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The Effect of Ethanol and Cycloheximide on the Vase Life of Cut Flowers Alstroemeria (*Alstroemeria hybrida*)

Dina Yaghoubi Kiaseh [1] and Mehrab Yadegari [2*]
[1] Department of Plant Protection, Arak Branch, Islamic Azad University, Arak, Iran
[2] Department of Agronomy and Medicinal Plants, Faculty of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

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*Corresponding author's email: Mehrabyadegari@gmail.com

Abstract

Alstroemeria is one of the most beautiful flowers in the family Alstroemeriaceae. One of the major problems of this cut flower is short life of leaves, this fact reduces the economic value of this cut flower. Abscission of petal before wilting is also considered as one of the most important problems of this flower. Petals of this flower are very sensitive to ethylene. A factorial experiment based on completely randomized design was conduced to study the interaction effect of ethanol and foliar spraying cycloheximide (CHI) on the vase life and postharvest characteristics of alstroemeria with four levels of ethanol (0, 2, 4 and 6%) and foliar spraying CHI in three levels (0, 0.5, 1 mM) in three replications and 36 experimental plots with five cut flowers per plot. ANOVA showed that the effects of interaction ethanol and CHI on the measured traits were significant at 5% for the vase life and 1% for the other traits. Results showed that the treatment of 1 mM CHI and 6% ethanol with 15.94 days has the maximum vase life in compared with the control (10.74 days).

Keywords: °Brix, Ethylene, Longevity, Ornamental plants, Postharvest.

INTRODUCTION

Alstroemeria (*Alstroemeria hybrida*) is one of the most popular flowers in the family Alstroemeriaceae that is grown due to the beautiful flowers with short life (Hofreiter and Rodriguz, 2006; Reid, 2002). The most important cut flowers in terms of marketing in Japan, the Netherlands, England and America has allocated to this flower in the last decade. In some countries, alstroemeria is used for landscape, lawns and flowering pot plant. Elegance, beauty, variety of colors, requiring less energy to produce and long life are many peiority for the success of this flower in international trade (Kim, 2005). Alstroemeria is one of the flowers that are sensitive to ethylene that decreases its vase life (Wagstaff *et al*., 2002). Although, the leaf senescence is genetically controlled using programmed processes. But the factors such as high temperature, dryness, scar and darkness increase the speed of this process (Ranwala and Miller, 2000).

Ethanol C_2H_5OH has been also known as ethyl alcohol and ethylic alcohol. Ethanol by preventiong of the producing ethylene has increased the longevity of carnation (Meng and Wang, 2004). Treatment of 2% ethanol + 2.5 % sucrose has the maximum impact on the longevity of lisianthus cut flowers (Farokhzad *et al.*, 2005). Hojjati *et al.* (2007) reported an increase in life of lisianthus flowers by using ethanol.

CHI ($C_{15}H_{23}NO_4$) at high concentrations has been known as an inhibitor of protein synthesis at the level of transcription and translation (Tabita and Shono, 2001). Gul and Tahir (2012) reported that CHI at high concentrations prevents flower opening and stimulates aging. Aging stimulation by CHI is due to the synthesis of some specific proteins (enzymes) that are responsible for development of programmed cell death in flower petals. CHI at low concentrations increased vase life of the flower, maintained flower opening speed and delayed aging (Gul and Tahir, 2012). The aim of this study is to investigate the use of CHI along with ethanol to improve vase life and electrolyte leakage during postharvest life of alstroemeria cut flowers.

MATERIALS AND METHODS

In January 2015, alstroemeria cut flowers harvested at commercial stage were prepared from Tehran and immediately transferred to the postharvest laboratory for treatment and evaluation characteristics. This study was performed in a factorial experiment based on CRD with two factors of ethanol at 4 levels (0, 2, 4, 6%) and CHI spraying at three levels (0, 0.5 and 1 mM) with 12 treatments, three replications, 36 plots, five cut flowers in each plot and overall 180 cut flowers. The vase life of cut flowers were evaluated in 12-hour photoperiod, light intensity of 12 μmol m^{-2} s^{-1}, relative humidity of 60 to 70% and the temperature of 20 ± 2 °C.

Characteristics that were measured include vase life, chlorophyll a and b, petal anthocyanins, °Brix, ratio of flowers opening, electrolyte leakage and dry matter percent.

In order to measure the amount of chlorophyll a and b, a cut flower was removed from every plot at the end of vase life of control. The amount of chlorophyll a and b was measured by using Mazumdar and Majumder method (2003).

To measure the anthocyanins, a cut flower was removed from each plot at the end of vase life of control and 0.5 g of petal was weighted and extracted. Anthocyanins were evaluated by method of Mazumdar and Majumder (2003).

In order to measure °Brix, a handheld refractometer was used. So that after recutting the stemend at the beginning and end of the vase life of the control, a drop of water in recut sections was pourd on the glass plate of refractometer (model N-1α manufactured by ATAGO, Japan) and °Brix was read. The difference between the values obtained by measuring average of Brix on the first day and the last day of vase life of control was considered as °Brix of the cut flowers.

To measure the ratio of open florets, counting open and close florets were performed once every two days and then ratio of flowers opening was calculated by the following equation:

Ratio of total open flowets = (Open flowers on the first day ÷ The total number of open and close flowers on the first day)/(Close flowers on the last day ÷ The total number of open and close flowers on the last day)

To measurement of electrolyte leakage at the end of vase life of the control, 0.5 g leaf of every plot with 50 ml of distilled water was placed into sealed containers at laboratory temperature for 24 hours and EC1 was measured with an EC meter. Then, to measure EC_2, 0.5 gram leaf was frozen at -20 °C for 24 hours and after 24 hours, they were again placed at room temperature for 24 hours; then the EC was measured and the electrolyte leakage was calculated using the following equation:

Electrolyte leakage = $EC_1 / EC_2 \times 100$

After ending the vase life of cut flowers, fresh weight of every flower was measured and at the end of the vase life, it was placed at 70 ° C for 24 hours. After ensuring complete drying of flowers, they were weighted by a digital scale. Dry matter percent was calculated from the following equation:

Dry matter percent = (dry weight / fresh weight of flowers at the last day of the control vase life) × *100*

Data analysis was performed by using SAS software and comparisions of data were performed according to LSD test.

RESULTS AND DISCUSSION

Vase life

Table of ANOVA shows that the simple effect of CHI and ethanol is significant at 1% level and the interation effect of them is significant at 5% (Table 1). Results indicated that the maximum vase life is related to the treatment of 1 mM CHI + 6% ethanol with 15.94 days and the minimum vase life is related to the control with 10.74 days (Fig. 1). Treatments of 4% ethanol without CHI (14.71 days), 0.5 mM CHI and 4% ethanol (15.55 days) and 0.5 mM CHI with 6% ethanol (15.37 days) have also no significant difference with the best treatment. In the mentioned treatments, concentrations of ethanol (with or without CHI) was high and it seems that ethanol has played a important role in increasing vase life.

It seems that ethanol and CHI acts as anti- aging by reducing the harmful effects of ethylene enhances the life of the flowers, which are sensitive to ethylene. Treatment of CHI on *Hemerocallis fulva* causes significant delay aging and also prolong the vase life of the flowers sprayed with CHI (Gulzar *et al*., 2005). In *Hemerocallis fulva* cut flowers that were treated with CHI after harvesting, rolling and wilting leaves were delayed two days and four days compared to the control, respectively (van Doorn *et al*., 1994). The use of ethanol in low concentrations as a preservative solution, increases the vase life of carnation cut flowers (Podd and van Staden, 1998). Sharif Hossein *et al*. (2007) also stated that using 8 and 10% ethanol is effective in delay aging of *Bougainvillea*. Wu *et al.* (1991) and Petridou *et al*. (1999) also reported the use of 2 to 8 % of ethanol and methanol treatments for increasing the life of carnation cut flowers. Petridou *et al*. (2001) and Pun *et al.* (1999) reported that ethanol by preventing ethylene production is effective in increasing vase life of carnation and chrysanthemum. In a study on the durability of carnation cut flowers with continuous treatment with 8% ethanol, the vase life was increased for two-fold (van Meeteren and van Gelder, 1999).

Chlorophyll a, b

ANOVA showed that the effects of CHI, ethanol and the interaction of them on chlorophyll a and b is significant at 1% level (Table 1). The mean comparison shows that the maximum amount of chlorophyll a and b are related to 1mM CHI + 6% ethanol with 6.33 and 2.883 mg g^{-1} FW, respectively and the minimum amount is related to the control flowers with 2.483 and 1.203 mg g^{-1} FW (Fig. 2 and 3).

It seems that the above anti-ethylene treatments cause the preservation of chlorophyll by inhibition of activity of chlorophyllase enzyme and development of chloroplasts. Yellowing of the leaves is a common phenomenon in many sensitive species such as alstroemeria, lily, chrysanthe-

Table 1. Analysis of variance of effects of ethanol and cycloheximide on vase life and quality of cut alstroemeria

S.O.V	df	Vase life	Chlorophyll a	Chlorophyll b	Petal anthocyanin	°Brix	Ratio of open florets	Electrolyte leakage	Dry matter
C (Cycloheximide)	2	6.05**	8.765**	0.332**	0.241*	0.175ns	0.088**	24.32**	2.83*
E (Ethanol)	3	13.16**	10.05**	1.997**	10.21**	0.156ns	0.062**	3.39ns	2.04ns
C*E	6	2.022*	0.838**	0.984**	3.83**	0.989**	0.055**	38.46**	4.219**
Error	24	0.552	0.003	0.003	0.047	0.235	0.010	4.02	0.783
CV (%)		5.299	1.306	2.618	5.343	12.418	17.944	24.723	7.514

*: Probability 5%   ns: Not significant   **: Probability 1%

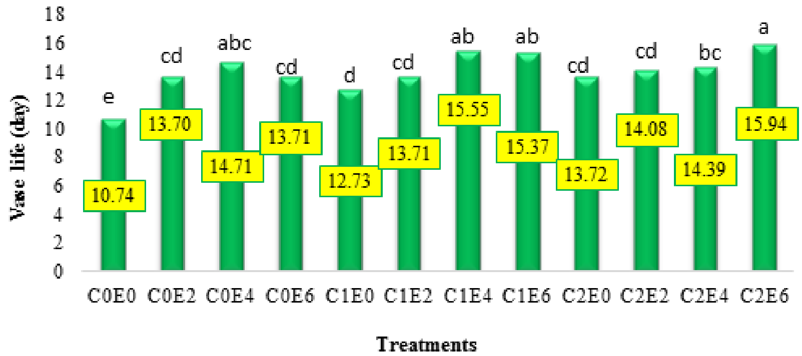

Fig. 1. Effect of ethanol and cycloheximide treatments on vase life.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

mum and stock. In many species, leaf yellowing may be caused by placing plants in the dark for a long time during storage or transport (Ferrant *et al*., 2003; Reid, 2002). Compounds such as CHI can prevent degradation of chlorophyll and can also delay the production of ethylene. CHI causes to delay the destruction of chlorophyll and protein in oats leaf and delay increasing respiration in leaf senescence (Thimann, 1987). Treatments ethanol and methanol at 2 % had the lowest rate of degradation of chlorophyll a and b. Petridou *et al.* (2001) showed that different concentrations of ethanol impact efficiently on the amount of chlorophyll and photosynthesis efficiency in chrysanthemum cut flower.

Petals anthocyanins

Analysis of variance showed that the effect of CHI is significant at 5% level, but effects of ethanol and interaction of them on anthocyanin of petals are statistically significant at 1% level (Table 1). The mean comparison shows that maximum anthocyanins of petals is related to the treatment of 0.5 mM CHI without ethanol with 6.156 µg g^{-1} FW and the lowest anthocyanin of petals is related with the treatment of 6% ethanol without CHI treatment with 2.556 µg g^{-1} FW (Fig. 4).

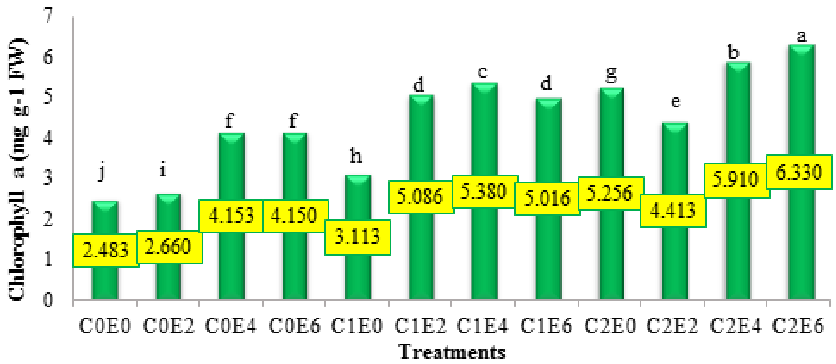

Fig. 2. Effect of ethanol and cycloheximide treatments on chlorophyll a.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

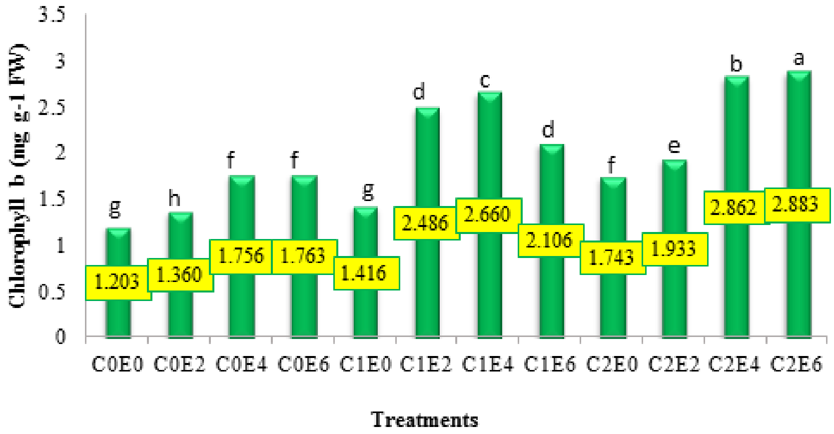

Fig. 3. Effect of ethanol and cycloheximide treatments on chlorophyll b.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

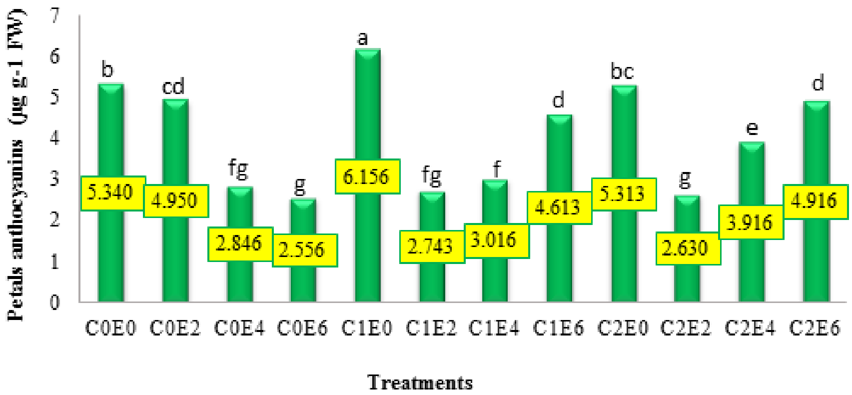

Fig. 4. Effect of ethanol and cycloheximide treatments on petals anthocyanins.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

Behavior of petals anthocyanins against treatments of CHI and ethanol does not follow from a specific pattern. It seems that the absence of ethanol in similar treatments had the maximum anthocyanin. Moreover, the amount of petals anthocyanins is enhanced by increasing ethanol from 2 to 6%. CHI at concentration of 0.5 mM had a positive effect on the petals anthocyanin (Fig. 4).

The presence of pigments in the petals of the plant is the indicator of quality after harvesting cut flowers that are a major cause of aging and vase life. Among these pigments, carotenoids and anthocyanins have a particular importance in the postharvest life of cut flowers. Collorless is one of the most common symptoms in many old flowers and changing the color of the old petals largely depends on pH changes in the vacuole (Edrisi, 2009). Petridou *et al*. (2001) showed that ethanol prevents formation of anthocyanins in the petals of chrysanthemum and provides the possibility to preserve natural white color of flowers during the vase life. The results are consistent with the results of current experiment.

Increasing ºBrix

Table of analysis of variance shows that the effect of ethanol and CHI is not significant, but the effect of interaction of them on the increasing °Brix is statistically significant at 1% level (Table 1). The mean comparison shows that the maximum increasing °Brix is related to the treatment of 1 mM CHI along with 6% ethanol with 3.86 % and the lowest increasing °Brix is related to of 2.25 % in the control treatment (Fig. 5).

The superior of these compounds can be anti-ethylene property of CHI and ethanol that in-

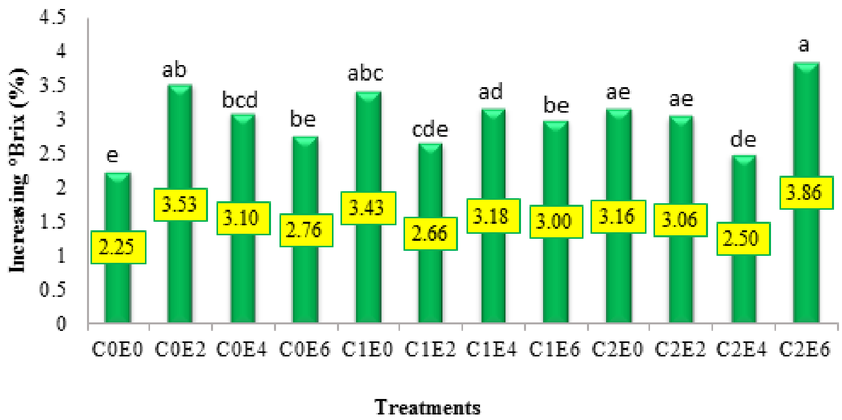

Fig. 5. Effect of ethanol and cycloheximide treatments on increasing °Brix.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

crease water absorption and sugar of the stem and decreases respiration rate. Any compound that can prevent from catabolism of carbohydrates, can improve the carbohydrate content and maintain these compounds in the stem. Carbohydrates are the main source of nutrition of flowers and source of required energy for all biochemical and physiological processes of cut flowers. The most important factor in delaying aging of cut flowers is increasing the amount of carbohydrates. Therefore, whatever the stored carbohydrate becomes more, the vase life is increased (Mutuie *et al.*, 2001).

Basiri *et al*. (2011) investigated the effect of anti-ethylene compounds on Brix degree of "White Liberty" of carnation and concluded that this compound is desirable to improve Brix degree. Podd and van Staden (1998) stated that alcohol prevents transferring carbohydrates from petal to ovary, as a result respiratory carbohydrates remain in the petals and are used for metabolism of the petals.

Ratio of open florets

Analysis of variance showed that the effects of ethanol, CHI and the interaction of them were statistically significant at the 1% level (Table 1). Comparison of the means of interaction of them showed that the maximum ratio of open flowvets is related to the treatment of 4% ethanol without CHI with 0.700 and the lowest ratio of open florets was related to the treatment of 1mM CHI with 6% ethanol with 0.370 (Fig. 6).

It seems that in this study, anti-ethylene treatments with reducing ethylene production, have caused the prevention of wilting flowers and have been accelerated flowers opening. Hashemabadi

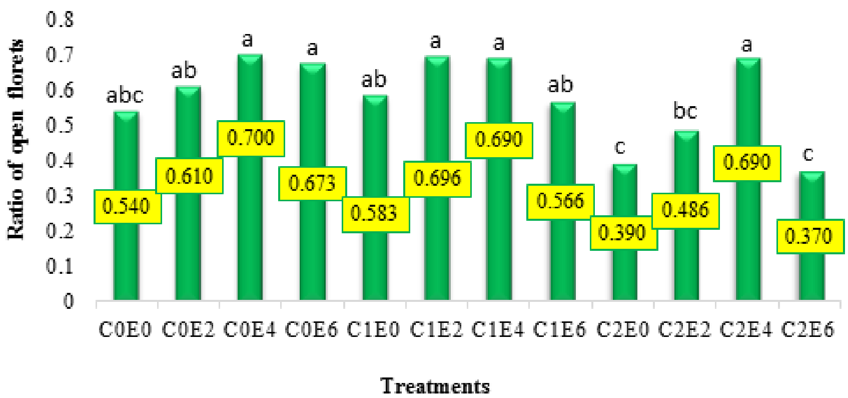

Fig. 6. Effect of ethanol and cycloheximide treatments on ratio of open florets.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

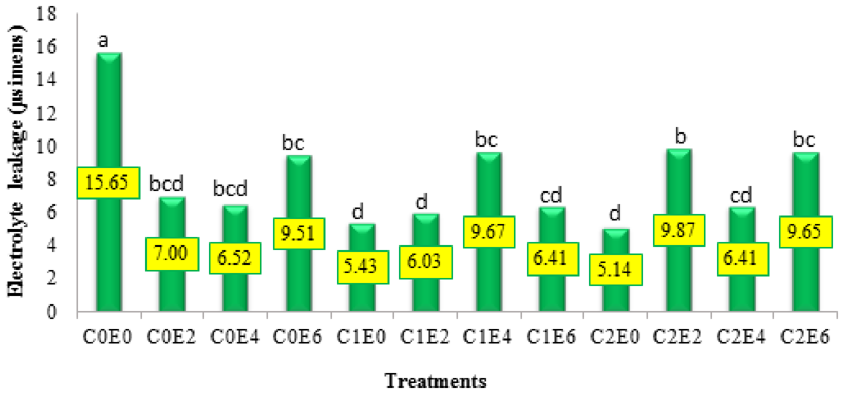

Fig. 7. Effect of ethanol and cycloheximide treatments on electrolyte leakage.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

(2006) also found that the use of anti-ethylene compounds reduced flowers opening process compared to the control by 17%. CHI relatively delayed opening of flowers due to control respiration (Gul and Tahir, 2012). It was found that CHI prevents from opening of flowers of carnation, lily and iris and delays aging (Shahri and Taher, 2010; Islam *et al*., 2011). Petridou *et al*. (2001) reported that ethanol treatments at low concentrations cause to maintain proper form of flower buds and improve the process of opening them.

Electrolyte leakage

Analysis of variance shows that the effect of ethanol is not significant, but the effects of CHI and interaction between them on electrolyte leakage is statistically significant at 1% level (Table 1). Results showed that the minimum electrolyte leakage is related to the treatment of 1 mM CHI without ethanol with 5.14 µsiemens and the maximum electrolyte leakage is related to the control with 15.65 µsiemens (Fig. 7). The highest electrolyte leakage is observed in treatments without CHI. Between two concentrations of 0.5 and 1 mM of CHI, 1 mM has higher electrolyte leakage than 0.5 mM. This means that CHI reduces electrolyte leakage, but it has a negative effect on electrolyte leakage at very high concentration.

It seems that of anti-ethylene compounds reduced ionic leakage because these compounds maintained the water balance and reduced water stress. The use of protein synthesis factors like CHI can help to maintain cell membrane. There are several data that show CHI effects on the transfer and absorption of ions (Wildes *et al*., 1970) and amino acids (Hunter *et al.*, 1973). Pulse treatment of narcissus with CHI increases dry and fresh weight of flowers and decreases electrolyte leakage (Gul and Tahir, 2012). Electrolyte leakage reduction in petal cells by CHI indicates that this substance prevents the synthesis of proteins that increase ions (van Doorn *et al.*, 1994).

Dry matter

Analysis of variance of data shows that the effect ethanol is not significant but CHI effect is significant at 5% level. The interaction of these two factors on dry matter is also statistically significant at 1% level (Table 1). Mean comparison shows the maximum dry matter is related to the treatment of 0.5 mM CHI along with 6% ethanol with 13.85 % and the minimum dry matter is related to the control treatment with 10.72 % (Fig. 8).

Increasing the volume of carbohydrates in cut flowers may be associated with the role of alcohol in decreasing respiratory rate and reducing ethylene production of the flower. The last stage of the development of flowers is along with degradation of dry weight and carbohydrate content of petals and life of cut flowers are improved by placing flowers in the vase solution containing carbohydrate (Bolandraftar, 2013). In cut flowers, the major dry matter component includes car-

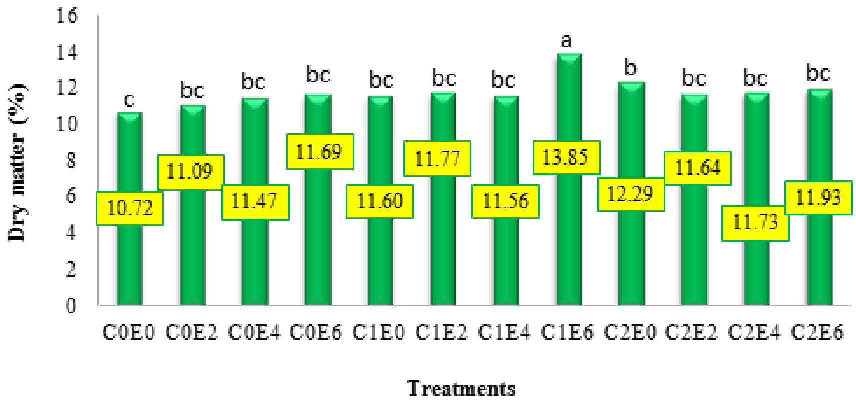

Fig. 8. Effect of ethanol and cycloheximide treatments on dry matter.
Cycloheximide (C0:0 Mm; C1:0.5 mM; C2:1 mM);
Ethanol (E0: 0 %; E2: 2 %; E4: 4 %; E6: 6 %)

bohydrates and proteins (Mostofi *et al.*, 2009). Pulse treatment of narcissus with CHI increased fresh and dry weight of this flower (Gul and Tahir, 2012). Blankenship and Dole (2003) reported that with decreasing in respiratory rate of cut flowers, carbohydrate catabolism is reduced and this leads to prevent degradation of sugars and higher dry weight percentage of cut flowers.

CONCLUSION

The results of this study showed that the use of anti-ethylene compounds of CHI and ethanol has improved the vase life of alstroemeria cut flowers by improving characteristics that related to vase life. The results showed that among different treatments, 1 mM CHI spraying along with 6% ethanol, 0.5 mM CHI along with 4% ethanol and 0.5 mM CHI along with 6% ethanol had the maximum increase of the vase life. Therefore, the treatment of 0.5 mM CHI along with 4% ethanol can be introduced as the best treatment.

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