



# Combination of Different Concentrations and Application Methods of Melatonin: Controls postharvest spoilage, maintains quality, and extends shelf-life of Cut Rose Flowers

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Original Article



**Citation:** Shamsinejad, S., Saffari, V.R., Pakkish, Z. and Saadati, S. 2024. Combination of Different Concentrations and Application Methods of Melatonin: Controls postharvest spoilage, maintains quality, and extends shelf-life of Cut Rose Flowers. Greenhouse Plant Production Journal, 1(4): 93-112.

 <https://10.61186/gppi.1.4.93>

## KEYWORDS

Ion leakage  
Longevity  
Melatonin  
Oxidative stress  
Treatment

## ABSTRACT

To date, various preservative solutions and chemicals have been introduced to extend the postharvest longevity of cut flowers, and research in this field holds significant economic and horticultural importance. In this study, the response of cut rose flowers to different application methods and concentrations of melatonin was investigated. The experiment was carried out based on a completely randomized design, with two treatment methods: short-term (pulse) and continuous application, using different concentrations and five replications per treatment. In the short-term (pulse) method, the samples were placed in a melatonin solution for a specified duration after re-cutting, and then each sample was transferred to a vase solution. The pulse treatments included melatonin concentrations of 0.1, 0.5, 1, and 2 mM, each applied for 30 minutes. Additionally, the 0.5 mM melatonin concentration was applied for 2 and 4 hours. Continuous treatments were applied at three concentrations (0.01, 0.1, and 1 mM) until the end of the vase life. The results indicated that melatonin treatments had a significant effect on vase life compared to the control. The longest vase life was observed with the 0.1 mM pulse treatment. It appears that melatonin application helps reduce ion leakage. Exhibited superior retention of water uptake, relative moisture content, chlorophyll, and carotenoid levels. The greater effectiveness of the pulse treatment compared to the continuous treatment may be attributed to the sensitivity and instability of melatonin.

## ARTICLE

### HISTORY

**Received:** 01 December 2024

**Revised:** 17 December 2024

**Accepted:** 31 December 2024

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## 1. Introduction

Flower senescence is the terminal phase of developmental processes that leads to the end of its life span. Since a number of developing countries are attracted to this global fresh flower trade for commercial purpose, this phenomenon is major obstacle for all the floricultural industries. Therefore, research related to postharvest changes was carried out to mitigate this problem. The post-harvest events in floricultural crops reflecting petal senescence are being reviewed in this paper, whereby various physiological and biochemical studies having data regarding lipid peroxidation, loss of membrane integrity and protein degradation central to petal senescence are included. With the expansion of urban life and the increasing work pressures in today's high-stress societies, the presence of nature in our daily lives has significantly diminished. However, reconnecting with nature remains one of the most effective ways to prevent and mitigate the harmful effects of our busy, industrial lifestyles (Rani and Singh, 2014). Flowers and plants offer a simple yet powerful means to achieve peace and soothe the human soul (Damunupola et al., 2010). As countries continue to industrialize and develop technologically, and as human lives become more mechanized and driven by advanced technology, the role of plants in people's lives becomes increasingly vital. Integrating more greenery into daily life can help reduce the detrimental impacts of industrial living (Abbasi et al., 2010). Despite their high economic value, cut flowers are highly perishable (Aba-Alkhail, 2005; Chang et al., 2008). Their high respiration rates and susceptibility to damage necessitate extensive postharvest care (Abd-El-Khair and Omima, 2006). The beauty of flowers lies in their ability to maintain freshness for an extended period (Aalifar et al., 2020). However, during the marketing stage, a significant portion of cut flowers up to 50% of total production—can be lost, resulting in substantial economic losses (Bleeksma and Van Doorn, 2003). The ability to preserve cut flowers is a critical necessity for marketing and exporting them. The vase life of cut flowers is the most important indicator of their value, significantly influencing consumer demand and the economic worth of the flowers (Cairns, 2005).

The rose, scientifically known as *Rosa hybrida* L., belongs to the Rosaceae family and includes numerous species and varieties, such as climbing, cluster, dwarf, and miniature roses (Anderson, 2016). Roses are the most exported cut flowers globally. An annual review revealed that roses, used as cut flowers, potted plants, and garden plants, have a market value exceeding \$10 billion. Despite the rose's exceptional popularity and export value, it has a relatively short vase life. Therefore, conducting research to extend its postharvest longevity is essential. Cut flowers are among the most important ornamental products in the global horticultural industry, with roses being one of the most popular and economically significant species. However, the short vase life of cut flowers is a major challenge that limits their marketability and consumer satisfaction (Eason et al., 2002; Gulluce et al., 2003; Hadizadeh et al., 2009). Postharvest longevity is influenced by various factors, including water relations, microbial activity, and oxidative stress (Elkovich, 2017). To address these issues, numerous preservative solutions and chemicals have been developed to extend the vase life of cut flowers (Asaoka and Heins, 1982; Dole et al., 2009; Chanasut et al., 2003; Genena et al., 2008; Rashidiani et al., 2020). Currently, in developed countries, instead of increasing agricultural land area, greater emphasis is placed on reducing postharvest losses through the adoption of modern technologies. In other words, the supply of flowers can be increased by minimizing storage losses and implementing new technologies (today, the focus is more on preserving the product after harvest rather than increasing production) (Akhtar et al., 2021; Lu et al., 2010; Mazrou et al., 2022). Although using improved handling and storage methods to reduce

losses incurs costs, these costs are significantly lower than the cost of production and the value of the product that would otherwise be lost due to negligence and lack of facilities. In addition to increasing production, another advantage of reducing losses is the better preservation of agricultural resources and soil, consumer health, and environmental hygiene (Halevy and Mayak, 2007; Hatamzadeh et al., 2012). To extend the vase life of cut flowers, various substances, including plant growth regulators, are used (Faragher, 1985; Hessini et al., 2022; Lezoul et al., 2022).

Melatonin is a pleiotropic molecule with multiple cellular and physiological effects, playing diverse roles in many aspects of plant growth and development. Research indicates that melatonin has a significant role in plant defense systems (Arnao and Hernández-Ruiz, 2017; Artea, 1996; Hernandez-Ruiz and Arnao, 2018; Mubarak et al., 2023; Hei et al., 2025). At appropriate concentrations, melatonin can mitigate nearly all abiotic stresses that induce oxidative stress. Researchers have demonstrated the potential mechanisms of melatonin's protective effects (Benner, 1993; Zhang et al., 2014). Melatonin (N-acetyl-5-methoxytryptamine, MT), a tryptophan derivative naturally occurring in many organisms, is recognized for its potent antioxidant properties. Melatonin plays regulatory and stimulatory roles in plants. Its regulatory functions include: improving seed germination, stimulating root formation, influencing geotropism, inducing senescence, affecting fruit ripening, enhancing immune responses in plants, and regulating plant hormone levels (Debnath et al., 2019; Fan et al., 2018). The stimulatory roles of melatonin include: acting as an anti-stress agent, stabilizing cell membranes, detoxifying harmful chemicals, regulating redox (oxidation-reduction) networks, optimizing photosynthesis, and increasing crop yield under drought stress and other stressful conditions (Arnao et al., 2013). Melatonin has been shown to play a role similar to cytokinins in delaying senescence (Aghdam et al., 2020; Mittler, 2002). The anti-aging effects of melatonin have been confirmed in species such as apple, cucumber, rice, peach, ryegrass, cassava, and Arabidopsis. In Arabidopsis, melatonin treatment leads to a reduction in chlorophyll-degrading enzymes (chlorophyllase and pheophorbide oxygenase) (Arnao and Hernández-Ruiz, 2017). One of the most well-known functions of melatonin is its antioxidant property against toxic oxygen and nitrogen species. The antioxidant activity of melatonin is significantly higher than that of vitamins C, K, and E. This phenomenon is likely due to melatonin being a potent antioxidant that easily penetrates cells, whereas the aforementioned vitamins are absorbed selectively (Bonnefont-Rousselot and Collin, 2010). Evidence suggests that environmental stresses increase endogenous melatonin levels (Li et al., 2007; Tan et al., 2012). It has been reported that exogenous application of melatonin in plants under cold stress increases germination rates (Posmyk et al., 2009). Additionally, studies have shown that exogenous melatonin application in wheat and rice enhances leaf chlorophyll content and prevents chlorophyll degradation under cold stress (Kang et al., 2010; Turk et al., 2014). Exogenous application of melatonin in maize under salt stress has been shown to increase the net photosynthetic rate, enhance the activity of antioxidant enzymes, and reduce ion leakage (Chaoqiun et al., 2016). The application of melatonin in postharvest gardenia led to an increase in chlorophyll content, chlorophyll fluorescence parameters, glutamine synthetase activity, water content, soluble protein, endogenous melatonin, and the expression of genes related to tryptophan decarboxylase, superoxide dismutase, catalase, and the ascorbate-glutathione cycle. Additionally, melatonin reduced carotenoid and flavonoid levels and programmed cell death. Melatonin also helped maintain the plant's external structure (Zhao et al., 2017). In another study on the effect of melatonin in postharvest anthurium, it was reported that melatonin application reduced chilling injury in cut anthurium flowers during cold storage (Aghdam and Fard, 2019).

Additionally, the application of different concentrations of melatonin led to an increase in vase life, a higher number of open florets in the inflorescence, reduced transpiration, improved water balance, and changes in chlorophyll, carotenoid, and endogenous melatonin levels in cut lily flowers (Mazrou et al., 2022). Short-term application of melatonin in the vase solution of roses resulted in an extended vase life, along with a significant increase in phenolic content, glutathione (GSH) levels, and the activity of CAT, APX, and GR enzymes (Debnath et al., 2019; Lezoul et al., 2022). The continuous application of different concentrations of melatonin on cut carnation flowers led to an increase in vase life, improved membrane stability index, and enhanced antioxidant activity (Zhou et al., 2023). Additionally, melatonin treatment combined with sucrose demonstrated an extended vase life and delayed degradation of flavonoids and anthocyanins in amaryllis (Brito et al., 2023; Pang et al., 2020).

This study aims to investigate the effects of different concentrations and application methods of melatonin on the postharvest longevity and physiological responses of cut rose flowers. By exploring the potential of melatonin to reduce oxidative stress and improve vase life, this research seeks to contribute to the development of effective postharvest treatments for the cut flower industry.

## 2. Materials and methods

Cut rose flowers of the *Samurai* cultivar were harvested in the morning of June 11 from a commercial greenhouse located 10 kilometers from Zarand city. The flowers were cultivated in the 'Aftab' Greenhouse, located in the city of Zarand. The flowers were transported to the Postharvest Physiology Laboratory at the Faculty of Agriculture, Shahid Bahonar University of Kerman. Fifty stems with uniform appearance, stem diameter and length, leaf number, and bud opening stage (consistent bud stage and level of opening) and free from diseases were selected. Four leaves were retained on each stem, and the rest were removed. The stems were cut to a length of 40 cm, and the ends were trimmed at a 45-degree angle. The stems were then placed in containers containing 500 mL of preservative solution according to the treatment type.

### 2.1. Preparation of Solutions and Application of Treatments

In this study, two treatment methods—short-term (pulse) and continuous—were investigated using different concentrations, with five replications per treatment. In the short-term (pulse) method, the samples were recut and placed in solutions with varying concentrations for the specified duration according to the treatments. Afterward, each sample was transferred to a vase solution containing distilled water, 2% sucrose, and 5% ethanol. The pulse treatments included melatonin concentrations of 0.1, 0.5, 1, and 2 mM, each applied for 30 minutes. Additionally, the 0.5 mM melatonin concentration was applied for 2 and 4 hours, and the desired traits were evaluated. In the continuous treatment method, the samples were recut and immediately placed in a solution containing the specified concentration of melatonin, quadruple-distilled water, 2% sucrose, and 5% ethanol. The continuous treatment concentrations included melatonin at 0.01, 0.1, and 1 mM. The control vase solution consisted of quadruple-distilled water, 2% sucrose, and 5% ethanol. Due to the application of melatonin, the treatments were carried out in labeled containers under dark conditions.

## 2.2. Evaluated Traits

### 2.2.1. Vase Life

Vase life refers to the duration during which the flowers retain their commercial value and are suitable for sale. Therefore, in experiments and research, vase life is defined as the number of days from the first day after applying the treatments until the flowers lose their commercial value. The end of vase life is determined by visual characteristics such as the appearance of color fading, petal drop, wilting, browning of petals, bending of the neck, and incomplete petal opening (Mousavi et al., 2014). In this study, vase life was calculated based on the number of days from placing the flowers in the vase solution (day 0) until more than 50% of the petals wilted and the stems bent.

### 2.2.2. Ion Leakage of Leaves

This trait is used to determine the permeability of the cell membrane of leaves or petals and assess the stability of leaf or petal cell membranes. To measure ion leakage, the following steps were performed: First, 0.5 grams of leaves were selected from each sample. Surface salts and electrolytes were removed using Whatman filter paper, and the cut surfaces were rinsed with distilled water. The samples were then placed in sealed tubes containing 10 mL of distilled water and kept in the dark at room temperature for 24 hours. The initial electrical conductivity (EC1) of the solution in contact with the samples was measured using an EC meter (Winlab Data Line Conductivity Meter: 1625036) at 21°C. To disrupt the cell walls and release the cell sap, the tubes containing the leaf segments were frozen at -20°C and then thawed at room temperature. The freezing and thawing process was repeated several times to rupture the cells and release their contents into the solution. The final electrical conductivity (EC2) of the solution was measured, and the membrane stability index and ion leakage percentage were calculated using the following formula:

$$\%EC = (EC1/EC2) \times 100$$

### 2.2.3. Solution Uptake Measurement

The containers used in this study held 500 mL of vase solution. On days 3, 4, and 5, as the solution volume decreased, the amount of quadruple-distilled water required to refill the containers back to 500 mL was recorded. The total volume of water added until the end of the vase life was calculated as the solution uptake.

### 2.2.4. Measurement of Relative Water Content (RWC)

To measure the relative water content (RWC), 0.2 grams of leaves were separated from each sample. After washing and drying, they were immediately weighed using a digital scale. Each sample was then placed in Petri dishes with a 10 cm diameter containing 10 mL of distilled water and kept in the dark for 4 hours to reach full saturation. The saturated weight was then measured.

The samples were dried in paper bags at 85°C for 24 hours and weighed again. The relative water content was calculated using the following formula:

$$\%RWC = \left( \frac{W_F - W_D}{W_S - W_D} \right) \times 100$$

RWC: Relative Water Content, WF: Fresh Weight of the Leaf, WD: Dry Weight of the Leaf, WS: Saturated Weight of the Leaf

### 2.2.5. Measurement of Visual Quality and Marketability

The assessment of visual quality and marketability was conducted through a scoring system ranging from 1 to 10 (7 to 10: excellent quality, 5 to 6: very good quality, 3 to 5: moderate quality, and below 3: poor quality). This evaluation was performed by experts up to the 11th day from the start of flower storage.

### 2.2.6. Flower Diameter

To evaluate the effect of preservative solutions on the opening of cut roses of the Samurai cultivar, the diameter of the flowers was measured using a digital caliper on days 3, 5, and so on, from the start of the experiment until the end of the vase life of the samples.

### 2.2.7. Chlorophyll Index (SPAD)

The chlorophyll index was measured and recorded using a handheld chlorophyll meter (SPAD-502- Made in Japan). Chlorophyll measurements were taken on days 3, 5, and so on, from the start of the experiment until the end of the vase life of the samples.

### 2.2.8. Measurement of Chlorophyll and carotenoid

For chlorophyll measurement, 0.1 g of fresh leaf sample was homogenized in 10 mL of 80% acetone. The resulting extract was centrifuged at 2700 rpm for 15 minutes. Subsequently, 2 mL of the supernatant was collected, and its absorbance was measured at wavelengths of 646.8 nm, 663.2 nm, and 470 nm using a BioTek-EPOCH spectrophotometer. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated using the following equations and reported as mg per gram of fresh weight.

$$\text{Chl}_a = 12.25 A_{663} - 2.79 A_{647}$$

$$\text{Chl}_b = 21.50 A_{647} - 5.10 A_{663}$$

$$\text{Chl}_t = C_a + C_b$$

$$\text{Car} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$$

### 2.2.9. Measurement of Anthocyanin

First, 0.1 grams of plant tissue were precisely weighed and ground in a mortar containing 5 mL of acidic methanol (99.5% ethyl alcohol and hydrochloric acid in a 99:1 ratio). Then, an additional 5 mL of acidic methanol was added, and the extract was transferred to a capped test

tube. The sample was kept in the dark at laboratory temperature for 24 hours. Afterward, it was centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was read at a wavelength of 550 nm using a spectrophotometer, and the anthocyanin concentration was calculated using the following formula (Wanger, 1979):

$$A = \epsilon \cdot B \cdot C$$

In this equation:

$B$  is the cavetti width,  $C$  is the anthocyanin concentration,  $A$  is the measured absorbance, and  $\epsilon$  is the molar extinction coefficient of anthocyanin, which is  $33,000 \text{ M}^{-1}\text{cm}^{-1}$ – $133,000 \text{ M}^{-1}\text{cm}^{-1}$ .

### 2.3. Statistical Analysis of Data

This study was conducted in a completely randomized design with five replications for each treatment. Data were analyzed using the SAS statistical software, and means were compared using the LSD test

## 3. Results

### 3.1. Effect on Vase Life

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.01$ ) on the vase life of roses (Table 1). The longest vase life was observed in the treatments of 0.1 mM melatonin (both continuous and pulsed), 1 mM (continuous), and 2 mM (pulsed), which showed a statistically significant difference compared to the control. These treatments (0.1 mM melatonin (both continuous and pulsed), 1 mM (continuous), and 2 mM (pulsed) resulted in a 22% increase in vase life compared to the control (Figure 1).

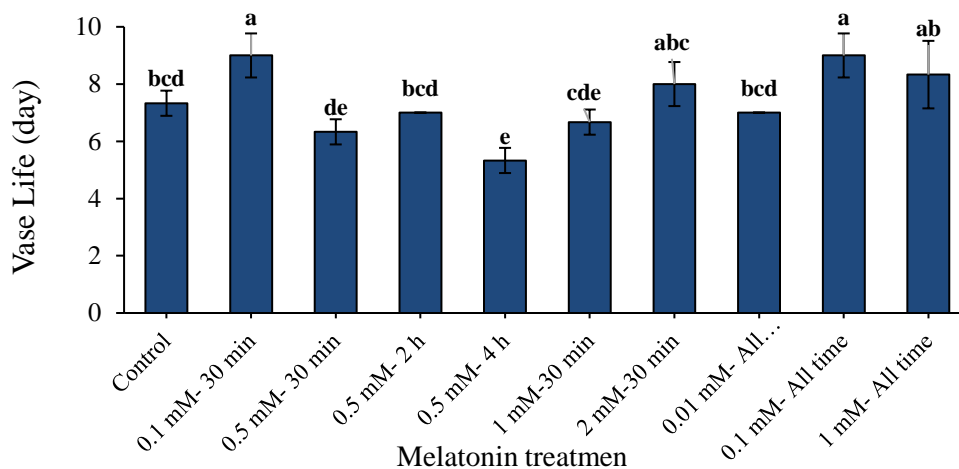


Figure 1. Mean vase life under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

**Table 1. Analysis of Variance (ANOVA) of the Effect of Melatonin on the Characteristics of Cut Rose Flowers.**

Source of Variation	Mean Squares												
	Df	Vase Life	Quality	Water Uptake	Flower Diameter	RWC	SPAD	Ion Leakage	Carotenoids	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Anthocyanin
Melatonin	9	4.207 ***	6.241 ***	55.481 ** *	6.796 ns	62.649 * *	13.809* **	228.57***	0.715 ***	1.549 ***	0.162 ns	0.811***	0.003 ns
Error	20	0.667	0.567	7.867	6.500	24.869	2.108	10.270	0.001	0.008	0.000	0.008	0.000
(CV%)	-	11.03	20.51	8.86	6.77	6.45	2.87	9.98	2.97	5.77	2.40	8.58	3.36
Total	29												

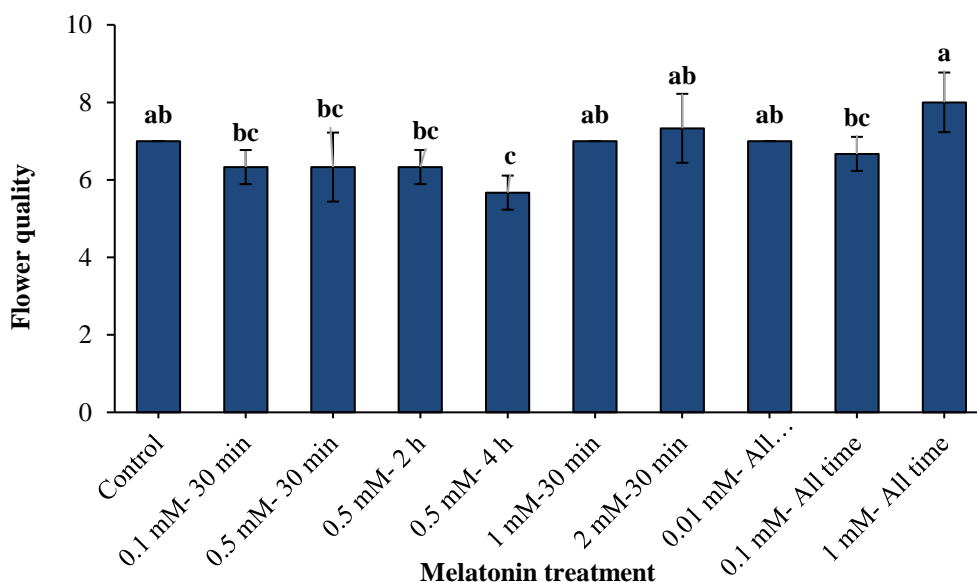
ns: No significant difference, \*: Significant at the 0.05 probability level, \*\*: Significant at the 0.01 probability level.

### 3.2. Effect on Quality

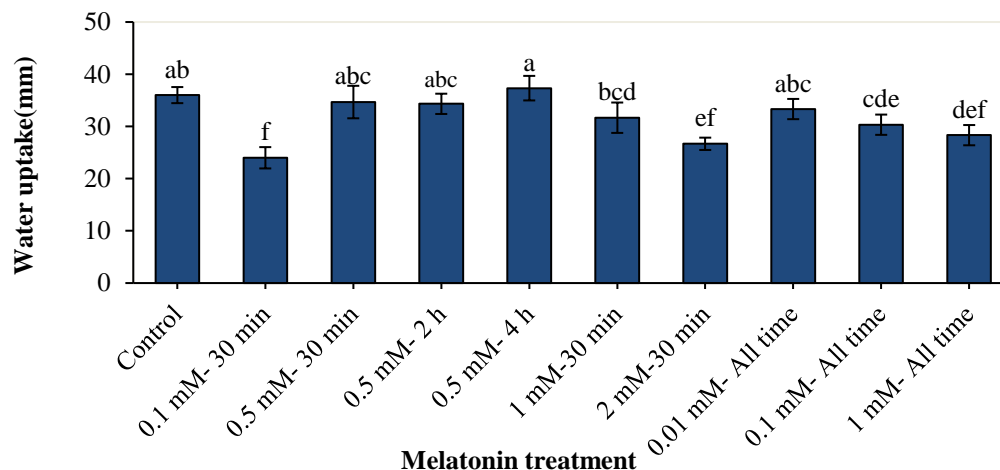
The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.01$ ) on the quality of cut rose flowers (Table 1). The highest quality was observed in the treatments of 1 mM and 0.01 mM melatonin (applied continuously) as well as 1 mM and 2 mM melatonin (applied pulsed), which showed a statistically significant difference compared to the control (Figure 2).

### 3.3. Effect on Water Uptake

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.01$ ) on the water uptake of cut rose flowers (Table 1). The lowest water uptake was observed in the treatments of 0.1 mM and 2 mM melatonin (applied pulsed) and 1 mM melatonin (applied continuously). However, no significant difference was observed between the control and other treatments for this trait. Overall, it can be concluded that water uptake in rose flowers did not increase significantly across all treatments compared to the control (Figure 3).



**Figure 2.** Mean quality under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).



**Figure 3.** Mean water uptake under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.4. Effect on Flower Diameter

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had no significant effect on the flower diameter of cut roses (Table 1). The largest diameter was observed in the treatment of 1 mM melatonin for 30 min (applied pulsed), although this increase was not statistically significant (Figure 4).

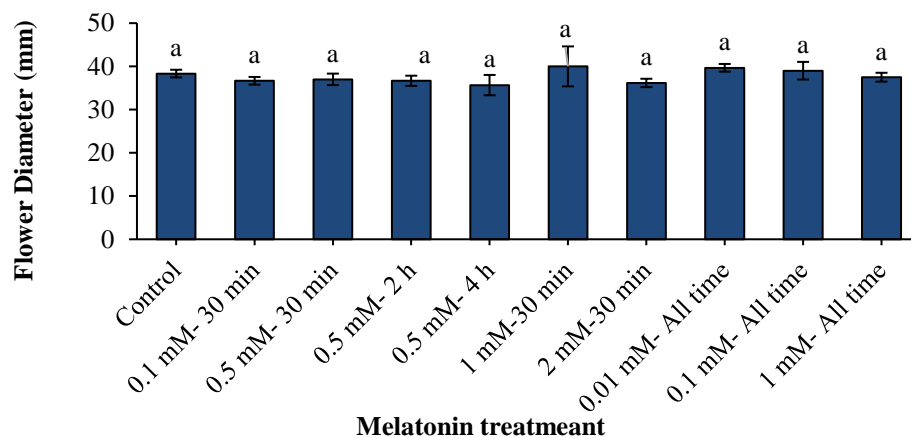


Figure 4. Mean flower diameter under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.5. Effect on Chlorophyll Index

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.01$ ) on the chlorophyll index of cut rose flowers (Table 1). The highest chlorophyll index was observed in the treatments of 1 mM and 0.5 mM melatonin (applied pulsed). However, no significant differences were observed among the treatments. (Figure 5).

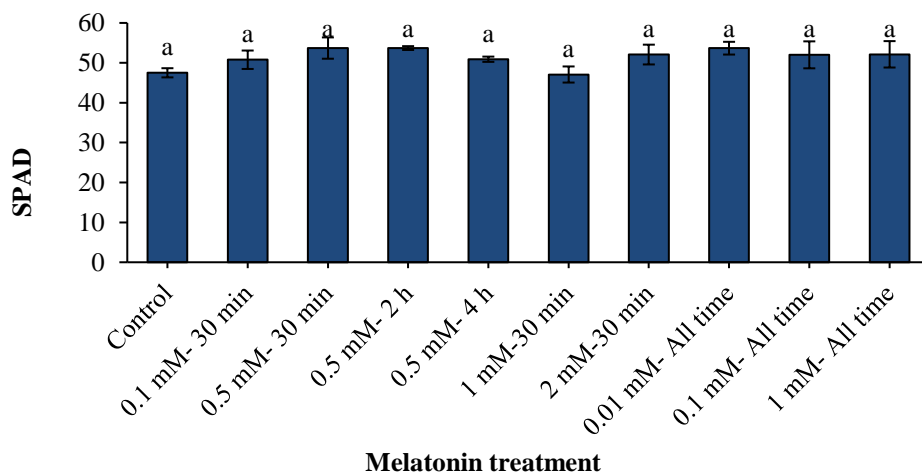
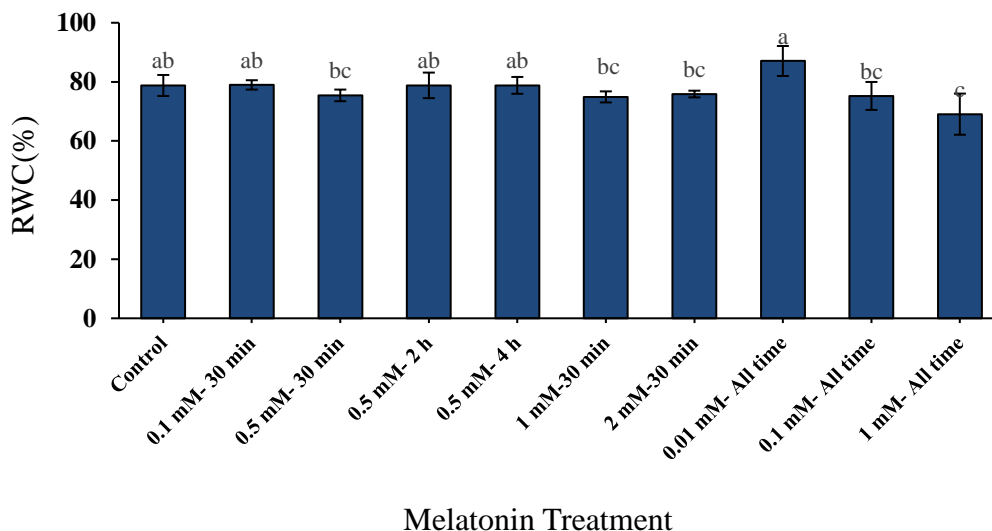


Figure 5. Mean chlorophyll index under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.6. Effect on Relative Water Content (RWC) of Leaves

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.05$ ) on the relative water content (RWC) of leaves (Table 1). The highest RWC was observed in the treatments of 0.01 mM melatonin (applied continuously) (Figure 6).



**Figure 6.** Mean relative water content (RWC) under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.7. Effect on Ion Leakage

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had a significant effect ( $p \leq 0.001$ ) on the ion leakage of cut rose flowers (Table 1). The highest ion leakage was observed in the control treatment and the treatment of 2 mM melatonin (applied pulsed), while the lowest ion leakage was observed in the treatment of 0.5  $\mu$ M melatonin (applied pulsed). However, no significant differences were observed among the treatments. (Figure 7).

### 3.8. Effect on Anthocyanin Content

The results of the analysis of variance (ANOVA) revealed that melatonin treatment had no significant effect on the anthocyanin content of cut rose flowers (Table 1). The highest anthocyanin content was observed in the treatment of 0.5 mM melatonin (applied pulsed), while the lowest was observed in the continuous application treatments. However, these differences were not statistically significant (Figure 8).

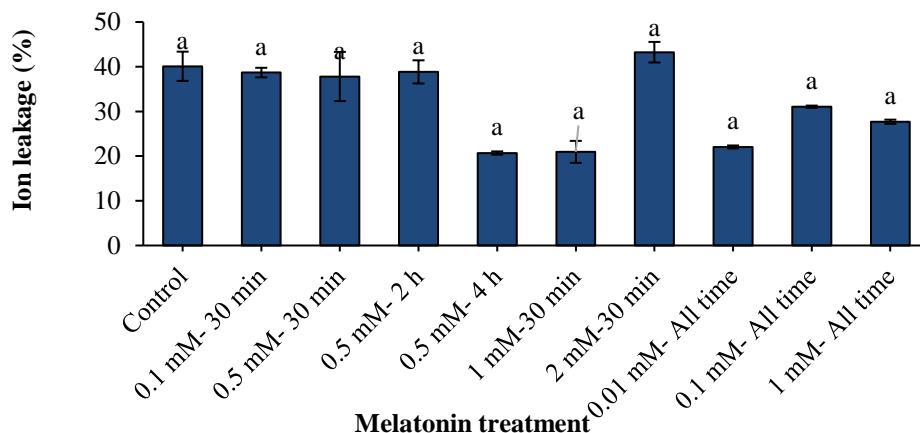


Figure 7. Mean ion leakage under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ .)

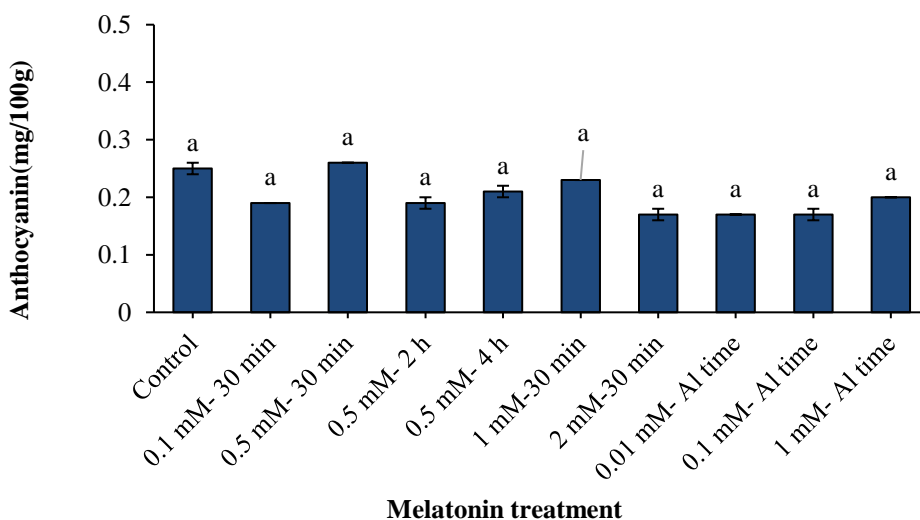
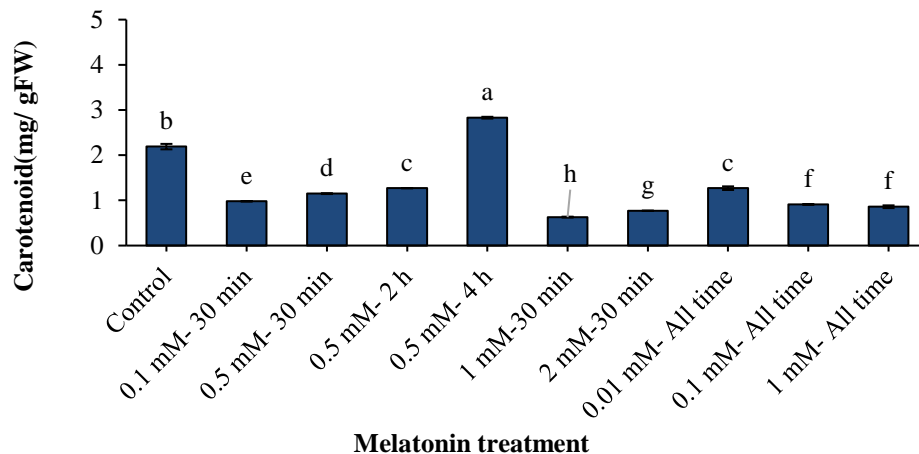


Figure 8. Mean anthocyanin content under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.9. Effect on Carotenoid Content

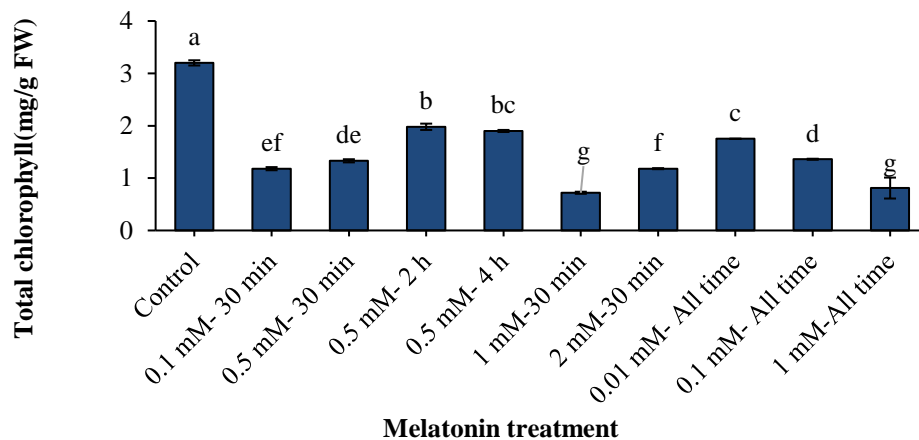
The results of ANOVA revealed that melatonin treatment had a significant effect ( $p \leq 0.01$ ) on the carotenoid content of cut rose flowers (Table 1). It can be concluded that the carotenoid level in rose flowers showed an overall decrease across all treatments compared to the control group. Only the 0.5 mM melatonin treatment maintained significantly higher carotenoid levels compared to the control and other treatments (Figure 9).



**Figure 9.** Mean carotenoid Content under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.10. Effect on Total Chlorophyll Content

The ANOVA results demonstrated that melatonin treatment had a significant effect ( $p \leq 0.001$ ) on the total chlorophyll content of cut rose flowers (Table 1). The highest total chlorophyll content was observed in the control treatment, while the lowest was recorded in the 1 mM pulsed melatonin treatments. In conclusion, the total chlorophyll content of rose flowers showed an overall decrease across all treatments compared to the control group (Figure 10).

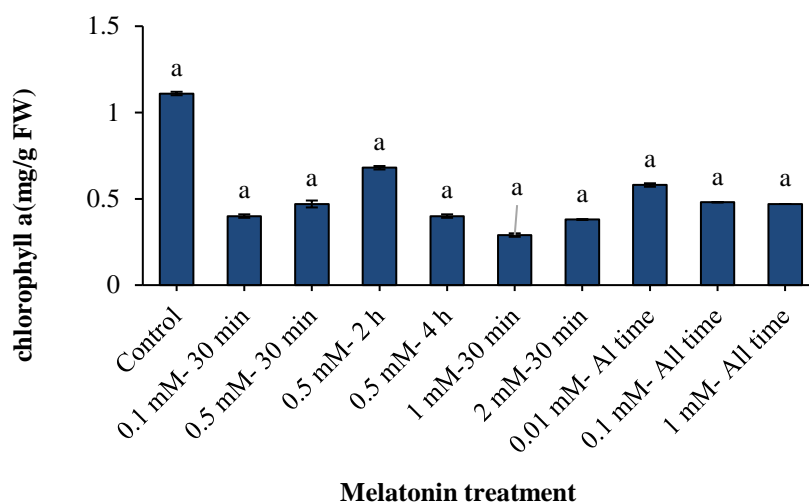


**Figure 10.** Mean total chlorophyll Content under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.11. Effect on Chlorophyll a Content

Statistical analysis indicated that melatonin application did not significantly influence chlorophyll a levels in cut rose stems (Table 1). A consistent reduction in chlorophyll a content

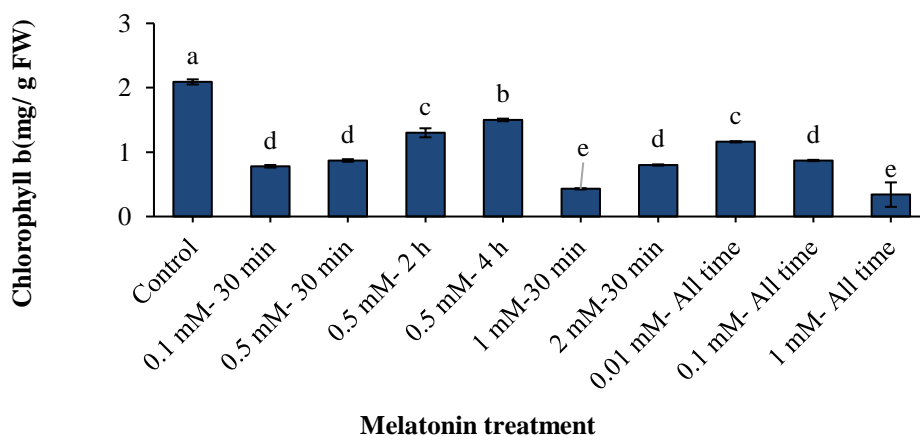
was observed in melatonin-treated flowers relative to control specimens; however, this trend lacked statistical significance (Figure 11).



**Figure 11.** Mean chlorophyll a Content under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

### 3.12. Effect on Chlorophyll b Content

Statistical analysis revealed a highly significant ( $p \leq 0.001$ ) impact of melatonin application on chlorophyll b levels in rose cut flowers (Table 1). Control plants maintained maximal chlorophyll b accumulation, whereas the 1 mM pulsed melatonin treatment resulted in minimal pigment retention. Comprehensive analysis demonstrated consistent chlorophyll b reduction in all melatonin treatments relative to untreated controls (Figure 12).



**Figure 12.** Mean chlorophyll b Content under different application methods and concentrations of melatonin. Mean values of 3 replicates  $\pm$  standard error (SE) are shown. Dissimilar letters above each column indicate significant differences based on the LSD test ( $p \geq 0.05$ ).

#### 4. Discussion

In addition, as a natural and safe preservative, it also exhibits strong antioxidant activity and can respond to biotic and abiotic stresses, ultimately enhancing crop yield and delaying fruit and flowers senescence (Mubarok et al., 2023; Wang et al., 2023). In this study, different application methods and concentrations of melatonin were investigated. Melatonin treatment significantly increased the vase life of cut rose flowers, with the highest improvement observed in treatments of 0.1 mM (continuous and pulsed), 1 mM (continuous), and 2 mM (pulsed). This suggests that melatonin may delay senescence by enhancing antioxidant activity and reducing oxidative stress (Mazrou et al. 2022). The quality of cut roses was significantly improved in treatments of 1 mM and 0.01 mM (continuous) and 1 mM and 2 mM (pulsed). This indicates that melatonin may help maintain cellular integrity and physiological functions, leading to better postharvest quality. While melatonin had a significant effect on water uptake, the overall increase was not substantial compared to the control. This suggests that melatonin's primary role may not be directly related to water absorption but rather to other physiological mechanisms. Melatonin treatment did not significantly affect flower diameter, although the largest diameter was observed in the 1 mM pulsed treatment. This implies that melatonin may not directly influence cell expansion in petals. The chlorophyll index was significantly higher in treatments of 1 mM and 0.5 mM (pulsed), indicating that melatonin may help preserve chlorophyll content by reducing degradation during senescence. Melatonin significantly increased RWC in treatments of 0.01 mM (continuous) and 0.1 mM and 0.5 mM (pulsed). This suggests that melatonin may enhance water retention in leaves, contributing to improved plant vitality. Ion leakage was significantly reduced in the 0.5  $\mu$ M pulsed treatment, indicating that melatonin may stabilize cell membranes and reduce electrolyte leakage, a marker of cellular damage. Melatonin did not significantly affect anthocyanin content, although the highest levels were observed in the 0.5 mM pulsed treatment. This suggests that anthocyanin synthesis may not be directly influenced by melatonin under these conditions. Senescence is a natural and programmed process that occurs in all living organisms, with over 800 genes implicated in its regulation. During senescence, macromolecules such as proteins and lipids are hydrolyzed, and the resulting compounds are transported to utilization sites within the plant (Ma et al., 2021; Wang et al., 2013; Wang et al., 2023). The breakdown of proteins like Rubisco and chlorophyll-binding proteins leads to chloroplast degradation. The disruption of Photosystems I and II is accompanied by the release and degradation of chlorophyll. In this context, melatonin may act as an antioxidant, preventing the accumulation of free radicals and thereby delaying the senescence process (Arnao et al., 2017; Reiter et al., 2015).

Postharvest quality and vase life of cut flowers are influenced by factors such as harvest time, genetic traits, environmental conditions, and proper management throughout the supply chain (Ban et al., 2023; Fanourakis et al., 2005; Mittler et al., 2002; Pomodakis et al., 2005; Zhao et al., 2017; Wu et al., 2023). Vase life is primarily affected by water uptake. Rapid physiological deterioration and induced senescence after harvest significantly reduce the quality and marketability of cut flowers, thereby limiting their commercial value (Hassan et al., 2020). It has been demonstrated that during the postharvest life of cut roses, reactive oxygen species (ROS) levels increase significantly, followed by a corresponding increase in antioxidant system activity (Hassan et al., 2020).

#### 4.1. Melatonin as an Antioxidant

In recent years, the application of melatonin as an antioxidant in postharvest plant management has gained increasing attention. The results of this study on melatonin treatments in the Samurai rose cultivar showed that melatonin significantly improved vase life. Melatonin likely helps reduce oxidative stress (by deactivating ROS) and decreases the oxidation of unsaturated lipids. Therefore, the resistance of unsaturated lipids to oxidation depends on melatonin's ability to scavenge ROS and prevent ROS production (Carrion-Antoli et al., 2022; Jannatizadeh et al., 2017; Zhou et al., 2023). These findings align with those of Mazrou et al. (2022), who reported that pulsed application of four melatonin concentrations for 30 minutes nearly doubled the vase life of cut roses compared to the control. The end of vase life is determined by the evaluation and rejection based on visual characteristics and subjective criteria, or in other words, marketability. Visual quality is highly valued by consumers, and higher quality attracts more customer attention. Therefore, the use of treatments to maintain quality is of great importance. The effects of melatonin on preserving the quality of cut roses are attributed to the enhancement of enzymatic and non-enzymatic antioxidant defense systems, which in turn reduce lipid peroxidation and H<sub>2</sub>O<sub>2</sub> accumulation, thereby maintaining membrane integrity. In this study, melatonin application had a significant effect on quality. It was observed that lower concentrations applied in a pulsed manner had a lesser impact on quality improvement. It has been reported that melatonin's mechanism of action involves maintaining water relations and antioxidant defense systems, which reduce oxidative damage (Xie et al., 2022; Zhao et al., 2017).

#### 4.2. Water Relations and Antioxidant Systems

The increased vase life of melatonin-treated roses in this study can be explained by melatonin's effect on maintaining proper water relations, leading to higher relative water content (RWC) compared to untreated flowers. It is well-established that cut roses are prone to water imbalance, making the maintenance of water relations crucial for extending vase life. The primary reason for the increased vase life of cut roses in response to melatonin application appears to be improved water relations. To regulate excessive ROS production under unfavorable conditions, plants possess an efficient antioxidant defense system comprising both enzymatic and non-enzymatic components. During flower senescence, the activation of ROS production, coupled with reduced ROS scavenging, leads to a significant increase in ROS, causing oxidative damage to lipids and membrane proteins and accelerating postharvest senescence. Increased ROS production has been documented in aging gladiolus and cut roses (Ba et al., 2021; Aalifar et al., 2020; Rashidiani et al., 2020). Similarly, research has shown that melatonin treatment maintains water relations and extends vase life in cut carnations (Lezoul et al., 2022; Wang et al., 2021; Wang et al., 2022).

#### 4.3. Analysis of Pigments

In a study, it was reported that melatonin, due to its anti-aging and hormone-like effects, prevents tissue senescence and chlorophyll degradation, thereby preserving product quality (Zheng et al., 2013). In plants, one of the most important roles of melatonin is its function as a powerful antioxidant and protector, safeguarding carotenoids against various stresses, particularly oxidative stress (Li et al., 2024; Aghdam et al., 2015; Li et al., 2017). It has been

reported that melatonin, in addition to acting as a potent antioxidant, is involved in processes such as growth and development induction, root and shoot development, increased leaf area and fresh weight, delayed leaf senescence, and consequently enhanced photosynthesis and carboxylation, chlorophyll and carotenoid content, thereby improving the quality and quantity of products. The application of melatonin on cucumber and one-year-old apple trees under drought stress conditions delayed leaf senescence and prevented chlorophyll degradation (Zhao et al., 2017), which aligns with the results of this study. In this study, despite the significant effect of melatonin on pigments, no significant increase compared to the control was observed. The results of this study showed that melatonin treatment had no significant effect on anthocyanin and chlorophyll a content in cut rose flowers. These findings are inconsistent with previous studies, such as Lezoul et al. (2022), which reported an increase in anthocyanin content at different melatonin concentrations.

## Conclusion

The significance of roses as ornamental plants is well recognized worldwide. In the present study, the application of melatonin and its effects on the postharvest characteristics of cut rose flowers were investigated. Given the increasing use of melatonin in recent years as an antioxidant in postharvest plant management, the importance of examining its effects becomes evident. Overall, the results of the experiments demonstrated that among the different concentrations and application methods of melatonin, the pulsed application of 0.1 mM melatonin was particularly effective in preserving the quality of cut roses. This treatment significantly influenced key characteristics such as vase life and the activity of catalase and peroxidase enzymes. The greater effectiveness of pulsed application compared to continuous application can be attributed to the sensitivity and instability of melatonin. In addition, considering the harmful environmental impacts of some chemicals used in floral preservative solutions, melatonin stands out as an environmentally friendly alternative. It lacks detrimental effects and can thus be safely used in the postharvest management of cut flowers. The findings of this study demonstrate that melatonin, particularly at specific concentrations and application methods (e.g., 0.5 mM pulsed), can significantly improve the postharvest quality and longevity of cut rose flowers. These effects are likely mediated through enhanced antioxidant activity, membrane stability, and water retention. Further research is recommended to explore the underlying molecular mechanisms and optimize melatonin application protocols for commercial use.

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