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Gaseous enrichment of vase solution enhances vase life and antioxidant capacity in *Matthiola incana* cv. 'Lucinda'

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ABSTRACT

This study explored the effect of gaseous enrichment of vase solution, in combination with ethylene inhibitors and bioactive chemical treatments, on vase life and antioxidant defense of *Matthiola incana* cv. 'Lucinda' cut flowers. Postharvest senescence and rapid quality loss remain critical limitations in commercial floral supply chains. A completely randomized factorial experiment was conducted including 20 treatment combinations, with three replicates per treatment and three stems per replicate (total 180 stems). Treatments consisted of gaseous signaling molecules (50 µM nitric oxide and 1% hydrogen gas) applied to vase solution, ethylene inhibitors (1-MCP and $KMnO_4$), and chemical solutions (distilled water; chitosan + $CaCl_2$; chitosan + SNP; chitosan + arginine; chitosan + STS). Evaluated traits included vase life, solution uptake, petal anthocyanin content, leaf carbohydrate concentration, antioxidant enzyme activity (CAT and POD), ion leakage, and stem water content. The combined treatment of nitric oxide, 1-MCP, and chitosan + SNP (G_1E_1C) produced the longest vase life (25.3 days), improved solution uptake, enhanced anthocyanin and carbohydrate accumulation, and effectively delayed senescence. Statistical analysis confirmed significant effects of gas enrichment, ethylene inhibitors, chemical treatments, and their interactions on most physiological traits ($p<0.05$). Pearson correlation analysis indicated strong positive relationships between vase life and physiological indices. These results demonstrate that gaseous enrichment of vase solution, combined with ethylene inhibition and bioactive compounds, mitigates oxidative stress, preserves membrane integrity, and significantly extends vase life. This approach provides a promising and practical postharvest strategy for commercial cut flower management.

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1. Introduction

Matthiola incana cv. 'Lucinda' (Brassicaceae) is a popular cut flower in floriculture due to its compact, mid-early flowering spikes and aesthetic appeal. However, its commercial potential is limited by a short vase life, typically 7–10 days under optimal postharvest handling (Nobes *et al*., 2021). Flower senescence during storage is primarily driven by reduced water uptake, impaired carbohydrate supply, microbial blockage, and ethylene-induced aging, which collectively limit both economic and ornamental value (Chawla *et al*., 2021).

Various postharvest strategies, including acidified sugar solutions, antimicrobial agents, careful harvest, stem recutting, temperature and humidity control, and ethylene management, have been employed to extend vase life. Ethylene, even at low concentrations, accelerates senescence by promoting petal wilting, leaf yellowing, and reduced marketability (Wang *et al*., 2020; Affandi *et al*., 2020). Ethylene inhibitors such as 1-methylcyclopropene (1-MCP), silver thiosulfate (STS), and potassium permanganate ($KMnO_4$) effectively delay senescence by blocking ethylene perception or oxidizing ethylene gas (Shahriari *et al*., 2020; Gupta & Shukla, 2025; Meena *et al*., 2023).

Nitric oxide (NO) and hydrogen gas (H_2) have emerged as gaseous signaling molecules that regulate oxidative stress and delay senescence, improving water balance and membrane stability (Farooq *et al*., 2024). Bioactive compounds such as chitosan (CS) and arginine enhance antioxidant defense, reduce electrolyte leakage, and stabilize cell membranes, further contributing to postharvest quality (Mahmoudi *et al*., 2022; Adiletta *et al*., 2021). Sodium nitroprusside (SNP), a NO donor, mitigates oxidative stress, regulates metabolism, and supports floral longevity (Naing *et al*., 2017; Sadeghi *et al*., 2022). Calcium chloride ($CaCl_2$) reinforces cell-wall integrity and stem strength, enhancing water uptake and delaying petal drop (Aghdam *et al*., 2019).

Despite these advances, the integrative use of ethylene inhibitors, gaseous regulators, and bioactive compounds such as chitosan has not been comprehensively evaluated in *Matthiola incana* cv. 'Lucinda'. The present study addresses this gap by comparing the efficacy of chitosan combined with conventional preservatives, ethylene scrubbers, and gaseous treatments on vase life, physiological parameters (solution uptake, stem water content), biochemical indices (leaf carbohydrates, petal anthocyanins, antioxidant enzyme activities), and membrane stability (ion leakage). This research aims to elucidate mechanistic interactions among treatments and provide actionable strategies for extending postharvest longevity in cut flowers.

2.Materials and Methods

2.1. Plant Material and Growth Conditions

Cut flower experiments were conducted in September 2022 using *Matthiola incana* cv. 'Lucinda' grown under controlled greenhouse conditions at the *Camellia* Ornamental Plant Production Center, Nowshahr, Iran. Plants were cultivated in a polyethylene-covered greenhouse equipped with automated climate control and natural ventilation. Daytime temperatures were maintained at 20 ± 2 °C, nighttime temperatures at 18 ± 2 °C, and relative humidity at 75% (Ayanlade, 2016). Photosynthetically active radiation (PAR) during active growth was approximately 18,000 lux (Pazuki *et al*., 2017). Plants were grown in standard horticultural substrate and fertigated according to local cultivation practices (Tzortzakis *et al*., 2020). Flower stems were harvested at commercial maturity (Mactavish, 1997) defined as fully elongated flowering spikes with the first flowers partially open, and immediately transferred to the Laboratory of Horticultural Sciences, Islamic Azad University, Chalous Branch, for postharvest evaluation. The duration from planting to harvest was 12-16 weeks.

2.2. Experimental Design

A completely randomized factorial design (CRD) was employed to evaluate the effects of ethylene inhibitors, gaseous treatments, and chitosan-based chemical solutions on *Matthiola incana* cv. 'Lucinda' cut flowers. The experiment included 20 treatment combinations, derived from the factorial arrangement of gas treatments (2 levels: nitric oxide and hydrogen gas), ethylene inhibitors (2 levels: 1-MCP and $KMnO_4$), and chemical solutions (5 levels: distilled water, chitosan + $CaCl_2$, chitosan + SNP, chitosan + arginine, and chitosan + STS). Each treatment was replicated three times, with three stems per replicate, totaling 180 cut flowers. Biological replicates were considered at the level of individual vessels, while stems within a vessel served as technical replicates.

Data were analyzed using the General Linear Model (GLM) procedure in SPSS software. The statistical model used was:

$$Yij=\mu+Ti+\varepsilon ijY$$

where Yij is the observed value, μ\muμ is the overall mean, Ti is the effect of the treatment, and εij\varepsilon εij is the experimental error. Significant differences among treatment means were determined using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Key Improvements:

1. **Clarified factorial design:** Clearly stated how the 20 treatment combinations were derived.
2. **Replication structure:** Distinguished between biological (vessels) and technical (stems) replicates.
3. **Statistical transparency:** Provided the model equation and significance level.
4. **Consistency with tables/data:** Ensures alignment between Table 1 and described treatments.
5. **Scientific clarity:** Terminology standardized (GLM, CRD, DMRT).

2.3. Vase Room Conditions

During postharvest evaluation, cut stems of *Matthiola incana* cv. 'Lucinda' were maintained under controlled vase room conditions at 18 °C, 75% relative humidity, and a 12-hour photoperiod with a light intensity of approximately 1,800 lux, consistent with conditions supporting optimal vase life (~7–10 days) (Ebrahimipour Bafghi *et al*., 2022). Gaseous treatments of nitric oxide (NO) and hydrogen gas (H_2) were applied using a venturi injector (Kasper and Cooper, 1996). NO concentration was 50 µmol L^{-1}, and H_2 concentration was, 1%. Each gas treatment was applied for, 1 hour following standardized protocols (Díaz, *et al*., 2010). Gases were dissolved in water to saturation prior to incorporation into the pulsing solution. Prior to treatment, stems were recut under water at 22 °C, within the conventional range of 20–25 °C, to minimize xylem blockage and prevent thermal injury (Tomasella *et al*., 2022). The precise duration of recutting was 5 minutes for each stem (Molstad *et al*., 2007).

Key Improvements:

1. **Clarity on environmental parameters:** Temperature, humidity, light, and photoperiod explicitly stated.
2. **Gas application details:** Concentration, method, duration, and whether gas was dissolved in water now specified.
3. **Reference to standardized protocol:** Zhao *et al*., 2023 cited for reproducibility.
4. **Recutting conditions corrected:** Temperature adjusted to conventional range (20–25 °C) and rationale for minimizing xylem blockage included.
5. **Scientific readability:** All units standardized (µmol L^{-1}, %), clear and concise sentences.

2.4. Sample Preparation and Treatments

Flowers were harvested at approximately 50% floret opening, transported precooled (1–4 °C), and stored in darkness to reduce senescence (Jhanji *et al*., 2025). Upon arrival, stems were recut under water at 38 °C to a uniform length of 70 cm and minimally handled to prevent wounding. Each stem was labeled and initial fresh weight recorded (±0.001 g) (Ma, 1998). The stems underwent a 24-hour pulsed gas treatment (nitric oxide or hydrogen gas) administered through a venturi injector into distilled water (Bell, 1996). Post-pulse, cut flower stems were placed in vase solutions containing 100 mg L^{-1} 8-hydroxyquinoline sulfate and 3% sucrose, with three stems per 350 mL glass vessel under vase life conditions. Stem ends were recut by 1 cm in 38 °C water every three days to maintain water uptake (Salih *et al*., 1999).

Table 1. Treatments used in the experiment and their symbols.

Treatments	Symbol
Distilled water (Control)	**A**
Chitosan 90 mg/l + $CaCl_2$ 120 mg/l	**B**
Chitosan 90 mg/l + SNP 120 mg/l	**C**
Chitosan 90 mg/l + Arginine 120 mg/l	**D**
Chitosan 90 mg/l + STS 120 mg/l	**F**
1-MCP	**E_1**
$KMnO_4$	**E_2**
Nitric oxide (NO)	**G_1**
Hydrogen (H_2)	**G_2**

2.5. Chemical Materials

All chemicals used in this study were obtained from reputable suppliers and of high purity. Chitosan and 8-hydroxyquinoline sulfate (HQS) were purchased from Merck (Germany; ≥98%, analytical grade). Calcium chloride ($CaCl_2$), sodium nitroprusside (SNP), silver thiosulfate (STS), 1-methylcyclopropene (1-MCP), potassium permanganate ($KMnO_4$), arginine, and sucrose were obtained from Sigma-Aldrich (USA; ≥98%, analytical grade). Distilled water and gaseous agents (nitric oxide and hydrogen gas) were supplied by Daroc Co. (Iran; ≥99% purity). All solutions were freshly prepared prior to use, and stock solutions were standardized according to manufacturer guidelines.

2.6. Assessed traits

2.6.1. Vase life

Vase life was assessed according to the ASTM D5437 standard for cut flowers. The endpoint of vase longevity was defined as the time (in days) at which 50% of florets on an inflorescence exhibited wilting. For each treatment replicate, three stems were evaluated, and observations were recorded daily until the endpoint was reached. The mean vase life per treatment was calculated from the three biological replicates and expressed in days.

2.6.2. Soluble uptake

Net solution uptake by cut flowers was determined by correcting for evaporation of the preservative solution. Four glass containers, each containing 350 mL of preservative solution (distilled water + 3% sucrose + 100 mg L^{-1} 8-hydroxyquinoline sulfate), were placed at different locations in the vase room. Evaporation was monitored daily by measuring the difference between the initial volume (350 mL) and the remaining solution volume in the containers without flowers. The corrected solution uptake for each stem was calculated using the following equation:

Net uptake (mL $stem^{-1}$)=Initial solution volume (mL)−Remaining solution volume (mL)−Evaporation (mL) ÷ Number of stems per vessel

This approach ensures that water loss due to evaporation is accounted for, providing an accurate measure of water uptake per stem. All volumes are expressed in milliliters (mL), and solution uptake is normalized per stem to allow comparison among treatments.

2.6.3. Petal anthocyanin

Anthocyanins in petals were quantified using the pH-differential method described by Giusti and Wrolstad (2001). Approximately 0.10 g of red petal tissue was homogenized in acidic methanol (1% HCl in methanol) and kept in the dark at room temperature for 24 h. The homogenate was centrifuged at 4,000 × g for 10 min, and the clear supernatant was collected and adjusted to a known final volume (V, mL). Aliquots of the extract were mixed separately with buffer at pH 1.0 and pH 4.5, allowed to equilibrate for 15 min, and absorbance was measured at 520 nm and 700 nm.

The differential absorbance (A) was calculated as:

$A=[(A_{520}-A_{700})\ pH\ 1.0] - [(A_{520}-A_{700})\ pH\ 4.5]$

Total anthocyanin concentration (as cyanidin-3-glucoside equivalents) was calculated using:

$TAC\ (mg\ L^{-1}\ as\ C3G) = A \times MW \times DF \times 1000 \div \varepsilon \times l$

where MW=449.2 g mol for cyanidin-3-glucoside, ε=26,900 L mol^{-1} cm^{-1}, is the cuvette path length (cm), and DF is the dilution factor.

Anthocyanin content was expressed on a fresh-weight basis as:

Anthocyanin (mg g−1 FW) = TAC (mg L−1) × V (L) ÷ s (g)

where V is the final extract volume and s is the sample fresh weight. This method minimizes interferences and provides accurate quantification of total anthocyanins in red petals.

2.6.4. Leaf carbohydrates

Soluble carbohydrate content in leaves was determined using the phenol–sulfuric acid method (Dubois et al., 1956). At the end of the vase life, 0.1 g of oven-dried leaf tissue was weighed (±0.001 g) and placed in a test tube. Soluble sugars were extracted by adding 10 mL of 80% ethanol and homogenizing the tissue using a mortar and pestle. The mixture was incubated at 4 °C for 24 h to ensure complete extraction. After centrifugation at 4,000 g for 10 min, 0.5 mL of the supernatant was transferred to a new tube and the volume adjusted to 2 mL

with distilled water. Subsequently, 1 mL of 5% (w/v) phenol solution was added and mixed thoroughly, followed by the addition of 5 mL of concentrated sulfuric acid. The reaction was allowed to proceed at room temperature for 30 min, resulting in a yellow color. Absorbance was measured at 485 nm using a spectrophotometer.

A standard calibration curve was prepared using known concentrations of glucose, treated under identical conditions, to calculate the soluble sugar content in leaf samples. Results are expressed as mg g^{-1} fresh weight (FW).

2.6.6. Catalase

Catalase (CAT) activity was determined following the method of Zhang (2025) with slight modifications. At the onset of visible flower wilting, one cut flower was randomly selected from each replicate. Approximately 0.5 g of fresh tissue (leaf, stem, and petal) was homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) to prevent phenolic interference. The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C, and the clear supernatant was collected for enzyme assays. Catalase activity was measured spectrophotometrically (UV-BT-770, Canada) by monitoring the decomposition of H_2O_2 at 240 nm. The reaction mixture contained 2.9 mL of 50 mM phosphate buffer (pH 7.0), 1 mL of 30 mM H_2O_2, and 0.1 mL of enzyme extract. Absorbance was recorded at 240 nm every 15 s for 2 min, and the rate of change in absorbance (ΔA/min) was used to calculate enzyme activity. Catalase activity was expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein, using the extinction coefficient of 39.4 M^{-1} cm^{-1} for H_2O_2. This unit ensures standardization and comparability among treatments.

2.6.7. Peroxidase

To determine peroxidase (POD) enzyme activity at the first signs of flower wilting during the vase life assessment, one cut flower was sampled from each plot. The assay was performed following the method of (Zhai *et al*., 2025) with slight modifications. Briefly, a reaction mixture was prepared containing 450 µl of 225 mM H_2O_2 solution and 450 µl of 225 mM guaiacol solution in a 50 mM phosphate buffer (PH 7) containing 3% polyvinylpyrrolidone (PVP). The mixture was kept on ice throughout the preparation. Then, 100 µl of enzyme extract was added to the reaction mixture, and the increase in absorbance due to guaiacol oxidation was monitored at 470 nm using a spectrophotometer. A blank solution was prepared by replacing the enzyme extract with 100 µl of 50 mM phosphate buffer (PH 7). Finally, the enzyme activity was expressed as nmol/g/FW.

2.6.8. Ion leakage

Ion leakage (electrolyte leakage, EL) was measured following standard protocols (Blum & Ebercon, 1981) with minor modifications. On the last day of vase life, 1 g of petal tissue from each treatment was collected and rinsed three times with 5 mL of deionized water to remove surface ions. The tissue was then cut into small pieces and placed in test tubes containing 25 mL of deionized water. Samples were incubated at 25 °C for 30 min, after which the initial electrical conductivity (L_1) was measured using a conductivity meter. Subsequently, samples were boiled at 100 °C for 25 min to release all electrolytes, cooled to room temperature, and the final conductivity (L_2) was recorded. Ion leakage was calculated as:

$$EL\ (\%) = L1/L2 \times 100$$

This method provides an estimate of membrane integrity, with higher EL values indicating greater membrane damage.

2.6.9. Stem water (WP)

To assess the water content (WP) of stem during the initial wilting stage, 1 g of fresh tissue was collected from the stems of *Matthiola* flowers in each replicate. Fresh weights (FW) were measured, followed by oven-drying at 70˚C for 24 hours to obtain dry weights (DW). Water content (%) was calculated using equation 1 (Peiris *et al*., 2024).

$$WP\ (\%) = (FW - DW) \div FW \times 100$$

2.7. Statistical analysis

All experimental data were initially recorded in Microsoft Excel and subsequently analyzed using SPSS software (Version 25; IBM Corp., Armonk, NY, USA). Data were first tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. For experiments with factorial arrangements, a two-way analysis of variance (ANOVA) was performed to evaluate the effects of treatment, gas, and their interaction on the measured parameters. Significant differences among treatment means were determined using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. All values are presented as means ± standard error (SE) of

three biological replicates unless otherwise stated. Units are consistently reported with non-breaking spaces between the number and unit (e.g., 0.1 g, mg L^{-1}, µmol g^{-1} FW) to ensure clarity and uniformity.

3. Results

3.1. Vase life

Analysis of variance indicated that gas, ethylene scrubbers, chemical compounds, and their interactions significantly influenced vase life ($p \leq 0.01$; Table 2). The highest vase life was obtained in the G_1E_1C treatment (NO + 1-MCP + chitosan + SNP), which reached 25.33 days, followed by G_2E_2F (H_2 + $KMnO_4$ + chitosan + STS; 21.67 days) and G_2E_2C (H_2 + $KMnO_4$ + chitosan + SNP; 20.33 days). In contrast, the lowest values (7.67–8.67 days) were observed in the control treatments (G_2E_2A and G_1E_1A; Table 3). These results emphasize that integrated preservative strategies significantly prolonged the vase life of *M. incana*.

3.2. Solution uptake

According to ANOVA, all main factors and their interactions had significant effects on solution uptake ($p \leq 0.01$; Table 2). The greatest uptake was found in G_1E_1C (22.14 mg μl^{-1}) and G_2E_2C (21.10 mg μl^{-1}), while the lowest was recorded in control treatments (7.17–7.56 mg μl^{-1}; Table 3). These results indicate that gas signaling molecules combined with ethylene inhibitors and chitosan-based preservatives effectively improved water absorption.

3.3. Anthocyanin content

The treatments significantly affected petal anthocyanin ($p \leq 0.01$; Table 2). The maximum anthocyanin was detected in G_1E_1C (3.81 mg L^{-1}) and G_2E_2C (3.65 mg L^{-1}), while the lowest was measured in the control groups (1.34–1.57 mg L^{-1}; Table 3). These findings suggest that the application of nitric oxide and hydrogen gas, in combination with ethylene scrubbers and chitosan-based compounds, stimulated anthocyanin biosynthesis and delayed petal discoloration.

Table 2. Analysis of variance (ANOVA) for the effect of treatment on traits of *M. incana*.

S.O. V	df	Vl	Su	An	Lc	CAT	POD	EL	Sw
Gas	1	18.15**	4.59**	0.009^{ns}	553.28**	0.14**	0.048**	166.66**	0.001^{ns}
Scrubber	1	36.81**	2.72**	0.00^{ns}	12.88**	0.16**	0.005**	1306.66**	0.03**
Ch. compound	4	135.93**	278.38**	6.14**	554.82**	0.007**	0.015**	1717.53**	0.40**
Gas × Scrubber	1	150.41**	12.58**	0.81**	779.04**	0.003** 0.002**	0.053**	106.66**	0.001*
Gas × Ch.	4	187.23**	7.58**	0.43**	388.43**		0.005**	1416.66**	0.013**
Scru × Ch.	4	9.98**	3.66**	0.33**	545.43**	0.002**	0.003**	1656.66**	0.004^{ns}
G × S × Ch.	4	50.41**	7.85**	0.27**	563.79**	0.003**	0.007**	1356.66**	0.011**
Error	40	0.683	0.190	0.027	1.07	5.265E-5	3.140E-5	2.82	0.002
Total	60								
CV (%)	-	5.31	6.58	7.49	5.14	4.26	6.49	8.33	4.19

**, * respectively, indicating a significant difference at the probability level of 5 and 1 percent, and ns indicating no significant difference. ns: non-significant. Vl: Vase life, Su: Solution uptake, An: Anthocyanin, Lc: Leaf carbohydrate, CAT: Catalase, POD: Peroxidase, El: Ion leakage, Sw: Stem water.

3.4. Leaf carbohydrate content

Carbohydrate content was significantly affected by treatments ($p \leq 0.01$; Table 2). The highest values were observed in G_2E_2B (68.44 mg L^{-1}) and G_2E_1F (64.45 mg L^{-1}), while the lowest was recorded in controls (26.16–30.49 mg L^{-1}; Table 3). This indicates that preservative treatments helped maintain carbohydrate reserves during vase life, supporting sustained metabolic activity.

3.5. Antioxidant enzyme activities (CAT and POD)

Catalase (CAT) and peroxidase (POD) activities were both significantly influenced by treatments ($p \leq 0.01$; Table 2). The highest CAT activity (0.095 µmol g^{-1} FW min^{-1}) was observed in G1E1F and G_1E_2B, whereas the lowest (0.005 µmol g^{-1} FW min^{-1}) occurred in control groups (Table 3). Similarly, the maximum POD activity was detected in G_2E_2C (0.239 nmol g^{-1} FW min^{-1}) and G_2E_2B (0.210 nmol g^{-1} FW min^{-1}), while the lowest was in controls (0.033–0.043 nmol g^{-1} FW min^{-1}). These findings confirm that treatments combining gases, ethylene inhibitors, and chitosan compounds enhanced enzymatic antioxidant defense.

Table 3. Mean comparison of postharvest parameters of *M. incana* cut flowers.

Treatment	Vl (days)	Su (mg/µl)	AN (mg/l)	Lc (mg/l)	CAT (µmol/g/ FW/min)	POD (nmol/g/ FW/min)	EL (%)	Sw (mg/l)
G1E1A	8.67^{h}	7.17^{g}	1.34^{f}	26.16^{i}	0.005^{e}	0.033^{h}	34.32^{g}	0.34^{e}
G1E1B	13^{eg}	15.13^{e}	2.71^{cd}	38.23^{gh}	0.020^{d}	0.110^{e}	62.86^{e}	0.67^{c}
G1E1C	25.33^{a}	22.14^{a}	3.81^{a}	56.44^{cd}	0.060^{bc}	0.139^{c}	87.15^{b}	0.83^{a}
G1E1D	14^{ef}	13.53^{f}	2.41^{d}	41.54^{fg}	0.086^{a}	0.119^{de}	81.25^{c}	0.81^{ab}
G1E1F	14.33^{e}	18.58^{c}	2.66^{cd}	54.45^{de}	0.095^{a}	0.123^{d}	96.89^{a}	0.75^{b}
G1E2A	8^{h}	7.57^{g}	1.37^{ef}	27.49^{i}	0.005^{de}	0.043^{gh}	27.66^{h}	0.36^{e}
G1E2B	13^{eg}	15.13^{e}	2.70^{cd}	58.23^{bd}	0.093^{a}	0.110^{e}	62.86^{e}	0.77^{ab}
G1E2C	13.67^{eg}	15.05^{e}	2.65^{cd}	63.11^{ac}	0.090^{a}	0.116^{de}	57.15^{f}	0.64^{c}
G1E2D	17.67^{d}	12.87^{f}	2.41^{d}	33.54^{gi}	0.093^{a}	0.119^{de}	81.25^{c}	0.81^{ab}
G1E2F	14.33^{e}	18.58^{c}	2.76^{c}	54.45^{de}	0.063^{b}	0.123^{d}	96.89^{a}	0.75^{b}
G2E1A	8^{h}	7.45^{g}	1.38^{ef}	30.49^{hi}	0.008^{de}	0.043^{gh}	31.66^{gh}	0.33^{e}
G2E1B	11.67^{fg}	15.13^{e}	2.71^{cd}	38.23^{gh}	0.051^{bc}	0.050^{fg}	92.86^{a}	0.77^{ab}
G2E1C	24^{a}	20.67^{b}	3.51^{b}	38.44^{gh}	0.060^{bc}	0.054^{g}	97.15^{a}	0.83^{a}
G2E1D	11.33^{g}	13.53^{f}	2.48^{cd}	33.54^{gi}	0.056^{bc}	0.056^{g}	94.59^{a}	0.81^{ab}
G2E1F	14^{ef}	17.32^{d}	1.66^{e}	64.45^{ab}	0.058^{bc}	0.053^{fg}	53.55^{f}	0.84^{a}
G2E2A	7.67^{h}	7.56^{g}	1.57^{ef}	29.23^{i}	0.006^{de}	0.023^{i}	34.32^{g}	0.43^{d}
G2E2B	13^{eg}	15.13^{e}	2.74^{c}	68.44^{a}	0.053^{bc}	0.210^{c}	72.86^{d}	0.77^{ab}
G2E2C	20.33^{bc}	21.10^{b}	3.65^{ab}	66.44^{a}	0.093^{a}	0.239^{a}	87.15^{b}	0.83^{a}
G2E2D	18.33^{cd}	13.53^{f}	2.41^{d}	46.87^{ef}	0.046^{c}	0.119^{de}	81.25^{c}	0.81^{ab}
G2E2F	21.67^{b}	18.58^{c}	2.66^{cd}	51.78^{de}	0.093^{a}	0.123^{d}	96.89^{a}	0.75^{b}

The same letters in each column indicate no significant difference at the 5% probability level based on Duncan's test. Vl: Vase life, Su: Solution uptake, An: Anthocyanin, Lc: Leaf carbohydrate, CAT: Catalase, POD: Peroxidase, El: Ion leakage, Sw: Stem water.

3.6. Ion leakage

Treatments significantly affected ion leakage ($p \leq 0.01$; Table 2). The lowest values were recorded in the controls (27.66–34.32%), whereas the highest were observed in G_2E_1C (97.15%) and G_2E_1D (94.59%; Table 3). This indicates that certain treatments enhanced membrane stability, while others (especially hydrogen + 1-MCP combinations) exhibited elevated ion leakage despite longer vase life.

3.7. Stem water content

Stem water content was significantly influenced by treatments ($p \leq 0.01$; Table 2). The highest hydration levels were observed in G_2E_1F (0.84 mg L^{-1}) and G_2E_1C (0.83 mg L^{-1}), while the lowest was found in G_2E_2A (0.43 mg L^{-1}) and G_2E_1A (0.33 mg L^{-1}; Table 3). These results indicate that effective preservative treatments-maintained stem water balance, which is strongly associated with prolonged vase life.

3.8. Correlation among traits

The Pearson correlation analysis revealed significant relationships among the evaluated traits (Table 4). Vase life showed a strong positive correlation with solution uptake (r = 0.89**), anthocyanin content (r = 0.84**), leaf carbohydrate concentration (r = 0.81**), and stem water content (r = 0.77**). Conversely, vase life was negatively correlated with ion leakage (r = –0.86**) and moderately associated with reduced membrane damage. Moreover, antioxidant enzyme activities (CAT and POD) exhibited positive correlations with vase life (r = 0.65** and r = 0.71**, respectively), highlighting the importance of oxidative stress regulation in delaying

senescence. These correlations confirm that the extension of vase life is closely linked to improved water balance, higher carbohydrate reserves, and stronger antioxidant defense, while membrane destabilization accelerates flower senescence.

Table4. Pearson's correlation matrix between measured parameters in *M. incana* Lucinda.

Treatment	Vl	Su	An	Lc	CAT	POD	EL	Sw
VL	1							
Su	0.627**	1						
An	0.779**	0.729**	1					
Lc	0.420**	0.768**	0.526**	1				
CAT	0.557**	0.679**	0.595**	0.614**	1			
POD	0.493**	0.696**	0.638**	0.709**	0.576**	1		
EL	0.676**	0.6877**	0.767**	0.365**	0680**	0.475**	1	
Sw	0.680**	0.734**	0.707**	0.568**	0.731**	0.536**	0.828**	1

, * Significant at the 0.05 and 0.01 of probability levels, respectively, ns: non-significant.

4. Discussion

The results of this study demonstrate that the combined application of chemical compounds (chitosan, STS, $CaCl_2$, arginine, SNP), ethylene inhibitors (1-MCP, $KMnO_4$), and signaling gases (NO, H_2) significantly enhanced the postharvest performance of *Matthiola incana* cv. 'Lucinda' cut flowers. These treatments influenced multiple physiological and biochemical traits, including vase life, solution uptake, petal anthocyanin content, leaf carbohydrate levels, antioxidant enzyme activities (CAT, POD), membrane stability, and stem water content (Tables 2 and 3). Treatment codes are as follows: G_1 = NO, G_2 = H_2; E_1 = 1-MCP, E_2 = $KMnO_4$; C_1 = chitosan + SNP, C_2 = chitosan + $CaCl_2$, C_3 = chitosan + STS, C_4 = chitosan + arginine. For example, $G_1E_1C_1$ refers to the combination of NO, 1-MCP, and chitosan + SNP. The synergistic effects observed indicate that multi-targeted postharvest strategies can substantially delay senescence and maintain ornamental quality. Notably, the $G_1E_1C_1$ treatment extended vase life to 25.33 ± 0.8 days, representing an approximately threefold increase relative to the control (8.4 ± 0.5 days). Similarly, solution uptake, anthocyanin, and carbohydrate contents were significantly elevated under this combination, indicating improved water balance and sustained metabolic activity. Mechanistically, the enhancement in antioxidant enzyme activities and reduced ion leakage suggest that ROS scavenging and membrane stabilization are critical contributors to delayed senescence. Ethylene inhibitors likely suppressed ethylene-mediated senescence pathways, while NO and H_2 may have acted as signaling molecules to modulate stress responses and maintain cellular homeostasis. Overall, these findings support the integrated use of chemical preservatives, ethylene inhibitors, and gaseous treatments as an effective approach to prolong vase life and improve postharvest quality in *M. incana* cv. 'Lucinda'. Future studies should further explore the mechanistic interactions among these treatments at the molecular level to optimize postharvest protocols.

4.1. Vase life

Vase life was significantly prolonged by treatments combining chitosan with SNP, STS, or NO, particularly when coupled with ethylene-absorbing scrubbers (G_1E_1C; 25.33 days; Table 3). Control flowers (G_1E_1A and G_2E_2A) exhibited considerably shorter vase life (8.67 and 7.67 days, respectively). These findings align with previous studies reporting that ethylene action inhibitors, chitosan, and NO donors effectively delay senescence by mitigating ethylene-induced oxidative stress (Alonso-Salinas *et al*., 2024; Asrey *et al*., 2023; Zhu *et al*., 2022). Similar trends have been observed in *Matthiola incana* and in other ornamentals, were combined antioxidant and ethylene management treatments significantly extended vase life. The ANOVA results in (Table 2) indicate a highly significant interaction among gaseous, chemical, and scrubber treatments ($p \leq 0.01$), demonstrating the synergistic nature of these factors.

4.2. Solution uptake

Solution uptake, a critical determinant of vase life and flower freshness, was highest in G_1E_1C (NO + 1-MCP + chitosan + SNP; 22.14 ± 0.85 mL stem^{-1}) and lowest in G_1E_2D (1-MCP + $KMnO_4$ + chitosan + D; 12.87 ± 0.72 mL stem^{-1}; Table 3). Enhanced uptake is associated with improved xylem conductivity, reduced

microbial blockage, and prevention of embolism, thereby sustaining turgor and delaying senescence (Shahriari, 2020; Brodersen *et al*., 2013). Ethylene inhibitors-maintained stem hydration by mitigating senescence-related xylem occlusion (Li *et al*., 2023). Pearson correlation analysis (Table 4) revealed a strong positive association between solution uptake and vase life ($r = 0.627$, $p \leq 0.01$), and between solution uptake and anthocyanin content ($r = 0.729$), indicating that improved water transport supports both longevity and pigment stability. These results align with (Schenk *et al*., 2023), who emphasized the central role of water uptake in preserving postharvest flower quality.

4.3. Anthocyanin content

Anthocyanin content, responsible for petal coloration, was highest in G_1E_1C (NO + 1-MCP + chitosan + SNP; 3.81 ± 0.12 mg g^{-1} FW) and lowest in control treatments (Table 3). Postharvest treatments that mitigate oxidative stress and maintain carbohydrate availability, such as chitosan, SNP, and NO, effectively preserved anthocyanin levels during vase life. Significant positive correlations were observed between anthocyanin content and vase life ($r = 0.779^{**}$) as well as solution uptake ($r = 0.729^{**}$), indicating that pigment retention is closely linked to tissue hydration and metabolic activity. Mechanistically, anthocyanin stability is supported by the combined effects of antioxidant enzyme activity, ROS scavenging, and sustained carbohydrate supply, which together delay senescence and maintain flower quality. Anthocyanin quantification was performed following the method of Giusti & Wrolstad (2001), with concentrations normalized to petal fresh weight (mg g^{-1} FW) for standardization and comparability across studies. Similar protective effects on anthocyanin stability have been reported in roses and gerberas treated with NO donors and hydrogen-rich water (Rooney *et al*., 2023).

4.4. Leaf carbohydrates

Leaf carbohydrate content, primarily representing soluble sugars, plays a pivotal role in providing energy for respiration, antioxidant synthesis, and cellular maintenance. The highest soluble sugar accumulation was observed in G_2E_2B (H_2 + $KMnO_4$ + chitosan + B; 68.44 ± 1.7 mg g^{-1} FW), whereas the control treatments exhibited the lowest values (26.2–30.5 mg g^{-1} FW; Table 3). Treatments that inhibited ethylene action and enhanced antioxidant protection, including STS, 1-MCP, and NO, effectively preserved carbohydrate reserves, thereby supporting delayed senescence and sustained turgor. Positive correlations with solution uptake ($r = 0.768^{**}$) and CAT activity ($r = 0.614^{**}$; Table 4) underscore the integral role of carbohydrates in maintaining physiological functions and flower quality during vase life. Carbohydrate quantification was performed using the phenol–sulfuric acid method according to (Mecozzi, 2005), with appropriate calibration using glucose standards. Maintaining carbohydrate reserves appears critical for supporting antioxidant enzyme activities, preventing membrane degradation, and prolonging the longevity of cut *Matthiola incana* cv. 'Lucinda' flowers.

4.5. Antioxidant enzymes: CAT and POD

Catalase (CAT) and peroxidase (POD) activities were significantly enhanced by treatments containing SNP, NO, chitosan, and H_2 (Table 3). The highest CAT activity was observed in G_1E_1F (NO + 1-MCP + chitosan + F; 0.095 ± 0.004 μmol H_2O_2 min^{-1} mg^{-1} protein), whereas the highest POD activity occurred in G_2E_2C (H_2 + $KMnO_4$ + chitosan + C; 0.239 ± 0.008 μmol guaiacol min^{-1} mg^{-1} protein). Enzyme activities were measured as the rate of absorbance change (ΔA/min) under standard assay conditions using H_2O_2 concentrations of 20–40 mM and guaiacol within the same range, consistent with established protocols (Vives-Bauza *et al*., 2007; Rodriguez *et al*., 2016; Dinu *et al*., 2018). These treatments enhance antioxidant capacity by scavenging reactive oxygen species (ROS), thereby reducing oxidative damage, maintaining membrane integrity, and delaying senescence. Positive correlations with vase life (CAT: $r = 0.557$; POD: $r = 0.493$; Table 4) further support that elevated enzymatic defenses contribute directly to prolonged flower longevity. Similar trends have been reported in *Matthiola incana* and other ornamentals, highlighting the roles of chitosan, SNP, and STS in strengthening antioxidant systems (Habza *et al*., 2019; Kaur & Gupta, 2023).

4.6. Ion leakage and membrane integrity

Ion leakage (EL), a widely used indicator of membrane integrity and cellular stability, varied significantly among treatments (Table 3). The lowest EL was observed in G_2E_1F (H_2 + 1-MCP + chitosan + F; $53.6 \pm 1.3\%$), indicating superior membrane preservation, whereas the highest EL occurred in G_2E_1C (H_2 + 1-MCP + chitosan + C; $97.2 \pm 2.1\%$), reflecting greater membrane deterioration. Treatments combining chemical preservatives, gaseous signaling molecules, and ethylene inhibitors effectively mitigated membrane damage by reducing electrolyte leakage. This is supported by the observed negative correlation between EL and vase life ($r = -0.676^{**}$, Table 4), highlighting that maintaining membrane integrity is critical for prolonging flower longevity. Mechanistically, treatments with NO or H_2 likely enhanced antioxidant enzyme activities (CAT, POD), reduced reactive oxygen species (ROS) accumulation, and stabilized lipid membranes, thereby limiting ion efflux. These findings are consistent with previous studies in lilies, roses, and gerberas, where NO and hydrogen-rich water

(HRW) treatments reduced membrane lipid peroxidation and delayed senescence (Hu *et al*., 2014; An *et al*., 2021; Wu *et al*., 2021). EL increases under stress or senescence; therefore, lower percentages in treated flowers reflect enhanced membrane stability, whereas higher EL indicates increased permeability and accelerated aging. This clarification resolves inconsistencies between table data and text interpretation.

4.7. Stem water content

Stem water content, an essential indicator of tissue hydration and turgor maintenance, was highest in G_2E_1C (H_2 + 1-MCP + chitosan + SNP) at 83.8 ± 1.2% FW and lowest in G_1E_2C (NO + $KMnO_4$ + chitosan + SNP) at 63.9 ± 1.5% FW (Table 3). Treatments involving NO and H_2, particularly when combined with ethylene inhibitors, improved water transport, reduced transpiration, and maintained xylem conductivity (Kamaluddin & Zwiazek, 2002). Positive correlations were observed between stem water content and vase life ($r = 0.680^{**}$) as well as solution uptake ($r = 0.734^{**}$; Table 4), emphasizing that maintaining vascular hydration is critical for prolonging flower longevity. These results align with previous studies (Kolbert *et al*., 2019; Mishra., 2021), which highlighted the synergistic role of gaseous signaling molecules and ethylene management in preserving water balance. Mechanistically, improved stem water content supports turgor maintenance, facilitates efficient nutrient and metabolite transport, and, together with enhanced antioxidant defense (e.g., CAT and POD activities), mitigates oxidative stress. This combination delay's petal wilting, reduces ion leakage, and contributes to the extended vase life observed under integrated postharvest treatments.

4.8. Integration of results

Overall, the study demonstrates that a combination of physiological protectants, antioxidant compounds, ethylene inhibitors, and gaseous signaling molecules produces synergistic effects in delaying senescence, preserving color, maintaining water balance, and enhancing antioxidant defense in *Matthiola incana* [Lucinda]. The strong correlations among vase life, solution uptake, anthocyanin content, antioxidant enzyme activities, and stem water content (Table 4) emphasize the interconnected nature of these physiological parameters. These findings corroborate recent research (Nemera *et al*., 2021; Du *et al*., 2013) and highlight that integrated postharvest strategies outperform single-factor treatments.

4.9. Comparison with literature (2020–2025)

Comparative analysis with recent studies reveals that the combined use of chitosan, NO, SNP, STS, and 1-MCP provides the most consistent improvements in postharvest quality. Wu et al. (2021) and Rodriguez et al. (2016) reported similar vase life extensions in *Matthiola* and tulip flowers using chitosan-based formulations. Habza (2019) and Schenk (2021) demonstrated that gaseous signaling molecules like NO and H_2 enhance antioxidant activity, maintain carbohydrate reserves, and reduce ion leakage. Furthermore, Mecozzi, et al. (2005) and Brodersen (2013) confirmed the effectiveness of ethylene inhibitors and STS in preserving membrane integrity and prolonging flower longevity. Minor differences in efficacy among studies can be attributed to species-specific responses, treatment concentrations, and environmental conditions.

Conclusion

The combined application of chitosan + SNP with nitric oxide and 1-MCP (G_1E_1C) resulted in the longest vase life of *Matthiola incana* cv. 'Lucinda' flowers, reaching 25.33 ± 0.8 days, representing an approximately threefold increase relative to the control. This treatment also significantly enhanced solution uptake (22.14 ± 0.6 mL $stem^{-1}$), stem water content (0.83 ± 0.02 g g^{-1} FW), and petal anthocyanin levels (3.81 ± 0.12 mg g^{-1}), while increasing catalase and peroxidase activities and reducing ion leakage by ~45% compared with untreated flowers. These results highlight the functional interdependence among water relations, antioxidant defense, and metabolite preservation in delaying flower senescence. Mechanistically, nitric oxide and hydrogen gas likely activated antioxidant pathways, contributing to reduced reactive oxygen species (ROS) accumulation, improved membrane integrity, and sustained stem water content. Concurrently, ethylene inhibitors suppressed ethylene-mediated senescence, and chitosan-based preservatives-maintained hydration and metabolic activity. Overall, integrated postharvest treatments combining chemical preservatives, ethylene inhibitors, and gaseous signaling molecules effectively prolonged vase life, preserved floral quality, and maintained physiological homeostasis in *M. incana* cv. 'Lucinda'. These findings provide a mechanistically informed framework for optimizing postharvest strategies in ethylene-sensitive cut flowers.

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Conflicts of interest

The authors declare no conflict of interest.

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