



ARTICLE

Eco-friendly or flower killer? Understanding the negative effects of essential oil on calla lily postharvest

Eco-friendly ou flower killer? Compreendendo os efeitos negativos do óleo essencial na pós-colheita de Zantedeschia

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Abstract: There is a demand for the development of sustainable postharvest conservation technologies for flowers, utilizing eco-friendly products to promote sustainability and reduce the production of toxic residues, such as essential oils. Although essential oils have been reported as effective for some species, in calla lily, some negative effects, such as stem strangulation, have been observed. Thus, the objective was to evaluate the effect of preservative solutions prepared with eucalyptus (*Eucalyptus citriodora*) essential oil on the postharvest quality of calla lily (*Zantedeschia aethiopica*), analyzing the biochemical and enzymatic processes of senescence. Flower stems, 50 cm in length, were placed in solutions prepared with pure water and various concentrations (0.1%, 0.25%, 0.75%, and 1.0%) of eucalyptus essential oil, with pure water as the control. The stems remained in contact with the solution in a cold chamber at 10 °C for 2, 4, 6, 8, and 10-h. Evaluations were carried out every 2 h, totaling 10-h. At each evaluation, samples were collected from three stem lengths (5, 10, and 15 cm) to determine the sucrose content. After 5 h, a stem section 10 cm in length was collected for electron microscopy analyses. The negative effects observed included the destruction of the conducting system, causing the death of all floral stems. The blockage in the stem limited sucrose absorption and stimulated the activity of antioxidant enzymes, leading to lipid peroxidation (MDA) and an increase in the activity of superoxide dismutase (SOD) and catalase (CAT).

Keywords: biochemical analyses, catalase, cut flower, superoxide dismutase, *Zantedeschia aethiopica*.

Resumo: Atualmente, há uma demanda pelo desenvolvimento de tecnologias sustentáveis para a conservação pós-colheita de flores, utilizando produtos ecológicos para promover a sustentabilidade e reduzir a produção de resíduos tóxicos, como os óleos essenciais. Embora os óleos essenciais tenham demonstrado eficácia para algumas espécies, em *Zantedeschia* foram observados alguns efeitos negativos, como o estrangulamento do caule. Assim, o objetivo foi avaliar o efeito de soluções preservativas preparadas com óleo essencial de eucalipto (*Eucalyptus citriodora*) na qualidade pós-colheita de *Zantedeschia* (*Zantedeschia aethiopica*), analisando os processos bioquímicos e enzimáticos de senescência. Hastes florais, com 50 cm de comprimento, foram colocadas em soluções preparadas com água pura e várias concentrações (0,1%, 0,25%, 0,75% e 1,0%) de óleo essencial de eucalipto, sendo a água pura utilizada como controle. As hastes permaneceram em contato com a solução em câmara fria a 10 °C por 2, 4, 6, 8 e 10 horas. As avaliações foram realizadas a cada 2 horas, totalizando 10 horas. Em cada avaliação, amostras foram coletadas de três comprimentos de haste (5, 10 e 15 cm) para determinar o teor de sacarose. Após 5 horas, uma seção de haste com 10 cm de comprimento foi coletada para análises de microscopia eletrônica. Os efeitos negativos observados incluíram a destruição do sistema condutor, causando a morte de todos os caules florais. O bloqueio no caule limitou a absorção de sacarose e estimulou a atividade das enzimas antioxidantes, levando à peroxidação lipídica (MDA) e ao aumento da atividade da superóxido dismutase (SOD) e da catalase (CAT).

Palavras-chave: análises bioquímicas, catalase, flor de corte, superóxido dismutase, *Zantedeschia aethiopica*.

Introduction

Extending the vase life of floral stems is one of the main challenges in postharvest flower handling (Paiva et al., 2024a), particularly in studies involving tropical species (Cunha Neto et al., 2023; Malakar et al., 2023). In the postharvest, innovation was considered of great importance, particularly for improving preservation and packaging techniques, extending the shelf life of the products (Paiva et al., 2024b). Although some research has been conducted on the postharvest treatment of *Zantedeschia aethiopica* (commonly known as calla lily), such as the application of sucrose (Almeida et al., 2009); preservative solutions (Almeida et al., 2007; Sales et al., 2018); pre-cooling and storage conditions (Almeida et al., 2008; Almeida et al., 2009; Almeida et al., 2011; Mattos et al., 2023), harvest stage optimization (Resende et al., 2014); and hydric relations (Sales et al., 2015; Sales et al., 2021), there are no records of the studies involving the use of natural products for this purpose.

The sustainable production of flowers and ornamental plants has become a global trend, emphasizing practices that reduce environmental impacts (Ribeiro et al., 2016). However, while traditional products are effective, particularly in minimizing microbial growth, they may leave residues that compromise the quality of agricultural products and harm the environment (Eldeeb and Adam, 2021; Thakur et al., 2023).

In sustainable and organic production, the use of natural products is essential at all stages, including in the formulation of preservative solutions

for postharvest treatments (Wisniewski et al., 2001). There is a growing demand for the development of sustainable postharvest conservation technologies for flowers, utilizing eco-friendly products to promote sustainability and minimizing the generation of toxic residues. Natural products such as essential oils offer a promising alternative to many toxic preservatives, due to their antifungal and insecticidal properties (Isman, 2006; Kaur et al., 2012; Gururani et al., 2023). As a result of this, the efficacy of new products should be tested (Verdonk et al., 2022).

Essential oils are volatile compounds produced in various parts of certain plants and can enhance postharvest durability in other species due to their antagonistic properties against microorganisms, thereby reducing losses (Wisniewski et al., 2001; Soliman and El-Sayed, 2023; Thakur et al., 2023). These properties are attributed to the high levels of phenolic compounds present in these oils (Bayat et al., 2011), which effectively control microorganisms and prevent xylem blockage (Soliman and El-Sayed, 2023).

In flowers, essential oils have already been successfully tested for postharvest conservation. For example, rosemary oil and ginger oil have been suggested for roses (Bastos et al., 2016), as well as peppermint and eucalyptus essential oils (Manfredini et al., 2017a), and eucalyptus essential oil alone (Almeida et al., 2020). However, the use of methyl jasmonate in roses was found to be ineffective (Manfredini et al., 2017b). In other species, positive effects have also been observed, such as thyme and clove oils in chrysanthemums (El-Sayed and El-Ziat, 2021), and

geranium, thyme, marjoram, and anise in carnations (Gururani et al., 2023). Lemon grass (*Cymbopogon citratus* (D.C) Stapf), known for its antimicrobial activity, has shown promising positive results in increasing the vase life of cut flowers such as gerbera, gladiolus, alstroemeria, and roses (Thakur et al., 2023).

The addition of chemical preservatives to the maintenance solution is beneficial, but beyond this, it is essential to understand the enzymatic and biochemical reactions triggered by products used. Essential oils act through mechanisms such as altering enzyme synthesis, inactivation, and promoting changes in plasma membrane permeability (Souza et al., 2005).

During the postharvest stage, the production of reactive oxygen species (ROS) becomes a critical factor. At high concentrations, ROS can damage membranes through lipid peroxidation leading to cell death. Essential oils may stimulate higher activity of antioxidant enzyme such as peroxidase (POD), catalase (CAT), or superoxide dismutase (SOD), thereby limiting further production of free radicals like H_2O_2 (Souza et al., 2021; Soliman et al. 2022, Soliman and El-Sayed, 2023).

One of the defense mechanisms employed by plants to eliminate ROS under stress conditions is the activation of the antioxidant enzyme system. This system includes enzymes such as ascorbate peroxidase (APX), (CAT), (POD), (SOD), and other reductases (Souza et al., 2021). The application of essential oils has been shown to reduce lipid peroxidation and free radical generation, as evidenced by decreased levels of malondialdehyde (MDA) and H_2O_2 , respectively. Additionally, essential oils promote increased production of total phenols, enhancing membrane stability. For instance, lemongrass essential has been reported to increase total soluble sugars and carotenoid content, while reducing MDA levels in postharvest gladiolus flowers (Thakur et al., 2023).

Although essential oils have been tested for postharvest applications in some ornamental plants, limited information is available regarding their effectiveness in calla lily and their impact on enzymatic and antioxidant activities.

Eucalyptus essential oil, previously tested successfully in roses, was selected for use in the preservative solution for calla lily based on these experiences (Manfredini et al., 2017a). Thus, the objective was to evaluate the effect of preservative solutions containing eucalyptus essential oil on the postharvest performance of calla lily, focusing on biochemical and enzymatic processes of senescence associated with senescence.

Material and Methods

Plant Material and Solution Preparation

Floral stems of calla lilies (*Zantedeschia aethiopica*) were harvested with open flowers and standardized to a length of 50 cm. The flowers were placed in a transparent plastic container containing 0.5 L of eucalyptus essential oil solution, with the floral stem being immersed in solution up to 10 cm and kept in a cold chamber at 10 °C. The preservative solutions were prepared by emulsifying the essential oil in Tween 20 and then diluting it in water to concentrations of 0%, 0.1%, 0.25%, 0.75%, and 1.0%, which constituted the treatments.

Experimental Design

The experiment was conducted using a complete randomized design with a factorial arrangement of 5 solutions (0, 0.1%, 0.25%, 0.75%, and 1.0%) and 5 collection times (2, 4, 6, 8, and 10 hours). Each treatment consisted of five repetitions, with 2 inflorescences per plot.

Biochemical Analysis

Samples for biochemical analysis were collected at 2, 4, 6, 8, and 10 hours after placing the stems in the preservative solution, totaling 5 collection times. To collect samples, cuts were made at three positions along the stem: 5 cm, 10 cm, and 15 cm from the base. The 5 cm position corresponded to the submerged part of the stem, the 10 cm position where the stems were in contact with the solution on their surface, and the 15 cm position above the solution. Three replicates were collected for each treatment, which included different concentrations of essential oil in the preservative solution, and for each cutting position. Samples were immediately placed in liquid nitrogen and stored in an ultra-freezer at -80 °C.

Biochemical analyses included determination of total sugars, quantification of malondialdehyde (MDA) to assess lipid peroxidation, and the determination of hydrogen peroxide (H_2O_2). The activity of

antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) was also analyzed.

Analysis of Total Soluble Sugars

The extraction of total soluble sugars was performed as follows: 200 mg dry stem mass was homogenized in 5 mL of 0.1 M potassium phosphate buffer (pH 7.0) and then placed in a water bath at 40 °C for 1,800 seconds. After homogenization, the material was centrifuged at $10,000 \times g$ for 1,200 seconds, and the supernatant was collected. This process was repeated, and the supernatants were combined. Sucrose quantification was conducted using the Anthrone method (Yemm and Willis, 1954).

Hydrogen Peroxide and Lipid Peroxidation

The extraction of hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA) was carried out as follows: 0.2 g of fresh stem material was macerated in liquid nitrogen and PVPP, then homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA). Subsequently, the samples were centrifuged at $12,000 \times g$ for 900 seconds at 4 °C, and the supernatant was collected and stored at -20 °C until further analysis.

Lipid peroxidation was quantified using the TBARS method (Buege and Aust, 1978): 125 μ L aliquots of the supernatant were mixed with 250 μ L of reaction medium containing 0.5% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA), followed by incubation at 95 °C for 1,800 seconds. After cooling rapidly with ice, absorbance was measured using an ELISA reader at wavelengths between 535 and 600 nm.

Hydrogen peroxide analysis was performed using the method described by Velikova et al. (2,000): 45 μ L aliquots of the supernatant were mixed with 180 μ L of a stock solution containing 10 mM potassium phosphate buffer at pH 7 and 1 M potassium iodide. Absorbance readings were taken at 390 nm using a spectrophotometer.

Antioxidant Enzymes

The extraction of antioxidant enzymes was performed as follows: 0.1 g fresh stem mass was macerated in liquid nitrogen and PVPP, then added to 1.5 mL of extraction buffer containing 400 mM potassium phosphate buffer (pH 7.8), 10 mM EDTA, 200 mM ascorbic acid, and water. The extract was then centrifuged at $13,000 \times g$ for 600 seconds at 4 °C. The supernatant was collected and stored at -20 °C until analysis. The supernatants were used to quantify the activities of superoxide dismutase (SOD) and catalase (CAT) (Biemelt et al., 1998).

SOD activity was assessed by the enzyme's ability to inhibit the photo-reduction of nitroblue tetrazolium (NBT). A 10 μ L aliquot of the enzyme extract was added to 190 μ L of incubation buffer containing 100 mM phosphate buffer (pH 7.8), 70 mM methionine, 10 μ M EDTA, water, 1 mM NBT, and 0.2 mM riboflavin. Samples were illuminated with a 20 W fluorescent lamp for 420 seconds, and absorbance readings were taken at 560 nm using a spectrophotometer (Giannopolitis and Ries, 1977).

CAT activity was measured using the method of Havir and McHale (1987). A 4 μ L aliquot of the sample was added to 167 μ L of incubation buffer containing 200 mM potassium phosphate buffer (pH 7.0), water, and 250 mM hydrogen peroxide at 30 °C. Absorbance readings were taken every 15 seconds for 180 seconds at 240 nm using a spectrophotometer. The molar extinction coefficient of $36 \text{ nM}^{-1} \text{ cm}^{-1}$ was used for calculations.

Visualization of stem bases using Scanning Electron Microscopy (SEM)

For the observation of stem characteristics, stem samples were collected by making cuts at a height of 0.1 m from the base. An initial sample (control) was collected from each treatment, followed by subsequent samples collected after 5 and 10 hours of exposure to the preservative solution, totaling 11 samples. The samples were sectioned and fixed in Karnovsky solution, then stored overnight in a refrigerator at 4 °C. Preparation involved washing the samples in 0.05 M cacodylate buffer (three washes, 10 minutes each).

Next, the samples were dehydrated in solutions with increasing acetone concentrations (25%, 50%, 75%, 90%, 100%), followed by drying in a critical point dryer using CO_2 . The dried samples were mounted on stubs and sputter-coated with gold using an SCD 505 apparatus at 50 mA for 180 seconds. The analyses were performed and photographed using a Zeiss EVO 40 VXP scanning electron microscope, following the protocol described by Alves (2004).

Statistical Analysis

The averages of the treatments were compared for significance using ANOVA, on a Sisvar, as described by Ferreira (2014). To compare means within treatments, the Scott-Knott test was applied at a significant level of $p < 0.05$.

Results and Discussion

The floral stems of calla lily were significantly affected by the use of eucalyptus essential oil as a floral preservative at all tested concentrations. The application of the preservative led to stem blockage and wilting. of the conducting vessels and for this, there is no data on the response of the flowers to the treatments, but only analyses of the stem to understand the causes of the blockage.

Analysis of Total Soluble Sugars

The addition of essential oils influenced the sucrose content in the stems compared to the water-control group (Fig. 1), although no

consistent pattern emerged across all treatments. In the initial hours after being placed in the solutions (2 and 4 hours), stems treated with lower concentrations of essential oil showed a tendency for increased sucrose content, particularly at the 5 cm and 10 cm cutting positions. A similar trend was observed at the 15 cm cutting position, where higher sucrose levels were recorded in stems treated with lower concentrations of essential oil.

However, in stems treated with higher concentrations of essential oil, sucrose levels were relatively low, even early in the treatment. This effect may be attributed to the rapid blockage of the stems, which restricted the transport of sucrose, leading to its accumulation in the basal parts of the stem.

In contrast, a study on carnations indicated that essential oils in preservative solutions enhanced carbohydrate content (Gururani et al., 2023). This suggests that, while eucalyptus essential oil may inhibit xylem flow in calla lily, essential oils in some species may promote it, highlighting species-specific responses to these treatments.

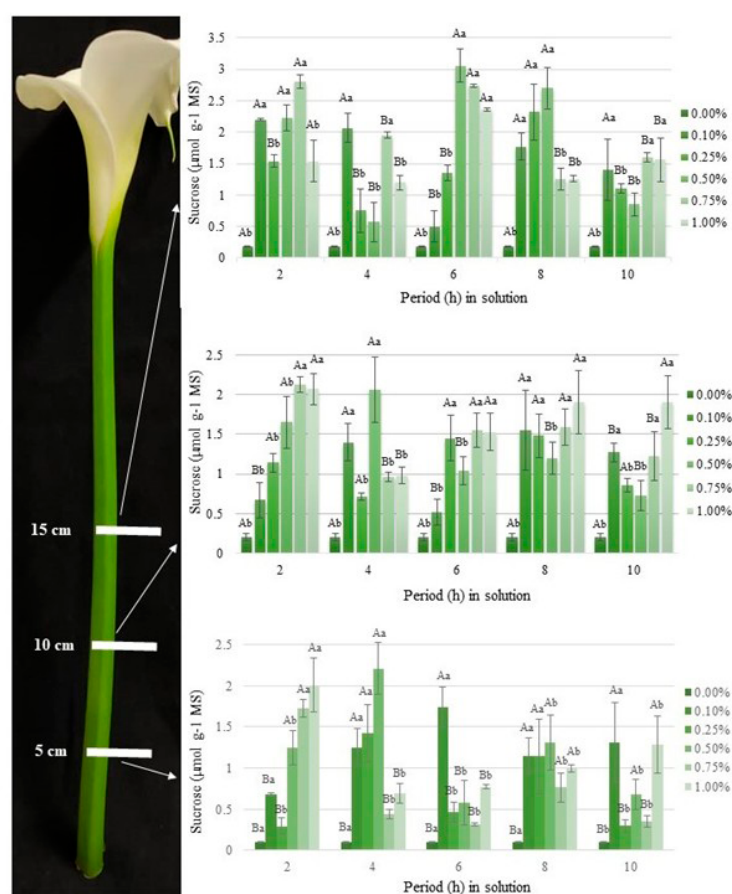


Fig. 1. Sucrose ($\mu\text{mol g}^{-1}$ MS) content in calla lily floral stems as a function of cutting position at different time intervals (h), and eucalyptus essential oil concentrations (%). Means with different capital letters (Period) or lowercase letters (essential oil concentration) significantly differ ($p < 0.05$ level) by Scott-Knott test.

An increase in sucrose content over time was expected, similar to what has been observed in other species like gladiolus, which were kept in solution with lemongrass essential oil (Thakur et al., 2023). However, contrary to this expectation, the observed stem blockage disrupted the absorption process, preventing the anticipated increase in sucrose.

Biochemical Analysis

The production of reactive oxygen species in plants is typically triggered by stress-inducing factors. To counteract the toxic effects of

ROS, plants have developed antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Deuner et al., 2008; Figueiredo et al., 2021).

Biochemical analyses of calla lily floral stem samples collected at a height of 5 cm from the base (the region immersed in the preservative solution) revealed signs of lipid peroxidation and the presence of the SOD enzyme (Fig. 2). This was likely due to the stem blockage observed in the upper part of the stem, which impedes the normal flow of water and nutrients, leading to oxidative stress.

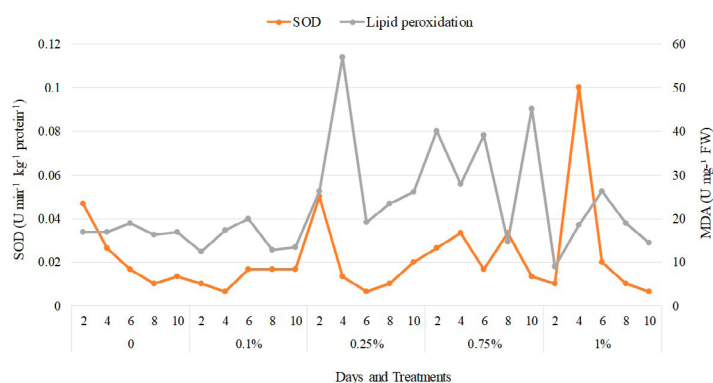


Fig. 2. Superoxide dismutase (SOD) activity and lipid peroxidation in calla lily floral stems with cuts made at 5 cm length immersed in different concentrations of eucalyptus essential oil solution.

The activity of SOD initially peaked in all tested concentrations of eucalyptus essential oil solutions, before stabilizing. This contrasts with the findings in gladiolus flowers exposed to lemongrass essential oil, where an increase in SOD activity was observed, with the highest activity recorded even on the 11th day (Thakur et al., 2023). This indicates that SOD activity tends to increase with the aging process of flowers.

The peak in SOD activity was most pronounced in stems treated with the control solution and the highest concentration of eucalyptus oil (1.0%) at 4 hours. SOD serves as the first line of defense against ROS (Souza et al., 2021), catalyzing the dismutation of O_2 into H_2O_2 and O_2 . At higher concentrations (0.75% and 1.0%), lipid peroxidation was more pronounced compared to lower concentrations, regardless of the duration

of exposure in the solution. Lower SOD activity was observed in stems treated with extreme concentrations like 0.25% and 1.0%, approaching the levels seen in control stems.

No significant catalase activity was detected at the 5 cm cutting position. CAT is an enzyme responsible for the breakdown of H_2O_2 , playing a critical role in detoxifying ROS, especially when H_2O_2 levels are elevated under stress conditions (Dubey, 2011).

When analyzing stems at the 10 cm cutting position, which corresponds to the part in contact with the preservative solution, significant CAT activity was detected, along with signs of lipid peroxidation (MDA). However, no hydrogen peroxide (H_2O_2) activity was detected at this position (Fig. 3).

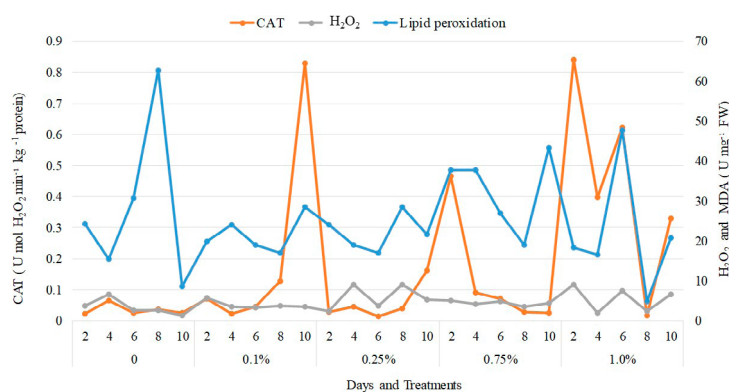


Fig. 3. CAT activity, hydrogen peroxide, and lipid peroxidation in calla lily floral stems with cuts made at 10 cm length immersed in different concentrations of eucalyptus essential oil solution.

CAT activity exhibited fluctuating values across treatments, with a general tendency to increase overtime exposure. Hydrogen peroxide quantification remained low in all treatments, including the control, suggesting its absence in the stem tissues. Lipid peroxidation, assessed by malondialdehyde levels, initially showed higher values, which decreased

with prolonged exposure (Fig. 4). In contrast, the control treatment maintained consistently low MDA levels. Similar results were observed in gladiolus flowers exposed to varying concentrations of lemongrass essential oil, where MDA content was lower compared to the control (Thakur et al., 2023).

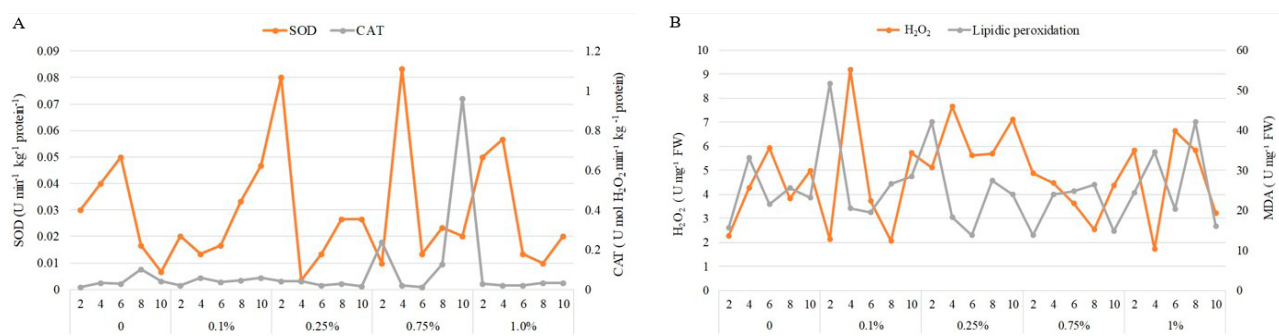


Fig. 4. SOD and CAT activity (A) and hydrogen peroxide and lipid peroxidation (B) in calla lily floral stems with cuts made at 15 cm length immersed in different concentrations of Eucalyptus essential oil solution.

The amount of MDA in the floral stems at the 15 cm length showed considerable fluctuation, with higher values observed across all treatments. The highest peak was recorded in the control treatment. CAT activity was initially very low in all treatments but showed a tendency to increase with longer exposure to the solution. A notable peak was observed in stems treated with the 0.75% concentration for 8 hours. In contrast, an increase in CAT activity was observed in gladiolus flowers exposed to different concentrations of lemongrass essential oil, compared to the control (Thakur et al., 2023).

Hydrogen peroxide levels remained constant and low across all treatments, indicating effective action of antioxidant enzymes. This aligns with previous studies showing that essential oils can reduce H $_2$ O $_2$ levels by enhancing antioxidant enzyme activities, such as POD, CAT, and SOD, thus preventing further free radical formation (Soliman et al., 2022). Phenolic compounds, which are antioxidants that neutralize the harmful effects of oxygen released from hydrogen peroxide, play a role in decomposing H $_2$ O $_2$ and slowing petal aging (Hopkins et al., 2007).

The accumulation of H $_2$ O $_2$ and MDA is indicative of cellular membrane degradation (Gururani et al., 2023). Free oxygen radicals, including H $_2$ O $_2$, are known to trigger lipid peroxidation, leading to increased MDA production (El-Sayed and El-Ziat, 2021). High

MDA levels in plant tissues reflect physiological stress tolerance and senescence (Geng et al., 2009). In the case of Zantedeschia stems, while MDA levels were high, H $_2$ O $_2$ activity remained at basal levels.

The response of antioxidant enzymes to abiotic stress helps explain the results observed at the 15 cm length, where stems experienced water deficit due to constriction at the 10 cm mark, limiting water uptake. This condition led to higher MDA and CAT activity, but low H $_2$ O $_2$ activity, which confirmed lipid membrane damage and compromised the effectiveness of the essential oil treatment.

Visualization of stem bases using Scanning Electron Microscopy (SEM)

Microscopic examination revealed constriction of the vascular vessels at the 10 cm length (immersed in the solution), with the severity of constriction increasing with higher concentrations of essential oil (Fig. 5). Cross-sectional images at various points along the Zantedeschia stem axis illustrated the effects of the treatments on cell and tissue damage. Contrary to expectations, based on the antimicrobial properties of essential oils, one would anticipate clear vascular vessels for water transport (Soliman and El-Sayed, 2023). However, the observed constriction suggests that essential oils may impair vascular function, potentially limiting the water transport capacity of the stems.

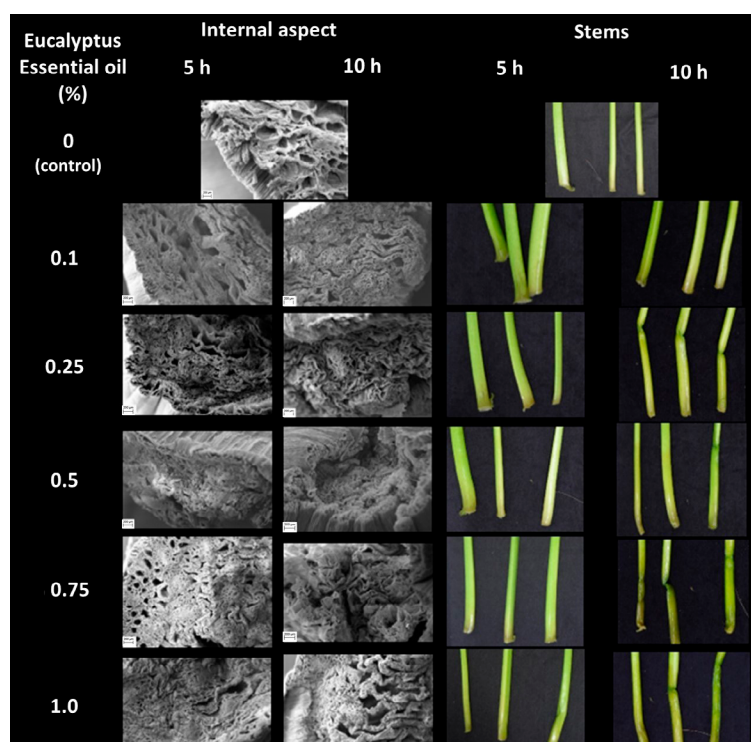


Fig. 5. Scanning Electron Microscopy (SEM) analysis of floral stems and visual analysis of the bases of calla lily inflorescences at different concentrations of eucalyptus essential oil after 5 and 10 hours of exposure to the solution.

Eucalyptus essential oils, with their hydrophobic properties and phenolic compounds, significantly altered the structure of cell membranes, making them more permeable. This dual action of eucalyptus essential oil, combined with the activity of antioxidant enzymes, contributed to the destruction of vascular vessels and membrane damage (El-Sayed et al., 2021; El Khetabi et al., 2022). As a result, the stems underwent strangulation, leading to wilting and the eventual death of the floral tissues.

The use of essential oils as a postharvest solution, while considered an eco-friendly alternative for several species, was found to be ineffective in maintaining the quality of *Zantedeschia*. Instead, it acted as a “flower killer.” The negative effects observed included the destruction of the vascular system, which impaired sucrose absorption and stimulated the activity of antioxidant enzymes, resulting in lipid peroxidation (MDA). This was accompanied by an increase in superoxide dismutase (SOD) and catalase (CAT) activity. However, despite these changes, hydrogen peroxide (H₂O₂) levels remained low or were undetectable, suggesting limited oxidative stress.

Conclusions

Eucalyptus essential oil not only was ineffective in maintaining the quality of *Zantedeschia* but also caused the death of all the floral stems. The use of eucalyptus essential oil led to an increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

Acknowledgements

The authors are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de apoio à Pesquisa do Estado de Minas Gerais (Fapemig) for the scholarships, grants, and financial support for the project.

Author Contribution

MCRS: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Formal Analysis, Validation, Visualization, Writing - Original Draft. **PDOP:** Conceptualization, Funding Acquisition, Project Administration, Methodology, Resources, Supervision, Validation, Writing - Review & Editing. **DPCS:** Conceptualization, Data Curation, Investigation, Methodology, Formal Analysis, Writing - Original Draft. **DGM:** Methodology, Resources, Validation, Writing - Review. **JRMF:** Data Curation, Investigation, Methodology, Writing - Original Draft, 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 upon request to the authors.

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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