



Research Article

The Effect of Rosemary Essential Oils and Thymol on Vase Life and Some Physiological Characteristics of *Alstroemeria* Cut FlowersMehrdad Babarabie^{1*}, Hossein Zarei² and Feryal Varasteh²¹MSc Former Student of Horticulture, Gorgan University of Agricultural Sciences, and Natural Resources, Gorgan, Iran; ²Department of Horticulture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

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ABSTRACT

In order to investigate the replacement of natural ingredients instead of chemicals in the preservative solutions of cut flowers, an experiment with factorial arrangement was carried out in a completely randomized design with three replications. In this study, the effect of concentrations of 4000, 2000, and 6000 mg l⁻¹ of thymol and Rosemary essential oil with 4% sucrose in preservative solutions were evaluated in order to study the *Alstroemeria* cut flower. Traits of vase life, solution uptake, flower diameter, total soluble solids, anthocyanin and chlorophyll was evaluated. The results showed that the most vase life of the flower was related to the treatment of 4000 mg l⁻¹ thymol with 14/33 days, while the control with 9 days of life had the least longevity. The greatest amount of flower diameter and total soluble solids was related to Rosemary essence and the greatest amount of solution uptake, anthocyanin and chlorophyll was observed in the flowers treated with thymol.

Key words: *Alstroemeria*, Rosemary essential oil, Thymol, Vase life

INTRODUCTION

Alstroemeria cut flower has been considered highly in Iran due to a variety of colors, beautiful flowers and high performance (Edalati Moraffah *et al.*, 2013). Today, some of the important problems with the flower industry are the postharvest waste, low quality, value of exports and marketing. The main cause of these problems is related to two general concerns: first, the development conditions of the plant. Second, the storage conditions after the harvest (Bani Jamal and Edrisi, 2009). Postharvest life of cut flowers is often affected by microbes, physiological occlusion and the presence of air in vessels due to the stem end occlusion and wooden vessels. It causes the non- absorption of water or the secretion of extracellular enzymes which can destroy the cell walls of vascular tubules (Damunupola *et al.*, 2010).

In order to increase the vase life of cut flowers, various combinations are used. Sucrose in preservative solutions improves the quality and longevity after the harvest. (Halvey and Mayak, 2003). but increases the microorganisms growth and thus occlusion the flow of water in the stem. So along with the use of sugar, the antimicrobials should be used in the preservative solutions

of cut flowers (Mir Saeed Ghazi *et al.*, 2013). Because of the toxicity of most chemicals and environmental pollution caused by them, the use of natural compounds that have no side effects on human health and the environment and are relatively cheap is very important (Okigbo and Ikediugwu, 2005). Such ingredients include some of the herbal extracts and essential oils that are safe ingredients and their antimicrobial properties and their effect on increasing life after the harvest of horticulture products have been proven. Thyme plant is a member of *Lamiaceae* family that has disinfectant effects. Thymol is a phenolic compound and the most important active ingredient of thyme and its other important compound is carvacrol that is dissolved well in alcohol (Momeni and Nobahar Shahrokhi, 1991). The study results of Oraee *et al.* (2011) indicated that the use of 100 mg l⁻¹ of thymol in the vase solution of gerbera cut flowers increases the vase life and decreases the number of dissolved bacteria. Mousavi bazaz and Tehranifar (2011) stated that the use of methanol and Herbal Essences of peppermint and thyme in *Alstroemeria* preservative solutions increases the vase life of flowers. *Rosemary* is a member of *Lamiaceae* family and its growth is common in many parts of Iran (Zargari, 1990). The main constituent of leaves and

flowering shoots of rosemary is essential oils and their main constituents are 1,8 -cineol, borneol, bornyl acetate and camphor (Steinegger and Hancel, 1998). Due to the use of treatment, 25% rosemary extract with 6% sucrose in the preservative solution of Dianthus, the vase life of flowers increased to 24 days (Basiri *et al.*, 2011).

The effect of *Daphne odora* essence on the increase of the storage life of sour cherry (*Prunus cerasus*) was examined (Azizi *et al.*, 2007). The results showed that *Daphne odora* essence has a significant effect on reducing the fungal contamination and increasing the storage life of sour cherry. Karimi *et al.* (2011) reported that the use of *Daphne odora* essence at concentrations of 600 and 800 mg l⁻¹ increased the vase life of rose cut flower.

The purpose of this experiment is to evaluate the effect of thymol and Rosemary essential oil on the vase life of *Alstroemeria* cut flowers and the possibility of replacing it with harmful chemicals to introduce the best concentration to increase its longevity.

MATERIALS AND METHODS

This experiment was performed in the Horticultural Laboratory of Gorgan University of Agricultural Sciences and Natural Resources in 2014. Flowers were provided from commercial greenhouse of Azin Behesht in Isfahan and were appropriately transferred to Gorgan. Flowers were harvested at the stage of commercial maturity when the color of most florets began to appear. (Chanasut *et al.*, 2003). Flowers were re-cut with 30 cm in length and then were placed in preservative solutions. The temperature of the experiment was 24 ± 2 ° C, the humidity was 60 ± 5 % and the light intensity was 850 lux. Thymol and Rosemary essential oil were purchased from Osareh Tabiat Company in Isfahan and were used at concentrations of 2000, 4000, and 6000 mg l⁻¹ with 4% sucrose in preservative solutions in flower preservative solutions. Also the treatment of control was included distilled water and 4% sucrose. The measured traits of this study were vase life, solution uptake, flower diameter; total soluble solids of petal, petal anthocyanins and leaf chlorophyll. Vase life of flowers was daily assessed and when 50% of florets fell down it was considered as the end of the flowers life (Ferrante *et al.*, 2002).

Flowers diameter was measured by a digital caliper on the second, sixth and tenth days. solution uptake was evaluated using the following formula and a graduated cylinder on the first, fifth and tenth days.

$$FW = \frac{(S_t - S_0)}{W_{t=0}}$$

In this formula, the mentioned symbols are as follows.

Fw: The amount of absorbed solution

S_t= Weight of solution (g) on the zero day, third day ...

S_{t-1}= Weight of solution (g) on the previous day

W_{t=0} = Flower fresh weight on the zero day

The total soluble solids were measured using the hand held refractometer HRN32 and for this purpose, 0/5 g petal was used. This was done on the first, fifth and tenth days.

To measure the anthocyanins of petals, 0/5 g petal with 10 ml of acidified methanol (99 ml of pure methanol

and 1 ml of hydrochloric acid) were pulverized in a porcelain mortar and the obtained extract was placed in laboratory plastic pipes for 24 hours in the dark at 4 ° C temperature. Then, it was centrifuged in Hermle-Z300 centrifuge machine for 10 minutes at around 4000 and the transparent solution was separated from the sample. Then, it was read using Unic-2800 refractometer device at the wavelength of 520 nm. This was done on the second, sixth and tenth days of the experiment.

To measure the chlorophyll in broadleaf plants, the chlorophyll meter device can be used. To this end, Hanstech-Cl-01 chlorophyll meter device was used to measure the chlorophyll of *Alstroemeria*'s leaf on the second, fifth, and eighth days. First, the device was calibrated and the leaf was placed between the two edges of the device and the appeared number was read. This was repeated for each sample at 32 points of the leaf and the mean of 3 numbers was recorded for a sample.

The experiment with factorial arrangement was performed in a completely randomized design with three replications. Data from the measured traits were analyzed with SAS software and the mean comparison was performed with LSD test and also the graphs were drawn using Excel software.

RESULTS

Vase life

The results of the analysis of variance for vase life of *Alstroemeria* flower showed that the effect of treatment was significant at 1% level (Table 1). The results of mean comparison showed that the highest flower longevity was related to the concentration of 4000 mg l⁻¹ of thymol with 14/33 days and the lowest vase life was observed in the control treatment with 9 days of longevity. (Table 3).

Table 1: Analyses of variance of effect treatment on vase life of cut *Alstroemeria*

S.O.V	df	Vase life
Treatment	6	11.63**
Error	14	0.71
Cv	-	7.09

** = P<0.01

Flower diameter

Analysis of variance of the effect of treatment and time on the diameter of *Alstroemeria* flower is shown in (Table 2). According to the results of this table, the effect of treatment, time and treatment and time interaction was significant at 1% level. According to the results of (Table 3), the highest and lowest flower diameter was achieved respectively in the treatment of Rosemary essential oil with concentration of 6000 mg l⁻¹ and control, while there was no significant difference between the different concentrations of thymol and control (Table 3). Also, the process of changes in flower diameter showed that the greatest flower diameter was achieved on the sixth day and then it had a downtrend (Figure 1).

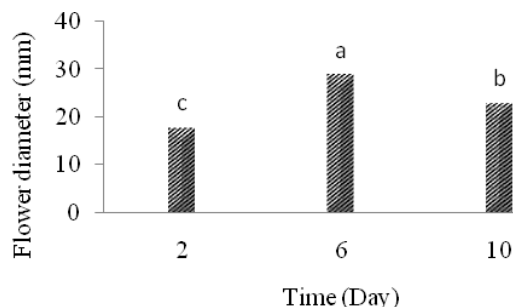
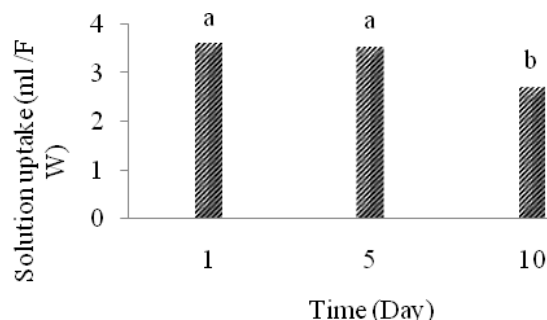
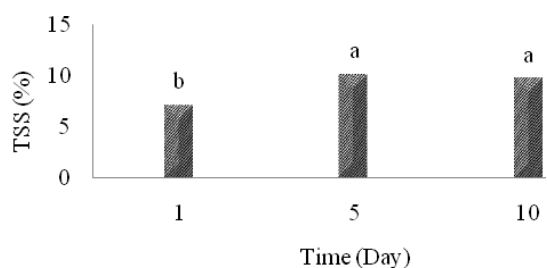
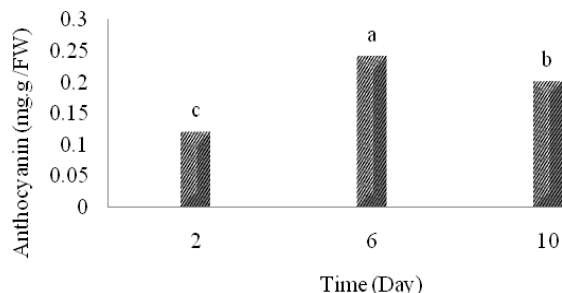
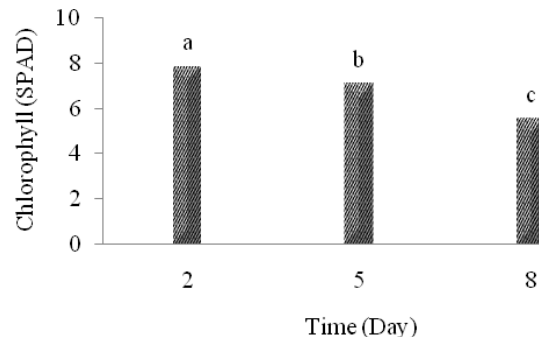
Solution uptake

The data in the analysis of variance (Table 2) show that the effect of treatment, time and treatment and time interaction was significant at 1% level. Table (3) shows

Table 2: Analyses of variance of treatment and time on physiological characteristics of cut *Alstroemeria*.

S.O.V	df	Flower diameter	Uptake solution	TSS	Anthocyanin	Chlorophyll
Treatment	6	167.25	3.28	8.62	0.007	4.45
Time	2	644.81	5.2	56.2	0.075	29.51
Treatment* Time	12	46.26	3.34	1.85	0.002	0.75
Error	40	5.34	0.93	0.48	0.0006	0.79
Cv	-	10.02	29.43	7.79	13.08	13.06

** = P<0.01, ns= no significant

**Fig. 1:** Changing process of flower diameter of cut *Alstroemeria* during the experiment.**Fig. 2:** Changing process of solution uptake of cut *Alstroemeria* during the experiment.**Fig. 3:** Changing process of TSS of cut *Alstroemeria* during the experiment.**Fig. 4:** Changing process of anthocyanin of cut *Alstroemeria* during the experiment.**Fig. 5:** Changing process of chlorophyll of cut *Alstroemeria* during the experiment.

that, the highest solution uptake was achieved in thymol 2000 mg l⁻¹ and the lowest value was observed in control. The process of changes in the solution uptake was shown in (Figure 2). The highest solution uptake was achieved on the first day and then it had a downtrend.

Total soluble solids

According to the results of (Table 2), the effect of treatment, time and time and treatment interaction in relation to the amount of total soluble solids was significant at 1% level. According to the results of mean comparison in (Table 3), the maximum amount of total soluble solids was related to the treatment of 6000 mg l⁻¹ Rosemary essential oil and the minimum amount was related to the treatment of 6000 mg l⁻¹ thymol. The results of (Figure 3) show that most total soluble solids were obtained on the fifth day.

Anthocyanin

The results of the analysis of variance related to Anthocyanin showed that the effect of treatment, time and time and treatment interaction was significant at 1% level (Table 2). Mean comparison of data showed that the concentration of 4000 mg l⁻¹ thymol had more ability to maintain Anthocyanin than other concentrations and control. The lowest level of anthocyanin was related to the treatment of 2000 mg l⁻¹ thymol and control (Table 3). The highest amount of Anthocyanin was observed on the sixth day (Figure 4).

Chlorophyll

The results of the analysis of variance for the data of chlorophyll measurement showed that the effect of treatment and time was significant at 1% level but the time and treatment interaction was not significant (Table 2). The results obtained from (Table 3) show that the highest and lowest amounts of chlorophyll were obtained in the treatment of 6000 mg l⁻¹ Rosemary essential oil and control (Table 3). The results showed that the highest

Table 3: Mean comparison the effect treatment on vase life and physiological characteristics of cut *Alstroemeria*.

Treatment	Vase life	Flower diameter	Uptake solution	TSS	Anthocyanin	Chlorophyll
T ₂₀₀₀	10d ^e	19.91 ^c	4.28 ^a	7.63 ^d	.15 ^c	6.14 ^{cd}
T ₄₀₀₀	14.33 ^a	20.24 ^c	3.58 ^{ab}	9.23 ^b	.23 ^a	7.41 ^{ab}
T ₆₀₀₀	11 ^{cd}	18.98 ^c	3.03 ^{bc}	8 ^{cd}	.19 ^b	6.62 ^{bcd}
R ₂₀₀₀	12.33 ^{bc}	25.69 ^b	3.37 ^{ab}	9.6 ^{ab}	.20 ^b	6.86 ^{bc}
R ₄₀₀₀	13.66 ^{ab}	28.15 ^a	3.03 ^{bc}	9.8 ^{ab}	.19 ^b	7.1 ^{ab}
R ₆₀₀₀	13 ^{ab}	28.7 ^a	3.01 ^{bc}	10.16 ^a	.18 ^b	7.82 ^a
C	9 ^e	19.55 ^c	2.31 ^c	8.35 ^c	.15 ^c	5.8 ^d

T: thymol, R: rosemary, C: control; In each column, means with the similar letters are not significantly different at 1% level of probability using LSD test.

chlorophyll was observed on the first day and then it had a downtrend (Figure 5).

DISCUSSION

The preservative solutions of flowers are a combination of different substances such as carbohydrates, microbiocides, or plant growth regulators to increase the longevity and maintain the quality of cut flowers (Sankla *et al.*, 2005). Hydrophobicity property of essential oils causes the penetration into the fat of cell membranes and mitochondria and the disruption of the structure and more penetration of them. These changes will lead to the leakage of ions and other cell contents. Finally, the loss of cell contents and the removal of vital molecules and ions will cause the death of microbes (Bakkali *et al.*, 2008). Finally, the prevention of vascular occlusion will lead to the increased flower longevity. Madad Zadeh *et al.* (2013) reported that the use of 100 mg l⁻¹ will lead to the increase of *Alstroemeria* longevity. The present study showed that the thymol and Rosemary essential oil increase the vase life and delay the senescence of *Alstroemeria*. This would be due to the effect of inhibiting the growth of bacteria and fungi which occlusion the vessels and subsequently attract more conservative solutions and the results of flower longevity and the solution uptake will confirm this issue.

Flower diameter has an important role in the marketing of cut flowers (Haji Reza *et al.*, 2013). Some antimicrobial compounds such as ethanol increase the fresh weight of plants and the Turgor of cell and also improve the growth of flower cup (Farokhzad *et al.*, 2005). In this study, thymol did not significantly increase the flower diameter. Kamyab *et al.* (2009) stated that the treatment of thymol and fennel essential oil on rose diameter had no significant difference compared to the control and their results are consistent with the present study. Meanwhile, the Rosemary essential oil significantly increased the flower diameter. The study results of Karimi *et al.* (2011) showed that the treatment of 600 and 800 mg l⁻¹ Rosemary essential oil increased the cut rose diameter that is consistent with the present study.

The role of exogenous carbohydrates to increase the flower longevity has been well known. Absorbed sugar solution is collected in the petal tissue, improves the osmotic potential and increases the amount of needed carbohydrates (Sarkka, 2004). It seems that due to the antibacterial activity of thymol and therefore less occlusion of vessels, the absorption of sucrose was performed better via the vase solution which can be a reason to the increase of petals total soluble solids compared to the control. In a study, the use of 100 mg l⁻¹

thyme essential oil with 1% sucrose led to the increase of the *Alstroemeria*'s petals total soluble solids (Mir Saeed Ghazi *et al.*, 2013).

Anthocyanins are the water soluble pigments that accumulate in the vacuole of petal epidermal cells and cause some colors in the range of orange to purple (Odonoghue *et al.*, 2002). The decrease in the amount of Anthocyanin after the flower harvest is a sign of senescence. The results showed that the concentrations of 4000 and 6000 mg l⁻¹ thymol which had more vase life compared to other concentrations and control, the decrease of Anthocyanin in its petals have been delayed. Ikani *et al.* (2013) indicated that the treatment of 75 mg l⁻¹ eucalyptus essential oil increased the amount of Anthocyanin in Gerbera cut flowers.

Alstroemeria produces very low amounts of ethylene but is sensitive to external ethylene. One of the major problems of *Alstroemeria* after the harvest is the yellowing of its leaves which reduces the quality of its appearance (Soleimany-fard *et al.*, 2013). The activity of microorganisms that grow in the solution causes the vascular occlusion, the release of harmful enzymes and the increase of the production of ethylene (Sarkka, 2004). It seems that the essential oils are able to reduce the ethylene production by reducing the microorganisms that are produced in the vase solutions of cut flowers in order to prevent the premature yellowing of leaves. However, little research has been done in this area.

Conclusion

Although the significant effect of chemicals such as silver compounds on the longevity and some quality traits of cut flowers have been demonstrated, since it has been known that these compounds are harmful to human health and the environment, it seems that further experiments on the use of essential oils and plant extracts in preservative solutions as alternative solutions are essential.

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