

Influence of exogenous melatonin and salicylic acid on shelf life and quality of banana Var. 'Ney Poovan (AB, *Musa* sp.)' during ambient and cold storage

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Abstract

The present work aimed to investigate the effect of exogenous melatonin and salicylic acid in post-harvest quality and shelf life of traditional banana variety Ney Poovan. The hands were dipped in melatonin, salicylic acid, and the combinations, along with absolute control (water dip) and control (alum and carbendazim dip) for 15 min, and then stored at ambient temperature and cold storage. The results revealed that minimum total soluble solids, titratable acidity, total sugars, and physiological loss in weight, and maximum ascorbic acid, total starch, firmness, and shelf life were recorded by the fruits treated with 1.0 mM melatonin. This could be attributed to delayed activity of ripening related enzymes *viz.*, pectin methyl esterase, polygalacturonase, amylase, cellulase, and β -glucosidase. The melatonin-treated fruits exhibited reduced anthracnose incidence due to the higher activity of antioxidant-related enzymes *viz.*, peroxidase, catalase, superoxide dismutase, and sustained firmness. The outcomes of the present work suggested that 1.0 mM melatonin can be effectively utilised to extend the shelf life, maintain the quality, and reduce the post-harvest disease incidence of banana, offering a sustainable way for the post-harvest handling of banana.

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Introduction

Banana is the fourth most important crop globally following rice, wheat, and maize. Its production has steadily increased from 70 million tonnes in 1999 to 131 million tonnes in 2020, with India, China, Indonesia, Brazil, and Ecuador as the leading producers (FAO, 2023). In 2021 - 2022, India cultivated bananas across 961,000 hectares, producing 35.08 million tonnes, which accounted for more than a quarter of global production. Banana is also the most traded fruit crop, and ranks fifth among traded agricultural commodities, following coffee, cereals, sugar, and cocoa. In 2021, global banana exports reached 20.4 million tonnes, with Ecuador, the Philippines, and Costa Rica as the top exporters (FAO, 2022). However, despite high production, only 15% of the world's bananas enter international trade (Sugianti *et al.*, 2022). While large scale commercial farms dominate banana cultivation in major exporting countries, India's banana industry relies on smallholder farmers, cultivating on lands typically

less than three acres. A significant portion of their produce is lost before reaching the market due to improper post-harvest handling and the highly perishable nature of bananas. In India, postharvest losses (PHL) account for nearly 25% of total production, valued at over INR 25 billion annually (Suresh Kumar *et al.*, 2022).

Scientific post-harvest management is essential to minimise losses, and extend banana shelf life. Strategies such as field sanitation, disease control, timely harvesting, proper packaging, and cold chain logistics play a crucial role. However, concerns over synthetic chemical residues in export markets, increasing legislative restrictions, and the emergence of resistant pathogens necessitate eco-friendly alternatives for post-harvest disease management.

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a ubiquitous tryptophan-derived compound present in all living organisms, from microorganisms to higher plants and animals. It serves as a potent antioxidant, protecting plants against biotic and

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abiotic stresses. Exogenous MT application has been shown to extend shelf life while preserving fruit quality in various perishable crops, including papaya (Borthakur *et al.*, 2024), lemon (Rastgoo *et al.*, 2024), and peach (Kucuker *et al.*, 2024). Similarly, salicylic acid (SA), a naturally occurring phenolic compound (2-hydroxybenzoic acid), plays a crucial role in post-harvest physiology. SA application has been reported to delay ripening, suppress ethylene synthesis, and reduce weight loss and decay, improving the post-harvest stability of peach (Zhang *et al.*, 2024) and grapes (Zebro and Heo, 2024). In mango, the individual effects of MT and SA on ripening regulation and shelf-life extension have been studied (Almasoud *et al.*, 2024). However, considering their combined effects, the present work was designed to evaluate the impact of post-harvest dipping of MT and SA on the shelf life and quality of Ney Poovan, a traditional banana variety, which has a greater scope to play a role in the international trade.

Materials and methods

Fruit and treatments

Ney Poovan banana hands, at 80% maturity (90 – 95 d after spike emergence with a pulp: peel ratio of around 2.0 ± 0.2), were purchased from a local banana market in Coimbatore, Tamil Nadu, India. After sorting to select hands of uniform size (13 ± 2 cm length and 10 ± 2 cm girth) and colour, with no visible cuts, blemishes, or rot, the fruits were immediately transferred to the laboratory. The experiment was conducted using a completely randomised design, with three replicates for each treatment. The banana hands were randomly divided into ten groups for two experiments: one conducted in ambient storage ($28 \pm 2^\circ\text{C}$), and the other in cold storage ($13 \pm 1^\circ\text{C}$). Each group was dipped in the respective concentration of melatonin, or salicylic acid, or both (T₁: absolute control; T₂: alum (10 g/L) + carbendazim (1.5 g/L); T₃: 1.0 mM melatonin; T₄: 1.5 mM melatonin; T₅: 1.0 mM salicylic acid; T₆: 1.5 mM salicylic acid; T₇: 1.0 mM melatonin + 1.0 mM salicylic acid; T₈: 1.0 mM melatonin + 1.5 mM salicylic acid; T₉: 1.5 mM melatonin + 1.0 mM salicylic acid; and T₁₀: 1.5 mM melatonin + 1.5 mM salicylic acid). The concentrations were based on findings from a preliminary study where concentrations ranging from 0.5 to 3 mM were tested. The 1- and 1.5-mM concentrations were found to be most effective in delaying ripening. The banana hands were dipped for

15 min in the respective solutions of melatonin and salicylic acid. For the preparation, 0.232 g of melatonin was first dissolved in 10 mL of ethanol, and diluted with distilled water to make 1 L of 1 mM solution. Similarly, 0.116 g of salicylic acid was dissolved in 10 mL of methanol, and then diluted with distilled water to make 1 L of 1 mM solution. After dipping, the treated hands were air-dried and stored in plastic crates under two conditions: ambient storage ($28 \pm 2^\circ\text{C}$) and cold storage ($13 \pm 1^\circ\text{C}$ and 85 – 90% relative humidity). Physiological and biochemical changes were recorded at 3-d intervals in ambient storage, and 7-d intervals in cold storage.

Weight loss and firmness

Weight loss (PLW) is the percentage difference between the initial weight and weight at the time of measurement, and was calculated using Eq. 1:

$$\text{PLW (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (\text{Eq. 1})$$

Fruit firmness was assessed using the Fruit Hardness Tester (FHT 1122, Bestone Industrial Ltd., Hong Kong, China), and expressed in kg/cm².

TSS, TA, and TSS/TA

Total soluble solids (TSS) were assessed using a hand refractometer (RHB-32 ATC, Erma Inc, Tokyo, Japan) with a measurement range of 0 to 32 °Brix. The pulp was finely ground using a pestle and mortar, and a small amount of juice (two to three drops) was placed on the prism for measurement. The obtained readings were then documented in °Brix.

Titrateable acidity (TA) was determined as per the method described by Ranganna (1977). Five grams of fruit sample was homogenised, and the volume was adjusted to 30 mL with distilled water. The resulting aliquot was filtered and 5 mL of the filtrate, accompanied by two to three drops of phenolphthalein indicator, titrated against 0.1 N sodium hydroxide until a pale pink colour emerged, and calculated using Eq. 2:

$$\text{Titrateable acidity (\%)} =$$

$$\frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Equivalent weight of citric acid} \times \text{Volume made up} \times 100}{\text{Volume of sample} \times \text{Weight of sample} \times 1000} \quad (\text{Eq. 2})$$

The TSS to acid ratio (TSS/TA) was obtained by dividing TSS (percentage) by titrateable acidity (percentage).

Ascorbic acid, total sugar, and total starch contents

To determine the ascorbic acid content, 5 g of pulp was homogenised and adjusted to 50 mL with a 4% oxalic acid solution. The resulting mixture was filtered, and 5 mL of the filtrate was combined with 10 mL of 4% oxalic acid. The titration was carried out against a dye solution (sodium bicarbonate and 2,6-dichloroindophenol dye) until a pink colour (V_2) appeared, persisting for a few minutes. A standard solution containing 1 µg/mL ascorbic acid was also titrated using the same procedure, yielding V_1 (Sadasivam and Balasubramanian, 1987). The ascorbic acid content was expressed in mg/100 g using Eq. 3:

$$\text{Ascorbic acid (mg/100 g)} = \frac{0.5 \times V_2 \times \text{Volume made up} \times 100}{\text{Weight of sample} \times \text{Volume of sample} \times V_1} \quad (\text{Eq. 3})$$

A 0.5 g sample of pulp was homogenised with 10 mL of 85% ethanol, and the sugars were extracted through centrifugation, repeating the process thrice. All supernatants were combined, and the volume adjusted to 100 mL with distilled water. A 0.5 mL aliquot of the sample was mixed with 0.5 mL of distilled water and 4 mL of pre-cooled anthrone reagent. This mixture was incubated for 10 min, followed by placement in a boiling water bath for 8 min, and subsequent cooling to room temperature. The absorbance was measured at 630 nm using a spectrophotometer, and the sugar content was calculated using a standard graph of D-glucose, expressed in g/100 g (Hedge and Hofreiter, 1962).

The residue obtained after the extraction of sugars was dried in a water bath, and then dissolved in 5 mL of distilled water and 6.5 mL of 52% perchloric acid. The resulting mixture was centrifuged, and this process was repeated twice. Subsequently, 0.2 mL of the supernatant was adjusted to 1 mL with distilled water, and 4 mL of anthrone reagent was added. The reaction mixture was boiled for 8 min in a water bath, and then measured at 630 nm using a spectrophotometer (Hedge and Hofreiter, 1962). The total starch content was determined from a standard graph of D-glucose and expressed in g/100 g.

*Enzyme activity**Enzyme extraction and assay*

The enzyme assay was prepared following the method outlined by Srivastava and Dwivedi (2000).

Two grams of pulp was homogenised in a chilled pestle and mortar with 15 mL of sodium phosphate buffer (0.2 M, pH 7.0) containing cysteine-HCl (0.2 M) and EDTA (0.2 M). The resulting mixture was then centrifuged at 15,000 rpm for 20 min. The clear supernatant was collected and used for the enzyme assay.

Ripening-related enzymes

To determine the PME (pectin methyl esterase) activity, a mixture consisting of 1 mL of pectin (0.01%), 0.2 mL of sodium chloride (0.15 M), and 0.1 mL of bromothymol blue (0.01%) was prepared in a cuvette, and placed in a spectrophotometer at 620 nm. After the addition of 0.1 mL of enzyme extract, the initial reading and the subsequent decrease in absorbance after 3 min were recorded, and the disparity in absorbance was used to calculate the enzyme activity based on the standard curve of galacturonic acid (Hagerman and Austin, 1986). The enzyme activity was expressed in milli equivalents of COOH released per minute per gram fresh weight of the sample.

For PG (polygalacturonase), a reaction mixture with a total volume of 1 mL contained 0.2 mL of sodium acetate buffer (0.2 M), 0.1 mL of sodium chloride (2 M), 0.3 mL of polygalacturonic acid (1%), and 0.4 mL of enzyme extract, and was incubated for 1 h at room temperature. The reaction was halted by placing the mixture in a boiling water bath and 1 mL of dinitrosalicylic acid (1%) was added. The absorbance was measured at 540 nm using a spectrophotometer against a blank, and the formed reducing group was quantified using a standard curve of galacturonic acid (Gayathri and Nair, 2017). The activity was expressed as µg/min/g fresh weight of the sample.

To assess amylase activity, a mixture containing 1 mL of substrate (0.5% potato starch), 0.5 mL of phosphate buffer (0.2 M), and 1 mL of enzyme extract was incubated at 37°C for 1 h, and the reaction was terminated by adding 1 mL of 1% dinitrosalicylic acid. The absorbance was measured at 540 nm against a blank that lacks enzyme extract. The activity was calculated using the standard graph of maltose, and expressed in µg/min/g fresh weight of the sample (Bernfeld, 1955).

Cellulase activity was determined using the assay mixture, comprising 1 mL of carboxymethyl cellulose, 1 mL of sodium acetate buffer (0.2 M), and 1 mL of enzyme extract, then incubated at 37°C for

60 min. The reaction was halted by placing the mixture in a boiling water bath for 5 min, and then 1 mL of 1% dinitrosalicylic acid was added. The absorbance was measured at 540 nm using a spectrophotometer against a blank. The activity was calculated using the standard graph of glucose (Li *et al.*, 2023), and expressed in $\mu\text{g}/\text{min}/\text{g}$ fresh weight of the sample.

The reaction mixture for β -glucosidase determination composed of 1 mL of enzyme extract and 1 mL of *p*-nitrophenyl- β -D-glucopyranoside (5 mM), and was placed in a shaking incubator at 45°C for 10 min. To stop the reaction, 1 mL of sodium carbonate (2 M) was added, and the absorbance was measured at 410 nm using a spectrophotometer against a blank. The activity was determined from the standard graph of *p*-nitrophenol (Chang *et al.*, 2011), and expressed in $\mu\text{g}/\text{min}/\text{g}$ fresh weight of the sample.

Antioxidant-related enzymes

Peroxidase (POX) activity was determined using 0.2 mL of hydrogen peroxide (0.1 M) which was added to a mixture containing 3.5 mL of phosphate buffer (0.2 M), 0.2 mL of enzyme extract, and 0.1 mL of *o*-dianisidine (1 mg/mL). The enzyme kinetics was read at 430 nm using spectrophotometer for 3 min at 30-sec intervals. The increase in absorbance was plotted in a graph against time, and the linear rise for 1 min was used for the estimation of peroxidase activity (Putter, 1974), and expressed in $\Delta\text{A}/\text{min}/\text{g}$ fresh weight of the sample.

For catalase (CAT), 50 μL of enzyme extract was added to a mixture of 1.5 mL of phosphate buffer (0.2 M), 1.5 mL distilled water, and 0.5 mL of hydrogen peroxide (12.5 mM). The enzyme kinetics was read at 240 nm for 1 min at 30-sec intervals. The decrease in absorbance was the activity of catalase (Aebi, 1984), and expressed in catalase activity/min/g fresh weight of the sample.

To determine super-oxide dismutase (SOD), assay mixture consisting of 1.2 mL of sodium pyrophosphate buffer (0.052 M), 0.1 mL of phenazine methosulphate (186 μM), 0.3 mL of nitroblue tetrazolium (300 μM), 0.2 mL of reduced nicotinamide adenine dinucleotide (780 μM), and 0.5 mL of enzyme extract in a total volume of 3 mL, along with a control lacking enzyme extract, was incubated at room temperature for 3 min. After incubation, 1 mL of glacial acetic acid and 4 mL of butanol were added with shaking. The chromogen layer was separated and read at 520 nm using a

spectrophotometer. The enzyme concentration required for 50% inhibition of nitroblue tetrazolium in 1 min was considered as one unit of enzyme activity (Kakkar *et al.*, 1984), and expressed in units/min/g fresh weight of the sample.

Anthraco-nose incidence

The incidence of anthracnose was calculated by giving visual score ratings for the sunken spots caused by *Colletotrichum* sp. on a scale of 1 to 5, and expressed in percentage (Sivakumar *et al.*, 2002) using Eq. 4:

$$\text{Anthraco-nose incidence (\%)} = \frac{\text{Sum of individual ratings}}{\text{Total number of fruits}} \times \frac{100}{\text{Maximum score}} \quad (\text{Eq. 4})$$

Shelf life

The fruits' shelf life was determined through visual assessments. The duration from harvest until the fruits reached an unmarketable state (defined as the stage where the hand displayed more than 50% blackening in the form of spots or lesions) was recorded and expressed in days.

Statistical analysis

The collected data were analysed using a completely randomised design with three replications, each consisting of five banana hands per replicate. Each treatment included 15 banana hands. The measurements were averaged per hand to obtain the data for analysis. A One-way analysis of variance (ANOVA) was performed to compare the means, and least significant difference (LSD) analysis was used to determine the significantly different treatments at a significance level of $p < 0.05$. All computations and analyses were conducted using the R software package. Additionally, principal component analysis (PCA) was performed to discern relationships among the variables using R Studio (version 4.3.1).

Results

Effect of melatonin and salicylic acid on weight loss and firmness

Weight loss increased in both storage temperatures regardless of treatment throughout the storage period. However, fruits treated with MT exhibited a significantly lower percentage of mean weight loss at the end of the storage period. The 1.0 mM MT treatment showed the lowest cumulative

weight loss, which was approximately 41% lower in ambient storage, and 53% lower in cold storage compared to the control. Similarly, fruits treated with 1.0 mM SA showed a 33.38% reduction in ambient storage, and a 45.59% reduction in cold storage compared to the control.

Fruit firmness declined over the storage period across all treatments. However, MT and SA treatments significantly slowed this decline. Notably, 1.0 mM MT maintained the highest firmness, recording 0.75 kg/cm² in ambient storage, and 0.79 kg/cm² in cold storage, which was higher than the control (0.59 and 0.58 kg/cm², respectively) and other treatment groups.

Effect of melatonin and salicylic acid on TSS, TA, and TSS/TA

The TSS and TA of the fruits showed an increasing trend as ripening progressed; however, the rate of increase was significantly lower in fruits treated with 1.0 mM MT. Among the stored fruits, the control group recorded the highest TSS values of 27.00 and 27.20 °Brix in ambient and cold storage, respectively, while fruits treated with 1.0 mM MT recorded the lowest values, at 23.80 and 25.00 °Brix, respectively. Fruits treated with 1.0 mM SA recorded intermediate values of 24.87 and 25.87 °Brix, respectively. Similarly, the TA of fruits treated with 1.0 mM MT was 24.76 and 34.44% lower than that of the control in ambient and cold storage, respectively, while fruits treated with 1.0 mM SA showed TA values 16.19 and 22.22% lower than the control in the same conditions. As shown in Figure 1, the TSS/TA ratio exhibited an increasing trend until the point of full ripening, after which it declined. The highest ratio was observed on the day of full ripening at both storage temperatures, regardless of treatment. Notably, fruits treated with 1.0 mM MT recorded the significantly highest TSS/TA ratio on day 6 of ambient storage (40.74), and day 21 of cold storage (43.29).

Effect of melatonin and salicylic acid on ascorbic acid, total sugar, and total starch contents

The 1.0 mM MT treatment retained 42.07% of the ascorbic acid available at harvest on day 9 in ambient storage, and 24.01% on day 21 in cold storage compared to the respective controls. The 1.0 mM SA dip retained 36.00 and 25.82% of ascorbic acid on day 21 under cold storage conditions.

Throughout the storage period, total sugar content exhibited an increasing trend, while total starch content decreased. Fruits treated with 1.0 mM MT recorded significantly lower total sugars (20.56 and 19.34 g/100 g) and higher total starch content (3.60 and 3.21 g/100 g) compared to the control fruits, which showed the highest total sugars (23.27 and 21.36 g/100 g) and the lowest starch content under ambient and cold storage, respectively (Figure 1).

Effect of melatonin and salicylic acid on ripening enzymes

As depicted in Figure 2, the activities of PME, PG, amylase, cellulase, and β -glucosidase gradually increased from day 0 until full ripening, followed by a subsequent decrease. However, in both storage conditions, treatments with 1.0 mM MT and SA significantly suppressed the activities of these ripening-associated enzymes.

Compared to the control, the activities of PME, PG, amylase, cellulase, and β -glucosidase were 15.36, 36.10, 45.07, 43.22, and 20.14% lower, respectively, on day 6 of ambient storage, and 11.14, 16.99, 28.72, 18.45, and 6.17% lower on day 21 of cold storage in fruits treated with 1.0 mM MT. Similarly, in fruits treated with 1.0 mM SA, the respective enzyme activities were 13.68, 23.25, 29.56, 22.17, and 12.68% lower under ambient storage, and 9.43, 11.32, 15.93, 15.96, and 3.37% lower in cold storage.

Fruits treated solely with MT or SA, as well as those treated with carbendazim and alum, displayed a sustained increasing trend in enzyme activity. In contrast, the remaining treatments exhibited an increasing trend followed by a decrease. This suggested that SA performed comparably to MT, whereas the combination of MT and SA showed a trend similar to that of the control fruits.

Effect of melatonin and salicylic acid on antioxidant enzymes

The activity of antioxidant enzymes increased during ripening, and began to decrease after full ripening in control fruits and those treated with combinations of MT and SA. However, a consistent increasing pattern only was observed in fruits treated with MT or SA alone, as well as in those treated with alum and carbendazim (Figure 3).

On day 6 of ambient storage, the activity of POX was 1.52 and 1.12 times higher in fruits treated

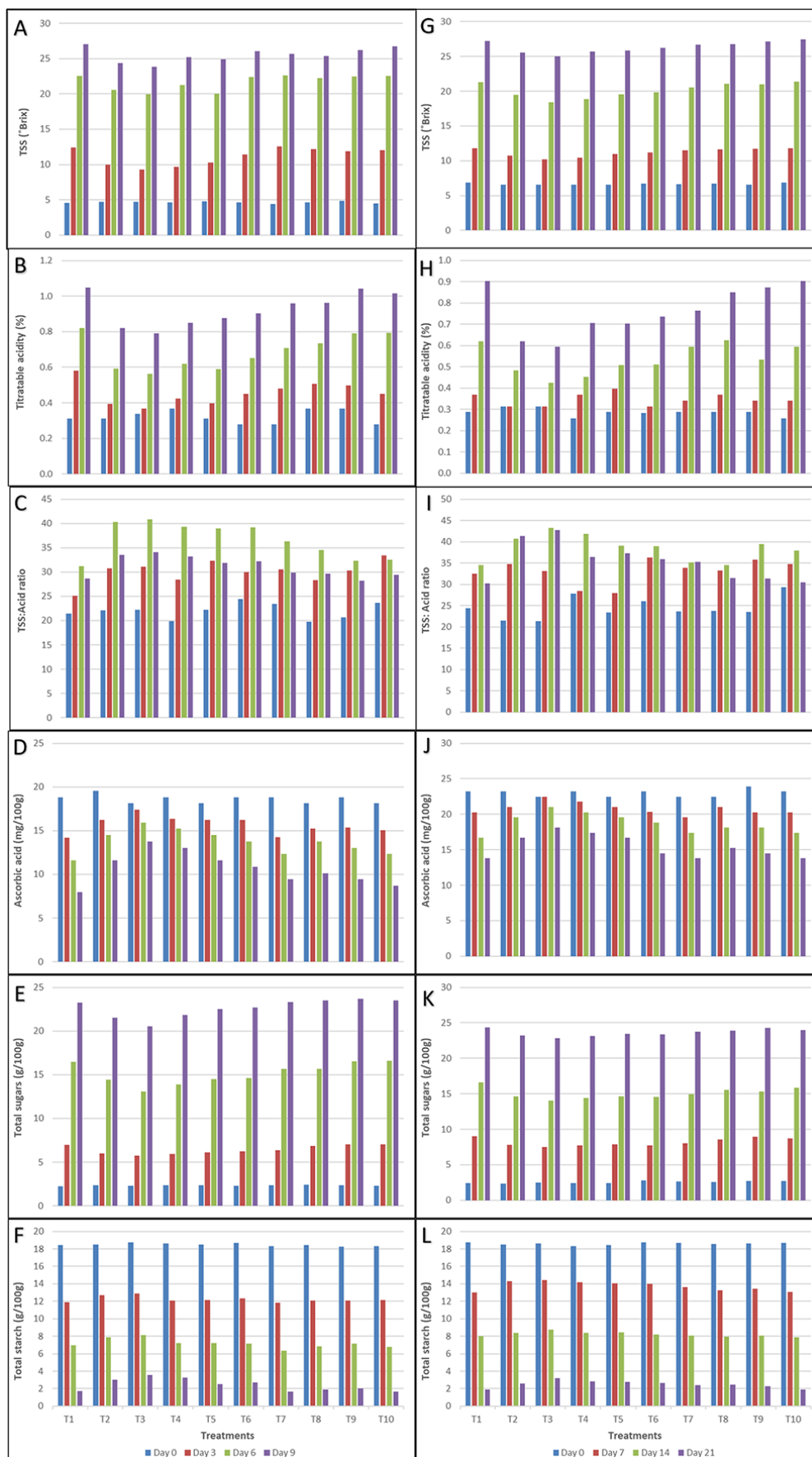


Figure 1. Effect of melatonin and salicylic acid on TSS, TA, TSS/TA ratio, ascorbic acid, total sugars, and total starch in traditional banana cv. Ney Poovan under ambient storage [(A) to (F)] and cold storage [(G) to (L)].

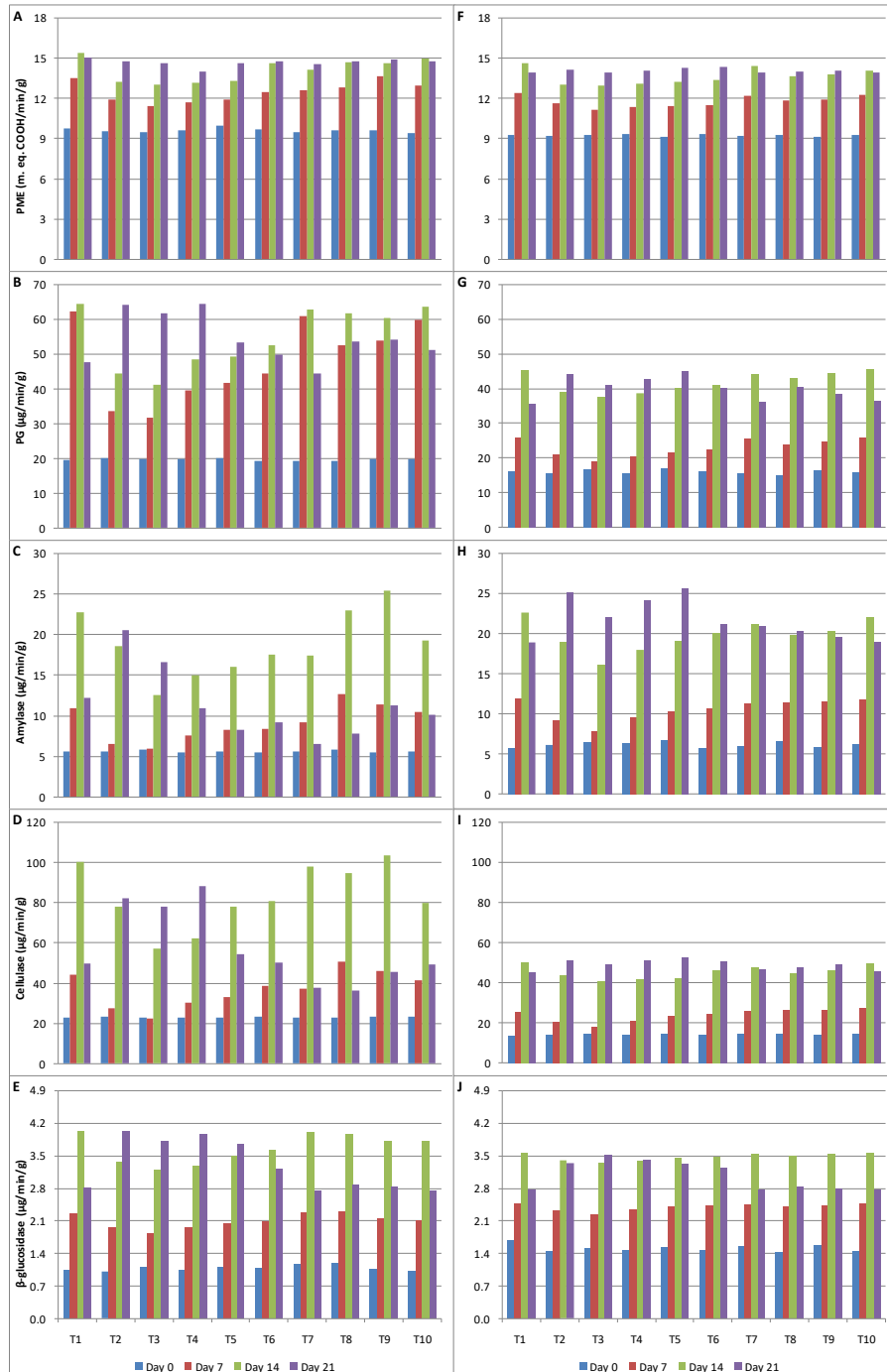


Figure 2. Effect of melatonin and salicylic acid on PME, PG, amylase, cellulase, and β -glucosidase in traditional banana cv. Ney Poovan under ambient storage [(A) to (E)] and cold storage [(F) to (J)].

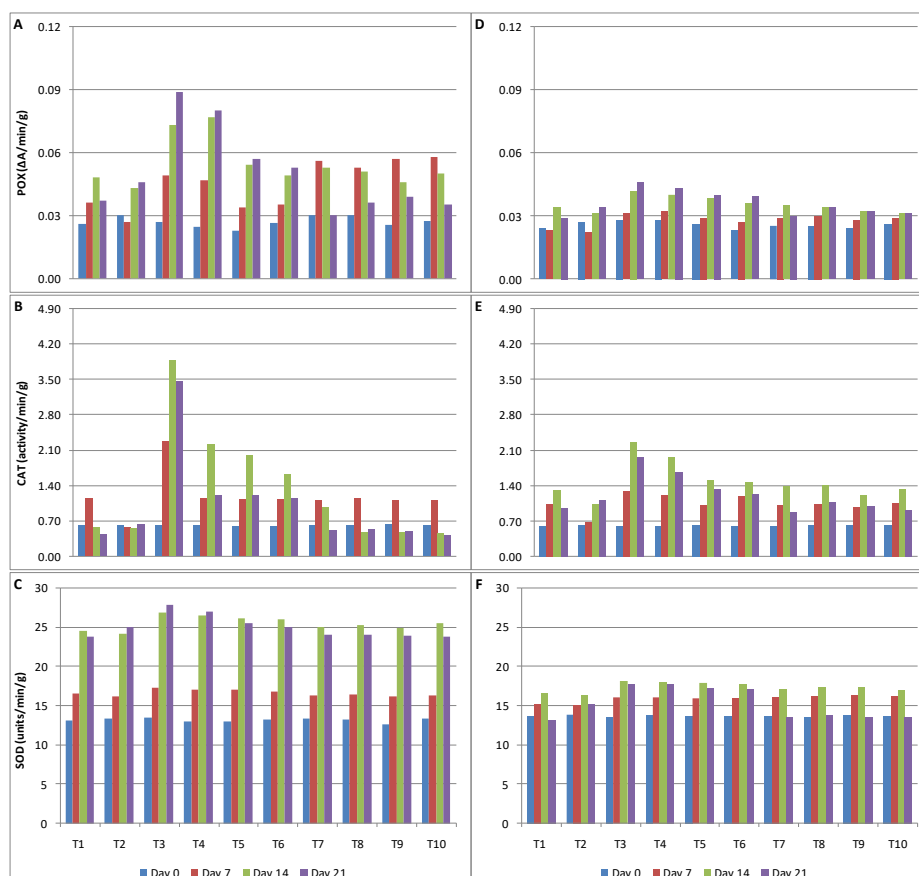


Figure 3. Effect of melatonin and salicylic acid on POX, CAT, and SOD in traditional banana cv. Ney Poovan under ambient storage [(A) to (C)] and cold storage [(D) to (F)].

with 1.0 mM MT and SA, respectively. Likewise, the activity of CAT was 6.00 and 3.47 times higher, and that of SOD was 1.09 and 1.06 times higher in fruits treated with 1.0 mM MT and SA, respectively. In cold storage on day 14, the activities of POX, CAT, and SOD were 1.10, 1.72, and 1.09 times higher, respectively, in fruits treated with 1.0 mM MT compared to the corresponding control fruits. Moreover, it is apparent from Figures 2 and 3 that the activity of ripening and antioxidant enzymes was consistently lower in cold storage compared to ambient storage throughout the entire storage period, irrespective of the treatments, suggesting that the enzyme activities were sensitive to temperature variations.

Effect of melatonin and salicylic acid on anthracnose incidence

The percent disease index was lowest in fruits treated with the carbendazim (28.89 and 13.33% in ambient and cold storage, respectively), which was statistically on par with the results obtained from 1.0 mM (28.89 and 17.78%) and 1.5 mM MT treatments (33.33 and 20.00%). SA was less effective than MT,

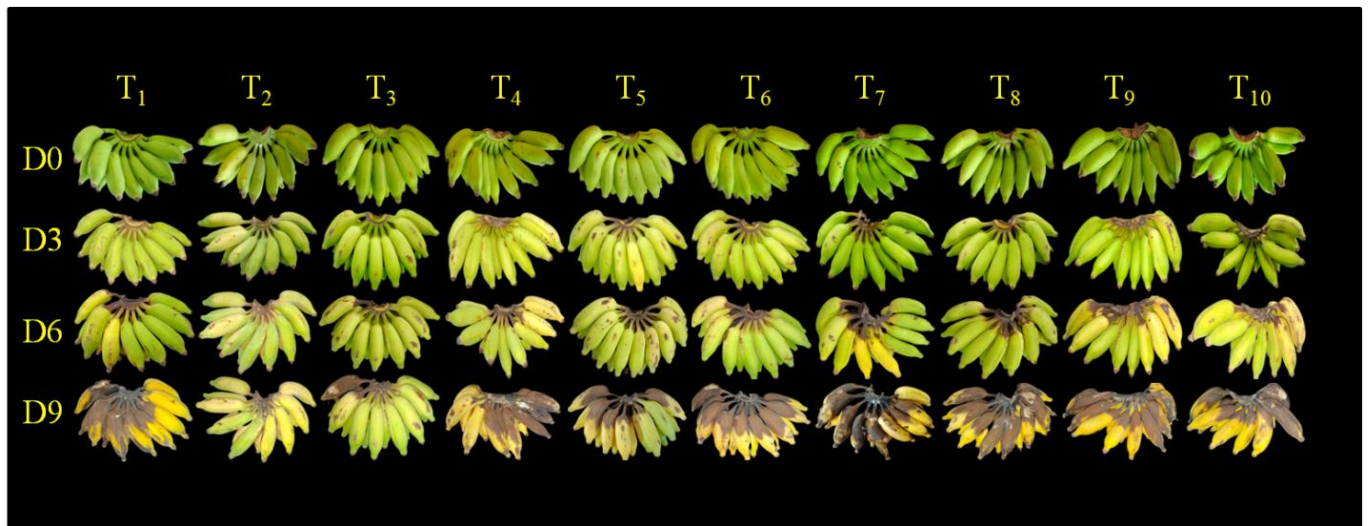
with disease incidence of 40.00 and 22.22% observed in fruits treated with 1.0 mM SA in ambient and cold storage, respectively.

Effect of melatonin and salicylic acid on shelf life

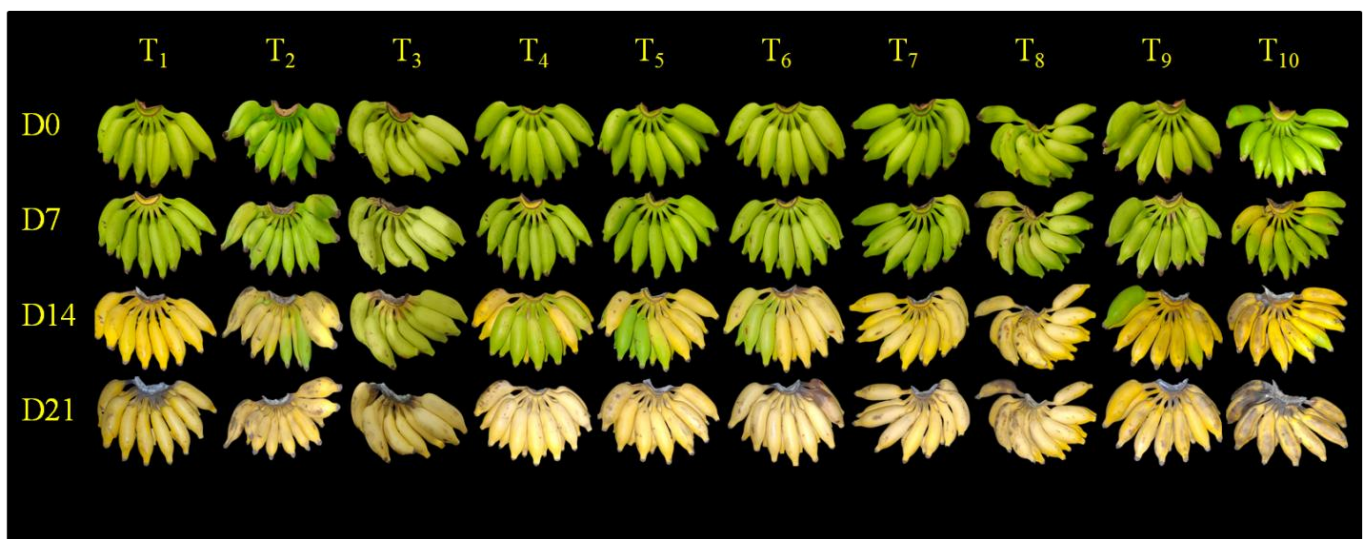
The average shelf life of control fruits was 8.33 d in ambient storage (Figure 4A), and 22.67 d in cold storage (Figure 4B). This shelf life was increased to 11.33 d in ambient storage, and 28.67 d in cold storage with the application of 1.0 mM MT. Also, the shelf life of 1.0 mM SA-dipped fruits increased up to 9.00 and 27.00 d in ambient and cold storage, respectively.

Principal component analysis

A principal component analysis (PCA) approach was employed to evaluate the impact of MT on the post-harvest quality of banana variety Ney Poovan fruit in ambient and cold storage (Figure 5), considering various physiological and biochemical parameters. The eigen values of the covariance matrix indicated that first two principal components (PCs) explained more than 85% of the total variance in the dataset for both ambient and cold storage. Notably,



(A)



(B)

Figure 4. Effect of melatonin and salicylic acid on ripening of traditional banana cv. Ney Poovan stored under ambient (A) and cold (B) condition.

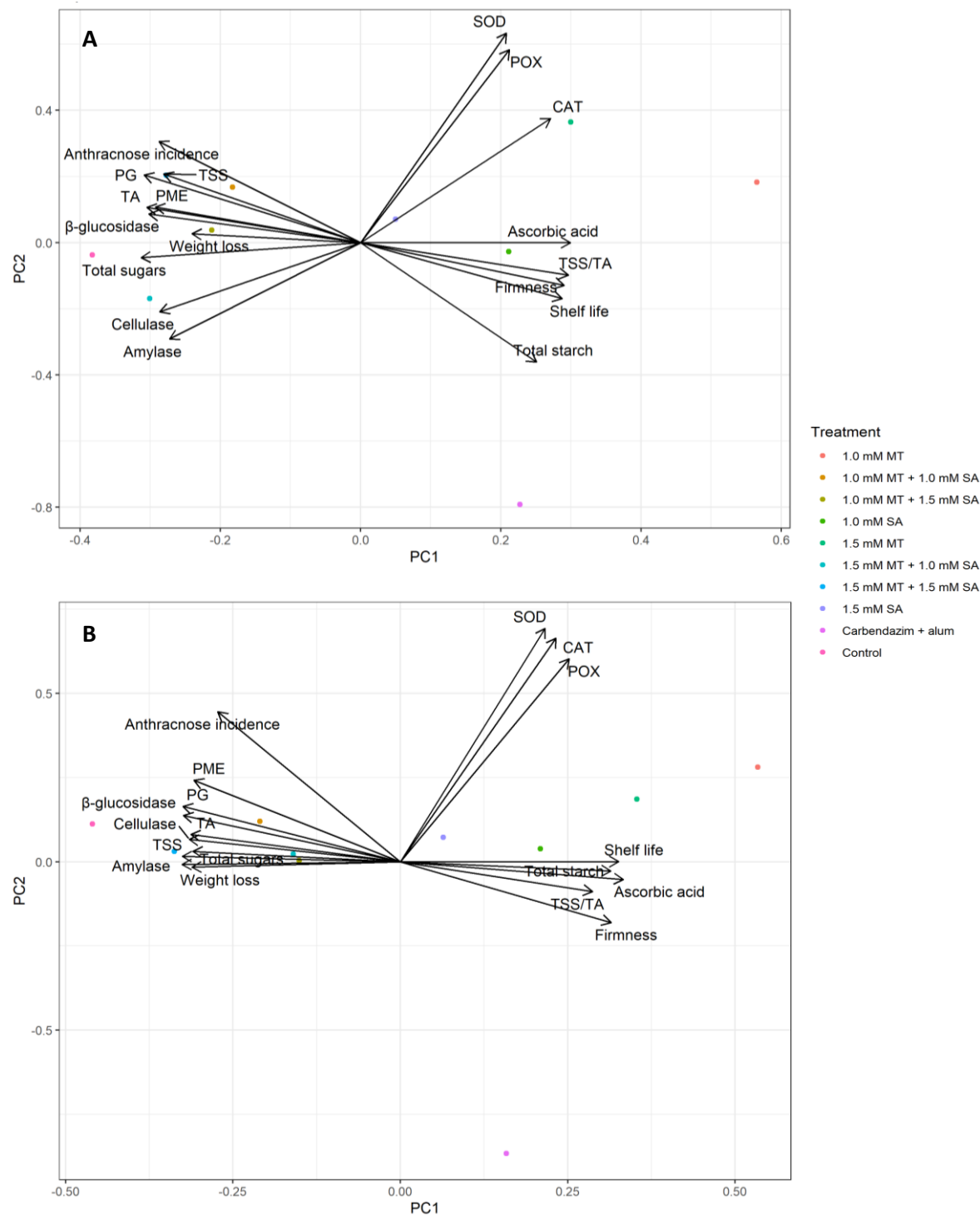


Figure 5. Principal component analysis of traditional banana cv. Ney Poovan stored under ambient condition (day 6) (A) and under cold storage (day 14) (B).

PC1 (76.72 and 81.13% in ambient and cold storage, respectively) exhibited positive correlations with firmness, TSS/TA ratio, ascorbic acid, total starch, peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), and shelf life. Conversely, the remaining parameters were defined by the negative axis of PC1. PC2, responsible for 9.42 and 8.64% variation in ambient and cold storage, respectively, and demonstrated positive correlations with all enzymes and negative correlations with ascorbic acid, total starch, total sugars, and firmness. The findings suggested a significant difference between the control and MT treatment.

Discussion

India is the leading producer of bananas globally, yet it ranks only 21st among exporting nations. This can be attributed to factors such as deceptive cultivation practices, inadequate post-harvest handling, inefficient domestic supply chain,

and limited adherence to international certification standards (Suresh Kumar *et al.*, 2022). Being a climacteric fruit, banana ripening is characterised by a sharp increase in respiration and a concurrent surge in ethylene production. Ethylene plays a central role in ripening by stimulating starch degradation, and promoting fruit softening through the upregulation of enzymes such as pectin methyl esterase (PME), polygalacturonase (PG), and cellulase (Lohani *et al.*, 2004).

In the present work, MT-treated fruits exhibited significantly lower weight loss, which was attributed to a decrease in respiration and transpiration rates (Table 1). The decrease in respiration is likely mediated by MT-induced nitric oxide (NO) synthesis, which directly inhibits cytochrome c activity (Brown, 2001). This inhibition downregulates metabolic activity, thereby reducing physiological losses, and contributing to the maintenance of fruit firmness during storage.

Table 1. Effect of melatonin on CO₂ and ethylene evolution rate under ambient storage conditions.

Parameter	Treatment	Day 0	Day 3	Day 6	Day 9
CO₂ (%)	Control	1.6 ± 0.42	6.7 ± 0.54 ^a	12.6 ± 0.61 ^a	7.2 ± 0.39 ^a
	1.0 mM melatonin		2.9 ± 0.49 ^b	4.5 ± 0.47 ^b	7.3 ± 0.43 ^b
Ethylene (ppm)	Control	0.3 ± 0.09	2.4 ± 0.19 ^a	7.6 ± 0.08 ^a	11.3 ± 0.06 ^a
	1.0 mM melatonin		1.1 ± 0.12 ^b	4.2 ± 0.09 ^b	9.2 ± 0.05 ^b

Values are mean of replications ± standard error of mean. Mean values in same column followed by similar lowercase superscripts are not significantly different at $p < 0.05$.

The higher TSS content in control fruits could be related to earlier ripening, and delayed ripening was apparent from lower TSS in treated fruits. The increase in total soluble solids (TSS) was primarily associated with degradation of starch to sugar (Panahirad *et al.*, 2020), and also contributed in part by the increase in organic acid content (Wyman and Palmer, 1964), and accumulation of protein (Dominguez-Puigjaner *et al.*, 1992). The content of organic acid (titratable acidity) continues to increase during ripening with malic and citric acids being the predominant organic acids in banana (Wyman and Palmer, 1964). Citric acid is synthesised during respiration as an intermediate in Krebs (TCA) cycle. The glyoxylate cycle, a special variant of TCA cycle governs the accumulation of malic acid in the pulp regulated by malate synthase, and induced by ethylene (Pua *et al.*, 2003). Thus, respiration is one of the co-factors for the accumulation of organic acids, and lower respiration rate might be the reason for the

lowest titratable acidity (0.79% in ambient storage and 0.59% in cold storage) in fruits subjected to 1.0 mM MT dip. The control fruits recorded lower TSS/TA ratio than treated fruits indicating that slower increase in TA (Figure 1) as a result of delayed ripening. As the TSS/TA ratio is a key character determining the taste and flavour of the fruit, the results indicated that post-harvest dip treatment with MT and SA had no influence in altering the taste and flavour of Ney Poovan banana fruits.

Ascorbic acid serves as a co-substrate in the biosynthesis of ethylene (Davey *et al.*, 2000). Its depletion during ripening is often aligned with the climacteric rise in ethylene production and ripening progression. In the present work, fruits treated with 1.0 mM MT retained significantly higher ascorbic acid content (Figure 1), which coincided with a reduced rate of ethylene evolution (Table 1). The disassembly of starch reserve leads to concomitant increase in sugars reaching up to 20% of pulp fresh

weight. The fruits dipped in 1.0 mM MT retained higher starch content, and recorded lower total sugar content, inferring slower conversion of starch which ultimately delayed ripening of treated fruits.

The activity of fruit ripening related enzymes is regulated by ethylene. In the present work, control fruits exhibited higher activity of ripening enzymes than fruits treated with 1.0 mM MT. Moreover, the peak enzyme activity in control fruits occurred earlier, whereas MT-treated fruits showed a delayed onset (Figure 2). This delayed enzymatic activity corresponded with reduced ethylene evolution, and supported the observed delay in fruit softening and the extended shelf life of MT-treated fruits. The inhibitory effect of MT on ethylene biosynthesis is likely mediated through both direct and indirect mechanisms. MT downregulates the expression of key ethylene biosynthetic genes, *ACS* (1-aminocyclopropane-1-carboxylic acid synthase), and *ACO* (1-aminocyclopropane-1-carboxylic acid oxidase) (Onik *et al.*, 2021). Additionally, MT-induced suppression of respiration - a process positively correlated with ethylene synthesis, further contributes to the reduced ethylene levels and slower ripening.

The level of antioxidant enzymes increases with ripening as ethylene production accelerates respiration, leading to increased ROS production in the inner mitochondrial wall during electron transport chain (Meitha *et al.*, 2020). Despite delayed ripening, the fruits dipped in 1.0 mM MT exhibited higher activity of antioxidant enzymes. This may be attributed to MT dual role in directly scavenging ROS and upregulating the expression of antioxidant-related genes, as well as its ability to suppress respiration, thereby limiting ROS generation (Manchester *et al.*, 2015). In contrast, the lower antioxidant enzyme activity observed in control fruits could result from oxidative inactivation caused by excessive ROS accumulation. Hydroxyl radicals and superoxide anions generated during advanced ripening stages may lead to structural damage of antioxidant enzymes by cleaving their peptide chains, thereby reducing their functional activity (Pigeolet and Remacle, 1991).

The minimal incidence of anthracnose was noticed in fruits treated with carbendazim, while the maximum symptom was exhibited by control fruits. The lesser incidence of anthracnose in fruits dipped in 1.0 mM MT was due to higher firmness inhibiting

penetration of mycelia and increased antioxidant activity brought about by post-harvest dip of MT.

The ripening pattern of fruits stored in ambient and cold storage conditions were similar, and the ripening period differed based on the storage temperature. The ripening rate was higher in higher temperature with a shorter shelf life and *vice versa*. This temperature-dependent variation can be attributed to the significant reduction in ethylene production and respiration rate under cold storage, effectively slowing down the metabolic processes involved in ripening (Orihuel-Iranzo *et al.*, 2010).

Both MT and SA had positive influence in enhancing the shelf life of the traditional banana variety 'Ney Poovan' compared to control. Although SA reduced the weight loss, activity of ripening related enzymes while increasing activity of antioxidant related enzymes, its effectiveness was lower than MT due to the absence of certain mechanism such as direct scavenging of ROS, and synthesis of NO which reduces respiration and ethylene synthesis. The putative mechanisms of action of MT and SA are given in Figure 6. The application of both MT and SA in combination did not significantly increase the shelf life, rather the response was similar to control fruits. Similar finding was reported by Awad and Al-Qurashi (2021) in mango. The factors responsible for the better performance of MT over SA in increasing shelf life needs further study.

Conclusion

The present work highlighted the role of exogenous melatonin in extending the shelf life of traditional banana variety 'Ney Poovan' while preserving the quality. Adopting 1.0 mM MT dip treatment as part of the post-harvest protocol significantly added up to three days of shelf life under ambient conditions, and six days under cold storage, while maintaining fruit quality. The findings underscored melatonin's potential as a viable and environmentally friendly alternative to carbendazim, a commonly used chemical fungicide. The diverse roles of melatonin in post-harvest preservation highlight its potential as a valuable tool for improving the storability and marketability of traditional banana varieties. Its application may contribute to enhancing the export quality of Indian bananas through better retention of fruit quality during extended storage and transit.

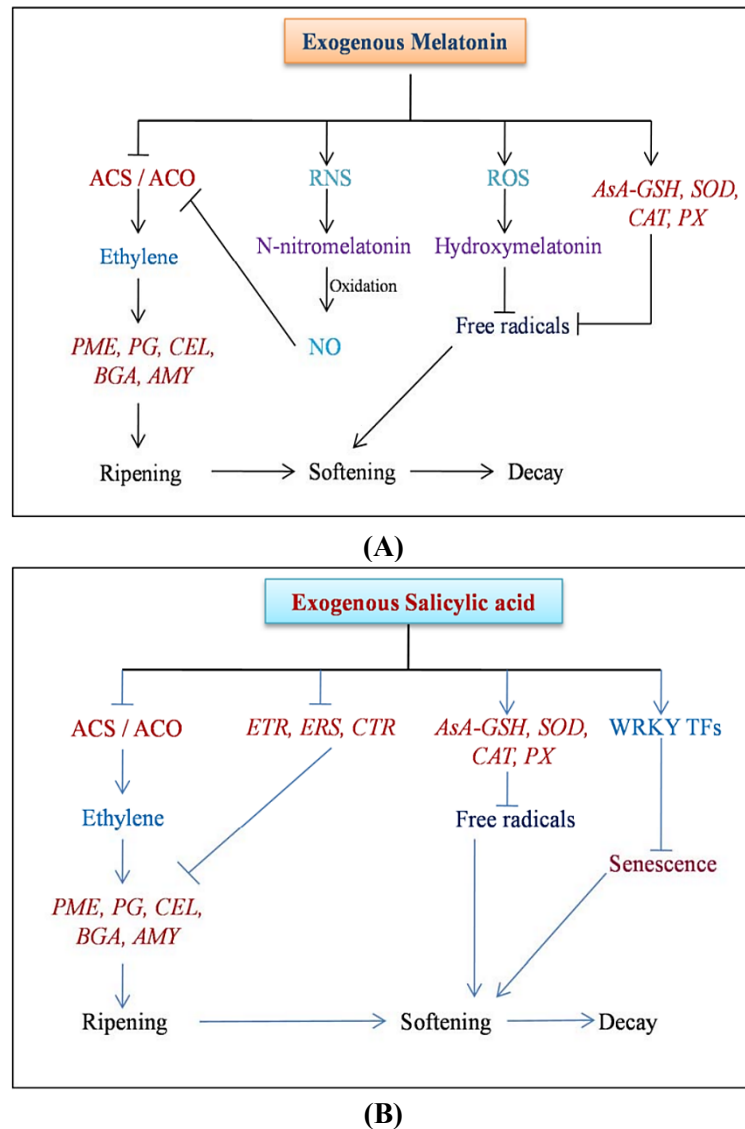


Figure 6. Putative mechanism of action of melatonin **(A)** and salicylic acid **(B)**. ACS: ACC synthase; ACO: ACC oxidase; RNS: reactive nitrogen species; ROS: reactive oxygen species; AsA-GSH: ascorbate glutathione; SOD: superoxide dismutase; CAT: catalase; PX: peroxidase; PME: pectin methylesterase; PG: polygalacturonase; CEL: cellulase; BGA: β -glucosidase; NO: nitric oxide; ETR: ethylene receptor; ERS: ethylene response sensor; CTR: constitutive triple response; WRKY TFs: WRKY transcription factors.

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