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The Effect of Natural Essential Oil Carvacrol and Some Growth Regulators on Vase Life of Cut Flowers of *Alstroemeria* cv. Bridal

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Abstract

The abscission of *Alstroemeria* petals is the serious problem at the industry of cut flowers of this plant. In this study, cut *Alstroemeria* cv. Bridal flowers were pulsed with solutions containing natural essential oil carvacrol, gibberellic acid and benzyladenine (50 and 100 mg L^{-1}), -5-sulfosalicylic acid (1 and 1.5 mM) and sucrose (5 and 10%) for 24 hours. The distilled water was used as control. After treatment, the flowers were placed in distilled water, and maintained at temperature of 22 ± 2°C, 70 ± 5% relative humidity, and 15 µmol m^{-2} s^{-1} light intensity 12 hours per day. The results showed that 50 and 100 mg L^{-1} gibberellic acid could significantly delay flower senescence with 3.33 and 3 days, respectively as compared to the other treatments. The highest petal anthocyanin content was found at gibberellic acid (50 and 100 mg L^{-1}), benzyladenine (100 mg L^{-1}) and 5-sulfosalicylic acid (1.5 mM) than other treatments. Conversely, lipid proxidation content and catalase enzyme activity was lower in these treatments as compared to the control. The protein content of gibberellic acid (50 and 100 mg L^{-1}) pulse treated flowers was higher than other treatments. In contrast, the flowers treated with 100 mg L^{-1} gibberellic acid showed the lowest peroxidase enzyme activity. Overall, the vase life of *Alstroemeria* cut flowers cv. Bridal increased in both gibberellic acid treatments (50 and 100 mg L^{-1}) than other studied solutions.

Keywords: *Alstroemeria*, Benzyladenine, Senescence, 5-Sulfosalicylic acid, Vase life.

INTRODUCTION

In the past two decades, *Alstroemeria* was one of the most popular cut flowers in the countries like Japan, Holland, England and America commercially (Ezhilmathi *et al.*, 2007). Although, *Alstroemeria* cut flowers have long vase life, but rapid leaf yellowing in postharvest and before that, the falling petals are the most important of limiting factors (Chanasut *et al,*. 2003). By adding some chemicals to the preservation solutions and provide suitable conditions for the flowers can delay decreasing of quality during postharvest (Ebrahim-Zadeh and Saifi,1999).

The flower preservation solutions are often acidic solution with microbicide to prevent growth of fungi and bacteria (Sobhani *et al.*, 2005), and thus that prevents the blocking of vessels that reduces water uptake by the flowers. Also, in order to improve the postharvest quality of cut flowers, plant growth regulators can add to preservative solution. Cytokinins, gibberellins, ethylene inhibitors and retardants of plants growth interfere in metabolic processing of plants, and cause the delaying senescence. Cytokinin have been identified as a retarder leaves of senescence processes, delaying the breakdown of proteins, reduced chlorophyll and increased the activity of many of the hydrolyzates (Skutnik *et al.*, 2001). Gibberellic acid increases the hydrolysis of starch and sucrose to glucose and fructose, also increases hasten flower opening, reducing the amount of dry matter in the stems and petals and delays in the loss and fading of petals (Emongor and Tshwenyana, 2004).

In recent years, the use of natural compounds such as plant essential oils as the new idea for control of bacterial and fungal contamination and reducing postharvest losses of horticultural crops such as fruits, vegetables and flowers is raised. Researches and commercial applications have revealed that natural compounds can be suitable replace for common chemical compounds (Solgi *et al.*, 2009). Hegazi and El-Kot (2009) showed that the essential oils of clove hindi, cinnamon, ginger, marjoram and fennel for gladiola reduce microbes accumulation in containers and increase the vase life. Solgi *et al*. (2009) showed that treatment with essential oils (thymol, carvacrol, garden thyme (*Thymus vulgaris*) and thyme (*Zataria multiflora*)) was significant effect in the solution uptake, fresh weight and vase life cut gerbera 'Dune'.

This study compared the effects of natural essential oils, carvacrol, with some growth regulators on postharvest life of cut *Alstroemeria* and finding the best concentrations treatments to enhance the vase life of cut flowers.

MATERIAL AND METHODS

Plant materials

Alstroemeria cut flowers cv. Bridal was obtained from commercial greenhouses in Pakdasht and immediately was transferred to postharvest labratoary; university of Guilan. The flower was harvested when the color was observed but the florets were not open. The flowers were pulsed in 250 ml chemical solution for 24 hours and then were transferred to containers containing 250 ml distilled water. Flowers were placed in vase life room with temperature of 22±2 °C, a relative humidity of 70±5%, light intensity of 15 µmol $m^{-2}s^{-1}$ and 12 hours day length. In this experiment vase life, anthocyanin content, lipid peroxidation, protein, peroxidase and catalase enzyme activity were evaluated.

The 12 different solutions was used in this experiment are:

1. Distilled water (DW)
2. Ethanol 1%
3. Sucrose 5% (S 5%)
4. Sucrose10% (S 10%)
5. 5-Sulfo salicylic acid 1 mM + Ethanol 1% (5-SSA 1)
6. 5-Sulfo salicylic acid 1.5 mM + Ethanol 1% (5-SSA 1.5)
7. Gibberellic acid 50 mg L^{-1} (GA 50)
8. Gibberellic acid 100 mg L^{-1} (GA 100)
9. Carvacrol 50 mg L^{-1}+ Ethanol 1% (Car 50)
10. Carvacrol 100 mg L^{-1}+ Ethanol 1% (Car 100)
11. Benzyladenine 50 mg L^{-1} (BA 50)
12. Benzyladenine 100 mg L^{-1} (BA 100)

Vase life

The end of *Alstroemeria* vase life was determined with yellowing of 50% leaves or falling 50% florets (Ferrante *et al*., 2002; Mutui *et al.,* 2006).

The total anthocyanin

In order to measure this trait, sampling was performed on the ninth day of the petals. Total anthocyanin content of petals was measured by the spectrophotometer according to the pH-differental method (Laitinen *et al.,* 2008). The acidified methanol (methanol with a volume ratio of 1% hydrochloric acid) was used for anthocyanin extraction. To determination total anthocyanin, two wavelengths (520 and 700 nm) were used.

Lipid proxidation

In order to measure this trait, sampling was conducted on the third day of the petals. Lipid proxidation was assayed by measuring the concentration of malondialdehyde according to the method of Heath and Packer (1968). This procedure produce red malondialdehyde–tio barbitoric acid (MDA-TBA) that quantified by spectrophotometry (PG Instrument + T80) at 532 nm. and other specific pigments were absorbed at wavelength of 600 nm.

Protein

In order to measure this trait, sampling was performed on the ninth day of the petals. Bradford method was used to measure protein content. The protein concentration of petals was assayed by the standard protein, bovine albumin serum (BSA) and the absorption was read at 595 nm by the spectrophotometer (PG instrument + T80). Standard curve were plotted according to the absorption standard protein and protein concentration in the samples was calculated by obtain the line equation (Bradford, 1976).

Peroxidase (POD) activity

In order to measure this trait, sampling was conducted on the third day of the petals. The activity of POD was determined according to the method of In *et al*. (2007). The reaction solution (1 mL) contained H_2O_2, guiacol and 50 µL of enzyme extract. Changes in absorbance at 470 nm were read every 10 s for 60 s using a spectrophotometer.

Catalase (CAT) activity

In order to measure this trait sampling was conducted on the sixth day of the petals. The activity of CAT was determined based on the oxidation of H2O2 using the method of Chance and Maehly (1955) with modifications. The reaction solution (0.5 mL) contained 25 mM phosphate buffer (pH = 7), 10 mM H_2O_2 and 10 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. Changes in absorbance at 240 nm were read every 10 s for 60 s using a spectrophotometer.

Statistical analysis

This experiment arranged based on RCD with 12 treatments, 3 replications, 36 plots and 108 cut flowers. In this experiment, distilled water and ethanol 1% were considered as control. Means comparison of data was performed using LSD test. Analysis of variance carried out with SAS software and diagrams were designed with Excel software.

RESULTS

Vase life

The results showed that, there is a significantly different preservative solutions and control

(distilled water and ethanol 1%) at 5% level (Table 1). The mean comparison showed that the highest flowers vase life was related to the gibberellic acid 100 and 50 mg L^{-1} with a mean vase life of 13.33 and 13 days, respectively. The vase life of control (distilled water and ethanol 1%) was 10.33 and 10 days respectively, while the best treatments with control treatments had not significant difference (Fig. 1).

Anthocyanin content

There was a significant difference for pulsed treatments and control on anthocyanin content of *Alstroemeria* petals at the 1% level (Table 1). The higher anthocyanin content was found in gibberellic acid (50 and 100 mg L^{-1}), compared to other treatments (Fig. 2).

Lipid peroxidation

Analysis of variance of pulsed flowers and control showed that there is a significant difference on lipid peroxidation at 1% level (Table 1). Ethanol 1% had highest levels of malondialdehyde, but gibberellic acid (50 and 100 mg L^{-1}) had lowest MDA (2.4215 and 2.4022 nmol g^{-1} FW), respectively.

Protein content

Analysis of variance of pulse treatment and control showed that there is a significant difference on protein content at 5% level (Table 1). The results showed that gibberellic acid (50 and 100 mg L^{-1}) had highest protein content, while, control (DW and ethanol 1%), and sucrose 5 and 10%, had lowest protein content (Fig. 4).

POD activity

There was a significant difference between pulsed and contol cut flowers for POD activity

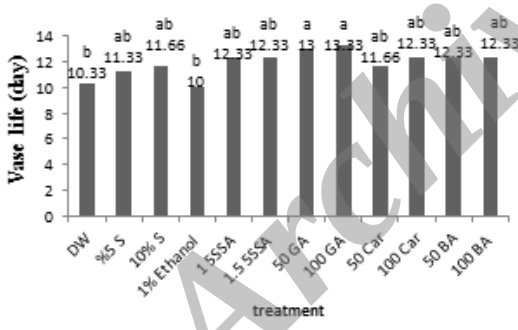

Fig. 1. Effect of different treatments on the vase life of cut *Alstroemeria* 'Bridal'

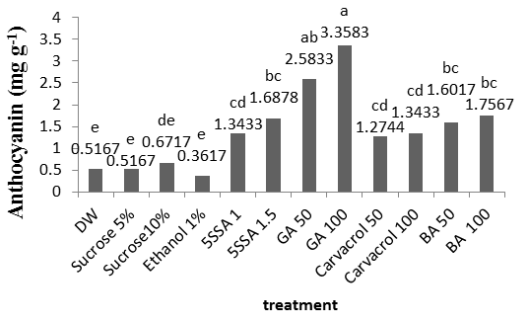

Fig. 2. Comparison of the treatments in anthocyanin of cut *Alstroemeria* 'Bridal'

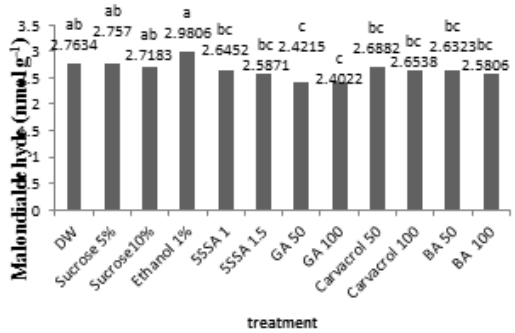

Fig. 3. Comparison of the treatments in the malondialdehyde amount of cut *Alstroemeria* 'Bridal'

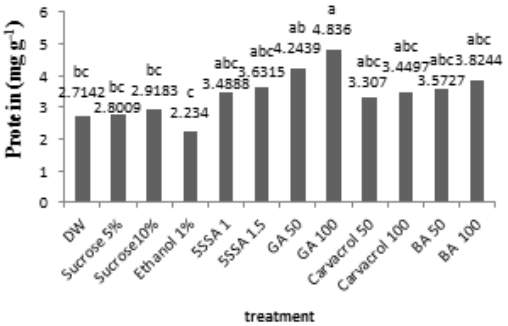

Fig. 4. Comparison of the treatments on the protein content of cut *Alstroemeria* 'Bridal'

at 1% level (Table 1). The ethanol 1% had significantly higher POD activity. In contrast, the lowest POD activity was found with 100 mg L^{-1} gibberellic acid (Fig. 5).

CAT activity

The analysis of variance showed that there is a significant difference at 5% level for CAT activity (Table 1). The highest CAT activity was found with ethanol 1%. While gibberellic acid (50 and 100 mg L^{-1}), benzyladenine (50 and 100 mg $L^{-1)}$ and 5-sulfosalicylic acid (1 and 1.5 mM) significantly reduced CAT activity compared to other preservation solutions (Fig. 6).

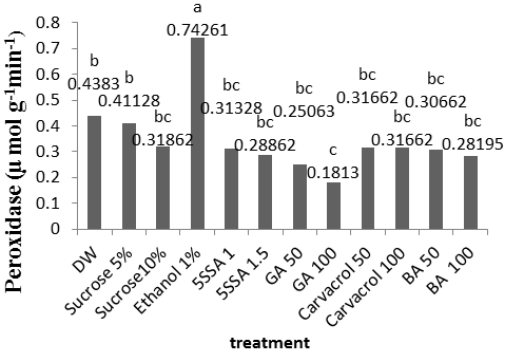

Fig. 5. Comparison of the treatments on the peroxidase activity of cut *Alstroemeria* 'Bridal'

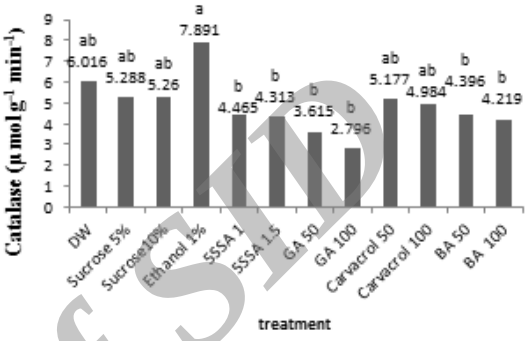

Fig. 6. Comparison of treatments on the catalase activity of cut *Alstroemeria* 'Bridal'

Table 1. Analysis of variance of treatments effect on the measured traits.

Sources of changes	Degrees of freedom	Mean square					
		Vase life	Anthocyanin	MDA	Protein	POD	CAT
Treatment	11	2.916*	2.365**	0.071**	1.492*	0.59**	4.874*
Error	34	1.277	0.106	0.016	0.641	0.007	1.993
CV (%)		9.485	9.668	4.827	7.889	8.072	16.746

* and** Significant at p ≤0.05 and p ≤0.01 level, respectively.

DISCUSSION

Positive effect of gibberellic acid on vase life could be related to maintaining chlorophyll content, soluble carbohydrates and improving the quality of flower color. Mutui *et al*. (2006) showed that GA_{4+7} delayed the start of leaf senescence to 7 days and falling petals to 2 days. Ezhilmathi (2001) showed that 5-sulfosalicylic acid make the most impact on the increased gladiolus cut flowers vase life. Solgi (2009) proved that 100 mg L^{-1} thymol and 50 mg L^{-1} carvacrol compared to the control treatment had most effective in increasing the vase life of cut gerbera. Negative effects of carvacrol and 5-sulfosalicylic acid in this experiment on vase life may be due to low concentration of them or ethanol used to dissolve it in distilled water. It appears that the cause of negative effect of ethanol on vase life of cut *Alstroemeria* is sensitivity of this flower to ethanol and 1% ethanol was toxicity and caused to early yellowing in the leaves. Hatamzadeh *et al.* (2012) showed that sucrose 1% in pulse solution is more effective on extending vase life of *Alstroemeria* cut flowers. So, we can conclude from this study that perhaps the use of sucrose 5 and 10% without antimicrobial compounds provides the conditions for the growth of microorganisms.

Mackay *et al.* (2005) showed that gibberellic acid with sucrose increased longevity of cut lupine, increased flower size and opening rate and improve the quality of petal color. Joyce *et al.*

(2004) showed that short-term treatments with 1 mM to 10 mM benzyladenine increased longevity and delayed the aging parameters such as loss of fresh weight, wilting, delayindg in opening of flower, colorless and abscission of cut *Grevillea* flowers.

Ghasemi Chlan and Haji Zadeh (2011) in their experiment on the rose 'Black Magic' concluded that the highest levels of anthocyanin leakage was observed in control flowers while flowers treated with 8-hydroxyquinoline citrate and silver nitrate were lowest anthocyanin leakage. It seems to be that two mentioned treatments are more effective in protecting cell membranes and prevent from electrolyte leakage. Bosma and Reid (2002) demonstrated that sugar increased number of open buds, bud opening speed, improved color of petals and extended longevity of cut *Campanula* flower. It seems that sucrose concentration used in our experiments provides an environment for growth of microorganisms and had negative effect on petals color.

Malondialdehyde accumulation is an index for degradation of the plasma membrane. Sing and Sharma (2003) in a study on the effects of plant growth regulators and sucrose on postharvest physiology, membrane stability and vase life of cut gladiolus, reported that gibberellic acid with sucrose reduced the lipid peroxidation, decreased lipoxygenase enzyme activity and improved the strength of the petals cell wall. The role of salicylic acid in plant protection against lipid peroxidation as an antioxidant compound is protect of the cell membrane. Salicylic acid is a plant hormone that stimulate resistance system. Significant difference was observed between carvacrol and control cut flowers in MDA content of cut *alstromeria* cv. Sukari (Solgi *et al.*, 2009, Madadzade *et al.*, 2012).

Eason *et al.* (2007) showed that gibberellic acid by delay in protease enzyme activity and protein breakdown, delayed the aging process of *Sandersonia* cut flowers. According to results of Ranwala and Miller (2000), oriental lily cut flowers that treated with GA_{4+7} had lower respiration than the control flowers and soluble carbohydrate levels of these plants was higher than control flowers. Lack of carbohydrates, destroyed the proteins so that the proteins can used as respiratory substrate, it seems that gibberellic acid with maintaining from soluble carbohydrates, prevented from protein degredation. According to Skutnik *et al.* (2001) cytokinins are known as delaying of the senescence process in leaves, and proteins degradation, reducing chlorophyll and increasing the activity of hydrolase. Benzyladenine, that was used in this experiment, had not significant effect on cut *Alstroemeria*. Also, the lack of a positive effect of 5-sulfosalicylic acid and carvacrol in maintaining the protein in *Alstroemeria* can be attributed to ethanol. In relation to carvacrol, our results is agreement with Solgi (2009) results on gerbera cut flowers and Madadzadeh (2012) on the *Alstroemeria* cut flowers, so these investigators reported that the preservative solutions containing silver nanoparticles, thymol and carvacrol with sucrose delayed senescence.

Gibberellic acid (100 mg L^{-1}) delayed the senescence and had lower peroxidase activity. But the ethanol 1%, distilled water and sucrose 5% treatments due to lower vase life, had more enzyme activity. Also, according to the Joyce *et al.* (2004), short-term treatment with 1-10 mM benzyladenine, increased vase life and delayed the senescence parameters such as loss of fresh weight, wilting, delaying in opening of flower, colorless, and abscission of *Grevillea* cut flowers, so we can conclude that the gibberellic acid and benzyladenine can reduce stress effects on plants with reduction of peroxidase and catalase antioxidant enzymes activities. Madadzadeh (2012) with the use of silver nanoparticles compound, thymol and carvacrol on *Alestroemeria* cv. 'Sukari' cut flowers showed that these compounds caused significantly reduced enzyme peroxidase activity compared to the control plants. These results are agreement with our results related to the use of carvacrol in two concentrations (50 and 100 mg L^{-1}) and 5-sulfosalicylic acid (1 and 1.5 mM), on cut *Alstroemeria* cv. Bridal. Indeed, salicylates as antioxidant compounds decreased ROS damages.

The significant difference was observed for catalase activity between 5-sulfo salicylic acid 1 and 1.5 mM with 1% ethanol. Ethanol could not to neutralize a positive impact 5-sulfosalicylic acid. The increase of antioxidant enzyme activity increases the senescence of flowers. Therefore,

the using higher concentrations of essential oils cause the decreasing the enzymes activity (Ponce *et al*., 2003), because phenolic compounds are one of the important antioxidant compounds that have an important role in ROS scavengering and protecting from the membrane leakage. As was described, gibberellic acid and benzyladenine by decreasing plant stresses, reduced antioxidant enzyme activity. Ethanol and sucrose, respectively, to creating a toxic and for microbial growth environment, were provided conditions to increase antioxidant enzyme activities.

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