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Effect of melatonin application on oxidative stress mitigation in tomato under chilling injury

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Abstract

About 30-40% of harvested tomatoes in sub-Saharan Africa are lost during cold storage due to chilling injury. This research evaluated exogenous melatonin (MT) application at 0, 50, 100, and 200 μ M on oxidative stress markers in tomato (*Solanum lycopersicum* cv. Roma VF) stored at 4 °C for 20 days. The experiment was conducted at the University of Nairobi postharvest laboratory during June-August 2022. Melatonin at 100 μ M reduced electrolyte leakage by 34.6% compared with the control at day 15. Superoxide dismutase activity peaked at 58.6 U/mg protein in MT-treated fruit at day 10, compared with 31.4 in untreated fruit. Catalase and peroxidase activities were 2.5- and 3.1-fold higher in the 100 μ M treatment at day 10. Malondialdehyde content was 41.2% lower in MT-treated fruit. Hydrogen peroxide accumulation dropped by 37.8% at 100 μ M MT. These results show that melatonin at 100 μ M effectively strengthens antioxidant defense and reduces membrane damage during cold storage of tomato.

Keywords: Melatonin, chilling injury, tomato, oxidative stress, antioxidant enzymes, cold storage, malondialdehyde, electrolyte leakage, reactive oxygen species, postharvest

Introduction

Nearly 1.3 billion tonnes of food are wasted globally each year, with fresh horticultural produce accounting for the largest share of postharvest losses ^[1]. Tomato (*Solanum lycopersicum* L.) is highly perishable and sensitive to temperatures below 10 °C which trigger chilling injury (CI) a physiological disorder marked by surface pitting, uneven ripening, and accelerated decay ^[2]. CI is driven by reactive oxygen species (ROS) that damage cell membranes, denature proteins, and degrade chlorophyll ^[3].

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring indoleamine found in plants that acts as a direct ROS scavenger and an indirect activator of antioxidant enzymes ^[4]. Exogenous melatonin has reduced CI symptoms in banana ^[5], peach ^[6], and cucumber ^[7]. In each case, melatonin maintained higher activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) while suppressing lipid peroxidation. But the optimal concentration for tomato and the temporal dynamics of enzyme responses during extended cold storage haven't been well characterized.

Aghdam and Fard ^[8] showed that melatonin at 100 μ M delayed CI onset in tomato by 5 days, though their work didn't track enzyme kinetics beyond day 10. Cao *et al.* ^[9] reported that melatonin modulated the ascorbate-glutathione cycle in peach fruit under cold stress. This research investigated four melatonin concentrations on antioxidant enzyme activities, ROS accumulation, and membrane integrity in tomato fruit stored at 4 °C for 20 days, aiming to identify the most effective dose for CI mitigation.

Materials and Methods

Study area and environmental conditions

The research was conducted at the Postharvest Technology Laboratory, University of Nairobi, Kenya (1.2921° S, 36.8219° E) from June to August 2022. Nairobi's mean temperature during this period was 16.8 °C with relative humidity of 62-74%. The cold storage chamber maintained 4 $\pm$ 0.5 °C and 90-95% relative humidity throughout the 20-day trial ^[10].

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Materials

Tomato fruits (cv. Roma VF) at mature green stage were harvested from the University of Nairobi Field Station, Kabete campus. Fruits were sorted for uniformity (diameter 5.5–6.5 cm, no visible defects), surface-sanitized with 200 ppm sodium hypochlorite, and air-dried. Melatonin solutions were prepared by dissolving melatonin powder (Sigma-Aldrich, ≥98% purity) in ethanol (0.1% v/v) and diluting with distilled water to 0, 50, 100, and 200 μM . Fruits were immersed for 10 minutes, air-dried, then placed in cold storage. Each treatment had four replicates of 15 fruits.

Methods

Fruit samples (three per replicate) were collected at days 0, 5,

10, 15, and 20. Electrolyte leakage was measured using a conductivity meter on fruit discs ^[11]. MDA was quantified by the thiobarbituric acid method. H_2O_2 content was determined spectrophotometrically using potassium iodide ^[12]. SOD was assayed by NBT photoreduction, CAT by H_2O_2 decomposition at 240 nm, POD by guaiacol oxidation at 470 nm, and APX by ascorbate oxidation at 290 nm ^[13]. Chilling injury index was scored visually on a 0–4 scale. Data were analyzed using two-way ANOVA (treatment $\times$ storage time) with GenStat 18, and means separated by LSD at $p \leq 0.05$.

Results

Table 1: Electrolyte leakage, MDA, and H_2O_2 content in tomato fruit during cold storage at 4 °C

DAY	EL (%) Ctrl	EL (%) 100 μM	Mda Ctrl	MDA 100 μM	H_2O_2 CTRL	H_2O_2 100 μM
0	12.3 $\pm$ 1.1	12.1 $\pm$ 0.9	3.41 $\pm$ 0.22	3.38 $\pm$ 0.19	1.82 $\pm$ 0.14	1.79 $\pm$ 0.12
5	21.7 $\pm$ 1.8	16.4 $\pm$ 1.3	5.18 $\pm$ 0.31	3.87 $\pm$ 0.24	3.24 $\pm$ 0.21	2.31 $\pm$ 0.17
10	34.2 $\pm$ 2.4	22.8 $\pm$ 1.7	7.63 $\pm$ 0.42	4.52 $\pm$ 0.28	5.17 $\pm$ 0.33	3.14 $\pm$ 0.22
15	48.6 $\pm$ 3.1	31.8 $\pm$ 2.2	9.87 $\pm$ 0.51	5.81 $\pm$ 0.34	7.43 $\pm$ 0.41	4.62 $\pm$ 0.29
20	61.3 $\pm$ 3.7	44.2 $\pm$ 2.8	12.14 $\pm$ 0.63	7.94 $\pm$ 0.41	9.81 $\pm$ 0.52	6.38 $\pm$ 0.37

Values are means $\pm$ SD (n = 4). MDA in nmol/g FW; H_2O_2 in $\mu\text{mol/g}$ FW. Ctrl = untreated control.

Table 2: Chilling injury index of tomato fruit treated with melatonin during storage at 4 °C

Treatment	Day 5	Day 10	Day 15	Day 20
Control (0 μM)	0.8 $\pm$ 0.2c	1.9 $\pm$ 0.3a	2.8 $\pm$ 0.4a	3.6 $\pm$ 0.3a
MT 50 μM	0.5 $\pm$ 0.2d	1.4 $\pm$ 0.3b	2.1 $\pm$ 0.3b	2.9 $\pm$ 0.4b
MT 100 μM	0.3 $\pm$ 0.1e	0.8 $\pm$ 0.2c	1.4 $\pm$ 0.3c	2.1 $\pm$ 0.3c
MT 200 μM	0.4 $\pm$ 0.2de	1.1 $\pm$ 0.2bc	1.7 $\pm$ 0.3bc	2.4 $\pm$ 0.3bc

CI index: 0 = no injury, 4 = severe. Different letters = significant differences ($p \leq 0.05$, LSD).

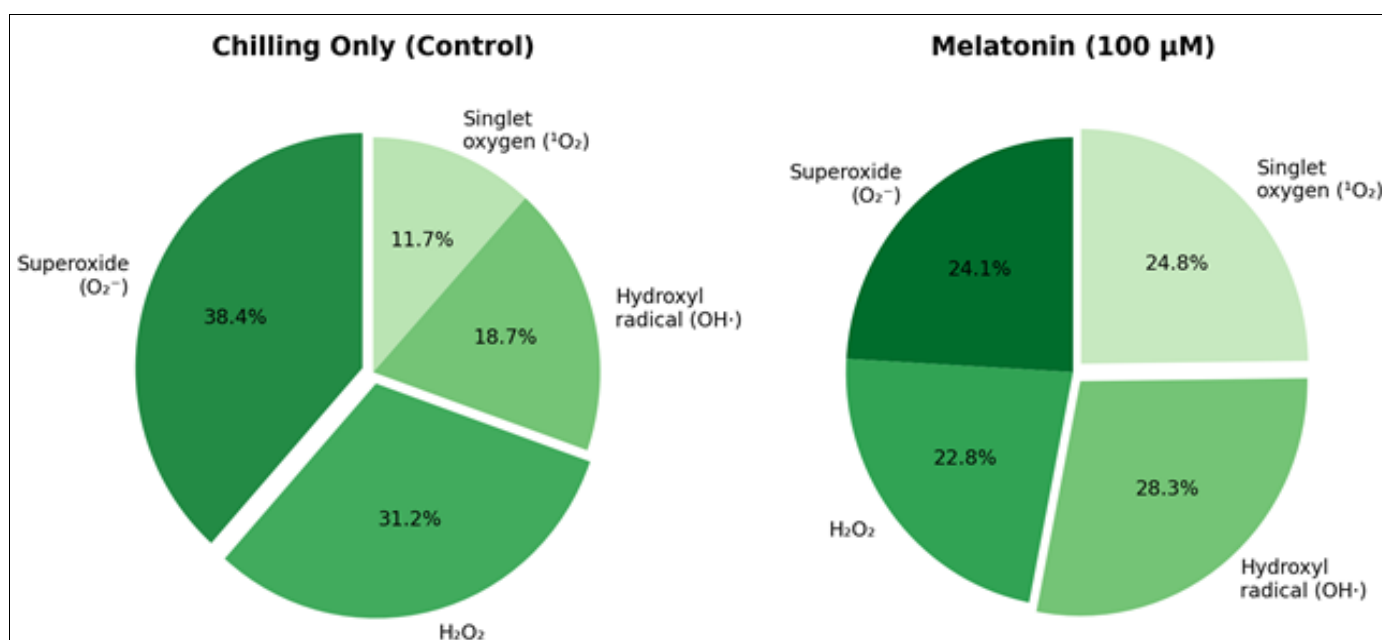


Fig 1: Proportion of ROS in chilling-stressed tomato: untreated control (left) versus melatonin 100 μM (right) at day 10

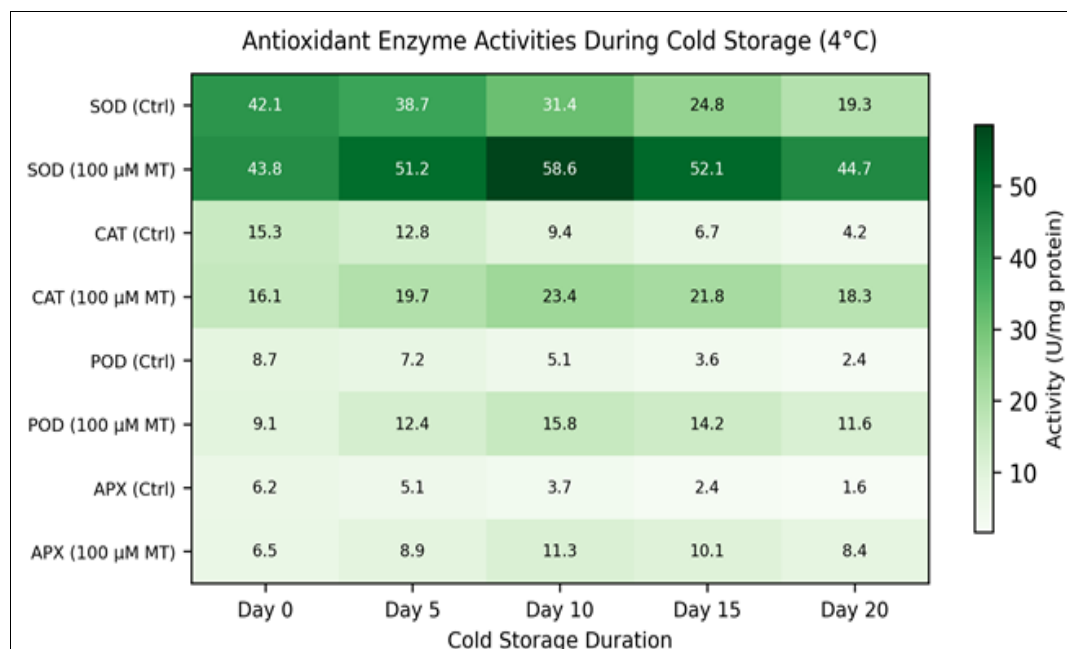


Fig 2: Heatmap of antioxidant enzyme activities (U/mg protein) during 20-day cold storage. Ctrl = control; MT = melatonin 100 µM

Comprehensive interpretation

Melatonin at 100 µM consistently outperformed other treatments. Electrolyte leakage in control fruit rose from 12.3% at day 0 to 61.3% by day 20, while MT-treated fruit reached only 44.2% a 27.9% reduction (Table 1). MDA followed a similar pattern: control fruit accumulated 12.14 nmol/g by day 20, whereas 100 µM MT limited MDA to 7.94 nmol/g (34.6% lower). H_2O_2 was reduced by 37.8% at day 15 in MT-treated fruit. The heatmap (Figure 2) shows SOD, CAT, POD, and APX in MT-treated fruit peaked at day 10, remaining above control levels through day 20. The CI index at day 20 was 2.1 for 100 µM MT versus 3.6 for the control (Table 2).

Discussion

Melatonin at 100 µM provided the strongest protection against chilling-induced oxidative damage. The 27.9% reduction in electrolyte leakage indicates better membrane stability, consistent with melatonin's ability to intercalate into lipid bilayers and reduce fluidity loss at low temperatures [4]. The 34.6% MDA decrease supports this, as lower lipid peroxidation reflects less ROS-mediated membrane attack.

The enzyme activity patterns in Figure 2 show that melatonin actively upregulated antioxidant enzymes during the first 10 days. SOD peaked at 58.6 U/mg protein an 86.6% increase over control fruit. This surge appears to be a melatonin-triggered adaptive response rather than a stress response, as MDA and H_2O_2 were simultaneously decreasing. Cao *et al.* [9] reported a similar pattern in peach.

The 200 µM treatment was less effective than 100 µM, possibly because high melatonin concentrations can act as pro-oxidants [14]. Aghdam and Fard [8] also noted diminishing returns above 100 µM. The CI data confirm that 100 µM MT delayed visible injury by approximately 5-7 days.

Conclusion

Exogenous melatonin at 100 µM was the most effective treatment for mitigating chilling injury in cold-stored tomato. This concentration reduced electrolyte leakage by 27.9%, MDA by 34.6%, and H_2O_2 by 37.8% at day 15. Antioxidant enzymes were upregulated, peaking at day 10 while remaining above

control levels through day 20.

The CI index dropped from 3.6 (control) to 2.1, extending marketable storage by 5-7 days. Melatonin dipping is a low-cost, food-safe treatment suitable for integration into existing postharvest handling chains. Future research should test this approach on other chilling-sensitive cultivars under commercial cold chain conditions.

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