



## Response of cut carnation (*Dianthus caryophyllus* L.cv. Tempo) to essential oils and antimicrobial compounds

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### Abstract

Carnation (*Dianthus caryophyllus* L. cv. Tempo) cut flower is sensitive to ethylene and bacterial infection on stem end. Bacterial contamination produces toxic compounds and these compounds stimulate the ethylene production that ultimately shortens vase life. Cut flowers were kept in vases containing *Artemisia* oil and *Anethum* oil (both at 4, 8 and 12%), 8-hydroxyquinoline sulfate (200, 400 and 600 mg l<sup>-1</sup>) and aluminum sulfate (100, 200 and 300 mg l<sup>-1</sup>). Results showed that the 100 mg l<sup>-1</sup> aluminum sulfate, 200 mg l<sup>-1</sup> 8-hydroxyquinoline sulfate, 12% *Artemisia* and *Anethum* essential oils induced the maximum vase life (17.9, 16.7, 16.1 and 15.1 days, respectively) of cut flowers. Vase life of control cut flowers was 13 days. Mentioned above treatments stimulate the minimum ethylene production and stem end bacterial colonies and maximum water uptake.

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## Introduction

Carnation (*Dianthus caryophyllus* L.), family Caryophyllaceae, is native to the Mediterranean region and Central Asia (Khalighi, 2010). Carnation is among the three top cut flowers around the world. The two species, *Dianthus barbatus* and *D. caryophyllus* are cultivated as commercial varieties (Matloubi, 2004; Fahmi and Hassan, 2005). Carnation cut flowers are so sensitive to ethylene that causes senescence and vase life reduction (Hashemabadi *et al.*, 2007). Carnation is also susceptible to the bacterial contamination on stem end that produces toxic compounds and affects ethylene production which reduces vase life (Lu *et al.*, 2010; Basiri and Zarei, 2011). Given the publicity of this problem among cut flowers, it seems to be necessary to find a solution. Essential oils act as incorporating environmentally friendly and highly effective on antimicrobial compounds (Edrisi, 2009; Kiamohammady, 2011). Herbal essential oils are natural compounds that are known as secondary metabolites which have powerful effect on pathogens control and their antimicrobial impact is proven as well. These compounds increase the vase life of cut flowers and their use is becoming widespread recently (Sharififar *et al.*, 2007; Svircev *et al.*, 2007). *Artemisia dracunculoides* and dill (*Anethum graveolens*) have medical properties (Omidbeygi, 2005). Mousavi Bazaz and Tehranifar (2011) investigated the effects of cumin, mint and thyme extracts (50 and 100 mg l<sup>-1</sup>) and ethanol (4 and 7%) on the vase life of cut *Alstroemeria* (*Alstroemeria* sp.) and concluded that all treatments had a positive effect on vase life and water uptake and also 50 mg l<sup>-1</sup> thyme extract extended vase life about 2 days more than the control. Solgi *et al.* (2009) studied on the effect of different herbal essential oils including carvacrol, thyme and zatariya, also silver nanoparticles on the vase life of gerbera (*Gerbera jamesonii* cv. Dune), and reported that treatment with 50 and 100 mg l<sup>-1</sup> carvacrol increased the vase life about 8 days as compared to the control. 8-hydroxyquinoline sulfate or citrate are used mainly to acidify the solution and combined with divalent ions such as iron and copper to prevent

vascular occlusion that can prevent enzymatic activities which lead to vascular blockage (Ichimura *et al.*, 1999; Edrisi, 2010). Tarand Hassan (2003) evaluated the effect of various levels of 8-hydroxyquinoline sulfate on the postharvest life of *Aster* sp. cut flower and concluded that the use of 400 mg l<sup>-1</sup> of this compound increased the vase life. It is proved that using calcium, aluminum, boron, nickel and zinc salts can extend vase life of cut flowers (Edrisi, 2010). In addition to the inhibitory effect of aluminum sulfate on microorganism's activity, it reduces the transpiration of cut flowers. Previous studies showed that the use of aluminum sulfate, especially at high concentrations may cause damage to the leaves of some flowers such as roses (*Rosa hybrida* L.) (Edrisi and Kalayie, 2005). Liao *et al.* (2001) investigated the effect of aluminum sulfate at different concentrations (50, 100 and 150 mg l<sup>-1</sup>) on the vase life of cut *Eustoma grandiflora* and concluded that the use of 150 mg l<sup>-1</sup> increased vase life up to 15.4 days. Additionally, aluminum sulfate enhanced water uptake and fresh weight. Hojjati *et al.* (2007) investigated different chemicals like cobalt chloride and aluminum sulfate on *Eustoma grandiflora* vase life and resulted that aluminum sulfate extends vase life of cut flowers. The purpose of the present study was to investigate the effect of different concentrations of herbal essential oils and other antimicrobial compounds on the vase life and some other characters of cut carnation (*Dianthus caryophyllus* L. cv. 'Tempo').

## Materials and methods

### Plant materials

Cut carnation (*Dianthus caryophyllus* L. Cv. 'Tempo') provided from a commercial manufacturer were equalized at 50 cm height, then they were re-cut from stem end; because of vascular blockage inhibition. Then they immediately transferred to the postharvest laboratory with 12 μMs<sup>-1</sup>m<sup>-2</sup> light intensity, 20 ± 2°C temperature, 12 hours day length and a relative humidity of 60 to 70% to assessment of the traits. The 5 cut flowers were placed in each plastic with 2 l volume vase and then were treated with specific concentrations of antimicrobial compounds.

### Experimental design and treatment

The experiment was carried out based on a completely randomized design with 13 treatments consisting *Artemisia* and *Anethum* essential oils at 3 levels (4, 8 and 12%), 8-hydroxyquinoline sulfate at 3 levels (200, 400 and 600 mg l<sup>-1</sup>) and aluminum sulfate at 3 levels (100, 200 and 300 mg l<sup>-1</sup>) along with control plots, in 3 replicates and 39 plots. The pulse treatment completed after 24 hours then flowers were transferred into other vases containing 500 ml 3% sucrose + 200 mg l<sup>-1</sup> 8-hydroxyquinoline sulfate preservative solution.

### Measurement of traits

The measured traits were vase life of cut flowers, fresh weight loss, water uptake, ethylene production and bacterial populations on the stem end. Vase life is characterized with leaf wilting index or leaf senescence and flowers wilting (Fig. 1) (Nabigol *et al.*, 2006). To evaluate the fresh weight loss, flowers fresh weight were measured at the first and the last day of vase life, and also considering the re-cut part weight, their difference were recorded. Due to the preservative solution initial content (500 ml), the absorption was measured according to each day reduction (both evaporation and stem uptake), the solution absorption was calculated and was divided to the initial flowers weight. To measure the amount of ethylene released from each pot, a flower was selected and after cut to 25 cm was weighted and then transferred into the smaller pots containing 30 ml 8-hydroxyquinoline citrate 250 mg l<sup>-1</sup> and each small pot was placed inside the jars and sealed firmly. In order to sample the air inside jars, wooden doors of each jar was equipped with a septum. Gas samples were taken to Tehran University Gas Analysis Laboratory. The released ethylene produced with the flower were measured with C-8 AIT measuring device, Shimadzu model. For stem end bacterial population counting, about 2 cm (0.5 g) were cut from the stem end 24 hours after treatment with antimicrobial compounds; also for preservative solution bacterial population counting, a 2 ml sample were isolated from each pot and the evaluation were performed according to Liu *et al.* (2009) method.

### Statistical analysis

The experimental design was R.C.B.D. Each experiment was carried out in three replicates and each replicate includes three explants. Analysis of variance (ANOVA) was done using SAS and SPSS statistical software and means were compared using the Least Significant Difference Test (LSD) at 5% probability level.

## Results and discussion

### Vase life

Analysis of variance showed that the effect of different treatments on vase life was statistically significant at 5% level. Mean comparisons revealed that 100 mg l<sup>-1</sup> aluminum sulfate, 200 mg l<sup>-1</sup> 8-hydroxyquinoline sulfate, 12% *Artemisia* and *Tarragon* essential oils were the most effective treatments with 17.9, 16.7, 16.1 and 15.1 days vase life, respectively (Table 1, Fig. 2). Vase life control cut flowers was 13 days. Enhancement of vase life can be described with antimicrobial properties of the mentioned above compounds, so that water absorption improved with prevention of vascular blockage and it delays water deficiency related wilting (Di, 2008; Jalili Marandi *et al.*, 2011). Jalili Marandi *et al.* (2011) investigated the effects of salicylic acid, herbal essential oils and silver thiosulfate (STS) on the vase life of cut roses and found that *Carum copticum* essential oil at the concentration of 500 mg l<sup>-1</sup> significantly improved the vase life as compared to the control. Mousavi Bazaz and Tehranifar (2011) with study on *Alstroemeria* and herbal essential oils, ethanol and methanol found that treatment with 50 and 100 mg l<sup>-1</sup> had the best effect on water absorption and vase life. Solgi *et al.* (2009) studied the effect of different compounds on cut gerbera cv. Dune and resulted that 50 and 100 mg l<sup>-1</sup> caused a two-fold increase on vase life compared to the control. Faraji (2005) found that 8-hydroxyquinoline citrate and aluminum sulfate had the best impact on vase life extend, flower diameter, opening percentage and flower quality of cut rose cv. Marosia. Liao *et al.* (2001) examined the effects of aluminum sulfate at concentrations of 50, 100 and 150 mg l<sup>-1</sup> on cut *Lisianthus (Eustoma grandiflora)*

and concluded that 150 mg<sup>-1</sup> of that extended vase life up to 15.4 days, additionally aluminum sulfate enhanced water uptake and fresh weight. Investigation of Hojjatiet al. (2007) with chemical treatments like cobalt chloride and aluminum sulfate and their effects on vase life of cut Lisianthus showed that aluminum sulfate can increase vase life. Khan et al. (2007) studied the effect of aluminum sulfate and sucrose on the properties of tulip (*Tulipahybrida*) and concluded that aluminum sulfate increased the relative water content in leaves

and petals 64.5% and 58.7%, respectively. Anju et al. (1999) examined the effect of 8-hydroxyquinoline citrate and sucrose on the vase life of cut chrysanthemum (*Chrysanthemum morifolium* L.) and revealed that these compounds caused an increase in fresh weight and vase life as compared to the control. Tar and Hassan (2003) investigated the impact of various levels of 8-hydroxyquinoline sulfate on *Aster* sp. vase life and concluded that the use of 400 mg<sup>-1</sup> increased vase life.

**Table 1.** Effect of antimicrobial compounds on measured traits of cut carnation cv. 'Tempo'.

Treatments	Traits				
	Vase life (day)	Water absorption (mlg <sup>-1</sup> F.W)	Fresh weight loss (g)	Ethylene (ml <sup>-1</sup> h <sup>-1</sup> g <sup>-1</sup> F.W)	Stem end bacterial clones (Log <sub>10</sub> CFU ml <sup>-1</sup> )
Control	13.00 <sup>b</sup>	1.39 <sup>c</sup>	7.80 <sup>a</sup>	0.614 <sup>a</sup>	135.50 <sup>a</sup>
Anethum oil 4%	14.8 <sup>b</sup>	1.75 <sup>ab</sup>	6.50 <sup>b</sup>	0.512 <sup>a</sup>	82.00 <sup>b</sup>
Anethum oil 8%	14.75 <sup>b</sup>	1.41 <sup>c</sup>	6.48 <sup>b</sup>	0.501 <sup>a</sup>	32.00 <sup>c</sup>
Anethum oil 12%	15.10 <sup>b</sup>	1.78 <sup>ab</sup>	4.31 <sup>c</sup>	0.313 <sup>c</sup>	30.40 <sup>c</sup>
Artemisia oil 4%	14.50 <sup>b</sup>	1.40 <sup>c</sup>	5.75 <sup>bc</sup>	0.381 <sup>c</sup>	64.00 <sup>b</sup>
Artemisia oil 8%	14.70 <sup>b</sup>	1.45 <sup>c</sup>	6.25 <sup>b</sup>	0.352 <sup>c</sup>	83.00 <sup>b</sup>
Artemisia oil 12%	16.10 <sup>b</sup>	1.80 <sup>ab</sup>	4.40 <sup>c</sup>	0.245 <sup>d</sup>	29.00 <sup>c</sup>
HQS 200 mg <sup>-1</sup>	16.70 <sup>a</sup>	1.94 <sup>a</sup>	4.20 <sup>c</sup>	0.214 <sup>d</sup>	25.00 <sup>c</sup>
HQS 400 mg <sup>-1</sup>	14.90 <sup>b</sup>	1.52 <sup>b</sup>	6.10 <sup>b</sup>	0.379 <sup>c</sup>	82.00 <sup>b</sup>
HQS 600 mg <sup>-1</sup>	13.90 <sup>b</sup>	1.61 <sup>ab</sup>	0.50 <sup>bc</sup>	0.298 <sup>d</sup>	42.10 <sup>b</sup>
Al <sub>2</sub> (SO <sub>4</sub> ) 100 mg <sup>-1</sup>	17.90 <sup>a</sup>	1.96 <sup>a</sup>	2.89 <sup>d</sup>	0.201 <sup>d</sup>	14.00 <sup>d</sup>
Al <sub>2</sub> (SO <sub>4</sub> ) 200 mg <sup>-1</sup>	13.30 <sup>b</sup>	1.59 <sup>b</sup>	4.80 <sup>c</sup>	0.598 <sup>a</sup>	47.10 <sup>c</sup>
Al <sub>2</sub> (SO <sub>4</sub> ) 300 mg <sup>-1</sup>	13.40 <sup>b</sup>	1.47 <sup>c</sup>	6.89 <sup>b</sup>	0.478 <sup>b</sup>	60.00 <sup>b</sup>

\*According to LSD test, in each column means with the same letter are not significantly different.

#### Fresh weight loss

Analysis of variance showed that the effects of different treatments on fresh weight reduction was significant at the 5% level. Mean comparison also revealed that 100 mg<sup>-1</sup> aluminum sulfate, 200 mg<sup>-1</sup> 8-hydroxyquinoline sulfate and 12% Artemisia and Dill essential oils were the most effective treatments with 2.89, 4.2, 4.4 and 4.3 g loss, respectively (Fig. 3, Table 1). Improvement of water uptake and water relations with antimicrobial compounds prevent fresh weight loss (Farrager et al., 1989; Van Doorn, 1997; Bayat et al., 2011). Liao et al. (2001) on their investigation on cut Lisianthus and applying different aluminum sulfate concentrations found that aluminum sulfate enhanced fresh weight as compared

to the control. These researchers also described this enhancement with a better corolla outreach. Hydroxyquinoline enhanced cut roses fresh weight and vase life significantly (Ichimura et al., 1999). These results are in accordance with Elgimabi and Ahmed (2009), Arab et al. (2006) and Kim and Li (2002).

#### Water absorption

Results showed that among all treatments, 100 mg<sup>-1</sup> aluminum sulfate, 200 mg<sup>-1</sup> 8-hydroxyquinoline sulfate and 12% Artemisia and Dill essential oils with 1.96, 1.94, 1.80 and 1.78 mlg<sup>-1</sup> F.W. were the best related to the water absorption (Fig. 4, Table 1). The analysis of variance showed that the effects of

different treatments on water absorption was significant at 5% level. Improving water relations and hydraulic conductivity in cut flowers arise from vascular obstruction prevention which ultimately will improve water absorption (Figueroa *et al.*, 2005). Another reason for superiority of mentioned compounds is microorganism's activity control (bacteria and fungi) that prevent vascular obstruction. Our results are in consistent with Jalili Marandi *et al.* (2011) and Burt (2004). Nabigol *et al.* (2006) showed that antiseptic and anti-ethylene compounds and also antibiotics increase water absorption, significantly. Anju *et al.* (1999) studied the effect of hydroxyquinoline and sucrose on vase life of chrysanthemum (*Chrysanthemum morifolium* L.) and concluded that these compounds caused an increasing in fresh weight and vase life and improving quality in comparison with the control. Khan *et al.* (2007) investigated the impact of aluminum sulfate and sucrose on the properties of tulip (*Tulipa hybrida*) and resulted that aluminum sulfate increased the relative water content in leaves and petals 64.5% and 58.7%, respectively.

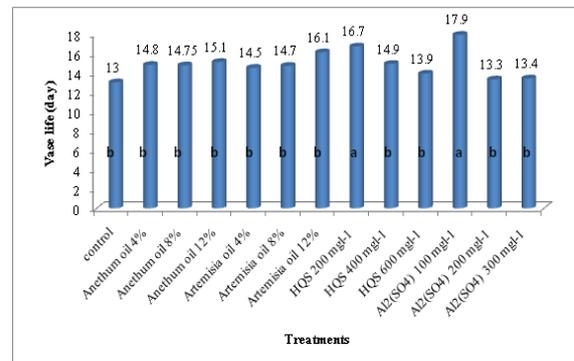


**Fig. 1.** Cut carnation cv. "Tempo" at the last day of vase life.

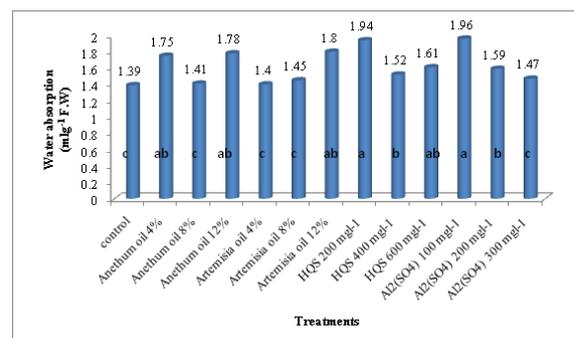
#### Bacterial population on stem end

Analysis of variance showed that the effects of different treatments on stem end bacterial population was significant at 1% level. Mean comparison of the data revealed that among all treatments, 100 mg/l aluminum sulfate, 200 mg/l 8-hydroxyquinoline sulfate and 12% Artemisia and Dill essential oils were the most effective treatments containing 14, 25, 29 and 30.4 log<sup>10</sup> CFU/ml<sup>-1</sup> population respectively that have a reduction as

compared to the control stem ends bacterial contamination (Fig. 5, Table 1). The effect of antimicrobial compounds used in these experiments is defined by disrupting the pathogens function and the respiratory chain which prevents pathogens activity and ultimately cause death (Solgi *et al.*, 2009; Hashemabadi, 2012).



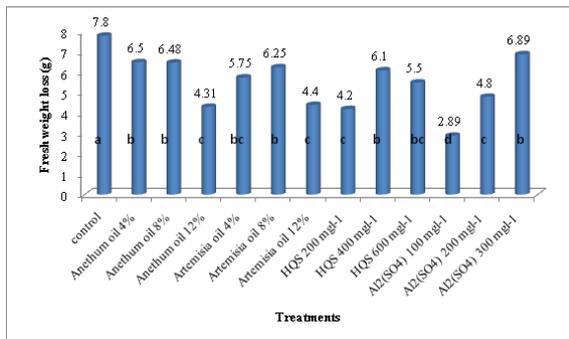
**Fig. 2.** Effect of different treatments on vase life of cut carnation cv. 'Tempo'.



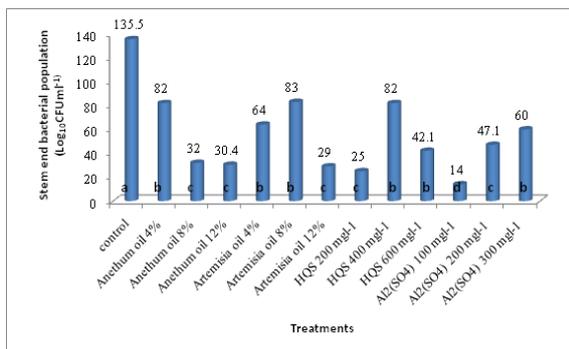
**Fig. 3.** Effect of different treatments on water absorption of cut carnation cv. 'Tempo'.

Study of Esfandiari *et al.* (2011) on cut lily (*Lilium longiflorum* cv. Shoching) showed that the antimicrobial compounds extend vase life by interfering bacterial cell division. These results are in accordance with Liu *et al.* (2009) and Shan (2012) about positive impact of antimicrobial compounds and herbal essential oils on pathogenic contamination control and vase life improvement. Kazemi and Ameri (2012) investigated the effects of herbal essential oils, silver nanoparticles and salicylic acid on bacterial contamination of carnation and found that all treatments significantly reduced bacterial populations much more than the control. Kazemi *et al.* (2011) showed that the use of vase life extending compounds at 1.5 and 3 mM decreased bacterial population of

carnation cut flower significantly (about 30-35%). Oraee *et al.* (2011) investigated the effect of different levels of silver nanoparticles and thyme essential oil on vase life and bacterial populations on stem end and in preservative solution of cut gerbera. They found that all these compounds had a positive effect in controlling bacterial contaminations, so 100 mg l<sup>-1</sup> thyme oil and 4 mg l<sup>-1</sup> silver nanoparticles with two-fold increase in vase life were the most effective treatments.



**Fig. 5.** Effect of different treatments on ethylene production of cut carnation cv. 'Tempo'.



**Fig. 6.** Effect of different treatments on stem end bacterial population of cut carnation cv. 'Tempo'.

#### Ethylene production

Mean comparison of the data revealed that among all treatments, 100 mg l<sup>-1</sup> aluminum sulfate and 200 mg l<sup>-1</sup> 8-hydroxyquinoline were the most effective treatments with 0.201 and 0.214 nll<sup>-1</sup> per hour per gram fresh weight significantly less ethylene concentration respectively (Fig 6, Table 1). Analysis of variance showed that the effects of different treatments on ethylene production were significant at 5% level. The impact of these antimicrobial compounds on cut carnation quality enhancement is obvious given the role of antimicrobial compounds on the uptake and

transport of nutrients, reduced respiratory rate and reduced the amount of ethylene. Our findings in reducing ethylene production are consistent with Goszcynska and Rudnicki (1981). Reid *et al.* (1980) found that the use of STS on cut carnation cv. "White Seam" in the 1 to 4 M ratio between silver and thiosulfate was satisfactory. Anti-ethylene and antimicrobial compounds due to their stem end bacterial contamination control, can stimulate ethylene production indirectly and can control ethylene production and extending vase life of cut carnation and gerbera (Kim and Lee, 2002; Basiri and Zarei, 2011; Basiri *et al.*, 2011). The results of mentioned studies about ethylene production control is consistent with the results of the current study.

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