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Improvement Postharvest Quality of Cut Alstroemeria (*Alstroemeria hybrida*) by Stem-End Splitting and Ethanol

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Abstract

For the improvement the postharvest longevity of cut alstroemeria (*Alstroemeria hybrida* L.), a factorial experiment was carried out based on RCD with 2 factors of stem end splitting at two levels (5 cm splitting and without splitting) and ethanol at five levels (0, 1, 2, 4 and 6%), 10 treatments, 3 replications and 30 plots. In this experiment, the estimated traits included vase life, water absorbtion, fresh weight, dry matter percent, ethylene, petal anthocyanin, electrolyte leakage and lipid peroxidation (MDA). ANOVA showed significant differences among treatments for vase life, water absorbtion, dry matter percent and electrolyte leakage at the 5% probability level and for other traits at the 1% probability level. Results showed that different treatments improved vase life as compared to control and maximum vase life of 19.77 days was achieved under the treatment of ethanol 1% + with 5 cm splitting compared to control (11.09 days).

Keywords: *Alstroemeria*, Ethanol, Stem end splitting, Vase life.

INTRODUCTION

Alstroemeria hybrida is one of the popular flowers of Alstroemeriaceae family that is grown because of its beautiful flowers with long life (Hofreiter and Rodriguz, 2006; Reid, 2002). Aging is a process of decay and a natural reason for death of an organ including cells, tissues and organs of organisms. Growth regulators, cell changes and hormone levels are the internal factors affecting the aging process (Thomas and Stoddart, 1980; Smart, 1994). The cut flowers are valuable among the garden products in terms of the economic value, but they need more post-harvest care due to quick decaying, high breathing and damage sensitivity (Chanasut *et al.*, 2003). Considering the importance of the quality of cut flowers in the flower trade, it should be tried to deliver high quality cut flowers to the consumer. One of the most important criteria for consumers to choose cut flowers is longevity and durability of the flower. For this reason, a suitable post-harvest program helps to maintain the quality of cut flowers for a longer time (Eason *et al*., 2001).

Ethanol with chemical formula of C_2H_5OH has been known as ethylic alcohol and ethyl alcohol. Nowadays, it is widely used in various industries and the importance of its application as a treatment to increase the life of ornamental flowers has been known. It is shown that ethanol increases the longevity of clove cut flowers by preventing the production and the effect of ethylene (Meng and Wang, 2004). Treatment of 2% ethanol along with 2.5% sucrose has the maximum impact on the longevity of Lisianthus cut flowers (Farokhzad *et al*., 2005). Hojjati *et al*. (2007) also reported an increase in the life of *Lisianthus* flower as a result of ethanol application. van Doorn and Dhort (1994) stated that cut flowers wilting during storage is due to inadequate absorption of water by the closure of the vessels as a result of the growth of bacteria, sedimentation of materials such as gums, formation of tyloses and the presence of air bubbles in the vascular system that is solved by the application of a split at the end of the branching phase. de Stigter and Broekhuysen (1986) reported that water exposure of the broad surface of the stem has a positive impact on increasing the side movement of water inside the xylem.

The aim of this study is to investigate the effect of a split in the stem end along with ethanol on the improvement of water absorption and the reduction of ethylene production of alstroemeria cut flowers.

MATERIALS AND METHODS

In January 2015, alstroemeria cut flowers harvested at the commercial stage were procured from Tehran and were immediately transferred to the post harvesting laboratory for treatment and evaluation of the properties. This study was continuously performed as a 2-factor factorial experiment based on a completely randomized design with two factors of a split in stem end at two levels (5 cm splitting and without splitting) and ethanol at five levels (0, 1, 2, 4 and 6%), with 10 treatments, 3 replications, 30 plots, 5 flowers in each plot and a total of 150 flowers in the laboratory under a 12-h photoperiod, light intensity of 12 µmol s^{-1} m^{-2}, relative humidity of 60 to 70 percent and the temperature of 2 ± 20°C for the evaluation of vase life. Flowers were uniformly recut

Fig.1. The first day of vase life (a), the end of vase life (b).

with a height of 50 cm and were put in prepared solutions after removing the leaves at the end of stem and weighing. Vase life was defined as the time from the start of treatment until the senescence of flowers (Fig. 1).

Considering initial volume of vase solution (600 mL) and the rate of evaporation in room and the reduction of the volume of the vase solution, water absorption was calculated by following equation:

Water absorption (ml g^{-1} FW) = 600 - (mean evaporation of room + remained solution at the end of vase life) / the average fresh weight of cut flowers

Regarding the final weight of flower in the last day, recut weight, weight of losses and weight of the first day, the increase in fresh weight was calculated according to the following equation:

Fresh weight increasing = (weight of losses + weight of recut+ final weight at last day of the control life) - initial weight.

At the end of the vase life of the control, the fresh weight of each 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 weighed 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.

To measure the amount of anthocyanins of petals on the fifth day of experiment, a branch was removed from each plot and the anthocyanin pigment was measured using Mazumder and Majumdar (2003)' method. To measure the amount of released ethylene in the second day, a branch was selected from each plot and the amount of ethylene was measured using Chamani (2004)'s method. In order to assess electrolyte leakage at the end of vase life of the control, 0.5 g leaf of each plot with 50 ml of distilled water was placed into sealed containers at laboratory temperature for 24 hours and its EC1 was then measured with an EC measuring device. Then, to measure EC2, 0.5 gram leaf was frozen at -20°C for 24 hours and afterwards, they were again placed at room temperature for 24 hours and then, its numbers were read with the EC device and the electrolyte leakage was calculated using the following formula:

Electrolyte leakage = $EC_1 / EC_2 \times 100$

For this purpose, a branch was removed at the end of vase life of the control and its petals were measured as peroxidation reaction product of membrane fatty acids to determine peroxidation of lipids and lipid peroxidation (MDA) using Heath and Parker (1986)'s method.

RESULTS AND DISCUSSION

Vase life

The results of analysis of variance showed that the simple effect of ethanol and split was statistically significant at the 1% level and the interaction of these two factors was statistically significant at the 5% level (Table 1). Comparison of the mean interaction of these two factors indicated that the maximum vase life was related to the treatment of one percent ethanol along with a split with 19.77 days and treatment of one percent ethanol without split with 19.12 days and the lowest

Table 1. ANOVA of effects of ethanol and splitting treatments on traits.

S.O.V.	df	Vase life	Water absorption	Fresh weight	Dry matter percent	Electrolyte leakage	Petal anthocyanin	Ethylene	Lipid peroxidation (MDA)
Ethanol (E)	4	48.42**	6.803**	21.36**	6.02**	3.150*	4.93**	0.315*	0.609**
Splitting (C)	1	27.37**	3.21**	18.47**	2.06*	5.418*	3.466**	0.227^{ns}	0.408**
E × C	4	1.47*	0.851*	15.37**	1.659*	3.440*	0.916**	0.517**	0.455**
Error	20	442	268	5.93761	0.466	0.920	0.00186	0.087	0.00423
CV (%)		4.403	6.96	6.61094	5.73	20.72	2.226	3.13	6.13

**: Significant at the 1% level; *: Significant at the 5% level; ^{ns}: Insignificant

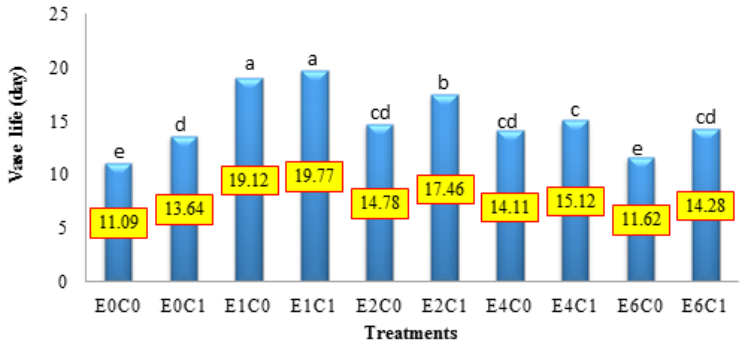

Fig. 2. Effects of ethanol and splitting treatments on vase life.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%

C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

life vase was related to control with 11.9 days and treatment of 6 percent ethanol without split with 11.62 days (Fig. 2). All treatments, as compared to the control group, increased the vase life.

One of the reasons for shorter vase life under the treatments of 2, 4 and 6 percent ethanol is high concentration of alcohol in continuous use. If treatments were applied as pulses, high concentrations of alcohol would certainly increase the vase life. In addition, a split in stem end did not have a significant effect on the generated polyethylene so that the least ethylene was obtained from 2% ethanol without split and higher concentrations of alcohol did not significantly affect ethylene production. It seems that ethanol acts as an aging deterrent by reducing the harmful effects of ethylene and prevents the obstruction of vascular at the stem end of flowers. The split at the end of the stem also resulted in the exposure of more surface of the stem xylem to the vase solution increasing the uptake of vase solution. Moreover, at the beginning of the experiment, 100 ml solution was added to the vase solution because of high rate of water uptake. Absorption of water neutralized the negative effects of ethylene produced as a result of the split and increased alstroemeria cut flower life (Mehri, 2014). The permanent and temporary use of ethanol with concentrations of 2 and 4 percent in chrysanthemum cut flower preservative solution increased the vase life (Petridou *et al*., 2001). It is also believed that ethanol hinders ethylene production by preventing the synthesis of ACC resulting in longer vase life (Wu *et al.*, 1992). Treatment of cloves cut flowers with low concentrations of ethanol dramatically increased their shelf life (Podd and Van Staden, 1998; Heins and Blakkey, 1980). In the case of alstroemeria flowers, the treatments of a split in the end of the stem had a constant effect on the vase life and water ratio. The positive effect shown in Mehri (2014)'s study was related to the exposure of a wide surface area of stem to water and also to the lateral movement of water within the xylem.

Water absorption

The results of analysis of variance showed that the simple effect of ethanol and a split was statistically significant on water absorption at the 1% level and their interaction was statistically significant for water absorption at the 5% level (Table 1). Comparison of the mean interaction indicated that the maximum water absorption is related to the treatment with 1% ethanol without split with 2.17 ml g^{-1} FW and 1% ethanol with split with 2.05 ml g^{-1} FW. The minimum water absorption is related to the control with 0.65 ml g^{-1} FW (Fig. 3).

It seems that disinfectant substances such as alcoholic solutions can increase the stem xylem hydraulic conductivity by controlling microorganisms activity relatively, and provides better conditions for the absorption of water in the stem, and so delays the aging process. The split in the end of the stem also increases water absorption as compared to the control by exposure of a broad surface of stem and xylem to vase solution (Bolandraftar, 2013). When the water absorption and

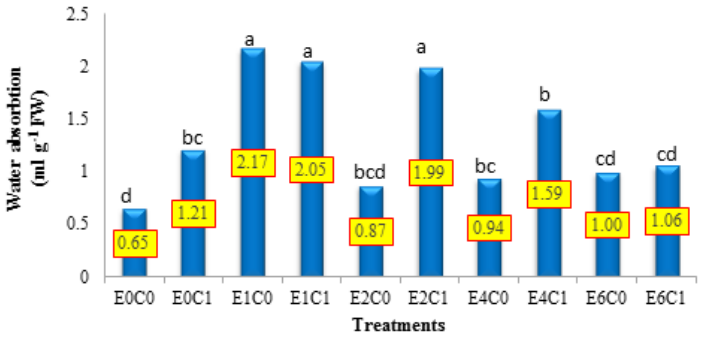

Fig. 3. Effects of ethanol and splitting treatments on water absorption.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%
C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

transpiration by cut flowers are unbalanced, they wilt prematurely as a result of the loss of cell turgor. Limited water absorption, which is caused by different factors including closure of stem xylem, can be considered as one cause of this imbalance and eventually causes irreversible wilt and the early end of the life of cut flowers (Van Meeteren *et al*., 2000). Hashemabadi *et al*. (2009) reported that the flowers of the cloves treated with anti-ethylene compounds caused a significant increase in water absorption as compared to the control treatment. In the present study, ethanol also increased water absorption as compared to the control. Ichimura *et al*. (2003) stated that hydraulic conductivity of cut flowers was reduced as a result of the bacterial proliferation and so the use of anti-ethylene compounds such as ethanol reduced bacterial proliferation and increased water absorption in cut flowers.

Increasing fresh weight

The results of analysis of variance showed that the simple effects of ethanol and split and their interaction were statistically significant for the increase in fresh weight at the 1% level (Table 1). Comparison of the mean interaction of these two factors indicated that the maximum increase in fresh weight was related to the treatment of 1% ethanol without split with 16.12g and 1% ethanol with a split with 16.09 g and that the minimum increase in fresh weight was related to the control treatment with 8.66 g (Fig. 4).

Overall, when cut flowers are placed in water, their fresh weight initially increases and

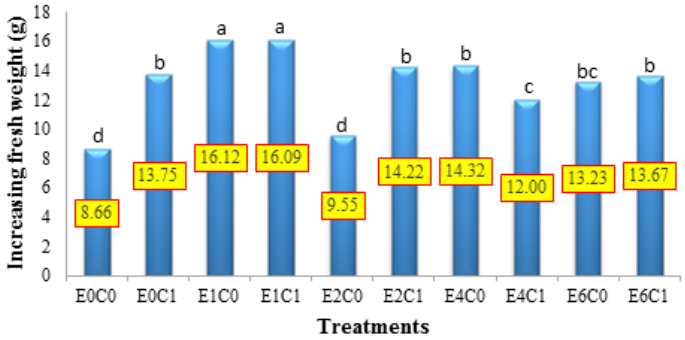

Fig. 4. Effects of ethanol and splitting treatments on increasing fresh weight.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%
C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

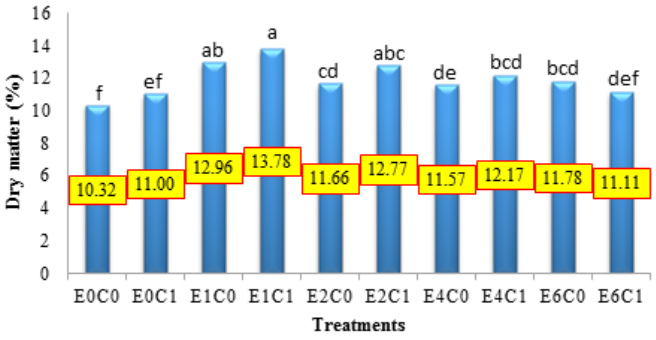

Fig. 5. Effects of ethanol and splitting treatments on dry matter percent.

C0= Without splitting
C1= With 5cm splittimg
E0= Ethanol 0%
E1= Ethanol 1%
E2= Ethanol 2%
E4= Ethanol 4%
E6= Ethanol 6%

then, start to decrease (Shimuzu-Yumoyo and Ichimura, 2005). It was shown that alcoholic compounds at low concentrations prevent vascular occlusion and maintain fresh weight at a higher level by maintaining the high volume of water and delaying the aging process of cells (Heins and Blakkey, 1980). Son *et al*. (2003) found that the treatment of "Red Sandra" rose cut flowers with anti-ethylene compounds increased water absorption and ultimately increased the fresh weight of these flowers. In fact, the fresh weight of cut flowers reduces as they approach the aging step which can be related to the reduction of water absorption and the loss of petals and leaves. Therefore, the cell turgor drops and the weight of cut flowers decreases with the loss of water (Edrisi, 2009).

Dry matter percent

The results of analysis of variance showed that the simple effect of ethanol and a split was statistically significant on dry matter at the 1 and 5% levels, respectively and their interaction was statistically significant for dry matter at the 5% level (Table 1). Comparison of mean interaction indicated that the maximum dry matter was related to the treatment with 1% ethanol with split with 13.78% and the minimum dry matter was related to the control treatment with 10.32% (Fig. 5).

An increase in the volume of carbohydrates of flower may be related to the role of alcohol in decreasing respiratory rate and reducing ethylene production of the flower (Bolandraftar, 2013). The split in the end of stem prevents oxidative stress by increasing water absorption and causes an increase in dry matter content by reducing protein degradation, decreasing respiratory rate and increasing the absorption of sucrose (Mehri, 2014). Sharif Hossain *et al.* (2007) reported an increase in the percentage of dry matter as a result of the treatment with 2% ethanol that is close to our findings. Blankenship and Dole (2003) reported that a decrease in respiratory rate of cut flowers reduced burning sugar which prevented the degradation of sugars and caused a higher level of dry weight percentage in cut flowers.

Petal anthocyanin

The results of analysis of variance showed that the simple effect of ethanol and split and their interaction was statistically significant for anthocyanin of petals at the 1% level (Table 1). Comparison of the mean interaction of these two factors indicated that the maximum anthocyanin of petals was related to the treatment with 1% ethanol without split with 4.137 µg g^{-1} FW and the minimum one was related to the control with 0.534 µg g^{-1} FW (Fig. 6).

Paleness is one of the most common symptoms in many old flowers and the change in the color of the old petals mainly depends on pH changes in the vacuole (Edrisi, 2009). Anti-ethylene compounds stabilize anthocyanin level in the petals. Ethanol prevents aging petals that is usually along with browning petals, and prevents ovarian development and its apparent death. Petridou *et*

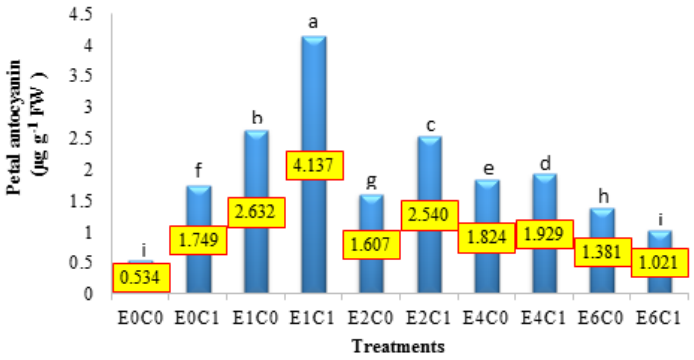

Fig. 6. Effects of ethanol and splitting treatments on petal anthocyanin.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%
C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

al. (2001) showed that ethanol prevents the formation of anthocyanin in the petals of chrysanthemum, and it may become possible that flowers maintain their natural color over the vase life.

Ethylene

The results of analysis of variance showed that the effect of the split was not significant on ethylene, but the effect of ethanol and the interaction of these two factors were statistically significant for ethylene at the 5 and 1% levels, respectively (Table 1). Comparison of the mean interaction indicated that the minimum ethylene was related to the treatments with 2% ethanol without split and 4% ethanol with split with 0.91 and 1.05 nl l^{-1} h^{-1} g^{-1} FW, respectively, and the differences in the rest treatments were not statistically significant (Fig. 7).

The effect of the split in the stem end and ethanol on ethylene production follows a specific pattern. It seems that high and very low concentrations of ethanol has the highest production of ethylene as compared to other treatments. Ethanol also has a more clearly effect on ethylene production as compared to the split in stem end. Alstroemeria flower is very sensitive to ethylene and the ethylene produced in the final stage of development of this flower decreases its vase life by perianth abscission (Wagstaff *et al*., 2002; Chanasut *et al*., 2003). Ethanol as same as silver nanoparticles because of controlling bacteria of stem end that can indirectly motivate to produce ethylene can control ethylene production and increase vase life of cut flowers of cloves and gerbera (Basiri *et al*., 2011). The above results about controlling the ethylene production using ethylene synthesis inhibitors is consistent with our results. Fahimi *et al.* (2005) examined the effect of anti-

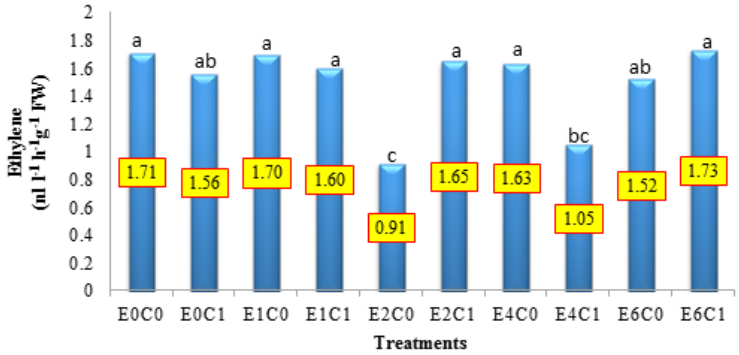

Fig. 7. Effects of ethanol and splitting treatments on ethylene.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%
C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

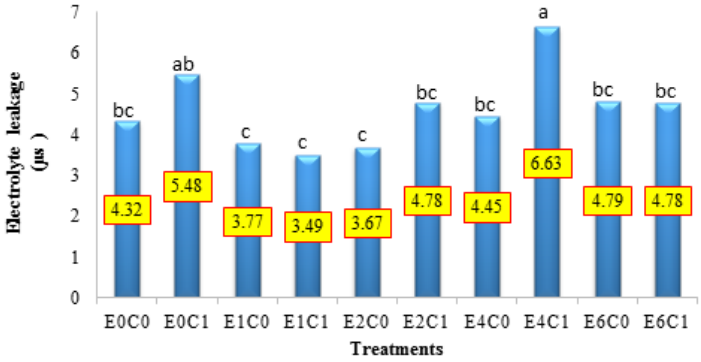

Fig. 8. Effects of ethanol and splitting treatments on electrolyte leakage.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%

C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

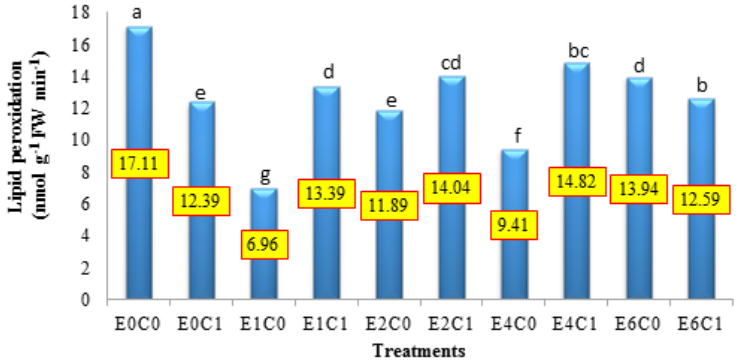

Fig. 9. Effects of ethanol and splitting treatments on lipid peroxidation.

C0= Without splitting
E0= Ethanol 0%
E2= Ethanol 2%
E6= Ethanol 6%

C1= With 5cm splittimg
E1= Ethanol 1%
E4= Ethanol 4%

ethylene compounds on the ethylene production of "First red" rose cut flowers and concluded that these compounds reduced ethylene production as compared to control and kept the ethylene production rate lower than that of control.

Electrolyte leakage

The results of analysis of variance showed that the simple effect of ethanol and split and their interaction were statistically significant for electrolyte leakage at the 5% level (Table 1). Comparison of the mean interaction of these two factors indicated that the minimum electrolyte leakage was related to the treatment of 1% ethanol along with a split with 3.49 µs and the maximum electrolyte leakage was related to the treatment of 4% ethanol along with a split with 6.63 µs (Fig. 8).

Lipid peroxidation (MDA)

Results of analysis of variance revealed that the effect of ethanol and split and their interaction were statistically significant for lipid peroxidation at the 1% level (Table 1). Comparison of the mean interaction of these two factors showed that the minimum amount of MDA was related to the treatment of 1% ethanol without split with 6.96 nmol g^{-1} FW min^{-1} and the maximum amount of MDA was related to control with 17.11 nmol g^{-1} FW min^{-1} (Fig. 9).

Since ethanol tends to dissolve lipids of the plant surface and the plasma membrane, it changes membrane permeability and as a result, it has a great impact on anatomical structure and

plant growth (Bolandraftar, 2013). Changes in cell membranes involved in the aging of flowers. This change is a result of active metabolic processes. There is some evidence that specific genes are responsible for controlling these processes (Isavand and Ashouri, 2010). With the development of the aging of plant organs and the positive role of ethylene and water stress in this process, oxygen radicals are generated that lead to peroxidation of membranes. MDA as a product of lipid peroxidation is increased. Anti-ethylene compounds like ethanol and a split in the stem end increase water absorption and reduce oxygen radicals and thus, MDA level decline (Bolanraftar, 2013).

CONCLUSION

Based on the results of the present study, among studied treatments, the treatment of 1% ethanol with a split in the end of stem showed the best performance as compared to other treatments. The use of alcoholic treatments along with a split significantly improved many characteristics such as vase life, water absorption, fresh weight, dry matter percentage, ionic leakage and anthocyanin of petal.

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