

Effects of 1-MCP and ethylene on preservation of quality and vase life of *Alstroemeria* (cvs. Hercules and Mayfair) cut flowers

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: In order to improve the vase life characteristics and postharvest quality of *Alstroemeria* cut flowers cv. Hercules and Mayfair, the effects of 1-methylcyclopropene (1-MCP) and ethylene have been investigated in a completely randomized design with three replications. First, cut flowers were fumigated at 0, 0.5, 1 and 1.5 $\mu\text{L L}^{-1}$ of 1-MCP concentrations for 24 h and then exposed to 1 $\mu\text{L L}^{-1}$ of ethylene for 18 h. This experiment was conducted at $20 \pm 2^\circ\text{C}$, 60-65% RH, and a 12/12 h light/dark photoperiod. The results showed that 1-MCP treatment significantly increased postharvest durability in both cultivars, compared to the control. Also, results of 1-MCP treatment on leaf chlorophyll index in both cultivars, confirmed the role of 1-MCP in preventing external ethylene action as the main factor of *Alstroemeria* leaf yellowness. Based on data, Mayfair cultivar was more sensitive to ethylene in comparing to Hercules cultivar, although 1-MCP treatment reduced the active oxygen species in both cultivars by reducing biosynthesis and ethylene action or by direct increase in antioxidant enzyme activity.

1. Introduction

Alstroemeria cultivars are consumed as cut flower due to their variation in the flower pattern and color which spotted with dark colors, and this caused an increase in their global trade during last decades (Ferrante *et al.*, 2002). *Alstroemeria* cut flower quality is often decreased by prematurity yellowing of leaves which reduce viability of leaves before appearing the secondary florets (Ferrante *et al.*, 2002).

From commercially point of view, the leaf green color preservation is one of the most important qualitative characteristics playing a role in aesthetic valuation of this ornamental plant (Mutui *et al.*, 2006). The leaf yellowing is affected by various factors such as poor storage conditions, deficiency of internal cytokinin, exposure to internal and/or external ethylene, darkness, accumulation of abscisic acid, leaf aging and damage (Ferrante *et al.*, 2009).

Ethylene is one of the most important limiting factors in maintaining

the quality and life of many cut flowers due to accelerating aging in the post-harvest stage. *Alstroemeria* cultivars showed varied range of sensitivity to ethylene. Although they produce very little ethylene, they are sensitive to external ethylene (Chanasut *et al.*, 2003) so that low concentration of this gaseous hormone causes leaf yellowing, abscission of petals and accelerating flower aging (Reid, 1989). For these reasons, many efforts are being made to gain more information about compounds inhibiting and blocking ethylene biosynthesis and action. 1-MCP patented by Edward Sisler, acts as a blocking agent of ethylene action by binding to ethylene receptors. In other words, 1-MCP binds competitively to ethylene binding site with affinity higher than that of ethylene and blocks downstream signal transduction, thereby prevents the expression of genes induced by ethylene, determining an extension of the shelf life of many climacteric cut flowers (Serek *et al.*, 1994; Ahmadi *et al.*, 2008, 2009; Daneshi Nergi and Ahmadi, 2014). Although some silver-containing compounds could inhibit ethylene action, they have toxicity effects on human health and the environment, limiting their application on crops. Moreover, to reduce the people concerns on these harmful and hazardous compounds, the demand of using safe compound to extend post-harvest life in crops especially edible products is also increasing.

The purpose of this study was to evaluate the efficacy of 1-MCP on extending the longevity of cut flowers and on reducing leaf yellowing in *Alstroemeria* cvs. Mayfair and Hercules. In most *Alstroemeria* cultivars the first aging symptom of cut flowers is the onset of yellowness in the leaves which occurs earlier than petals aging or abscission. The activity of antioxidant enzymes in petals and ethylene production of both cultivars were also evaluated.

2. Materials and Methods

In this experiment, cut flowers of *Alstroemeria* cvs. Mayfair and Hercules were harvested based on commercial indices from a greenhouse located in Pakdasht, Tehran, Iran. After harvesting, cut flowers were immediately transported dry to the Postharvest Laboratory at Tarbiat Modarres University. Stems were re-cut under water to a length of 45 cm and held in 500 ml vase solution containing 200 mg/L 8-hydroxyquinoline sulfate (8-HQS), and 3% sucrose. (Rasouli *et al.*, 2015). Then cut flower stems were placed in 200 L glass chambers and treated with 1-MCP at concentrations of 0, 0.5, 1, and 1.5 $\mu\text{L L}^{-1}$ for

24 hours (Daneshi Nergi and Ahmadi, 2014).

Ethyl Bloc powder prepared from US AgroFresh was used for applying 1-MCP treatment. Considering the given concentrations, certain amounts of Ethyl Bloc were weighed and placed in Petri dishes, then warm water was added to the Petri dishes inside glass chambers. Immediately, the lids of glass chambers were hermetically sealed by adhesive tape (Daneshi Nergi and Ahmadi, 2014). After 24 hours of treatment with 1-MCP, the lids of the chambers were removed and cut flowers were ventilated with fresh air for one hour. Then, by closing and sealing the lids again, ethylene was injected into each chamber, to expose all flowers to 1 μLL^{-1} exogenous ethylene for 18 h. When the ethylene treatment was completed, the lid of glass containers was opened and the vases containing flowers were placed on the laboratory table. The experiment was conducted at $20 \pm 2^\circ\text{C}$, 60-65% RH and a 12/12 h light/dark photoperiod at an illumination of 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Vase life

One of the most important criteria for evaluation of postharvest quality is the viability of cut flowers. In this study, the flower life is the time interval between the beginning of treatment until the cut flowers lose their ornamental value. When 50% of petals falling down in cut *Alstroemeria*, it was considered as the end of vase life quality (Mutui *et al.*, 2006).

Relative fresh weight and chlorophyll content

In order to measure this trait, the weight of flower was measured using digital scale (KERN model) with an accuracy of ± 0.001 g. This trait was calculated during the evaluation period using equation: relative fresh weight (percent) equal to $(\text{wt}/\text{wt} = 0)$ in this regard wt: flower weight (g) on day 0, 3, 6, 9 and 12, $\text{wt} = 0$: flower weight g per day (He *et al.*, 2006). To measure the chlorophyll content, 0.2 g of leaf sample was used to extract its chlorophyll in 80% acetone. Then, in a volumetric flask, 25 ml of the extract was filtered, acetone reached 25 ml volume and chlorophyll was completely extracted (Arnon, 1949).

Evaluation of catalase activity

About 200 mg of frozen petal cell mass, sampled on day 6 of the experiment, was extracted in 3 ml of 25 mM sodium phosphate buffer (pH = 6.8) and centrifuged for 4 min at 15°C . The enzyme solution was used to measure enzyme activity. Then, the reaction mixture was prepared including 25 mM of sodium phosphate buffer (pH = 6.1), 10 mM of hydrogen peroxide and enzymatic extract. Catalase activity was

measured at the wavelength of 240 nm and calculated as a delta of absorbance at 240 nm per mg protein. All enzymatic extraction stages were performed on ice (Cakmak and Horst, 1991).

$$\text{CAT activity} = (\text{final Abs} - \text{Initial Abs}) / \text{protein}$$

Evaluation of superoxide dismutase activity

About 200 mg of petal tissue, sampled on day 6 of experiment, was extracted in 3 ml of HEPES-KOH buffer containing 0.1 mM of sodium EDTA (pH = 7.8). The resulting homogenate was centrifuged for 30 minutes at 12000 rpm at 4°C. The resulting supernatant was used to measure superoxide dismutase activity. The reaction composition in the final volume of three ml is as follows:

HEPES-KOH buffer (50 mM) containing 0.1 mM of sodium EDTA at pH = 7.8, sodium carbonate (50 mM) at pH = 10.2, L-methionine (12 mM), Nitro Blue Tetrazolium (NBT) (75 mM), Riboflavin (1 mM), and the enzyme extract were appropriately considered as one unit of superoxide dismutase activity as an enzyme that results in 50% inhibition of nitrobutetrazolium at 560 nm (Swanson, 1955). Adsorption of the reaction mixture was measured using BIO-RAD spectrophotometer.

Measurement of ethylene

After finishing ethylene treatment three flowers from each treatment were placed in a 1.8 L sealed glass bottle and kept at $20 \pm 2^\circ\text{C}$ for 48 h, same as experimental condition. By a gas-tight syringe, gas samples were taken through head spaces and were injected into a GC manufactured by Agilent America Co. Model GC-6890N fitted with a capillary column and a flame ionization (FID) detector. The carrier gas was helium at 6.5 mL min^{-1} , injection temperature was 180°C and column temperature was 60°C (Daneshi Nergi and Ahmadi, 2014).

Statistical analysis of the data

The experiment was conducted in a completely randomized design (CRD) with four treatments and three replications. The data were analyzed using statistical software SAS version 9 and the means were compared by the least significant difference (LSD) test ($P=0.05$). The figures were drawn using Excel software.

3. Results

Vase life

This experiment showed that the vase life of cut

flowers cv. Hercules was longer than cv. Mayfair, although vase life of both cultivars was affected by 1-MCP application. Maximum plant longevity was 12 days for cv. Hercules under $1.5 \mu\text{L L}^{-1}$ 1-MCP treatment and the longevity of cv. Mayfair was extended to 10.8 days under $1 \mu\text{L L}^{-1}$ of 1-MCP, which were significantly different at level as indicated by Least significant difference (LSD), comparing to the control. No significant differences were observed between 1-MCP treatment levels in any of cultivars at 1% level by an LSD (Fig. 1).

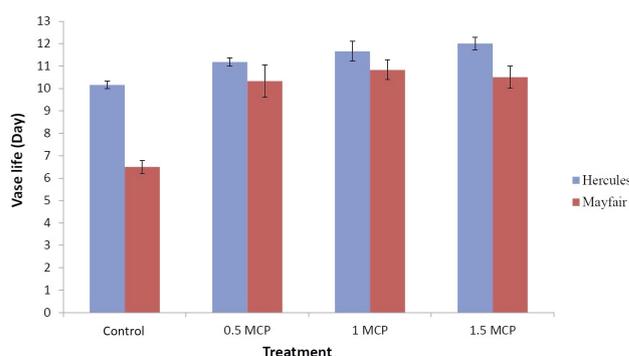


Fig. 1 - Vase life of *Alstroemeria* treated with concentrations of 1-MCP at 0, 0.5, 1 and $1.5 \mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

Relative fresh weight

The results of analysis of variance and mean comparison of relative fresh weight of cut flower of *Alstroemeria* cv. Hercules showed that the highest relative fresh weight (119.5%) was obtained on the sixth day after applying treatment, which was statistically significant at 1% probability level. The lowest relative fresh weight (90.2%) was obtained on the twelfth day after applying treatment, which was not significantly different from the relative fresh weight on day 0, but it was significantly different with relative fresh weight at other times, at probability level 1% (Fig. 2). In cv. Mayfair, the highest relative fresh weight (106.32%) was showed at $1 \mu\text{L L}^{-1}$ of 1-MCP treatment, which was statistically significant at 1% probability level with 0.5 and $1.5 \mu\text{L L}^{-1}$ of 1-MCP treatments. Control samples revealed the lowest relative fresh weight (62.9%) (Fig. 3).

Chlorophyll content

Data showed that 1-MCP treatments affected significantly the chlorophyll content in cv. Hercules. The highest content of chlorophyll (1.13 mg) was revealed at $1 \mu\text{L L}^{-1}$ of 1-MCP concentration which

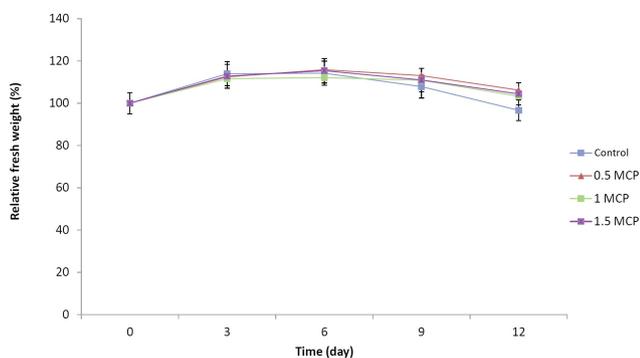


Fig. 2 - Relative fresh weight of cut flower Alstroemeria cv. Hercules treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 $\mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

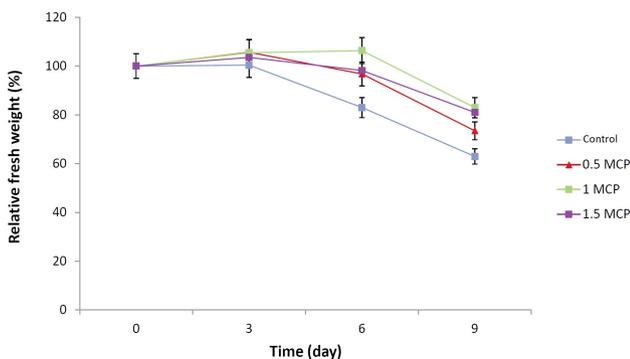


Fig. 3 - Relative fresh weight of cut flower Alstroemeria cv. Mayfair treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 $\mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

was not significantly different from 1.5 $\mu\text{L L}^{-1}$ of 1-MCP. The lowest chlorophyll content (0.94 mg) was measured in leaves treated without any 1MPC (Fig. 4).

In Mayfair cultivar, the effect of treatment on chlorophyll content was significant at 5% probability level so that the maximum chlorophyll content (1.63 mg) was revealed at 1.5 $\mu\text{L L}^{-1}$ of 1-MCP treatment which was statistically significant at 5% probability level, comparing to control (Fig. 5).

Endogenous ethylene production

Ethylene production was measured 48 hours after ethylene treatments. Cut flowers cv. Hercules showed the lowest endogenous ethylene production at 1 $\mu\text{L L}^{-1}$ of 1-MCP, which was not significantly different to ethylene biosynthesized under 1.5 $\mu\text{L L}^{-1}$ of 1-MCP (Fig. 6). Cut flower cv. Mayfair revealed lowest ethylene production under 1.5 $\mu\text{L L}^{-1}$ of 1-MCP

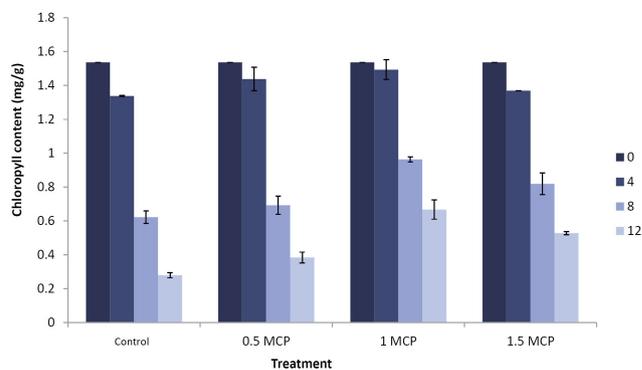


Fig. 4 - Relative chlorophyll content on days 0, 4, 8 and 12 of cut flower of Alstroemeria cv. Hercules treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 $\mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

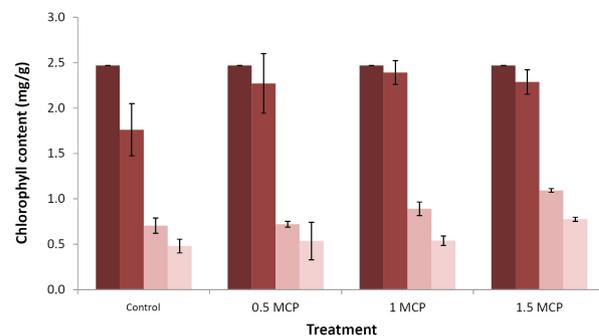


Fig. 5 - Relative chlorophyll content on days 0, 3, 6 and 9 of cut flower of Alstroemeria cv. Mayfair treated with concentrations of 1-MCP at 0, 0.5, 1 and 1.5 $\mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

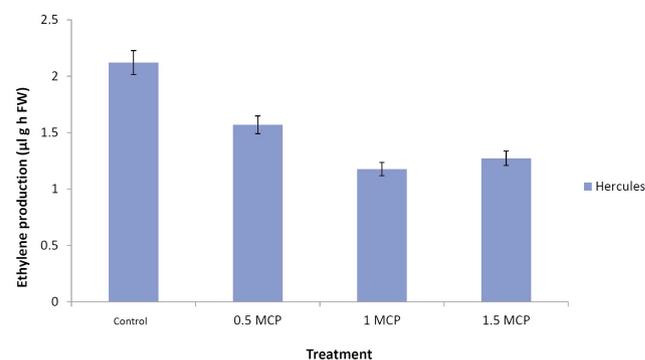


Fig. 6 - Ethylene production of cut flower of Alstroemeria cv. Hercules 48 h after finishing ethylene treatment. Vertical bars represent standard deviation.

application, showing significant differences in respect to other treatments (Fig. 7). Data showed that cv. Hercules produced higher content of ethylene in comparison to cv. Mayfair.

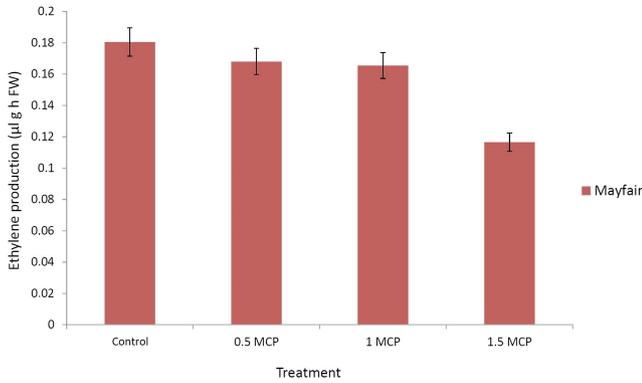


Fig. 7 - Ethylene production of cut flower of *Alstroemeria* cv. Mayfair 48 h after finishing ethylene treatment. Vertical bars represent standard deviation.

Catalase activity

Data on cut flowers *Alstroemeria* cv. Hercules showed that 1-MCP had a positive effect on catalase activity, so that the highest activity was evaluated under $1 \mu\text{L L}^{-1}$ (55/98 absorption delta/mg protein) concentration, which was significantly different at 1% probability level, comparing to other treatment based on LSD test (Fig. 8).

Flower petals of cv. Mayfair showed the highest level of catalase activity under 1 and $1.5 \mu\text{L L}^{-1}$ of 1-MCP concentration, without any significant difference. The lowest catalase activity was revealed in control sample which did not receive any 1-MCP (27.79 absorption delta / mg protein) (Fig. 8).

Superoxide dismutase activity

The analysis of data in cut flowers *Alstroemeria* cv. Hercules showed that the maximum activity of superoxide dismutase was appeared in 1-MCP at concentration of $1.5 \mu\text{L L}^{-1}$ (82.34 enzyme units/mg protein) according to LSD test, without any significant difference with $1 \mu\text{L L}^{-1}$ concentration (Fig. 9).

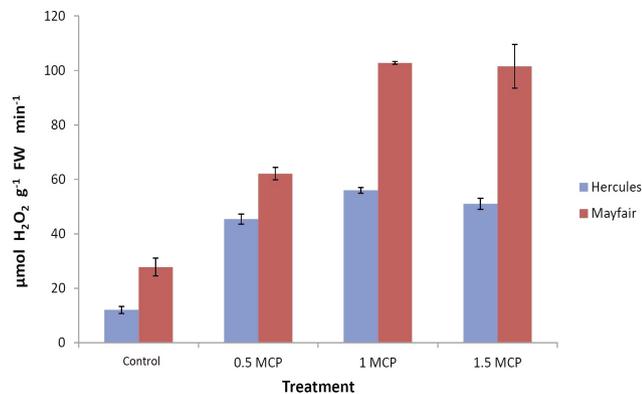


Fig. 8 - Catalase activity in *Alstroemeria* flowers treated with concentrations of 1-MCP at 0, 0.5, 1 and $1.5 \mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

In the cut flower of *Alstroemeria* cv. Mayfair, the maximum activity of superoxide dismutase was obtained from the treatment of 1-MCP at a concentration of $1 \mu\text{L L}^{-1}$ (90.97 units of enzyme/mg protein) which was no significantly different from other 1-MCP concentrations according to LSD test (Fig. 9). The lowest level of superoxide dismutase activity was related to control samples, which had a significant difference at 1% probability level with other treatments.

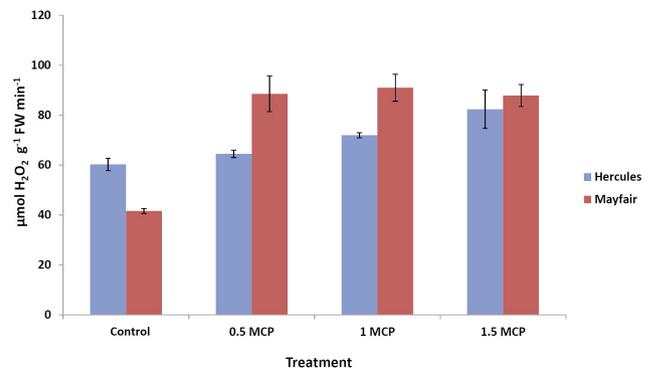


Fig. 9 - Superoxide dismutase activity in *Alstroemeria* flowers treated with concentrations of 1-MCP at 0, 0.5, 1 and $1.5 \mu\text{L L}^{-1}$. Vertical bars represent standard deviation.

4. Discussion and Conclusions

Vase life is one of the most important factors for evaluating the quality of cut flowers. The role of ethylene as the most important accelerating factor in aging of plant organs has been entirely investigated, especially in climacteric crops. In this experiment, 1-MCP was used to reduce the deleterious effects of ethylene (Fig. 1). Based on the results, ethylene accelerated quality deterioration and leaf senescence in the flowers of two *Alstroemeria* cultivars, while 1-MCP increased vase life of both *Alstroemeria* cvs. by alleviation of the harmful effect of ethylene. 1-MCP treatment preserves quality and prolongs post-harvest life by preventing ethylene action and subsequently preventing endogenous ethylene production.

1-MCP compound, like other ethylene action inhibitors such as silver thiosulfate, delayed flower aging when the flowers were exposed to $1 \mu\text{L L}^{-1}$ of ethylene (Serek *et al.*, 1995). This experiment showed that 1-MCP treatment decreased the indigenous ethylene biosynthesis in cut flowers of *Alstroemeria* (Fig. 6 and 7). Accordingly, it seems that the prolonged vase life of cut flowers of *Alstroemeria*

by application of 1-MCP could be related to the role of 1-MCP in inhibition of internal ethylene production. Similar to our results, Chutichudet *et al.* (2011) reported that 1-MCP can prevent ethylene production and increase the life of cut flowers. In carnation cut flower cv. Fortune, it was shown that vase life increased with increasing concentration of 1-MCP (Ranjbar and Ahmadi, 2015).

The results of the present study showed that the relative fresh weight of cut flowers increased and then reduced over time, which was higher in control samples, comparing to 1-MCP-treated cut flowers (Fig. 2 and 3). The reduction in flowering stem weight of flowers treated with 1-MCP during storage may be affected by the interference of 1-MCP in ethylene self-production system (Porat *et al.*, 1995). The report of Daneshi Nergi and Ahmadi (2014) on rose cut flowers cv. Sparkle treated with 1-MCP and ethylene showed that the effects of treatment on the relative fresh weight were significant over time (Daneshi Nergi and Ahmadi, 2014).

According to the present results, 1-MCP at 1 and 1.5 $\mu\text{L L}^{-1}$ concentration had the better yield on chlorophyll content in cv. Hercules (Fig. 4). Also, in cv. Mayfair, 1.5 $\mu\text{L L}^{-1}$ of 1-MCP treatment performed better than control samples (Fig. 5). Chlorophyll, as the main pigment involved in light harvesting, plays essential role in terms of absorption and utilization of light energy in photosynthesis. Basically, the leaf aging is associated with a reduction in chlorophyll content. It was reported that reduction of chlorophyll content of *Alstroemeria* leaves coincided with leaves aging (Ferrante *et al.*, 2002). Ethylene accelerates chlorophyll degradation in the leaves of many cut flowers. The results of both cultivars showed that 0.5 $\mu\text{L L}^{-1}$ of 1-MCP could not prevent chlorophyll degradation, which was consistent with some reports (Çelikel *et al.*, 2002; Seglie *et al.*, 2010). It seems that protection of chlorophyll in leaves of cut flowers *Alstroemeria* under 1-MCP treatment could be related to preventing the action of external ethylene, known as the main cue of leaf yellowing in ornamental plants. In Asian pear, 1-MCP treatment delayed chlorophyll degradation, which was attributed to the effect of 1-MCP treatment on suppression of enzymes involved in on ethylene production pathway (Cheng *et al.*, 2012). The process of leaves yellowing in cut *Alstroemeria* is under the control of genetic background and environmental conditions. Data of Hercules cultivar showed that 1 $\mu\text{L L}^{-1}$ of 1-MCP resulted in lower ethylene synthesis than other treatments (Fig. 6). At the concentration of 1.5 $\mu\text{L L}^{-1}$ in

Mayfair cultivar, 1-MCP resulted in lower ethylene synthesis than other treatments (Fig. 7). In both cultivars, 1-MCP treatments prevented ethylene production. The pre-treatment with 1-MCP for preventing ethylene production is consistent with the study results of rose cv. Samantha (Xue *et al.*, 2008) and clove (Seglie *et al.*, 2010).

Catalase is considered an important biological factor and its major function is in the decomposition of hydrogen peroxide to water and oxygen and preventing creation of hydroxyl radicals (Spanou *et al.*, 2012). Superoxide dismutase like Cu-Zn superoxide dismutase, Mn superoxide dismutase and outside cell superoxide dismutase play a critical role in inhibition of superoxide (Miao and St Clair, 2009).

In fact, catalase and superoxide dismutase play roles in protecting the metabolism balance of oxygen in plant tissues (Xie *et al.*, 2003). Superoxide causes lipid peroxidation, cell membrane damage and finally senescence; 1-MCP can affect enzyme activities, which remove superoxide (Li *et al.*, 2007).

In the cut flower of Hercules, no significant difference was found between 1-MCP treatments, but the activity of catalase was significantly increased compared to control (Fig. 8). The results of Mayfair cultivar cut flower showed that no significant difference was found between 1 and 1.5 μL of 1-MCP but catalase activity increased significantly compared to control (Fig. 8). Ethylene-stimulated aging could change the cell structure and increased the concentration of reactive oxygen species such as superoxide radicals, hydroxyl radicals and hydrogen peroxide. Therefore, 1-MCP treatment reduced the reactive oxygen species and consequently delayed aging by reducing the biosynthesis and action of ethylene or by directly increasing the activity of antioxidant enzymes (Fig. 9). Consistent with the results of this experiment, an increase was observed in antioxidant enzymes activity of 1-MCP -treated *Gladiolus* flowers (Hassan and Ali, 2014). It seems that 1-MCP treatment reduced the oxidative stress in cut flowers. In other words, activity of these enzymes is a factor for the protection of cells against oxidative stresses (Zhou *et al.*, 2014). In asparagus, 1-MCP hindered the ethylene signal transduction and resulted in a delay by affecting ethylene biosynthesis, and enhancing superoxide dismutase activity (Zhang *et al.*, 2012). In addition, it has been found that applying 1-MCP delayed aging, but its effect varied depending on the flower organ, cultivar, and development stage and treatment concentration. It seems that catalase activity depends on the plant species, plant type and experimental condi-

tions during aging. Finally, it can be concluded that increasing the activity of antioxidant enzymes reduces the aging of flowers.

In general, 1-MCP treatment had a positive effect on improving physiological and biochemical characteristics resulted in an extended postharvest longevity of *Alstroemeria* cultivars. The higher concentrations of 1-MCP revealed better effect in comparing to low concentrations, especially on ethylene production. According to the results, 1-MCP treatments, by increasing the protein content and the activities of catalase and superoxide dismutase, improved the quality and increased vase life of cut flowers of *Alstroemeria*.

In conclusion, based on this study, the application of 1-MCP acting as ethylene action inhibitor, could be recommended to increase postharvest life and extended the longevity in *Alstroemeria* cut flowers.

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