

Improving *Alstroemeria* Vase Life by Plant Extracts and 8-Hydroxyquinoline Sulfate

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Received: 06 November 2018

Accepted: 13 January 2019

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The effects of savory, caraway, and rose extracts and 8-HQS were studied on the vase life of *Alstroemeria* cut flowers. The plant extracts at three levels (10, 20, and 40 %), 8-HQS at three levels (100, 200, and 300 mg l⁻¹), and control (distilled water) with 3 % sucrose were continuously applied on the basis of a Completely Randomized Design with three replications. It was found that the treatments were more effective on sustaining vase life as compared to control. The application of 8-HQS at the rates of 200 and 300 mg l⁻¹ resulted in the longest vase life of 19.83 and 19.66 days, respectively. Among plant extracts, 20 % savory showed the longest vase life (18 days). The highest solution uptake (1.81 ml g⁻¹ FW) was associated with the application of 20% savory extract. The treatments of 10, 20, and 40 % savory extract and 200 mg l⁻¹ 8-HQS were the most successful treatments in sustaining fresh weight. The lowest bacterial population in vase solution (3 Log₁₀ CFU ml⁻¹) was observed in the treatment of 200 mg l⁻¹ 8-HQS. The treatments of 20 % savory extract and 200 mg l⁻¹ 8-HQS had the lowest electrolyte leakages of 4.46 and 4.93 %. The treatments of 40 and 20 % savory extract were the most effective treatments in reducing MDA accumulation. Given these results, it is recommended to use savory extract as a natural, environment-friendly compound to improve post-harvest traits and extend the longevity of *Alstroemeria* cut flowers.

Abstract

Keywords: *Carum carvi*, Rose water, *Satureja hortensis*, Vase solution.

INTRODUCTION

Alstroemeria is one of the most important cut flowers in the world and a newly introduced flower in Iran whose stunning beauty of petals has attracted a lot of people. Leaf yellowing and petal shedding are the major factors limiting its post-harvest longevity (Bagheri and Sedaghatour, 2013; Isapareh *et al.*, 2014).

Post-harvest longevity is a critical factor in the perceived quality of cut flowers and has a significant role in their economical value and marketability (Soleimany Fard *et al.*, 2013). Since the detachment of cut flowers from the maternal plant is the main cause of their senescence and quality loss, providing conditions similar to the pre-detachment conditions in terms of, say, water, energy or carbohydrate availability can decelerate their senescence and degradation (Goszynska *et al.*, 1990; Solgi and Ghorbanpour, 2015; Monterio *et al.*, 2002).

In addition to supplying the energy to the cut flowers, the application of sugar in vase solution can also be a good nutritional source for microorganisms that can, in turn, block the vessels, reduce the solution uptake, and shorten the longevity of the cut flowers (Jahanifar *et al.*, 2016). So, it is necessary to apply disinfectants along with sugars (Williamson *et al.*, 2002; Balestra *et al.*, 2005).

Numerous disinfectants have been already proposed to be applied with sucrose, two of the most commonly used ones being 8-hydroxyquinoline citrate (8-HQC) and sulfate (8-HQS) that are used as strong disinfectants in the suppression of vase solution microorganisms (Singh and Sharma, 2003). Tar and Hassan (2003) reported that the application of 8-hydroxyquinoline (8-HQ) in preservative solution of *Cosmos sulphureus* cut flowers reduced microorganisms in vase solution and stem end and improved their longevity significantly. There are numerous reports confirming that the application of 8-HQ in the preservative solution of the cut flowers is a good way to reduce microbial populations in vase solution and improve the longevity of the cut flowers (Gilman and Steponks, 1972; Masoodi *et al.*, 2012; Hassan, 2005).

After the recognition of adverse impacts of chemicals on human health and environment, the removal and reduction of their use were put on the agenda of the post-harvest physiologists of agricultural and horticultural products. In this respects, plant essential oils and extracts have been used as natural disinfectants in vase solution. Many studies show their positive effects (Solgi *et al.*, 2009; Ponce *et al.*, 2004; Bakkali *et al.*, 2007). According to Sheniyor *et al.* (2010), amaryllis cut flowers showed longer longevity in vase solution containing essential oil of thyme than in that containing 8-HQS. Similar studies have persuaded researchers to look for the influence of different plant essential oils and extracts on post-harvest life of cut flowers. The present study used the extracts of rose, savory, and caraway to improve the longevity of *Alstroemeria*.

Rose extract that is taken from the petals and sepals of roses has antibacterial and antioxidant properties. It contains diverse compounds including geranium (30-40 %), citronellol (40-60 %), linalool (20-30 %) and strapen (20-25 %) (Ataee Bojd *et al.*, 2014).

Savory (*Satureja hortensis*) is a medicinal herb belonging to Lamiaceae family and the main ingredients of the essential oil of its shoot are carvacrol (30-40 %), thymol (20-30 %) and other phenolic compounds (Fathi *et al.*, 2013; Mohammadhosseini and Biranvand, 2013). Various studies have mentioned numerous biological activities for savory essential oil and extract including antibacterial, antioxidant, and antifungal properties (Mohammadhosseini and Biranvand, 2013). Zakerin and Golban (2015) found that the use of savory extract postponed the wilting of rose cut flowers. Bayat *et al.* (2012) observed the longest vase life of the cut flowers of carnation cv. 'Yellow Candy' in vase solution containing 100 mg l⁻¹ savory essential oil.

Caraway (*Carum carvi*) belongs to Umbelliferacea family. Limonene and carvone are the main constituents of caraway seed essential oil (Jiang *et al.*, 2011). Some researchers suggest that the application of S-carvone (as the principal constituent of caraway oil) in vase solution extends

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cut flower longevity via sustaining water uptake and fresh weight (Damunpola *et al.*, 2010; He *et al.*, 2006).

In this experiment, we aimed at studying the influence of plant extracts (of rose, savory and caraway) on sustaining vase life of *Alstroemeria* and comparing their impacts with the effect of 8-hydroxyquinoline sulfate (8-HQS).

MATERIALS AND METHODS

The study was carried out on vase life of *Alstroemeria* cut flowers on the basis of a Completely Randomized Design with 13 treatments and 3 replications. The flowers were procured from a commercial greenhouse in Tehran. They were harvested at commercial maturity, that is, when the color of most florets was visible (Chanasut *et al.*, 2003), and were immediately transferred to laboratory with 22 ± 2 °C, 60-70 % RH. The extracts were procured from Barij Essence Co. 8-HQS was a product of Merck Co. (Germany). The experimental treatments included control (distilled water), rose, savory and caraway extracts (10, 20, and 40 %), and 8-HQS (at three rates of 100, 200, and 300 mg l⁻¹) applied with sucrose 3 % continuously. The plants were sampled every other day. The following traits were measured as described.

Vase life

It was the number of days from treatment until 50% floret desiccation (Mutui *et al.*, 2006).

Solution uptake

It was estimated by the following equation:

$$\text{Solution uptake (ml g}^{-1}\text{ FW)} = \frac{Vt0 - (Et + Vt1)}{FW}$$

Where, Vt0 represents initial volume of vase solution (500 cc), Et represents mean surface evaporation of the solution, Vt1 represents the volume of solution on the last day, and FW represents the fresh weight of flowers on the first day.

Fresh weight loss

The fresh weights of cut flowers were measured at the end of vase life by a digital scale and then, the fresh weight loss was calculated by the following equation:

Fresh weight loss = Initial fresh weight – (Final fresh weight + Recut weight + Desiccants weight)

Percent dry matter

After the fresh weights of cut flowers were determined on the last day, the flowers were oven-dried at 70 °C for 24 hours. Then, percent dry matter was estimated by the following equation:

$$\text{Percent dry matter} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100$$

Degrees Brix (°Brix) loss

Degrees Brix was measured on the small re-cuts from the stem end on the first and last days of the vase life. One or two drops of water in these parts were soaked on reflectometer (model N-1α, ATAGO, Japan), and °Brix was read. The loss of °Brix was calculated as the difference of °Brix on the last and first day of the vase life.

Flower opening index

To estimated flower opening index, the highest flower diameter and the perpendicular diagonal to that were measured by a caliper every other day under the full opening of the flowers. Then, they were averaged and entered into the following equation to estimate flower opening

$$\text{Flower opening index} = \frac{\frac{D_n + 2}{D_n} + \frac{D_n + 4}{D_n + 2} + \frac{D_n + 6}{D_n + 4}}{3}$$

Where, D_n represents the number of days when flower diameter was measured, $D_n + 2$ represents the diameter of the flower on the second day and so on.

Vase solution bacteria enumeration

The vase solution was sampled 24 hours after the treatments and the bacteria colonies in vase solution were enumerated by Liu *et al.* (2009).

Petal's carotenoids

The petals were sampled at the end of vase life of control to measure petal carotenoid. It was read at 440, 645 and 663 nm wavelengths by spectrophotometer, and the pigment concentration was calculated by the following equation expressed in $\mu\text{g g}^{-1}$ DW:

$$\text{Petal's carotenoids} = 4.69 \times A_{440} - 0.268 \times (20.2) A_{645} + (8.02) A_{663}$$

Electrolyte leakage and malondialdehyde (MDA)

To estimate electrolyte leakage and malondialdehyde (MDA), young leaves of the plants were sampled on the fifth day of the experiment. The former was measured by Kaya *et al.* (2001)'s method and the latter by Heath and Parker (1969)'s method.

After the measurement of the traits and collection of the data, they were statistically analyzed by MSTATC Software Package and the means were compared by LSD test at the 1 and 5 % probability levels.

RESULTS

Vase life

All treatments changed vase life of *Alstroemeria* significantly at the 5 % probability level (Table 1). It was revealed that the treatment with disinfectants significantly extended vase life as compared to control so that control plants had the shortest vase life of 13.33 days and the treatments with 200 and 300 mg l^{-1} 8-HQS were associated with the longest vase life of 19.83 and 19.66 days, respectively. Among plant extracts, the longest vase life was observed in the treatment of 20 % savory extract (18 days), but it did not show statistically significant differences with those observed in the treatments of 200 and 300 mg l^{-1} 8-HQS. The treatments of 40 % rose extract, 10 and 40 % savory extract, and 40 % caraway extract were among the best treatments for ensuring the longevity of *Alstroemeria* (Table 2).

Solution uptake

According to the analysis of variance, solution uptake was influenced by the treatments at the 1% probability level (Table 1). As means comparison indicated, the application of plant extracts and 8-HQS at all rates increased solution uptake as compared to control. As is evident in Table 2, the highest solution uptake of 1.81 ml g^{-1} FW was observed in the treatment of 40 % savory extract followed by the treatments of 200 and 300 mg l^{-1} 8-HQS which did not show significant differences to each other. Control plants showed the lowest solution uptake of 0.446 ml g^{-1} FW (Table 2).

Fresh weight loss

Analysis of variance revealed significant differences among different treatments in *Alstroemeria* fresh weight (FW) loss at the 1 % probability level (Table 1). Means comparison pointed to the treatment of 22 mg l⁻¹ 8-HQS as the most successful one in hindering FW loss. It showed an FW loss of 2.94 g, but it did not show significant differences with the treatments of 10, 20, and 40 % savory extract. Control (with an FW loss of 14.9 %) as well as the treatments of 10 and 20 % rose extract were linked with the highest FW loss and they were not appropriate for sustaining FW (Table 2).

Percent dry matter

Percent dry matter of *Alstroemeria* was significantly impacted by different treatments at the 5% probability level (Table 1). As can be seen in Table 2, percent dry matter was higher in all treatments than in control (11.52 %). The highest dry matter was related to the treatments of 10 and 20% rose extract, 10 % savory extract and 300 mg l⁻¹ 8-HQS, which were significantly different from the treatments of 40 % rose extract, 20 and 40% savory extract, 40 % caraway extract, and 100 and 200 mg l⁻¹ 8-HQS (Table 2).

Flower opening index

Analysis of variance indicated that the effect of different treatments was significant on flower opening index of *Alstroemeria* at the 1 % probability level (Table 1). Means comparison revealed that the treatment of 20 % savory extract and 20 % caraway extract were related to lowest rate of flower opening (0.41 and 0.42, respectively). The highest opening rate of 0.72 was obtained from the treatment of 100 mg l⁻¹ 8-HQS which was not significantly different from the treatments of 200 mg l⁻¹ 8-HQS (0.68), 40 % savory extract (0.70) and 10 % rose extract (0.63) (Table 2).

Degrees Brix loss

The effects of plant extracts and 8-HQS were shown by analysis of variance to be significant on °Brix loss of *Alstroemeria* at the 1% probability level (Table 1). Means comparison revealed that all disinfectants inhibited the loss of °Brix as compared to control (1.63). The impacts of 10% rose extract (1.36) and 40% caraway extract (1.166) were not statistically significant compared to control. The lowest loss of °Brix (0.60 and 0.66) was linked with the treatments of 300 and 200 mg l⁻¹ 8-HQS which prevented the reduction of °Brix significantly as compared to control. Among plant extracts, 10% and 40% savory extract and 10% caraway extract were the best in the inhibition of °Brix loss (0.75, 0.81, and 0.85, respectively) (Table 2).

Vase solution bacteria

Plant extracts and 8-HQS changed vase solution bacteria population significantly at the 1 % probability level (Table 1). Means comparison revealed that all disinfectants inhibited the growth and propagation of bacteria in vase solution significantly as compared to control, so that control showed the highest number of bacteria in vase solution (52.33 Log₁₀ CFU ml⁻¹). Though reduced the bacteria population in vase solution to 47 Log₁₀ CFU ml⁻¹, the application of 40% rose extract did not show significant differences with control. The best compound in reducing bacteria population was 8-HQS at all rates, especially at the rate of 200 mg l⁻¹ (3 Log₁₀ CFU ml⁻¹). Among plant extracts, the application of 40% savory extract (13 Log₁₀ CFU ml⁻¹) was the most effective compound in controlling bacteria population (Table 2).

Table 1. Analysis of variance of the effect of 8-HQS and rose, savory and caraway extracts on the measured traits.

S.o.V	df	Vase life	Solution uptake	Dry matter	Fresh weight loss	Brix loss	Flower opening index	Vase solution bacteria	Electrolyte leakage	MDA
Treatments	12	11.59*	0.546**	2.43*	43.71**	0.246**	0.029**	832.1**	8.50**	17.1**
Error	26	4.84	0.119	1.29	6.20	0.079	0.004	19.82	1.16	7.69
cv (%)		12.99	30.7	8.37	33.29	28.78	11.51	16.47	15.6	19.65

* and ** show significance at the 5 and 1% probability levels in the LSD test, respectively.

Table 2. Means comparison for the effect of 8-HQS and rose, savory and caraway extracts on the measured traits.

Treatments	Vase life (day)	Solution uptake (ml g ⁻¹ F.W.)	Loss F.W.(g)	Flower opening index	Dry matter (%)	Brix loss	Bacteria in the vase solution (Log10 CFU ml ⁻¹)	Petals carotenoid (µg g ⁻¹ F.W.)	Electrolyte leakage(%)	MDA (nmol g ⁻¹ F.W.)
Control	13.33 ^e	0.44 ^f	14.9 ^a	0.55 ^{cd}	11.52 ^c	1.63 ^a	52.33 ^a	0.042 ^b	10.23 ^a	17.01 ^a
10 % Rose water	14.00 ^{de}	0.67 ^{ef}	11.41 ^{ab}	0.63 ^{abc}	14.09 ^a	1.36 ^{ab}	44.33 ^{bc}	0.092 ^c	5.52 ^{ef}	16.23 ^{ab}
20 % Rose water	14.83 ^{cde}	0.523 ^f	11.79 ^{ab}	0.49 ^{de}	14.57 ^a	1.01 ^{bcd}	25.66 ^d	0.079 ^{de}	7.7 ^{bc}	15.33 ^{abc}
40 % Rose water	17.16 ^{a-d}	0.96 ^{def}	8.93 ^{bc}	0.57 ^{cd}	13.94 ^{ab}	1.06 ^{bcd}	47.00 ^{ab}	0.065 ^e	9.45 ^{ab}	16.49 ^{ab}
10 % Satureja	17.00 ^{a-e}	1.13 ^{b-e}	3.94 ^e	0.53 ^{cd}	14.14 ^a	0.98 ^{bcd}	23.33 ^d	0.066 ^e	6.12 ^{c-f}	17.63 ^a
20 % Satureja	18.00 ^{abc}	1.21 ^{b-e}	3.74 ^e	0.41 ^e	14.03 ^{ab}	0.75 ^{cd}	18.66 ^{de}	0.124 ^b	4.46 ^f	11.29 ^{cd}
40 % Satureja	17.83 ^{abc}	1.81 ^a	3.88 ^e	0.70 ^{ab}	13.89 ^{ab}	0.81 ^{cd}	13.00 ^{ef}	0.139 ^a	7.52 ^{cd}	10.19 ^{cd}
10 % Carum	17.83 ^{abc}	0.926 ^{ef}	10.49 ^b	0.51 ^{de}	12.94 ^b	0.85 ^{cd}	39.00 ^c	0.076 ^e	5.95 ^{c-f}	13.75 ^{a-d}
20 % Carum	16.00 ^{b-e}	1.02 ^{c-f}	8.69 ^{bcd}	0.42 ^e	12.16 ^{bc}	0.96 ^{bcd}	43.33 ^{bc}	0.085 ^{cd}	7.67 ^{bc}	14.16 ^{a-d}
40 % Carum	16.83 ^{a-e}	1.20 ^{b-e}	6.16 ^{bd}	0.60 ^{bcd}	13.54 ^{ab}	1.16 ^{abc}	25.33 ^d	0.075 ^{cf}	7.30 ^{cde}	15.09 ^{abc}
100 mg l ⁻¹ HQS	17.83 ^{abc}	1.50 ^{a-d}	4.66 ^{de}	0.72 ^a	14.06 ^{ab}	0.81 ^{cd}	10.00 ^{fg}	0.067 ^{fg}	7.27 ^{cde}	11.91 ^{bd}
200 mg l ⁻¹ HQS	19.83 ^a	1.63 ^{ab}	2.94 ^e	0.68 ^{ab}	13.26 ^{abc}	0.66 ^d	3.00 ^g	0.085 ^{cd}	4.93 ^f	12.03 ^{bd}
300 mg l ⁻¹ HQS	19.66 ^{ab}	1.57 ^{abc}	5.66 ^{cde}	0.60 ^{bcd}	14.44 ^a	0.60 ^d	6.33 ^{fg}	0.134 ^a	5.79 ^{cde}	13.45 ^{a-d}

With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test.

Petal's carotenoids

Analysis of variance for petal carotenoid of *Alstroemeria* cut flowers revealed significant differences among the treatments at the 1% probability level (Table 1). According to means comparison, all treatments sustained petal carotenoid as compared to control. Carotenoid content was the lowest ($0.042 \mu\text{g g}^{-1}$ FW) in control. The treatments of 40 % savory extract and 300 mg l^{-1} 8-HQS produced the highest petal carotenoid contents of 0.139 and $0.134 \mu\text{g g}^{-1}$ FW, respectively (Table 2).

Electrolyte leakage

Analysis of variance showed that different treatments significantly changed electrolyte leakage of *Alstroemeria* cut flowers at the 1 % probability level (Table 1). The study of means comparison revealed that all disinfectants reduced electrolyte leakage significantly as compared to control. The highest leakage (10.23 %) was related to control with no statistically significant differences with that of 40 % rose extract (9.45 %). The lowest electrolyte leakage was observed in the treatment of 20 % savory extract (4.46 %) and 200 mg l^{-1} 8-HQS (4.93 %) which had no significant difference to each other (Table 2).

Malondialdehyde (MDA)

The influence of different treatments was significant on MDA accumulation at the 1% probability level (Table 1). Table 2 shows that MDA was the highest in control ($17.01 \text{ nmol g}^{-1}$ FW) and 10 % savory extract ($17.63 \text{ nmol g}^{-1}$ FW), which showed no statistically significant differences with that under the treatment of 10, 20 and 40% rose extract, 10, 20 and 40 % caraway extract, and 300 mg l^{-1} 8-HQS. The lowest MDA was related to the treatment of 40 % and 20 % savory extract (10.19 and $11.29 \text{ nmol g}^{-1}$ FW, respectively) (Table 2).

DISCUSSION

Maintaining water uptake and controlling microbial population in vase solution are two key factors in extending the longevity of cut flowers. The present study showed that 8-HQS had the strongest effect on vase life, solution uptake, percent dry matter, microbial population suppression, and fresh weight loss inhibition. Plant extracts like savory did not show significant differences in these traits with 8-HQS and improved membrane structure maintenance, electrolyte leakage inhibition, and MDA accumulation reduction of *Alstroemeria* as compared to other extracts and control.

Although 200 and 300 mg l^{-1} 8-HQS were more effective than other treatments in longevity improvement, the application of 20 % savory increased the longevity of *Alstroemeria* cut flowers by 4.67 days as compared to control. It can be related to the antibacterial, antifungal, and antioxidant effect of savory extract. In fact, plant essential oils and extracts help sustaining the solution uptake and freshness of cut flowers through inhibiting the growth and activity of microbes and hindering vascular blockage (Bayat *et al.*, 2012; Hashemi and Mirdehghan, 2014).

It is suggested that the antibacterial property of plant essential oils and extracts is related to phenol compounds as well as alcohols, aldehydes, ketone, etc. (Jalili Marandi *et al.*, 2011; Farrag *et al.*, 1989; Bounatirou *et al.*, 2007). Abdolahi *et al.* (2010 a, b) reported that the highest content of compounds found in the essential oil of such plants as savory is phenol. Therefore, it can be concluded that the phenol compounds in savory extract is the reason for its strong antimicrobial property which is, in turn, the reason for the decrease in microbial load and preservation of solution uptake and longer longevity of *Alstroemeria*. In Jalili Marandi *et al.* (2011) too, the application of *Carum copticum* and *Satureja hortensis* essential oils sustained the fresh weight and freshness of rose cut flowers significantly.

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It is suggested that the plant essential oils and extracts containing carvacrol and thymol possess a strong antibacterial property due to having a hydroxyl group on phenol ring (Bounatiro *et al.*, 2007). The present study showed that savory extract was more effective than other extracts in suppressing the microbial population in vase solution of *Alstroemeria*, which is not surprising given the fact that the main constituent of savory essential oil and extract is carvacrol (30-40 %). Singh and Sharma (2003) reported that the application of 8-HQ salts as a strong disinfectant inhibited the activity of microbes in vase solution and increased solution uptake, which is consistent with our results.

All disinfectants reduced electrolyte leakage and MDA as compared to control, among which 200 and 300 mg l⁻¹ 8-HQS and 20 % savory extract were the most effective compounds. The post-harvest stresses imposed on cut flowers trigger the activity of antioxidants. Lower stresses, especially lower water stress, can result in the production of less free oxygen radicals and lower MDA as the product of lipid peroxidation, which in turn inhibits the wilting of cut flowers (Hasanpoorasil *et al.*, 2015). Phenol compounds, which are the main constituents of medicinal herbs' essential oil and extracts, sweep out the active oxygen species and help sustaining membrane structure, hindering the oxidation of membrane lipids and reducing MDA accumulation (Hasanpoorasil *et al.*, 2015; Upadhyaya and Panda, 2004).

Hasanpoorasil *et al.* (2015) reported the lowest MDA concentration in the cut flowers of gladiolus 'White' treated with 300 and 400 mg l⁻¹ 8-HQ, 100 mg l⁻¹ *Zataria multiflora*, and 3 mg l⁻¹ silver nanoparticles. Kazemi *et al.* (2014) obtained the lowest MDA from the application of 200 and 300 mg l⁻¹ 8-HQ and 300 mg l⁻¹ *Daphne odora*. They suggested that MDA concentration was reduced at higher concentrations of essential oils. In the present study, higher concentration of savory extract was associated with lower MDA.

Researchers believe that essential oils can hinder the oxidation of saturated compounds due to their antioxidant and free oxygen radical neutralization properties and can inhibit MDA production by sustaining membrane structure (Kazemi *et al.*, 2014; Bradley and Min, 1992). Jahanifar *et al.* (2016) observed the lowest electrolyte leakage of *Alstroemeria* cut flowers in those treated with 600 mg l⁻¹ peppermint. As we know, phenol compounds in plant essential oils and extracts prevent membrane degradation because of their antibacterial property. Therefore, it can be said that essential oils reduce electrolyte leakage and extend the longevity of *Alstroemeria* cut flower through conserving cell wall integrity.

CONCLUSION

The freshness and longevity of *Alstroemeria* cut flowers were sustained by all studied disinfectants, among which 8-HQS and savory extract were the best treatments. Given the importance of curbing the use of chemicals and replacing them with natural, environment-friendly compounds, it is recommended to use savory extract to extend the longevity of *Alstroemeria* cut flowers.

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How to cite this article:

Mohammadi Kabari, S.F. and Jadid Solimandarabi, M. 2019. Improving *Alstroemeria* vase life by plant extracts and 8-Hydroxyquinoline Sulfate. *Journal of Ornamental Plants*, 9(1), 1-11.

URL: http://jornamental.iurasht.ac.ir/article_664522_e64fabe0b79f12299b39b3949d69bd85.pdf

