J. BIOL. ENVIRON. SCI.,
2011, 5(14), 41-46

Effect of ethanol, methanol and essential oils as novel agents to improve vase-life of Alstroemeria flowers

Azadeh Mousavi Bazaz* **and Ali Tehranifar**
Department of Horticulture, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN

ABSTRACT

The production of Alstroemeria flowers has been rapidly increasing in the world. In this research effect of different concentrations of ethanol (4, 7, 10%) methanol (4, 7, 10%) as pulse treatments and some essential oils (50 or 100 mg L^{-1} peppermint (*Mentha piperata* L.), thyme (*Thymus vulgaris* L.) and black cumin (*Bunium persicum* (Boiss.) B. Fedtsch)) on flower longevity, solution uptake, fresh weight and SPAD value as a measure of leaf greenness of *Alstroemeria peruviana 'santorini'* were analyzed. Results showed alcohol treatments had no positive effect on increasing vase life of Alstroemeria. Applying essential oils could extend the vase-life. The greatest longevity of vase life was related to 50 mg L^{-1} of thyme essential oil treatment and approximately it improved inflorescence cut vase life more than 2 days longer than control treatment. The greatest solution uptake and decrease in fresh weight were seen in 100 mg L^{-1} peppermint essential oil and 100 mg L^{-1} thyme essential oil, respectively. Essential oils could not maintain SPAD value in higher amount than control treatment but these compounds particularly 50 mg L^{-1} thyme, peppermint and black cumin essential oil are useful for increasing vase life of Alstroemeria.

Keywords: Alstroemeria, Essential oils, ethanol, methanol, vase life

INTRODUCTION

During the last two decades, Alstroemeria has been one of the most commercially successful ornamental cut flowers in the Netherlands, U.K., Japan and the USA (Kim 2005). The production of Alstroemeria flowers has been rapidly increasing in the world (Spence et al. 2000). In 2003, Alstroemeria cut flowers ranked in the 9th position of the annual turnover (Kim 2005). One of the first indicators of the deterioration of Alstroemeria flower stems is leaf yellowing (Chanasut et al. 2003). Leaf yellowing occurs quickly if cut stems are held or transported in the dark (Dai and Paull 1991; Hicklenton 1991 and van Doorn et al. 1992). It was demonstrated that the onset of yellowing is associated with chlorophyll breakdown, and treatment with gibberellins or cytokinins delayed senescence, but treatment with auxins or polyamines had no effect (Ferrante et al. 2002). Indeed, the vase life of cut flowers is often limited by an accumulation of bacteria in hydration solutions and flower stems (Macnish et al. 2008). Bacteria block stem xylem vessels and thereby reduce rates of water supply to flowers (Macnish et al. 2008). Inclusion of various antimicrobial compounds such as chlorine, metal salts, quaternary ammonium salts and quinoline esters in vase water can reduce the number of bacteria and thereby extend flower longevity (Macnish et al. 2008). But, effective concentrations of these biocides can be toxic to flowers (van Doorn et al. 1990 and Knee 2000).

Essential oils (EOs) are organic natural substances that are not only safe but environmentally friendly (Solgi et al. 2009). The EOs have strong antimicrobial properties against some pathogens because they have high levels of phenolic compounds such as carvacrol, thymol and eugenol (Bounatirou et al. 2007 and Sharififar et al. 2007). Thymol, thyme oil, and zataria oil are effective against some bacteria and fungi, and are used for controlling plant diseases, particularly on fruit (Svircev et al. 2007; Braga et al. 2008 and Yahyazadeh et al. 2008). Also the essential oil of Salvia ringens and Salvia tomentosa exhibit antimicrobial activity against Gram-positive and Gram- negative microorganisms (Rowshan et al. 2010). Vase life of *Gerbera jamesonii* cv. 'Dune improved by addition of 100mg L−1 essential oils (Solgi et al. 2009). However, despite the antimicrobial effect of *Mentha piperita* (Fadaei et al. 2010), *Bunium persicum* (Bahador et al. 2009) and *Thymus vulgaris* (Bounatirou et al. 2007) essential oils, there is no information on the use of these essential oils for control of microbial contaminations and extending the vase-life of Alstroemeria cut flowers.

On the other hand, ethanol and methanol have also been tested successfully in prolonging the vase life of cut carnations, the concentration that was effective in increasing vase life of carnation flowers ranged from 2% to 8% (Heins and Blakely 1980; Wu et al. 1992 and Petridou et al. 1999). Ethanol and methanol also improve vase life of cut chrysanthemum (Petridou et al. 2001). It has been reported that using 8% and 10% ethanol extend vase life of bougainvillea sp. by causing delay senescence. Low concentration of ethanol decreased the formation of ethylene, because it inhibited the action of ACC synthase thereby affecting flower wilting, abscission, scar and color chang (Sharif Hossain et al. 2007). Also it has been reported that

* Corresponding author: mosaviazadeh@yahoo.com

Alstroemeria is sensitive to exogenous ethylene (Chanasut et al. 2003). We hypothesized that the inclusion of essential oils, ethanol and methanol in Alstroemeria vase water would extend flower longevity by reducing the accumulation of stem-plugging bacteria in solutions, and in this paper was searched the effect of different concentrations of ethanol, methanol and some essential oils on flower longevity of *Alstroemeria peruviana* 'santorini'.

MATERIALS AND METHODS

Plant material

Flowering stems of *Alstroemeria peruviana* 'santorini' were grown in standard hydroponic greenhouse conditions in Mashhad, harvested at commercial maturity (oldest buds about to open) and immediately transported to the horticultural laboratory at Ferdowsi University of Mashhad. Stems were graded for uniform quality and then re-cut to 45 cm-length in accordance with commercial practice.

Treatments

Experiment 1

Treatments were set following completely randomized design. Each treatment was repeated by 8 replications. Total 56 flowers were used for 7 treatments. The treatments were distilled water (control treatment), ethanol (4, 7, and 10 %) and methanol (4, 7, and 10 %) as puls treatments. Flower stems were placed in solution containing different concentrations of ethanol and methanol and after 24 hours were placed in distilled water until the end of the experiment. The flowers were kept in a controlled room at 22±2 ^{0C} with 60% humidity (RH) and continuous fluorescent lighting (12Mmolm^{-2} s^{-1} light intensity). After recording the initial fresh weight, flowers were placed in 300 mL capacity glass vases containing 250 mL of distilled water and weights of initial glass combining soluble were recorded at the start of the experiment.

Experiment 2

Treatments were set following completely randomized design by 8 replications. Total 56 flowers were used for 7 treatments. The treatments were distilled water (control treatment), 50 and 100mg L^{-1} of thyme (*Thymus vulgaris* L.), black cumin (*Bunium persicum* (Boiss.) B. Fedtsch) and peppermint (*Mentha piperata* L.) Essential oils (Eos). The EOs of the dry herb (thyme and peppermint) and seed (black cumin) were extracted by a Clevenger apparatus (Moghtader et al., 2009). In each case, 50 g of the plant material was distilled in 700 ml H2O in a 1000-ml flask for 3 hours. EOs samples were stored at 4°C until using. Flower stems were placed individually in solution containing different concentrations of Essential oil (EO) until the end of the experiment in a controlled room at 22±2 ^{0C} with 60% humidity (RH) and continuous fluorescent lighting (12Mmolm^{-2} s^{-1} light intensity). After recording the initial fresh weight of cut flowers, they were placed in 300 mL capacity glass vases containing 250 mL of distilled water and essential oils for Eo treatments. Also initial glass combining soluble weights were recorded at the first of the experiment.

In both experiments, vase mouths were covered with a sheet of Aluminum foil to minimize evaporation and contamination. Glass vases were placed in an autoclave at 120 ^{0C} for 20 minutes before starting two experiments and immediately used for experiments. Solution uptake, fresh weight of flowering stem, weight of glass vase containing solution (in order to calculating solution uptake) were measured every other day during the vase period. SPAD value as a measure of leaf greenness was assessed on day 5 and 9 on the uppermost fully expanded leaf from each replication. Vase life of Alstroemeria cut flowers was measured by determining the number of days from onset of the experiment until 50% petals fall or wilt. In experiment 1 and 2 solution uptake and fresh weight were analyzed over the first six or seven days of the experiments, respectively (difference between amount of solution uptake and fresh weight at first and day 6 or 7). Data were analyzed using one-way ANOVA in MSTAT-C. Means were compared by the LSD test at P = 0.05.

RESULTS

Experiment 1: effect of alcohol on vase life and other traits of Alstroemeria

The treatments had a clear effect and that was a reduction in vase life and the longevity of flowers remaining on control treatment was approximately 4 days longer than other treatments (Table 1). It was seen most of the flower stems were treated with alcohol started to rot after about four days and their vase life were terminated after about six days. The greatest and lowest amount of solution uptake was seen in control and ethanol 10% treatments, respectively, but there was no significant difference between control treatment with ethanol 4% or methanol 10% (Table 1). The greatest and lowest decrease of fresh weight were related to ethanol 10% and control treatments, respectively (Table 1). Amount of SPAD for methanol 4% treatment was higher compare to other treatments, but it had no significant difference with control treatment.

Table 1. Effects of different concentration of methanol and ethanol on flowering stem Alstroemeria vase life, solution uptake, fresh weight decrease and SPAD value (The data with the same letter are not significantly different at P≤5%).

Treatment	Vase life (day)	Solution uptake (g stem^{-1})	Fresh weight decrease (g)	SPAD value
Ethanol 4%	6.750 b	44.28 ab	4.841 bc	41.21 ab
Ethanol 7%	6.00 b	39.46 bc	5.446 bc	36.81 bcd
Ethanol 10%	7.00 b	36.51 c	8.498 a	35.59 cd
Methanol 4%	6.00 b	41.41 bc	2.355 de	42.08 a
Methanol 7%	6.00 b	39.53 bc	3.909 cd	37.96 abc
Methanol 10%	6.00 b	45.53 ab	6.564 ab	32.79 d
Distilled water	10.38 a	49.25 a	0.82 e	40.90 ab
LSD	1.743	6.284	2.231	5.017

Experiment 2: effect of essential oils on vase life and other traits of Alstroemeria

Results showed some essential oils could extend the vase-life of Alstroemeria cut flowers than control treatment. The greatest longevity of vase life was related to 50 mg L^{-1} of thyme EO treatment and approximately it improved stem cut vase life more than 2 days longer than control treatment (Table 2). However, vase life of flowers which had been treated with black cumin EO had no significant difference compared to control treatment (Table 2).

The greatest and lowest solution uptake was seen in 100 mg L^{-1} peppermint Eo and 50 mg L^{-1} black cumin Eo, respectively (Table 2) and there was no significant difference between 100 mg L^{-1} peppermint Eo with control treatment. The greatest decrease in fresh weight was related to 100 mg L^{-1} thyme oil. There was no significant difference in amount of SPAD between treatments at first stage of measuring, but at the second stage there were significant differences between treatments and the greatest and lowest amount of SPAD was related to control and 100 mg L^{-1} thyme EO treatment, respectively (Table 3). During the first five days of the experiment, cut Alstroemeria in 100mgL^{-1} thyme oil, 100 and 50 mg L^{-1} peppermint EOs maintained fresh weights higher than initial fresh weights (Figure 1).

Table 2. Effect of different concentration of essential oils on flowering stem Alstroemeria vase life, solution uptake, fresh weight decrease (The data with the same letter are not significantly different at P≤5%).

Treatment	Vase life (day)	Solution uptake (g stem^{-1})	Fresh weight decrease (g)
Peppermint EO 50 mg L^{-1}	12.50 a	63.28 b	3.234 bc
Peppermint EO 100 mg L^{-1}	12.13 ab	71.13 a	3.737 bc
Thyme EO 50 mg L^{-1}	13.03 ab	66.60 ab	3.433 bc
Thyme EO 100 mg L^{-1}	12.25 ab	62.70 b	4.770 a
black cumin EO 50 mg L^{-1}	12.50 abc	53.92 c	4.233 ab
black cumin EO 100 mg L^{-1}	11.88 bc	61.71 b	3.777 abc
Distilled water	11.00 c	64.53 ab	3.037 c
LSD	1.236	6.791	1.012

Table 3. Effect of different concentration of essential oils on flowering stem Alstroemeria SPAD value (The data with the same letter are not significantly different at P≤5%).

Treatment	SPAD (day five)	SPAD value (day nine)
Peppermint EO 50 mg L^{-1}	40.73 a	28.40 ab
Peppermint EO 100 mg L^{-1}	42.47 a	30.02 ab
Thyme EO 50 mg L^{-1}	43.43 a	29.80 ab
Thyme EO 100 mg L^{-1}	41.75 a	25.72 b
black cumin EO 50 mg L^{-1}	45.70 a	29.47 ab
black cumin EO 100 mg L^{-1}	41.08 a	28.63 ab
Distilled water	43.77 a	33.66 a
LSD	5.387	6.833

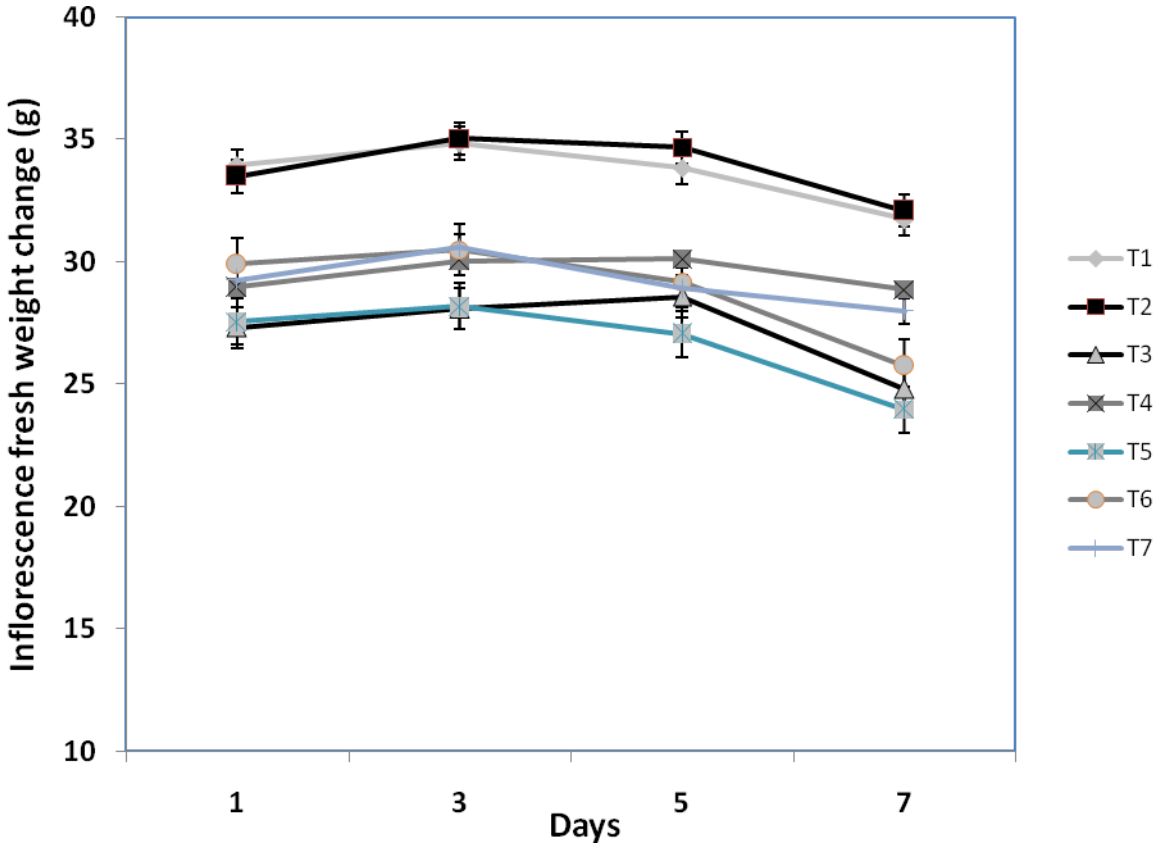

Figure 1. Effect of different concentrations of essential oils on inflorescence fresh weight changes during first seven days of *Alstroemeria* flowers. Vertical bars show standard errors of means. (T1= peppermint EO 50 mg L^{-1}, T2= peppermint EO 100 mg L^{-1}, T3= thyme EO 50 mg L^{-1}, T4= thyme EO 100 mg L^{-1}, T5= black cumin EO 50 mg L^{-1}, T6= black cumin EO 100 mg L^{-1}, T7= control treatment), each point contains 8 replication.

DISCUSSION

In spite of the important role of alcohol on flower longevity of some cut flowers such as carnation (Heins and Blakely, 1980), chrysanthemum (Petridou et al., 2001) and bougainvillea (Sharif Hossain et al., 2007), use of low concentrations of ethanol and methanol, not only had no positive effect in postharvest longevity of Alstroemeria, but also decrease quality and vase life of this cut flower compare to control treatment and caused stem rotting in cut flowers. Some essential oils such as carvacrol and 100mg L−1 thymol, thyme oil or zataria oil significantly improved the vase-life of gerbera by 6–7.5 days (Solgi et al., 2009) and some EO treatments in experiment 2 increased vase life of Alstroemeria compare to control treatment. Vascular blockage by bacteria causes decreasing water uptake and finally results in stem breaking or bending and petal wilting in some cut flowers (Balestra et al., 2005; Meman and Dabhi, 2006). These essential oils maybe could increase vase life of this cut flower by antimicrobial activities and maintaining water turgidity and balance for extending vase life. Except of 50 mg L^{-1} black cumin Eo, there was no significant difference between control treatment with other essential oils for solution uptake, but pepprmint EO (100 mg L^{-1}) and thyme EO (50 mg L−1) had higher solution uptake than distilled water. As shown in Table 3, these compounds could not maintain spad value at higher amount compare to control treatment.

In conclusion, using various concentrations of ethanol and methanol tested in this experiment did not increase Alstroemeria vase life. However EOs particularly 50 mg L^{-1} thyme, peppermint and black cumin are useful for increasing vase life of cut Alstroemeria, they maybe could increase vase life of this cut flower by antimicrobial activities and maintain water turgidity and balance for extending vase life. More research need for exploring the effect of these compounds on other cultivars of Alstroemeria.

REFERENCES

Bahador S, Negari AK and Abbaspoor M (2009). The effect of planting depth and corm weight on yield and agronomical characteristics of *Bunium persicum* (Boiss.) B. Fedtsch. Iranian Journal of Medicinal and Aromatic Plants. 25(3): 321-332.

Balestra GM, Agostini R, Bellincontro A, Mencarelli F and Varvaro L (2005). Bacterial populations related to gerbera (*Gerbera jamesonii* L.) stem break. Phytopathol. Mediterr. 44: 291–299.

Bounatirou S, Simitis S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, Costa MM, Figueiredo AC, Barroso JG and Pedro LG (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et link. Food Chem., 105: 146–155.

Braga PC, Culici M, Alferi M and Dal Sasso M (2008). Thymol inhibits Candida albicans biofilm formation and mature biofilm. Int. J. Antimicrob. Agents. 31: 472–477.

Chanasut U, Rogers HJ, Leverentz MK, Griffiths G, Thomas B, Wagstaff C and Stead AD (2003). Increasing flower longevity in *Alstroemeria*. Postharvest Biol. Technol., 29: 324-332.

Dai JW and Paull RE (1991). Postharvest handling of Alstroemeria. HortScience. 26: 314.

Fadaei S, Aberoomand A, Sharifan P, and Larijani K (2010). Evaluation of Antimicrobial Activity of Mentha piperita L. Essential Oil and Its Comparison with Sodium Benzoate. Food Technology & Nutrition. 8 (1): 34-42.

Ferrante A, Hunter DA, Hackett WP and Reid MS (2002). Thidiazuron- a potent inhibitor of leaf senescence in *Alstroemeria*. Postharvest Biol. Technol., 25: 333–338.

Heins RD and Blakely N (1980). Influence of ethanol on ethylene biosynthesis and flower senescence of cut carnation. Sci. Hortic., 13: 361–369.

Hicklenton PR (1991). GA3 and benzylaminopurine delay leaf yellowing in cut *Alstroemeria* stems. HortScience. 26: 1198-1199.

Kim JB (2005). Development of efficient regeration and transformation systems in *Alstroemeria.* PhD thesis, Wageningen University, The Netherlands, 160P

Knee M (2000). Selection of biocides for use in floral preservatives. Postharvest Biol. Technol., 18: 227–234.

Macnish AJ, Leonard RT and Nell TA (2008). Treatment with chlorine dioxide extends the vase life of selected cut flowers. Postharvest Biol. Technol., 50: 197–207.

Meman MA and Dabhi KM (2006). Effects of different stalk lengths and certain chemical substances on vase life of gerbera (*Gerbera jamesonii* Hook.) cv. 'Savana Red'. J. Appl. Hortic., 8: 147–150.

Moghtader M, Mansori I, Salari A and Farahmand H (2009). Chemical composition and antimicrobial activity of the essential oil of Bunium persicum Boiss. Seed. Iranian Journal of Medicinal and Aromatic Plants. 25(1): 20-28.

Petridou M, Voyiatzi C and Voyiatzis D (1999). Aspirin, methanol and some antibacterial compounds prolong the vase life of cut carnations. Adv. Hort. Sci., 13: 161–164.

Petridou, M, Voyiatzi C and Voyiatzis D (2001). Methanol, ethanol and other compounds retard leaf senescence and improve the vase life and quality of cut chrysanthemum flowers. Postharvest Biol. Technol., 23: 79–83.

Rowshan V, Khosh Khoi M, and Javidnia K (2010). Effects of Salicylic Acid on Quality and Quantity of Essential oil Components in Salvia macrosiphon. J. Biol. Environ. Sci., 4(11), 77-82.

Sharif Hossain ABM, Boyce AN and Osman N (2007). Postharvest Quality, Vase Life and Photosynthetic Yield (Chlorophyll Fluorescence) of Bougainvillea Flower by Applying Ethanol. Australian Journal of Basic and Applied Sciences. 1(4): 733-740.

Sharififar F, Moshafi MH, Mansouri SH, Khodashenas M and Khoshnoodi M (2007). In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. Food Control. 18: 800–805.

Solgi M, Kafi M, Taghavi TS and Naderi R (2009). Essential oils and silver nanoparticles (SNP) as novel agents to extend vase-life of gerbera (*Gerbera jamesonii* cv. 'Dune') flowers. Postharvest Biol. Technol., 53: 155-158.

Spence NJ, Mills PR and Barbara DJ (2000). A survey of viruses of Alstroemeria in the UK and the characterisation of carlaviruses infecting Alstroemeria. European Journal of Plant Pathology. 106: 843-847.

Svircev AM, Smith RJ, Zhou T, Hernade M, Liu W and Chud CL (2007). Effects of thymol fumigation on survival and ultrastracture of Monilinia fructicola. Postharvest Biol. Technol., 45: 228–233.

Van Doorn WG and Perik RRJ (1990). Hydroxyquinoline citrate and low pH prevent vascular blockage in stems of cut rose flowers by reducing the number of bacteria. J. Am. Soc. Hort. Sci., 115: 979–981.

Van Doorn WG, Hibma J, and De Wit J (1992). Effect of exogenous hormones on leaf yellowing in cut flowering branches of *Alstroemeria pelegrina* L. Plant Growth Regul., 11; 445-448.

Wu MJ, Lorenzo Z, Saltveit ME and Reid MS (1992). Alcohols and carnation senescence. HortScience. 27: 136-138.

Yahyazadeh M, Omidbaigi R, Zare R and Taheri H (2008). Effect of some essential oils onmycelial growth of Penicillium digitatum Sacc.World J.Microbiol. Biotechnol., 24: 1445–2145.