

Use of ethanol, methanol and essential oils to improve vase-life of chrysanthemum cut flowers

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ABSTRACT: The production of chrysanthemum cut flowers has been rapidly increasing in the world. However, a relatively limited vase life reduces its marketability. An experiment was conducted to research the 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 vase life, water uptake rate, water loss rate, water balance, relative fresh weight and SPAD value as a measure of leaf greenness of chrysanthemum (*Dendranthema grandiflora* (Ramat.) Kitamura, cv. Reagan White). Results showed 100 mg L⁻¹ of thyme essential oil and ethanol 7% treatments were the most effective in extending vase life of chrysanthemum which were almost 6 and 5.5 days greater than that of the control, respectively. The 100 mg L⁻¹ of thyme essential oil and ethanol 7% had a clear effect that was increase relative fresh weight. Water balance declined almost linearly with vase time and was slightly faster for the methanol 4% flowers. The amount of SPAD value by the 100 mg L⁻¹ of thyme essential oil was greater than all treatments and in general essential oil treatments had greater SPAD value than alcohol treatments and the lowest SPAD value was seen by methanol 10%.

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

INTRODUCTION

With an increasing pressure on ornamental and horticultural plants for better quality and production, various techniques have been developed for the achievement of these goals (Kim et al., 2010). Cut flowers develop water deficit stress even when standing in water (Damunupola et al., 2010). Stem cut flowers that are placed in water often develop a negative water balance and their rate of water uptake becomes lower than the transpiration rate (Damunupola et al., 2010). Reductions in stem hydraulic conductivity and the negative water balance is due to an occlusion in the xylem in the basal part of the stem by microbes and their products (Loubaud and van Doorn, 2004; Meeteren et al., 2006) by physiological plugging processes (van Doorn and Cruz, 2000) and by disruption of water columns in xylem vessels (Nijssse et al., 2000). 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). Chrysanthemum is ranked as the second most economically important cut flower in the world, after rose. However, it has a relatively short vase life and finding methods to increase flower longevity is of great importance (Vahdati et al., 2012).

Water uptake and 'rehydration' of chrysanthemum stems has been facilitated by postharvest manipulations (D'Hont et al., 1991). Also, the addition of antibacterial agents in the holding solutions has been recommended (Van Doorn, 1997). 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; Knee, 2000). Thus, there is a growing interest on the research on the possible use of natural products such as plant based essential oils and extracts, which may be less damaging for pest and disease control (Bajpai and Kang, 2012).

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 thymol, carvacrol and eugenol (Bounatirou et al., 2007; Sharififar et al., 2007). Thyme oil, thymol 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). Vase life of *Gerbera jamesonii* cv. 'Dune and carnation cut flowers improved by addition of 100mg L⁻¹ essential oils and coriander essential oil, respectively (Solgi et al., 2009 and Nermeen et al., 2010).

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 extending the vase-life of Chrysanthemum cut flowers.

On the other hand, 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. Ethanol and methanol have also been tested successfully in prolonging the vase life of cut carnations and the concentration that was effective in increasing vase life of carnation flowers ranged from 2% to 8% (Heins & Blakely 1980; Wu et al., 1992 and Petridou et al., 1999). 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 change (Sharif Hossain et al., 2007).

SPAD value is a measurement of chlorophyll content from the last expanded leaf that assesses the relative greenness of plants in a rapid and nondestructive manner SPAD value Readings were taken on the uppermost fully expanded leaf from each replication (Debaeke et al., 2006).

We hypothesized that the inclusion of essential oils on Chrysanthemum vase water would extend flower longevity of cut chrysanthemum and we compared the effect of different concentration of ethanol, methanol and some essential oils on flower longevity of Chrysanthemum.

MATERIALS AND METHODS

Cut Chrysanthemum (*Dendranthema grandiflora* (Ramat.) Kitamura, cv. Linda) flowers were purchased from a wholesale cut flower market in Mashhad city, Iran. They immediately transported to the Horticulture department laboratory at Ferdowsi University of Mashhad. Stems were graded for uniform quality and then re-cut to 40 cm-length in accordance with commercial practice.

Treatments

Treatments were set following completely randomized design by 7 replications. The treatments were distilled water (control treatment), 50 and 100 mg L⁻¹ of thyme (*Thymus vulgaris* L.), black cumin (*Bunium persicum* (Boiss.) B. Fedtsch), peppermint (*Mentha piperata* L.) Essential oils (EOs) as continues treatments and also ethanol (4, 7, 10 %) and methanol (4, 7, 10 %) as pulse treatments. 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 in solution containing different concentrations of Essential oil (EO) until the end of the experiment. Also ethanol and methanol were used as pulse treatments and after 24 hours cut flowers were placed in distilled water until the end of the experiment. All cut flowers were kept in a controlled room at 22±2 °C 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 alcohol and EO treatments, respectively. Also initial glass containing soluble weights were recorded at the start of the experiment.

Vase mouths were covered with a sheet of Aluminum foil to minimize evaporation and contamination. Glass vases were placed in an autoclave at 120 °C for 20 minutes before starting experiment and immediately used. Solution and water uptake, fresh weight of flower stem, weight of glass vase containing solution (in order to calculating solution uptake) were measured every other day during the vase period. Readings were avoided that would be directly on the leaf midrib. SPAD value measured using the Minolta SPAD meter (Minolta Camera Co., Osaka, Japan). Vase life of Chrysanthemum cut flowers was measured by determining the number of days from onset of the experiment until 50% florets fall or wilt. Average water uptake was calculated as: water uptake (g stem⁻¹) = (St⁻¹ - St); where, St is weight of vase combining water (g) at t = days 3, 5, 7 and St⁻¹ is weight of vase combining water (g) on 1, 3 and 5 day, respectively. Average water loss was calculated as: water loss (g stem⁻¹) = (Ct⁻¹ - Ct); where, Ct is the combined weights of the cut stem and vase (g) at t = days 3, 5, 7 and Ct⁻¹ is the combined weights of the stem and vase (g) on 1, 3 and 5 day, respectively. Water balance was calculated as water uptake from the vase minus water loss from the stem. Relative fresh weight (RFW) of stems was calculated as:

RFW (%) = $(W_t/W_{t=0}) \times 100$; where, W_t is weight of stem (g) at $t = \text{day } 7$ and $W_{t=0}$ is weight of the same stem (g) at $t=\text{day } 1$.

Statistical Analysis

Data were subject to analysis of variance (one-way ANOVA) using MSTAT-C program version 1.42. Means were compared by the least significant difference (LSD) test at the 0.05 probability level.

RESULTS

All substances used except ethanol 10% significantly extended the vase life of cut chrysanthemum flowers compared to the control (Table 1). 100 mg L⁻¹ of thyme EO and ethanol 7% treatments were the most effective in extending vase life which were almost 6 and 5.5 days greater that of the control, respectively (Table 1).

The lowest relative fresh weight was related to control treatment. The 100 mg L⁻¹ of thyme EO, ethanol 7%, 50 mg L⁻¹ of thyme EO and methanol 10% treatments increased relative fresh weight (RFW) of cut chrysanthemum significantly compare to control treatment (Table 1).

Table 1. Effect of different concentration of ethanol, methanol and essential oils on cut Chrysanthemum vase life, relative fresh weight and spad value.

Treatment	Vase life (day)	Relative fresh weight (g stem ⁻¹)	Spad value
Ethanol 4%	12.29 abc	58.61 e	42.47 bc
Ethanol 7%	14.00 ab	71.13 ab	42.64 bc
Ethanol 10%	10.71 cd	62.68 bcde	38.91 cd
Methanol 4%	12.00 bc	62.20 cde	44.67 abc
Methanol 7%	12.29 abc	67.11 abcde	42.45 bc
Methanol 10%	12.29 abc	67.90 abcd	36.46 d
Peppermint EO 50 mg L ⁻¹	11.71 bc	64.16 bcde	47.77 ab
Peppermint EO 100 mg L ⁻¹	12.86 abc	64.13 bcde	48.47 a
Thyme EO 50 mg L ⁻¹	11.29 c	69.02 abc	46.50 ab
Thyme EO 100 mg L ⁻¹	14.71 a	74.21 a	50.36 a
black cumin EO 50 mg L ⁻¹	12.14 abc	59.40 de	47.63 ab
black cumin EO 100 mg L ⁻¹	12.86 abc	60.80 cde	47.87 ab
Distilled water	8.57 d	59.99 de	46.31 ab

(Data with the same letter are not significantly different at $P \leq 5\%$)

The amount of average water uptake by cut flowers for methanol 4% treatment was greater than that of the flowers with other treatments (Figure 1). On the other hand the average amount of water loss for methanol 4% had an additive rhythm at first seven days (Figure 2). Average water loss by cut flowers for ethanol 7%, ethanol 10%, methanol 7% and methanol 10% decreased in first five days of the experiment (Figure 2).

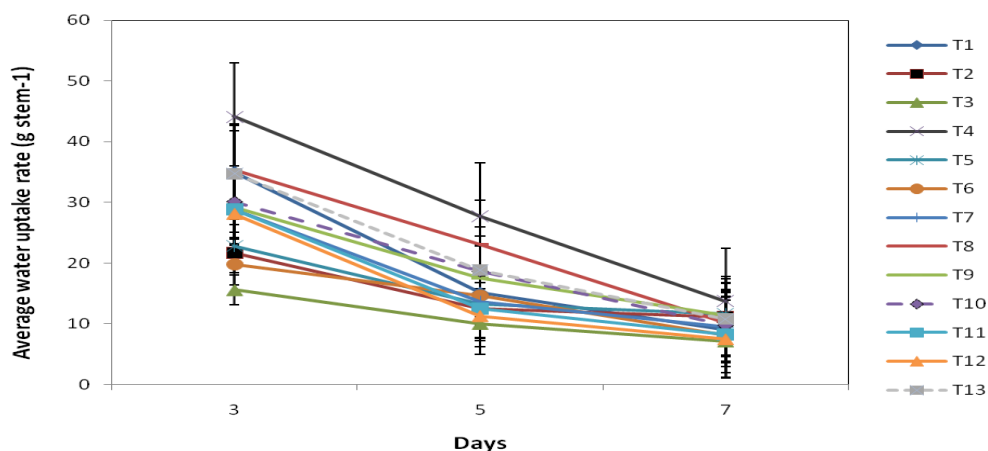


Figure 1. Average water uptake of cut Chrysanthemum over time. Vertical bars show standard errors of means. (T1= Ethanol 4%, T2= Ethanol 7%, T3= Ethanol 10%, T4= Methanol 4%, T5= Methanol 7%, T6= Methanol 10%, T7= Peppermint EO 50 mg L⁻¹, T8= Peppermint EO 100 mg L⁻¹, T9= Thyme EO 50 mg L⁻¹, T10= Thyme EO 100 mg L⁻¹, T11= Black cumin EO 50 mg L⁻¹, T12= Black cumin EO 100 mg L⁻¹, T13=Control treatment), each point contains 7 replication.

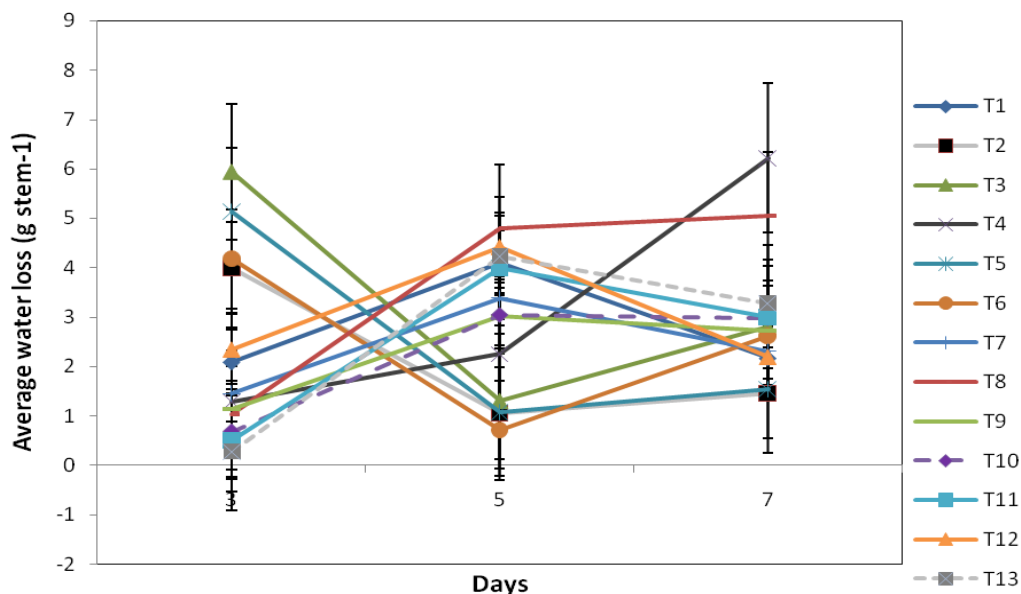


Figure 2. Average water loss of cut Chrysanthemum over time. Vertical bars show standard errors of means. (T1= Ethanol 4%, T2= Ethanol 7%, T3= Ethanol 10%, T4= Methanol 4%, T5= Methanol 7%, T6= Methanol 10%, T7= Peppermint EO 50 mg L⁻¹, T8= Peppermint EO 100 mg L⁻¹, T9= Thyme EO 50 mg L⁻¹, T10= Thyme EO 100 mg L⁻¹, T11= Black cumin EO 50 mg L⁻¹, T12= Black cumin EO 100 mg L⁻¹, T13=Control treatment), each point contains 7 replication.

Water balance declined almost linearly with vase time and was slightly faster for the methanol 4% flowers (Figure 3). Also the water balance for ethanol 7% and 100 mg L⁻¹ of thyme EO was slower than control treatment and even increased slightly for ethanol 7% at the end of day 7 (Figure 3).

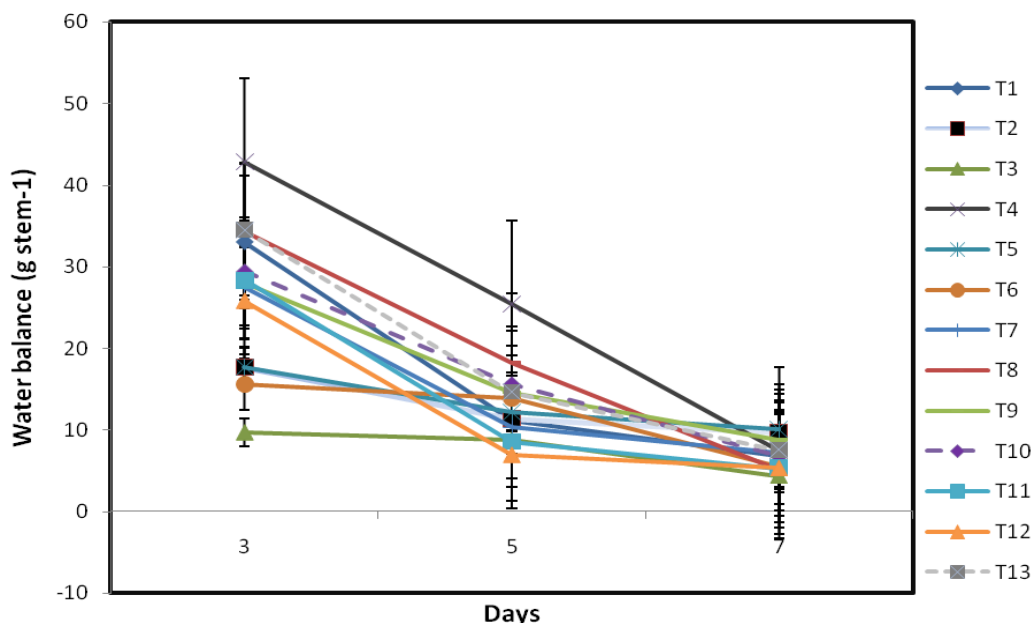


Figure 3. Water balance of cut Chrysanthemum over time. Vertical bars show standard errors of means. (T1= Ethanol 4%, T2= Ethanol 7%, T3= Ethanol 10%, T4= Methanol 4%, T5= Methanol 7%, T6= Methanol 10%, T7= Peppermint EO 50 mg L⁻¹, T8= Peppermint EO 100 mg L⁻¹, T9= Thyme EO 50 mg L⁻¹, T10= Thyme EO 100 mg L⁻¹, T11= Black cumin EO 50 mg L⁻¹, T12= Black cumin EO 100 mg L⁻¹, T13=Control treatment), each point contains 7 replication.

Leaves of cut chrysanthemum flowers deteriorated earlier than inflorescences and at day 7 only cut flowers of seven treatments (black cumin EO 50 mg L⁻¹, methanol 4%, thyme EO 50 mg L⁻¹, peppermint EO 50 mg L⁻¹, thyme EO 100 mg L⁻¹, black cumin EO 100 mg L⁻¹, control treatment) had fresh and turgid leaves and other treatments had wilt leaves and between alcohol treatments only methanol 4% had fresh leaves at day 7.

The amount of SPAD value by the 100 mg L⁻¹ of thyme EO was greater than all treatments, but there was no significant difference between these treatments and control treatment (Table 1). In general EOs treatments had greater SPAD value than alcohol treatments and the lowest SPAD value was seen by methanol 10% (Table 1).

DISCUSSION

Water deficit in a cut stem flower in vase solution will develop when the rate of water uptake is lower than the rate of transpiration (van Doorn, 1997). In the current experiments, methanol 4% and 100 mg L⁻¹ of peppermint EO treatments maintained a more favorable water balance than control flowers (Figure 3). These results were related at first seven days, because vase life of cut flowers by control treatment, terminated faster (about 8.5 day) and comparison in later days between control treatment and other treatments was impossible. Water balance decline was slightly faster by control treatment than ethanol 7% and methanol 10% (Figure 3).

Methanol and ethanol as pulse treatments has already been shown to prolong the vase life of cut chrysanthemum (Petridou et al., 2001)) and bougainvillea (Sharif Hossain et al., 2007), also methanol and ethanol used to prolong the vase life of cut carnations, successfully (Petridou et al., 1999) and to improve vegetative growth in geranium and centaurea (Devlin et al., 1995). It has been suggested that it acts by providing a readily available carbon source and by limiting carbon loss by photorespiration in C-3 plants (Devlin et al., 1995; McGiffen and Manthey, 1996).

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). Positive effects on extending vase life of cut carnation was seen by EOs such as dill and coriander (Nermeen et al., 2010) and Some Eos treatments in this experiment increased significantly flower longevity of chrysanthemum 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 maintain water turgidity and balance for extending vase life. Based on our results, new antimicrobial agents such as peppermint oil, thyme oil and black cumin oil had a positive effect on the vase-life, relative fresh weight and relative water uptake (Table 1 and Figure 1).

Due to differences in leaf chlorophyll concentration, SPAD readings vary among chrysanthemum cut flowers. In wheat, SPAD readings were directly related to chlorophyll and nitrogen (N) leaf content for a single cultivar (Debaeke et al., 2006). Fox et al. (1994) and Peltonen et al. (1995) reported good correlations between SPAD readings and plant N concentration from GS 30 to GS 41. Giunta et al. (2002) found that SPAD readings were positively correlated with leaf N per unit of leaf area in a population of 17 cultivars of durum wheat. It is possible that EOs maintained leaf nitrogen content better than some methanol and ethanol treatments, thus SPAD value were greater in EOs treatments (Table1).

In conclusion, using various concentrations of ethanol, methanol and EOs tested in this experiment, the vase life of cut chrysanthemum increased. However ethanol 7% as pulse treatment and 100 mg L⁻¹ thyme were more effective for increasing vase life, relative fresh weight on cut chrysanthemum. They may increase vase life of this cut flower by antimicrobial activities and maintain water turgidity and balance. To find out the proposed mechanism of action, e.g. inhibition of the growth of bacteria in the vase solution or inside the xylem vessels of the flower stem needs further elucidation. Also additional experiments need to explore the effect of combination of these compounds together and other floral preservatives on the vase life of chrysanthemum cut flowers.

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