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Increasing the Vase Life of Alstroemeria Cut Flowers Using Cycloheximide, Benzyladenine and Coconut Juice

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In order to improve the vase life of alstroemeria cut flowers (Alstroemeria aurantiaca), an experiment was performed based on completely randomized design with 10 treatments including cycloheximide (50, 100 and 200 mg l⁻¹), benzyladenine (50, 100 and 200 mg l⁻¹), coconut juice (5, 10 and 20 %) and control (distilled water) in three replications. In this study, characteristics such as vase life, dry matter, increasing ° Brix, the percentage of flower opening, chlorophyll a and b and ionic leakage were evaluated. The results showed that coconut juice at a concentration of 5% has the maximum vase life (17.39 days), although it has no significant difference with different levels of benzyladenine and 10 and 20% coconut juice treatments. 10% coconut juice with 2.58 and 0.88 has the highest ° Brix and flower opening index, respectively. Treatments of 50 mg l-1 benzyladenine, 5 and 10% coconut juice had the maximum impact on the amount of dry matter. The most successful treatments for reducing ionic leakage are two treatments of 5% coconut juice and 100 mg l⁻¹ benzyladenine. All treatments cause maintenance of chlorophyll a and b compared to the control, and two treatments of 50 mg l-1 cycloheximide and 5% coconut juice had the highest content of chlorophyll a and b, respectively among treatments. According to the obtained results, using 5% coconut juice in the vase solution can effectively improve the vase life of alstroemeria cut flowers.

Abstrac

Keywords: Benzyladenine, Chlorophyll, Cytokinin, Ionic leakage, Vase life.

INTRODUCTION

Alstroemeria is one of cut flowers belonging to Alstroemeriaceae family that in recent years, because of the beauty and variety of flower colors as well as relatively easy culturing, has attracted the attention of producers and enthusiasts of ornamental plants particularly cut flowers and every year, a high number of exporting cut flowers is related to this cut flower (Ferrante *et al.*, 2002). Since most important and economical component in the supply and demand for cut flowers to the international market is their post-harvest vase life, so, reducing waste and increasing the vase life of cut flowers are very important. The limiting factors of the vase life of alstroemeria cut flower, which is sensitive to ethylene, are early yellowing leaves and petal fall. In previous studies, it has been found that the yellowing leaves of alstroemeria that are mainly along with the degradation of chlorophyll is the most important factor of reduction of post-harvest quality of this cut flower. Therefore, delaying aging and wilting of leaves can increase vase life and marketability of this plant in commercial markets (Chanasut *et al.*, 2003; Dai and paull, 1991). One of the methods suggested for maintaining freshness of alstroemeria leaves is the use of growth regulators such as cytokinins.

Cytokinins postpones yellowing leaves by preventing the degradation of chlorophyll, the evolution of chloroplasts, leaf growth and development, and ultimately delaying the process of aging (Penner and wiely, 2008; Han and miller, 2003). Ojaghi *et al.* (2013) believe that the use of cytokinin stimulates proteins, enzymes involved in the biosynthesis of chlorophyll and production of chloroplasts and prevents declining chlorophyll.

Researchers believe that artificial cytokinins are more effective in delaying aging plants due to greater stability; benzyladenine is a synthetic cytokinin whose impact has been reported for reducing aging of cut flowers (Davis, 1998; Dudareva *et al.*, 1998). Mutui *et al.* (2004) reported that benzyladenine effectively increases the vase life of cut flowers and reduces aging of cut flowers with preventing the degradation of proteins and chlorophyll. Some researchers believe that benzyladenine delays senescence of cut by avoiding ethylene activities (Huang and chen, 2002; Han and miller, 2003). The positive effect of benzyladenine has been reported on improvement the quality and vase life of cut flowers of *Lisianthus* (Hassanpour Asil and Karimi, 2010), *Lilium* (Arefnia *et al.*, 2013) and *Alstroemeria* (Ojaghi *et al.*, 2013). Coconut juice is a rich natural source of carbohydrates, electrolytes and growth regulator such as cytokinins whose effect has been reported positive in the extension of vase life of cut flowers (Jayalekshmy *et al.*, 1986; Mamaril *et al.*, 1986; Nair *et al.*, 2000 Agampodi and jayawardena, 2007). Agampodi and jayawardena (2007) reported that 5% coconut juice treatment of cut *Anthurium* (Wild Pink variety) flowers improves the vase life compared to the control. Treatment of gerbera cut flowers (Nair *et al.*, 2000) with coconut juice increased post-harvest longevity and quality.

The use of protease inhibitor is a reasonable solution to preserve the life of leaves and cut flowers. Researchers believe that cycloheximide prevents the destruction of the petals by preventing the synthesis of proteins, which are responsible for aging. In fact, cycloheximide by preventing the synthesis of enzymes responsible for the destruction of membrane integrity, causes maintenance of membrane health and longevity of cut flowers (Halaba and Rudnicki, 1986; Suttle and kende, 1980; Jones *et al.*, 1994). Results of the study conducted by Jones *et al.* (1994) showed that increasing the vase life of cut flowers by treatment with cycloheximide has not been due to an increase in water absorption and controlling the microorganisms, but rather because of inhibition of the synthesis of new proteins. Shahri and tahir (2010) believe that the use of 0.05 mM cycloheximide before transferring cut flowers to distilled water enhances the vase life of cut *Ranunculus* flowers is. Increasing the vase life of cut carnation (cv. 'White Sim') flowers (Drory *et al.*, 1995; Wulster *et al.*, 1982) and *Lilium* (Bieleski and Reid, 1991; Lay yee *et al.*, 1992) has been reported with the use of cycloheximide.

The aim of this study is to investigate the effects of cycloheximide, benzyladenine and coconut juice on the vase life of leaves and cut flowers of alstroemeria.

MATERIALS AND METHODS

Alstroemeria cv. 'Camilla' cut flowers harvested in the commercial step were purchased from Tehran and in order to test, they were immediately transferred to a laboratory with 2 ± 20 ° C temperature, the relative humidity of 65 to 75% and photoperiod of 12 hours per day with the amount of light of 15 μ mol m⁻² s⁻¹. This experiment was performed based on completely randomized design with 10 treatments in three replications including cycloheximide in three levels (50, 100 and 200 mg l⁻¹), benzyladenine at three levels (50, 100 and 200 mg l⁻¹), coconut juice at three levels (5, 10 and 20%) and control (distilled water), 30 plots and 150 flower branches. The traits such as vase life, flower opening index, increasing °Brix, dry matter, ionic leakage and chlorophyll a, b were evaluated. The vase life was calculated by counting the days from the time of placing flowers in the vase solution until wilting leaves and petals (Fig. 1).

Fresh weight of cut flowers was measured on the last day using a digital scale and then the flowers were dried in the oven at 70 $^{\circ}$ C for 24 hours and their dry matter percentage was calculated using the following formula:

Dry Matter Percentage =(Dry Weight)/(Fresh Weight) ×100

To measure ° Brix, a refractometer model N-1 α manufactured by ATAGO Company of Japan was used and increasing ° Brix was calculated by deducting ° Brix of the last day from ° Brix of the first day. To measure the flower opening index, the largest flower diameter and the diameter perpendicular to it every were measured by a digital caliper once every two days and then the obtained values were averaged and flower opening index was calculated by the following formula:

Flower Opening Index = $(D_n + 2/D_n + D_n + 4/D_n + 2 + D_n + 6/D_n + 4)/3$

Where, D_n is the number of days that flower diameter has been evaluated and D_n+2 is flower diameter evaluated on the second day and so on.

For the measurement of chlorophyll, the leaves were separated from the flower branch and then on the fifth day of trial and the process of measuring chlorophyll was conducted using the method of Mazumdar and Majumdar (2003). For this purpose, extraction was performed using 80% acetone and the amount of pigment was read using a spectrophotometer at wavelengths of 660 and 642 nm, and finally chlorophyll content in mg g-1 FW was calculated using the following formula:

Chlorophyll a = $9.93 (A_{660}) - 0.777 (A_{624.5})$

Chlorophyll $b = 17.6 (A_{624.5}) - 2.81 (A_{660})$

The method of Kaya *et al.* (2001) was used for measuring ionic leakage and finally ionic leakage was calculated using the following formula:

Ionic Leakage = $EC_1/EC_2 \times 100$

At the end of experiments, measured data were analyzed using statistical software of MSTATC, and comparison of data was performed using LSD test and Excel graphs.



Fig. 1. Alstroemeria cut flowers on the first day (right) and the end of the day vase life (left).

RESULTS AND DISCUSSION

Vase life

Analysis of variance showed that the use of cycloheximide, benzyladenine and coconut juice in the preservative solution significantly increases the vase life of alstroemeria cut flowers with the probability level of 1% compared to the control (Table 1). Although the effects of the 5% coconut juice treatment on increasing the vase life with 17.39 days was more, but this treatment had statistically no significant difference with treatments of 10 and 20% coconut juice, 50, 100 and 200 mg l⁻¹ benzyladenine. Although cycloheximide levels were significantly increased Alstroemeria vase life compared to the control, but it was less effective than treatments of benzyladenine and coconut juice in improving the post-harvest life of alstroemeria cut flowers (Fig. 2).

The use of cytokinins in flowers such as alstroemeria, which face with early chlorosis of the leaves, is very important. In fact, cytokinins with a role in cell division, preventing the degradation of chlorophyll, chloroplast evolution, growth and development of leaf, opening and closing of stomata, cause a delay in senescence of cut flowers and leaf. In addition, cytokinins have the anti-ethylene property and cause prolonged vase life of cut flowers by preventing ethylene activity (Gilbart and dedolph, 1965; Gulzar *et al.*, 2005; Penner and wiely, 2008). As mentioned in results, the vase life of alstroemeria cut flowers was significantly increased with the use of benzyladenine and coconut juice. In fact, it can be said that the coconut juice is a rich source of glucose, electrolytes and growth regulators and by providing a set of factors required for cut flowers after separation from the mother flower, reduces the tension on cut flowers and so it has increased significantly vase life compared with other treatments. The positive effect of coconut juice on the vase life of anthurium has been reported (Agampodi and Jiyawordena, 2007) that agrees with the

S.O.V	df	Vase life	Increasing °brix	Flower opening index	Dry matter	Chlorophyll a	Chlorophyll b	lonic leakage
Treatment	9	10.96 **	1.24**	0.043**	4.98**	4.63**	0.859**	12.52*
Error	20	2.78	0.357	0.0053	1.32	0.0055	0.0033	3.89
CV (%)	29	11.34	3.58	10.71	9.53	1.78	3.4	6.23

*: Significant at p< 0.05; **: Significant at p< 0.01.

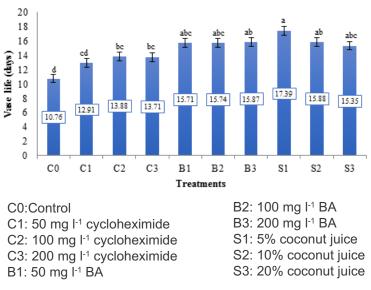


Fig. 2. The effect of different treatments on vase life of cut flowers alstroemeria.

results of current study. Researchers believe that benzyladenine by preventing the conversion of ACC to ethylene and producing it in the petals, maintains cut flowers and increases the vase life of cut flowers (Huang and Chen, 2002; Han and Miller, 2003). Hassanpour Asil and Karimi (2010) reported that benzyladenine by reducing ethylene production, increasing water absorption and preventing fresh weight loss, increases the vase life of Lisianthus cut flowers and similar results have been reported for cut flowers of heliconia (Kumar *et al.*, 2008), alstroemeria (Mittler, 2002) and gladiolus (Singh *et al.*, 2008) reported that are in accordance with the results of current study.

Different concentrations of cycloheximide increase vase life of alstroemeria cut flowers compared to control, but it has less effect on vase life compared to benzyladenine and coconut juice that its resone can be attributed to the continuous use of cycloheximide as cycloheximide shows better effects in pulse treatments. Scientists believe that increasing vase life of cut flowers occurs by using inhibitors of synthesis of certain proteins. Cycloheximide is an inhibitor for synthesizing new destructive proteins and a blocker of synthesis of enzymes responsible for the destruction of membrane that effectively delays the aging of cut flowers (Gul and Tahir, 2013; Wulster *et al.*, 1982; Lukaszewski and Reid, 1989; Shahri and Tahir, 2010, Suttle and Kende, 1980).

Gulzar *et al.* (2005) reported that treatment of common day-lily cut flowers with cycloheximide and cytokinins delays aging and fading of flowers. In the study conducted by van Doorn *et al.* (1994), treatment of iris cut flowers with cycloheximide delays rolling leaves and wilting flowers. Gul and Tahir (2013) reported similar results about cycloheximide positive effect on vase life and quality characteristics of narcissus cut flowers that agrees with the results of present study.

Increasing ° brix

According to the results of analysis of variance of data, the effect of cycloheximide, benzyladenine and coconut juice on increasing ° Brix was significant at 1% level (Table 1). The use of 10% coconut juice had the maximum ° Brix (2.58%) of alstroemeria cut flowers that did not differ significantly from treatments of 20 and 5% coconut juice and 100 and 200 mg l⁻¹ benzyladenine. The minimum increase of ° Brix was obtained with the control treatment (0.68) that had no significant difference with two treatments of 50 and 100 mg l⁻¹ cycloheximide (Fig. 3).

The most important factor in delaying aging cut flowers is preserving and increasing the amount of carbohydrates of the flower. In fact, soluble carbohydrates are the main source of energy required for all biochemical and physiological processes of cut flowers that result in delaying aging

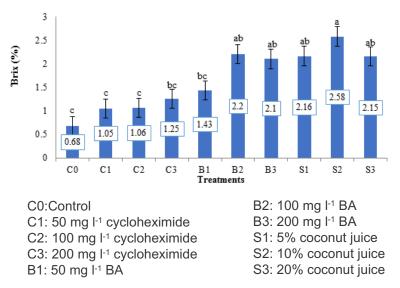


Fig. 3. The effect of different treatments on increasing ° brix of cut flowers alstroemeria.

(Mutui *et al.*, 2001). In this study, ° Brix was increased in all treatments compared to the control, and treatments of coconut juice and benzyladenine were more successful than cycloheximide in increasing °Brix of alstroemeria cut flowers. The researchers believe that cytokinins resulted in transferring produced material from leaves to different parts of the flower and increase soluble carbohydrates content of petals. Increasing total soluble solids of petals along with increasing petal osmotic pressure increases absorption of water and preservative solution and so, causes a delay in wilting cut flowers by maintaining carbohydrates and supplying energy required for flowers to survive. Chanasut *et al.* (2003) believe that treatment of cut flowers with growth regulators such as cytokinins and gibberellins along with sucrose is effective in maintaining carbohydrates of al-stroemeria cut flowers.

Flower opening index

The effect of cycloheximide, benzyladenine and coconut juice on the opening of alstroemeria cut flowers were significant at 1% level (Table 1). Two treatments of 10% coconut juice (0.88) and 5% coconut juice (0.77) were the most successful treatments in flower opening trait that they had statistically no significant difference with the control (0.76). As seen in Fig. 3, the different levels of cycloheximide and benzyladenine and 20% coconut juice decreased flower opening compared to the control and the minimum flower opening ratio was related with the treatment of 200 mg l⁻¹ cycloheximide (0.50) (Fig. 4).

Gul and Tahir (2012) believe that cycloheximide delays flower opening process due to control the breathing. Treatment of cut flowers of Cloves (Drory *et al.*, 1995; Wulster *et al.*, 1982) and *Lilium* (Lay yee *et al.*, 1992; Bieleski and Ried, 1991) with cycloheximide delays in wilting and opening the petals. In these research, it was mentioned that cycloheximide prevents the spread of petals by preventing the synthesis of enzymes responsible for the release of osmotic salts. As mentioned in the statement of results, coconut juice increased flower opening, it seems that coconut juice by maintaining carbohydrates, avoiding burning sugars as well as anti-ethylene activity improves the petals opening and on the other hand, prevents buds wilting. Researchers believe that cytokinins delay aging of cut flowers (Shahri and tahir, 2010; Islam *et al.*, 2011). In different studies, benzyladenine by delaying buds fully opening, increased the vase life of cut flowers (Salisbury and Ross, 1986; Halevy and Mayak, 1981; Hichlenton, 1991; Mutui *et al.*, 2004) that agrees with the results of present research.

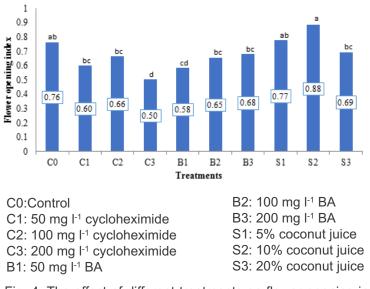


Fig. 4. The effect of different treatments on flower opening index of cut flowers alstroemeria.

Dry matter

Results of table ANOVA indicated that the use of cycloheximide, benzyladenine and coconut juice at all used concentrations significantly increases dry matter of alstroemeria cut flowers with the probability level of 1 % (Table 1). As shown in Fig. 4, the lowest dry matter is related to the control treatment (10.14%). The most successful treatments for increasing dry matter were three treatments of 10% coconut juice (13.71%), 5% coconut juice (13.52%) and 100 mg l⁻¹ benzyladenine (13.06 %), but they statistically were not significantly different from treatments 100 and 200 mg l⁻¹ benzyladenine and 20% coconut juice. Although cycloheximide levels compared to the control increased dry matter of alstroemeria cut flowers, but it was in second place compared with different levels of benzyladenine and coconut juice (Fig. 5).

One of the most important effects of natural and synthetic cytokinins is delaying aging and accelerating transition of nutrition and organic materials (Kioshi, 2003). In fact, it can be said that coconut juice and benzyladenine through maintaining and increasing carbohydrates of shoot have increased dry matter of alstroemeria cut flowers. Mutui *et al.* (2001) reported that the use of 75 to 100 mg l⁻¹ benzyladenine can increase dry matter of alstroemeria cut flowers. Gul and Tahir (2013) also stated that treating narcissus cut flowers with cycloheximide pulse treatment increases dry matter that is in accordance with the results of current research.

Chlorophyll a

According to the results of table ANOVA, the effect of different levels of cycloheximide, benzyladenine and coconut juice on chlorophyll a was significant at 1% level (Table 1) and alstroemeria leaves chlorophyll a content has been significantly increased with the use of above treatments. Fig. 5 shows the minimum and maximum chlorophyll a are related to the control treatment (1.81 mg g⁻¹ FW) and 50 mg l⁻¹ cycloheximide (6.01 mg g⁻¹ F.W.), respectively. In different levels of cycloheximide and coconut juice, chlorophyll a was significantly reduced with increasing concentrations. However, in different levels of benzyladenine, the amount of chlorophyll a was increased with increasing concentration (Fig. 6).

Chlorophyll b

The results of the data corresponding the effect of different treatments on chlorophyll b showed a significant difference at 1% level between treatments of cycloheximide, benzyladenine and coconut juice (Table 1). Chlorophyll b had the maximum amount in treatments of 50 mg l^{-1}

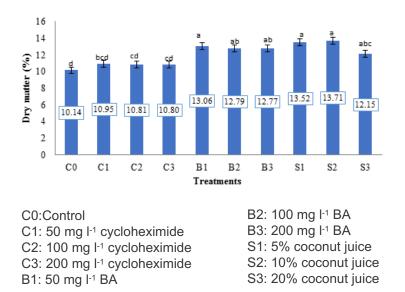


Fig. 5. The effect of different treatments on dry matterof cut flowers alstroemeria.

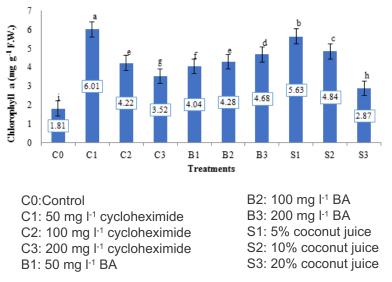


Fig. 6. The effect of different treatments on chlorophyll a of cut flowers alstroemeria.

cycloheximide (4.2 mg g⁻¹ FW) and 5% coconut juice (2.36 mg g⁻¹ FW). In general, all treatments used in this study significantly increased chlorophyll b of alstroemeria leaves compared to the control (71.0 mg g⁻¹ FW) (Fig. 7).

The use of cytokinins has been recommended to reduce decomposition of chlorophyll in cut flowers. Mortazavi *et al.* (2007) believe that cytokinins prevent degradation and deterioration of proteins, chlorophyll and also prevented ribonucleic acid through impacting on the activity of enzymes such as proteases and chlorophyllase, and thereby delay the aging process. In the current study, the role of benzyladenine and coconut juice as a mixture of cytokinins was proven on chlorophyll a and b of alstroemeria cut flowers. So that flowers treated with these substances had more chlorophyll than the control. A group of researchers believe that cytokinins through delaying in the decomposition of proteins involved in the synthesis of chlorophyll (Mutui *et al.*, 2001) and activating the enzymes involved in biosynthesis of chlorophyll (ferrante *et al.*, 2002), prevent the degradation of chlorophyll and delay aging. Hassanpour Asil and Karimi (2010) and Ojaghi *et al.* (2013) stated that benzyladenine can increase chlorophyll and the vase life.

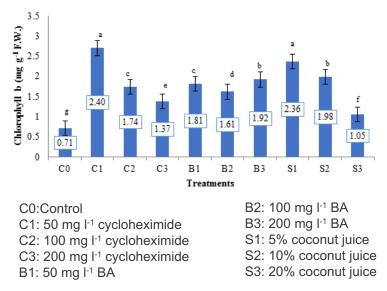


Fig. 7. The effect of different treatments on chlorophyll b of cut flowers alstroemeria.

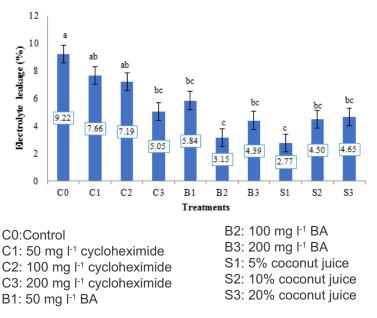


Fig. 8. The effect of different treatments on ionic leakage of cut flowers alstroemeria.

Jones *et al.* (1994) reported that cycloheximide avoids starting the aging and degradation of chlorophyll by preventing the synthesis of certain proteins. Thimann (1987) also believes that protein synthesis inhibitors such as cycloheximide can prevent the degradation of chlorophyll, this researcher stated that cycloheximide would delay the decomposition of chlorophyll and proteins in oat leaves that is in accordance with the results of current study.

Ionic leakage

The results of analysis of variance showed that there is a significant difference at the 5% level between the effects of different levels of cycloheximide, benzyladenine and coconut juice on ionic leakage (Table 1). Results of mean comparison showed that all applied treatments reduce ionic leakage of alstroemeria cut flowers, although there was statistically no significant difference between treatments of the control (9.22 %) and 50 and 100 mg l⁻¹ cycloheximide. The most successful treatments of ion leakage reduction were treatments of 5% coconut juice (2.77 %) and 100 mg l⁻¹ benzyladenine (3.15 %) that had statistically no significant difference (Fig. 8).

Lesham (1988) reported that cytokinins reduce aging and assist the ability of transferring and accumulation of metabolites in tissues and organs as well as maintaining the structure of the membrane. Arefnia *et al.* (2013) stated that spraying *Lilium* cut flowers with benzyladenine can maintain cell membrane stability and increase shelf-life. Penner and Wiely (2008) believe that cytokinins delay a preliminary aging of petals by maintaining the cell health. Abd El Aziz (2007) reported that benzyladenine can increase the vase life of cut flowers by improving the durability of the cell membrane and delaying cell membrane lipid peroxidation that agrees with the results of current study. Kazemi *et al.* (2011) believe that losing the health of membrane and membrane process increases permeability and ionic leakage in old cut flowers. Hunter et al. (1973) showed that protein synthesis controllers like cycloheximide are effective in maintaining membrane health. Van Doorn *et al.* (1994) believe that the decrease in ionic leakage by cycloheximide in petal cells is due to the prevention of synthesis of proteins, which increase ions. In a study counducted by Gul and Tahir (2012), it was found that pulse treatment of narcissus cut flowers with cycloheximide reduces the ionic leakage that is in accordance with the results of current research.

CONCLUSIONS

According to the results of this study, the use of cycloheximide, benzyladenine and coconut juice in alstroemeria cut flower vase solution prevents the degradation of chlorophyll and cell membrane structure and by increasing dry matter and ° Brix of flowers has provided more favorable conditions for longer durability of alstroemeria cut flowers than the control. Among abovementioned treatments, the treatment of 5% coconut juice was the superior treatment for most of the traits so that it increased vase life by 6.63 days compared to the control group. Therefore, the use of 5% coconut juice in the vase solution of alstroemeria cut flowers is recommended for achieving a longer post-harvest life.

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