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RESEARCH ARTICLE

Effect of bioactive compounds on enzymatic regulation and shelf life of mango during post-harvest

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Abstract

The effects of melatonin, salicylic acid (SA) and brassinosteroids on the post-harvest quality and shelf life of two mango varieties, Alphonso and Sindhura, were investigated with respect to physiological, biochemical and enzymatic changes. The study assessed physiological loss in weight (PLW), Total Soluble Solids (TSS), titratable acidity, sugars, total phenolics, carotenoids, ripening related enzymes (pectin methylesterase, polygalacturonase, amylase and cellulase) and antioxidant enzymes (peroxidase, catalase and superoxide dismutase). According to the results, the shelf life of mango was considerably increased by 200 µM melatonin, 2.0 mM salicylic acid and 25 ppm brassinosteroids, which also decreased PLW to 9.81 %, 10.12 % and 9.79 % in Alphonso and 9.48 %, 9.51 % and 9.92 % in Sindhura. These treatments maintained a balance between TSS, sugars and acidity, while preserving higher concentration of total phenolics and carotenoids. Treated fruits exhibited lower ripening enzyme activity, particularly polygalacturonase, which declined by 16.7 %, 11.2 % and 17.0 % in Alphonso and 11.5 %, 10.9 % and 11.2 % in Sindhura with 200 µM melatonin, 2.0 mM salicylic acid and 25 ppm brassinosteroids treatments, respectively. Similarly, pectin methyl esterase (PME) was reduced by 12.2 %, 11.6 % and 10.5 % in Alphonso and 12.1 %, 10.3 % and 12.1 % in Sindhura corresponding to these treatments, delaying cell wall degradation and maintaining fruit firmness. Additionally, antioxidant enzymes were upregulated, mitigating oxidative stress by reducing reactive oxygen species (ROS) and preserving fruit quality. These treatments were effective in delaying ripening, enhancing stress resistance and extending the marketability of mangoes during storage. This study highlights the potential of melatonin, salicylic acid and brassinosteroids as eco-friendly alternatives to synthetic chemicals for improving post-harvest mango management.

Keywords

brassinosteroids; enzymes; mango; melatonin; past-harvest; salicylic acid

Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits globally, renowned for its rich flavour, nutritional value and economic significance (1). As a staple fruit in many countries, mangoes play a pivotal role in agricultural production, trade and dietary intake. Beyond their delightful taste, mangoes are a vital source of vitamins A and C, dietary fibre and antioxidants, making them highly valued in both fresh and processed forms (2).

Despite their importance, mangoes are highly perishable and face significant challenges in post-harvest handling. Issues such as rapid ripening, susceptibility to mechanical damage, microbial infection and post-harvest diseases drastically reduce fruit quality, shelf life and marketability. These issues result in substantial economic losses for producers and traders, particularly in tropical regions where proper storage and transportation infrastructure are limited (3).

In recent years, the use of bioactive compounds has emerged as a promising approach to mitigate post-harvest issues and extend the shelf life of fruits like mangoes. Compounds such as melatonin (4), salicylic acid (5) and brassinosteroids (6) have shown potential in enhancing fruit quality, delaying ripening and improving stress resistance. These natural compounds, often found in plants, offer an eco-friendly alternative to synthetic chemicals traditionally used in past-harvest treatments. The use of eco-friendly alternatives in past-harvest treatments is increasingly relevant in the context of sustainable agriculture and environmental conservation. Although traditional synthetic chemicals are effective, they pose risks such as environmental pollution, chemical residues on produce and potential harm to human health. (7). In contrast, natural bioactive compounds like melatonin, salicylic acid and brassinosteroids offer safe and sustainable solutions.

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring hormone in plants and animals, acting as an antioxidant and regulating plant growth, stress responses and defence mechanisms. Salicylic acid is a phenolic compound that plays a crucial role in plant defence against pathogens and in modulating stress tolerance. Brassinosteroids, a class of polyhydroxysteroids, are essential plant hormones that promote growth, development and enhance resistance to various environmental stresses. The exogenous application of these compounds has been proven to increase the shelf life and maintain quality in many fruits (8-10).

While there are numerous bioactive compounds with potential applications in post-harvest management, this study focuses on comparing the effectiveness of melatonin, salicylic acid and brassinosteroids. By evaluating their roles in improving the post-harvest quality and longevity of mangoes, this research aims to provide insights into the most effective treatments for minimizing post-harvest losses and maintaining fruit quality during storage.

Materials and Methods

Mature green mangoes of the cultivars Alphonso and Sindhura, both known for their commercial importance, were collected from a farmer's field in Muthalamada, Palakkad district, Kerala. These fruits were carefully transported to the Postgraduate Analytical Laboratory, Horticultural College and Research Institute (HC&RI), Tamil Nadu Agricultural University (TNAU), Coimbatore. Upon arrival, the mangoes were cleaned with distilled water to remove any field debris before applying treatments. The experiment was laid out in a completely randomized design (CRD) with ten treatments, including a control group and three replications. The treatments encompasses: T_1 - Control (distilled water), T_2- melatonin (150 µM), T_3 - melatonin (200 µM), T_4 - melatonin (250 µM), T_5 - salicylic acid (1.5 mM), T_6 - salicylic acid (2.0 mM), T_7 - salicylic acid (2.5 mM), T_8- brassinosteroids (10 ppm), T_9 - brassinosteroids (25 ppm) and T_{10} - brassinosteroids (50 ppm). Each treatment involved dipping the fruits for 30 seconds before allowing them to air-dry and storing them at an ambient temperature of 27± 2 ˚C. Observations were made on various physiological and biochemical parameters at regular intervals.

Shelf Life

Shelf life was recorded based on visual observations, noting the number of days the fruits remained marketable from the time of harvest. This parameter indicated the duration the fruits stayed marketable.

Physiological loss in weight (PLW)

PLW was determined as the percentage reduction in the initial weight of the fruit compared to its weight at each measurement point. This calculation was made every three days and expressed as a percentage.

Total Soluble Solids (TSS), Titratable acidity (TA), TSS/TA

Total Soluble Solids (TSS) were measured in °Brix using a hand refractometer (0-32 °Brix, Erma Inc., Tokyo) by placing 2-3 drops of mashed pulp juice on the prism. Titratable acidity (TA) was assessed by homogenizing 5 g of fruit with 30 ml of distilled water, filtering the mixture and titrating 5 ml of the filtrate against 0.1 N NaOH with phenolphthalein as an indicator until a pale pink colour appeared. TA was expressed as a percentage of citric acid using a standard formula (11). The TSS: acid ratio was calculated by dividing TSS by TA.

Total sugars

Total sugars were estimated using the anthrone method and expressed in g/100 g. A 0.5 g sample was homogenized with 10 mL of 85 % ethanol, centrifuged at 10,000 rpm for 10 min and the supernatant was diluted to 100 mL. From this, 0.5 mL was mixed with 0.5 mL of distilled water and 4 mL anthrone reagent. After 10 min of incubation and heating in a boiling water bath for 8 min, the mixture was cooled to room temperature and absorbance was measured at 640 nm. Sugar content was calculated using a standard D-glucose calibration curve (12).

Total phenol content

A 5 g sample was homogenized using 80 % methanol (or ethanol) for two to three grinding cycles. The homogenate was centrifuged at 10,000 rpm for 20 min, and the supernatant was collected and diluted to 50 mL with distilled water. A 0.5 mL aliquot of the supernatant was further diluted to 3 mL with distilled water, followed by the addition of 0.5 mL Folin-Ciocalteu reagent and a 3-min incubation. Subsequently, 2 mL of 20 % sodium carbonate solution was added, mixed thoroughly and incubated in a boiling water bath for 10 min. After cooling, the solution developed a blue colour and its absorbance was measured at 700 nm using a spectrophotometer (13).

Carotenoids

Total carotenoid content was determined by homogenizing a 1 g sample with 10 mL of a 3:2 petroleum ether: acetone mixture, followed by centrifugation. The supernatant was diluted to 50 mL with the same solvent and the absorbance was measured at

450 nm using a UV spectrophotometer. Carotenoid concentration was calculated and expressed as micrograms per 100 g of sample (14).

Enzyme Activity

Two grams of pulp were homogenized in a chilled mortar and pestle with 15 mL of sodium phosphate buffer (0.2 M, pH 7.0), supplemented with 0.2 mL EDTA and 0.2 mL cysteine-HCl. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 ˚C and the clear supernatant was collected for enzyme activity assays, as per the method described by Srivastava and Dwivedi (15). Sodium phosphate buffer provides a stable pH environment to maintain enzyme activity during extraction. EDTA chelates divalent metal ions, preventing unwanted enzyme activation, while cysteine-HCl acts as a reducing agent to protect enzymes from oxidation. These components together stabilize the enzymes, ensuring accurate activity assays.

Ripening Enzymes

***Pectin Methyl Esterase (PME) activity*:** The activity of PME activity was conducted using a spectrophotometer set at 620 nm. A mixture of 0.1 mlLbromothymol blue (0.01 %), 0.2 ml sodium chloride (0.15 M) and 1 ml pectin solution (0.01%) was prepared in a cuvette. The initial absorbance was recorded before adding 0.1 ml of enzyme extract. After the addition of the enzyme extract, absorbance was measured after three minutes. The change in absorbance was used to calculate enzyme activity, referencing a galacturonic acid standard curve (16). The results were expressed as milli equivalents of COOH released per minute per gram of fresh sample weight.

***Polygalacturonase activity*:** To measure polygalacturonase activity, a reaction mixture was prepared by combining 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, making a total volume of 1 ml. This mixture was incubated at room temperature for one hour. The reaction was then halted by adding 1 ml of 1% Di Nitro Salicylic acid (DNS) and placing the mixture in a boiling water bath. The absorbance of the solution was measured at 540 nm using a spectrophotometer against a blank. Enzyme activity was calculated using a galacturonic acid standard curve, as described in a study (17) and expressed in µg/min/g of the fresh weight of the sample.

***Amylase activity*:** To determine amylase activity, a reaction mixture consisting of 0.5 ml phosphate buffer (0.2 M), 1 ml of 0.5% potato starch substrate and 1 ml of enzyme extract was incubated at 37 ºC for one hour. The reaction was stopped by adding 1 ml of 1% Di Nitro Salicylic acid (DNS). Absorbance was then measured at 540 nm using a spectrophotometer, with a blank sample as the reference. The activity was calculated from a standard maltose curve, following the method of Bernfeld (18) and expressed in micrograms per minute per gram (µg/min/g) of fresh weight of the sample.

***Cellulase activity*:** The cellulase activity was determined by incubating a reaction mixture containing 1 ml of carboxymethyl cellulose, 1 ml of sodium acetate buffer (0.2 M) and 1 ml of enzyme extract at 37 ºC for 60 minutes. To stop the reaction, 1 ml of 1% Di Nitro Salicylic acid (DNS) was added after placing the mixture in a boiling water bath for 5 minutes. Absorbance was recorded at 540 nm using a spectrophotometer, with a blank sample as the reference. The enzyme activity was calculated from a standard glucose curve (19) and expressed as micrograms per minute per gram (µg/min/g) of fresh weight of the sample.

Antioxidant enzymes

***Peroxidase*:** To initiate the reaction, 0.2 ml of hydrogen peroxide (0.1 M) was added to a cuvette 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 activity was monitored using a spectrophotometer at 430 nm for three min, with readings taken every 30 sec. The increase in absorbance over time was plotted, and the linear rise within one minute was used to calculate peroxidase activity (20). The activity was expressed as ΔA/min/g of fresh sample weight.

***Catalase activity*:** To initiate the reaction, 0.5 ml of hydrogen peroxide (12.5 mM) was added to a cuvette containing 1.5 ml phosphate buffer (0.2 M), 50 µl of enzyme extract and 1.5 ml of distilled water. The cuvette was placed in a spectrophotometer and the enzyme activity was monitored at 240 nm for one min, with readings taken every 30 sec. The catalase activity, which caused a decrease in absorbance, was determined following the method of Aebi (21) and expressed as catalase activity/ min/g of fresh sample weight.

***Superoxide dismutase activity*:** To perform the reaction, 0.5 ml of enzyme extract, 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 1.2 ml of sodium pyrophosphate buffer (0.052 M) were combined in a cuvette. The mixture was incubated at room temperature for 3 minutes. Following incubation, 4 ml of butanol and 1 ml of glacial acetic acid were quickly added while shaking vigorously. After allowing the layers to separate, the upper butanol phase was transferred to a clean cuvette and absorbance was measured at 520 nm with a spectrophotometer. Superoxide dismutase activity was calculated following the method of Kakkar et al. (22).

Statistical Analysis

The experiment followed a completely randomized design (CRD). A one-way analysis of variance (ANOVA) was conducted to compare the treatment means, and the Least Significant Difference (LSD) test was applied to determine which treatments showed significant differences. Statistical significance was set at $p \leq 0.05$. All data analyses were performed using R Studio (version 4.3.1, released 2023-06-16) with relevant R packages for statistical computing.

Results

Shelf life

All treatments significantly influenced the storability of the fruits. Specifically, the treatments with 200 µM melatonin (T_3), 25 ppm brassinosteroid (T_9) and 2.0 mM salicylic acid (T_6) led to the longest shelf life for both varieties compared to the control and other treatments (Table 1). In the Alphonso variety, T_3 resulted in the greatest increase in mean shelf life, extending it by 12.67 days, followed closely by T_6 and T_9, each extending shelf life by 12.00 days. In the Sindhura variety, T_3 also provided the longest shelf life extension of 12.00 days, with T_6 and T_9

Table 1. Effect of bioactive compounds on shelf life (days) in mango varieties under ambient storage

Treatments	Alphonso	Sindhura
T_1	10.67^{c}	9.33^{e}
T_2	11.33^{bc}	9.67^{de}
T_3	12.67^{a}	12.00^{a}
T_4	11.33^{bc}	10.33^{cd}
T_5	11.00^{bc}	10.33^{cd}
T_6	12.00^{ab}	11.67^{ab}
T_7	11.33^{bc}	10.67^{c}
T_8	11.00^{bc}	10.33^{cd}
T_9	12.00^{ab}	11.67^{ab}
T_{10}	11.33^{bc}	11.00^{bc}
SE(d)	**0.58**	**0.42**
CD	**1.20**	**0.88**

T_1-Control (water dip); **T_2** - Melatonin (150 µM); **T3** - Melatonin (200 µM); **T_4** - Melatonin (250 µM); **T_5** - Salicylic acid (1.5 mM); **T_6**-Salicylic acid (2.0 mM); **T_7**- Salicylic acid (2.5 mM); **T_8**- Brassinosteroid (10ppm); **T_9**- Brassinosteroid (25ppm); **T_{10}** - Brassinosteroid (50 ppm). Each value is a mean for three replicates ($p = 0.05$).

closely following at 11.67 days at the end of the storage period.

Physiological loss in weight

The percentage loss in weight (PLW) for Alphonso and Sindhura mangoes, as shown in Table 2, revealed that PLW increased progressively throughout the storage period. All treatments significantly reduced weight loss compared to the control, which exhibited the highest weight loss of 11.06% for Alphonso on the 12th day. In Alphonso, the treatment that was most effective in minimizing weight loss was T9 (Brassinosteroid 25 ppm) with a loss of 9.79%, followed by T3 (Melatonin 200 µM) with 9.81% and T6 (Salicylic Acid 2.0 mM) with 10.12%. Similarly, for Sindhura, only these treatments (T_3, T_6 and T_9) maintained fruit quality up to the 12th day, while all other treatments led to deterioration by day 9. The 9th day, Sindhura exhibited the lowest weight losses in T_3 (7.09%), followed by T_9 (7.59%) and T_6 (8.63%).

Total Soluble Solids (TSS)

Total Soluble Solids (TSS) increased during storage in both Alphonso and Sindhura (Table 3), but treatments T_3, T_6 and T_9 effectively maintained lower Brix values. By day 12 in Alphonso, T_3, T_6 and T_9 recorded 20.07, 21.00 and 21.50°Brix, respectively while control had 25.37°Brix. In Sindhura, on day 9, T_3, T_6 and T_9 showed significantly lower readings (20.50,

Table 2. Effect of bioactive compounds on physiological loss in weight (%) in mango varieties under ambient storage

Treatments	Alphonso				Sindhura			
	Day 3	Day 6	Day 9	Day 12	Day 3	Day 6	Day 9	Day 12
T_1	0.48^{b}	7.68^{a}	9.11^{f}	11.06^{a}	3.08^{a}	7.05^{a}	10.85^{a}	NA
T_2	0.35^{e}	6.24^{e}	9.33^{f}	10.42^{bc}	2.62^{c}	5.42^{d}	8.62^{d}	NA
T_3	0.26^{g}	5.29^{g}	8.12^{g}	9.81^{d}	1.75^{g}	5.13^{e}	7.09^{f}	9.48^{b}
T_4	0.44^{c}	5.83^{f}	9.88^{e}	10.27^{bc}	2.29^{e}	5.69^{bc}	10.41^{b}	NA
T_5	0.39^{d}	6.57^{cd}	10.30^{d}	10.17^{c}	2.49^{d}	5.63^{c}	9.57^{c}	NA
T_6	0.26^{g}	5.85^{f}	10.34^{d}	10.12^{cd}	1.89^{f}	5.02^{e}	8.63^{d}	9.51^{b}
T_7	0.35^{e}	6.29^{de}	10.61^{cd}	10.31^{bc}	2.26^{e}	5.84^{b}	9.69^{c}	NA
T_8	0.39^{d}	7.12^{b}	10.88^{bc}	10.36^{bc}	3.06^{ab}	5.67^{bc}	8.55^{d}	NA
T_9	0.31^{f}	5.77^{f}	11.15^{b}	9.79^{d}	2.01^{f}	5.11^{e}	7.59^{e}	9.92^{a}
T_{10}	0.91^{a}	6.69^{c}	12.04^{a}	10.61^{b}	2.96^{b}	5.60^{cd}	10.62^{ab}	NA
SE(d)	**0.01**	**0.15**	**0.20**	**0.17**	**0.058**	**0.09**	**0.17**	**0.09**
CD	**0.02**	**0.31**	**0.41**	**0.35**	**0.120**	**0.19**	**0.36**	**0.19**

T_1-Control (water dip); **T_2** - Melatonin (150 µM); **T3** - Melatonin (200 µM); **T_4** - Melatonin (250 µM); **T_5** - Salicylic acid (1.5 mM); **T_6**-Salicylic acid (2.0 mM); **T_7**- Salicylic acid (2.5 mM); **T_8**- Brassinosteroid (10ppm); **T_9**- Brassinosteroid (25ppm); **T_{10}** - Brassinosteroid (50 ppm). Each value is a mean for three replicates ($p = 0.05$).

Table 3. Effect of postharvest treatments on Total Soluble Solids (°Brix) in mango varieties under ambient storage

Treatments	Alphonso					Sindhura				
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 0	Day 3	Day 6	Day 9	Day 12
T_1	6.00^{b}	9.00^{b}	15.00^{b}	19.00^{a}	25.37^{a}	7.00^{b}	13.00^{a}	19.00^{a}	24.00^{a}	NA
T_2	5.00^{c}	8.00^{d}	13.50^{d}	17.00^{c}	23.11^{cd}	6.00^{c}	10.50^{ef}	16.00^{d}	21.00^{cd}	NA
T_3	5.00^{c}	7.00^{e}	12.50^{e}	15.00^{e}	20.07^{h}	7.00^{b}	10.00^{f}	15.00^{e}	20.50^{de}	22.00^{b}
T_4	5.20^{c}	7.00^{e}	14.50^{c}	16.00^{d}	22.00^{ef}	6.00^{c}	9.00^{g}	15.00^{e}	21.80^{bc}	NA
T_5	7.00^{a}	10.00^{a}	13.50^{d}	18.00^{b}	22.50^{de}	7.00^{b}	11.00^{de}	17.00^{c}	22.00^{b}	NA
T_6	6.00^{b}	7.00^{e}	11.00^{g}	15.00^{e}	21.00^{g}	7.00^{b}	10.50^{ef}	15.50^{de}	20.00^{e}	23.00^{a}
T_7	7.00^{a}	8.00^{d}	13.50^{d}	16.00^{d}	23.50^{bc}	8.00^{a}	12.00^{bc}	16.00^{d}	22.50^{b}	NA
T_8	6.00^{b}	8.50^{c}	16.50^{a}	17.00^{c}	24.00^{b}	6.00^{c}	12.50^{ab}	18.00^{b}	23.50^{a}	NA
T_9	5.00^{c}	7.00^{e}	12.00^{f}	15.00^{e}	21.50^{fg}	6.00^{c}	10.50^{ef}	14.00^{f}	21.00^{cd}	22.00^{b}
T_{10}	5.00^{c}	8.00^{d}	12.40^{ef}	15.00^{e}	22.00^{ef}	7.00^{b}	11.50^{cd}	16.00^{d}	22.00^{b}	NA
SE(d)	**0.12**	**0.18**	**0.23**	**0.36**	**0.42**	**0.16**	**0.26**	**0.29**	**0.46**	**0.20**
CD	**0.25**	**0.37**	**0.48**	**0.76**	**0.88**	**0.33**	**0.54**	**0.60**	**0.95**	**0.43**

Treatment details: **T_1**-Control (water dip); **T_2** - Melatonin (150 µM); **T_3** - Melatonin (200 µM); **T_4** - Melatonin (250 µM); **T_5** - Salicylic acid (1.5 mM); **T_6**-Salicylic acid (2.0 mM); **T_7**- Salicylic acid (2.5 mM); **T_8** - Brassinosteroid (10 ppm); **T_9**- Brassinosteroid (25 ppm);**T_{10}** - Brassinosteroid (50 ppm). Each value is a mean for three replicates ($p = 0.05$).

20.00 and 21.00°Brix) than the control (24.00).

Titratable acidity (TA)

Titratable acidity (TA) decreased throughout storage in both Alphonso and Sindhura, but treatments with melatonin, salicylic acid, and brassinosteroids significantly slowed the rate of decline as given in Table 4. Control fruits (T_1) showed the lowest TA, with 0.23% on day 12 for Alphonso and day 9 for Sindhura. In Alphonso, T_3 and T_6 maintained higher TA levels (0.46% and 0.38%), while in Sindhura, T_3 (0.84%), T_9 (0.77%), and T_6 (0.69 %) were most effective in preserving acidity.

TSS:TA

The TSS: acid ratio increased steadily during storage in both Alphonso and Sindhura mangoes (Table 5). Treatments with melatonin (200 µM), salicylic acid (2 mM) and brassinosteroids (25 ppm) significantly influenced this ratio, maintaining lower values compared to the control. By the end of storage, the highest ratios were in control fruits (110.11 for Alphonso and 95.48 for Sindhura). T_3 and T_6 maintained lower ratios in Alphonso (50.99 and 52.27), while in Sindhura, T_3 (24.26), T_9 (27.34) and T_6 (28.93) were most effective on day 9.

Total sugar

Total sugar content increased linearly from unripe to ripe stages across all treatments. The highest sugar content was observed in the control fruits on day 12 for Alphonso (20.05 g/100 g) and day 9 for Sindhura (13.33 g/100 g) as given in Table 6. The lowest sugar content was recorded in Alphonso on day 12 for T9 (16.10 g/100 g), T3 (16.22 g/100 g), and T6 (16.64 g/100 g). For Sindhura, the lowest content on day 9 was in T3 (10.19 g/100 g), followed by T6 (10.33 g/100 g) and T9 (10.51 g/100 g).

Total phenol content

Total phenol content decreased during storage but remained higher in treated fruits compared to the control as given in Table 7. By day 12 in Alphonso, T3 (20.05), T9 (19.40) and T4 (19.25) showed significantly higher phenol levels than the control (13.05). In Sindhura, on day 9, T3 (16.00), T6 (15.65) and T9 (15.45) maintained higher phenol content, while the control recorded the lowest (10.15).

Carotenoids

Carotenoid content increased in both treated and untreated Alphonso and Sindhura mangoes during storage, with treated fruits showing lower levels than the control (Table 8). By day 12 in Alphonso, T3 (21,133.43 µg/100g), T6 (21,773.26 µg/100g) and T9 (21,288.92 µg/100g) recorded the lowest carotenoid levels. In Sindhura, viable treatments on day 12 included T3 (19,880.33 µg/100g), T6 (20,468.76 µg/100g) and T9 (20,666.03 µg/100g), while the highest carotenoid content was observed

Table 4. Effect of postharvest treatments on titratable acidity (%) in mango varieties under ambient storage

Treatments	Alphonso					Sindhura				
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 0	Day 3	Day 6	Day 9	Day 12
T_1	3.23^{de}	1.99^{g}	1.38^{f}	0.61^{g}	0.23^{d}	2.53^{c}	1.69^{f}	0.99^{f}	0.23^{g}	NA
T_2	3.45^{c}	2.61^{cd}	1.69^{e}	0.84^{e}	0.31^{c}	2.61^{bc}	1.99^{cd}	1.46^{a}	0.38^{f}	NA
T_3	3.69^{b}	2.99^{a}	2.15^{a}	1.22^{a}	0.46^{a}	2.69^{ab}	2.22^{a}	1.46^{a}	0.84^{a}	0.38^{a}
T_4	3.30^{cde}	2.69^{c}	$1.92c^{d}$	1.07^{c}	0.31^{c}	2.61^{bc}	2.07^{bc}	1.38^{b}	0.54^{e}	NA
T_5	3.15^{e}	2.30^{f}	1.69^{e}	0.92^{d}	0.31^{c}	2.53^{c}	1.92^{de}	0.92^{g}	0.61^{d}	NA
T_6	3.45^{c}	2.84^{b}	1.99^{bc}	1.15^{b}	0.38^{b}	2.76^{a}	2.15^{ab}	1.38^{b}	0.69^{c}	0.23^{c}
T_7	3.45^{c}	2.99^{a}	1.84^{d}	1.07^{c}	0.23^{d}	2.69^{b}	1.92^{de}	1.30^{c}	0.53^{e}	NA
T_8	3.38^{cd}	2.46^{e}	1.84^{d}	0.69^{f}	0.23^{d}	2.61^{bc}	1.84^{e}	1.07^{e}	0.38^{f}	NA
T_9	3.76^{ab}	2.84^{b}	2.07^{ab}	1.23^{a}	0.31^{c}	2.69^{ab}	2.07^{bc}	1.46^{a}	0.77^{b}	0.31^{b}
T_{10}	3.92^{a}	2.53^{de}	1.92^{cd}	1.07^{c}	0.23^{d}	2.61^{bc}	1.99^{cd}	1.15^{d}	0.61^{d}	NA
SE(d)	**0.07**	**0.06**	**0.04**	**0.02**	**0.01**	**0.07**	**0.04**	**0.04**	**0.02**	**0.00**
CD	**0.16**	**0.12**	**0.08**	**0.05**	**0.02**	**0.14**	**0.09**	**0.07**	**0.03**	**0.01**

Treatment details: T_1 - Control (water dip); T_2 - Melatonin (150 µM); T_3 - Melatonin (200 µM); T_4 - Melatonin (250 µM); T_5 - Salicylic acid (1.5 mM); T_6 - Salicylic acid (2.0 mM); T_7 - Salicylic acid (2.5 mM); T_8 - Brassinosteroid (10 ppm); T_9 - Brassinosteroid (25 ppm);T_{10} - Brassinosteroid (50 ppm). Each value is a mean for three replicates ($p = 0.05$).

Table 5. Effect of postharvest treatments on TSS: acid ratio in mango varieties under ambient storage

Treatments	Alphonso					Sindhura				
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 0	Day 3	Day 6	Day 9	Day 12
T_1	1.86^{c}	4.50^{a}	10.85^{a}	30.92^{a}	110.11^{a}	2.76^{b}	7.69^{a}	19.03^{a}	95.48^{a}	NA
T_2	1.45^{f}	3.06^{a}	7.99^{a}	20.12^{a}	75.23^{cd}	2.29^{e}	5.26^{e}	10.96^{f}	54.69^{c}	NA
T_3	1.36^{g}	2.38^{a}	5.81^{a}	12.20^{a}	50.99^{e}	2.60^{cd}	4.49^{h}	10.28^{g}	24.26^{h}	57.29^{c}
T_4	1.57^{e}	2.60^{a}	7.55^{a}	14.88^{a}	71.61^{d}	2.29^{e}	4.34^{h}	10.85^{f}	40.55^{de}	NA
T_5	2.22^{a}	4.34^{a}	7.99^{a}	19.53^{a}	73.24^{d}	2.76^{b}	5.73^{d}	18.44^{b}	35.81^{f}	NA
T_6	1.74^{d}	2.46^{a}	5.51^{a}	13.02^{a}	52.27^{e}	2.53^{d}	4.88^{g}	11.21^{f}	28.93^{g}	91.15^{a}
T_7	2.03^{b}	2.67^{a}	7.32^{a}	14.88^{a}	91.15^{b}	2.98^{a}	6.25^{c}	12.25^{e}	41.85^{d}	NA
T_8	1.78^{d}	3.46^{a}	8.95^{a}	24.59^{a}	93.32^{b}	2.29^{e}	6.78^{b}	16.74^{c}	61.19^{b}	NA
T_9	1.33^{gh}	2.46^{a}	5.79^{a}	12.21^{a}	78.13^{c}	2.23^{e}	5.06^{f}	9.59^{h}	27.34^{g}	68.36^{b}
T_{10}	1.28^{h}	3.16^{a}	6.46^{a}	13.95^{a}	95.49^{b}	2.68^{bc}	5.76^{d}	13.89^{d}	39.06^{e}	NA
SE(d)	**0.04**	**1.16**	**4.38**	**12.49**	**2.16**	**0.06**	**0.08**	**0.26**	**1.23**	**0.63**
CD	**0.08**	**2.42**	**9.13**	**26.06**	**4.51**	**0.12**	**0.17**	**0.54**	**2.56**	**1.30**

Treatment details: T_1 - Control (water dip); T_2 - Melatonin (150 µM); T_3 - Melatonin (200 µM); T_4 - Melatonin (250 µM); T_5 - Salicylic acid (1.5 mM); T_6 - Salicylic acid (2.0 mM); T_7 - Salicylic acid (2.5 mM); T_8 - Brassinosteroid (10 ppm); T_9 - Brassinosteroid (25 ppm); T_{10} - Brassinosteroid (50 ppm). Each value is a mean for three replicates ($p = 0.05$).

Table 6. Effect of postharvest treatments on total sugar content (g/100g) in mango varieties under ambient storage

Treatments	Alphonso					Sindhura				
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 0	Day 3	Day 6	Day 9	Day 12
T_1	4.85^{a}	6.29^{a}	8.83^{a}	14.17^{a}	20.05^{a}	4.25^{abc}	5.65^{a}	8.05^{a}	13.33^{a}	NA
T_2	4.78^{ab}	5.78^{bc}	8.42^{b}	11.21^{d}	18.92^{b}	4.43^{a}	5.42^{b}	7.39^{bc}	11.58^{de}	NA
T_3	4.45^{c}	5.54^{d}	6.83^{d}	10.12^{e}	16.22^{g}	4.10^{cd}	4.51^{e}	6.60^{f}	10.19^{f}	16.60^{c}
T_4	4.42^{c}	5.53^{d}	8.25^{b}	11.11^{d}	17.02^{ef}	4.21^{bcd}	5.07^{c}	7.16^{cd}	11.18^{e}	NA
T_5	4.60^{bc}	5.85^{b}	8.39^{b}	13.10^{bc}	18.38^{bc}	4.03^{d}	5.11^{c}	7.42^{bc}	11.86^{cd}	NA
T_6	4.76^{ab}	5.60^{cd}	7.46^{c}	10.48^{e}	16.64^{fg}	4.37^{ab}	5.02^{c}	6.78^{ef}	10.33^{f}	17.99^{b}
T_7	4.75^{ab}	5.83^{b}	8.26^{b}	13.46^{b}	18.05^{cd}	4.05^{d}	5.22^{c}	7.51^{b}	12.10^{c}	NA
T_8	4.74^{ab}	6.21^{a}	8.39^{b}	14.15^{a}	18.84^{bc}	4.42^{a}	5.49^{ab}	7.89^{a}	12.67^{b}	NA
T_9	4.44^{c}	5.41^{d}	7.69^{c}	9.25^{f}	16.10^{g}	4.12^{cd}	4.72^{d}	6.98^{de}	10.51^{f}	18.75^{a}
T_{10}	4.62^{bc}	5.60^{cd}	8.39^{b}	12.86^{c}	17.48^{de}	4.12^{cd}	5.48^{ab}	7.49^{b}	11.80^{cd}	NA
SE(d)	**0.11**	**0.10**	**0.16**	**0.25**	**0.38**	**0.09**	**0.10**	**0.15**	**0.23**	**0.18**
CD	**0.22**	**0.21**	**0.34**	**0.52**	**0.80**	**0.19**	**0.20**	**0.30**	**0.49**	**0.38**

Treatment details: T_1-Control (water dip); T_2 - Melatonin (150 µM); T_3 - Melatonin (200 µM); T_4 - Melatonin (250 µM); T_5 - Salicylic acid (1.5 mM); T_6-Salicylic acid (2.0 mM); T_7- Salicylic acid (2.5 mM); T_8 - Brassinosteroid (10 ppm); T_9- Brassinosteroid (25 ppm); T_{10} - Brassinosteroid (50 ppm). Each value is a mean for three replicates (p = 0.05).

Table 7. Effect of postharvest treatments on total phenol content in mango varieties under ambient storage

Treatments	Alphonso					Sindhura				
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 0	Day 3	Day 6	Day 9	Day 12
T_1	34.55^{a}	28.30^{c}	23.90^{f}	21.70^{b}	13.05^{f}	33.20^{a}	21.50^{e}	16.35^{g}	10.15^{f}	NA
T_2	34.70^{a}	29.05^{bc}	25.70^{cd}	22.00^{b}	18.45^{b}	33.55^{a}	24.50^{cd}	18.15^{d}	12.95^{d}	NA
T_3	35.45^{a}	30.45^{a}	27.55^{ab}	24.50^{a}	20.05^{a}	33.45^{a}	28.15^{a}	19.55^{a}	16.00^{a}	12.95^{a}
T_4	35.90^{a}	29.10b^{c}	26.85^{bc}	22.25^{b}	19.25^{ab}	32.65^{a}	23.70^{d}	17.85^{de}	11.20^{e}	NA
T_5	34.30^{a}	29.15b^{c}	25.30^{de}	20.10^{c}	16.55^{c}	33.00^{a}	24.45^{d}	18.85^{bc}	14.95^{c}	NA
T_6	35.30^{a}	29.85^{ab}	27.40^{ab}	24.00^{a}	18.45^{b}	32.80^{a}	26.10^{b}	19.30^{ab}	15.65^{ab}	12.40^{b}
T_7	35.50^{a}	23.15^{e}	25.75^{cd}	21.90^{b}	15.05^{de}	33.30^{a}	23.85^{d}	17.25^{f}	10.45^{f}	NA
T_8	35.05^{a}	25.45^{d}	24.15^{ef}	21.30^{b}	14.65^{e}	33.60^{a}	23.70^{d}	17.00^{f}	10.30^{f}	NA
T_9	34.95^{a}	29.15^{bc}	28.40^{a}	24.50^{a}	19.40^{a}	33.50^{a}	25.35^{bc}	18.75^{c}	15.45^{abc}	11.95^{c}
T_{10}	35.15^{a}	28.75^{bc}	25.05^{def}	22.00^{b}	15.75^{cd}	33.65^{a}	24.20^{d}	17.45^{ef}	15.10^{bc}	NA
SE(d)	**0.95**	**0.54**	**0.57**	**0.55**	**0.39**	**0.85**	**0.43**	**0.23**	**0.29**	**0.16**
CD	**1.98**	**1.13**	**1.18**	**1.15**	**0.82**	**1.77**	**0.90**	**0.49**	**0.61**	**0.33**

Treatment details: T_1-Control (water dip); T_2 - Melatonin (150 µM); T_3 - Melatonin (200 µM); T_4 - Melatonin (250 µM); T_5 - Salicylic acid (1.5 mM); T_6-Salicylic acid (2.0 mM); T_7- Salicylic acid (2.5 mM); T_8 - Brassinosteroid (10 ppm); T_9- Brassinosteroid (25 ppm);T_{10} - Brassinosteroid (50 ppm). Each value is a mean for three replicates (p = 0.05).

Table 8. Effect of postharvest treatments on carotenoid content (µg/100g) in mango varieties under ambient storage

Treatments	Alphonsa					Sindhura				
	Day0	Day 3	Day 6	Day 9	Day12	Day0	Day 3	Day 6	Day 9	Day12
T_1	4674.62^{a}	8578.9^{a}	13575.37^{a}	17671.91^{a}	23004.91^{a}	3425.30^{a}	7595.81^{a}	13533.72^{a}	18142.12^{a}	NA
T_2	4688.64^{a}	8138.12^{bc}	12137.32^{cde}	16780.31^{bcd}	22375.34^{ab}	3429.31^{a}	6555.19^{c}	12781.19^{b}	16706.73^{b}	NA
T_3	4686.83^{a}	7790.40^{d}	11615.73^{e}	16020.03^{e}	21133.43^{c}	3426.44^{a}	6057.02^{d}	11669.70^{c}	15990.03^{c}	19880.33^{a}
T_4	4686.36^{a}	8057.30^{bcd}	11803.40^{de}	16324.98^{cde}	22432.47^{ab}	3422.97^{a}	6188.53^{d}	11928.96^{c}	16352.16^{bc}	NA
T_5	4688.95^{a}	8117.86^{bc}	12517.01^{bc}	16970.80^{bc}	22436.25^{ab}	3422.76^{a}	6663.12^{bc}	12809.60^{b}	16722.68^{b}	NA
T_6	4687.27^{a}	7945.39^{cd}	11762.15^{de}	16185.76^{de}	21773.26^{bc}	3423.81^{a}	6164.52^{d}	11898.13^{c}	16272.69^{bc}	20468.76^{a}
T_7	4672.43^{a}	8072.05^{bcd}	12258.09^{cd}	16470.01^{bcde}	22233.27^{ab}	3425.20^{a}	6218.64^{d}	12149.47^{c}	16693.11^{bc}	NA
T_8	4688.81^{a}	8362.71^{ab}	12938.41^{b}	17047.00^{ab}	22984.11^{a}	3424.57^{a}	6953.95^{b}	12829.26^{b}	17766.35^{a}	NA
T_9	4687.30^{a}	7977.36^{cd}	11791.61^{de}	16072.06^{e}	21288.92^{c}	3427.91^{a}	6151.29^{d}	11885.42^{c}	16191.93^{bc}	20666.03^{a}
T_{10}	4683.28^{a}	8067.18^{bcd}	12683.63^{bc}	16446.96^{bcde}	22586.90^{ab}	3426.54^{a}	6207.52^{d}	12022.41^{c}	16418.94^{bc}	NA
SE(d)	**78.86**	**151.94**	**280.5015**	**333.11**	**423.44**	**62.77**	**140.95**	**232.11**	**341.76**	**495.16**
CD	**164.50**	**316.93**	**585.1158**	**694.85**	**883.28**	**130.93**	**294.01**	**484.18**	**712.90**	**1211.62**

Treatment details: T_1-Control (water dip); T_2 - Melatonin (150 µM); T_3 - Melatonin (200 µM); T_4 - Melatonin (250 µM); T_5 - Salicylic acid (1.5 mM); T_6-Salicylic acid (2.0 mM); T_7- Salicylic acid (2.5 mM); T_8 - Brassinosteroid (10 ppm); T_9- Brassinosteroid (25 ppm);T_{10} - Brassinosteroid (50 ppm). Each value is a mean for three replicates (p = 0.05).

in the control for both varieties.

Enzyme Activity

Ripening enzymes

Pectin methyl esterase activity: Pectin methyl esterase (PME) activity in mangoes increased as the fruits ripened, as represented in Fig. 1. The highest PME activity was observed on the 12th day for Alphonso (466.00 m.eq. COOH/min/g) and on the 9th day for Sindhura (107.00 m.eq. COOH/min/g). All treatments significantly reduced PME activity compared to the control, with 200 µM Melatonin (T_3), 2.0 mM Salicylic Acid (T_6) and 25 ppm Brassinosteroids (T_9) showing the lowest levels by the end of the storage period. Specifically, PME activities for Alphonso were 409.00, 412.00 and 417.00 m.eq. COOH/min/g, while for Sindhura, they were 94.00, 96.00 and 94.00 m.eq. COOH/min/g on the 12th and 9th days, respectively.

Polygalacturonase activity: Polygalacturonase (PG) activity in the mango varieties Alphonso and Sindhura increased with storage duration. The highest enzyme activity was recorded in the control fruits on the last day of storage, at 79.43 µg/min/g for Alphonso and 77.24 µg/min/g for Sindhura. In contrast, the lowest PG activity for Alphonso was found in T_9 at 65.91 µg/min/g, followed by T_3 at 66.15 µg/min/g and T8 at 66.27 µg/min/g. For Sindhura, the lowest values were similar across treatments, with T_3 at 68.34 µg/min/g, T_6 at 68.83 µg/min/g and T_9 at 69.27 µg/min/g (as shown in Fig. 2.).

Amylase activity: Amylase activity, as shown in Fig. 3, increased significantly during ripening before declining toward the end of storage in both mango varieties. For Alphonso, peak activity

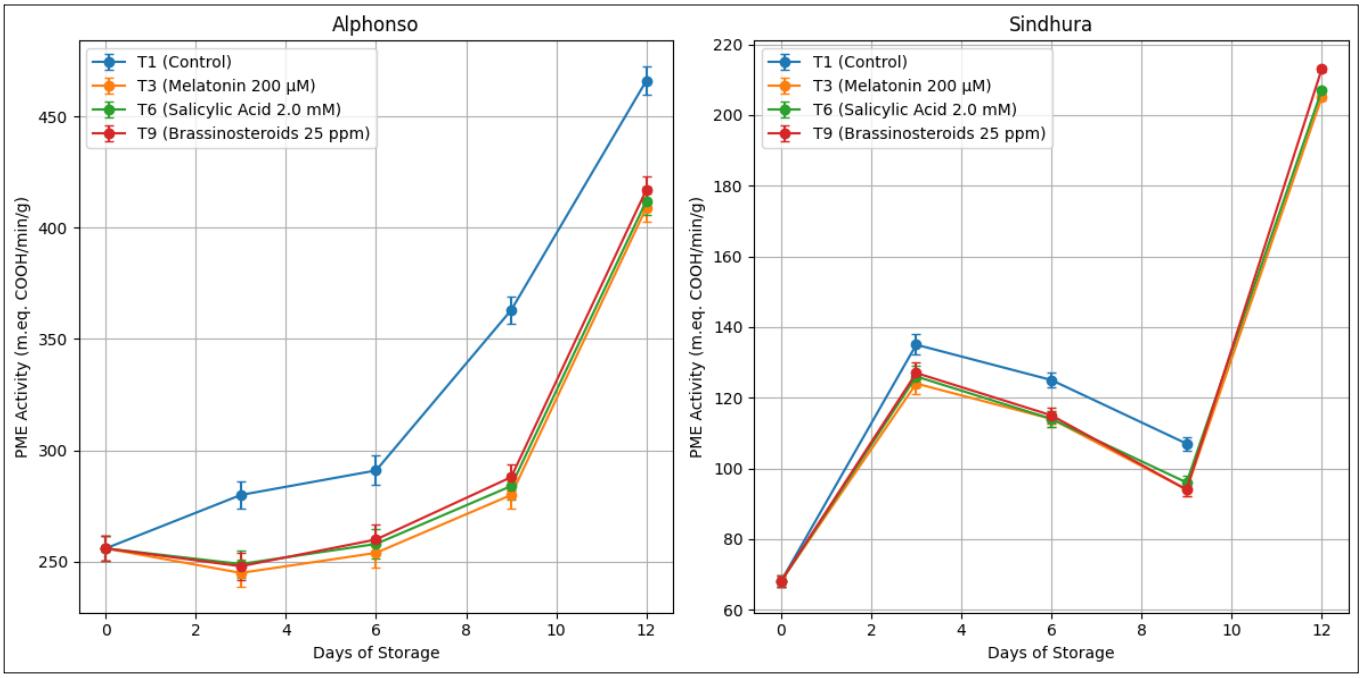

Fig. 1. Effect of bioactive compounds on PME activity (%) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); **T_3** - Melatonin (200 µM); **T_6** -Salicylic acid (2.0 mM); **T_9**- Brassinosteroid (25 ppm). Each value is a mean for three replicates (p = 0.05).

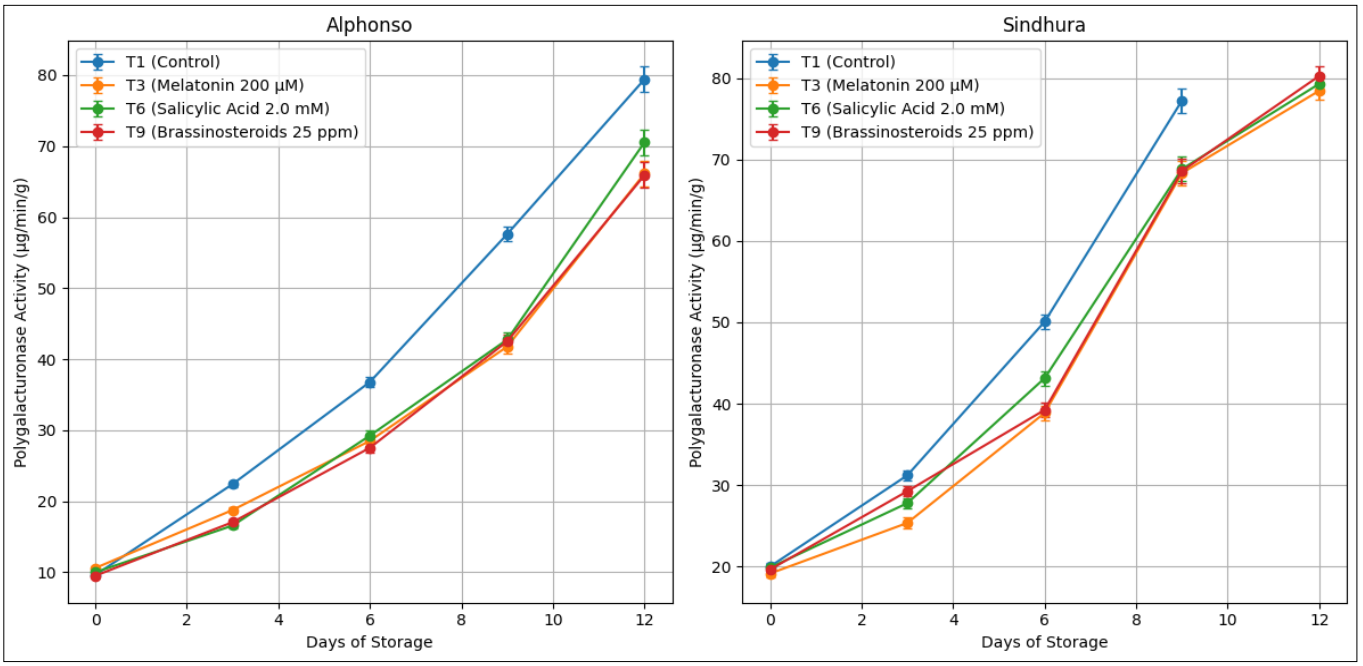

Fig. 2. Effect of bioactive compounds on polygalacturonase activity (µg/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. **T_1**- Control (water dip); **T_3**- Melatonin (200 µM); **T_6**- Salicylic acid (2.0 mM); **T_9**- Brassinosteroid (25 ppm). Each value is a mean for three replicates (p = 0.05).

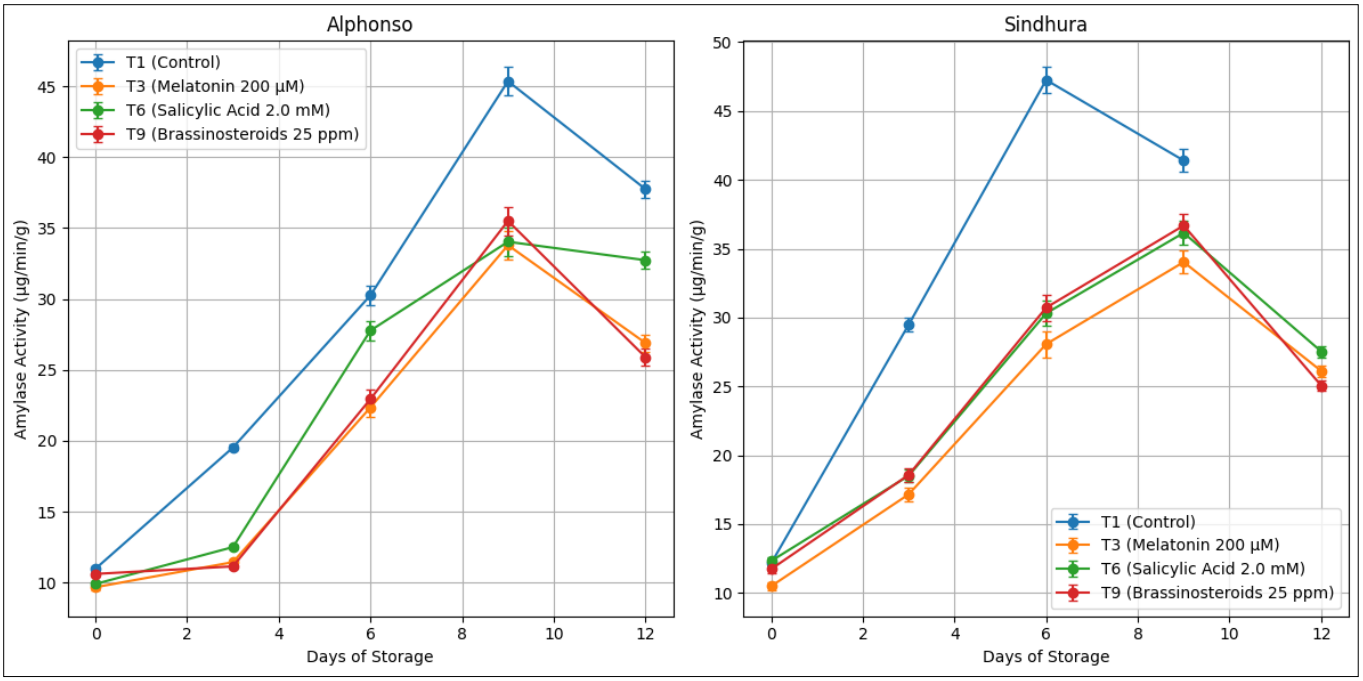

Fig. 3. Effect of bioactive compounds on amylase activity (µg/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); **T_3**- Melatonin (200 µM); **T_6**- Salicylic acid (2.0 mM); **T_9**- Brassinosteroid (25 ppm). Each value is a mean for three replicates ($p = 0.05$).

occurred on the 9th day in the control group (45.35 µg/min/g), while the lowest activity was found in T_3 (33.79 µg/min/g), T_6 (34.03 µg/min/g), and T_9 (35.50 µg/min/g) on the same day. Enzyme activity declined by the final storage day. In Sindhura, peak activity was noted on the 6th day for the control fruits, with treated groups experiencing a delay of nearly three days. The control recorded a peak of 47.25 µg/min/g, while the lowest activity among treatments was in T_3 (34.03 µg/min/g), followed by T_{10} (35.56 µg/min/g) and T_6 and T_9, which were comparable at 36.15 µg/min/g and 36.68 µg/min/g, respectively.

***Cellulase activity*:** Cellulase activity for Alphonso and Sindhura is presented in Fig. 4. In Alphonso, enzyme activity increased steadily, peaking on the last day of storage at 101.20 µg/min/g in the control group. In contrast, Sindhura's peak activity in treatments T_3, T_6 and T_9 was delayed by three days, while other treatments reached their peak on the 9th day. The lowest cellulase activities for Alphonso were noted in T_3 (72.39 µg/min/g), T_9 (75.23 µg/min/g) and T_6 (75.37 µg/min/g). For Sindhura, the control recorded a peak of 81.90 µg/min/g, with the lowest values seen in T_3 and T_6 on the 9th day.

Antioxidant enzymes

***Peroxidase activity*:** Fig. 5 shows a gradual increase in peroxidase activity during storage for both mango varieties, peaking before declining at the end. Alphonso reached its peak on the 9th day, while Sindhura peaked on the 6th day. The control group displayed significantly lower peroxidase activity at 0.30 ΔA/min/g on their respective peak days compared to the treated fruits. Notably, the highest peroxidase activity was

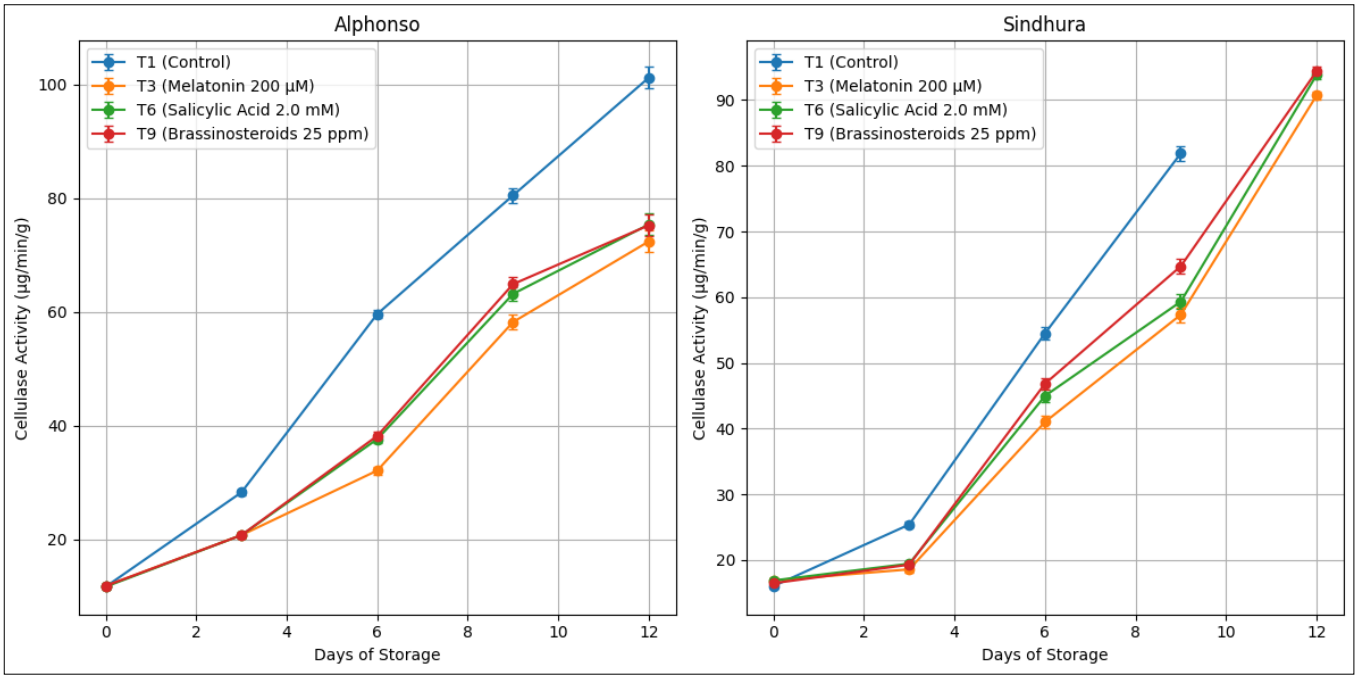

Fig. 4. Effect of bioactive compounds on cellulase activity (µg/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); **T_3**- Melatonin (200 µM); **T_6**- Salicylic acid (2.0 mM); **T_9**- Brassinosteroid (25 ppm). Each value is a mean for three replicates ($p = 0.05$).

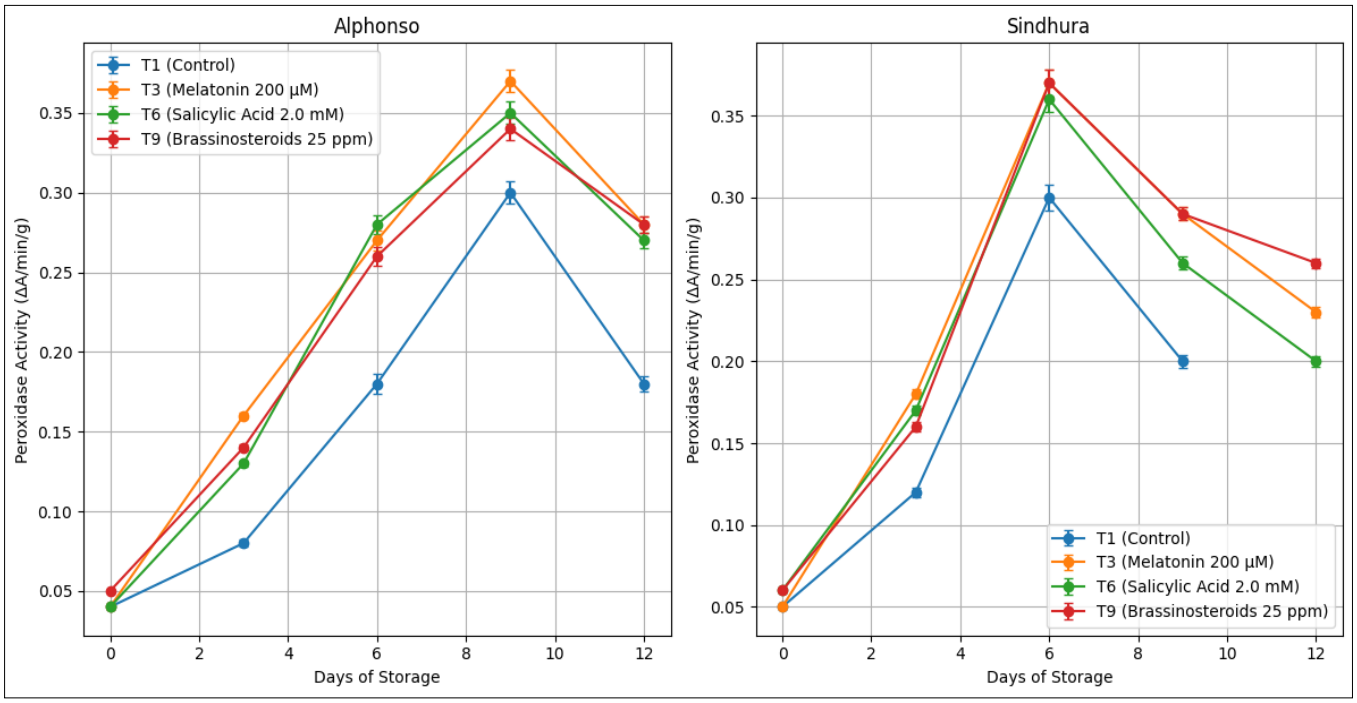

Fig. 5. Effect of bioactive compounds on peroxidase activity (ΔA/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); T_3- Melatonin (200 µM); T_6- Salicylic acid (2.0 mM); T_9- Brassinosteroid (25 ppm). Each value is a mean for three replicates ($p = 0.05$).

found in fruits treated with 200 µM Melatonin (T_3), 2.0 mM Salicylic Acid (T_6) and 25 ppm Brassinosteroid (T_9).

***Catalase activity*:** Fig. 6 presents the changes in catalase activity during storage for Alphonso and Sindhura mangoes. Catalase activity initially increased, peaking on the 9th day before declining through the 12th day across all treatments. Only three treatments, 200 µM Melatonin (T_3), 2.0 mM Salicylic Acid (T_6) and 25 ppm Brassinosteroid (T_9), remained effective in Sindhura until day 12. These treatments exhibited significantly higher catalase activity, with values of 4.87, 3.80 and 4.39 activity/min/g in Alphonso and 5.96, 5.79 and 5.92 activity/min/g in Sindhura. In contrast, the control group displayed lower levels of catalase activity at 2.68 and 4.34 activity/min/g for Alphonso and Sindhura, respectively.

***Superoxide dismutase activity*:** Fig. 7 shows the superoxide dismutase (SOD) activity for Alphonso and Sindhura mangoes. SOD activity steadily increased throughout the storage period, peaking on the last day. Control fruits had lower SOD activity, measuring 69.85 units/min/g for Alphonso and 58.49 units/min/g for Sindhura. In contrast, fruits treated with 200 µM melatonin (T_3), 25 ppm brassinosteroid (T_9) and 2.0 mM salicylic acid (T_6) exhibited significantly higher SOD activity. In Alphonso, the recorded values on the 12th day were 81.56, 81.02 and 79.36 units/min/g, respectively, while in Sindhura, the

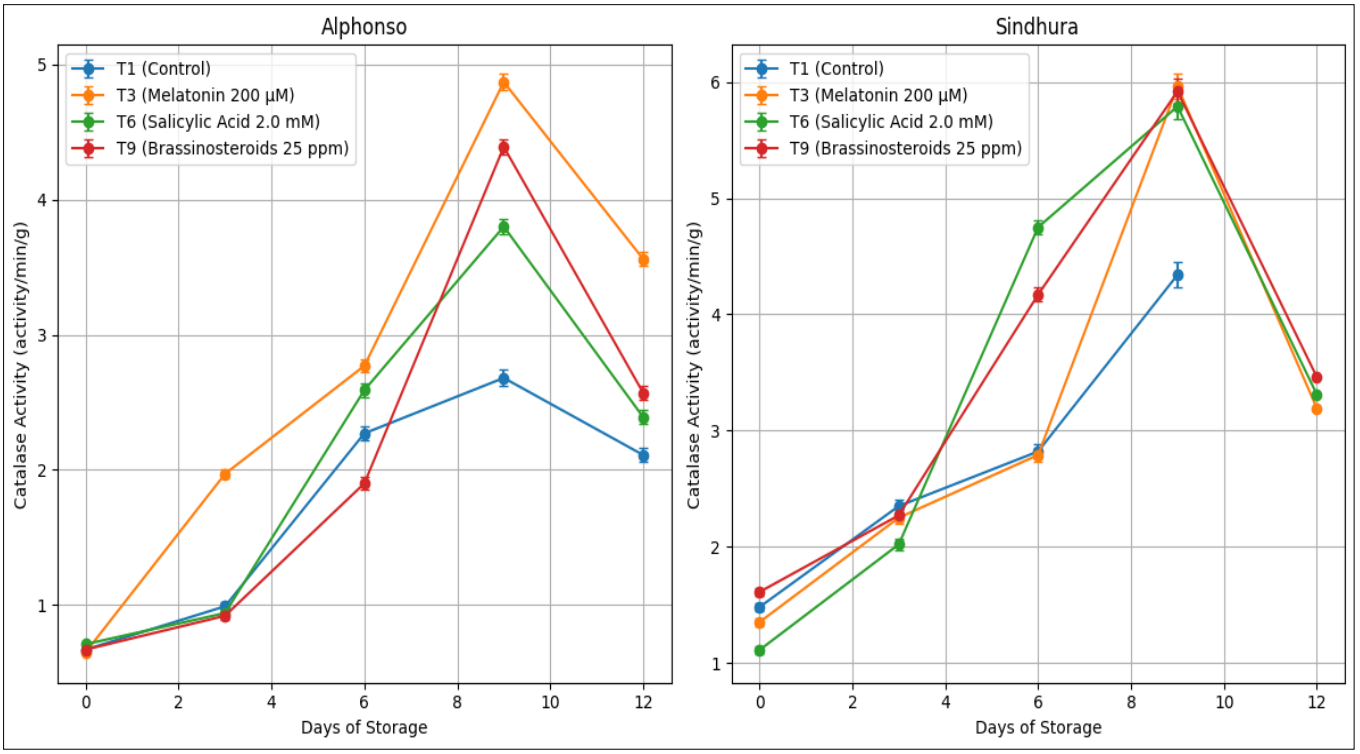

Fig. 6. Effect of bioactive compounds on catalase activity (activity/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); T_3- Melatonin (200 µM); T_6- Salicylic acid (2.0 mM); T_9- Brassinosteroid (25 ppm). Each value is a mean for three replicates ($p = 0.05$).

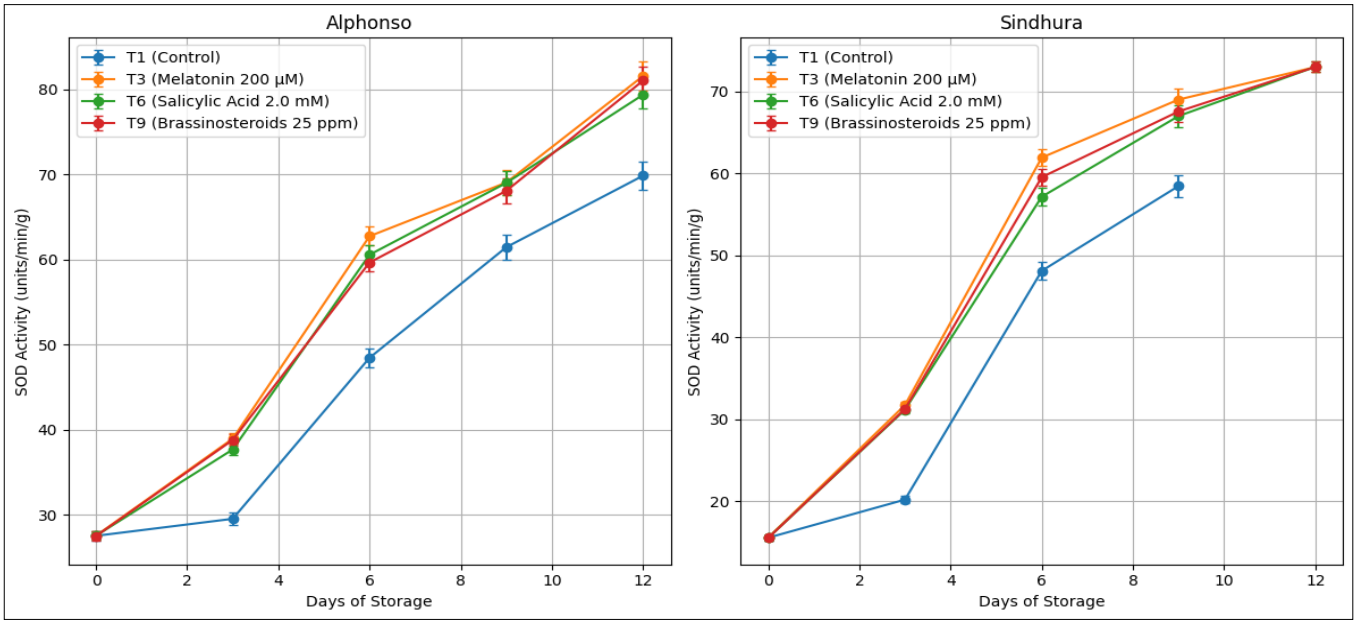

Fig. 7. Effect of bioactive compounds on superoxide dismutase activity (units/min/g) in mango varieties A) Alphonso and B) Sindhura under ambient storage. T_1- Control (water dip); T_3- Melatonin (200 µM); T_6- Salicylic acid (2.0 mM); T_9- Brassinosteroid (25 ppm). Each value is a mean for three replicates ($p = 0.05$).

corresponding values on the 9th day were 69.03, 67.58 and 67.02 units/min/g.

Discussion

The increase in shelf life and quality of Alphonso and Sindhura mangoes treated with melatonin, salicylic acid and brassinosteroids can be attributed to several key factors related to the modulation of ripening, enzymatic activity and antioxidant capacity. Melatonin (200 µM), salicylic acid (2.0 mM) and brassinosteroids (25 ppm) demonstrated the ability to delay fruit ripening by reducing ethylene biosynthesis and respiration rates (23). Ethylene accelerates ripening and triggers various ripening-related processes such as enzymatic activities, softening and colour change (Fig. 8). By lowering ethylene levels, these treatments were found to slow down these processes, extending the shelf life of fruit. Exogenous application of melatonin, salicylic acid and brassinosteroids has been reported to increase storability in fruits (9, 10, 24) by regulating quality parameters and ethylene production.

The physiological loss in weight (PLW) was significantly lower in treated fruits, which retained firmness over a long duration. These findings align with those of Ali et al. (25), Kaur et al. (26) and others (27). These results are likely due to the reduced activity of ripening enzymes in treated fruits. Enzymes such as pectin methylesterase (PME), polygalacturonase (PG), cellulase (CEL) and amylase are involved in the breakdown of cell wall components, leading to fruit softening and textural changes (28). In treated fruits, these enzymes exhibited lower activity, especially in the T_3 (Melatonin), T_6 (Salicylic acid) and T_9 (Brassinosteroid) treatments. This lower activity reduces the degradation of pectin and cellulose, thereby maintaining fruit firmness and weight loss for a longer period (Fig. 8). This is likely due to the ability of melatonin, salicylic acid and brassinosteroids to reduce water loss and slow down the physiological processes that lead to fruit dehydration (29). Reduced PLW also means less susceptibility to shrivelling and spoilage.

The interplay between TSS, acidity and total sugars is critical for the maintenance of quality and shelf life. A balanced increase in TSS during storage indicates ripening and controlled TSS levels help maintain fruit quality over extended periods (30). Low sugar levels slow the conversion of starches to sugars, reducing the metabolic activity associated with ripening. It reduces the activity of cell wall-degrading enzymes (31). While high acidity helps maintain firmness, preventing excessive softening of the fruit over time and it balances flavour during storage (2). It suppresses the growth of spoilage-causing microorganisms, effectively extending its shelf life.

Higher total phenol content contributes significantly to shelf life by delaying deterioration, maintaining quality, and reducing vulnerability to spoilage. Phenols neutralize free radicals, reducing oxidative stress in fruit tissues. This preserves cellular integrity, slowing down ripening and senescence. Additionally, phenols inhibit enzymes like polygalacturonase and cellulase, which degrade cell walls, thereby reducing fruit softening and maintaining texture (32). Phenols also exhibit antimicrobial properties that inhibit spoilage causing microorganisms. High carotenoid content further enhances

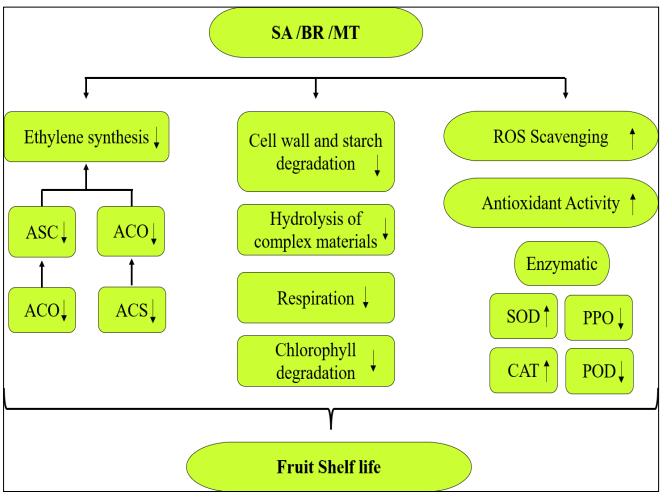

Fig. 8. Probable mode of action of bioactive compounds in postharvest fruits.

mangoes shelf life by boosting antioxidant capacity and improving fruit quality. As potent antioxidants, carotenoids help neutralize free radicals, reducing oxidative stress and delaying senescence in fruit tissues. It also provides a vibrant yellow to orange colour to the fruits, which enhances marketability (33).

Reduced activity of cell wall degradation enzymes might be due to the lower ethylene levels and respiration rates, as ethylene acts as a cascade and is important for the activation of genes responsible for the synthesis of key enzymes (34). PME hydrolyses pectin, a major component of the cell wall, causing fruit softening. The activation of PG is a direct response to increased ethylene levels. Cellulase and hemicellulase break down cellulose and hemicellulose in the cell wall, while PME modifies pectin by demethylating it, making it more susceptible to degradation by PG. Ethylene regulates the activity of PG, PME and cellulase (34). In our study on melatonin, salicylic acid and brassinosteroid application the activity of these enzymes was found to be reduced. The results are in line with previous studies of Wang et al. (35), Amiri et al. (36) and Meena et al. (37).

Fruit ripening is a complex oxidative process involving reactive oxygen species (ROS), which can accelerate fruit deterioration if not controlled. Ethylene production during ripening elevates respiration, increasing ROS levels. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) play crucial roles in scavenging ROS and minimizing oxidative damage (38). Melatonin, salicylic acid (SA) and brassinosteroids (BRs) have been shown to enhance the activity of these enzymes, reducing oxidative stress and extending fruit shelf life. Melatonin boosts the expression of ROS-related genes, increasing the activity of SOD, CAT and ascorbate peroxidase (APX), thereby lowering lipid peroxidation and preserving fruit quality (39). Similarly, SA and BRs regulate antioxidant defences by increasing the levels of SOD, CAT and PAL (phenylalanine ammonia-lyase), reducing the accumulation of ROS and oxidative stress markers like malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) (7, 40). Collectively, these treatments delay ripening, maintain fruit quality and extend shelf life by bolstering the fruit's natural antioxidant defence systems.

The use of bioactive compounds like melatonin, salicylic acid and brassinosteroids in post-harvest management offers significant environmental and economic benefits. These natural compounds provide a sustainable alternative to synthetic chemicals, reducing pollution, soil degradation and chemical residues on fruits while supporting environmental conservation. Economically, they minimize past-harvest losses, extend shelf life, and maintain fruit quality, enabling access to distant markets and reducing costs associated with refrigeration and chemical inputs. By enhancing fruit appearance, taste and nutritional value, these treatments meet consumer demands for premium-quality produce, increasing profitability while promoting sustainable and eco-friendly agricultural practices (41, 42).

The combined effects of melatonin, salicylic acid and brassinosteroids in regulating ripening enzymes, enhancing antioxidant defences, maintaining firmness and controlling chemical changes are key reasons for the observed increase in shelf life and improved quality in mangoes. These treatments effectively delay the ripening process, slow down enzymatic activities related to softening and protect the fruit from oxidative damage, thereby extending its marketability and storage potential.

Future research should focus on optimizing the dosage, timing and application methods of melatonin, salicylic acid and brassinosteroids for different mango varieties and storage conditions. Integrating these treatments with existing past-harvest technologies such as cold storage and modified atmosphere packaging, could enhance scalability. Additionally, developing cost-effective formulations and evaluating their feasibility for large-scale adoption will be critical for bridging the gap between laboratory research and commercial application in post-harvest management systems.

Conclusion

The present study demonstrates that, among the various treatments tested at different concentrations, melatonin (200 µM), salicylic acid (2.0 mM) and brassinosteroids (25 ppm) proved to be the most effective in extending the shelf life and improving the quality of Alphonso and Sindhura mango varieties. These treatments successfully delayed the ripening process by regulating key ripening enzymes, such as pectin methylesterase, polygalacturonase, cellulase and amylase, which are responsible for softening and starch degradation. Additionally, they enhanced the activity of antioxidant enzymes, including superoxide dismutase, catalase and peroxidase, thereby reducing oxidative damage during storage. This resulted in better retention of fruit firmness, reduced weight loss and favorable chemical changes like reduced TSS and sugar content and high acidity. Moreover, these treatments maintained non-enzymatic antioxidants, such as phenols and carotenoids. Overall, melatonin (200 µM), salicylic acid (2.0 mM) and brassinosteroids (25 ppm) provided superior post-harvest benefits, making them the best treatments for maximizing mango shelf life and quality under ambient storage conditions. The beneficial effects of these bioactive compounds demonstrate significant potential for scalability in commercial mango production. The use of these natural bioactive compounds offers an eco-friendly and cost-effective solution suitable for integration into existing post-harvest management practices. With minimal infrastructure requirements and broad applicability, these treatments could be adopted by both small-scale and large-scale producers, enhancing the marketability and export potential of mangoes while reducing post-harvest losses on a commercial scale.

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Authors' contributions

AB planned the formal analysis and carried out the investigation, formulated and adopted the standard methodology, software and wrote the original draft. SV contributed to the conceptualisation, framing the methodology, obtaining resources, funding acquisition, supervision and writing, reviewing and editing the manuscript. KC helped in framing the

methodology, employing software and writing, reviewing and editing the manuscript. VAG contributed to writing, reviewing and editing the manuscript. RM also participated in writing, reviewing and editing the manuscript. 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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