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Vase Life and Quality of Cut Alstroemeria as Affected by Integrated Application of Plant Essential Oils and Non-Chemical Treatments

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Alstromeria has beautiful flowers that is popular in the world. One of the most problem in commercial of this flower is ethylene sensitivity and bacterial contamination in stem end of cut flowers. In this experiment, effect of essential oils of bitter orange (0, 25 and 50 mg l⁻¹) and lawson cypress (0, 100 and 200 mg l⁻¹) and nonchemical treatment (with and without) with 18 treatments, 3 replications and 54 plots were tested on vase life and quality of cut *Alsteromeria*. ANOVA showed that interaction effect of mentioned treatments were significant on all of traits except loss of fresh weight. Results showed that $L_{200}B_{50}S_0$ (200 mg l⁻¹ essential oil lawson cypress × 50 mg l⁻¹ bitter orange without splitting) and $L_{200}B_{50}S_1$ (200 mg l⁻¹ essential oil lawson cypress × 0 mg l⁻¹ bitter orange with splitting) had highest vase life. Also, lowest bacterial population found in $L_{200}B_{50}S_1$ (200 mg l⁻¹ essential oil lawson cypress × 50 mg l⁻¹ bitter orange with splitting).

Abstract

Keywords: Bitter orange (*Citrus aurantium*), Essential oils, Lawson cypress (*Chamaecyparis lowsoniana*), Vase life.

INTRODUCTION

Alstroemeria belongs to the family of Amaryllidaceae and is native to South America. It is monocotyledon, annual, perennial, and cold-sensitive. It is featured with the yellow color of neck and the occurrence of dark spots on the bottom part of neck and petals (Ghasemi Ghahsareh & Kafi, 2005).

The post-harvest quality loss in most ornamental plants can be caused by several factors including water stress, vascular blockage, nutrient deficiencies, and the adverse effect of ethylene (Edrisi, 2009). To cope with vascular blockage, compounds like essential oils of bitter orange and lawson cypress are used to increase the vase life of this plant. In a study on the impact of plant essential oil and silver nanoparticles on vase life of gerbera, Solgi et al. (2009) concluded that the treatment with 1 or 2 mg L⁻¹ silver nanoparticles with 50 or 100 mg L⁻¹ carvacrol extended vase life up to 8 days as compared to control. Also, Torabi Jafjiri et al. (2010) revealed that the treatment with lawson cypress increased vase life of chrysanthemum cut flowers. Thyme essential oil extended the vase life of narcissus cut flowers (Shaniwar et al., 2009). Bayat et al. (2012) stated that essential oils are safe natural compounds that can substitute chemicals used for extending vase life of cut flowers. It is reported that injuries to plant tissues increases the expression of various genes and induces a range of enzyme including phenylalanine ammonia lyase (Lois et al., 1989), peroxidase (Lagrimini, 1991), and ACC oxidase (Pech-Scott & Kende, 1999). Some enzymes are involved in the biosynthesis of compounds related to lignin and suberin (Espelie et al., 1986). These responses are induced by wounds to prevent the entrance of microorganisms into the opened tissue (Bucciarelli et al., 1998). It has been proven that injuries results in xylem blockage. The blockage may be related to the precipitation of substances like resin in xylems (Davies et al., 1981) or to the formation of tylose - balloon-like bulges in the cells adjacent to an injury (Tyree and Zimmermann, 2002). These blockage inhibit the entrance of microorganisms, too. In some species, wounds to stems may cause several types of blockages including tylose and slime deposition (Weiner & Liese, 1995).

The objective of the present study was to extend post-harvest life of alstroemeria cut flowers, to inhibit bacterial activities on stem ends, and to introduce organic, environment-friendly compounds to substitute chemicals. The present paper studies the effect of stem-end splitting as a physical treatment and the application of essential oils of lawson cypress and bitter orange on extending post-harvest life of alstroemeria cut flowers.

MATERIALS AND METHODS

The present study was carried out as a factorial experiment with three factors on the basis of a completely randomized design with 18 treatments, 3 replicates, 54 plots and 270 cut flowers. The factors included mechanical treatment at two levels (splitting in 40°C water and no splitting) (Fig. 1) and the essential oils of lawson cypress and bitter orange at three levels (0, 100 and 200 mg L⁻¹). The stem end of alstroemeria cut flowers was re-cut and all leaves that might have entered



Fig.1. Recut of the stem end.

in solution were removed. All cut flowers were cut at the height of 40 cm. The volume of the preservative solution was 500 mL. Then, all cut flowers were individually weighed before they are soaked in the solutions. Five cut flowers were placed in 2-litre plastic pots. Then, they were continuously treated with essential oils of bitter orange and lawson cypress. The post-harvest laboratorial conditions were 12 hours of illumination supplied by a florescent lamp with the intensity of 12 μ mol m⁻² s⁻¹. The room temperature was set to 20±2°C with 60-70% relative humidity. The recorded traits included vase life, flower opening index, fresh weight loss, bacteria count in preservative solution, ethylene content, and chlorophyll a and b.

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Vase life was measured on the basis of the wilting and yellowing of leaves and the abscission of the flowers (Mensuali Sodi & Ferrante, 2005). To measure flower opening index, the highest flower diameter and the perpendicular diameter were measured by a digital caliper every other day until the full opening of the flower. Then, the data were averaged and the flower opening index was determined by the following equation:

Flower opening index = $(D_n+2/D_n+D_n+4/D_n+2+D_n+6/D_n+4)/3$

where, D_n shows flower diameter on the first day of the experiment and $D_n + 2$ shows the flower diameter read on the third day, and so on.

In order to measure chlorophyll a and b, the leaves were detached from cut flowers on the fifth day and then, chlorophyll was measured by Mazumdar and Majumdar (2003)'s method.

To measure vase solution bacteria content, the pot solution was sampled 24 hours after the treatment and was diluted with 0.9% normal saline to achieve 30-300 bacterial colonies in all petri dishes. Then, 0.1 mL of the resulting solution was spread on agar, and bacterial colony was enumerated 24 hours after incubation at 37°C (Liu *et al.*, 2009).

Fresh weight loss (in terms of g) was calculated by the following equation given the initial fresh weight, final fresh weight, and re-cut and abscission weights:

Fresh weight loss = initial fresh weight – (final fresh weight + recut weight + abscission weight) To measure released ethylene, one cut flower was selected from each treatment 24 hours after the treatment. Then, it was weighed, placed in air tight jar. The gas samples were sent to Gas Analysis Laboratory to analyze the ethylene content. It was measured by GC-8 AIT, model Shimadzu.

Data were analyzed by SPSS and SAS statistical software packages, data means were compared by LSD method, and graphs were drawn by MS-Excel Software Package.

RESULTS AND DISCUSSION

Vase life

Analysis of variance showed that the simple effects of lawson cypress and bitter orange essential oils were significant on vase life at the one percent level, but the effect of splitting was insignificant. Also, the interactions of bitter orange essential oil × lawson cypress essential oil, lawson cypress essential oil × splitting, and bitter orange essential oil × splitting were significant for vase life at the one percent statistical level. The interaction of bitter orange essential oil × lawson cypress

Table 1. ANOVA of lawson cypress essential oil, bitter orange essential oil and splitting on the studied traits.

S.o.V	df	Vase life	Fresh weight loss	Flowers opening index	Chlorophyll a	Chlorophyll b	Ethylene content	Vase solution bacterial population
L	2	25.17**	30.25**	0.0129 ^{ns}	0.284**	0.0729**	2.22**	43838**
В	2	9.48**	6.39**	0.0042 ^{ns}	1.001**	0.346**	1.18**	25062**
S	1	1.36 ^{ns}	0.414 ^{ns}	0.0945**	3.641**	0.957**	0.016 ^{ns}	1944**
L×B	4	1.94**	2.26**	0.023**	0.329**	0.212**	0.119 ^{ns}	2711**
L×S	2	3.73**	1.181 ^{ns}	0.0073 ^{ns}	1.444**	0.403**	0.0964 ^{ns}	388.3 ^{ns}
B×S	2	3.67**	0.652 ^{ns}	0.0446**	0.4002**	0.068**	0.082 ^{ns}	1261.0*
L×B×S	4	1.06*	0.443 ^{ns}	0.0192**	0.750**	0.038**	0.197*	916.02*
Error	36	0.3852481	0.5078352	0.0045685	0.0085356	0.0023	0.0498	249.0741
CV (%)		5.650188	13.99388	10.10494	4.671657	5.284677	15.45589	10.16256

ns:Not significant *:Probability 5%L: Lawson cypress essential oil S: Splitting

Probability 1%
B: Bitter orange essential oil

Table 2. Means comparison of the interaction of lawson cypress essential oil, bitter orange essential oil and splitting on the studied traits.

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ^{.1} FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
$L_0B_0S_0$	7.74 ⁱ	8.120 ª	0.656 ^{c-g}	1.339 ^k	0.481 ^h	2.00 b	260.3 _a
$L_0B_0S_1$	9.80 ^{gh}	7.510 ª	0.673 ^{c-f}	1.980 ^{ef}	0.887 ^{de}	2.50 ª	270.3 _a
$L_0B_{25}S_0$	9.37 ^h	6.327 a	0.756 abc	1.680 ^{hi}	0.792 f	1.65 ^{bc}	226.6 ^b
$L_0B_{25}S_1$	10.39 ^{e-h}	5.580 ª	0.556 ^g	3.196 ª	1.367 ^b	1.22 ^{de}	214.3 ^b
$L_0B_{50}S_0$	9.78 ^{gh}	6.340 ª	0.860 ª	1.401 ^{jk}	0.680 ^g	1.00 e	154.3 ^{cd}
$L_0B_{50}S_1$	9.78 ^{gh}	5.443 a	0.690 ^{c-f}	2.721 ^b	1.492ª	1.23 ^{de}	147.6 ^{de}
$L_{100}B_0S_0$	11.38 ^{cde}	4.690 a	0.706 ^{cde}	1.552 ^{ij}	0.799 f	1.20 ^{de}	174.0 °
$L_{100}B_0S_1$	11.30 ^{cde}	4.410 ^a	0.616 ^{d-g}	1.779 ^{gh}	0.770 f	1.48 ^{cd}	147.0 ^{de}
$L_{100}B_{25}S_0$	10.89 ^{c-f}	5.033 ª	0.826 ab	2.004 ef	0.938 ^{cd}	1.93 ^b	119.6 ^{fgh}
$L_{100}B_{25}S_1$	11.73 bcd	4.933 a	0.600 efg	2.710 ^b	1.406 ^b	1.23 ^{de}	106.0 ^{ghi}
$L_{100}B_{50}S_0$	12.71 ^{ab}	3.867 ª	0.590 fg	2.106 de	0.840 ef	1.75 ^{bc}	120.6 ^{fgh}
$L_{100}B_{50}S_1$	10.74 ^{c-g}	4.937 ^a	0.623 ^{d-g}	2.131 de	0.959 ^{cd}	1.50 ^{cd}	96.67 ^{hi}
$L_{200}B_0S_0$	10.06 ^{fgh}	5.310 ª	0.586 ^{fg}	1.328 ^k	0.942 ^{cd}	1.52 ^{cd}	142.6 def
$L_{200}B_0S_1$	11.41 ^{cde}	4.517 ª	0.726 bcd	2.434 °	1.017 °	1.82 ^{bc}	180.3 °
$L_{200}B_{25}S_0$	11.77 bc	3.607 ^a	0.656 ^{c-g}	1.852 ^{fg}	0.894 de	0.950 °	124.0 efg
$L_{200}B_{25}S_1$	11.44 ^{cd}	3.960 ^a	0.556 ^g	1.799 ^{gh}	0.915 ^{de}	1.65 ^{bc}	96.33 ^{hi}
$L_{200}B_{50}S_0$	^{13.70} a	3.327 ª	0.756 abc	2.196 d	0.663 ^g	1.80 bc	129.3 ^{d-g}
$L_{200}B_{50}S_{1}$	12.73 ab	3.753 ª	0.600 efg	1.383 ^k	0.613 ^g	1.02 ^e	85.00 ⁱ

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

$L_0B_0S_0$: control

L₀B₀S₁: 0 mg L⁻¹ Lawson cypress essential oil × 0 mg L⁻¹ bitter orange essential oil × splitting L₀B₂₅S₀: 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil × no-splitting L₀B₂₅S₁: 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil × splitting L₀B₅₀S₀: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil × no-splitting L₀B₅₀S₁: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil × splitting $L_{100}B_0S_0$: 100 mg L⁻¹ Lawson cypress essential oil × 0 mg L⁻¹ bitter orange essential oil × no-splitting L100B0S1: 100 mg L-1 Lawson cypress essential oil × 0 mg L-1 bitter orange essential oil × splitting L100B25S0: 100 mg L-1 Lawson cypress essential oil × 25 mg L-1 bitter orange essential oil × no-splitting L₁₀₀B₂₅S₁: 100 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil × splitting L100B50S0: 100 mg L-1 Lawson cypress essential oil × 50 mg L-1 bitter orange essential oil × no-splitting L₁₀₀B₅₀S₁: 100 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil × splitting L₂₀₀B₀S₀: 200 mg L⁻¹ Lawson cypress essential oil × 0 mg L⁻¹ bitter orange essential oil × no-splitting L200BoS1: 200 mg L-1 Lawson cypress essential oil × 0 mg L-1 bitter orange essential oil × splitting L₂₀₀B₂₅S₀: 200 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil × no-splitting L₂₀₀B₂₅S₁: 200 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil × splitting L₂₀₀B₅₀S₀; 200 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil × no-splitting L₂₀₀B₅₀S₁: 200 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil × splitting

essential oil × splitting was significant at the five percent statistical level (Table 1). Means comparison of the interaction between lawson cypress and bitter orange essential oils and splitting for vase life revealed that the longest vase life (13.70 days) was obtained from the treatment of 200 mg L⁻¹ lawson cypress essential oil, 50 mg L⁻¹ bitter orange essential oil and no splitting, and the shortest vase life (7.7 days) was observed in control (Table 2). The positive effect of essential oils on vase life was related to their antimicrobial activity. Acting as disinfector in vase solution, they helped preserving vascular conductivity and water uptake by reducing bacterial population in solution and stem end resulting in longer vase life of cut flowers (Jalili Marandi *et al.*, 2011). Mousavi Bazzaz *et al.* (2011) reported that the use of mint essential oil at the rate of 50 mg L⁻¹ extended vase life of alstroemeria by 2.03 days. According to Torabi Jafjiri *et al.* (2010), 20 mL L⁻¹ lawson cypress+ 5% sucrose had the highest effect on improving vase life of chrysanthemum cut flowers.

Fresh weight loss

According to analysis of variance, the simple effects of lawson cypress and bitter orange essential oils were significant on the loss of fresh weight at the one percent statistical level, but splitting had no significant effect on fresh weight loss. The interaction of the essential oils of bitter orange and lawson cypress was significant for fresh weight loss at the one percent level, but the interaction of essential oil of lawson cypress or bitter orange with splitting were insignificant for this trait (Table 1).

Also, the interaction of bitter orange essential oil × lawson cypress essential oil × splitting was insignificant for fresh weight loss. Means comparison of the effect of bitter orange essential oil × lawson cypress essential oil × splitting for fresh weight loss indicated that the lowest fresh weight loss (3.327 g) was related to the treatment of 200 mg L⁻¹ lawson cypress essential oil \times 50 mg L^{-1} bitter orange essential oil \times no splitting which yielded the longest vase life, although treatments exhibited insignificant differences (Table 2). As means comparison of the interaction of essential oils showed, the lowest fresh weight loss (3.540 g) was associated with the treatment of 200 mg L⁻¹ lawson cypress essential oil and 50 mg L⁻¹ bitter orange essential oil, and the highest one (7.815 g) was observed in control (Table 3). Orace et al. (2011) reported the positive impact of the use of essential oil on vase life of rose cv. 'Raibon' cut flowers. They found that essential oil improved water uptake by inhibiting the growth of bacteria in vase solution and stem end and diminished the loss of fresh weight. Solgi et al. (2009) evaluated the effect of plant essential oils to be positive on fresh weight improvement of gerbera cv. 'Dune' cut flowers. Also, our results are in agreement with Morones et al. (2005). Mousavi Bazzaz et al. (2011) reported the improvement of fresh weight of alstroemeria cut flowers as affected by the application of essential oils in vase solution. The application of essential oil in preservative solution of rose cv. 'Grand' cut flowers resulted in higher fresh weight through improving water relations (Shanan, 2012). The application of 500 mg L⁻¹ essential oil of Corum copticum improved fresh weight of rose cut flowers as compared to control (Jalili Marandi et al., 2011). Fresh weight loss is an indicator of cut flower's senes-

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ^{.1} FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
L ₀ B ₀	8.77 d	7.815 ^a	0.665 ab	1.660 °	0.684 ^{de}	1.178 ª	265.3 ª
L_0B_{25}	9.88 °	5.953 ^b	0.656 ^b	2.438 ^a	1.142 ab	1.515 ª	220.5 b
L_0B_{50}	10.25 °	5.891 ^b	0.775 ^a	2.061 abc	1.023 abc	1.881 ª	151.0 °
L100B0	11.34 ^b	4.550 ^{cd}	0.661 ab	1.666 °	0.785 ^{cde}	1.178 ª	160.5 °
$L_{100}B_{25}$	11.31 ^b	4.983 °	0.713 ab	2.357 ab	1.172 ^a	1.515 ª	112.8 ^d
L ₁₀₀ B ₅₀	11.73 ^b	4.401 ^{cd}	0.606 ^b	2.118 abc	0.899 bcd	2.048 a	108.6 ^d
$L_{200}B_{0}$	10.74 bc	4.913 °	0.656 ^b	1.881 abc	0.980 abc	1.178 ª	161.5 °
$L_{200}B_{25}$	11.60 ^b	3.783 ^{de}	0.606 ^b	1.825 bc	0.905 bcd	1.515 ª	110.1 ^d
$L_{200}B_{50}$	13.21 ^a	3.540 °	0.678 ab	1.789 °	0.638 ^e	1.715 ª	107.1 ^d

Table 3. Means comparison of the interaction of lawson cypress essential oil and bitter orange essential oilon the studied traits.

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

L₀B₀: control

 L_0B_{25} : 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil L_0B_{50} : 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{100}B_0$: 100 mg L⁻¹ Lawson cypress essential oil × 0 mg L⁻¹ bitter orange essential oil $L_{100}B_{25}$: 100 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{100}B_{50}$: 100 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{100}B_{50}$: 200 mg L⁻¹ Lawson cypress essential oil × 0 mg L⁻¹ bitter orange essential oil $L_{200}B_0$: 200 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{200}B_{25}$: 200 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{200}B_{25}$: 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 25 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil $L_{200}B_{50}$: 0 mg L⁻¹ Lawson cypress essential oil × 50 mg L⁻¹ bitter orange essential oil

cence which is accompanied with water loss. Van Meeteren *et al.* (2000) showed the gradual loss of fresh weight in flowers during aging. Water loss in petals usually increases as plants age due to the increase in membrane permeability. Therefore, conservation of petal's water with different treatments can play an important role in avoiding senescence (Armitage and Laushman, 2003).

Flower opening index

According to analysis of variance, the simple effect of essential oils of lawson cypress and bitter orange were not significant on flower opening index, but the trait was significantly influenced by splitting at the one percent statistical level. Also, interactions of bitter orange essential oil \times lawson cypress essential oil, bitter orange essential oil \times splitting, and bitter orange essential oil \times lawson cypress essential oil × splitting were significant for flower opening index at the one percent level, but the interaction of lawson cypress× splitting was insignificant (Table 1). Means comparison of the data for the interaction between lawson cypress and bitter orange essential oil and splitting for flower opening index revealed that the highest index (0.860) was related to the treatment of 0 mg L⁻¹ lawson essential oil \times 50 mg L⁻¹ bitter orange essential oil \times no-splitting, and the lowest one (0.556) was related to the treatment of 0 mg L^{-1} lawson essential oil \times 25 mg L^{-1} bitter orange essential oil × splitting and the treatment of 200 mg L⁻¹ lawson essential oil × 25 mg L⁻¹ bitter orange essential oil × splitting (Table 2). In a study on essential oils of coriander and savory, Jalili Marandi et al. (2011) revealed that the use of essential oils improved flower opening index of rose cut flowers as compared to control. Liu et al. (2009) reported that essential oils had positive effect on the diameter of rose cut flowers. Hosseini Darvishian et al. (2011) found that the use of rosemary and thymes essential oil had positive, significant influence on the opening of rose cut flowers.

Chlorophyll a content

Analysis of variance showed that the simple effects and interactions of lawson cypress and bitter orange essential oils and splitting were significant on chlorophyll a content at the one percent statistical level (Table 1). Means comparison for the interaction of lawson cypress and bitter orange essential oils and splitting for chlorophyll a content revealed that the highest chlorophyll a content (3.196 mg g⁻¹ fresh weight) was obtained from the treatment of 0 mg L⁻¹ lawson essential oil × 25 mg L⁻¹ bitter orange essential oil × splitting and the lowest one (1.328 mg g⁻¹ fresh weight) was related to the treatment of 200 mg L⁻¹ lawson essential oil × 0 mg L⁻¹ bitter orange essential oil × no-splitting and control (Table 2).

Chlorophyll b content

As analysis of variance showed, the simple effects and interactions of lawson cypress and bitter orange essential oils and splitting were significant for chlorophyll b content at the one percent level (Table 1). Means comparison of the interaction of lawson cypress and bitter orange essential oils and splitting for chlorophyll b content indicated that the highest chlorophyll b content (1.492 mg g⁻¹ fresh weight) was obtained from the treatment of 0 mg L⁻¹ lawson essential oil × 50 mg L⁻¹ bitter orange essential oil × splitting and the lowest one (0.481 mg g⁻¹ fresh weight) was related to control (Table 2). Higher dosages of both essential oils had adverse impact on chlorophyll a and b, and their lower dosages were better than control. Also, splitting had positive impact on them. In a study on the effect of plant essential oils on carnation cut flowers, Kazemi and Ameri (2012) showed that the use of these compounds increased chlorophyll in carnation cut flowers significantly as compared to control. The use of essential oils at lower rates increased pigments in gladiolus cut flowers (Mohammadi *et al.*, 2011). Kazemi *et al.* (2011) reported that the introduction of essential oil to preservative solutions of cut flowers increased chlorophyll content. They suggested that thymes essential enhanced total chlorophyll content in lisianthus cut flowers as compared to control. Geng *et al.* (2009) revealed that the loss of green color of some vegetables like broccoli is as-

sociated with the loss of water during storage, so that if water loss was controlled by fog system, yellowing and chlorophyll loss would be limited. They stated that chlorophyll loss was also related to lipid peroxidation which resulted in the destruction of cell membrane. Yamauchi *et al.* (2004) stated that peroxidase had a significant role in injuring the chlorophyll of broccoli florets. Lawson cypress essential oil inhibits or retards chlorophyll loss probably through inhibiting the formation of free radicals and lipid peroxidation.

Vase solution bacteria population

Analysis of variance revealed that the simple effects of lawson cypress and bitter orange essential oils and splitting and the interaction of lawson cypress and bitter orange essential oils were significant for vase solution bacteria count at the one percent statistical level, and the interactions of bitter orange essential oil \times splitting and bitter orange essential oil \times lawson cypress essential oil \times splitting were significant at the five percent statistical level, but the interaction of lawson cypress essential oil \times splitting was insignificant (Table 1).

Means comparison of the interaction of lawson cypress and bitter orange essential oils and splitting for vase solution bacteria count indicated that the highest number of bacteria in vase solution was related to the treatment of 0 mg L^{-1} lawson essential oil \times 0 mg L^{-1} bitter orange essential oil × splitting (270.3 Log₁₀ CFU mL⁻¹) and control (260.3 Log₁₀ CFU mL⁻¹) and the lowest one (85 Log₁₀ CFU mL⁻¹) to 200 mg L⁻¹ lawson essential oil \times 50 mg L⁻¹ bitter orange essential oil \times splitting (Table 2). Plant essential oils inhibit bacterial activity and finally, kill them by their antimicrobial effect on pathogens and disruption of their respiindexn chain yield (Solgi et al., 2009; Hashemabadi, 2011), which is consistent with our findings. Orace et al. (2011) reported that antibacterial essential oils reduced bacterial infection in gerbera vase solution. A study on the effect of plant essential oils and salicylic acid on carnation cut flowers showed that all treatments reduced microbial population in stem end and vase solution (Kazemi & Ameri, 2012). Jalili Marandi et al. (2011) related the positive effects of plant essential oil on durability of rose cut flowers to their antimicrobial activity and suggested that plant essential oils acted as antibacterial compounds in vase solution reducing the population of bacteria in preservative solution. As a result, vascular conductivity, water uptake and finally, vase life were increased. Shabani et al. (2011) showed that the treatment of bird-of-paradise cut flowers with essential oils reduced the number of bacteria in their stem as compared to control. Our results about the positive influence of essentials oils on the

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log₁₀ CFU mL⁻¹)
L0S0	8.967 ^c	6.929 ^a	0.758 ^a	1.473 d	0.651 d	1.521ª	213.8 ª
L0S1	10.308 ^b	6.178 ^a	0.640 ^a	2.632 a	1.248 ^a	1.542ª	210.8 ª
L100S0	11.665 ^a	4.530 ^a	0.708 ^a	1.887 bc	0.859 ^{bc}	1.543ª	138.1 ª
L100S1	11.261 ^{ab}	4.760 ^a	0.613 ^a	2.206 b	1.045 ^b	1.653ª	116.6 ª
L200S0	11.845 ^a	4.081 ^a	0.667 ^a	1.792 cd	0.833 ^{cd}	1.519ª	132.0 ª

Table 4. Means comparison of the interaction of lawson cypress essential oil and splitting on the studied traits.

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

L₀S₀: control

L₀S₁: 0 mg L⁻¹ Lawson cypress essential oil × splitting

 $L_{100}S_0$: 0 mg L⁻¹ Lawson cypress essential oil × no-splitting $L_{100}S_1$: 100 mg L⁻¹ Lawson cypress essential oil × splitting $L_{200}S_0$: 100 mg L⁻¹ Lawson cypress essential oil × no-splitting

 $L_{200}S_1$: 100 mg L⁻¹ Lawson cypress essential oil × splitting

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

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Table 5. Means comparison of the interaction of bitter orange essential oil and splitting on the studied traits.

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
B ₀ S ₀ B ₀ S1 B ₂₅ S ₀ B ₅₀ S ₀	9.73 c 10.84 abc 10.67 bc 11.18 ab 12.06 a	6.040 ^a 5.479 ^a 4.989 ^a 4.824 ^a 4.511 ^a	0.6500 bc 0.6722 ab 0.7466 a 0.5711 c 0.7355 a	1.406 ° 2.065 b 1.845 b 2.568 ° 1.901 b	0.7410 ° 0.8916 bc 0.8752 bc 1.2713 ° 0.7277 °	1.206 ^a 1.150 ^a 1.536 ^a 1.493 ^a 1.780 ^a	192.3 ^a 199.2 ^a 156.7 ^{ab} 138.8 ^{bc} 134.7 ^{bc}

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

B₀S₀: control

 $B_0S1: 0 \text{ mg } L^{-1}$ bitter orange essential oil × splitting

 $B_{25}S_0{:}\ 0\ mg\ L^{{\scriptscriptstyle -}1}$ bitter orange essential oil \times no-splitting

 $B_{25}S_1$: 100 mg L⁻¹ bitter orange essential oil × splitting

 $B_{50}S_0$: 100 mg $L^{\text{-1}}$ bitter orange essential oil \times no-splitting

 $B_{50}S_1$: 100 mg L⁻¹ bitter orange essential oil × splitting

Table 6. Means comparison of the interaction of lawson cypress essential oil on the studied traits.

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ^{.1} FW)	Chlorophyll b (mg g ^{.1} FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
L0	9.63⁵	6.55 ^a	0.698 ^a	2.05 ^a	0.950ª	1.881ª	212.2ª
L100	11.46ª	4.64 ^b	0.660 ^{ab}	2.04 ^a	0.952ª	1.515⊳	127.3⊳
L200	11.85ª	4.07 ^c	0.647 ^b	1.83 ^b	0.841 ^b	1.178∝	126.2⊳

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

L₀: control

L100: 100 mg L-1 Lawson cypressessential oil

L200: 200 mg L-1 Lawson cypressessential oil

control and removal of pathogens and the increase in vase life are in agreement with Liu *et al.* (2009) and Shanan (2012).

Ethylene content

Analysis of variance showed that the simple effects of lawson cypress and bitter orange essential oils were significant on ethylene content at the one percent statistical level and the interaction of lawson cypress essential oil × bitter orange essential oil × splitting was significant at the five percent statistical level (Table 1). Means comparison for the interaction of the studied factors on ethylene content showed that the highest ethylene content (2.5 nL h⁻¹ g⁻¹ fresh weight) was related to 0 mg L⁻¹ lawson essential oil × 0 mg L⁻¹ bitter orange essential oil × splitting and the lowest one (0.950 2.5 nL⁻¹ h⁻¹ g⁻¹ fresh weight) was related to 200 mg L⁻¹ lawson essential oil × 25 mg L⁻¹ bitter orange essential oil × no-splitting (Table 2). The superior effect of disinfecting compounds on ethylene content can be related to antibacterial property of these compounds which reduced ethylene production by reducing bacterial population in vase solution. Zagory and Ried (1986) suggested that microbial infections of stem end and vase solution induced the production of senescence stimulating gas, through which it reduced the quality and durability of cut flowers. Kazemi and Ameri (2012) examined the effect of vase life extending compounds on carnation cut flowers and reported that the use of silver nanoparticles and plant essential oils alleviated ACC-oxidase enzyme activity that catalyzes ethylene biosynthesis, which is in agreement with our findings. Table 7. Means comparison of the interaction of bitter orange essential oil on the studied traits.

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ^{.1} FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
B0	10.28°	5.75ª	0.661ª	1.735°	0.816 ^c	1.801ª	290ª
B25	10.93 ^b	4.90 ^b	0.658ª	2.207ª	1.07ª	1.478♭	247⁵
B50	11.73ª	4.61 ^b	0.686ª	1.989⁵	0.853 ^b	1.295°	189°

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

B₀: control

B₂₅: 25 mg L⁻¹ bitter orange essential oil

B₅₀: 50 mg L⁻¹ bitter orange essential oil

Table 8. Means comparison of the interaction of splitting on the studied traits.

Treatments	Vase life (day)	Fresh weight loss (g)	Flowers opening index	Chlorophyll a (mg g ^{.1} FW)	Chlorophyll b (mg g ⁻¹ FW)	Ethylene content (nl ⁻¹ l ⁻¹ h ⁻¹ g-1 FW)	Vase solution bacterial population (Log ₁₀ CFU mL ⁻¹)
S ₀	10.82ª	5.18ª	0.710ª	1.71 ^b	0.781 ^b	1.542ª	161.2ª
S ₁	11.14ª	5.00ª	0.627 ^b	2.23ª	1.047ª	1.507ª	149.2 ^b

Means with similar letter(s) in each column showed insignificant differences according to LSD test.

S₀: control) no-splitting)

 S_1 : splitting

CONCLUSION

L200B5S0 (200 mg L⁻¹ lawson essential oils \times 50 mg L⁻¹ bitter orange essential oils without splitting) had highest vase life and this treatment had agreeable results in other traits. We can recommended L200B50S0 for preservative solution for cut *Alestroemeria*.

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