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Effect of 1-Methylcyclopropene and Methyl Jasmonate on Post-harvest Life of Alstroemeria cv. "Calgary" Cut Flowers

Atiqullah Sediqy[1], Ali Tehranifar[1*], Mahmoud Shoor[1], Hossein Nemati[1]

[1]Department of Horticultural Science and Landscape Architecture, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

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***Corresponding author's email:** tehranifar@um.ac.ir

Alstroemeria is one of the most important cut flower crops globally, known for its diversity in color, various cultivars, and overall beauty, contributing to its success in the global market. The use of different preservative solutions at various stages of cut flower supply to the market plays a critical role in preventing ethylene production and maintaining the water balance and energy supply required for the plant after being separated from the mother plant. Therefore, this investigation effect of 1-methylcyclopropene (1-MCP) and methyl jasmonate (MeJA) on the post-harvest life of alstroemeria (cv. Calgary) cut flowers was conducted in the Department of Horticultural Science and Landscape Architecture, Faculty of Agriculture, Ferdowsi University of Mashhad. The experiment was done in a factorial design (3 × 3) with five repetitions for each treatment. The results of the main effect of methyl jasmonate (MeJA) application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, catalase, peroxidase, and superoxide dismutase were observed at a concentration of 0.2 µL/L of MeJA. The main effect of 1-MCP application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, catalase, peroxidase, and superoxide dismutase were observed at a concentration of 1 µL/L of 1-MCP. The results of the interaction effect between MeJA and 1-MCP application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, and antioxidant enzymes were observed in the treatment with 4% sucrose + 300 mg l^{-1} 8-HQS + 1 µL/L 1-MCP + 0.2 µL/L MeJA.

Abstract

Keywords: Anthocyanin, Chlorophyll, Hydroxyquinoline, MeJA, 1-MCP.

INTRODUCTION

Alstroemeria, scientifically known as *Alstroemeria aurantica*, belongs to the Alestromeriaceae family and is native to South America. It is cultivated for the production of cut flowers and potted plants. Most species of alstroemeria have been identified in Chile and Brazil. Today, alstroemeria is primarily grown as a cut flower, with significant cultivation taking place in the Netherlands, Colombia, and the United States. In recent decades, alstroemeria has become one of the most important cut flowers globally due to its beautiful blooms, wide range of patterns and colors, and long-vase life quality. Over the past 20 years, various commercial hybrids of alstroemeria species, often referred to as Alstroemeriaceae or Peruvian lily, have become a significant part of the world cut flower trade (Nikbakht *et al*., 2020).

Alstroemeria flowers are highly sensitive to ethylene, and the ethylene produced in the late stages of their growth can lead to a reduction in their vase life, often causing petal drop (Wagsttaf *et al*., 2005; Chanasut *et al*., 2003). In flowers, which are ethylene-sensitive, ethylene has a major impact on petal senescence. It interacts with other phytohormones to start a signaling pathway and control different developmental stages (Dar *et al*., 2021). Many researchers have demonstrated that the appropriate chemistry of vase solutions can reduce respiration rate and senescence, thus increasing the longevity of cut flowers. These preservative solutions include carbohydrates, ethylene biosynthesis inhibitors such as silver thiosulfate (STS), aminoethoxy vinyl glycine, and antimicrobial agents (Vehniwal and Abbey, 2019).

Multiple stresses and their temporarily nature limit the vase life of cut flowers. The main symptoms of flower senescence are decreased water absorption, depletion of stored carbohydrates, and increases in ethylene production and respiratory activity. There are numerous methods for prolonging the preservation of cut flowers, such as controlling temperature and flower dehydration, using growth regulators, ethylene action inhibitors, and flower preservative treatments. Adding sugar to pulse solution or vase solution prolongs the postharvest life of cut flowers by enhancing water balance and energy or delaying senescence by lowering ethylene production (da Costa *et al*., 2021). To extend the vase life of cut flowers, using sucrose in the holding solution has been shown to increase the level of glucose and fructose in the petals. Sugars as a source of carbon and energy for cut flowers. Sugars also maintain the osmotic balance, promote water uptake, inhibit ethylene production and protein degradation, and ultimately postpone the senescence process (Alam *et al*., 2023).

One of the most crucial preservatives in the floral business is the germicide 8-hydroxyquinoline sulfate (8-HQS), which also serves as an antibacterial and enhances water absorption by decreasing "physiological" stem blockage in sterile tissues (Monya *et al*., 2021). However, adding sugar to 8-HQS improved the efficiency of this treatment. The application of 8-HQS + 3% sucrose at rates of 200 and 300 mg/l caused the longest vase life, lowest bacterial population in the vase solution, and least electrolyte leakages in cut *Alstroemeria* (Mohammadi Kabari and Jadid Soleimandarabi, 2019).

1-Methylcyclopropene (1-MCP) influences various aspects of aging and ripening in plants, including pigments, softening, cell wall metabolism, scent, and aroma. The aim of using 1-MCP for ornamental plants (cut flowers and potted plants) is to increase their vase life.1-MCP may increase JA content by increasing the BrWRKY12-BrLOX4 module-mediated JA biosynthesis in Chinese cabbage. Having potential uses for improving bioactive components, prolonging shelf life, and raising the market value of harvested horticulture products (Yue *et al*., 2023). The application of 1-MCP in lilies inhibits wilting and complexity response, extending the flower's life by half a day (Kim *et al*., 2010). However, the effects of 1-MCP treatment on potted plants like kalanchoe may vary depending on the cultivar or environmental factors.

Limiting effects on the post-harvest life of some , varieties have been observed (Park, 2012).

Jasmonates are derived from jasmonic acid and are a naturally occurring plant volatile compound. They act similarly to jasmonic acid in plants and are active in fruits such as strawberries, plums, lychees, apples, grapes, and peaches, increasing total phenolic content after harvest by activating the enzyme phenylalanine ammonia-lyase responsible for phenolic biosynthesis. Pre-harvest application of methyl jasmonate in grapes leads to the accumulation of anthocyanins in cells by activating proteinase inhibitors and chitinase gene expression (Ruiz - Garcia and Gomez-Plaza, 2013). Methyl jasmonate (MeJA) is favored as a vapor treatment to enhance the post-harvest lifespan of cut roses (Foukaraki *et al*., 2017).

Therefore, we design this research to evaluate effectiveness of 1-methylcyclopropane and methyl jasmonate on morphophysiological and biochemical characters to increase postharvest life of alstroemeria 'Calgary' cut flowers.

MATERIALS AND METHODS

In September 2022, alstroemeria 'Calgary' cut flowers were harvested in the commercial stage from the greenhouse located in Mashhad and transferred to the horticultural lab, Faculty of Agriculture, Ferdowsi University of Mashhad. The flowers were recut to 50 cm in height, and after weighing, they were placed in a 750 ml volume of vase solution containing 4% sucrose and 300 mg l^{-1} 8-hydroxyquinoline sulfate. Then cut flower stems were placed in 200-liter glass aquariums and treated with 1-MCP at concentrations of 0, 0.5, and 1 µL /L for 24 hours (Nergi and Ahmadi, 2014). Ethyl bloc powder prepared from US AgroFresh was used for applying the 1-MCP treatment. Considering the given concentrations, certain amounts of Ethyl Bloc were weighed and placed in Petri dishes, and then warm water (40–50 ˚C) was added to the Petri dishes inside glass aquariums. Immediately, the lids of glass aquariums were hermetically sealed with adhesive tape. For the steam treatment with methyl jasmonate at concentrations of 0, 0.1, and 0.2 µL L^{-1}, the flower stems were placed inside 200 L glass aquariums for 24 hours. Then, depending on the desired treatment concentration, the appropriate amount of liquid methyl jasmonate was mixed with 20 µL L^{-1} of ethanol. The mixture was poured onto filter paper inside the aquarium, and the lids of the aquariums were immediately sealed completely using special adhesive tapes (Darras *et al*., 2005).

During testing, the flowers were kept at 22 ± 2 ˚C with a relative humidity (RH) of 60–70%. At the end of the experiment, various traits such as water uptake, relative fresh weight, vase life, chlorophylls, anthocyanin, catalase, peroxidase, and superoxide dismutase enzyme activity were measured. The experiment was conducted in a factorial (3 × 3) design based on a completely randomized design with 5 replications for each treatment.

Vase life is defined as the leaves yellowing and petals wilting process and is expressed as days.

To measure the chlorophyll content in the leaves, follow the method of Lichtenthaler and Wellburn (1983). Anthocyanin content in petals was carried out according to the method of Liao *et al*. (2013). Catalase enzyme activity was followed by the Cakmak and Horst (1991), method. Measurement of peroxidase enzyme activity was conducted using the method described by Tabatabaei and Ehsanzadeh (2016).

The activity of the superoxide dismutase (SOD) enzyme was determined similarly to the method mentioned for the catalase enzyme (Chance and Maehly, 1995).

The data were analyzed by SPSS software, and the means were compared by the least significant difference (LSD) test ($P < 0.01$ and 0.05). The figures were drawn using Excel software.

RESULTS

The results of the analysis of variance indicate that the main effects of 1-MCP and MeJA, as well as the interaction effect between 1-MCP and MeJA, were significantly different at a 1% probability level for water absorption, relative fresh weight, chlorophyll a, b, and antioxidant enzymes (Table 1). The results of the analysis of variance also show that the main effects of 1-MCP and MeJA were significantly different at a 1% probability level for vase life (Table 1). The results of the analysis of variance also demonstrate that the main effect of 1-MCP was significant at a 1% probability level for anthocyanin (Table 1).

Table 1. Analysis of variance for the effects of 1-MCP and MeJA on measured traits.

S.o.V	df	MS								
		Water uptake	Relative fresh weight	Vase life	Chl. a	Chl. b	Anthocyanin	CAT	POX	SOD
1-MCP (A)	2	1351.57**	806.39**	19.09**	32.02**	30.25**	0.048**	2631.34**	4.01**	3280.19**
MeJA (B)	2	386.48**	485.05**	9.68**	13.89**	10.79**	0.007^{ns}	651.39**	1.83**	828.00**
A×B	4	33.62**	101.38**	0.25^{ns}	4.19**	2.86**	0.0024^{ns}	44.17**	0.61**	74.83**
Error	36	2.91	1.09	0.24	0.11	0.08	0.0016	3.86	0.024	3.73
CV (%)		1.51	1.07	4.69	5.62	5.78	2.81	3.37	8.83	2.96

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test, respectively.

The results of the main effect of MeJA on water uptake, and relative fresh weight, vase life, chlorophyll a, b, and anthocyanin, and antioxidant enzymes showed that with an increase in the concentration of MeJA, there was an increasing trend in the levels of water uptake, relative fresh weight, vase life, pigments, and antioxidant enzymes. The highest and lowest levels of these factors were obtained in treatments with 0.2 and 0 µl/L MeJA, respectively (Table 2).

Table 2. Comparison of the mean main effects of MeJA on measured traits.

MeJA	Water uptake	Relative fresh weight	Vase life	Chl. a	Chl. b	Anthocyanin	CAT	POX	SOD
(µl/L)	(g/cut flower)	(%)	(days)	(µg/mg FW)		(µg/mg FW)	(unit mg^{-1} protein)		
0	99.18c	89.68c	9.533c	4.5991c	3.6339c	0.2402a	43.840c	1.2360c	49.315c
0.1	111.83b	100.18b	11.067b	6.0981b	5.1897b	0.1269c	61.475b	1.7313b	67.895b
0.2	117.76a	103.80a	11.733a	7.5664a	6.4697a	0.1929b	69.775a	2.2693a	78.533a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

The results of the mean main effect of 1-MCP on water uptake and relative fresh weight showed that with an increase in the concentration of 1-MCP, there was an upward trend in water absorption and relative fresh weight. The highest levels of these factors were observed at a concentration of 1 µl/L 1-MCP (Table 3). There were no significant differences between the treatments with 1 and 0.5 µl/L 1-MCP in terms of water absorption and relative fresh weight (Table 3). The shortest vase life was observed in the control treatment, while the treatment with 1 µl/L 1-MCP had the longest vase life. The results of the mean main effect of 1-MCP on

pigments and antioxidant enzyme levels showed that with an increase in the concentration of 1-MCP, the pigments and antioxidant enzyme levels increased. The highest levels of chlorophyll a, b, and antioxidant enzymes were observed at a concentration of 1 µl/L 1-MCP (Table 3).

Table 3. Comparison of the mean main effects of 1-MCP on measured traits.

1-MCP (µl/L)	Water uptake (g/cut flower)	Relative fresh weight (%)	Vase life (days)	Chl. a (µg/mg FW)	Chl. b (µg/mg FW)	Anthocyanin (µg/mg FW)	CAT (unit mg^{-1}protein)	POX (unit mg^{-1}protein)	SOD (unit mg^{-1}protein)
0	103.77b	91.32b	9.933c	4.9878c	4.1532c	0.1747b	50.889c	1.4027c	56.858c
0.5	111.88a	101.18a	10.867b	6.5009b	5.3455b	0.2129a	60.865b	1.7320b	67.892b
1	113.11a	101.16a	11.533a	6.7749a	5.7946a	0.1725b	63.337a	2.1020a	70.994a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

The results of the interactive effect of 1-MCP and MeJA on water absorption, relative fresh weight, and vase life showed that with an increase in the concentration of 1-MCP in all concentrations of MeJA, water absorption, relative fresh weight, and vase life of the cut flower of alstroemeria increased. The highest level of water absorption, relative fresh weight, and vase life were observed in the treatment with a concentration of 1 µL/L 1-MCP+0.2 µL/L MeJA. While the lowest level of measured traits was observed in the control treatment, there were no significant differences between the treatments (0.1 µL/L MeJA & control) and (1 µL/L 1-MCP+0.1 µL/L MeJA & 0.5 µL/L 1-MCP+0.2 µL/L MeJA) in terms of vase life (Fig. 1).

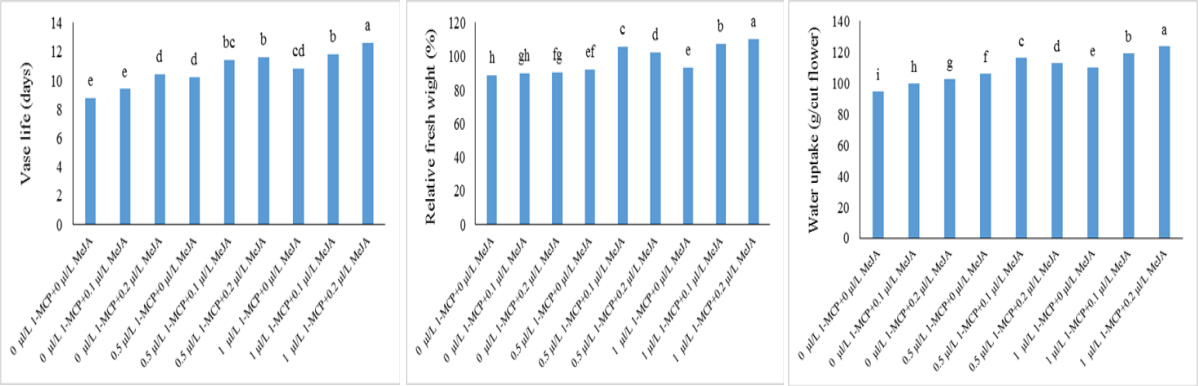

Fig. 1. Interactive effect of 1-MCP and MeJA on uptake of water, relative fresh weight and vase life.

The results of the interactive effect of 1-MCP and MeJA showed that chlorophyll a and b and anthocyanin increased with the increase in the concentration of 1-MCP and MeJA. The highest chlorophyll a and b and anthocyanin content was observed in the treatment with 1 µL/L 1-MCP+ 0.2 µL/L MeJA. The lowest pigment content was observed in the control (Fig. 2).

The results of the interactive effect of 1-MCP and MeJA showed that catalase, peroxidase, and superoxide dismutase increased with the increasing concentration of 1-MCP and at concentrations of 0 µL/L and 0.2 µL/L MeJA. In the presence of 0.1 µL/L of MeJA with concentrations of 0.5 and 1.0 µL/L of 1-MCP, catalase, peroxidase, and superoxide dismutase were higher than at the zero concentration of 1-MCP. However, there were no significant differences between the concentrations of 0.5 and 1.0 µL/L of 1-MCP. The highest levels of catalase, peroxidase, and superoxide dismutase were observed in the treatment with 1.0 µL/L 1-MCP+0.2 µL/L MeJA, while the lowest levels were observed in the control (Fig. 3).

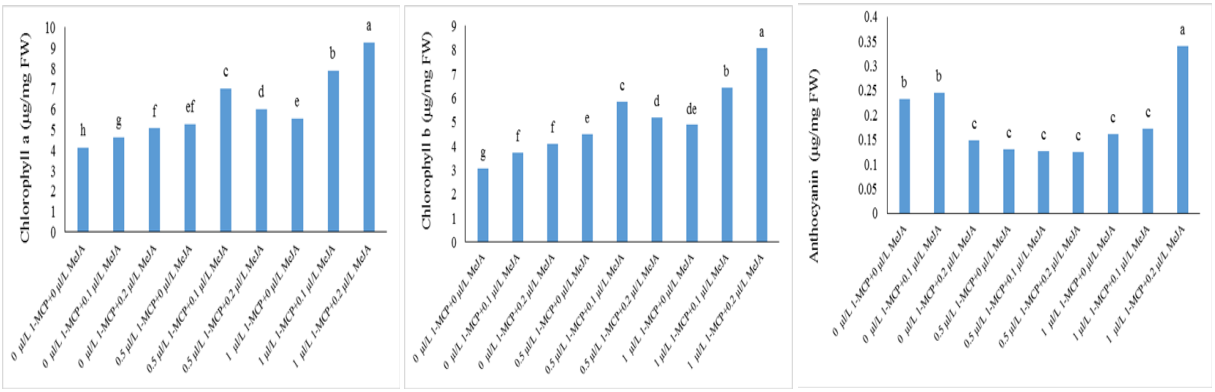

Fig. 2. Interactive effect of 1-MCP and MeJA on chlorophyll a, b and anthocyanin.

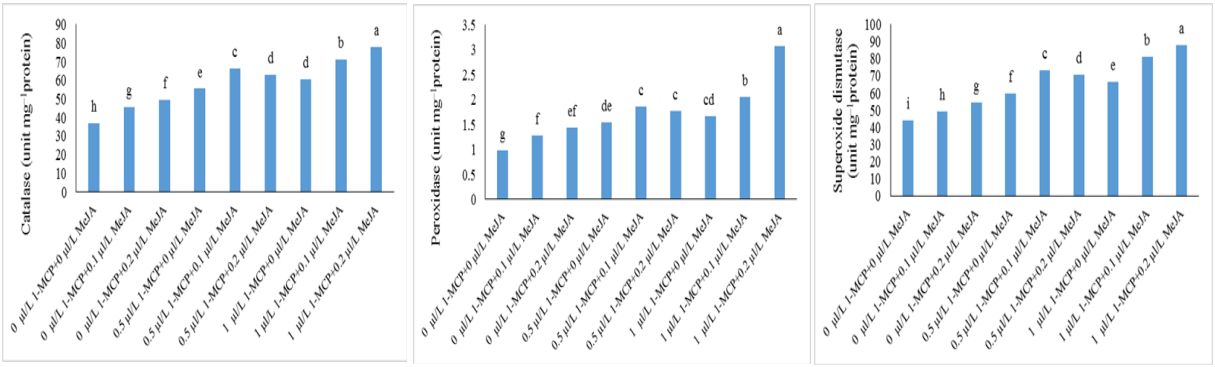

Fig. 3. The interactive effect of 1-MCP and MeJA on antioxidant enzymes.

DISCUSSION

The wilting of cut flowers is related to a hormonal mechanism that involves changes in the physical and biochemical properties of cell membranes. This process is characterized by a rapid decrease in the levels of phospholipids and proteins, an increase in the activity of degrading enzymes, the dispersion of macromolecules, increased respiration activity, and decreased membrane stability. Ultimately, this leads to the uncontrolled loss of soluble substances and water, resulting the wilting and death of the cut flowers (Dhindsa *et al*., 1981). Blockage of the stem by bacteria reduces water uptake, leading to bending and wilting of the stem as well as withering of the petals (Solgi *et al*., 2009). Gray mold fungus is a common pathogen in cut flowers, and it plays a significant role in the onset of aging and determining the vase life of cut flowers. Gray mold fungus can block the cut stems, reduce water uptake, and contribute to wilting (Capdeville *et al*., 2003). The cut flower continues to photosynthesize even after it has been removed from the mother plant; therefore, the cut flower requires a food source to maintain its health and stay fresh for a longer period of time. Treating a cut flower with floral preservation extends its vase's life. These floral preservatives can help keep cut flowers fresher for longer. A flower preservative is a complicated mixture containing sucrose (the primary food material), an acidifier (to lower the pH of the solution), a microorganism inhibitor (to act as a germicide), and a respiratory inhibitor (to decrease the impact of ethylene). A clean vase should be utilized (Monya *et al*., 2021).

Methyl jasmonate application induces the production of secondary metabolites, the expression of defense genes, and the induction of systemic resistance in plants against pathogens (Ahmad *et al*., 2016). The concentration of 0.1 µl MeJA vapor treatment after harvest also had similar results, increasing vase life and quality by reducing the growth of microorganisms in

the holding solution (Son *et al*., 2003). Methyl jasmonate had a positive effect on reducing the activity of gray mold and significantly reducing the growth and activity of microorganisms in the holding solution, as well as reducing petal spotting in cut *Freesia* flowers. It induced the expression of defense genes and the synthesis of some defensive compounds, resulting in decreased growth and activity of microorganisms (Darras *et al*., 2005).

Regarding the increase in chlorophyll content as a result of treatment with methyl jasmonate, it can be explained that MeJA leads to an increase in the level of beta-carotene in cells. The primary protective role of beta-carotene and xanthophylls in photosynthetic tissue may involve preventing the production of singlet oxygen, thus protecting chlorophyll from oxidative damage (Farooq *et al*., 2009).

According to research, external application of MeJA can cause an increase in anthocyanin accumulation in plants. Anthocyanins and phenols are plant secondary metabolites that inhibit reactive oxygen species and protect against photodynamic damage. Research has demonstrated that using naturally occurring chemicals, such as methyl jasmonate, might enhance secondary metabolites. For example, preharvest application of 200 µM MeJA on strawberry fruits improved ascorbic acid, total anthocyanin contents, and total antioxidant activity (Asgari *et al*., 2023). Treating blackberry plants with methyl jasmonate significantly increased the flavonoid content in these plants (Wang *et al*., 2008). Gun *et al*. (2023) discovered that nanofiber mats created by utilizing methyl jasmonate and nanosilver active compounds were effective in suppressing microbial growth and prolonging the vase life of cut rose flowers. This study's findings are comparable to those of Hasanzadeh-Naemi *et al*. (2021). They found that MeJA treatments improve post-harvest quality and cut flower vase life. 0.2 mM MeJA enhanced the level of CAT and SOD activity, membrane stability index, anthocyanin, total soluble sugar, and relative water content.

Since post-harvest senescence plays a limiting role in product supply and the marketability of many cut flowers, the application of methods with high reliability has become crucial. Regarding the use of 1-MCP as an ethylene antagonist, it has been established that this compound can effectively compete with ethylene for binding to ethylene receptors, thereby preventing an ethylene response (Seglie *et al*., 2011). 1-MCP has prevented petal abscission in chrysanthemums, although its efficacy depends on transportation conditions, storage temperature, and the number of applications (Djanaguiraman *et al*., 2011). In a study on cut cluster amaryllis flowers, treatment with 1-MCP reduced ethylene production at all concentrations, delaying chlorophyll degradation compared to control plants (Seglie *et al*., 2011). Meng *et al*. (2023) found that fruits treated with 1-MCP or SA alone had increased resistance to pathogen infection, while 1-MCP combined with SA therapy improved self-resistance. Using the preservation approach in combination with 1-MCP and SA could be a viable strategy to extend shelf life. Lilies treated with 1-MCP for 8 hours had maximum anthocyanin content until the twelfth day of storage (Chutichudet *et al*., 2010). The application of 1-MCP to soybean plants reduced hydrogen peroxide levels compared to untreated plants. It also reduced ethylene production and free radicals and increased antioxidant enzyme activity. This treatment extended the post-harvest life of the flowers by inhibiting external ethylene action. Research on cut lilies also suggests that 1-MCP preserves the quality by inhibiting internal ethylene production (Djanaguiraman *et al*., 2011). Therefore, increasing the post-harvest life of cut flowers using 1-MCP is attributed to the inhibition of ethylene action and consequently the suppression of ethylene biosynthesis (Mojdeh, 2020). Application of 0.5 and 1 µL L^{-1} 1-MCP significantly extended vase life, water uptake, and relative fresh weight of *N. tazetta* cut flowers compared to the control. The effects of 1-MCP in preserving chlorophyll content result from inhibiting

ethylene action and, consequently, ethylene biosynthesis, which is the most crucial factor in leaf yellowing in ornamental plants. In a study on cut cluster amaryllis flowers, treatment with 1-MCP reduced ethylene production and delayed chlorophyll degradation compared to control plants (Asil *et al*., 2013). Bayat and Moradinezhad (2020) found that the application of 0.5 and 1 µL L^{-1} 1-MCP significantly extended vase life, water uptake, and relative fresh weight of *N. tazetta*-cut flowers compared to the control. This result is similar to the current study. Additionally, 1-MCP prevented yellowing in *Dendrobium* and *Amaryllis* varieties (Serek *et al*., 1998), with its effects attributed to blocking ethylene receptors.

Sucrose has been demonstrated to enhance glucose and fructose levels in petals, lending support to the use of sugar-based external holding solutions to extend the vase life of cut flowers. Sugars are a source of energy and carbon for cut flowers and serve a vital role in reducing protein breakdown and ethylene generation, maintaining osmotic equilibrium, enhancing water intake, and finally delaying the senescence process (Sharafshah Rostami and Kaviani, 2023). The addition of sucrose in pulse solution or as a component of vase solution improves flower vase life by enhancing water balance and energy or delaying senescence through reductions in ethylene production (da Costa *et al*., 2021). According to Sun *et al*.'s (2022) results, sugar can improve the quality of cut flowers by affecting the hormone balance of flower tissues and delaying the breakdown of mitochondria in senescent petals. Studies have shown that providing sucrose in the vase solution for cut flowers leads to improved quality and better post-harvest longevity (Gebremedhin, 2020). The use of sugar solutions in the vase water leads to a reduction in ethylene production and enhances the quality and post-harvest longevity of cut flowers, including lilies (Verlinden and Garcia, 2004). The positive effect of sucrose in extending the vase life of other cut flowers such as roses, marigolds, chrysanthemums, waxflowers, snapdragons, lisianthus, statice, alstroemeria, and gladiolus has also been demonstrated (Manzoor *et al*., 2018).

CONCLUSIONS

Finally, it can be concluded that increasing the activity of antioxidant enzymes reduces the aging of flowers. In general, 1-MCP and MeJA vapor treatments had a positive effect on improving physiological and biochemical characteristics, resulting in an extended postharvest longevity of alstroemeria cut flowers. The higher concentrations of 1-MCP and MeJA revealed a better effect in comparison to low concentrations. According to the results, 1-MCP and MeJA vapor treatments, by increasing the protein content and the activities of catalase, peroxidase, and superoxide dismutase, improved the quality and increased the vase life of alstroemeria cut flowers. Based on this study, the postharvest application of 1-MCP acting as an ethylene action inhibitor and MeJA induces the production of secondary metabolites, the expression of defense genes, and the induction of systemic resistance in plants against pathogens, which could be recommended to increase postharvest life and extend the longevity of alstroemeria cut flowers.

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