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# Application of Various Concentrations of Essential Oils of Savory, Ajowan and Thyme to Maintain Quality and Shelf Life of Gladiolus Cut Flower

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**Abstract**
Two experiments were designed to evaluate the addition of essential oils to preservative solution and subsequent effects on postharvest quality and vase life of *Gladiolus* 'Sorati' cut flowers. In first experiment, the solutions of savory (*Satureja hortensis.*), ajowan (*Trachyspermum ammi*) and thyme (*Thymus vulgaris*) were applied at the concentrations of 2, 4, and 6 (mg $L^{-1}$), distilled water + 2% sucrose were considered as control treatment. In second experiment, the cut flowers pulsed with $CaCl_2$ and $AgNO_3$ for 1 h and then transferred to preservative solution contain 2 mg $L^{-1}$ of savory, ajowan, thyme and distilled water (control). All cut flowers were kept at 25±2$^{o}$C for 20 days and qualitative properties were evaluated during postharvest periods. Savory 2 mg $L^{-1}$ exhibited as the most effective treatment for minimizing dehydration of the gladiolus cut flowers by enhancing the water absorption and flower weight rate than other treatments. The interaction of pulsing with $CaCl_2$ and essential oils could maintain the quality of cut flowers.

**Keywords:** Antioxidants, ethylene, medicinal plant, vascular blockage, vase life.

## Introduction

*Gladiolus* belongs to Iridaceae family and was used extensively as cut flower in Iran. *Gladiolus* flower is a spike inflorescence and normally harvested with relatively few open florets, and the life of the flower depends on the life and opening of individual florets remaining on the spike which needed water absorption to continue until the end. Flowers are compressed shoots made up of specialized foliar parts that are adapted for reproduction. They represent a diverse group varying in size, structure, young and metabolically active tissues typically with little stored carbon. Almost invariably, floral products are highly perishable, seldom lasting more than a few weeks after harvest (Kays, 1991).

It is well known that conditions during postharvest greatly affect longevity of flowers. Temperature, relative humidity, light, air velocity and ethylene concentration plays key role in determining postharvest quality and vase-life, while the other contributing factors are composition of holding solution, proliferation of bacteria and other microbes in preservative solution and the quality of water (Halevy and Mayak, 1981).

Some chemical substances have been used to extend the vase life of rose cut flower containing calcium and silver (Capdeville *et al.*, 2003), cut gerbera (Geshnizjany *et al.*, 2014) and tuberose (Bahrehmand *et al.*, 2014). Silver nitrate

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and 8-HQC (8-hydroxyquinoline citrate) are in sucrose solution as antibacterial agents to enhance cut flowers vase-life (Nair *et al.*, 2003; Meman and Dabhi, 2006). Ketsa *et al.* (1995) have shown that $AgNO_3$ was more effective than silver thiosulfate (STS) in controlling microbial growth and vase life of *Dendrobium* 'Pompadour' flowers. Environmental and safety issues in relation to chemicals forced the scientist to look for good alternative substances in preservative solution of cut flowers.

The efficacy of essential oils (EOs) as novel antimicrobial agents was studied in extending the vase-life of *gerbera* cut flowers (Solgi *et al.*, 2009) and also sanitation towards grey mould of pepper fruit (Tzortzakisa *et al.*, 2016). Addition of 50 mg $L^{-1}$ carvacrol and 100 mg $L^{-1}$ thymol, thyme oil or zataria oil significantly improved the vase-life by 6–7.5 d (Solgi *et al.*, 2009). They concluded that improving the vase-life in preservative solutions containing carvacrol and thymol might be due to the fact that these are very effective antimicrobial agents, which inhibited the microbial growth and prevented bacterial plugging of water conducting tissues.

Reactive oxygen species increased DNA fragmentation and petal senescence of *Osmanthus fragrans* which considered another reason of senescence of cut flowers petals (Zoua *et al.*, 2014). Ezhilmathi *et al.* (2007) showed that 5-sulfosalicylic acid (5-SSA) in vase solution increased cumulative uptake of vase solution, vase life, number of opened florets and decreased the number of unopened florets in cut flowers of *Gladiolus grandiflora* 'Green Willow' compared to the controls. Spikes kept in vase solution containing 5-SSA also exhibited lower respiration rates, lipid peroxidation and lipoxygenase activity, and higher membrane stability, soluble protein concentration, and activity of superoxide dismutase and catalase. Results suggest that 5-sulfosalicylic acid increases vase life by increasing the reactive oxygen species scavenging activity of the *Gladiolus* cut flowers. Therefore compounds related to antioxidant system could affect the senescence process in plant tissues. Besides the antimicrobial property of essential oils, many studies have shown that essential oils could consider as a good antioxidants (Amarowicz, *et al.*, 2009; Chen *et al.*, 2009). This study was conducted to evaluate the possible role of EOs in holding solution and the interaction of these compounds with $CaCl_2$ and $AgNO_3$ on the quality and vase-life of cut *Gladiolus*.

## Materials and Methods

### *Plant material and treatments*

The *Gladiolus grandiflora* 'Sorati' cut flowers used for the experiment were cultivated in a commercial greenhouse located near Rafsanjan city in Kerman province of Iran. In the morning of fall season, flowers were harvested near the ground surface with leaves at horticultural maturity (when the basal florets start to open). Flowers were held in water immediately and then transported within 1 h to the laboratory. Basal end of spikes (70-80 cm in length) were recut under water to remove air emboli and all leaves were removed except a pair of them on stem. A factorial design with three replications was used for two separate experiments and each replicate (experimental unit) contained 3 cut flowers.

In experiment 1, different preservatives as treatments considered as first factor and include: different concentrations (2, 4, and 6 mg $L^{-1}$) of savory, ajowan and thyme and distilled water as control. In Experiment 2 the cut flowers were pulsed with silver nitrate at 2 mg $L^{-1}$ and calcium chloride (1%) for 1 h and then transferred to different preservatives including thyme (2mg $L^{-1}$), savory (2 mg $L^{-1}$), ajowan (2 mg $L^{-1}$) and distilled water (control). All the holding solutions contain 2% sucrose. Cut flowers introduced into these solutions and kept at 25±2°C and relative humidity 70 ± 5% under continuous illumination of fluorescent lamp during the day. Different parameters were evaluated at 4, 8, 12, 16, 20 days during storage and considered as second factor in factorial design.

### *Flower wilting and opening*

Visual rating scale for wilting of flowers was evaluated based on visual quality indexing using a scoring system as follows: 0= without wilting symptoms, 1= 20% wilting, 2= 40% wilting, 3= 60% wilting, 4= 80% wilting and 5= 100% wilting. The same method was used to estimate flower opening. The above-mentioned parameters were measured at 4-d intervals during storage periods.

### *Flower weight and relative fresh weight*

Fresh weight was measured for each treatment by a digital scale at 4, 8, 12, 16, 20 days of storage. Relative fresh weight of flowers (RFW) was calculated using the formula: RFW(%) = (Wt/Wt0) × 100; where Wt is weight of flowers (g) at t = days, 4, 8, 12, 16, 20. and Wt0 is weight of the same flower (g) on day 0.

### *Flower longevity*

The interval between the transfer of flowers to the preservative solutions and 80-85% of petal wilting was considered as the vase-life duration.

### *Statistical analysis*

All data were treated by analyses of variance and statistical differences determined between means by Duncan's Multiple Range Test at P ≤0.05 and ≤0.01 by MSTATC software. Differences are reported only for treatment main effects since interactions were not significant.

## Results

### *Experiment 1*

A summary of the statistical results in first experiment is shown in Table 1. Treatments with different concentration of essential oils had no significant effect on flower opening but were significant on flower wilting at P≤0.05 and at P≤0.01 on solution uptake, flower weight and relative flower weight during storage at 25°C. The flower opening, flower wilting and solution uptake were increased over the 12-16 d after harvest and remained nearly constant until 20 d after harvest, but the flower weight and relative flower weight were decreased (Fig. 1).

**Table 1. Effect of pulsing with $AgNo_3$ and $CaCl_2$ on vase life, flower wilting, flower opening, flower weight and preservative absorption of gladiolus cut flower**

| | Treatments | Vase Life (d) | Flower Wilting (%) | Flower Opening (%) | Preservative Absorption (g) | flower weight (g) |
|---|---|---|---|---|---|---|
| 1 | $CaCl_2$+ Savory | 16.1[†] [a] | 52.44 [a] | 71.56 [a] | 302.907 [ab] | 243.71 [b] |
| 2 | $CaCl_2$+Ajowan | 16.22 [a] | 53.78 [a] | 72.05 [a] | 332.463 [a] | 263.79 [a] |
| 3 | $CaCl_2$+Thyme | 15.1 [a] | 53.33 [a] | 70.22 [a] | 298.78 [ab] | 266.93 [a] |
| 4 | $CaCl_2$ | 14.1 [a] | 53.77 [a] | 72.44 [a] | 265.03 [b] | 211.06 [c] |
| 5 | $AgNO_3$ + Savory | 15.77 [a] | 54.22 [a] | 72.89 [a] | 286.561 [b] | 216.90 [c] |
| 6 | $AgNO_3$ + Ajowan | 15.77 [a] | 53.33 [a] | 70.22 [a] | 262.97 [b] | 222.12 [c] |
| 7 | $AgNO_3$ +Thyme | 15.22 [a] | 55.56 [a] | 70.22 [a] | 222.75 [c] | 221.22 [c] |
| 8 | $AgNO_3$ | 14.88 [a] | 51.98 [a] | 68.89 [a] | 282.36 [b] | 223.79 [c] |

† Values with similar letters in each column are not significantly different (P < 0. 05).

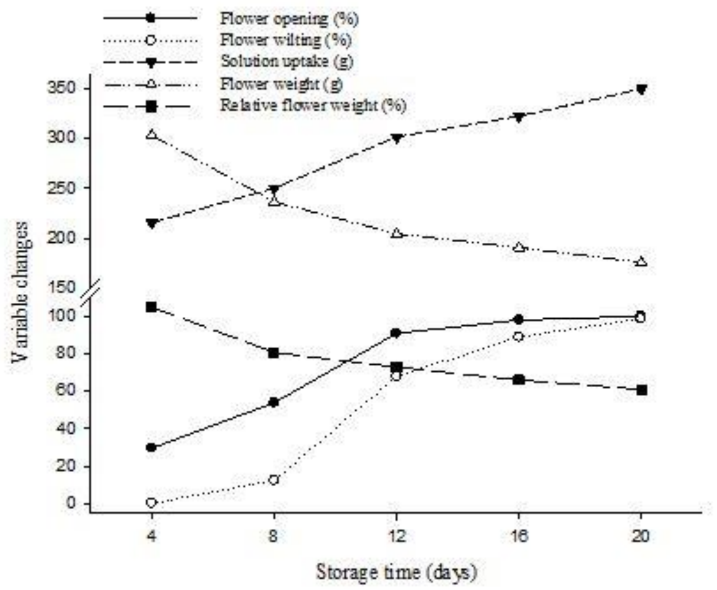


**Fig. 1. Changes of flower opening, flower wilting, solution uptake, flower weight, and relative flower weight during storage at 25°C**

The vase solution containing savory at 2 and 4 mg L$^{-1}$ significantly increased the cumulative uptake of vase solution (388.99 and 336.42 g respectively) compared to the control (288.41 g) resulting in 35.1% and 16.8% increase in vase solution uptake respectively (Fig. 2). The solution uptake of cut *Gladiolus* was enhanced with increasing of ajowan concentration in vase solution and at 6 mg L$^{-1}$ the differences were significant compare to control while there was no significance difference between solution uptake of this treatment at 2 and 4 mg L$^{-1}$. Application of 2, 4 and 6 mg L$^{-1}$ of thyme essential oil decreased the solution uptake compared to control.

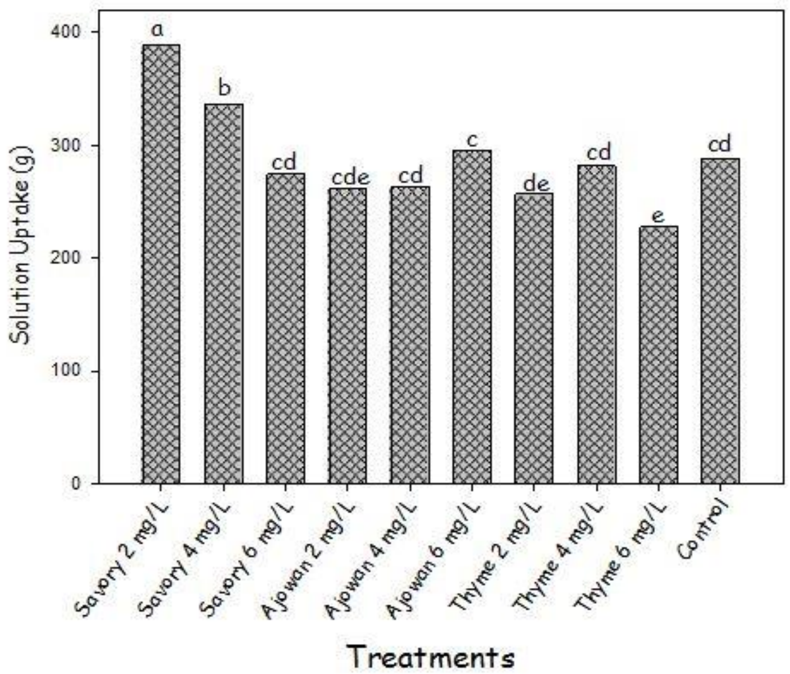


**Fig. 2. Effects of different concentration of essential oils on solution uptake (g) of cut *Gladiolus* during storage at 25±2°C. Values with similar letters in each column are not significantly different (P < 0.05).**

The data on the effect of different concentration of essential oils on flower opening flower wilting flower relative weight and flower weight of cut flower is shown in Figure 3. Flower weight of cut flower of *Gladiolus* in vase solution containing 2 and 4 mg $L^{-1}$ of savory was higher compared to control but these variables was worse in treatment with high concentration of thyme compared to other treatments and control (Fig. 3).

Addition of 2, 4 and 6 mg $L^{-1}$ of EOs of savory, 2 mg $L^{-1}$ of ajowan and 2 mg $L^{-1}$ of thyme, to preservative solutions could extend significantly the vase-life compared to control (Fig. 4). However, no significant ($P \leq 0.05$) differences were found among other concentrations of EOs in extending vase-life. These data implied this hypothesis that higher concentration of EOs especially ajowan and thyme could damage the cut flowers.

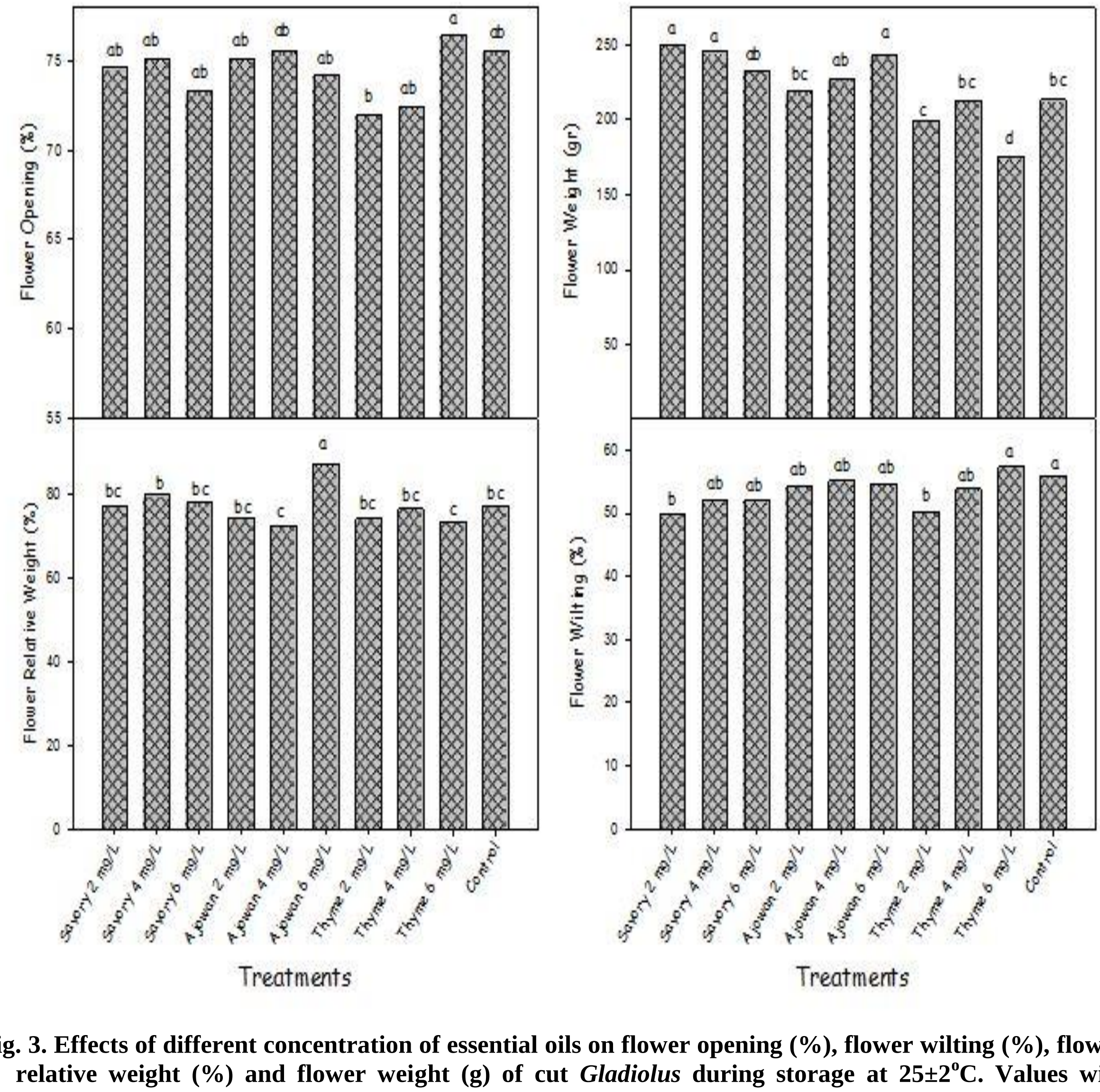


**Fig. 3. Effects of different concentration of essential oils on flower opening (%), flower wilting (%), flower relative weight (%) and flower weight (g) of cut *Gladiolus* during storage at 25±2°C. Values with similar letters in each column are not significantly different (P < 0.05).**

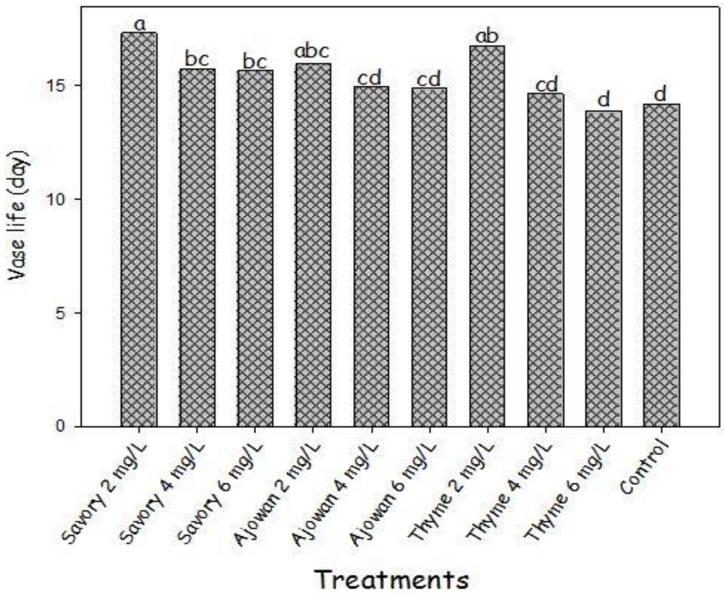


**Fig. 4. Effects of different concentration of essential oils on vase life (day) of cut *Gladiolus* during storage at 25±2°C. Values with similar letters in each column are not significantly different ($P < 0.05$).**

Solgi *et al.* (2009) in a study on gerbera showed that the thymol, carvacrol, thyme oil and zataria oil could be a new antimicrobial agent that in combination with 6% sucrose had a positive effect on the vase-life, relative fresh weight and relative solution uptake of gerbera cut flowers. They concluded that addition of 50 mg $L^{-1}$ carvacrol and 100 mg $L^{-1}$ thymol, thyme oil or zataria oil significantly improved the vase life by 6–7.5 days.

### *Experiment 2*

The results of different interactions of $CaCl_2$ and $AgNO_3$ with essential oils on vase life, flower wilting and opening, preservative absorption and flower weight of cut gladiolus has shown in Table 1. The treatment of $CaCl_2$ + ajowan increased the solution uptake and flower weight of gladiolus significantly compared to $CaCl_2$ treatment (control). In contrast, the interaction of $AgNO_3$ with essential oils was not effective in improve the quality of the flower. In addition, the overall quality of cut flowers diminished in this combination. The results also revealed a negative effect of pretreatment with $AgNO_3$, followed by preserving in solution containing essential oils on preservative solution and flower weight of cut flowers. On the basis of above mentioned results, it is concluded that pulsing with $CaCl_2$ and $AgNO_3$, accompanied by placing in preservative containing essential oils are not suitable to enhance the postharvest life and quality of *Gladiolus* cut flowers. Furthermore, it is suggested that either the pulsing time or $AgNO_3$ and $CaCl_2$ concentration may be decreased to evaluate its effect in commercial extension of postharvest life and quality of cut flowers.

## Discussion

Senescence is the final phase in the ontogeny of an organ or whole plant involving a series of irreversible events that leads to death (Zoua *et al.,* 2014). The results of different studies on cut flowers has shown that vascular blockage by bacteria and other microorganisms could decrease water uptake of preservatives and finally results in stem breaking and petal wilting (Van Doorn and De Witte, 1994; Nair *et al.*, 2003). Therefore, water balance and turgidity are important in extending cut flower vase-life. The essential oils of medicinal plant contain bioactive compounds such as menthol, thymol, carvachrol and it has been shown that these compounds have an antimicrobial and antioxidant activity (Solgi *et al.*, 2009; Jia *et al.*, 2010).

It seems that higher concentration of essential oils could damage the cut flower of *Gladiolus*. This could be concluded because savory and thyme 6 mg $L^{-1}$ decreased the uptake of solution and in consequences the flower weight and even flower opening was affected in these treatments. Furthermore, the combination of these essential oils with $AgNO_3$ diminished the overall quality of the cut flower by injury to flower stem. Thyme at 6 mg $L^{-1}$ increased the wilting of flower and decreased the flower weight of cut flowers indicating that this treatment was even worse than control and other treatment. The calcium application improved postharvest quality of rose cut flowers by reducing petal senescence and advanced the life of petals by increasing the relative water content, keeping turgidity of leaf cells and avoiding the cell walls from deformation (Mortazavi *et al.*, 2007). Also Geshnizjany *et al.* (2014) showed that $CaCl_2$ postharvest spray, nano silver in a vase solution, as well as their combinations could significantly increase the vase life of gerbera cut flowers.

Various studies have demonstrated that the EOs of medicinal plant contain different chemicals which shows antimicrobial and antioxidant activity (Ozcan and Erkmen, 2001; Chen *et al.*, 2009). Jia *et al*, (2010) studied the chemical compounds and antioxidant activity of EOs in *Thymus marschallianus* Will. The main components were Thymol (28.0% to 32.9%), p-Cymene (7.7% to 25.4%), and γ -Terpinene (18.0% to 22.4%). They also concluded that the EOs from *T. marschallianus* and *T. proximus* had antioxidant and antimicrobial properties. The extracts obtained from above 2 *thymus* species, which contained higher concentration of phenolic components, were effective scavengers of free radicals. The tested EOs represented an inexpensive source of natural antimicrobial substances for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of the processed food. Ozcan and Erkmen (2001) showed that the EOs of savory were effective against all bacterial species. The antifungal activity of savory depends on species in such a manner that it was ineffective against *Rhizopus oryzae* and *Aspergillus niger* but was effective against *Saccharomyces cerevisiae* and *Candida rugosa*.

Besides the antimicrobial activity of EOs, the antioxidant activity of these compounds was studied during recent years (Amarowicz, *et al.*, 2009; Chen *et al.*, 2009). Activated oxygen species such as $O_2^-$ or $H_2O_2$ and their interaction product, hydroxyl radical (OH), react with and degrade proteins, lipids and nucleic acids leading to senescence (Arora *et al.*, 2002; Thompson *et al.*, 1987). Numerous studies have shown that a strong antioxidant and antiradical capacities are generated by EOs from thyme, oregano, and marjoram (Amarowicz *et al.*, 2009). Sage exhibits one of the strongest antioxidant activities among medicinal herbs (Santos-Gomes *et al.*, 2002). Therefore the beneficial effect of EOs could be related to their antioxidants activity and also to their antimicrobial properties.

According to our study, addition of different concentration of EOS especially savory at 2 and 4 mg $L^{-1}$ in holding solution of cut flowers could extend the vase life and maintain the better quality of *Gladiolus* but besides these effects the concentration of Eos is very critical and need further elucidation. Also Pulsing treatment of $AgNO_3$ did not increase the vase life of *Gladiolus* cut flowers. This suggests that pulsing with $AgNO_3$ followed by keeping in EOs may cause damage to cut flowers. The interaction was better in $CaCl_2$, where the combination of this treatment with Eos increase the flower weight, preservative absorption significantly and also vase life of cut flower although it was not significant.

## Conclusions

With regards to practical alternatives to compounds containing silver and other chemical preservatives, it seems that EOs may be commercially acceptable as it is less expensive than other chemical and also there is not the danger of damage to environments but the interaction of EOs with $CaCl_2$ and inhibitors of ethylene production need further investigation. In conclusion, our results indicate that the addition of EOs to holding solution is effective in extending the longevity of cut gladiolus flower and may be useful for commercial application as a new preservative of cut flowers.

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