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Scientific Article

Application of melatonin and sucrose in prolonging the vase life of amaryllis cut flowers (*Hippeastrum Hybridum* Herb)

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Abstract

The effects of melatonin application by itself and mixed with sucrose solution on vase life and physicochemical changes in amaryllis cut flowers were investigated. Amaryllis stems with flower buds at harvest points were placed into vases containing different maintenance solutions: Melatonin (Mt; 0.01 mM), Sucrose (Suc; 4%), Melatonin + Sucrose (Mt + Suc; 0.01 mM + 4%) and distilled water (Control). Each treatment consisted of five repetitions, with one inflorescence stem per vase. The vases remained at 22 ± 3 °C and constant lighting of 13 µmol m^{-2} s^{-1} for 14 days. Immersion of stems in Suc or Mt + Suc delayed the initial senescence processes of the amaryllis cut flower. Vase life was prolonged for additional 2 days compared to stems immersed in water (control), increasing from 10 to 12 days of conservation. Floral stem mass was kept more stable; the degradation of anthocyanin and flavonoid pigments was delayed; and the stability of phenolic compounds and total carbohydrates was maintained for 14 days. These findings confirm the role of melatonin as a preservative in cut flowers. However, its preservative effect was potentialized by the addition of sucrose to the water of the amaryllis cut flowers. More work needs to be carried out to investigate the physiological mechanisms promoted by the interaction of melatonin and sucrose in the regulation of senescence in amaryllis cut flowers, including the analysis of gene expression and activity of the antioxidant system.

Keywords: flower longevity, phenolic compounds, phytoregulator, vase life.

Resumo

Aplicação de melatonina e sacarose no prolongamento da vida de vaso de flores de corte de amarilis (*Hippeastrum hybridum* Herb)

O presente trabalho investigou os efeitos da aplicação de melatonina individualmente e misturada com solução de sacarose na vida de vaso e nas alterações físico-químicas de flores de corte de amarílis. Hastes de amarilis com botões florais em pontos de colheita foram colocadas em vasos contendo diferentes soluções de manutenção: Melatonina (Mt; 0,01 mM), Sacarose (Suc; 4%), Melatonina + Sacarose (Mt + Suc; 0,01 mM + 4%) e Água destilada (Controle). Cada tratamento constou de 5 repetições, sendo uma haste de inflorescência por vaso. Os vasos permaneceram a 22 ± 3° C e iluminação constante de 13 µmol $m^{-2}.s^{-1}$ durante 14 dias. A imersão das hastes em Suc ou Mt + Suc retardou os processos iniciais de senescência de flor de corte de amarílis, prolongando por mais 2 dias sua vida de vaso, em relação às hastes sem uso desses conservantes (controle). Aumentando de 10 para 12 dias de conservação. Pois mantiveram mais estáveis a massa fresca, retardaram a degradação de pigmentos antocianinas e flavonoides, bem como, mantendo a estabilidade dos compostos fenólicos e carboidratos totais ao final de 14 dias. Esses achados confirmam o papel da melatonina como conservante de flores de corte. Todavia, o seu efeito conservante foi potencializado em amarílis, associado à sacarose. Mais trabalhos necessitam ser realizados para investigar mais profundamente quais mecanismos fisiológicos promovidos pela interação melatonina e sacarose na regulação da senescência das flores de amarilis cortadas, incluindo análise de expressão de genes e atividade do sistema antioxidante.

Palavras-Chave: compostos fenólicos, fitoregulador, longevidade de flores, vida de vaso.

Introduction

Shelf life of cut flowers is quickly compromised by floral senescence events. The exogenous application of organic compounds aimed at prolonging vase life for commercialization is common (Costa et al., 2021). Sucrose addition has been extensively used in order to maintain the water balance of cut flowers (Lin et al., 2019). The addition

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of sucrose in the vase solution aims at replenishing reserves spent by respiratory metabolism, as well as reducing the synthesis and sensitivity of the hormone ethylene, which helps to increase the longevity of flowers after the cut. Among other effects, the application of sucrose can improve the visual appearance of flowers by increasing anthocyanins (Ichimura et al., 2022), the opening rate of flower buds, petal size, and reducing open flower abscission (Norikoshi et al., 2016).

Among other organic compounds, melatonin (*N*-acetyl-5-methoxytryptamine) (Mt) comes from the amino acid tryptophan and occurs naturally in plants and animals (Murch, 2000). In plants, it is involved in several responses against abiotic and biotic stresses, regulating signaling and combating reactive oxygen species (ROS) (Arnao & Hernández-Ruiz, 2019). Melatonin may provide tolerance to toxic metals such as Cr by inducing anthocyanin biosynthesis in tomato plants (Sun et al., 2023). The use of melatonin is associated with increased activity of the antioxidant apparatus and reduced enzymatic browning in minimally processed vegetables (Li et al., 2022). Xiang et al. (2020) working with exogenous melatonin on the vase life of uncut snapdragon flowers (*Antirrhinum majus* L.), demonstrated its positive effects on inflorescence length, flower quality and size. Among the few studies involving the role of exogenous melatonin in the post-harvest of cut flowers, Lezoul et al. (2022) observed that the vase life of carnation *Dianthus caryophyllus* L. was increased, associated with the control of membrane stability and potentiation of the antioxidant capacity of the flowers. In a study with the same species, in pulsing solutions with melatonin in cut roses, Mazrou et al. (2022) highlighted the role of melatonin in controlling water loss in the induction of phenolic compounds, associated with the activation of the enzymatic antioxidant system in the membrane integrity of these flowers. However, there is still little information about the use of melatonin with cut flowers, considering that its exogenous use could result in positive responses controlling stress in plants.

Amaryllis (*Hippeastrum* sp.), also known as hypeaster or lily, is a bulbous plant economically important in the world market of ornamental plants (Azimi and Alavijeh, 2020). Amaryllis is a plant with wide adaptation to tropical conditions and its commercialization can be carried out in the form of bulbs with the inflorescence stem or as cut flower, due to its long stems and distal inflorescence (Marasek-Ciolakowska et al., 2021). Among the few studies on the conservation of amaryllis cut flowers, AbdelKader (2012) demonstrated that the joint action of 8-hydroxycholine sulfate (8-HQS) and sucrose prolonged the vase life and increased the weight of the cut stems, in comparison to those maintained under control conditions (water) and submitted only to 8-HQS. This author also highlights the relevance of marketing amaryllis in the form of cut flowers due to its high economic importance and high demand.

Although studies have been carried out on the positive effects of melatonin against stress in plants and the postharvest quality of fruits and vegetables, there is not, as far as we know, sufficient knowledge about the possible effects of melatonin alone and the combination of melatonin and sucrose, especially on cut amaryllis flowers. Thus, the present work proposed to investigate the effects of exogenous application of melatonin solution by itself and melatonin + sucrose on the quality of amaryllis cut flowers in vase condition. Flower opening, color variations, sugars, pigments and antioxidant compounds were quantified.

Material and Methods

Amaryllis bulbs (*Hippeastrum hybridum* Herb) cv. Minerva with an average of 6.5 cm in diameter were acquired in a seedling nursery (Terra Viva, LTDA) in the city of Santo Antônio de Posse, in the state of São Paulo, Brazil, located at coordinates 22° 36′ 24″ S and 46° 55′ 9″ W. These bulbs were transferred to the experimental station of the Academic Unit of Serra Talhada, of the Universidade Federal Rural de Pernambuco (UAST/UFRPE), located in the municipality of Serra Talhada, Pernambuco, Brazil, at coordinates 07º 59' 31" S and 38º 17' 54" W.

The bulbs were planted in a greenhouse located at the UAST in one-liter plastic pots filled with a substrate consisting of soil and cattle manure in the proportion of 1:1 (v/v). The bulbs were irrigated every two days, based on the reference evapotranspiration (ETo), calculated using the Penman-Monteith equation (Allen et al., 1998). The plants were kept in a greenhouse for 50 days, where they had an average radiation of 5.83 kWh m^{-2}.day, relative humidity of 54.6% and average temperature of 29.7 °C.

The stems of the amaryllis inflorescence had reached the point of commercial harvest 50 days after planting, with the inflorescences still closed. The stems were then manually cut, maintaining a total length of 21 cm from the base to the apex, placed in vases containing distilled water and transferred to the post-harvest laboratories of the Postgraduate Program in Vegetal Production (PGPV/UAST). Subsequently, the stems were distributed among 500 mL vases containing one of the following treatments: melatonin (Mt) at 0.01 mM; sucrose (Suc) at 4%; mt 0.01mM + 4% sucrose (Mt + Suc) and distilled water (control). The melatonin was formulated by the company Formula Godoy LTDA. The solutions were not changed throughout the experiment, and the stems remained in the same solution for 14 days under a controlled environment with a temperature of 22 ± 3 °C and 65% RH and constant lighting at 13 µmol m^{-2} s^{-1}.

Every two days, the number of fully open flowers (FOF) and longevity evaluations were carried out and every seven days, flowers and stems were collected for physical-chemical characterization.

The color variations of the petals were measured using a portable digital colorimeter (RS – 232, RGB serial output - 1002) with values obtained in the RGB system. The data obtained by the colorimeter were converted to the CIE color scale *L*, a*, b**. *L** corresponds to lightness (0 -100, darkest to brightest), *a** corresponds to variations from green (- a) to red (+ a), and *b** is attributed to variations from blue (- b) to yellow (+ b). Value conversion was performed using online software available on a public website (Convert | EasyRGB, n.d. 2023). The *a** and *b**

data were subsequently converted into Chroma saturation values (*C**) according to the methodology by Brito et al. (2022). Five flowers of each treatment were used, from which 3 RGB color measurements were taken from each petal, from the initial, middle and final portions, and, thus, the average color per flower was estimated.

The pH and electrical conductivity (EC) were measured in the solution used to treat the stems. A pH meter (TECNAL, TEC-5, Piracicaba, Brazil) was used at a temperature of 25 °C. The electrical conductivity of the solution was measured using a bench conductivity meter (model PHS-12 dW). The results were expressed in mS cm^{-1}.

Membrane damage was determined based on the quantification of cellular content leakage, as described by Singh et al. (2008), with some modifications. For each repetition of the treatment, five petal discs measuring 1 cm in diameter were placed in a test tube containing 10 mL of distilled water (DW). The initial reading was performed using a bench conductivity meter (TECNAL, TEC-4MP, Piracicaba, Brazil), after incubating the test tubes at room temperature for 3 h. Then, the samples with the solution were heated at 95 ºC in a water bath (TECNAL, TE-056 mag, Piracicaba, Brazil) for 10 min and the final conductivity was recorded.

The extraction and determination of total soluble carbohydrates was performed according to (DuBois et al., 1956). A 0.05 g aliquot of the petal was homogenized in 1.3 mL of potassium phosphate buffer (100 mM, pH 7.0) and liquid nitrogen. The extract was centrifuged at 13,000 x *g* for 21 min at 4 °C. After centrifugation, 25 µL of supernatant was collected and prepared in test tubes for the quantification of sugars. For the reaction, 475 µL of distilled water, 500 µL of 5% phenol and 2.5 mL of sulfuric acid PA were added and maintained for 10 min at a temperature of 25 ºC. The absorbance readings were taken in a spectrophotometer (Libra S8 Biochrom, Cambridge, England) at 490 nm. Soluble carbohydrates were quantified using an anhydrous glucose calibration curve. Results were expressed in mg g^{-1} FW.

The determination of the content of total phenolic compounds was performed according to (Eugênio et al., 2021), with some modifications. The extraction was carried out with the maceration of by macerating 0.10 g of amaryllis petal in 1.5 mL of methanol. After 12 hours in the dark at 4 °C, the extract was centrifuged at 10,000 rpm at 2 °C for 21 minutes.

The assay was performed with a mixture of 5 µL of the supernatant, 2400 µL of distilled water, and 5 µL of Folin Cioucauteu (0.25 N). Then, 300 µL of sodium carbonate (1 N) was added and the tubes were kept in the dark at room temperature for 2 hours. Absorbance readings were taken with a spectrophotometer (Libra S8 Biochrom, Cambridge, England) at 725 nm and the results were expressed in mmol of gallic acid kg^{-1} WF, quantified based on a standard curve of gallic acid.

The content of total flavonoids and anthocyanins was determined through an adaptation by Francis (1982) methodology. To obtain the extract, it was necessary to carry out the entire process in the dark. A 0.1 g aliquot of the petals was macerated with 7 mL of ethanol (99.4%) and HCl (1%) solution. Then, the samples were kept at resting for 12 hours at 8 ± 2 ºC, protected from light. Subsequently, absorbance readings were taken with a spectrophotometer (Libra S8 Biochrom, Cambridge, England) at wavelengths of 535 nm for anthocyanins and 374 nm for flavonoids.

For the determination of chlorophylls, *a* (Chl *a*), *b* (Chl *b*), total (Chl total), and carotenoids (Car) 0.1 g of fresh matter (FM) of the petals of each inflorescence was weighed, macerated in 5 mL of acetone 80%, protected from light. The samples were centrifuged at 6000 g for 15 min at 4 °C. Measurements of chlorophyll *a*, *b*, total, and carotenoid contents were carried out in a spectrophotometer (Libra S8 Biochrom model, Cambridge, England) at wavelengths of 470, 645 and 663 nm, according to the method proposed by Lichtenthaler (1987). Chl *a*, Chl *b*, total Chl, and total Car concentrations were estimated in mg g^{-1} FW.

The experiment data were defined by a completely randomized design (DIC) with 4 treatments (1. Melatonin; 2. Sucrose; 3. Melatonin + Sucrose and 4. Distilled water, control), with 5 repetitions. Each repetition was considered as one stem per vase. Data were submitted to normality tests, analysis of variance and Tukey's test at 5% probability with the aid of R 4.3.0 software.

Results

There was no interaction effect between storage days and preservatives solutions to flower opening and membrane stability index analysis.

The control stem presented the fastest change in visual evaluation score (Figure 1 A). The floral opening was smaller in control, showing a slight tendency of delay in opening at 4 days compared to those stems submitted to the preservative solution (Figures 1B and D). At 6 and 8 days, stems were vigorous and perfect for sale, regardless of the treatments, all were marketable (Figure 1 D). At 10 days, the control flower began to lose marketing quality, starting with wilting symptoms, which was intensified at 12 days, confirmed by the greatest reduction of fresh weight (Figure 1 C). In addition, was observed a visual yellowing of the petals, followed by loss of flavonoids and anthocyanins (Figure 1 D, Figures 3 B and D, respectively). These changes were evidenced by the high values of *L** and *b** (Table 1). On the other hand, in this same period, the flowers submitted to melatonin alone and Mt + Suc, were ready for commercialization (Figure 1D).

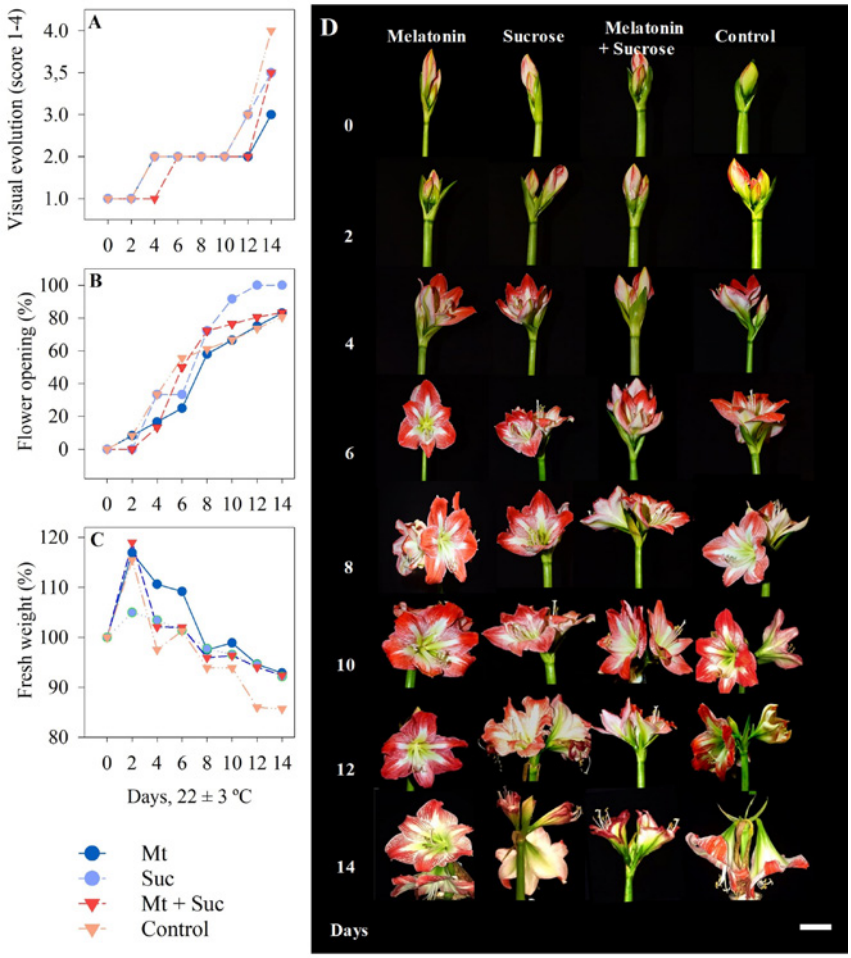

Figure 1. Visual evolution (A), flower opening (B), fresh mass (C) and visual aspect (D) of stems and flowers of amaryllis cv. Minerva in preservation solutions with Melatonin (Mt, 0.01 mM), Sucrose (Suc, 4%), Melatonin (0.01 mM) + Sucrose (4%) (Mt + Suc), and distilled water (Control), at 0, 2, 4, 6, 8, 10, 12 and 14 days of conservation. Horizontal bar represents 5 cm scale.

Color variation occurred for all cut flowers for all treatments (Table 1) and the components that corresponded to the final color value comprised the analysis of the *L*a*b** set. Flowers treated with Mt, Suc or Mt + Suc presented a darker color, close to reddish purplish, at 14 days of storage. This contrasted with the untreated flowers, which presented high *L**, reduction of *a** (- red) and increase of *b** (+ yellow) (Table 1).

Table 1. Color in Standard Luminosity (*L**), *a**, *b** and Chroma (*C**) in stems of amaryllis cv. Minerva in preservation solutions with Melatonin (Mt, 0.01 mM), Sucrose (Suc, 4%), Melatonin (0.01 mM) + Sucrose (4%) (Mt + Suc), and distilled water (Control). Colors represent the CieLAB variation.

Treatment	Color	Days		
		0	7	14
	**			
Mt	*L**	32.32 ± 6.89 Ba	14.88 ± 2.86 Bb	33.40 ± 0.96 Ba
	*a**	34.19 ± 1.51 Aa	31.30 ± 1.23 Aa	16.92 ± 0.39 Ab
	*b**	26.73 ± 5.17 Aa	22.34 ± 4.01 Aa	6.73 ± 2.12 Bb
	*C**	43.62 ± 4.41 Aa	38.85 ± 1.54 Ab	18.40 ± 1.15 Ac
	**			
Suc	*L**	25.82 ± 6.06 Ba	8.11 ± 1.29 Bb	22.96 ± 5.08 Ba
	*a**	36.92 ± 0.86 Aa	27.41 ± 1.99 Aa	17.45 ± 8.08 Ab
	*b**	24.51 ± 1.14 Aa	12.74 ± 2.03 Aa	16.91 ± 1.73 Ba
	*C**	44.32 ± 1.31 Aa	30.26 ± 2.65 Ab	25.16 ± 6.85 Ac
	**			
Mt + Suc	*L**	48.46 ± 8.36 Aa	15.26 ± 4.27 Bb	25.01 ± 3.79 Bb
	*a**	30.25 ± 5.47 Aa	29.82 ± 1.71 Aa	16.74 ± 2.24 Ab
	*b**	20.45 ± 2.51 Aa	17.01 ± 1.13 Aa	12.82 ± 2.75 Ba
	*C**	36.63 ± 5.68 Aa	34.35 ± 1.90 Ab	21.12 ± 3.44 Ac
	**			
Control	*L**	25.04 ± 0.99 Bb	29.73 ± 4.15 Ab	51.36 ± 2.62 Aa
	*a**	31.33 ± 4.10 Aa	27.33 ± 4.10 Aa	7.52 ± 2.17 Ab
	*b**	20.93 ± 0.88 Aa	19.49 ± 1.49 Aa	33.63 ± 4.13 Aa
	*C**	37.98 ± 1.48 Aa	33.63 ± 4.13 Ab	25.89 ± 7.34 Ac

* Values with different letters between the columns show a significant difference (P< 0.05); Capital letters in the columns compare all treatments within the day of analysis, lowercase letters in the lines compare each treatment individually between analysis days.

** Color standard (RGB) - Hex. code: Mt-0 = #833122; Mt-7 = #4F0901; Mt-14 = #6B04444; Suc-0 = #731C18; Suc-7 = #390101; Suc-14 = #53281C; Mt+Suc-0 = #A95D52; Mt+Suc-7 = #4E0C0B; Mt+Suc-14 = #583128; Control-0 = #6A231D; Control-7 = #723229; Control-14 = #937450

In the preservation solutions containing Suc and Mt + Suc, the flowers presented pH values significantly lower compared to the Melatonin and distilled water after 2 days (Figure 2 A). On the other hand, the electrical conductivity (EC) of these same solutions was significantly higher (Figure 2B). Regardless of treated was preservation solution there was no membrane damage to the stems when compared to control (Figure 2D). The difference in membrane damage was observed only by comparing the samples at the beginning and the end of the storage period (Figure 2C).

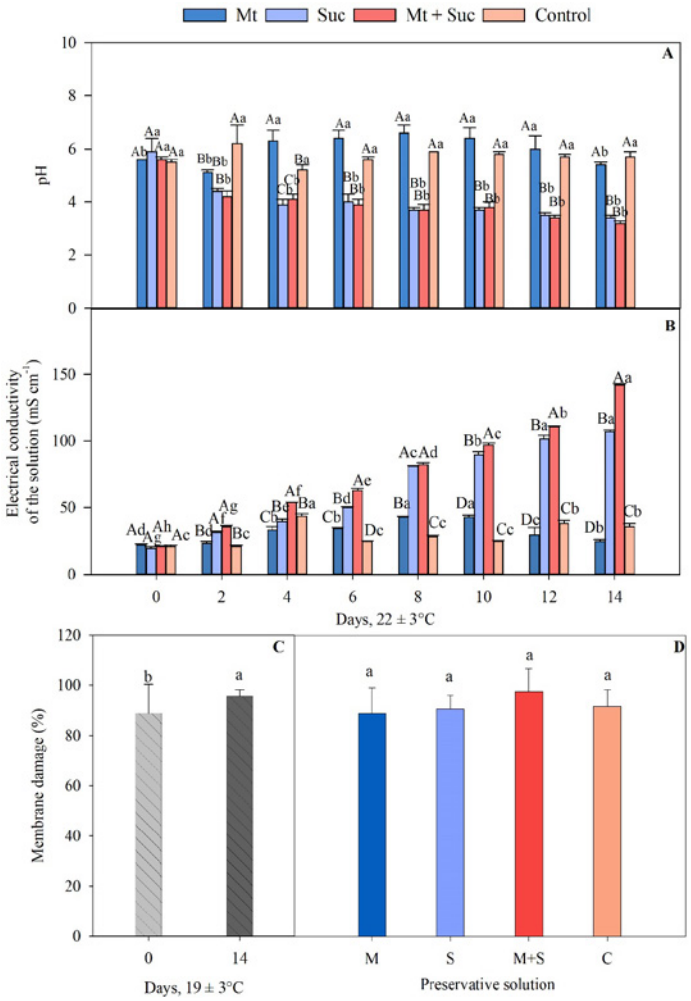

Figure 2. pH (A), Electrical conductivity (EC) of the solution (B); and Membrane damage (C and D) in stems of Amaryllis cv. Minerva in preservation solutions with Melatonin (Mt, 0.01 mM), Sucrose (Suc, 4%), Melatonin (0.01 mM) + Sucrose (4%) (Mt + Suc), and distilled water (Control). The flowers were kept at 22 ± 3 °C and 65% RH. The bars represent the standard error of the mean. Similar letters represent no statistically significant differences by Tukey's test ($p < 0.05$). Capital letters compare all treatments within the day of analysis. Lowercase letters compare each treatment individually between analysis days.

At the beginning of the experiment, the amount of phenolic compounds and flavonoids were higher in stems submitted to Mt + Suc (Figures 3 A and B). When the stems reached 7 and 14 days, the phenolic and flavonoid compounds were significantly higher in the stems treated with Suc or Mt + Suc (Figures 3 A and B); in the case of 14 days, these increases were significant. The soluble carbohydrate content decreased, but was higher for stems treated with sucrose and Mt+Suc (Figure 3C). The levels of anthocyanins at 7 and 14 days always had the lowest levels in the control flowers (Figure 3D).

Flowers treated with the Mt + Suc had higher levels of chlorophyll a, b, total, and total carotenoids at day O (Figures 4 A, B, C and D). At day 7, surprisingly, the control flowers showed the highest levels of these pigments (Figures 4 A, B, C and D). At day 14, control, Mt and Mt+Suc treated flowers had the highest levels of these pigments (Figures 4 A, B, C and D).

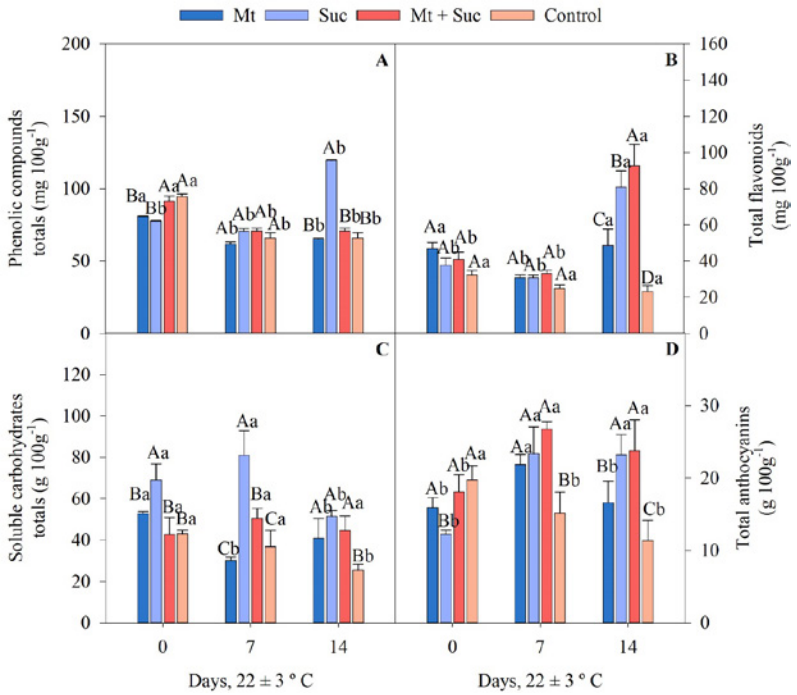

Figure 3. Total phenolic compounds (A); Total flavonoids (B); Total soluble carbohydrates (C) and total anthocyanins (D) from stems of Amaryllis cv. Minerva in preservation solutions with Melatonin (Mt, 0.01 mM), Sucrose (Suc, 4%), Melatonin (0.01 mM) + Sucrose (4%) (Mt + Suc), and distilled water (Control). The flowers were kept at 22 ± 3 °C and 65% RH. The bars represent the standard error of the mean. Similar letters represent no statistically significant differences by Tukey's test ($p < 0.05$). Capital letters compare all treatments within the day of analysis. Lowercase letters compare each treatment individually between analysis days.

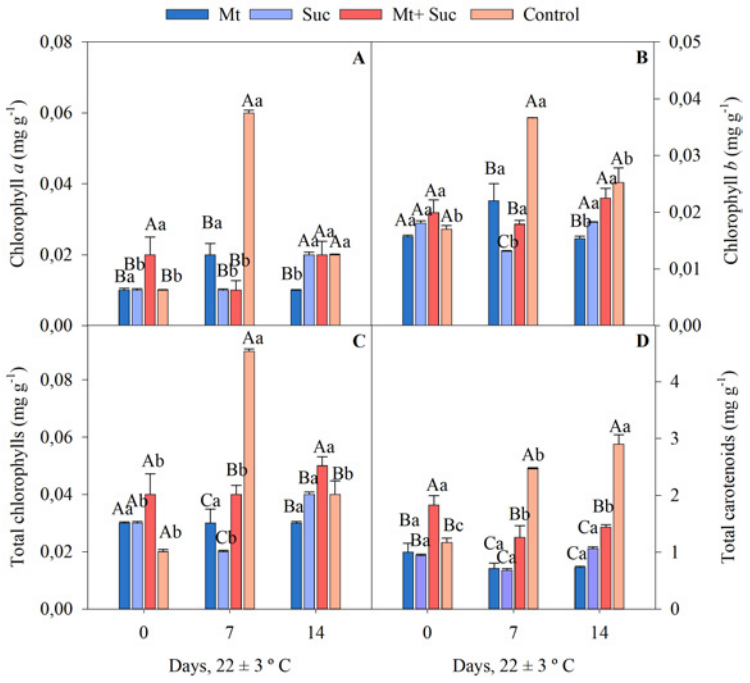

Figure 4. Content of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and total carotenoids (D) in petals of Amaryllis cv. Minerva in preservation solutions with Melatonin (Mt, 0.01 mM), Sucrose (Suc, 4%), Melatonin (0.01 mM) + Sucrose (4%) (Mt + Suc), and distilled water (Control). The flowers were kept at 22 ± 3 °C and 65% RH. The bars represent the standard error of the mean. Similar letters represent no statistically significant differences by Tukey's test ($p < 0.05$). Capital letters compare all treatments within the day of analysis. Lowercase letters compare each treatment individually between analysis days.

Discussion

The results presented here demonstrate that conservation solutions containing sucrose (Suc), melatonin (Mt) and melatonin + sucrose (Mt + Suc) played an important role in the maintenance and conservation of amaryllis cut flowers. The solutions used isolated or combined, increased the conservation of amaryllis cut flower by two days at a marketable level, which is a significant time for commercialization. In addition, the sucrose and Mt + Suc solutions prolonged the opening of the flowers compared to control flowers, after 8 days of conservation, although the total floral opening was not significantly different among the treatments. The control treatment induced greater loss of fresh mass of the stems, compared to the application of Mt, Suc and Mt+Suc solutions. The dehydrated appearance of the stems 10 days after treatment was already noticeable. Studies indicate that sucrose solutions can increase carbohydrate levels in petal vacuoles, which helps in water absorption and maintenance of the water status of cut flowers (Norikoshi et al., 2016). Carbohydrate levels in amaryllis petals remained higher in stems maintained in Mt, Suc and Mt+Suc solutions, compared to control flowers (Figure 3C). Melatonin also has shown playing an important role in regulation of enzymes activity involved in sucrose metabolism, inhibiting sucrose degradation enzymes and increasing the synthesis of this sugar in apple fruit (Fan et al., 2022). Furthermore, the increase of shelf life of cut flowers has been mentioned in studies with melatonin in an isolated use (Lezoul et al., 2022) and in synergistic action with Se (Zhou et al., 2023) in a vase solution. Studies have pointed out that the effective action of melatonin on the delay of senescence in cut flowers may depend on the dose applied. The loss of quality of cut carnations was delayed by 10 days when 0.1 mM of melatonin was applied, compared to distilled water (Lezoul et al., 2022). Among the general effects, melatonin had the power to delay the effects of senescence, especially when combined with sucrose (Figure 1). Further studies with greater interactions between doses and the vase life of amaryllis cut flowers may increase data precision.

The preservative solutions that manage to remain stable under low pH conditions are those that have the most influence on the post-harvest conservation of flowers, given that an acidic environment reduces microbial growth in the solution and in the vascular tissues of the stems, maintaining the water status of flowers (Carlson et al., 2015; Spricigo et al., 2021). The media containing Suc and Mt + Suc, the pH was smaller than the other treatments (Figure 2 A). In addition to the nature of the preservative solution, the pH and EC variation of the solution depends on interaction with the cut stems. The effects of sucrose on the acidification of the preservative solution induced increases in EC compared to solutions without sucrose (Figure 2 B). Increases in electrical conductivity over time can be promoted by degradation of the cellular components of the stems that are released into the media; furthermore, as an organic substrate of metabolism, this may cause increase of bacteria and bacteria secretion (Carlson et al., 2015), even though a lower pH was maintained in the solution (Figure 2 A). Microbial growth may not have been enough to compromise the conservation of petals for a longer period. Indeed, the petals suffered little effect from the treatments in terms of membrane damage, remaining statistically the same (Figure 2D).

Maintaining the colors of the petals is a decisive factor for the commercialization of cut flowers. In our study, the amaryllis control flowers showed a higher loss of color, compared to the other treatments, becoming lighter in color as evidenced in increments in L^*, and yellowish, by increasing the values positive in b^*, compared to the flowers treated with other solutions 14 days after cutting (Table 1). These variations in the reddish color of the petals are caused by the accumulation and degradation of anthocyanins in the flowers (Lezoul et al., 2022). Corroborating these results, the levels of flavonoids and anthocyanins in the control amaryllis petals were smaller, 14 day of storage, compared to the flowers treated with preservative solutions (Figures 3 B and D). The Mt, Suc and Mt + Suc solutions maintained the highest levels of flavonoids and anthocyanins in amaryllis cut flowers, when compared to the control, at 14 days (Figures 3 B and D). Flavonoid and anthocyanin pigments, as well as lignin and phenolic compounds are originate from the activity of the phenylpropanoid pathway, activated mainly by the key enzyme phenylalanine ammonia lyase, PAL (Dixon and Paiva, 1995). Previous studies have shown that melatonin upregulates anthocyanin biosynthesis genes, including increasing transcripts and PAL activity, among other anthocyanin and phenol pathway genes in different plant organs (Mazrou et al., 2022). Interestingly, total phenol levels decreased over 7 days for all preservative solution treatments; control flowers however, showed the greatest reductions in phenols, flavonoids, carbohydrates and anthocyanins at 14 days (Figures 3A, B and D). Anthocyanins, flavonoids and phenols are related to their protective role against free radicals, highlighting their activity in maintaining the antioxidant capacity and integrity of cell membranes, especially under stress conditions. Increased anthocyanins levels have been associated with exogenous applications of melatonin in reducing symptoms of post-harvest senescence in strawberries (Aghdam and Fard, 2017), plums (Arabia et al., 2022) and lychee (Zhang et al., 2018). Recent studies report that the foliar application of melatonin in cut carnations (*D. caryophyllus* L.) induct phenolic metabolites production to help delay foliar senescence, however, procyanidin levels decreased in melatonin treatments (Zhou et al., 2023). The authors reported that significant increases in procyanins in control flowers may be related to the high degree of senescence, while melatonin application may induce lignins to maintain water status (Zhou et al., 2023). Mazrou et al. (2022) reported the protective role of pulsing solutions containing melatonin, inducing higher levels of phenols and improving the ability to remove ROS in cut roses. Furthermore, melatonin induced PAL-mediated synthesis of phenolic compounds, helping anthurium cut flowers to increase vase life under cold stress (Aghdam et al., 2019).

Suc, Mt and Mt + Suc solutions effects on anthocyanin increments and delay in color decay can also be explained by the role of sucrose in the induction of anthocyanins in cut flowers (Ichimura et al., 2021; Lin et al., 2019). The conservation of vase life associated with the addition of sucrose in preservative solution has been reported to increase anthocyanins in carnations, even in combination with nanosulfur (Lin et al., 2019). In addition, Zhang et al. (2015) reported the role of sugars, including sucrose, in the induction of anthocyanin biosynthesis genes in cut flowers of *Paeonia suffruticosa*, providing greater coloration and delay of senescence. Further advances in understanding the effects of melatonin and sucrose on the regulation of the phenylpropanoid and anthocyanin pathway in amaryllis cut flowers are important tools for the development of new cultivars with longer vase life.

The yellowing of petals is an event consistent with the senescence processes of plant tissues. The amaryllis cut flowers increased in *b** coloring, mainly in control flowers (Table 1). The levels of chlorophyll *b* and total carotenoids showed significant increases during storage of control flowers, which may have influenced the greater yellowing level, compared to the preservation solutions containing Mt + Suc (Figures 4 B and D). High levels of chlorophyll *a* were also observed for Mt + Suc. It is worth mentioning that the amaryllis flowers used in our experiment were of a bicolor variety, from which small amounts of chlorophylls were obtained. The senescence process in cut white carnations, on the other hand, has been related to the reduction of total chlorophylls in control flowers, compared to flowers treated with 0.1 mM melatonin (Lezoul et al., 2022). These authors also attributed the melatonin-induced maintenance of chlorophylls in the preservation of cut flowers to photochemical protection of chloroplasts, reducing damage to membranes. This corroborates findings by Hu et al. (2023) who highlighted the role of melatonin protecting the photosystems, preventing the destruction of chlorophylls in carnation leaves subjected to heat. Mt reduced the effects of senescence in cut gardenia leaves (*Gardenia jasminoides* Ellis), induced by low light, through the decrease of flavonoids and carotenoids, to the detriment of the increase of chlorophylls (Zhao et al., 2017). Since the flowers of the amaryllis cv. Minerva had bicolored petals, the chlorophyll levels may not have been so determinant for the degree of senescence. This suggests that carotenoid levels are a possible indicator of post-harvest life decay of amaryllis cut flowers.

Conclusions

We demonstrated that melatonin and sucrose, alone or in combination, extended vase life and improved the quality of amaryllis cut flowers by two days, compared to stems kept in only water (control), increasing from 10 to 12 days of storage. The fresh mass remained stable, degradation of anthocyanin and flavonoid pigments was delayed, and the stability of phenolic compounds and total carbohydrates was maintained to the end of 14 days kept in preservative solution. Overall, we provide evidence that melatonin reduces the senescence effects of amaryllis cut flowers, adding to the known effect of sucrose on flowers. We suggest that more work needs to be done to investigate which physiological mechanisms are promoted by the melatonin and sucrose interaction in the regulation of senescence in amaryllis cut flowers, including determination of endogenous melatonin concentrations and synergistic action with preservative organic molecules and activity of the antioxidant system.

Author Contribution

FALB: Wrote and critically analyzed the manuscript, co-supervisor in which he assisted in the conception and design of the research and interpretation of the data. **NRCMJ**: Collected the data, wrote the monograph that led to the aforementioned manuscript. **LDCSM and MBT**: Assisted with data collection and critical evaluation of the manuscript. **L.F.S.**: Wrote and critically analyzed the manuscript, co-supervising which helped with the conception and design of the research. **TMG**: Assistance with data collection, statistical analysis and critical evaluation of the manuscript. **ANS**: Advisor who assisted in the conception and design of the research, in the analysis and interpretation of data and in writing the manuscript.

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