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Proline and Arginine Improves the Vase Life of Cut *Alstroemeria* 'Mars' Flowers by Regulating Some Postharvest Physiochemical Parameters

Dina Yaghoubi Kiaseh[1], Davood Hashemabadi[2*] and Behzad Kaviani[2]

[1] Ph.D. Student, Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[2] Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran

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*Corresponding author's email: davoodhashemabadi@yahoo.com

Abstract

To study the effect of "cycloheximide" and amino acids of arginine and proline on post-harvest longevity of cut *Alstroemeria × hybrid* 'Mars' flowers, a factorial experiment was conducted based on a randomized complete design with 3 replications and 20 treatments. The experiment includes CHI at 4 levels (0, 50, 100, 1000 µM), amino acid (AA) at 5 levels [0, 5, 10 mM (Arg) and 5, 10 mM proline] in terms of 24 h pulse. The results showed that flowers treated with "CHI_{50} x Pro_{10}" had the most vase-life (9.3 days), water uptake (1.771 ml g^{-1} F.W.), and dry material (13.56 %), the least MDA aggregation (1.32 nmol g^{-1} F.W.), and the most CAT enzyme activity (5.68 nmol g^{-1} F.W. min^{-1}). The treatment of "CHI_{10} x Arg_5" was one of the most successful treatments in water uptake, wet weight, dry material, and total protein preservation, and MDA aggregation decrease, SOD enzyme activity increase, and took the second place in vase-life increase (9.08 days). Therefore, the 2 treatments of "CHI_0 x Arg_5" and "CHI_{50} x Pro_{10}" are presented as the most successful treatments in quantitative and qualitative trait's improvement of cut *Alstroemeria* flowers. The lowest positive impact on the majority of analyzed traits belongs to the treatment of 1000 µM CHI. Therefore, the application of high levels of CHI (1000 µM) on vase-life solution of cut *Alstroemeria × hybrid* 'Mars' is not recommended due to negative and toxic impact on post-harvest traits.

Keywords: Amino acid, Polyphenol oxidase, Proline, Protein demolition, Senescence.

INTRODUCTION

Alstroemeria, is one of the most important cur flowers among global markets which has numerous aficionados because of its beautiful flowers and various colors. Petal shedding and yellowing of leaves are signs of senescence and end of vase-life in cut alstroemeria. Senescence delay and vase-life preservation –similar to all cur flowers- play significant roles in preserving economic value of this cut flower (Naghiloo *et al*., 2020; Ferrante *et al*., 2002; Aros *et al*., 2019)

Senescence and end of life are natural and genetic happenings in plants, but this process in accelerated in cur flowers due to separation from maternal base. It has been reported that senescence and end of vase-life in cur flowers are accompanied by detrimental protein synthesis which is responsible for membrane and macromolecules degradation. Therefore, delaying the synthesis of these proteins by applying detrimental protein synthesis inhibitors, can hinder the progress of senescence and premature decay in cut flowers (Thomas and Stodart, 1980; van Meetren *et al*., 2001; Shahri and Tahir 2010).

CHI is a chemical compound and a detrimental protein synthesis inhibitor in terms of translations and duplication. This substance hinders membrane-degradation enzyme synthesis and has an inhibitory impact on the progress of senescence of cut flowers (Jones *et al*., 1994; Islam *et al*., 2011; Gul and Tahir, 2013). Jones *et al*. (1994) believe that CHI causes senescence delay and pigment preservation in many cut bulbous flowers by detrimental protein synthesis preservation. The results of Gulzar *et al*. (2005) study indicated that CHI and cytokinins application causes protein degradation delay, membrane structure preservation, and carbohydrates dissolved in petals of cut *Hemerocallis* flowers. In Shahri and Tahir (2010) research, CHI at the level of 0.05 mM caused vase-life increase in cut *Ranunculus* flowers. Gul and Tahir (2013) recorded increased vase-life, preserved dry and wet weight, and maintained membrane structure in cut *Narcissus* flowers treated with CHI. The positive effect of CHI on quality and longevity of cut *Dianthus* (Wulster *et al*., 1982), *Hemerocallis* (van Doorn *et al*., 1994), and *Alstroemeria* (Yaghubi Kiaseh and Yadegari, 2016; Alizadeh Matak *et al*., 2017) flowers has been reported.

Reduction of proteins are outstanding signs of senescence in cut flowers. Also, total protein level reduces excessively in cut flowers after separation from stock plant. Amino acids are components of proteins. Most amino acids are transferred from primary absorption places to different organs of plants by xylem and phloem in order to participate in constructing proteins and other vital essential macromolecules. Therefore, external use of them can prevent total protein reduction in plant tissue and petal of cut flowers (Caputo and Barneix, 1999; Ortiz-Lopez *et al*., 2000). There are some records that indicate that amino acids are able to postpone senescence process by increasing mRNA duplication, photosynthesis activation, and increasing the amount of proteins. The anti-oxidant effect of amino acids, and their role in membrane stability preservation against oxidative stress has also been reported.

Proline is an amino acid with a unique structure which is involved in protein construction in free forms. Proline is a non-enzymatic anti-oxidant which has been recorded as an anti-stress, smolite compatible, and free radical scavenger (Szabados and Savoure, 2009; Kumar *et al*., 2010). Kumar *et al.* (2010) reported that a short term treatment of roses treated with 5 mM proline has increased vase-life significantly; whereas high concentrations of CHI (8, 10 mM) cause vase-life decrease in their research. Alipour *et al.* (2016) used 0,1,10 µM concentrations of proline in order to increase vase-life in cut tuberose. Results of this research showed that the most vase-life, ascorbate peroxidase enzyme activity, and the lowest PPO enzyme activity are required by applying 10 mM of proline.

Arginine is one of the components of protein and one of the widely-used amino acids in living cells. Arginine is the precursor of polyamines, proline, nitric oxide, and glutamine (Liu *et al*., 2006). It also plays a major role in the regulation of plant growth process (Galston and Sawh-

ney, 1990), and resistant infusion against stress (Abdul Qados, 2009; Khalil *et al*., 2009). Asadi Karam and Asrar (2015) reported that arginine contributes in scavenging of ROS through polyamines and nitric oxide synthesis. Nasibi *et al*. (2014) used arginine, cysteine, and 5-salicylic sulfur acid in vase-life solution of cut *Tuberosa* flowers and reported that these compounds have positive effect on electrolyte leakage reduction, lipid peroxidation decrease, anti-oxidant enzyme activity increase and vase life increase in cut flowers. In Alipour *et al*. (2013) research, the impact of arginine (0, 5, 10 µM) on the vase-life of cut *Tuberosa* flowers was studied. Results of this paper indicated that the most vase-life, guaiacol peroxidase, ascorbate peroxidase, and CAT enzymes activity was obtained by flowers treated with 10 µM arginine. The positive effect of arginine on proteins preservation, stress reduction in legume (Amira and Abdul, 2010), photosynthesis pigments preservation, and longevity increase in strawberry (Bidaki *et al*., 2018) and pigment preservation, protein preservation, lipid peroxidation decrease, anti-oxidant activity increase, and post-harvest quality preservation in green asparagus (Wang *et al*., 2017) has been recorded.

With due attention to the fact that senescence process in accompanied by proteins consumption increase and detrimental proteins synthesis increase (Borochov and Woodson, 1989), this research was executed with the aim of senescence delay and vase-life improvement in cut *Alstroemeria* × *hybrid* 'Mars' by means of CHI application as detrimental proteins synthesis inhibitor, and arginine and proline amino acids application as anti-oxidant compounds and protein's components.

MATERIALS AND METHODS

To study the effect of CHI and amino acids (AA) on vase-life of *Alstroemeria* × *hybrid* 'Mars', a factorial experiment was conducted based on a randomized complete design with 3 replications and 20 treatments. Experimental treatments include 4 levels of CHI (0, 50, 100, 1000 µM), and 5 levels of amino acid (0, 5, 10 mM arginine and 5, 10 mM proline) in terms of 24-hour pulse.

Cut *Alstroemeria* flowers were bought from a greenhouse in Mahalat, Iran and were transferred to the laboratory in the shortest period of time in compliance with the rules of transportation. In order to enforce the pulse treatments, first, *Alstroemeria* flowers were recut under running water with a height of 40 cm, and then instantly treated with the forgoing solution. After the accomplishing the pulse treatments, flowers were transferred to 500 ml vases containing 3 % sucrose, then preserved in a room with a temperature of 20 ± 2 °C, 60-75 % relative humidity, and 12 hours of lighting with an intensity of 15 µmol m^{-2} s^{-1} until the end of the experiment.

Assessment of traits

Vase-life

Vase-life was estimated by counting the number of days from day zero (first day of the experiment) to the 50% wilting of petals (Mutui *et al*., 2006).

Water uptake

Water uptake was estimated by the following equation:

$$\text{Water uptake (ml g}^{-1}\text{ FW)} = \frac{V_{t0} - (E_t + V_{t1})}{FW}$$

Where; V_{t0} is the initial volume of vase solution, E_1 is average evaporation from the solution surface, V_{t1} is how's the volume of solution remaining on the last day, and FW in the flower fresh weight on the first day.

Fresh weight loss

Fresh weight of the flowers on the first day (FW_0) and the last day of the experiment (FW_2) was measured by a digital scale with an accuracy of 0.001 grams. In order to prevent vessel obstruction, one centimeter from the bottom of the flower stems was recut under running water once every 3 days, and the recuts were measured (FW_1). Eventually, fresh weight loss of cut *Alstroemeria* flowers was estimated by the following equation:

$$\text{Fresh weight loss (g)} = FW_0 - (FW_1 + FW_2)$$

Dry matter

On the last day of the experiment (end of vase-life), fresh weight of 2 flower branches from each replication was measured by a digital scale with an accuracy of 0.001 g. Cut flowers were dried in electric oven with a temperature of 72 °C for 48 hours. The weight of dried flowers was measured, and dry material was calculated by the following equation:

$$\text{Dry matter} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Chlorophyll a and b

In order to measure chlorophyll a and b Mazumdar and Majumder (2003) method was utilized. Immediately after observing first signs of wilting, sampling of *Alstroemeria* leaves was enforced in the vase-life room. 0.5 g of leaves sample was extracted by 8% acetone. Absorption content of resultant samples was read by the Shimadzu UV-120-02 spectrophotometer at 642 and 660 nm, and the following equation was applied to determine a and b chlorophyll in mg g^{-1} F.W.

$$\text{Chlorophyll a} = 9.93\ (A_{660}) - 0.777\ (A_{642})$$
$$\text{Chlorophyll b} = 17.6\ (A_{642}) - 2.81\ (A_{660})$$

Total protein

Immediately after observing first signs of wilting, petals were sampled, and the indirect Kjeldahl method was utilized to measure total protein. First, N content (equation 1), and then total protein content were calculated by the following equation:

Equation 1 $$\text{Nitrogen (\%)} = \mathbf{0.56} \times \mathbf{t} \times (\mathbf{a} - \mathbf{b}) \times \frac{\mathbf{V}}{\mathbf{W}} \times \frac{\mathbf{100}}{\mathbf{DM}}$$

Equation 2 $$\text{Total protein (\%)} = \mathbf{nitrogen} \times \mathbf{6.25}$$

Where; **t** is the concentration of acid used for titration in mol L^{-1}, **a** represents the amount of acid used for sample, **b** is the amount of acid used for control in mL, **V** is the volume of extract taken from digestion in mL, **W** is the sample weight for digestion in g, and **D.M.** is the present of plant dry matter.

Malondialdehyde (MDA)

In order to measure MDA the Heath and Parker (1968) method was utilized. Therefore, as soon as observing first signs of wilting in flowers, sampling the petals was enforced. Next, 0.5 g of petal tissue was extracted by liquid nitrogen. Then, 1 mL of potassium phosphate buffer and 0.5 M EDTA were added. Resultant solution was centrifuged at 14000 rpm at 4°C for 20 minutes. Afterwards, the supernatant was added with 1000 µL of TCA and TBAS. The resultant sample

was placed in a hot bath at 95°C for 30 minutes followed by cooling in a container with ice. Samples re-centrifuged until the red-colored malondialdehyde thiobarbituric acid emerged. The absorbance of the samples was read at 532 and 600 nm and the petal MDA content was estimated by the following equation:

$$MDA(nmol\ g^{-1}\ FW)=A_{532nm}\ -A_{600nm}$$

Anti-oxidant enzymes

In order to measure the activity of anti-oxidant and PPO enzymes, petals were sampled immediately after observing first sign of wilting in vase-life room. Petals were collected and placed in liquid N. Samples were extracted by 50 mM of potassium phosphate buffer. The resultant extract was centrifuged at 10500 rpm at 4°C for 25 minutes. Afterwards, the transparent solution generated on the surface was extracted by means of a sampler, then it was used as the enzyme extract.

SOD enzyme activity

SOD enzyme activity measurement was executed by Giannopolitis and Ries (1997) method. For that matter, 0.1 mL enzymatic extract, 25 mM NBT, 13 mM methionine, 0.1 mM EDTA, 50 mM sodium carbonate, and 50 mM potassium phosphate buffer were gently mixed by a shaker in florescence light and a temperature of 22°C. The blended samples were kept in a dark room for 30 minutes. Then, the absorption rate of the samples were read by spectrophotometer (Shimadzu UV-120-02) at 560 nm for 2 minutes. SOD enzyme activity was reported in a measurement unit of enzyme g^{-1} F.W. min^{-1}.

CAT enzyme activity

The activity rate of CAT in cut *Alstroemeria* petals was measured by the Beers and Sizer (1952) method. 400 µL of 15 mM hydrogen peroxide, 2.6 mM of 50 mM (pH=7) potassium phosphate buffer, and 40 µL of enzymatic extract were used as reaction mixture. Samples were gently blended by a shaker for 30 minutes, CAT enzyme activity was determined by reading absorption rate of the samples at 240 nm by spectrophotometer (Shimadzu UV-120-02) for 2 minutes.

PPO enzyme activity

For this purpose, Raymond *et al.* (1993) method was used. 60 test tubes (samples numbers) were put up in a warm water bath (40°C). 2.5 mL potassium phosphate buffer (0.2 M with pH=6.8) and 0.2 mL pyrogallol (0.02 M) were added into the tubes. When the temperature of the insider solution reached 40°C, 200 µM enzymatic extract was added. Absorption rate of the samples was read by spectrophotometer (Shimadzu UV-120-02) at 430 nm. PPO enzyme activity was reported a measurement unit of nmol g^{-1} F.W.

Statistical analysis

Data collected from daily harvests and laboratory measurements were analyzed by SPSS16 software package and means were compared by the LSD test.

RESULTS

Vase-life

The interaction of CHI and amino acid was significant ($P < 0.05$) for the vase-life of cut *Alstroemeria* × *hybrid* 'Mars' flowers (Table 1). Table 1 shows that applying a level of 1000 µM of CHI decreases vase-life more than the other levels. The longest vase-life (9.3 days) belongs to "CHI_{50}×Pro_{10}" treatment, which didn't differ from the application of 5 or 10 mM of arginine and proline in combination with 0, 50, and 100 µM CHI or the treatment of "CHI_{100}×AA_0" signifi-

cantly. The shortest vase-life (6.52 days) was obtained by "CHI_{1000}×AA_0" which indicates the negative impact of CHI at higher rates on the vase-life of *Alstroemeria* × *hybrid* 'Mars' (Table 1) (Fig. 1).

Table 1. Analysis of variance for the interactive effect of 'cycloheximide × amino acid' on the recorded traits.

S.o.V	df	Vase life	Water uptake	Fresh weight loss	Dry matter	Chlorophyll a	Chlorophyll b	Total protein	MDA	SOD	CAT	PPO
Cycloheximide (CHI)	3	8.59**	1.32**	317**	1.17^{ns}	16.13**	8.83**	19.4*	0.486**	2.99*	1.26**	0.004**
Amino acid (AA)	4	0.19^{ns}	0.14**	46.5**	4.17**	1.99**	0.10^{ns}	6.25^{ns}	0.455**	49.65**	5.74**	0.002**
CHI×AA	12	0.94*	0.10**	32.4**	1.50*	2.28**	1.24**	13.84*	0.756**	3.22*	4.17**	0.0009**
Error	40	0.35	0.01	0.497	0.49	0.45	0.060	4.84	0.024	0.721	0.021	0.0001
CV (%)		7.18	10.44	8.31	5.97	10.83	17.2	7.27	6.655	7.43	7.73	12.29

*, ** and ^{ns}: significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

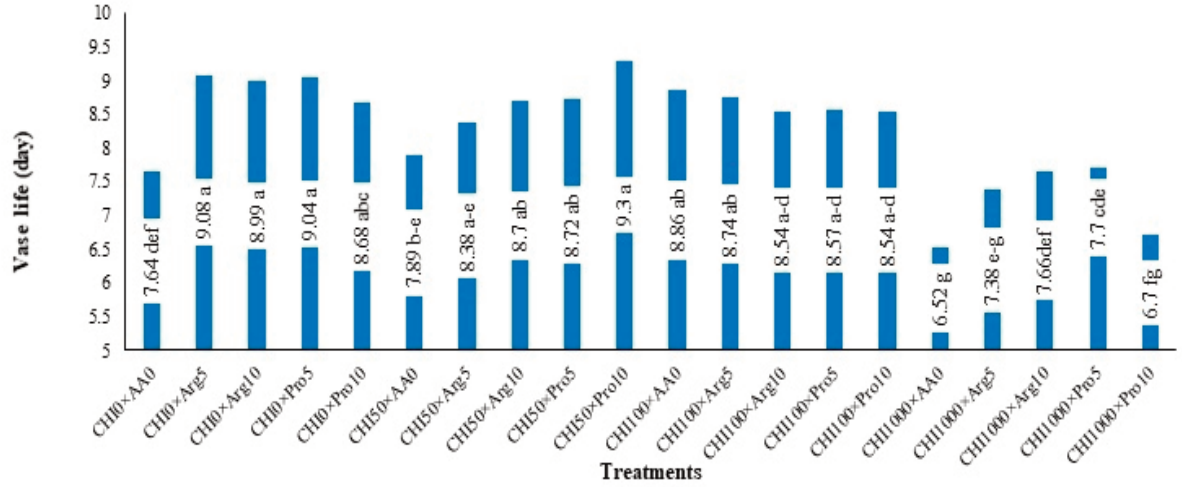

Fig. 1. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on vase life of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

Water uptake

The interactive effect of "CHI×AA" on water uptake was significant with a possibility of 1% (Table 1). The comparison of means indicated that similar to vase-life, water uptake was also decreased by an application of 1000 µM CHI with or without amino acid combination. The lowest water uptake (0.655 ml g^{-1} FW) is related to the treatment of "CHI_{1000}×AA_0". The treatment of "CHI_{50}×Pro_{10}","CHI_0×Arg_5", and "CHI_{100}×Arg_5" didn't significantly differ from one another, and appropriated the highest water uptake (Fig. 2).

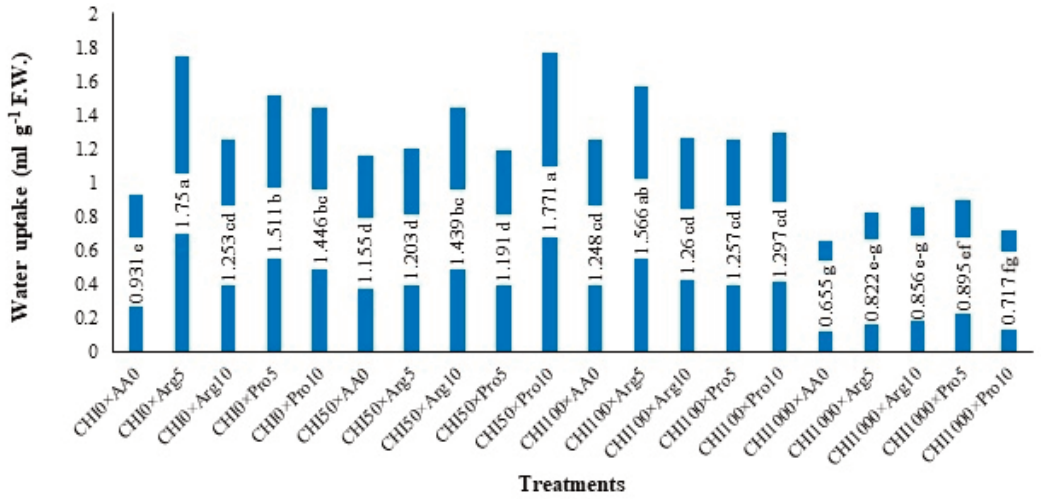

Fig. 2. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on water uptake of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

Fresh weight loss

The interactive effect of "CHI×AA" on fresh weight loss was significant with a possibility of 1% (Table 1). The comparison of the means indicated that fresh weight decreases significantly by increasing CHI concentration. Arginine and proline application at the levels of 0, 50, and 100 µM CHI hindered fresh weight loss. At a 1000 µM level of CHI, all concentrations of amino acids except proline 10 mM preserved fresh weight significantly. Treatments of "CHI_{1000}×Pro_{10}" and "CHI_{1000}×AA_0" had the most fresh weight loss. The lowest fresh weight is related to "CHI_0×Arg_5" and "CHI_{50}×Pro_{10}" treatment respectively (Fig. 3).

Dry matter

The interaction of "CHI×AA" on dry matter was significant with a probability of 5 % (Table 1). The comparison of the means showed that the most dry matter of cut *Alstroemeria* × *hybrid* "Mars" (13.65 %) was obtained by the "CHI_{50}×Pro_{10}" treatment, which did not significantly differ from "CHI_0×Arg_5", "CHI_{100}×Pro_5", "CHI_{100}×Pro_{10}", and "CHI_{100}×Arg_{10}" treatments.

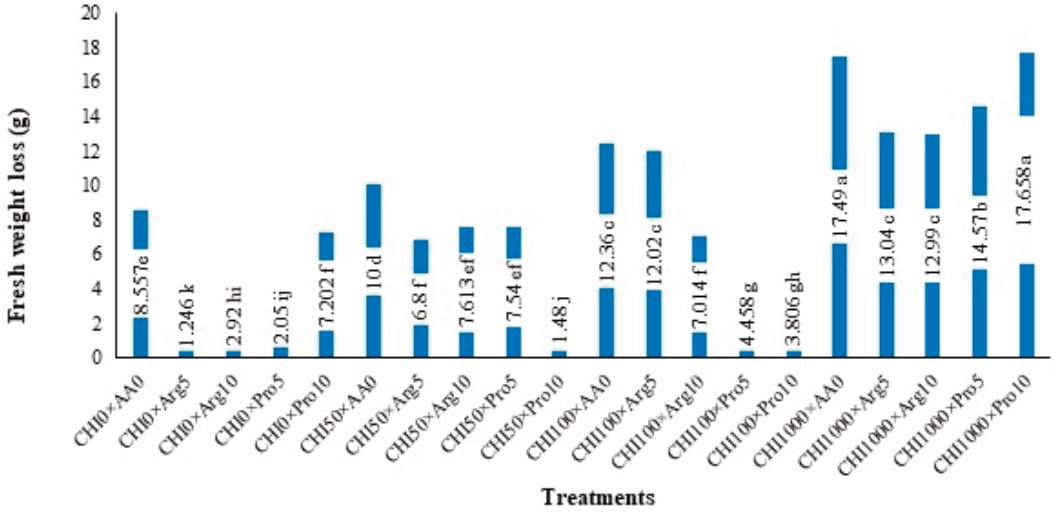

Fig. 3. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on fresh weight loss of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

The lowest dry matter belonged to treatments of "CHI_{1000}×AA_0", "CHI_{50}×AA_0", and "CHI_0×AA_0" that were not proper treatments in dry matter increase an preservation in cut *Alstroemeria* × *hybrid* 'Mars' (Fig. 4).

Chlorophyll a

The interactive impact on the chlorophyll a content was significant with a probability of 1% (Table 1). Among all interaction treatments, the most chlorophyll a (7.82 mg g^{-1} F.W.) is related to "CHI_{100}×Arg_5" treatment which didn't significantly differ from "non-CHI at all 5 levels of amino acid" and "CHI_{50}×Pro_{10}" treatments. The lowest a chlorophyll content (3.98 mg g^{-1} F.W.) was obtained by "CHI_{1000}×AA_0" treatment (Fig. 5).

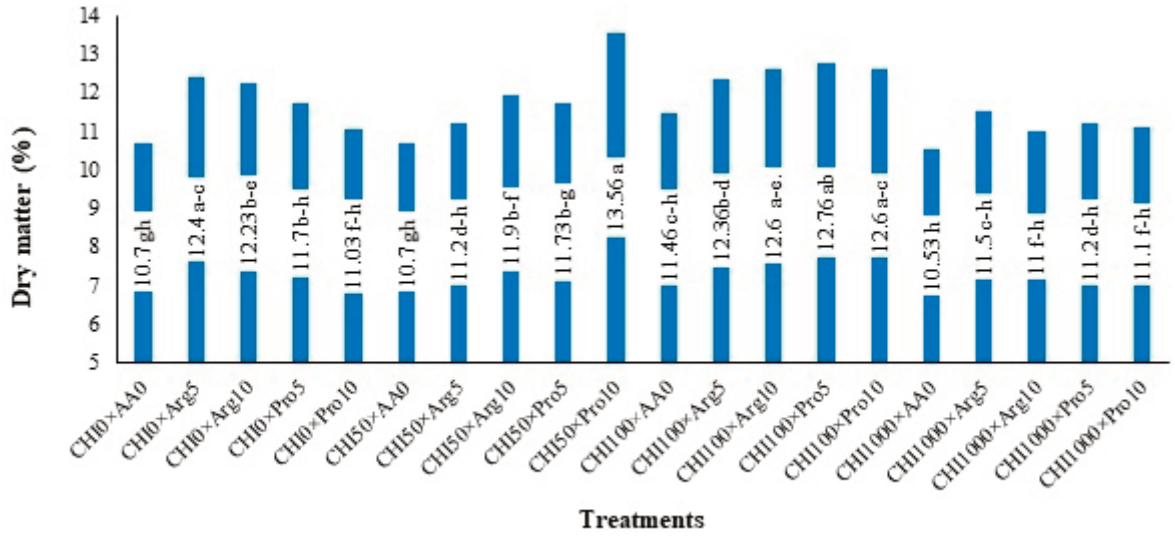

Fig. 4. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on dry matter of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

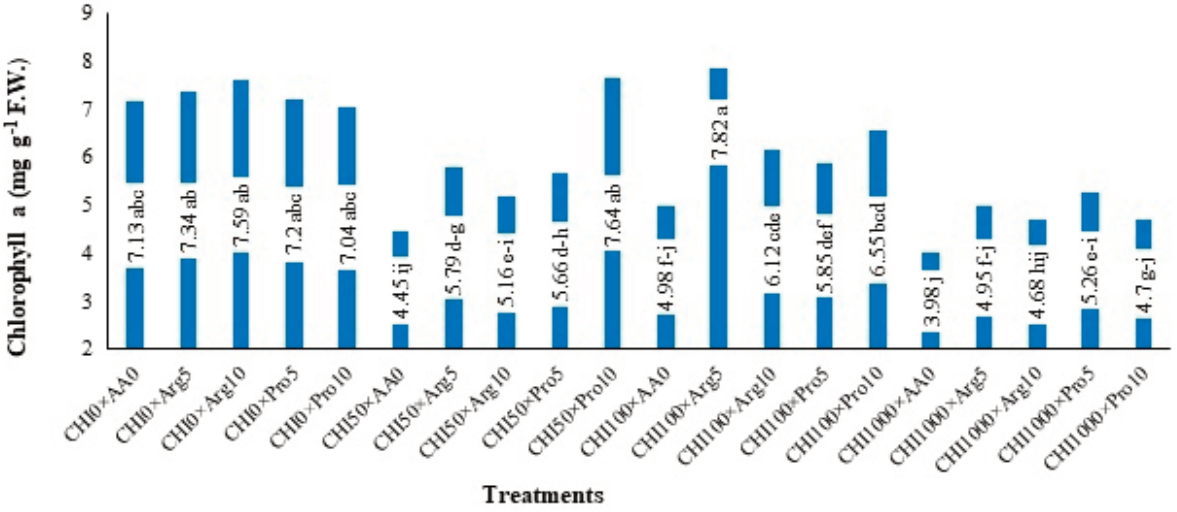

Fig. 5. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on chlorophyll a of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

Chlorophyll b

The interaction of "CHI×AA" on chlorophyll b content was significant whit a probability of 1% (Table 1). According to the results of means comparison, the lowest b chlorophyll content was obtained by an application of "CHI_{1000}×Pro_{10}", "CHI_{1000}×Arg_5", and "CHI_{1000}×AA_0". The treatment of "CHI_0×Arg_{10}" (5.12 mg g^{-1} F.W.) was the most successful treatment in chlorophyll b preservation and didn't significantly differ from the treatments of "CHI_{50}×Pro_{10}", "CHI_0×AA_0", "CHI_0×Arg_5", "CHI_{50}×Pro_5 and 10", "CHI_{100}×Arg_5 and 10", and "CHI_{100}×Pro_{10}" (Fig. 6).

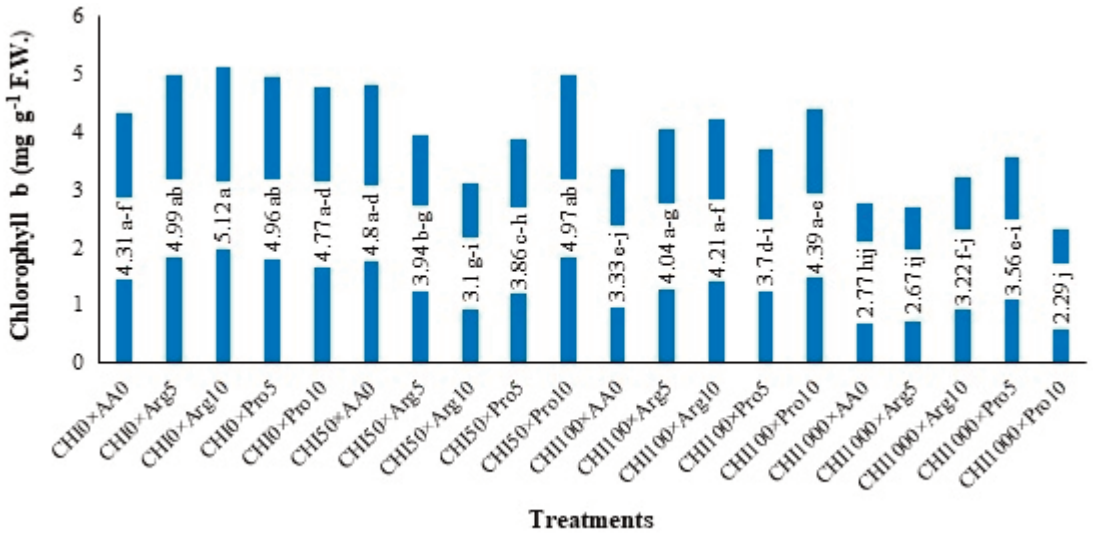

Fig. 6. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on chlorophyll b of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

Petal protein content

The interactive effect of treatments had a significant impact on petal protein content ($P<0.05$) (Table 1). Comparison of the means indicated that the application of 5 mM Arg more successful in petal protein preservation. Among all treatments, the most protein petal content belonged to flowers treated with "$CHI_{0,\ 50,100\ and\ 1000}$×$Arg_5$" statistically didn't differ from each other. Treatments of "CHI_{1000}×AA_0" (7.56 %) and "CHI_{1000}×Pro_{10}" (7.69 %) didn't have a significant different with one another and appropriated the lowest petal protein (Fig. 7).

Malondialdehyde (MDA)

ANOVA data indicated that interactive effect of treatments on MDA aggregation in *Alstroemeria* petals was significant with a probability of 1% (Table 1). Comparison of the means revealed that the most MDA content is related to flowers treated with "CHI_{1000}×AA_0" (3.55 nmol g^{-1} F.W.). The application of amino acid at different levels of CHI caused MDA decrease. The most successful treatments in MDA aggregation decrease were treatments of "CHI_{50}×Pro_{10}", "CHI_0×Pro_5", and "CHI_0×Arg_5" respectively, which hindered membrane lipids peroxidation and MDA aggregation in cut *Alstroemria* 'Mars' petals (Fig. 8).

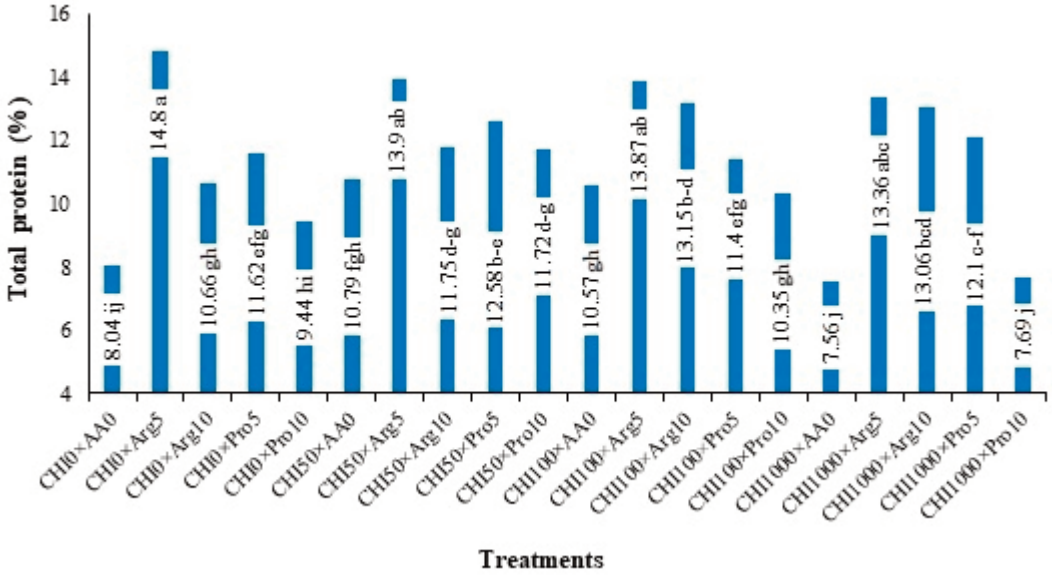

Fig. 7. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on total protein of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

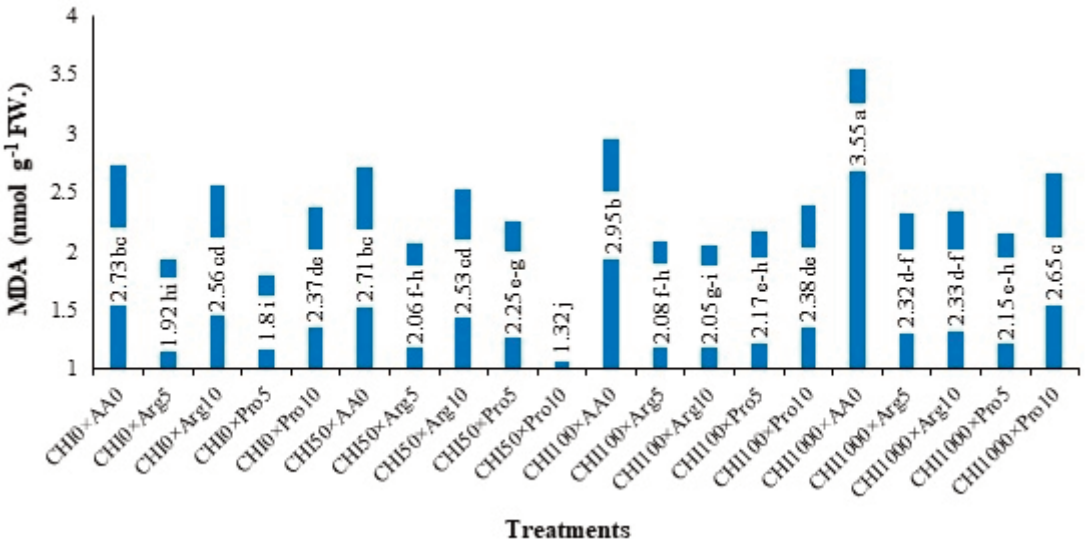

Fig. 8. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on MDA of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

SOD enzyme activity

The interactive effect of "CHI×AA" on SOD enzyme activity was a significant with a probability of 5 % (Table 1). Comparison of the means indicated that application of high levels of CHI (1000 µM) has negative impact on antioxidant SOD enzymes. The lowest activity of this enzyme is related to "CHI_{1000}×AA_0" (24.94 IU g^{-1} F.W. min^{-1}) treatment. The treatment of "CHI_0×Arg_5" with 34.39 IU g^{-1} F.W. min^{-1} obtained the most SOD enzyme activity among all treatments which didn't have a significant difference with treatments of "CHI_{50}×Pro_{10}", "CHI_0×AA", "CHI_0×Pro_5 and 10", "CHI_0×Arg_{10}", and "CHI_{100}×Pro_{10}" and the set of these treatments were successful in increasing SOD enzyme activity (Fig. 9).

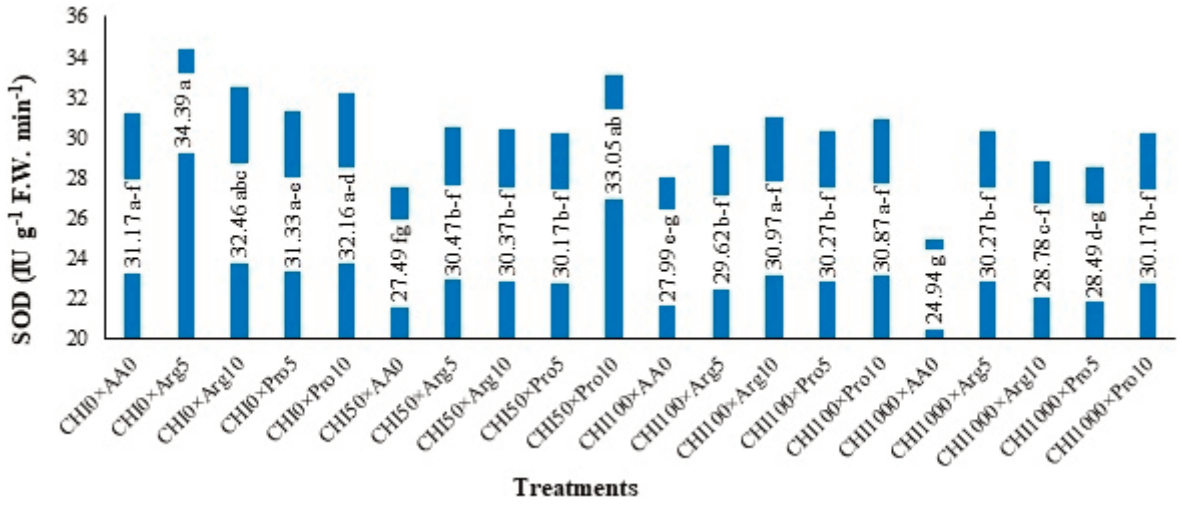

Fig. 9. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on SOD activity of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

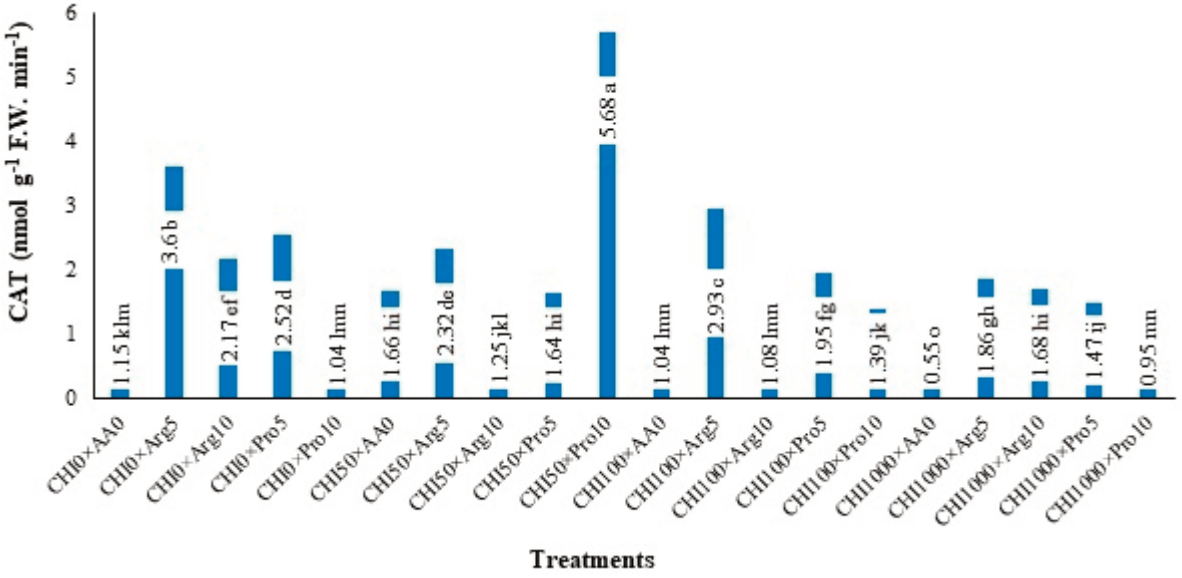

Fig. 10. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on CAT activity of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

CAT enzyme activity

The interactive impact of "CHI×AA" on CAT enzyme activity what is the significance with a probability of 1% (Table 1). Comparison of the means showed that, treatments of "$CHI \times AA_0$" (0.55 nmol g^{-1} F.W. min^{-1}) and "$CHI_{1000} \times Pro_{10}$" (0.95 nmol g^{-1} F.W. min^{-1}) had the lowest CAT enzyme activity, and weren't proper treatments in preserving this enzyme activity. The most CAT enzyme activity is related to treatment of "$CHI_{50} \times Pro_{10}$" (5.68 nmol g^{-1} F.W. min^{-1}) and "$CHI_0 \times Arg_5$" (3.60 nmol g^{-1} F.W. min^{-1}) respectively. Also, these treatments were the most successful ones in CAT enzyme activity increase (Fig. 10).

PPO enzyme activity

The interactive effect of treatments on PPO enzyme activity was significant with a probability of 1% (Table 1). Results of means comparison indicated that by increasing CHI concentration

and without amino acid consumption, PPO enzyme activity was increased. As fig. 11 shows the most PPO enzyme activity is related to treatments of "$CHI_{0, 50, 100 \text{ and } 1000}\times AA_0$". Treatments of "$CHI_0\times Arg_{10}$" and "$CHI_{50}\times Pro_{10}$" did not significantly different from each other and appropriated the lowest PPO enzyme activity among all treatments (Fig. 11).

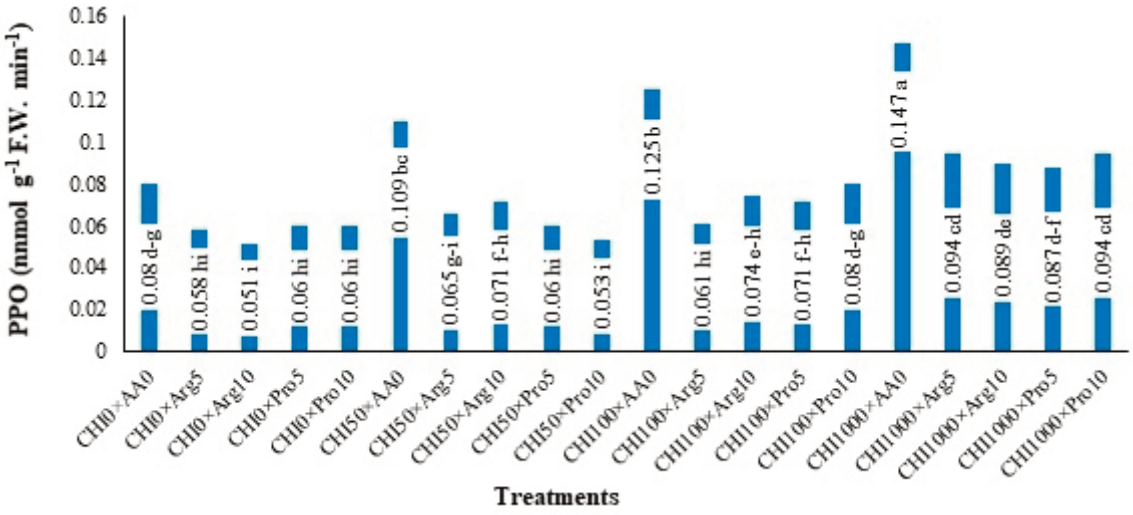

Fig. 11. The interactive effect of cycloheximide (CHI) and amino acids of arginine (Arg) and proline (Pro) on PPO activity of cut *Alstroemeria* × *hybrid* 'Mars' flowers. AA0: No amino acid.

DISCUSSION

Senescence and life termination of cut flowers begins with synthesis of a special proteins which are responsible for senescence beginning and membrane demolition and macromolecules. The application of detrimental proteins synthesis inhibitors, can hinder senescence progress in cut flowers in this research (Gulzar *et al*., 2005; Shahri and Tahir, 2010; Islam *et al*., 2011). Application of CHI as a detrimental protein synthesis inhibitor at low concentrations (up to 100 µM) exclusively or combined with amino acid was successful in vase-life preservation in cut *Altromeria* 'Mars' flowers in comparison to control treatments. Application of Arg and Pro amino acids exclusively or combined with CHI caused vase-life improvement compared to not using amino acids, or to control treatment. Also, amino acids application decreased negative effects of high concentration of CHI on vase-life. In former researches, positive impacts of CHI (Shahir and Tahir, 2010; Islam *et al*., 2011), arginine (Nasibi *et al*., 2014), and proline (Kumar *et al*., 2010; Alipour *et al*., 2016) on the vase-life of different cut flowers was recorded which conforms to the present research.

The positive effects of CHI on vase-life progress can be attributed to the impact of this substance on senescence starter proteins synthesis enzymes responsible for membrane demolition (Suttle and Kende, 1980; Jones *et al*., 1994), anti-ethylene impact (Eason and de Vre, 1995), and anti-fungal and anti-microbial impact of this substance (Jones *et al*., 1994). Although, the anti-fungal and anti-microbial impact of CHI on flowers longevity has not been studied, results of the present research indicated that at 50 and 100 µM concentrations of CHI which has positive impacts on vase-life progress, water uptake was more than the control treatment which could be caused by anti-microbial impact of this substance and its effect on water uptake continuity preservation; hence, this theory needs further investigation. A group of researchers believe that CHI causes cellular turgidity by affecting new proteins synthesis which probably plays a role in water stress effects (Drory *et al*., 1995). Positive effect of CHI on water uptake increase in cut *Iris* (van Doorn

et al., 1994) and *Alstroemeria* (Yaghoubi Kiaseh and Yadegari, 2016; Alizadeh Matak *et al*., 2017) flowers was recorded which conforms to the present research.

The positive impact of amino acids on water uptake improvement at all three concentrations of CHI was clear. Researchers recorded that application of amino acids as nitrogen and energy resource has positive impact on growth and post-harvest features of plants (Geshnizjani and Khosh-Khui, 2018; Chang et al., 2010). In Bidaki *et al.* (2018) research the application of 500 mM Arg hindered fresh weight loss in strawberry fruits. Generally, positive effect of Arg on vase-life preservation can be attributed to anti-stress and anti-oxidant effect of resultant metabolic compounds of this amino acid. Positive effect of amino acids on water uptake in cut roses and gerbera flowers has been reported which conforms to the present research. Results of Alipour *et al*. (2016) research indicated that cut tuberose flowers treated with proline were less fresh weight loss in comparison to the control. In this paper, the lowest fresh weight loss was obtained by flowers treated with 10 mM proline. Proline is one of the smolites which causes cellular turgidity preservation by osmotic pressure adjustment (di Stasio et al., 2018). As mentioned in the statement of results, arginine and proline were effective in water uptake, fresh weight, and dry matter preservation. The positive impact of proline on maintained features improvement can be attributed to smolite an anti-oxidant effect of this substance (Szabados and Savoure, 2009; Yang et al., 2009).

Leaves yellowing which is followed by chlorophyll demolition and breakdown, is one of the first signs of senescence in cut *Alstroemeria* flowers, which reduces the commercial value of these cut flowers (Ferrante *et al*., 2002; 2005). Researchers believe that CHI causes pigment preservation by postponing the senescence of leaves and flowers (Thimann, 1987; Jones *et al*., 1994). In the present research, CHI application caused chlorophyll a increase exclusively, and also caused chlorophyll b increase by only an application of 50 mM concentration of it. Although, the combination of CHI and amino acids wasn't successful in many cases, the treatment of "$CHI_{50}\times Pro_{10}$" which had the most vase-life, was successful in chlorophyll a and b preservation. Positive effects of CHI on leaves chlorophyll preservation in cut *Alstroemeria* flowers was recorded (Yaghoubi Kiaseh and Yadegari, 2016; Alizadeh Matak *et al*., 2017). Eason and de Vre (1995) reported that the demolition of pigment in aged flowers requires detrimental protein synthesis, and CHI as detrimental protein synthesis inhibitor, hinders increase or demolition of pigments in cut flowers. Thimann (1987) reported that CHI prevents chlorophyll demolition in *Avena* leaves but there no sign of significant increase of chlorophyll a and b by CHI application in the present research. Preservation of chlorophyll in rose (Farshid *et al*., 2013) and periwinkle (Talaat *et al*., 2005) flowers with application of amino acids was reported which corresponds to the present research. Poursoltan Hojagan *et al.* (2017) reported that the positive effect of proline and arginine on photosynthetic pigment is related to anti-oxidants and anti-stress effect of these amino acids and also is related to metabolic products of these compounds.

Total protein decreases in cut flowers after separation from stock plant is due to proteolysis and protein degradation (Rabiza Swider *et al*., 2003). Sultan and Farooq (1997) believe that separating flowers from stock plant and their confrontation with water stress causes protein decrease in petal tissue. Paulin (1983) believes that water stress in cut flowers causes protein synthesis decrease and subtraction of total protein. In the present research, the treatments of "$CHI_{100}\times Pro_{10}$" and "$CHI_{1000}\times AA_0$" which had the lowest water uptake, also had the lowest total protein. CHI at both levels of 50 and 100 mM, exclusively and combined with amino acids caused total protein increase compared to control treatment ("$CHI_0\times AA_0$"). Researchers have recorded that CHI causes protein and membrane stability and hinders senescence progress by affecting protease activity and detrimental proteins synthesis prevention (Sultan and Farooq, 1997; Borochov and Woodson, 1989; Shahri and Tahir, 2010). Preservation and increase of protein in cut *Ranunculus* flowers (Shahri and Tahir, 2010) by an application of CHI have been reported which corresponds to the present research.

Arginine and proline amino acids in combination with all four levels of CHI cost total protein increase in petals of *Alstroemeria* flowers. Asghari *et al.* (2006) reported that the application of external amino acids through increasing nitrogen percentage and free amino acids, causes protein increase in plant tissue which conforms to the results of the present research. In the time of senescence and environmental stresses, the activity of oxygen free radicals increases. One of the destructive impact of the activity of oxygen free radicals is demolition of proteins (Davies *et al*., 1987). Proline amino acid is a non-enzymatic antioxidant, with smolite effect, which plays a significant role in decreasing destructive stress applied to the plant, membrane structure preservation, and stabilization of protein structure (Szbados and Savoure, 2009).

Generally we can say that the external application of amino acids as the main components of proteins, and supplier source of nitrogen (Karima *et al*., 2005; Haj Seyed Hadi *et al*., 2011), provides the energy and amino acids needed for proteins formation and life continuation in cut *Alstroemeria* flowers; and arginine 5 mM was the most successful treatment in protein preservation in petals of this cut flower. As we know arginine is one of the main protein components, and its positive effect on petal proteins preservation can be related to anti-oxidant an anti-aging effect of metabolic products of this amino acid.

MDA aggregation in plant tissue is a sign of senescence, lipid peroxidation, and membrane damage (Ezhilmathi *et al*., 2007). In the present research, increase of CHI concentration caused MDA increase and decreased SOD enzymes activity in compared to control treatment. Results of Alizadeh Matak *et al*. (2017) indicated that CHI application on vase-life solution of cut *Alstroemeria* flowers caused MDA aggregation decrease and subtraction of anti-oxidant activity of POD enzyme. In addition, CHI at 50 mg L^{-1} cause significant decrease in SOD enzyme activity that conforms to the results of the present research.

As it was mentioned in the results, amino acids at all 4 levels of CHI caused membrane stability and vase-life increase in cut *Alstroemeria* flowers by decreasing lipid peroxidation and MDA aggregation. Also, in the present research, treatments with the most MDA had less SOD activity. SOD and CAT are enzymatic defense system of plants. It has been proved that preserving anti-oxidant enzymes activity through scavenging of oxygen free radicals causes MDA aggregation decrease and membrane structure preservation (Nasibi *et al*., 2016). In the present research, amino acids at all 4 levels of CHI caused increase in SOD enzyme activity, and the most SOD activity was obtained by superior treatments of vase-life.

The effect of experimental treatments on CAT enzyme activity was variable, although the most CAT enzymes activity was obtained by the treatment of "CHI_{50}×Pro_{10}" which had the most vase-life content, arginine 5 mM at all 4 levels of CHI did not have an acceptable impact on anti-oxidants enzyme activity preservation. This result indicates the positive effect of arginine and proline amino acids on anti-oxidant enzyme activity and MDA aggregation decrease, in the set of these factors cause post-harvest vase-life increase. Proline has anti-oxidant impact, and the protective effect of this amino acid against ROS damage has been reported in prior research (Alia and Saradhi, 1991; Matysik *et al*., 2002). Results of Alipour *et al*. (2016) showed that treating tuberose flowers with proline causes increase in CAT, guaiacol peroxidase, and ascorbate enzymes activity and decrease in PPO enzyme activity. In Kumar *et al*. (2010) research, application of proline decreased free radicals and increased SOD enzyme activity in petals of cut rose flowers which conforms to the result of the present research. Alia and Saradhi (1991) reported that proline decreases oxygen free radicals damages by physiological shut down of oxygen signals and reaction with hydroxyl radicals.

Alipour *et al.* (2013) indicated that the application of arginine in vase-life solution of cut tuberose flowers increases longevity through antioxidant enzyme activity increase which corresponds with results of the present research. Positive effect of arginine on anti-oxidant enzyme ac-

tivity preservation an MDA aggregation decrease seems to be related to the role of this amino acids in polyamines biosynthesis. Polyamines as anti-aging factors (Kandil *et al*., 2011), osmotic pressure regulator, oxygen free radicals scavenging, hinder membrane demolition and preserve membrane stability (Bouchereau *et al.,* 1999; Pandey *et al.,* 2000).

PPO is a strong oxidizing agents which causes decay and brown color in petals and fruit by oxidation of phenols, therefor decreasing the activity of this enzyme causes post-harvest longevity increase (Dubravina *et al*., 2005). In the present research, use of amino acids caused decrease in PPO enzyme activity which was acceptable according to the positive effects of these compounds on water uptake and cellular turgidity preservation. High concentration of CHI (1000 µM) which had the weakest revenue in vase-life preservation, also had the most PPO enzyme activity. Alipour *et al*. (2016) reported that proline is an anti-oxidant compound which causes vase-life increase in cut tuberose flowers by increasing antioxidant enzymes activity and decreasing PPO enzyme activity. In Nasibi *et al*. (2014) research the application of arginine caused PPO enzyme activity decrease in cut tuberose flowers which corresponds to the present research. As it was mentioned in results, application of high concentration of CHI (1000 mM) had negative impact on all studied traits, and decreased vase-life and related traits in comparison to the control treatment. The negative effect of high concentrations of CHI on vase-life and related traits in cut *Ranunculus* (Shahri and Tahir, 2010), *Hemerociallis* (Islam *et al.,* 2011) and *Nerine sarniesis* (Gul *et al*., 2012) also has been reported in prior research which confirmed to the results of the present research.

CONCLUSION

Interactive impact of "CHI × amino acid" on vase-life of cut flowers hasn't been studied before. Results of our study indicated that the treatment of "CHI_{50}×Pro_{10}" and "CHI_{0}×Arg_{5}" had the most proper revenue in the assessed traits, and their application on cut *Alstroemeria* "Mars" flowers in order to increase vase-life and related traits, is recommended. Applying high levels of CHI (1000 µM) is not recommended though, due to it' negative effects.

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