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The Impact of Pre and Postharvest Treatments on the Quality and Vase Life of Cut Lisianthus Flowers

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ABSTRACT

Lisianthus (Eustoma grandiflorum), a member of the Gentianaceae family, is a commercially important cut flower. This study investigated the effects of pre-harvest (salicylic acid, vermicompost, humic acid, and their combination) and postharvest treatments (melatonin, rosemary essential oil, and tragacanth gel) on the activity of antioxidant enzymes and the vase life of cut lisianthus flowers. The experiment was conducted as a completely randomized design with 12 treatments and 3 replications. The evaluated enzymatic traits included catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) activities. The results demonstrated that all combined treatments significantly influenced the biochemical characteristics at the 1% probability level. Among all treatments, the melatonin-vermicompost combination was identified as the most effective. It resulted in the lowest degradation, as indicated by the smallest increase in PPO activity, alongside favorable outcomes for CAT and APX enzymes, ultimately leading to the greatest extension of vase life. In conclusion, the application of these treatments, particularly the combined melatonin-vermicompost treatment, effectively enhances the postharvest longevity of cut lisianthus flowers and is highly recommended.

Keywords: Ascorbate peroxidase, Vase life, Melatonin, Salicylic acid, Catalase, Polyphenol oxidase

INTRODUCTION

Lisianthus, scientifically known as *Eustoma grandiflorum*, belongs to the family Gentianaceae and is native to South America and Mexico, thriving in conditions compatible with temperate and warm regions (Barbosa *et al*., 2024). Lisianthus is an annual or biennial plant; tall varieties are cultivated for cut flower production, while dwarf varieties are suitable for pot cultivation (Ataii *et al*., 2015). Furthermore, the longevity of cut flowers is one of the most important commercial requirements worldwide. The future of the international market heavily relies on adopting new technologies that enhance the longevity of cut flowers (Rezai *et al*., 2024). To ensure postharvest longevity, processes that affect flower senescence must be identified, followed by the establishment of effective methods for evaluating cut flowers. After harvest, cut flowers can only absorb water or nutrient solutions present in the vase from the stem, and water uptake and transport can be limited due to blockages at the stem cut caused by microbial growth, tyloses formation, deposition of secondary materials, and the presence of air in the vascular system. Meanwhile, transpiration and environmental humidity can lead to water loss, causing wilting of the flower, which occurs when water uptake cannot keep pace with water loss.

Preharvest Treatments

a) Salicylic acid is one of the key compounds in the ethylene synthesis process in plants. Salicylic acid prevents the conversion of aminocyclopropanecarboxylic acid to ethylene (Bayat & Aminifard, 2017). Salicylic acid is a phenolic compound found in many genera and plays significant roles in growth and metabolism, as well as in regulatory responses to stress and developmental processes in plants, including heat, photosynthesis, stomatal conductance, transpiration, ion uptake and transport, disease resistance, crop yield, and glycolysis. The role of salicylic acid and its potential to preserve postharvest characteristics of vegetables and cut flowers has been previously reported.

b) Humic acids are macromolecules that include humic substances, which are organic materials distributed in terrestrial soils, natural waters, and sediments. Unlike other components of humic acid (fulvic acid and humin), they are soluble in alkaline environments, partially soluble in water, and insoluble in acidic environments. Due to their amphiphilic characteristics, they form micelle-like structures in neutral to acidic conditions, making them useful in agriculture, pollution remediation, medicine, and beneficial drugs (de Melo *et al*., 2016). Humic acid increases the quantity and quality of plants by providing more accessible essential elements, especially micronutrients and macronutrients such as iron, potassium, phosphorus, and nitrogen, altering primary and secondary metabolism, reducing the pH of alkaline soils, enhancing plant resistance to various biotic and abiotic stresses, retaining water in the soil, and reducing temperature fluctuations (Zhi *et al*., 2024).

c) Vermicompost, produced through the non-thermophilic biological decomposition of organic waste by earthworms and associated microbes, is an important biofertilizer that sustainably enhances crop yield and helps adapt to environmental stresses (Manzoor *et al*., 2024). The use of vermicompost reduces soil acidity, increases porosity, and enhances water retention capacity, microbial activity, and overall soil health. These advancements lead to better germination, growth, development, and performance of plant products.

Postharvest Treatments

a) Melatonin can increase the synthesis of total phenolic compounds in plants by enhancing the activity of glucose-6-phosphate dehydrogenase, shikimate dehydrogenase, and phenylalanine ammonia-lyase. When applied externally, melatonin leads to an increase in internal melatonin levels in plants, which is not only harmless but can also be beneficial (Delgado-Vargas *et al*., 2022). Melatonin also reduces malondialdehyde concentration, a key indicator of lipid peroxidation and cellular damage, with positive effects observed in tuberous flowers (Zulfiqar *et al*., 2023).

b) Tragacanth is a gum that is naturally exuded from the stem tissues of certain species of *Astragalus* when they are split or wounded. This plant is common in Iran, Syria, and Turkey. Tragacanth gum is one of the most important and primary sources of commercial gum worldwide, with numerous applications in the pharmaceutical, health, and food industries (BeMiller, 2018). Tragacanth gum is a high molecular weight anionic polymer. Due to its renewability, availability, cost-effectiveness, non-allergenic, non-carcinogenic, non-toxic nature, and environmental compatibility, it has garnered significant attention. These characteristics make tragacanth an essential polysaccharide that offers remarkable opportunities for beneficial applications in wastewater treatment, extending the shelf life of agricultural products, green chemistry, health, and other industrial settings (Boamah *et al*., 2023).

c) Essential oils are oil-like liquids with a strong aroma and distinct flavor extracted from plants, possessing very potent physiological and medicinal properties. These compounds are complex mixtures of hydrocarbons and their oxygenated derivatives, derived from two different isoprenoid pathways, and are used in medicine, cosmetics, and the food industry (Akman *et al*., 2023; Sharifi-Rad *et al*., 2017).

MATERIALS AND METHODS

Location and Method of Experimentation

This research was conducted in 1403 (2024) at the Honar Rooz greenhouse, covering an area of 1000 square meters with a semi-gothic structure 5.5 meters high, located in Najafabad County, Isfahan Province, with a maximum temperature of 30°C and a minimum temperature of 18°C. The air circulation system in this greenhouse included a roof vent, and the greenhouse covering was plastic, with a drip irrigation system (tape and hose). The seeds used were of the Mariachi variety, purchased from Sabz Royesh Damoon (the representative of Sakata Company in Iran). This experiment was designed as a completely randomized design with 12 different pre- and postharvest treatments, 3 replications, and a total of 36 experimental units (plots). Evaluations were conducted on 340 plants one week after the final application of treatments and before the actual harvest.

Treatments

Salicylic Acid: The salicylic acid used was obtained under the trade name Salix from Sabz Royesh Damoon. The components of this product included 2% salicylic acid, 3% potassium nitrate, and 2% other compounds.

Vermicompost: The vermicompost was sourced from Majidi Nejad Brothers in Najafabad, fed with livestock manure (cow) + 4 to 5% chopped corn.

Humic Acid: The humic acid used as a trench fertilizer was obtained under the trade name Parsileon S2 from Pars Kimia Kasht Agripars Yazd. The components of this product included 2% sulfur, 25% organic matter, and 12% organic carbon. The humic acid used in irrigation was under the trade name Humic Power from the Spanish company FuturaCo, which contained granulated humic acid with potassium and was fully water-soluble. The components of the fertilizer included: 60% total humic extract, 53% humic acids, 7% fulvic acids, 8% water-soluble potassium, and humic extract derived from leonardite.

Melatonin: The melatonin used in this study was obtained from Merck Germany with 100% purity from Shayan Chemie Isfahan.

Rosemary Essential Oil: The rosemary essential oil was obtained from Giah Royan Mehr Company.

Tragacanth: The tragacanth used in this study was purchased from a herbal shop.

Preparation of Enzyme Extracts for Measuring Antioxidant Enzymes

To extract and measure the enzymes, 0.5 grams of plant tissue was ground in a porcelain mortar in the presence of liquid nitrogen at the end of the experiment. Then, 4 milliliters of extraction buffer (containing 50 mM potassium phosphate (pH = 7.8), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% polyvinylpyrrolidone (PVP), 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF)) was added to the plant sample. After that, the homogenate was centrifuged at 13,000 × g for 5 minutes at 4°C. After completing the centrifuge, the supernatant was collected and used to measure the catalase enzyme activity (Correa de Souza *et al*., 2014).

Measurement Method for Catalase Enzyme (CAT)

To measure the catalase enzyme activity, 100 microliters of the plant tissue enzyme extract were added to 3 milliliters of the reaction solution containing 45 mM hydrogen peroxide and 50 mM phosphate buffer (pH= 7). Enzyme activity was assessed using a spectrophotometer and absorption at a wavelength of 240 nm for 1 minute. The unit of measurement was considered to be enzyme units per milligram of fresh weight (Courtney *et al*., 2016).

Measurement Method for Ascorbate Peroxidase Enzyme (APX)

The activity of the ascorbate peroxidase enzyme in all treatments was measured using the method of Nakano and Asada (1981). The reaction mixture consisted of 2.5 milliliters of 50 mM phosphate buffer with pH= 7 (containing 0.1 mM EDTA and 1 mM sodium ascorbate), 0.1 milliliters of the extracted enzyme, and 0.2 milliliters of 1% hydrogen peroxide. Then, ascorbate peroxidase enzyme activity was calculated as a reduction in absorption within one minute at a wavelength of 290 nm using a spectrophotometer. To measure ascorbate peroxidase activity, the extinction coefficient (2.8 mM^{-1} cm^{-1}) was used. The enzyme activity was obtained using the following equation:

Unit (µmol/min/mL) = (ΔOD/min × Assay Volume (mL)) / (Extinction Coefficient (2.8 mM^{-1} cm^{-1}) × Sample Volume (mL))

Measurement Method for Polyphenol Oxidase Enzyme (PPO)

For this purpose, two milliliters of the reaction mixture containing an amount of extract with 40 mg of protein, 20 microliters of proline solution, and 1.86 milliliters of 25 mM citrate-phosphate buffer (pH 6.4) was completely mixed in a test tube. This mixture was aerated using a vortex for two minutes, and then the spectrophotometer was zeroed using this mixture. Immediately thereafter, 40 microliters of 100 mM pyrocatechol solution was added to the reaction mixture, mixed quickly, and the changes in light absorbance at a wavelength of 515 nm were measured for one minute at 10-second intervals. The enzyme activity was calculated based on the changes in light absorbance per minute per milligram of protein.

RESULTS

The analysis of variance of the data showed that the effect of different treatments on the levels of catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) enzymes was significant at the 1% probability level (Table 1).

Table 1. Analysis of Variance Results for the Effect of Treatments on Some Biochemical Characteristics

Mean Squares				
Polyphenol	Ascorbate	Catalase	Degrees of Freedom	Sources of Variation
**0.27369	**1.16757	**0.02846	23	Treatment
0.01818	0.01645	0.00557	48	Experimental Error
24.55	11.34	21.80		Coefficient of Variation (%)

**Significant at 1% probability level

Catalase Enzyme

The comparison of mean data indicates that the highest level of catalase enzyme was related to the vermicompost treatment (2 kg/m²) before harvest and tragacanth gel (2%) after harvest on day zero, measuring 0.62 mg/g of fresh weight. The lowest level was related to the humic acid treatment (2 kg/m²) before harvest and melatonin (50 µM concentration) after harvest on day six, measuring 0.15 mg/g of fresh weight (Figure 1).

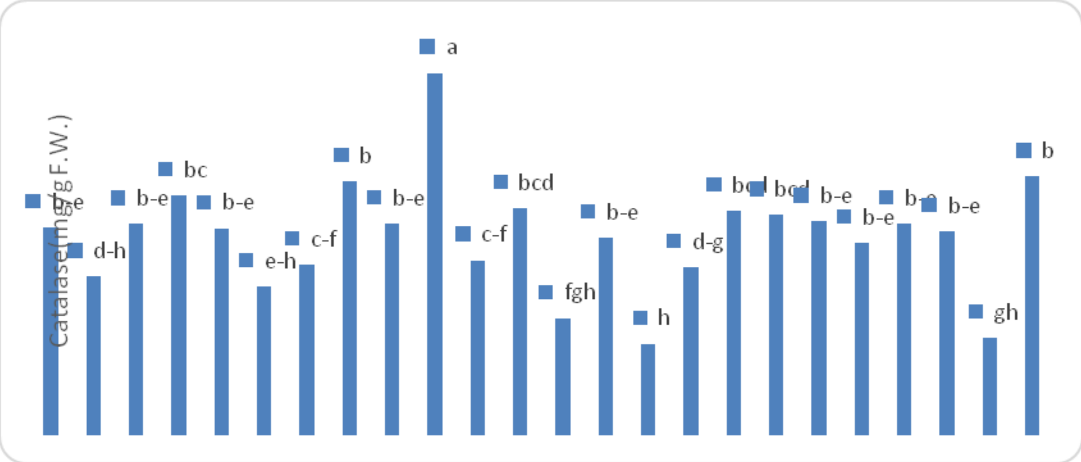

Figure1.Comparison of Mean Catalase Enzyme Levels in mg/g Wet Weight Across Different Treatments

Ascorbate Peroxidase Enzyme

The comparison of mean data showed that the highest level of ascorbate peroxidase enzyme was related to the humic acid treatment (2 kg/m²) before harvest and rosemary essential oil (3 ppm concentration) after harvest on day six, measuring 2.88 mmol/min. The lowest level was related to the humic acid treatment (2 kg/m²) before harvest and melatonin (50 µM concentration) after harvest on day six (Figure 2).

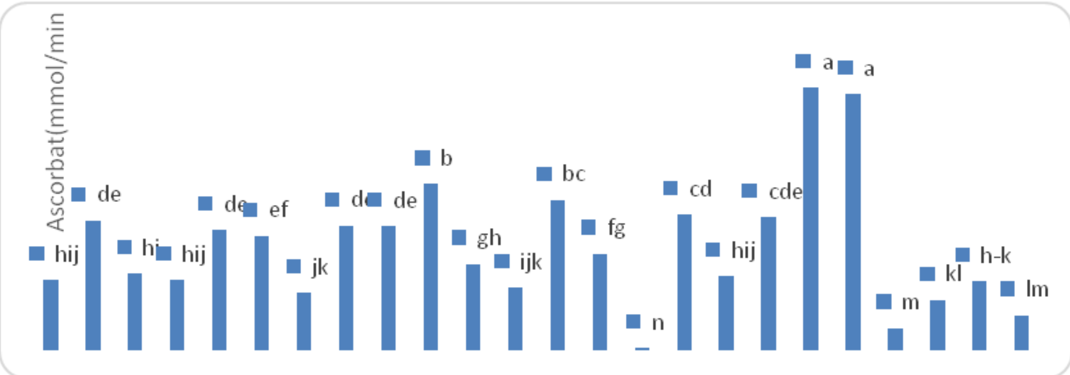

Figure 2. Comparison of Mean Ascorbate Peroxidase Enzyme Levels in mmol/min Across Different Treatments: MSA0 (Melatonin Salicylic Day Zero); MVER0 (Melatonin Vermi Day Zero); MHU0 (Melatonin Humic Day Zero); MHV0 (Melatonin Humic Vermi Day Zero); RSA0 (Rosemary Salicylic Day Zero); RVER0 (Rosemary Vermi Day Zero); RHU0 (Rosemary Humic Day Zero); RHV0 (Rosemary Humic Vermi Day Zero); GCSA0 (Tragacanth Salicylic Day Zero); GCVER0 (Tragacanth Vermi Day Zero); GCHU0 (Tragacanth Humic Day Zero); GCHV0 (Tragacanth Humic Vermi Day Zero); MSA6 (Melatonin Salicylic Day Six); MVER6 (Melatonin Vermi Day Six); MHU6 (Melatonin Humic Day Six); MHV6 (Melatonin Humic Vermi Day Six); RSA6 (Rosemary Salicylic Day Six); RVER6 (Rosemary Vermi Day Six); RHU6 (Rosemary Humic Day Six); RHV6 (Rosemary Humic Vermi Day Six); GCSA6 (Tragacanth Salicylic Day Six); GCVER6 (Tragacanth Vermi Day Six); GCHU6 (Tragacanth Humic Day Six); GCHV6 (Tragacanth Humic Vermi Day Six).

Polyphenol Oxidase Enzyme

The comparison of mean data also indicated that the highest level of polyphenol oxidase enzyme was associated with the humic acid + vermicompost treatment (1+1 kg/m²) before harvest and tragacanth gel (2% concentration) after harvest on day six, measuring 0.88. The lowest level was related to the humic acid + vermicompost treatment (1+1 kg/m²) before harvest and rosemary essential oil (3 ppm concentration) after harvest on day zero, measuring 0.014 (Figure 3).

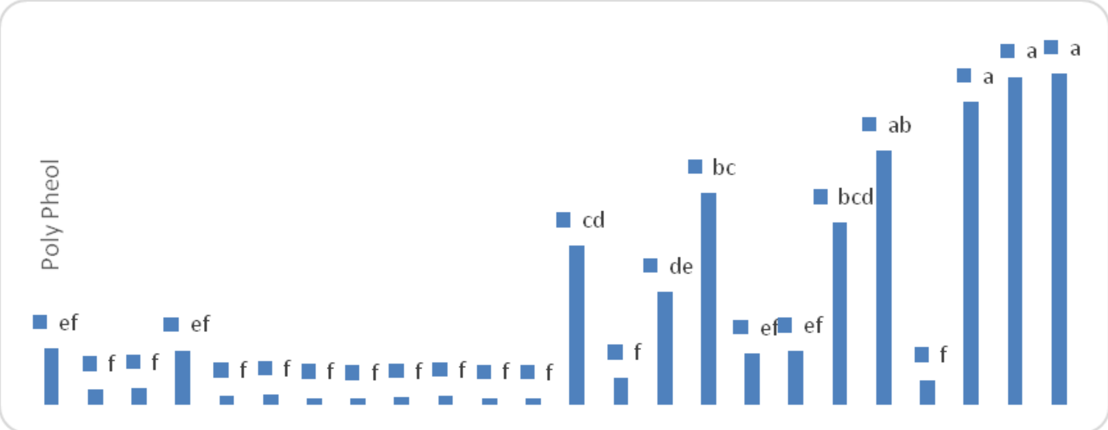

Figure 3. Comparison of Mean Polyphenol Oxidase Enzyme Levels Based on Changes in Light Absorbance per Minute per Milligram of Protein Across Different Treatments: MSA0 (Melatonin Salicylic Day Zero); MVER0 (Melatonin Vermi Day Zero); MHU0 (Melatonin Humic Day Zero); MHV0 (Melatonin Humic Vermi Day Zero); RSA0 (Rosemary Salicylic Day Zero); RVER0 (Rosemary Vermi Day Zero); RHU0 (Rosemary Humic Day Zero); RHV0 (Rosemary Humic Vermi Day Zero); GCSA0 (Tragacanth Salicylic Day Zero); GCVER0 (Tragacanth Vermi Day Zero); GCHU0 (Tragacanth Humic Day Zero); GCHV0 (Tragacanth Humic Vermi Day Zero); MSA6 (Melatonin Salicylic Day Six); MVER6 (Melatonin Vermi Day Six); MHU6 (Melatonin Humic Day Six); MHV6 (Melatonin Humic Vermi Day Six); RSA6 (Rosemary Salicylic Day Six); RVER6 (Rosemary Vermi Day Six); RHU6 (Rosemary Humic Day Six); RHV6 (Rosemary Humic Vermi Day Six); GCSA6 (Tragacanth Salicylic Day Six); GCVER6 (Tragacanth Vermi Day Six); GCHU6 (Tragacanth Humic Day Six); GCHV6 (Tragacanth Humic Vermi Day Six).

DISCUSSION AND CONCLUSION

Catalase Enzyme Activity

The comparison of mean data indicates that the highest level of catalase enzyme was found in the tragacanth-vermicompost treatment on day zero, measuring 0.62 mg/g of fresh weight, while the lowest level was observed in the melatonin-humic acid treatment on day six, measuring 0.15 mg/g of fresh weight. On the other hand, the results showed that in some treatments, there was a significant increase in catalase enzyme activity over time. In the melatonin-vermicompost treatment, the enzyme activity on day zero was 0.273 mg/g of fresh weight, which increased to 0.339 mg/g of fresh weight by day six. Similarly, in the rosemary-vermicompost treatment, the enzyme activity increased from 0.256 mg on day zero to 0.379

mg/g of fresh weight on day six. In the rosemary-humic treatment, the activity increased from 0.294 mg/g of fresh weight on day zero to 0.331 mg on day six. Therefore, the greatest significant difference in the increasing the amount of enzyme activity in treatment based on day zero to day six is in the rosemary-vermicompost.

Studies show that the increased catalase enzyme activity in cut flowers after harvest is associated with increased antioxidant activity and improved membrane stability, which can be affected by treatments such as tragacanth gel, vermicompost, rosemary essential oil, and humic acid. In a study on cut carnation flowers by investigating the sudden increase of free radicals during the post-harvest period, it was found that catalase enzyme activity also increased, which is related to the reduction in polar lipids, a slight increase in peroxides, and the onset of a sudden electrolyte flow, which has led to a correlation between free radical production and membrane integrity loss, which has led to an increase catalase activity (EC 1.11.1.6) (Droillard *et al*., 1987). In another research on the post-harvest life of cut rose flowers, it was found that increased catalase enzyme activity is associated with an increase in membrane stability index (Hasanzadeh-Naemi *et al*., 2021).

Increased catalase activity following the use of vermicompost after harvesting plants can be attributed to several biochemical and microbial advancements in the soil. Vermicompost enriches soil with organic materials and nutrients, which in turn stimulates microbial activity and enzyme production, including catalase. This enzyme plays an important role in the decomposition of hydrogen peroxide, thus contributing to plant stress responses and overall soil health. The application of vermicompost significantly increases microbial biomass and activity, leading to increased catalase production (Toor *et al*., 2024). Higher levels of dissolved organic carbon and nutrients in the soil are positively correlated with catalase activity. Additionally, vermicompost improves substrates nutrient profiles, including nitrogen and phosphorus, which are essential for microbial growth and enzyme activity (Zhang *et al*., 2014). Increased availability of nutrient increase plant growth, which can stimulate microbial communities and catalase production in the rhizosphere (Toor *et al*., 2024). Vermicompost can reduce abiotic stress on plants, increase healthier growth and increase catalase levels as a protective response (Santos *et al*., 2022). Collectively, the findings from these previous studies are consistent with the results obtained in the present research regarding the enhancement of catalase activity.

Ascorbate Peroxidase Enzyme Activity

Comparing the mean data indicates that the highest level of ascorbate peroxidase enzyme activity was observed in the rosemary-humic acid treatment on the sixth day at 2.88 mmol/min, and in the rosemary-humic acid-vermicompost treatment on the sixth day at 2.81 mmol/min, and the lowest level of this enzyme activity was observed in the melatonin-humic acid treatment on the sixth day Also, the activity of this enzyme in melatonin-salicylic acid treatments showed a significant increase from 0.76 on day 0 to 1.64 mmol/min on the sixth day, and in melatonin-humic acid-vermicompost treatments from 0.76 on day 0 to 1.48 on the sixth day. In total, the highest increase in ascorbate peroxidase enzyme activity was observed between on the zero to sixth days in the rosemary-humic acid treatment (from 0.62 on the zero day to 2.88 mmol/min on the sixth day).

The results of the research indicate that the application of humic acid in cut flowers leads to an increase in antioxidant enzyme activity (Fan *et al*., 2015). Rosemary essential oil has also been proven to have antioxidant properties (Erkan *et al*., 2008; Mezza *et al*., 2018), which due to the presence of phenolic compounds rich in essential oil compounds (Lemos *et al*., 2015). Antioxidant activity has been confirmed as a free radical regulating capacity, along with its effect on lipid peroxidation in rosemary essential oil (Bozin *et al*., 2007). Moreover, the application of rosemary essential oil and its effect on membrane stability and increased ascorbate peroxidase activity in cut gladiolus flowers has been established (Mousavi *et al*., 2024).

At the cellular level, rosemary essential oil significantly increased cell survival, catalase, superoxide dismutase, and glutathione, while reducing exposure to hydrogen peroxide. Mechanistically, rosemary essential oil alleviated oxidative stress by activating the NRF2 signaling pathway and enhancing the expression of NRF2, NQO-1, and HO-1 proteins, further confirmed by molecular interaction with the main component 1.8 cineole. Therefore, rosemary essential oil can be considered a strong natural antioxidant with potential strategies in the food and pharmaceutical industries (Li *et al*., 2024). The nuclear factor erythroid 2-related factor 2 (NRF2) plays a crucial role in regulating the transcription of genes encoding antioxidant proteins involved in glutathione synthesis, xenobiotic detoxification, and free radical scavenging (Li *et al*., 2019).

Polyphenol Oxidase Enzyme Activity

The comparison of mean data indicates that the highest level of polyphenol oxidase enzyme activity was observed in the tragacanth-humic acid-vermicompost treatment on day six, measuring 0.88, while the lowest level was found in the rosemary-humic acid-vermicompost treatment on day zero, measuring 0.014, based on changes in light absorbance per minute per milligram of protein. Most treatments showed a significant increase in polyphenol oxidase enzyme activity on day six, with the greatest increase between day zero and day six occurring in the tragacanth-humic acid-vermicompost treatment. The least increase (best result) during the experimental period was observed in the melatonin-vermicompost treatment, which increased from 0.38 to 0.69, with a difference of 0.31 based on changes in light absorbance per minute per milligram of protein (difference between day zero and day six).

These studies indicate that melatonin and vermicompost treatments reduce polyphenol oxidase activity by enhancing antioxidant systems, inhibiting phenolic metabolism, and preventing enzymatic browning, thereby improving the quality and shelf life of fruits and vegetables. The primary agent of polyphenol oxidase (PPO, EC 1.14.18.1) is a copper-containing oxidoreductase that involves two different enzymatic reactions, which include oxygen and phenolic substrates that convert to dark polymer pigments. This process can be controlled by nutritional treatments that lead to increased antioxidant production and activity (Tinello & Lante, 2018). Melatonin can also regulate the metabolism of phenolic and reactive oxygen species in plants (Panadare & Rathod, 2018). Reports indicate that melatonin treatment causes an overproduction of polyphenols, flavonoids, anthocyanins, and ascorbic acid, thereby increasing antioxidant activity. Enzymatic browning is controlled by measuring the content of malondialdehyde and hydrogen peroxide, polyphenol oxidase, guaiacol peroxidase, and lipoxygenase activities that are slowed under melatonin treatment (Magri & Petriccione, 2022). Also, the results of one study showed that melatonin treatment inhibits polyphenol oxidase activity (Gao *et al.*, 2018). It has also been found that melatonin reduces the accumulation of phenolic compounds. This effect, combined with the activities of low polyphenol oxidase and peroxidase, is likely lead to the long-term quality maintenance by maintaining cellular redox homeostasis (E Silva *et al.*, 2024). Vermicompost treatments sometimes reduce the polyphenol oxidase activity in plants. This reduction can be attributed to variety of factors, including changes in nutrient availability such as nitrogen (Tikoria *et al.*, 2022). Vermicompost also changes the physical and chemical properties of the environment, which can impact the abundance and activity of various microbial communities. It can affect enzyme activity, potentially leading to a reduction in the polyphenol oxidase activity (Enebe & Erasmus, 2023; Leaseburg *et al.*, 2022). These results are consistent with the findings of this research.

The results of this study demonstrate that the integrated application of pre- and postharvest treatments significantly affects the biochemical properties and vase life of cut lisianthus flowers. The melatonin-vermicompost combination emerged as the most effective treatment, exhibiting the least increase in PPO activity (from 0.38 to 0.69 based on changes in absorbance/min/mg protein), an increase in CAT activity (from 0.273 to 0.339 mg/g fresh weight), and consequently, the best vase life performance. In contrast, the melatonin-humic acid treatment generally yielded the weakest results. Furthermore, the rosemary-vermicompost treatment induced the greatest increase in CAT (from 0.256 to 0.379 mg/g fresh weight) and APX activity (from 0.62 to 2.88 mmol per minute). While the main effects of pre-harvest treatments were sometimes overshadowed by the postharvest applications and their interactions, the synergy between vermicompost (as a pre-harvest treatment) and melatonin (as a postharvest treatment) was remarkable. Therefore, the application of these treatments, particularly the combined melatonin-vermicompost treatment, is recommended to enhance the vase life and reduce postharvest losses in cut lisianthus flowers. Further research is suggested to explore the sole effects of these pre-harvest treatments in more detail.

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