

The Effect of Different Polyamines on Some Physiological Traits as ACC Oxidase and Superoxide Dismutase Enzymes Activity in *Chrysanthemum morifolium* cv. 'Bright Golden Ann'

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Abstract

This experiment was conducted to determine the effect of foliar spray with polyamines on postharvest life of chrysanthemum cut flowers in the horticulture laboratory of Islamic Azad University of Rafsanjan in 2015. For this purpose, an experiment was conducted based on a completely randomized design with three replications with spermidine, spermine and putrescine treatments at 1, 2 and 3 mM concentrations. Different parameters such as vase life, quality, weight loss, dry matter percentage, chlorophyll, total soluble dissolved, protein, water uptake, ACC oxidase and SOD were measured. The results showed that all treatments significantly increased the survival and quality of flowers as compared to control. Spermidine (3 mM) resulted in maximum vase life so that after 9 days, the flowers of this treatment were of a good quality. The lowest weight loss and highest level of dissolved solids were observed in 3 mM spermidine and then, in 2 mM spermidine. ACC oxidase enzyme activity was reduced by a factor of quarter in 3 mM spermidine treatment as compared to control. On the other hand, superoxide dismutase activity in this treatment was 3 times higher than that in control. The highest amount of chlorophyll was observed in the treatment of 3 mM spermidine and putrescine. Solution uptake was increased when polyamines were applied so that the highest solution uptake was observed in the first stage of measurement in relation to 3 mM putrescine. But in the second stage of measurement, 1 mM spermine outperformed. Treatments had no significant effect on dry matter percentage and protein amount in leaves of cut flowers. So, according to the results of this test, treatment with 3 mM spermidine is recommended as the best treatment to enhance the quality and vase life of chrysanthemum (*Chrysanthemum morifolium* cv. 'Bright Golden Ann') cut flowers.

Abbreviations: ACC oxidase: 1- aminocyclopropan -1- carboxylic acid oxidase; SOD: Superoxide dismutase.

Keywords: Putrescine, Quality, Spermidine, Spermine, Vase life.

INTRODUCTION

Chrysanthemum morifolium belongs to Asteraceae family. This genus contains 200 species of annual, perennial, herbaceous and greenhouse plants. This plant is one of the most important ornamental plants in the world (Ghasemi Ghahsare and Kafi, 2009).

Postharvest senescence is the main constraint on selling most of cut flowers, and efforts have been made to discover and develop postharvest treatments to increase shelf life of cut flowers (Bowyer, 2003). Because ethylene hormone plays an important role in advancing the process of aging in horticultural crops, compounds that inhibit the production and activity of ethylene are very impressive in increasing the life and keeping the quality of cut flowers. Polyamines are polycationic compounds with low molecular weight that have linear carbon chains with two terminal amine groups. The most important polyamines include putrescine (with two amine groups), spermidine (with three amine groups) and spermine (with four amine groups). Other polyamines that are present at lower levels in organisms include homospermidine, homospermine, caldo-pentamine, thermospermine and cadaverine (Asnaashari and Khosroshahi, 2009).

It is believed that polyamines have anti-aging properties in plants. Polyamines, particularly Put, are more abundant in young organs and decreases in tissues with age. Since exogenous treatment of polyamines reduces the signs of aging such as the reduction of the amount of chlorophyll and proteins, it is suggested that aging may be caused by the reduced activity of the arginine decarboxylase enzyme, thereby reducing the production of polyamines (Galston and Kaur-Sawhney, 1990).

The precursor of polyamine and ethylene is S-adenosylmethionine, and the two compounds have opposite effects on ripening and aging. Research shows that ripening and aging are the result of the balance between these two compounds (Bregoli *et al.*, 2002). It is reported that in tomatoes, polyamines prevent the transcription, production and activity of ACC-synthase enzyme and thus, reduce the level of ACC leading to the reduced enzyme activity of ACC-oxidase and a decrease in ethylene production (Li *et al.*, 1992). Polyamine concentration decreases with the crop's aging, and this reduction increases the production of ethylene by stimulating the ACC-synthase enzyme. It is known that a reduction in polyamine levels increases the sensitivity of crop tissues to ethylene. Similarly, polyamines may be needed to prevent the expression of ACC-synthase gene (s). So, those tomatoes that have high levels of polyamines produce lower levels of ethylene and have longer postharvest life (Asnaashari and Khosroshahi, 2009). The results of a research showed that ethionine or AOA increases the concentration of putrescine and spermidine but decreases the concentration of spermine during aging. AOA acts as inhibitor of arginine decarboxylase and ACC-synthase activities and reduces the concentration of endogenous putrescine in petals (Ki Cheol *et al.*, 1995).

Increased activity of antioxidant enzymes such as glutathione reductase, superoxide dismutase, catalase and ascorbate peroxidase has been observed in rose leaves after exogenous application of spermine and spermidine (Sood and Nagar, 2003). These enzymes play an important role in reducing oxygen free radicals. Thus, by increasing the activity of these enzymes, vase life of cut flowers will be increased. It was also reported that the use of polyamines could significantly increase fresh weight, uptake of preservative solution, opening of flowers and vase life of gladiolus flowers (Dantuluri *et al.*, 2008). In another study, cut flower of carnation cultivar 'Riko' and gerbera cultivar 'Lisa' were treated with various concentrations of polyamines in a preservative solution. In carnation, the biggest delay in senescence was achieved in 10 mM L⁻¹ spermidine treatment in preservative solution. In gerbera flowers, the best result was achieved in spraying 0.1 mM L⁻¹ and 10 mM L⁻¹ spermidine in preservative solution (Bagni and Tassoni, 2006). It has been reported that treatment with 0.1 mM spermine inhibited reducing sugars and soluble proteins during the vase life of cut rose and reduced ethylene production, but spermidine showed no considerable effect (Chen Wei *et al.*, 2000).

As already mentioned, chrysanthemum is one of the most popular cut flowers. So, the aim of the present study was to improve the vase life of chrysanthemum cv. 'Bright Golden Ann' with new compounds such as polyamines which are more environment-friendly than other substances and are effective at low concentrations.

MATERIALS AND METHODS

In this study, the effect of foliar application of spermidine, putrescine and spermine on quality and vase life of cut flowers of *Chrysanthemum morifolium* cv. 'Bright Golden Ann' was compared to control. For this purpose, an experiment was conducted based on a completely randomized design with three replications with spermidine, spermine and putrescine treatments at 1, 2 and 3 mM concentrations. Cut flowers were taken from a greenhouse located in Mahalat city. These flowers were cut with the length of 35 cm and were put in 500 ml glass flasks containing 3% sucrose. Polyamines spermine, spermidine and putrescine were sprayed on cut flowers. Treated flowers were kept in a germinator at $25\pm 1^{\circ}\text{C}$, 65-75% relative humidity and $15\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ of light (day/night duration of 12/12 hours). Control flowers were sprayed with distilled water.

Flower vase life was recorded since the beginning of the experiment until the wilting of petals or when the petals began to fall out (Fig. 1).

To determine the quality of flowers on the third, sixth and ninth day of experiment, panel test was used with 7 replicates. Numbers 1 to 4 were used in order to rank the quality of flowers. So that the numbers 1 through 4, respectively, represent poor, fair, good and excellent. For this purpose, 7 people tested the morphological characteristics of flowers such as shape, color and scent and then ranking was done (Heintz, 1983).

At the end of the experiment, cut flowers were put in an oven at 120°C for 24 hours and then, dry weight was measured using a 0.001-precision digital scale. To measure the percentage of dry matter, shoot dry weight was divided by the initial weight and the result was multiplied by 100 (Hant, 1982).

For the measurement of dissolved solids and protein in the leaves, anthrone and biure reagents were used and then, light absorption was read at wavelengths of 595 and 625 nm by a spectrophotometer (Bradford, 1979; Somogy, 1952). For measuring the chlorophyll content of leaves, acetone was used for extraction and then, the light absorption of solution was read using a spectrophotometer at wavelengths of 646.6 and 663.6 nm. The total chlorophyll concentration was calculated using the following equation (Haghighi, 2009):

Total chlorophyll ($\mu\text{m}/\text{ml}$): $[(17.76 \times \text{OD}_{646.6}) + (7.37 \times \text{OD}_{663.6})] \times V/W$

OD: The read absorbance;

V: Acetone volume;

W: Fresh weight of sample (g).

The amount of solution uptake was calculated on the third and sixth day of experiment by

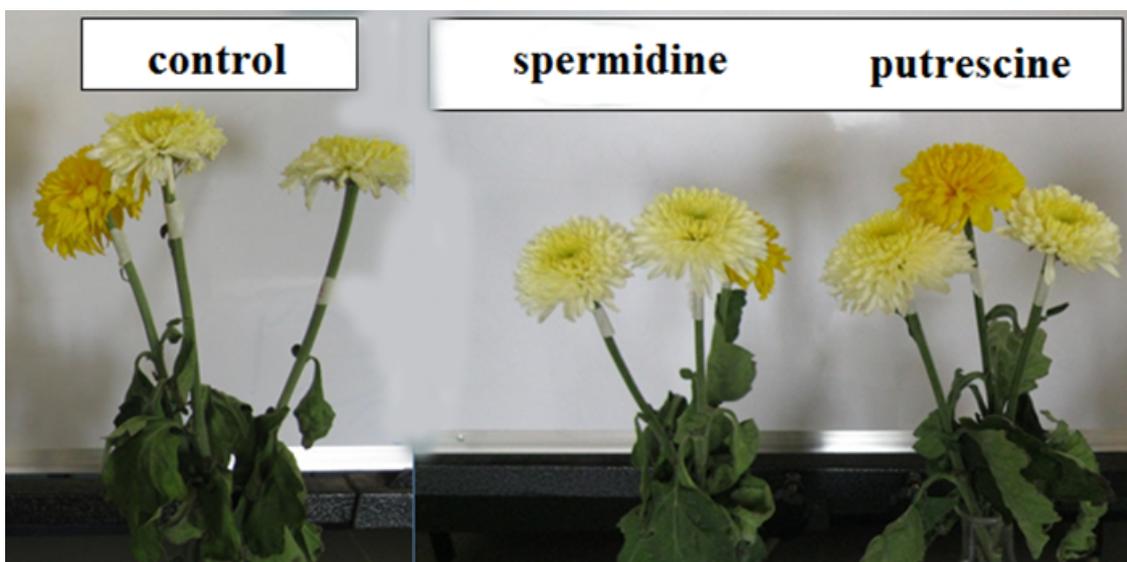


Fig. 1. Comparison of the effect of spermidine (9th day) and putrescine (6th day) on vase life and quality of chrysanthemum as compared to control (4th day, end of vase life)

the following formula (5):

Solution uptake (ml day⁻¹ g⁻¹ F.W.)= (S_{t-1}-S_t)/ W_t

S_t: Solution weight (g) at different days of measurement;

S_{t-1}: Solution weight (g) one day ago;

W_t: Shoot fresh weight on the first day.

On the second day of experiment, ACC oxidase and SOD enzymes activity in leaves of cut flower were measured. Superoxide dismutase was measured by the method described by Gianopolitis and Ries (1977). ACC oxidase activity was assayed by measuring according to the method described by Maye-Lean and John (1994).

To determine the weight loss, cut flowers were weighed before putting in preservative solution and then, weighed again at the end of experiment. For calculating the amount of weight loss, the final weight was subtracted from the initial weight.

The effects of putrescine, spermidine and spermine were statistically analyzed in a completely randomized design with three replications. Totally, 10 treatments in 30 experimental units were compared to each other. In order to analyze the data, SAS software package was used and the means were compared by LSD multiple range test. The graphs were plotted using MS-Excel software package.

RESULTS

Results of ANOVA showed that the effects of different treatments were significant on total chlorophyll, solution uptake (1 and 2), ACC-oxidase, and super oxide dismutase at the 5% level, and on vase life, weight loss, total dissolved solids and quality at the 1% level. They did not significantly impact dry matter and protein (Table 1).

Vase life

As can be seen in Table 2, flower durability at 2 and 3 mM spermidine and 3 mM putrescine was significantly different from control but the difference was not significant to each other. The highest durability was related to 3 mM spermidine.

Quality

On the third day, all treatments had high quality as compared to control. But on the sixth day, the highest quality of flowers was seen initially in the treatments of spermidine and putrescine at 3 mM concentrations and then, in the treatments of spermidine and putrescine at 2 mM concentration. On the ninth day of the measurement, flower quality was decreased in all treatments except for 2 and 3 mM spermidine. And spermidine 3 mM was the only treatment that had a good quality on the ninth day (Table 2 and Fig. 2).

Weight loss

According to Table 2, it was shown that weight loss at 2 and 3 mM concentrations of spermidine and 2 mM concentration of putrescine was significantly lower than that of control. The lowest weight loss was related to the treatment of spermidine at 3 mM concentration.

Solution uptake

According to Table 2 and Fig. 3, at the first stage of measurement, absorbance was maximal in putrescine treatment at 3 mM concentration and the difference was significant as compared to control. Minimum water absorption at the first stage was related to the treatment of 1 mM spermine but the difference was insignificant as compared to control. Water absorption in the second stage had the lowest value in control whose difference was significant with other treatments except 3 mM spermine and 1 mM putrescine. The highest rate of water absorption at the second stage was

Table 1. Analysis of variance for some traits evaluated in *Chrysanthemum morifolium* cv. 'Bright Golden Ann'

S.O.V	df	Quality 3 th day	Quality 6 th day	Quality 9 th day	Vase life	Weight loss	Dry matter	Total dissolved solids	Protein	Total chlorophyll	Solution uptake (1)	Solution uptake (2)	Solution uptake (2)	ACC-oxidase	SOD
Treatment	9	0.133**	4.59**	1.54**	7.59**	9.43**	1.63 ^{ns}	59.25**		4.2717 ^{ns}	8958*	128.3*	213.2*	2398.1*	4254.9*
Error	18	0.037	0.862	0.252	0.722	0.97	1.451	769.3		75.182	27126	50.4	76.55	1511.9	2321.3
CV (%)		4.92	43.39	125.46	12.81	11.49	6.75	48.23		12.59	13.19	12.82	21.39	9.2	7.5

*, ** represents significant effects at the 0.05 and 0.01 probability levels, respectively; ns means non-significant

Table 2. The effect of different treatments of polyamines spermine, spermidine and putrescine on some postharvest traits of cut *Chrysanthemum morifolium* cv. 'Bright Golden Ann'

Treatments (mM)	Vase life (day)	Quality 3 th day (1-4)	Quality 6 th day (1-4)	Quality 9 th day (1-4)	Weight loss (g)	Solution uptake(1) (ml g ⁻¹ FW)	Solution uptake(2) (ml g ⁻¹ FW)
Control	4.0 e	3.3 b	0.7 cd	0.1 c	9.8 ab	0.52 bc	0.23 c
Spd (1)	5.0 de	3.8 a	0.3 d	0.3 c	10.6 a	0.5 bc	0.41 ab
Spd (2)	8.3 ab	4.0 a	2.9 ab	1.3 b	6.9 d	0.54 bc	0.46 ab
Spd (3)	9.0 a	4.0 a	3.7 a	3.0 a	4.5 e	0.56 b	0.49 ab
Spr (1)	6.0 cd	4.0 a	1.3 bcd	0.3 c	9.3 abc	0.49 c	0.53 a
Spr (2)	6.0 cd	4.0 a	2.0 abc	0.3 c	9.3 abc	0.56 b	0.43 ab
Spr (3)	6.0 cd	4.0 a	1.9 bc	0.3 c	9.3 abc	0.52 bc	0.37 abc
Put (1)	6.3 cd	4.0 a	2.2 abc	0.3 c	9.8 abc	0.55 bc	0.32 bc
Put (2)	7.3 bc	4.0 a	3.1 ab	0.3 c	7.9 cd	0.56 b	0.4 ab
Put (3)	8.3 ab	4.0 a	3.7 a	0.7 bc	8.2 bcd	0.72 a	0.41ab

*Means sharing similar letters in a column are statistically non-significant at P < 0.01

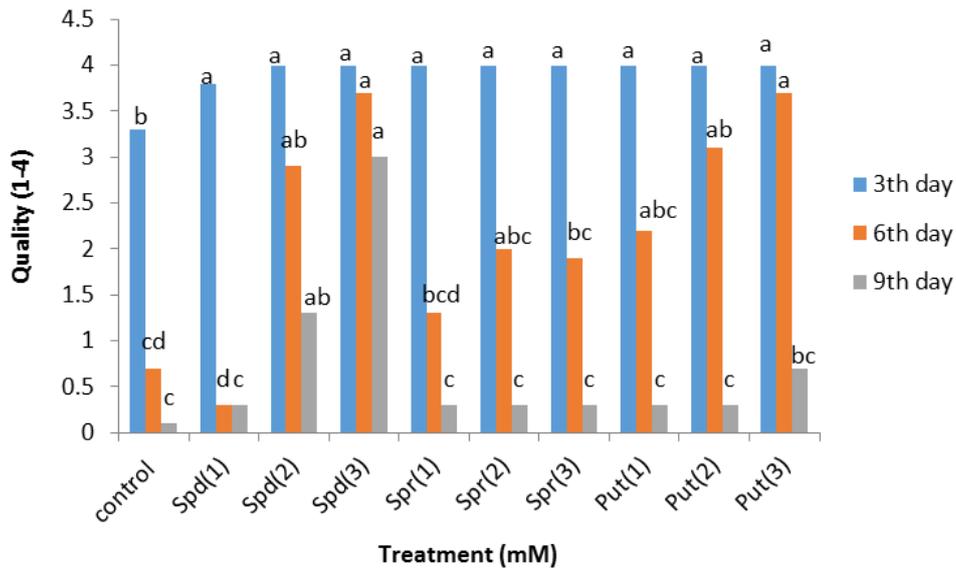


Fig. 2. The effect of different treatments on the quality of *Chrysanthemum morifolium* cv. 'Bright Golden Ann' on different days

related to 1 mM spermidine. The difference was not significant with other treatments except for the treatment with 3 mM spermine and 1 mM putrescine and control.

Total soluble solids (TSS)

According to Table 3, TSS in 3 mM spermidine was the highest showing significant differences with all other treatments as well as control. The concentration of sugar in 2 mM spermidine, 1, 2 and 3 mM spermine, and 3 mM putrescine treatments was significantly higher than that in control.

Chlorophyll

Putrescine in all concentrations and 3 mM Spd were the most effective treatments for the chlorophyll content of chrysanthemum leaves. Spermine at 3 mM concentration had the lowest effect on chlorophyll content which was not significantly different from control. All treatments except 3 mM spermine increased the chlorophyll content of the leaves significantly (Table 3).

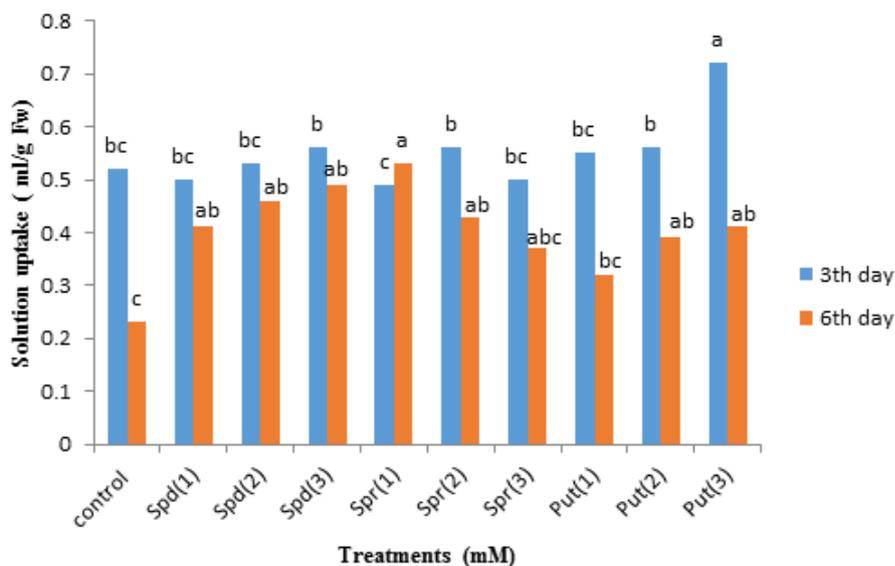


Fig. 3. The effect of different treatments with polyamines on the solution uptake in *Chrysanthemum morifolium* cv. 'Bright Golden Ann'

Table 3. The effect of different treatments with polyamines on some physiological traits of *Chrysanthemum morifolium* cv. 'Bright Golden Ann'

Treatments (mM)	Total soluble solids (mg g ⁻¹ FW)	Total chlorophyll (µM ml ⁻¹)	ACC oxidase (nmol ml ⁻¹ h ⁻¹)	Super oxide dismutase (unit g ⁻¹ FW)
Control	3.6 ^d	774.6 ^c	196.9 ^a	70.0 ^c
Spd (1)	7.1 ^{bcd}	1097.6 ^{ab}	190.0 ^a	75.0 ^c
Spd (2)	10.5 ^b	1091.3 ^{ab}	50.4 ^d	150.3 ^{ab}
Spd (3)	13.9 ^a	878.4 ^{bc}	45.1 ^d	180.0 ^a
Spr (1)	9.6 ^b	1151.5 ^{ab}	180.0 ^{ab}	78.34 ^c
Spr (2)	7.2 ^{bc}	1155.9 ^{ab}	160.0 ^b	80 ^{bc}
Spr (3)	8.2 ^{bc}	1250.0 ^a	150.0 ^b	90.2 ^{bc}
Put (1)	5.6 ^{cd}	1216.4 ^a	90.7 ^{bc}	100 ^{bc}
Put (2)	6.8 ^{bcd}	1249.7 ^a	80.0 ^c	120.4 ^b
Put (3)	10.1 ^b	1308.6 ^a	70.4 ^c	130.9 ^b

*Means sharing similar letters in a column are statistically insignificant at P < 0.01

ACC-oxidase and superoxide dismutase enzymes

Spermidine at 2 and 3 mM concentrations significantly increased the activity of superoxide dismutase in leaves of chrysanthemum as compared to other treatments, whilst all spermine treatments were not significantly different from control in terms of the impact on the activity of this enzyme (Table 3). ACC-oxidase enzyme activity in all treatments except 1 mM spermine was significantly decreased as compared to control. The lowest activity of ACC-oxidase enzyme was initially observed in the treatment of 3 mM spermidine that was not significantly different from the treatment with 2 mM spermidine; and then in the different treatments of putrescine and finally in the spermine treatments at 2 and 3 mM concentrations, respectively (Table 3).

DISCUSSION

One of the important post-harvest factors of cut flowers is the preservation of their quality. Polyamines, including putrescine (diamine), spermidine (triamine) and spermine (tetramine), are a new group of plant growth regulators that are important in various processes, such as increased cell division, increased biosynthesis of enzymes, regulation of different processes of development, differentiation, flowering, embryogenesis, rooting, ripening, and aging (Kakkar, 2002). According to the results of this experiment, all treatments significantly increased post-harvest life of cut flowers as compared to control. Spermidine (3 mM) resulted in the highest vase life, so that after 9 days, the flowers applied with this treatment showed good quality, whilst other treatments and control were lost on the 9th day. Spermidine increased the vase life of flowers by about 5 days. Polyamine can delay aging by preventing ethylene production. The results of the present study, also, show that the use of these three types of polyamines reduced ACC-oxidase activity. Spermidine especially at 2 and 3 mM concentration resulted in the largest decline in the activity of this enzyme. As is known, ACC-oxidase enzyme is the main enzyme in the biosynthesis of ethylene. Reduced activity of this enzyme after polyamine application shows the anti-ethylene effect of these compounds as there are several reports in this regard (Li *et al.*, 1992; Ki Cheol *et al.*, 1995; Chen Wei *et al.*, 2000).

The increase in polyamines content may be one of the mechanisms responsible for the anti-aging effect of polyamines by preventing lipid peroxidation (Yang *et al.*, 2000). Polyamines can reduce ROS by their antioxidant properties on the one hand and can reduce membrane lipid degradation by reducing the lipoxygenase enzyme activity on the other hand. So, membrane permeability is maintained and therefore, the quality and vase life of flowers are increased (Lee *et al.*, 1997) which was confirmed by the results of the present study too. Spermidine treatment caused maximum vase life of cut flowers of chrysanthemum. On the other hand, in this treatment, the highest

increase in the superoxide dismutase enzyme activity was observed in the leaves of flowers. This enzyme plays an important role in reducing oxygen free radicals and thus, can increase the postharvest life of cut flowers of chrysanthemums.

In another study, delay in aging by polyamines was reported in cut flowers of gladiolus through membrane stability (Kakkar, 2002). After studying the effect of spermidine and calcium sulfate on quantitative and qualitative features, and vase life of rose flowers, similar results were reported (Farhi *et al.*, 2014). Measuring the solution uptake on the third day showed that putrescine 3 mM made the most solution uptake by the flowers and in this treatment a significant difference was observed with control, while other treatments were not significantly different from control. Measuring the solution uptake on the sixth day showed significant effect of most treatments on increasing solution uptake. The main factor determining the quality and vase life of cut flowers is water balance (Singh and Kumar, 2008). When transpiration is greater than water absorption, cut flowers are faced with water loss and flower wilting develops. Inability to absorb water is one of the causes of flower wilting that may be due to the blocking of vessels (Halevy and Mayak, 1981). The research showed that the use of polyamines in gladiolus flower increased water absorption (Sivaprakasam *et al.*, 2009). Cut flowers are sensitive to gravity. During the keeping of a branch of cut flowers, bent neck happened and water flow was blocked to half-open buds. Geotropism resistance depends on the strength and turgor of the flower stems and branches which are determined by solution absorption (Singh *et al.*, 2005). Weight loss at 2 and 3 mM spermidine and 2 mM putrescine were lower than that at control. The lowest weight loss was related to spermidine treatment with concentration of 3 mM. One of the most important factors in determining the quality and vase life of flowers is their fresh weight. The mechanism proposed for the polyamines can be the role of these compounds to reduce evaporation from the tissues of cut flowers and also to decrease its respiration that prevents weight loss and wilting of cut flowers and maintains its quality (Kar *et al.*, 2013). Also, another factor in fresh weight gain can be the increase in absorption of preservative solution by polyamine treatments.

Application of the three polyamines caused a significant increase in the amount of soluble solids in the leaves of flowers. Spermidine (3 mM) resulted in the highest amount of soluble solids in the leaves of flowers. In most cut flowers, there are still some soluble sugars in the petals suggesting that the cells have some sugar in store even at the time of wilting. It is likely that there is a high concentration of sugar in the vacuoles, but cell organelles such as mitochondria are not able to use it. The inability of the cell organelles in sugar consumption reduces the durability of the flowers. It is believed that many plants that have the ability to increase soluble sugar in their body have the ability to deal with adverse environmental conditions. Soluble sugars are involved in membrane stability and reduce the flower wilting (Hashemi *et al.*, 2013). In this experiment, the flowers that had a greater survival rate had more soluble sugars too. Spermidine affects the ethylene production and decreases it, thus reducing the intake of protein and soluble sugars during the process of respiration.

Putrescine and spermidine treatments at the concentrations of 3 mM were the most effective treatments for chlorophyll content in chrysanthemum leaves. The results showed that the use of polyamines on cut flowers of chrysanthemum increased the chlorophyll content of leaves. Ethylene causes chlorophyll degradation and polyamines (spermidine), due to their anti-ethylene role, prevent the production of enzymes that are involved in the production of ethylene. They prevent the production of free radicals that cause degradation of chlorophyll. Also, the polyamines (spermidine) prevent chlorophyll degradation by reducing the activity of hydrolytic enzymes in thylakoid membrane (Valero *et al.*, 2002). It is known that polyamines prevent aging, reduce the chlorophyll content, and reduce the synthesis of ethylene (Asnaashari and Khosroshahi, 2009).

CONCLUSION

Given the economic importance and valuable features of chrysanthemum, it is necessary to apply treatments to control ethylene production and to offer this flower to the consumer with a

better quality. On the other hand, because polyamine treatment is performed as spraying on flowers and is used at a very low concentration, it has been cost-effective as compared to the use of the preservative solution. Also, florist can quickly spray it on the flowers without the need for labor. So the life of flowers in flower shops will be increased by a factor of approximately two. According to the results of this study, spermidine at the concentration of 3 mM is recommended among different polyamines as the best type of polyamine for spraying to enhance the quality and vase life of cut *Chrysanthemum morifolium* cv. 'Bright Golden Ann'. It can be used as a method to extend the vase life of cut flowers for sellers and for consumers.

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