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ARTICLE

# Melatonin regulates oxidative stress to delay senescence in *Hemerocallis fulva* L.

A melatonina regula o estresse oxidativo para retardar a senescência em *Hemerocallis fulva* L.

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**Abstract:** Melatonin has emerged as a potent plant growth regulator, significantly enhancing the postharvest quality of horticultural products. Structurally analogous to the plant hormone indole-3-acetic acid, it regulates key processes like seed germination, growth, flowering and defense against abiotic stresses. Its powerful antioxidant properties make it an effective free radical scavenger, boosting plant resilience to oxidative stress and positioning it as an innovative phytohormone for postharvest treatments. The current study explores the efficacy of melatonin in delaying senescence in detached scapes of *Hemerocallis fulva*. Scapes were harvested when the most mature bud was one day before anthesis and treated with different concentrations of melatonin (40, 80, 120, and 160 µM). Results indicate that 120 µM melatonin treatment significantly delayed senescence, extending the vase life of the flower scapes (12 days) relative to untreated controls (7 days), which showed accelerated senescence. Melatonin application was associated with enhanced antioxidant enzyme activity, reduced lipoxygenase activity and diminished hydrogen peroxide ($H_2O_2$) levels, collectively alleviating oxidative stress. Furthermore, melatonin enhanced the content of soluble proteins, phenols, sugar fractions and proline content within the tepal tissues. The treatment also effectively suppressed bacterial proliferation and improved solution uptake in the flower scapes. These findings suggest that melatonin modulates the senescence of *H. fulva* by orchestrating oxidative stress responses and enhancing postharvest quality, offering a holistic and innovative approach to postharvest management.
**Keywords:** antioxidant enzymes, hydrogen peroxide, lipoxygenase, proline, vase life.

**Resumo:** A melatonina emergiu como um potente regulador do crescimento vegetal, melhorando significativamente a qualidade pós-colheita de produtos hortícolas. Estruturalmente análoga ao hormônio vegetal ácido indol-3-acético, regula processos importantes como a germinação de sementes, o crescimento, a floração e a defesa contra estresses abióticos. Suas poderosas propriedades antioxidantes a tornam um eficaz eliminador de radicais livres, aumentando a resiliência das plantas ao estresse oxidativo e a posiciona como um fitorregulador inovador para tratamentos pós-colheita. O presente estudo explora a eficácia da melatonina em retardar a senescência de escapos destacados de *Hemerocallis fulva*. Os escapos foram colhidos na fase em que o botão mais maduro estava um dia antes da antese e tratados com diferentes concentrações de melatonina (40, 80, 120 e 160 µM). Os resultados indicam que o tratamento com 120 µM de melatonina retardou significativamente a senescência, prolongando a vida de vaso dos escapos florais (12 dias) em comparação aos controles não tratados (7 dias), que apresentaram senescência acelerada. A aplicação de melatonina foi associada ao aumento da atividade de enzimas antioxidantes, redução da atividade de lipoxigenase e níveis reduzidos de peróxido de hidrogênio ($H_2O_2$), atenuando coletivamente o estresse oxidativo. Além disso, a melatonina aumentou o conteúdo de proteínas solúveis, fenóis, frações de açúcares e prolina nos tecidos das tépalas. O tratamento também suprimiu efetivamente a proliferação bacteriana e melhorou a absorção de solução nos escapos florais. Esses resultados sugerem que a melatonina modula a senescência de *H. fulva* ao orquestrar respostas ao estresse oxidativo e melhorar a qualidade pós-colheita, oferecendo uma abordagem holística e inovadora para o manejo pós-colheita.
**Palavras-chave:** enzimas antioxidantes, lipoxigenase, peróxido de hidrogênio, prolina, vida em vaso.

## Introduction

Flower senescence is a critical developmental process in ornamental plants, characterized by the progressive degradation and eventual death of petal tissues. This process, which varies significantly across flower species, is genetically programmed and involves a complex sequence of biochemical changes. A key factor driving flower senescence is the accumulation of reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), which induces oxidative stress within cells (Zentgraf et al., 2022). Oxidative stress is known to deactivate enzymes, disrupt organelles, compromise membrane integrity and initiate harmful interactions with essential biomolecules, including proteins, lipids, pigments and DNA (Nousis et al., 2023). Such oxidative damage can eventually cause cell death, playing a key role in triggering flower senescence. In plants, ROS are generated in different cellular compartments, including mitochondria, chloroplasts, peroxisomes and the endoplasmic reticulum (Phua et al., 2021). The buildup of ROS can result from either excessive production or inadequate detoxification. To mitigate the harmful effects of ROS, plants rely on an array of antioxidant defense mechanisms, which include both enzymatic antioxidants such as catalase, superoxide dismutase and peroxidases and non-enzymatic antioxidants like glutathione, ascorbate, flavonoids and phenolic acids (Hasanuzzaman et al., 2020).

Recently, melatonin (N-acetyl-5-methoxytryptamine), a low-molecular weight bioactive molecule, has emerged as a significant player in plant physiology. Ubiquitously distributed among living organisms, melatonin is recognized for its pleiotropic functions and is increasingly acknowledged as a novel phytohormone and master regulator in plants (Jindal et al., 2024). Melatonin is involved in regulating numerous physiological processes, including growth, development and postharvest physiology (Bose and Howlader, 2020). Its powerful free radical scavenging and antioxidative properties are particularly notable, as they enhance plant resilience against oxidative stress. Studies have shown that melatonin not only directly scavenges ROS but also upregulates the transcription and activity of key antioxidant enzymes, further reinforcing the plant's defense against oxidative damage (Lu et al., 2022). Additionally, melatonin has been observed to delay leaf senescence in various horticultural crops. External application of melatonin positively regulates both dark-and age-induced leaf senescence by reducing ROS accumulation and modulating abscisic acid and auxin biosynthesis (Jing et al., 2022). Despite these findings, the specific mechanisms by which melatonin mediates its anti-senescent effects in *Hemerocallis fulva* remain poorly understood.

*Hemerocallis fulva* L., belonging to the Asphodelaceae family, is a popular ornamental plant renowned for its vibrant and attractive cymose

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inflorescence. However, the commercial appeal of *H. fulva* is limited by the short vase life of its detached scapes, which capitulate to premature senescence (Rodriguez-Enriquez et al., 2013). This fast-postharvest decline represents a significant challenge for the cut flower industry, leading to substantial economic losses. Enhancing the postharvest longevity of *H. fulva* by delaying senescence is therefore crucial for preserving its aesthetic qualities and improving its market viability. This study aims to investigate the regulatory role of melatonin in delaying flower senescence by modulating oxidative stress in *H. fulva* scapes. By elucidating the underlying physiological and biochemical mechanisms through which melatonin influences senescence, this research seeks to provide insights that could provide strategies for extending the vase life of *H. fulva* and other ornamental plants, thereby enhancing their commercial value.

## Material and methods

Uniform, freshly harvested flower scapes of *H. fulva*, each bearing a bud in its penultimate stage of development (the day preceding anthesis), were collected from the Kashmir University Botanical Garden (KUBG). These scapes were promptly transferred to the laboratory in distilled water (DW) to ensure minimal physiological disturbance. Upon arrival, the scapes were pruned to a consistent length of 30 cm and randomly segregated into five distinct groups (Fig. 1). Four groups were treated with varying concentrations of melatonin, specifically 40, 80, 120, and 160 µM. An additional set was kept in distilled water, as the control group. The initiation of treatments was designated as day zero (0). To prevent solution evaporation, Aluminium foil was used to seal the tops of the flasks. Laboratory conditions were stringently controlled, with relative humidity set at 62% ± 10%, a photoperiod of 12 hours per day, and a mean ambient temperature of 24 ± 2 °C. Various physiological and biochemical parameters, including vase life, solution uptake, bacterial proliferation, soluble protein levels, proline content, sugar fractions (reducing sugars), total phenolic content, membrane stability index (MSI), hydrogen peroxide ($H_2O_2$) concentration and enzymatic activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and lipoxygenase (LOX), were meticulously evaluated on the third (D3) and seventh (D7) days following the initiation of treatments.


**Fig. 1.** The impact of melatonin treatments on the senescence and postharvest performance of flowers from scapes of *H. fulva* was assessed on the day (A), day 2 (B) and day 12 (C) after the scapes were placed in the treatment solutions.

**Parameters evaluated**

**Vase life**

The vase life was recorded from the first day of postharvest treatment application until the last flower on the scape lost its ornamental quality.

**Bacterial load and solution uptake**

Bacterial density was measured by recording the optical density (OD) at 600 nm using a Uv-VIS spectrophotometer (Systronics). For this, 1 mL samples were collected from each treatment group, including the control. The assessment was performed following the method outlined by Naing et al. (2017), using *Escherichia coli* as a reference (OD 1 = $8\times10^8$ CFU $mL^{-1}$). Solution uptake was calculated based on the difference between the initial solution volume in the flask and the volume remaining after the experiment.

**Membrane stability index (MSI)**

MSI was evaluated by measuring solute leakage, indicated by the conductivity of tepal tissues, following the method proposed by Sairam (1994). Conductivity readings were obtained using an Elico CM 180 conductivity meter. MSI was computed using the given equation:

$$MSI = (1 - C1/C2) \times 100$$

where C1 and C2 correspond to the conductivities recorded at 25 °C and 100 °C, respectively.

**Hydrogen peroxide ($H_2O_2$) content**

$H_2O_2$ content was measured following the method described by Alexieva et al. (2011). 1 g from tepal tissue was homogenized in 0.1% ($wv^{-1}$) trichloroacetic acid (TCA) buffer, and the mixture was centrifuged at 12,000 g for 15 minutes. To 0.5 mL of the supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI) were added. The absorbance of the resulting solution was recorded at 390 nm. The concentration of hydrogen peroxide was calculated using a standard curve created from known $H_2O_2$ concentrations.

**Superoxide dismutase (SOD) activity**

SOD activity was determined following the protocol established by Dhindsa et al. (1981), which is based on the enzyme's ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Absorbance was measured at 560 nm, and SOD activity was expressed $min^{-1}$ $mg^{-1}$ protein.

**Catalase (CAT) activity**

CAT activity was evaluated using the method described by Aebi (1984), which is based on the enzyme's ability to decompose hydrogen peroxide ($H_2O_2$). Absorbance was measured at 240 nm and enzyme activity was calculated and expressed as micromoles of $H_2O_2$ degraded $min^{-1}$ $mg^{-1}$ protein.

**Ascorbate peroxide (APX) activity**

APX activity was measured following the method of Chen and Asada (1989), which tracks the decrease in absorbance at 290 nm resulting from the oxidation of ascorbate (0.1 Mm). Enzyme activity was expressed in $\mu M^{-1}$ $min^{-1}$ $mg^{-1}$ proteins.

**Lipoxygenase (LOX) activity**

LOX was determined using the method outlined by Axerold et al. (1981). Absorbance was measured at 234 nm over a 5-minute period. The enzymatic activity was calculated and reported µM $min^{-1}$ $mg^{-1}$ protein.

**Total phenols and reducing sugars**

To quantify sugar fraction and phenolic compounds, 1 g of finely chopped tepal tissue was immersed in hot 70% ethanol, macerated and subjected to centrifugation three times. The supernatant obtained was used to measure total phenols and reducing sugars. Total phenols were determined using the method described by Swain and Hills (1959), with gallic acid as the standard. Reducing sugars were quantified following Nelson's method (1994), using D-glucose as the standard.

**Proline content and soluble proteins**

To estimate proline content, 1 g of plant tissue was homogenized in 4 mL of sulfosalicylic acid then centrifuged for 10 minutes at 1000 rpm. The supernatant was treated with ninhydrin reagent and glacial acetic acid. Proline levels were quantified using the method of Bates et al. (1973), with an appropriate volume of the supernatant and L-proline as the reference standard. To quantify soluble proteins, 1 g of tepal tissue was homogenized in a 100 mM phosphate buffer (pH 7.2) containing 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, 10% polyvinyl pyrrolidone and 1 Mm dithioreitol. The homogenate was then centrifuged at 12,000 g for 15 minutes at 4 °C using a refrigerated centrifuge. Protein determination was done on an aliquot of the supernatant Lowry et al. (1951) method. Soluble protein content was recorded as mg $g^{-1}$ fm.

**Statistical analysis**

Statistical analysis for this study was conducted using a completely randomized design. Data were analyzed with analysis of variance (ANOVA) through SPSS software (SPSS version 16; Chicago, USA) to compare the treatment means. Standard errors among replicates were calculated and Duncans Multiple range test (DMRT) was used post hoc analysis to evaluate differences between treatment groups, with a significance threshold set at $p < 0.05$. The experiment was conducted three times to confirm the reliability and consistency of the results.

## Results

**Vase life**

While control flower scapes showed a vase life of 7 days, scapes treated with different concentrations of melatonin 40, 80, 120, and 160 µM exhibited a vase life of 9, 10, 12, and 11 days, respectively (Fig. 2). Among these, the 120 µM showed the most pronounced effect in delaying senescence, resulting in the longest vase life among the treated groups.

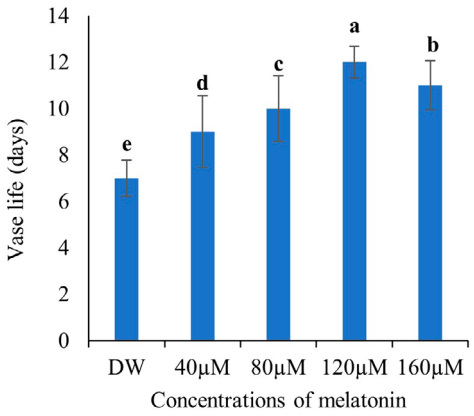


**Fig. 2.** Effect of various melatonin concentrations on vase life of *H. fulva*. Error bars represent ± SE (standard error) calculated from the mean of five replicates. Bars labelled with different letters indicate significant differences at $p < 0.05$.

**Bacterial load and solution uptake**

Melatonin treatments led to a significant reduction in bacterial growth within the vase solutions, with the most pronounced effect observed in scapes treated with 120 µM melatonin. This concentration also enhanced solution uptake, as evidenced by the highest absorption rates in these scapes, followed by those treated with 80 µM. In contrast, the control group showed the highest bacterial proliferation and the lowest solution uptake, as illustrated in Fig. 3A and 3B.

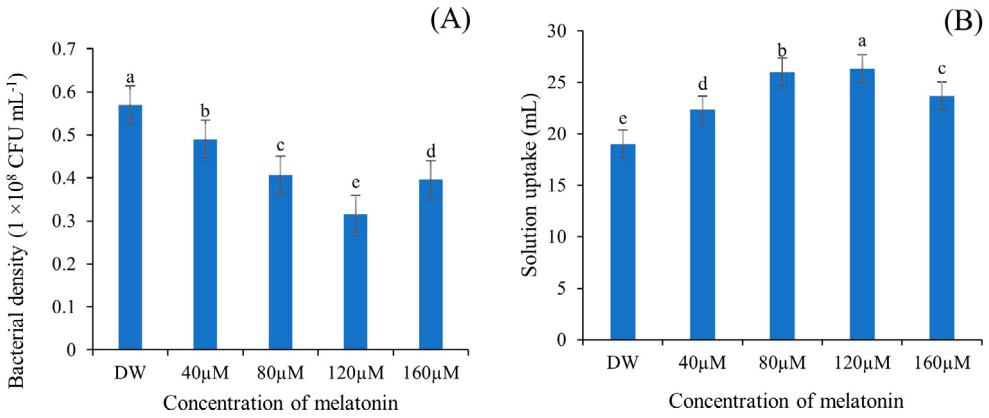


**Fig. 3.** Effect of various melatonin concentrations on bacterial density (A) and solution uptake (B) in *H. fulva*. Error bars denote ± SE (standard error) from mean values of five replicates. Bars marked with different letters represent statistically significant differences at $p < 0.05$.

**Antioxidant enzyme activities and hydrogen peroxide levels**

Tepal tissues from the flowers of scape treated with melatonin showed enhanced activities of the antioxidant enzymes SOD, CAT and APX, with the highest activity observed in tissue samples from scapes of flowers treated with 120 µM melatonin, followed by those treated with 80 µM. However, these enzyme activities decreased significantly as the flowers developed from day 3 to day 7. Notably, melatonin-treated tepals exhibited a marked reduction in $H_2O_2$ levels compared to the control flowers held in distilled water (DW), which had the highest $H_2O_2$ content. Among the treatments, 120 µM melatonin was the most effective in limiting $H_2O_2$ accumulation compared to the other treatments (Fig. 4A – 4D).

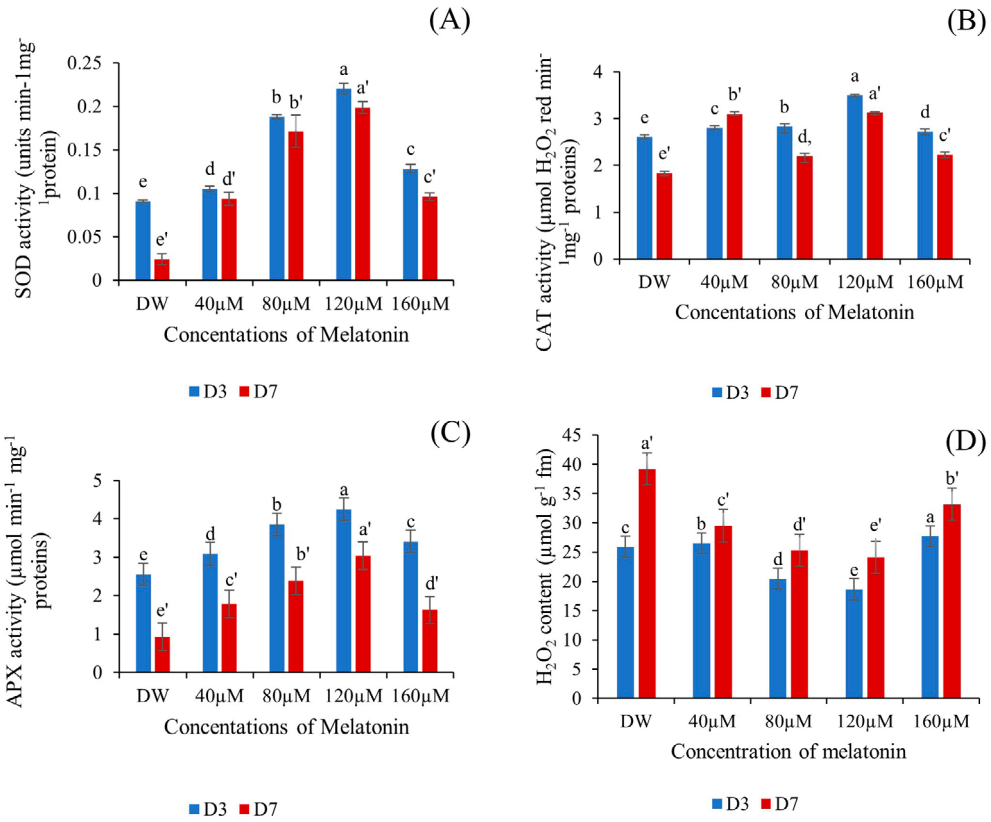


**Fig. 4**. Effect of various concentration of melatonin on the SOD activity (A) CAT activity (B) APX activity (C) and $H_2O_2$ content (D) of *H. fulva*. Error bars denote ± SE (standard error) from mean values of five replicates. Bars marked with different letters represent statistically significant differences at $p < 0.05$.

**Membrane stability index (MSI) and lipoxygenase (LOX) activity**

The tepal tissues from the flowers of scapes treated with various concentrations of melatonin showed a marked increase in MSI compared to the control group. Tepal tissues from flowers of scapes held in distilled water (DW) exhibited the lowest MSI, while those treated with 120 µM melatonin demonstrated the highest MSI. However, as flower development progressed from day 3 (D3) to the day 7 (D7), there was a notable decline in MSI, with the smallest decrease observed in the tepal tissues treated with 120 µM melatonin (Fig. 5A). In contrast, the tepal tissues from flowers of scapes treated to varying concentration of melatonin displayed a significant reduction in lipoxygenase (LOX) activity. The lowest LOX activity was recorded in the tepal tissues treated with 120 µM melatonin, while the highest LOX activity was observed in the flowers held in DW. Unlike MSI, LOX activity increased dramatically as flower development advanced from D3 to D7, with the smallest increase in LOX activity seen in tepal tissues treated with 120 µM compared to the other treatments (Fig. 5B).

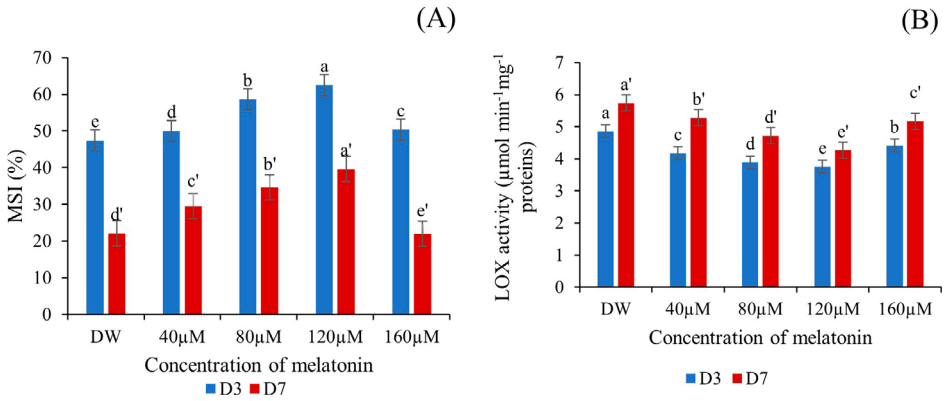


**Fig. 5**. Effect of various concentrations of melatonin on membrane stability index (MSI) (A) and lipoxygenase (LOX) activity (B) in *H. fulva*. Error bars represent ± SE (standard error) calculated from the mean of five replicates. Bars with different letters show statistically significant differences at $p < 0.05$.

**Reducing sugars and total phenols**

Flower scapes supplemented with melatonin exhibited an increase in reducing sugars content compared to the control, with the highest levels observed in tissue samples from scapes treated with 120 µM melatonin. A similar trend was seen for total phenols, with the highest phenolic content recorded in 120 µM-treated flowers on days 3 and 7, followed by those treated with 40 µM. However, both reducing sugars and total phenol levels declined as the flowers matured from day 2 to day 6 of the experiment, as illustrated in Fig. 6A - 6B

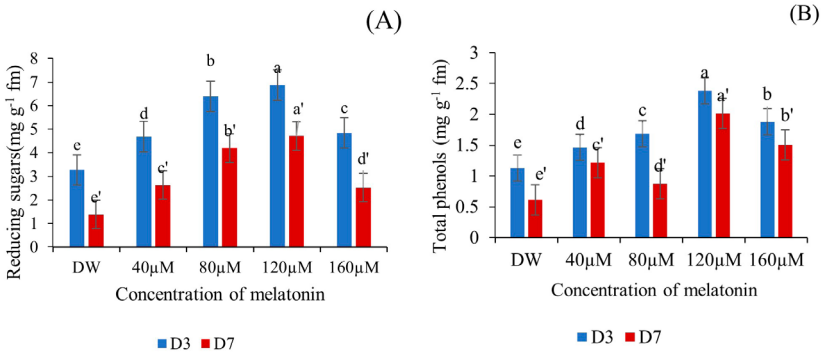


**Fig. 6**. Effect of varying concentrations of melatonin on reducing sugars (A) and total phenols (B) in the tissue samples from flowers of scapes of *H. fulva*. Error bars represent ± SE (standard error) calculated from the mean of five replicates. Bars with different letters show statistically significant differences at $p < 0.05$.

**Soluble protein and proline content**

Melatonin application resulted in a significant increase in protein content in the tepal tissues compared to the control. On day 3, scapes treated with 120 µM melatonin exhibited the highest soluble protein levels, exceeding those of the control and other treatment groups. However, as the scapes matured, a decline in soluble protein content was observed in tepal tissues across all groups, with the control group showing the steepest reduction (Fig. 7A). Additionally, melatonin treatments significantly elevated proline content in the flower scapes, with the 120 µM melatonin group displaying the highest proline accumulation, followed by the 160 and 80 µM treatments. A gradual decline in proline content was noted across all treatments, including the control, as the experiment progressed (Fig. 7B).

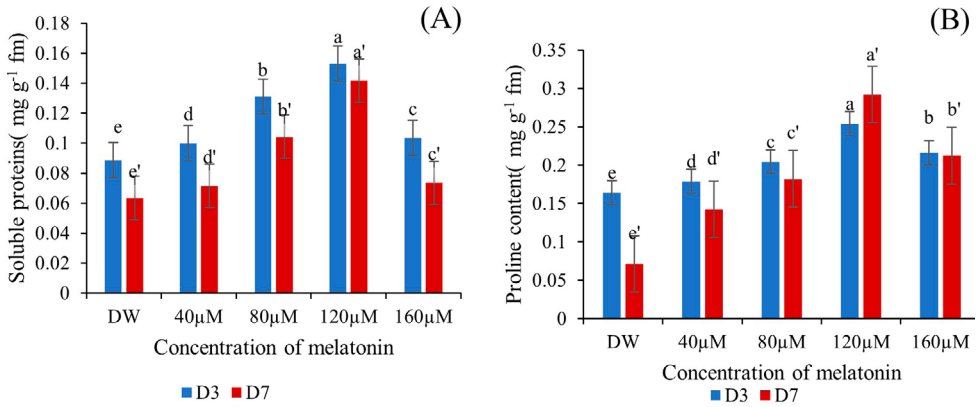


**Fig. 7**. Effect of varying concentrations of melatonin on soluble protein content (A) and proline content (B) in tissue samples from flowers of scapes of *H. fulva*. Error bars denote ± SE (standard error) based on mean values from five replicates. Bars labelled with different letters indicate statistically significant differences at $p < 0.05$.

## Discussion

Melatonin, a versatile plant growth regulator, has emerged as a powerful agent in delaying flower senescence, an essential yet complex process often triggered by increased oxidative stress, membrane lipid peroxidation and decline in antioxidant defense mechanisms. The intricacies of this phenomenon are shaped by a cascade of genetic reprogramming, driven by various regulatory elements, which ultimately leads to cell death and the onset of petal senescence (Lone et al., 2024). In this study, we explore the physio-chemical pathways through which melatonin exerts its protective effects on *H. fulva* scapes, demonstrating its significant ability to delay senescence. Our findings reveal that melatonin treatment notably enhances key antioxidant enzyme activities, such as SOD, CAT and APX, while concurrently reducing LOX activity, which is closely associated with lipid peroxidation and cellular damage. The most pronounced effects were observed with a 120 µM melatonin concentration, underscoring its potent role in enhancing the plant's antioxidative defenses and mitigating oxidative damage. These results align with previous studies where melatonin enhanced antioxidant enzyme activities in other flower species, including *Paeonia lactiflora* (Wang et al., 2024a) further validating its critical role in flower senescence.

This study also demonstrated that the increase in total phenolic content in response to melatonin treatment may play a crucial role in reducing lipid peroxidation, thereby preserving membrane integrity. This result is consistent with previous research indicating that rose flower senescence is caused by membrane disruption from lipid peroxidation (Mazrou et al., 2022), highlighting the protective role of total phenolics against oxidative stress. Overall, these findings reinforce the idea that exogenous melatonin strengthens the antioxidant defense system, potentially delaying senescence by stabilizing cellular membranes and reducing oxidative damage.

In this study, melatonin treatments effectively decreased LOX activity, indicating a reduction lipid peroxidation in cut flowers, thereby preserving membrane integrity. This observation is consistent with other studies showing that melatonin reduces lipid peroxidation while maintaining the MSI (Zulfiqar et al., 2023). The decrease in LOX activity associated with melatonin is plausible, as melatonin inhibits the formation of free radicals, which in turn enhances membrane stability (Shreya et al., 2022). Additionally, melatonin application upregulated the antioxidant defense system including both enzymatic and non-enzymatic component, which plays a crucial role in scavenging toxic ROS (Shreya et al., 2022). Moreover, melatonin enhances the quality of peony cut flowers by reducing ion leakage, hydrogen peroxide ($H_2O_2$), lipid peroxidation and LOX activity, highlighting its ability to alleviate oxidative stress and stabilize cellular membranes (Wang et al., 2024a).

Hydrogen peroxide ($H_2O_2$), recognized as a relatively stable ROS, undergoes detoxification via the catalytic actions of APX and CAT, which facilitate its conversion into water ($H_2O$) and molecular oxygen ($O_2$) (Wang et al., 2024b). Our results demonstrate a pronounced upregulation

of APX and CAT activities compared to control, suggesting an enhanced antioxidative defense mechanism. This observed upsurge in antioxidant enzyme activity is consistent with findings from Ahmad et al. (2020), who reported similar trends during the leaf senescence process in maize. Notably, treatment with melatonin significantly augmented APX and CAT activities, resulting in a marked attenuation of $H_2O_2$ accumulation. This modulation by melatonin likely potentiates the antioxidative defense system, thereby mitigating oxidative stress and limiting ROS accumulation (Ahammed et al., 2020).

Air embolism and bacterial growth are known to cause vascular blockages, which can significantly reduce water uptake in the stems of cut flowers (Naing et al., 2017). In the current study, melatonin treatment markedly enhanced water conduction within the flower scapes. This improvement is likely attributed to the bactericidal properties of melatonin, as evidenced by reduced bacterial growth observed in the test solutions containing melatonin. Melatonin's antibacterial effects have been documented against various pathogens, including *P. aeruginosa*, *A. baumannii* and *S. aureus* (Tekbas et al., 2008). These findings suggest that melatonin not only enhances water uptake by minimizing vascular blockage but also contributes to the overall health and longevity of cut flowers by mitigating microbial-induced deterioration.

Protein content is widely recognized as a reliable indicator of a plant's physiological state. In the current study, a significant increase in protein content was observed in melatonin-treated scapes compared to control group. These findings are consistent with those reported by Kaya et al. (2022), who found enhanced protein levels in pepper plant exposed to arsenic stress after melatonin application. The elevated levels of soluble proteins resulting from melatonin treatment likely contribute to enhanced cellular resilience through osmotic regulation, functioning synergistically with sugars. Under stress conditions, plants accumulate osmoprotectants like proline to counteract environmental stress and mitigate ROS-induced damage by restoring osmotic balance (Zulfiqar and Ashraf, 2023; Haq et al., 2024). Melatonin enhances the synthesis of osmoprotective compounds, including proline, aiding in stress alleviation by maintaining cellular water content (Kaya et al., 2022). Our findings indicate that melatonin treatment significantly boosted proline biosynthesis, likely through upregulation of proline biosynthetic pathways (Altaf et al., 2023). This enhanced proline accumulation may have improved the osmotic status of melatonin-treated scapes, contributing to delayed senescence and increased stress resilience.

## Conclusions and perspectives

This study underscores melatonin's pivotal role in delaying flower senescence by fortifying antioxidative defenses, mitigating oxidative stress, stabilizing cellular membranes, enhancing water uptake and promoting the accumulation of osmoprotectants. These multifaceted effects highlight melatonin's potential as a promising bio-regulator in floriculture, significantly enhancing the vase life of cut flowers and alleviating senescence driven by oxidative damage. The outcomes of this research not only reinforce melatonin's utility in postharvest management but also pave the way for further inquiry into its underlying molecular mechanism. Future studies could delve into the specific signalling pathways and regulatory networks through which melatonin modulates antioxidant enzyme activity, membrane stability, and sugar metabolism, providing deeper insights into its role in senescence regulation. Moreover, exploring the interactions between melatonin and other plant growth regulators (PGRs) could yield a more comprehensive understanding of the hormonal crosstalk that governs flower development and longevity. Finally, research addressing the practical aspects of melatonin application, such as optimal concentration ranges, delivery methods, and its compatibility with other postharvest treatments, is essential for its integration into commercial floriculture, offering substantial benefits to both growers and the floral industry.

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**Author Contribution**

**HYL:** Writing- Original Draft, Data Curation, Investigation, Visualization, Validation. **MA:** Data Curation, Methodology, Resources, Software. **WWT:** Data Curation, Methodology, Resources, Software. **AAW:** Investigation, Visualization, Formal Analysis. **MAZ:** Investigation, Visualization, Formal Analysis. **IT:** Conceptualization, Resources, Supervision, Writing - Review & Editing.

**Conflict of Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data Availability Statement**

Data will be made available on request.

**Declaration of generative AI and AI-assisted technologies in the writing process**

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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