Journal of Ornamental Plants
*Available online on: www.jornamental.com*
*ISSN (Print): 2251-6433 ISSN (Online): 2251-6441*

# Study on Effects of Ascorbic Acid and Citric Acid on Vase Life of Cut Lisianthus (*Eustoma grandiflorum* 'Mariachi Blue'

Farnaz Sheikh[1], Seyed Hossein Neamati[2], Navid Vahdati[3] and Ali Dolatkhahi[3]
[1] MSc, Horticulture Department, Ferdowsi University of Mashhad.
[2] Assistant Professor, Horticulture Department, Ferdowsi University of Mashhad.
[3] PhD Student, Horticulture Department, Ferdowsi University of Mashhad.

Received: 15 July 2014 Accepted: 10 January 2015
*Corresponding author's email: nvnavid345@gmail.com

Abstract

The postharvest life of cut Eustoma grandiflorum flowers is limited in open flowers. Therefore a factorial experiment based on a completely randomized design with ascorbic acid (AsA) at 4 levels (0, 100, 200, 300 mg $L^{-1}$) and citric acid (CA) at 3 levels (0, 100, 200 mg $L^{-1}$) with 3 replications and 3 samples for each replications, was conducted for this purpose. Results indicated that a significant increase with applying ascorbic and citric acid nearly in all traits both individually and in combination, with higher concentrations imposing greater effects ($p\leq0.05$ and $p\leq0.01$). The highest vase life (17.6 days) and petal water content (68.9%) was observed for the interaction of ascorbic acid (300 mg $L^{-1}$) and citric acid (100 mg $L^{-1}$) and ascorbic acid (300 mg $L^{-1}$) and citric acid (200 mg $L^{-1}$), respectively, which shows a 94 and 252% increase compared to control (9.1 days and 27.3%). Along with this, relative water content and petal water content raised with AsA and CA increase. Water content also showed a similar manner. Fresh weight decreased in all treatments during experiment, but this reduction was much less in AsA (300 mg $L^{-1}$) alone and in interactions with CA levels. According to the results of this experiment, ascorbic acid and/or citric acid as cheap, safe and biodegradable compounds are suitable alternatives for chemical treatments in order to prolong vase life of cut flowers of Eustoma. Commercialization of these compounds for optimum formulations needs further experiments.

**Keywords:** Cut flowers, Organic Acids, Postharvest life, Preservative solution.

## INTRODUCTION

Cut flowers are precious products in horticulture. Maintaining good quality of cut flowers and extending the vase life, is considered important and practical for having acceptable products for the markets especially for sensitive species. In general, short vase life of cut flowers is attributed to several factors e.g. carbohydrate depletion (Ketsa, 1989); water stress (Sankat and Mujaffar, 1994), microorganisms (especially bacteria) (van Doorn and Witte, 1991) and ethylene (Wu *et al.*, 1991). In addition, a major cause of deterioration in cut flowers is blockage of xylem vessels by microorganisms that accumulate in the vase solution or in the vessels themselves (Danaeeet al, 2011). For many years, floral preservatives have been acidified and have usually included biocides to inhibit bacterial proliferation (Nowak and Rudnicki, 1990).

Lisianthus is one of the newly introduced cut flowers and is ranked as one of the top ten economically important cut flower in the world (Dole and Wilkins, 1999). Mean vase life of lisianthus cut flowers is considered to be 2 weeks; however it varies greatly among its cultivars (Dole and Wilkins, 1999).

Ascorbic acid (AsA) is a bacteria growth inhibitor commercialized recently, which has been extensively evaluated for protecting cut flowers from physical plugging (Jin *et al*., 2006, Hatami *et al.*, 2010). AsA is synthesized in higher plants and affects plant growth and development. It plays an important role in the electron transport system (El-Kobisy *et al.,* 2005). Robinson (1973) reported that AsA acts as a co-enzymatic reaction by which carbohydrates; proteins are metabolized and involved in photosynthesis and respiration processes. Bolkhina *et al.* (2003) stated that AsA is the most abundant antioxidant which protects plant cells, and it is currently considered to be a regulator on cell division and differentiation and added to that, it is involved in a wide range of important functions such as antioxidant defense, photoprotection, regulation of photosynthesis and growth. Pretreatment of cut rose with AsA for 12 h prolonged vase life of plant exposed to water deficit stress (Jin *et al.*, 2006).

Besides ascorbic acid, citric acid is also known as a potential component of vase solution in order to enhance cut flowers vase life (Vahdati *et al.,* 2011). Citric acid (CA), is as well a wide spread organic acid in plant kingdom and makes a weak acid when dissolved in water. CA is used to adjust water pH and to control the growth of microorganisms. CA is commercially advised for a number of cut flowers like chrysanthemum (Dole and Wilkins, 1999). Also, CA reduces the risk of vascular blockage in cut flowers through its anti-embolism habit (Bhattacharjee *et al.,* 1993). Adding CA to vase solution decreased pH and microbial activity and hence increased vase life of cut carnation flowers by 30-40% over control (Ebrahimzadeh *et al*., 2003). An enhancing effect of CA was also observed in 'Bougati' and 'Rollet' cultivars of cut rose flowers (Lechinani, 2006).

The main purpose of this work was to determine the response of cut lisianthus flowers to ascorbic and citric acid preservatives and their effects on vase life and qualitative characteristics of cut flowers.

## MATERIALS AND METHODS

### Plant materials

Lisianthus (*Eustoma grandiflorum*. Cv. Mariachi Blue) flowers grown in a standard greenhouse were supplied from a local grower. Cut flowers were trimmed to 30 cm after arrival to the laboratory and were placed in prepared solutions. Experimental conditions were carefully monitored at 20 ± 2 °C mean temperature, 60 ± 10 % RH and 15 µmol $m^{-2}s^{-1}$ with a 12 hour photoperiod using florescent lamps.

### Treatments

AsA and CA are soluble in water and are easily dissolved in optimum concentrations. Four levels of AsA as follow; 0, 100, 200, 300 mg $L^{-1}$ and three levels of CA at 0, 100, 200 mg $L^{-1}$ were

used alone and in combination. A 300 ml preservative solution was used for each replication and cut flowers were placed in the solutions after cutting to 30 cm long. Sucrose at 4% was added in all treatments as a base solution.

**Vase life**

The postharvest life of cut *Eustoma grandiflorum* flower is limited by poor bud opening and bent neck in open flowers. The vase life of the inflorescence was considered terminated when 50% of the open florets had wilted (Cho *et al*., 2001).

**Relative water content (RWC)**

Relative water content (RWC) from each sample was determined according to Barros and Virtly method (1962). For this purpose, two excised leaves per plant were weighed (fresh weight, FW) and placed in water in the dark with their petioles plunged in distilled water for 6 hours to allow them to reach full turgidity and, hence, to determine their turgid weight (TW). These leaves were then dried at 70 ºC for 24 h and their dry weight (DW) was recorded. Finally, RWC was calculated using the below equation:

$$\% RWC= (FW-DW)/ (TW-DW) \times 100$$

**Petal water content**

For this trait, on the 12$^{th}$ day from the start of the experiment, 1g of petals from all replications was sampled and each sample were taken as FW and then dried at 70 ºC for 24 h and their DW was recorded. Petal water content (% WP) was then determined with the below equation (Kalate Jari *et al*., 2008):

$$\%WP= FW-DW/DW \times 100$$

**Fresh weight loss**

Weight reduction and relative fresh weight of cut flowers were measured in one day intervals through the experiment from day 1 to 10 (Karimi *et al.*, 2008). Weight decrease compared to day one (initial) was then calculated using equation below:

$$\text{Weight decrease compared to initial (\%)} = (Wt-W0/W0) \times 100$$

Which in the equation:

Wt = fresh weight in day ten

W0 = initial fresh weight (day one)

The opening of each vase was covered in order to limit vase solution evaporative loss and to allow determination of the uptake by stems of the different treatments. Solution uptake volume was calculated by subtracting the volume of evaporated solution from flasks of the same volume without cut flowers.

**Water content of cut flower**

At the end of vase life period of the examined flowers, they were oven dried for 48 hr at 72 ºC. Dry weight was measured after 2 days and water content was calculated using equation below:

$$WC = (FW-DW/FW) \times 100$$

Total water content was determined by subtracting the dry weight of whole flower from their corresponding fresh weight. Results were divided to dry weight using the below formula (Kalate Jari *et al.,* 2008).

$$\text{Water content} = (FW-DW)/DW$$

**Statistical analysis**

This experiment was conducted as a factorial experiment based on completely randomized

Table 1. Analysis of variance for measured traits in this experiment.

| | Vase life | Relative water content | Petal water content | Fresh weight loss | Water content |
|---|---|---|---|---|---|
| Ascorbic acid (AsA) | 32.24** | 717.96* | 861.62* | 270.21* | 41.39 * |
| Citric acid (CA) | 31.17** | 410.23* | 782.25* | 254.95* | 71.2* |
| (AsA) × (CA) | 12.71* | 321.87* | 208.1 ns | 72.69 ns | 64.92* |
| Error | 4.29 | 210.51 | 278.07 | 81.01 | 23.78 |
| CV (%) | 26.75 | 22.12 | 25.79 | 29.37 | 27.27 |

*, ** and ns represent significance at 0.05 , 0.01 and non-significant.

design with 3 replications and 3 samples (individual flowers) for each replication. Data were analyzed as factorial ANOVAs using JMP4.

## RESULTS

### Vase life

According to the results shown in table 1, using AsA and/or CA and their interactions as preservatives, significantly increased the vase life of lisianthus cut flowers, over control ($p \leq 0.01$ and $p \leq 0.05$ respectively). Highest vase life (16.81 and 15.77 days) was observed in solutions containing AsA (300 mg $L^{-1}$) and CA (200 mg $L^{-1}$), respectively. Highest vase life of interaction treatments (17.6 days) was observed in AsA 300 and CA 100 mg $L^{-1}$ which shows a 94% increase compared to control (Table 2).

### Relative water content (RWC)

According to the results, effects of AsA and CA treatments alone and in interaction on RWC are shown significant ($p \leq 0.05$) (Table 1). RWC increases up to (74.22%) in 200 mg $L^{-1}$AsA but decreases to (61.26%) again in 300 mg $L^{-1}$ AsA. Highest interaction value (81.7%) is observed in 200 mg $L^{-1}$ AsA and 200 mg $L^{-1}$ CA and shows a 39% enhancement over control (Table 2).

### Petal water content

Results from petal water content measurement shows a significant difference in AsA and CA treatments over control ($p \leq 0.05$) (Table 1). Highest measured petal water content is shown

Table 2.Mean comparison of different ascorbic and citric acid treatments on measured traits.

| AsA (mg $L^{-1}$) | CA (mg $L^{-1}$) | Vase Life (day) | Relative water content (%) | Petal water content (%) | Fresh weight loss (g) | Water content (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 12.51 $^{b}$ | 52.55 $^{b}$ | 37.89 $^{b}$ | 41.86 $^{a}$ | 77.02 $^{b}$ |
| 100 | 0 | 13.33 $^{b}$ | 64.14 $^{ab}$ | 40.93 $^{ab}$ | 37.96 $^{ab}$ | 77.5 $^{b}$ |
| 200 | 0 | 13.55 $^{b}$ | 74.22 $^{a}$ | 57.3 $^{a}$ | 33.07 $^{b}$ | 77.46 $^{b}$ |
| 300 | 0 | 16.81 $^{a}$ | 61.26 $^{ab}$ | 54.99 $^{a}$ | 29.36 $^{b}$ | 78.65 $^{a}$ |
| 0 | 0 | 12.58 $^{b}$ | 59.74 $^{b}$ | 39.01 $^{b}$ | 39.57 $^{a}$ | 74.85 $^{b}$ |
| 0 | 100 | 13.8 $^{b}$ | 59.87 $^{b}$ | 49.42 $^{ab}$ | 36.6 $^{ab}$ | 78.91 $^{ab}$ |
| 0 | 200 | 15.77 $^{a}$ | 69.79 $^{a}$ | 54.91 $^{a}$ | 30.53 $^{b}$ | 79.21 $^{a}$ |
| 0 | 0 | 9.1 $^{f}$ | 58.9 $^{cd}$ | 27.3 $^{abcde}$ | 42.4 $^{ab}$ | 76 $^{abc}$ |
| 0 | 100 | 10.9 $^{ef}$ | 44.3 $^{d}$ | 38.7 $^{abcd}$ | 48.7 $^{a}$ | 75.1 $^{bc}$ |
| 0 | 200 | 17.6 $^{a}$ | 64.6 $^{bc}$ | 47.7 $^{abc}$ | 34.5 $^{abc}$ | 80 $^{abc}$ |
| 100 | 0 | 12.3 $^{def}$ | 59.6 $^{c}$ | 37.8 $^{abcd}$ | 41.7 $^{ab}$ | 79.9 $^{ab}$ |
| 100 | 100 | 13 $^{cde}$ | 70.1 $^{abc}$ | 36.2 $^{abcd}$ | 40.1 $^{ab}$ | 78.2 $^{ab}$ |
| 100 | 200 | 15.3 $^{abcd}$ | 77.5 $^{ab}$ | 48.8 $^{abc}$ | 32 $^{abc}$ | 74.4 $^{bc}$ |
| 200 | 0 | 12.9 $^{cde}$ | 66.1 $^{bc}$ | 55.2 $^{ab}$ | 40.4 $^{ab}$ | 68.9 $^{c}$ |
| 200 | 100 | 13.8 $^{bcde}$ | 78.5 $^{ab}$ | 62.4 $^{a}$ | 34.6 $^{abc}$ | 79.9 $^{ab}$ |
| 200 | 200 | 13.3 $^{cde}$ | 81.7 $^{a}$ | 54.3 $^{ab}$ | 24.2 $^{abcd}$ | 83.6 $^{a}$ |
| 300 | 0 | 16 $^{abc}$ | 75.7 $^{ab}$ | 35.7 $^{abcd}$ | 33.8 $^{abc}$ | 74.7 $^{bc}$ |
| 300 | 100 | 17.6 $^{a}$ | 68.5 $^{abc}$ | 60.4 $^{a}$ | 23 $^{abcd}$ | 82.5 $^{ab}$ |
| 300 | 200 | 16.9 $^{ab}$ | 58.3 $^{cd}$ | 68.9 $^{a}$ | 31.4 $^{abc}$ | 78.8 $^{ab}$ |

*In each column, means with the similar letters are not significantly different at 5% level.

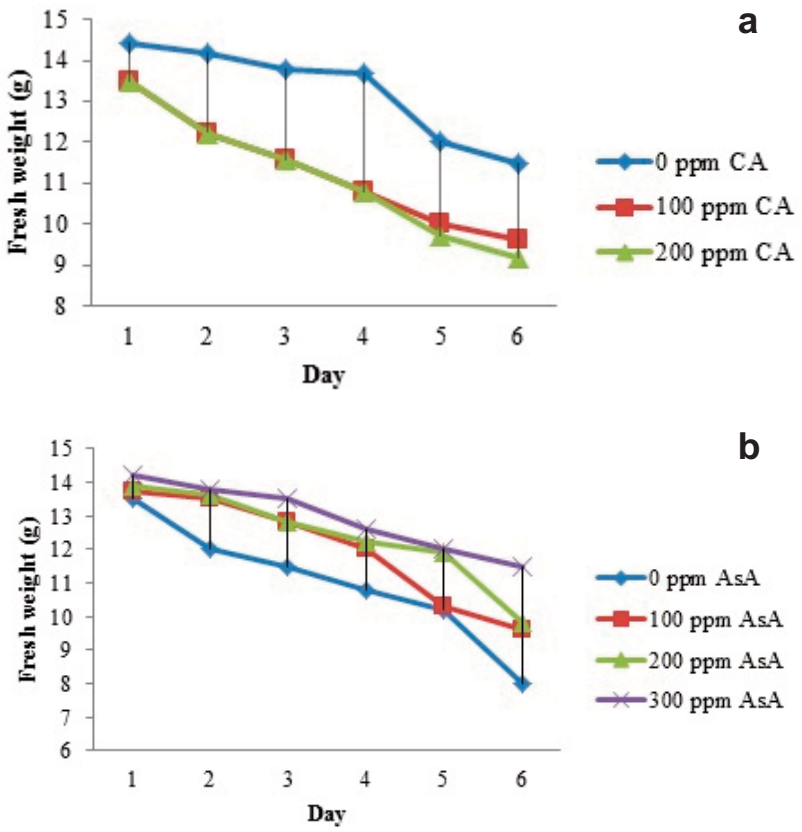


Fig. 1. Change in fresh weight of cut flowers during the first six days of the experiment in AsA (a) and CA (b) treatments.

AsA and CA 200 mg $L^{-1}$. Interaction treatments were not significant in any statistical level, but a considerable increase was observed compared to control (Table 2).

**Fresh weight loss**

Effects of AsA and CA on initial weight variations (day 10 to day 0) were significant ($p \leq 0.05$), but however, interaction effects were not significant (Table 1). The least weight decrease (23%) of cut flowers during the experiment was observed in interaction of AsA 300 mg $L^{-1}$ and CA 100 mg $L^{-1}$ (Table 2). Fig. 1 shows the weight decrease of cut flowers in different treatments used for this experiment. According to them an increase in acid concentration results in a lower weight decrease.

**Water content of cut flower**

According to the results, AsA showed no significant difference with control but CA and interaction treatments on WC are significant ($p \leq 0.05$) (Table 1). Highest WC measured (83.6%) of interaction treatments was observed in AsA 200 mg $L^{-1}$ and CA 200 mg $L^{-1}$ (Table 2).

**DISCUSSION**

In the present study, it was demonstrated that AsA and CA treatments significantly extends vase life in cut lisianthus flowers. Many researches have revealed that the presence of microorganisms in water can cause vascular blockage of cut flower stems (Van Doorn and Witte, 1991).AsA has been shown to have an improving role in postharvest vase life of cut flowers (Jin *et al.*, 2006; Sujata *et al.*, 2003).

AsA is the most abundant antioxidant which protects plant cells and involved in a wide range of important functions as antioxidant defense, photoprotection, regulationof photosynthesis and growth (Bolkhina *et al.,* 2003; El-Kobisky *et al*., 2005). It was reported that AsA pretreatment increased an-

tioxidant activity of some enzymes (superoxide dismutase and ascorbate peroxidase), which leads to improved vase life of cut rose (cv. Samantha) exposed to water deficit stress (Jin *et al.,* 2006).

In addition, it is well known that acidic solutions inhibits bacterial growth and proliferation (Raskin, 1992b), thus considering AsA and CA as weak acids, extended vase life of lisianthus cut flower in this experiment can be attributed to inhibit the growth of microorganisms in the treatments mentioned above. In addition, CA can alleviate water uptake and extend vase life due to its anti-embolism trait which is due to a decrease in microorganisms and low vascular blockage risk with it (Bhattacharjee *et al*., 1993). Other investigators also suggested that reduced pH generally has been considered to improve flower vase life and most flower preservatives contain an acidifier to reduce the pH of the vase solution (Halvey and Mayak, 1980).

Petals of a cut flower are the main ornamental parts and turgidity of this part is important for a good looking and marketable product. Petal turgidity depends largely on water uptake and maintenance in treatments suggested (Vahdati *et al,* 2011). Results of this experiment show a significantly higher water uptake and turgid petals which subsequently increases the cut flower fresh weight. The increases in water uptake and subsequently cut flower fresh weight, is apparently considered due to the acidifying and stress alleviating properties of AsA (Hatami *et al*., 2010). According to our results, it can generally be discussed that, the major part of the water uptake is gathered in the petals which in fact helps to have a better visual quality in treated cut flowers. CA in another study showed to be effective on decreasing water potential in cut rose cv. Cara Mia peduncle and a higher turgidity was observed (Durkin, 1979). AsA alleviates water stress in plants and prevents cell membrane from induced damages and cut flower deterioration by the stress (Jin *et al.,* 2006). Similar to Jin *et al.* (2006), a very small decrease in cut flowers fresh weight over control in this experiment is observed in AsA treatments. AsA prevents senescence and chlorophyll degradation which agree with our results.

Relative water content (RWC) is an index representing the amount of water in the plant organs and shows the ability of a plant in maintaining water under stress conditions (Abbaszadeh *et al.,* 2008). Hence, in a controlled environment for an experiment, the measured RWC shows the response of a plant and the higher the measured amount, the greater the ability of a treatment for keeping water (Abbaszadeh *et al*., 2008). Therefore, according to these results, it seems that at day 10 of the experiment (end of control vase life duration), samples placed in control treatments were under severe stress and could not take up and keep water properly, whereas AsA treatments in comparison, at the same time were in normal non stress conditions which can be the main reason for better looking flowers.

## CONCLUSION

In conclusion, adding AsA and CA to cut flower preservation solutions, increased vase life and preserved cut flowers for a longer period due to an acidic and anti-stress properties. Acidic solution, on the other hand, prevented bacterial growth and proliferation (vascular blockage) and facilitated water uptake in cut flowers. AsA showed a greater effect rather than CA treatments and a significant difference in vase life of lisianthus cut flowers was observed with higher concentrations performing better. According to the results it can generally be mentioned that AsA and CA as cheap, safe and biodegradable compounds can be suitable alternative chemical treatments in order to prolong vase life of cut lisianthus (*Eustoma grandiflorum* cv. Mariachi Blue (flowers which is a fact that would be much appreciated by the growers and handlers of cut flowers. Commercialization of these compounds for optimum formulations of course needs further experiments.

## Literature Cited

Abbaszadeh1, B., Ashourabadi, Sharifi, E., Lebaschi, M. H., Hajibagher Kandy, M. N. and Moghadami, F. 2008. The effect of drought stress on proline contents, soluble sugars, chlorophyll and

relative water contents of balm (*Melissa officinalis* L.). Iranian Journal of Medicine and Aromatic Plants. 23: 504-513.
Barrs, H.D. and Weaterley, P.E. 1962. A re-examination of the relative turgidity techniques for the estimating water deficit in leaves. Australian Journal of Biological Sciences. 15: 413-428.
Bhattacharjee, S. K., Singh, V. and Saxena, N. K. 1993. Studies on vegetative growth, flowering, flower quality and vase life of roses. Journal of Primary Industries.21 (2): 67-71.
Blokhina, O., Virolainen, E. and Fagerstedt, K.V. 2003. Antioxidant, oxidative damage and oxygen deprivations stress: A review. Annual Botany. 91:179-194.
Cho, M.S., Celikel, F., Dodge, L. and Reid, M.S. 2001. Sucrose enhances the postharvest quality of cut flowers of *Eustoma grandiflorum* (Raf.) Shinn. Proceeding. VII International Symposium on Postharvest. Acta Horticulture. 543.
Danaee, E., Mostofi, Y. and Moradi, P. 2011. Effect of GA3 and BA on postharvest quality and vase life of gerbera (*Gerbera jamesonii.* cv. Good Timing) cut flowers.Horticulture, Environment, and Biotechnology. 52 (2), 140-144.
Dole, J. M. and Wilkins, P. 1999. Floriculture principles and species. Prentice Hall Inc. Upper Saddle River, New Jersey.
Durkin, D. J. 1979. Effect of millipore filtration, citric acid and sucrose on peduncle water potential of cut rose flowers. Journal of American Society of Horticultural Sciences. 104 (6):860-863.
Ebrahimzadeh, A., Masiha, C., Nazemieh, A. and Valizade, M. 2003.Studying the effects of preserving solutions on longevity and some other qualitative traits of *Dianthus caryophyllus* cut flower. Iranian Journal of Horticultural Science and Technology, (4): 31-42.
El-Kobisy, D.S., Kady, K.A., Medani, R.A. and Agamy, R.A. 2005. Response of pea plant (*Pisum sativum* L.) to treatment with ascorbic acid. Egyptian Journal of Applied Science, 20:36-50.
Halevy, A. H. and Mayak, S. 1980. Senescence and postharvest physiology of cut flowers.Part 2. In. J. Janick (ed). Horticultural Reviews 2. AVI Pub. Westport, Conn.
Hatami, M., Ghasemnezhad, M., Hatamzadeh, A. and Omran, S. G. 2010. Effect of ascorbic acid on antioxidant capacity during flower development in 'Royal Class' rose cut flowers. Acta Horticulturae. 877.
Jin, J., Shan A, N., Maa, N., Bai, B.J. and Gaoa, J. 2006. Regulation of ascorbate peroxidase at the transcript level is involved in tolerance to postharvest water deficit stress in the cut rose (*Rosa hybrida* L.) cv. Samantha. Postharvest Biology and Technology, 40:236–243
Kalate Jari S., Khalighi A., Moradi F. and Fatahi Moghadam M. R. 2008. The effect of calcium chloride and calcium nitrate on quality and vase life of rose flowers cv. Red Giant. Iranian Journal of Horticultural Science and Technology. No. 3: 163-179.
Karimi., M., Asil., M.H. Lahiji., H. S. and Sasani., S.T. 2008. Effects of temperature and different chemical treatments for extending *Lilium* cut flower shelf life. Iranian Journal of Horticultural Science and Technology. No. 43: 1-9.
Ketsa, S. 1989.Vase life characteristics of inflorescences of dendrobium 'Pompadour'. Hort Science, 64: 611–615.
Lechinani, A. 2006. Effects of different treatments on vase life of two rose cut flower cultivars. Proceeding of Fifth Iranian Horticultural Congress, Shiraz.
Nowak, J. and Rudnicki, R.M. 1990. Postharvest handling and storage of cut flowers, florist greens and potted plants, p: 210. Timber Press, Porthand, Oregan.
Raskin, I. 1992b. Salicylate, a new plant hormone. Plant Physiology, 99: 799–803.
Robinson, F.A. 1973. Vitamins. In Phytochemistry. Vol. III: 195-220. Lawrence, Miller, P., (Ed) Van- Reinhold Co., New York.
Sankat, C.K. and Mujaffar, S. 1994. Water balance in cut anthurium flowers in storage and its effect on quality. Acta Horticulturae. 368: 723–32.
Sujata, A. N., Vijai Singh, T.V. and Sharma. R.S. 2003. Effect of chemical preservatives on enhancing

vase-life of gerbera flowers. Journal of Tropical Agriculture. 41: 56-58.
Vahdati, N., Tehranifar, A., Bayat, H. and Selahvarzi, Y. 2012. Salicylic and citric acid treatments improve the vase life of cut *Chrysanthemum* flowers. Journal of Agriculture and Science Technology. Vol. 14, 879-887.
Van Doorn, W.G. and De Witte, Y. 1991. The mode of action of bacteria inthe vascular occlusion of cut rose flowers. Acta Horticulturae. 298: 165–7.
Wu, M.J., Van Doorn, W.G. and Reid, M.S. 1991.Variation in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars. II.Comparison of sensitivity to exogenous ethylene and of ethylene binding. Scientia Horticulturae. 48: 109–16.