney Poovan (AB)	Grand Naine (AAA)	Poovan (AAB)	Ney Poovan (AB)
T_1	1	1	1	8	10	4
T_2	1	1	1	7	9	3
T_3	1	1	1	6	7	2
T_4	1	1	1	5	8	1
T_5	1	1	1	4	6	1
T_6	1	1	1	4	6	1

V_1 - Grand Naine (AAA); V_2 - Poovan (AAB); V_3 - Ney Poovan (AB); T_1 - Control (Untreated); T_2 - Melatonin @ 200 µM; T_3 - Melatonin @ 400 µM; T_4 - Melatonin @ 600 µM; T_5 - Melatonin @ 800 µM and T_6 - Melatonin @ 1000 µM. Treatment groups are scaled from 1 to 10 on different storage conditions. The data shown was one of the four replicates.

Conclusion

Bananas have a short postharvest shelf life due to climacteric ripening, making effective techniques crucial for extending shelf life and maintaining its quality. Recent studies showed that melatonin can influence postharvest physiology and prolong shelf life in various crops. Our findings revealed that melatonin significantly prolonged shelf life and maintained the morpho-physiological, biochemical and fruit quality characteristics of Grand Naine, Poovan and Ney Poovan varieties compared to untreated control. The 1000 µM melatonin concentration produced the most substantial effect across all three banana varieties. These results suggest that postharvest melatonin treatment could be a promising approach for enhancing bananas' quality and storage potential.

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Authors' contributions

MD, PJ, VR, LA, MB and MK conceived the concept, designed the study and prepared the manuscript. MD carried out the experimental procedures and statistical analyses. All authors reviewed and endorsed the final manuscript.

Compliance with ethical standards

Conflict of interest: No potential conflict of interest was reported by the author(s).

Ethical issues: None

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