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Improvement Vase Life of Chrysanthemum (*Dendranthema grandiflorum* L.) Cut Flowers Using Essential Oils of *Geranium*, *Eucalyptus* and *Myrtus*

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Abstract

This study carried out based on a randomized completely design with 10 treatments geranium, eucalyptus and myrtus extraction, in 3 levels (2, 6 and 10 ml in 600 ml of distilled water) and control (distilled water) with 3 replications in 30 plots and each plot with 4 cut flowers. In this experiment traits such as vase life, water absorbtion, fresh weight, dry matter percent, petal protein content, petal carotenoids, leaf chlorophyll content, lipid peroxidation (MDA), SOD and POD enzymes activity and bacterial population were measured. The results showed that 2 ml myrtus the per 600 ml was the longest vase life compared to control (9.74 days) increased the vase life of 5.99 days. The highest bacterial population was observed in control plants. Also, the lowest MDA was found in 2 ml myrtus oil.

Keywords: *Chrysantemun*, *Eucalyptus, Geranium*, *Myrtus*, Vase life.

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* L.) is one of the most popular cut flowers in the world (Dole and Wilkins, 2004; Hashemi *et al.*, 2013). Studies on postharvest life of chrysanthemum cut flowers have shown that the vase life of this cut flowers is long because it is non-climacteric. However, water stress as a result of vascular occlusion have been reported as an important factor in reducing the quality of chrysanthemum cut flowers (Halevy and Mayak, 1979; Adachi *et al.,* 1999; Nabigol *et al.,* 2005). Microorganisms, especially fungi and bacteria that grow in the vase solution, block the stem end and limit water uptake by cut flowers with the production of chemical compounds.

Disinfectants are used long years to remove microorganisms of vase solution (Roychowdhury and Sarkar, 1995; Nowak and Rudnicki, 1990; Zagory and Reid, 1986). In recent years, the use of natural compounds has been preferred instead of chemicals to control pathogens of fruits and vegetables as well as disinfectants in preservative solution of cut flowers (Burt, 2004; Thanberg *et al.*, 2002; Valero and Frances, 2006; Solgi *et al*., 2009). Among these materials can be pointed to the active ingredients of medicinal plants or essential oils, which are completely natural and biodegradable. These compounds due to having high concentrations of phenolic compounds, have antimicrobial properties and their use in the vase solution has been evaluated positive on the postharvest life of cut flowers (Bounatirou *et al*., 2007; Lambert *et al*., 2001; Mihajilov Krstev *et al*., 2010).

Solgi *et al.* (2009) reported that the use of essential oils of *Zataria multiflora* and *Thymus vulgaris* as well as their active ingredients in the preservative solution increase the vase life of gerbera cut flowers. Mousavi Bazaz and Tehranifar (2011) found that the effect of essential oils of cumin, mint and thyme on alstroemeria cut flower life is positive. Similar results have been reported for the positive effects of herbal essential oils on the increase of the vase life of gladiolus (Hegazi and Gan, 2009), rose (Jalili Marandi *et al.*, 2011) and cloves (Kazemi and Ameri, 2012) cut flowers.

The aim of this study is to evaluate the effect of essential oils of geranium, eucalyptus and myrtus on the postharvest quality and vase life of chrysanthemum cut flowers.

MATERIALS AND METHODS

The study of chrysanthemum cut flowers was performed as a randomized complete block design with 10 treatments and 3 replicates. In the laboratory, cut flowers were arranged under water with a height of 50 cm. Essential oils of geranium, myrtus and eucalyptus used in this study were purchased from Barij Essence Company. The used treatments were herbal essential oils of geranium, eucalyptus and myrtus in 3 levels (2, 6 and 10 ml in 600 ml of distilled water with the control treatment that along with 3 % sucrose were permanently used. Chrysanthemum cut flowers were maintained until the end of the experiment under controlled conditions with a 12-hour photoperiod, light intensity of 12 µmol m^{-2} s^{-1}, relative humidity 60 to 70 % and temperature of 20 ± 2 °C.

Vase life was calculated by counting the days since applying the treatment (first day) until wilting the leaves and flowers (Nabigol *et al.*, 2005). Water uptake of flowers was calculated using the following formula (Hashemi *et al*. 2013):

Water uptake = [initial volume of vase solution - (solution remaining at the last day + average evaporation of room)] / fresh weight of cut flowers at the first day

In order to measure a, b and total chlorophyll and carotenoid of petals, sampling was performed in the fifth day of the test and the process of measuring chlorophyll and carotenoid of petals was performed using Mazumdar and Majumdar method (2003) and finally, the amount of leaf chlorophyll content suggested in mg g-1 of fresh weight and carotenoids of petals in µg g-1 of fresh weight were calculated using the following formula:

chlorophyll a = 9.93 (A_{660}) - 0.777 ($A_{642.5}$)

chlorophyll b = 17.6 ($A_{642.5}$) - 2.81 (A_{660})

total chlorophyll = 7.12 (A_{660}) – 16.8 ($A_{642.5}$)

carotenoid = 4.69 + A_{440} – 0.268 × (20.2) (A_{645}) + (8.02) (A_{663})

24 hours after applying the treatments, sampling of the stem end and the vase solution was performed and the bacterial colonies of stem end was counted according to the method of Oraee *et al*. (2011) and bacteria in the vase solution was counted using Liu *et al*. (2009) method. Kjeldahl method was used to measure protein. The level of malondialdehyde (MDA), as a product of prox-idation reaction of membrane fatty acids, was measured using Heath and Parker (1968). In *et al.* (2007) method was used to measure the peroxidase enzyme activity and Giannopolitis and Reis (1997) method was used to measure superoxide dismutase activity.

At the end of the experiment, data analysis was performed using MSTATC software and data mean was compared using LSD method.

RESULTS AND DISCUSSION

The results of analysis of variance show that the effect of geranium, myrtus and eucalyptus essential oils on all measured traits except POD activity was significant at level of 1%. The effects of treatments on POD activity was significant at 5% level (Table 1).

Table 1. ANOVA effects of various essential oils on the measured traits.

S.O.V	df	Vase life	Dry matter	Water uptake	Carotenoid	Chlorophyll a	Chlorophyll b	Total chlorophyll
Treatments	9	9.20**	11.20**	1.66**	0.031**	2.095**	0.555**	4.705**
Error	20	0.805	1.207	0.237	0.008	0.001	0.009	0.011
CV (%)		6.50	4.52	10.81	1.80	6.66	3.08	1.16

**: Probability 1%

Continues Table1. ANOVA effects of various essential oils on the measured traits.

S.O.V	df	Petal's Protein	Bacterial on vase solution	Bacterial on stem end	POD	SOD	MDA
Treatments	9	5.18**	778**	1654**	0.139*	2.330**	1.890**
Error	20	1.067	108	219	0.042	0.142	0.421
CV (%)		9.28	13.27	12.51	11.11	3.46	4.61

**: Probability 1% *: Probability 5%

Vase life

According to the results of Table 2, it could be stated that the use of eucalyptus, myrtus and geranium essential oils in the vase solution compared to control significantly increased the vase life of chrysanthemum cut flowers. Among all applied treatments, the most durability was related with treatment of 2 ml of myrtus (15.73 days) that has statistically no significant difference with treatments of 10 ml of geranium (14.74 days), 6 ml of myrtus (14.75 days), 2 ml of eucalyptus (15.13 days) and 6 ml of eucalyptus (4.75 days). The control treatment with 9.74 days had the minimum vase life among all treatments (Table 2).

In previous studies, it has been reported that the use of herbal essential oils can delay aging of flower and increase durability through reducing the microbial population of the solution and stem end and improve water uptake (Mousavi Bazaz and Tehranifar, 2011; Solgi *et al.*, 2009). The antimicrobial effect of essential oils on increasing the vase life of cut flowers of cloves (Bayat *et al.*, 2011) and gerbera (Ziyaei Movahed *et al.*, 2010; Solgi *et al.*, 2009) has been reported that is in accordance with the results of current study.

Water uptake

The result of mean comparison showed that water uptake is significantly increased compared to the control by using herbal essential oils. As the control with 3.19 ml g^{-1} F.W. had the lowest water uptake. Among the herbal essential oils, eucalyptus treatments of 6 and 10 ml with

5.39 and 5.14 ml g^{-1} F.W., respectively, and 2 and 6 ml of geranium with 5.40 and 5.04 ml g^{-1} F.W., respectively, have no statistically significant difference and have the maximum water uptake, Although, 2 and 10 ml of myrtus with 4.52 and 4.51 ml g^{-1} respectively, had not different significant with mentioned treatments (Table 2).

Water balance is the most important factor in determining the quality and postharvest life of cut flowers (da Silva, 2003). As mentioned in the statement of results, treatment of chrysanthemum cut flowers with herbal essential oils, improved water uptake compared to the control. In fact, essential oils by controlling bacterial and fungal contamination of the vase solution, prevents obstruction of vascular and causes balance of water relations of cut flowers. Similar results have been reported that the positive effect of herbal essential oils on water uptake of cut flowers of alstroemeria (Mousavi Bazaz and Tehranifar, 2011) and cloves (Bayat *et al*, 2011) and these results are in accordance with the results of current study.

Dry matter

By using herbal essential oils, the percentage of dry matter is increased compared to the control. So that the control treatment with 21.39 % had the lowest dry matter that of course has no statistically significant difference with the treatment of 2 and 10 ml of myrtus and 6 ml eucalyptus (Table 2). The maximum amount of the dry matter was related to the treatment of 6 ml of myrtus with 27.42 %, which has no statistically significant difference with the treatments of 10 ml of geranium and 2 ml of eucalyptus (Table 2). As mentioned in the statement of results, the use of herbal essential oils improved dry matter weight of chrysanthemum cut flower by increasing water relations and fresh weight. Similar results have been reported that the positive effect of herbal essential oils on increasing dry matter of gladiolus cut flowers (Hegazi and Gan, 2009) and these results are in accordance with the results of the this study.

Petal's carotenoids content

Based on the mean of comparison, the amount of carotenoids in petals was increased in all used treatments compared to the control. As seen in Table 2, there is no statistically significant difference between used essential oils in terms of the amounts of carotenoids in petals. The control treatment with 4.77 µg g^{-1} F.W has the least amount of carotenoids in petals (Table 2).

Carotenoids and anthocyanins are the main index durability and marketing of cut flowers. In previous studies, it has been found that treatment of cut flowers with a vase solution containing sugar or disinfectants causes maintaining cell turgidity by improving water uptake and thus prevents destruction of important pigments such as carotenoids (Amarjit, 2000; Hassanpour Asil and Karimi, 2010, Edrisi, 2008).

Petal's protein content

The results showed that petal's protein content in the control treatment (9.07 %) was less than other treatments. Maximum petal's protein content was related to treatments of 2 ml of geranium (13.16 %) and 2 ml of eucalyptus (12.91 %) that have no statistically significant difference with treatments of 2, 6 and 10 ml of myrtus and 10 ml of geranium (Table 2).

Researchers reported that herbal essential oils increase vase life through reducing the breakdown of protein. They stated that herbal essential oils have antioxidant properties that prevent activity of oxygen free radicals and damage of proteins caused by free radicals and protect the protein in cut flowers (Kazemi *et al.*, 2014; Rajasekaran *et al.*, 2002). Maintaining and increasing the protein in Lisianthus cut flowers (Kazemi *et al*., 2014) have been reported with the use of herbal essential oils in the vase solution that these results are in accordance with the results of the present research.

Chlorophyll a, b and total

Based on the results, content f chlorophyll a, b and total has been increased compared to

the control by using essential oils. So that the control treatment has the minimum amount of chlorophyll a, b and total with 4.72, 2.44 and 7.16 mg g^{-1} F.W, respectively. The maximum content of chlorophyll a (7.59 mg g^{-1} F.W) was obtained for treatment of 2 ml of eucalyptus essential oil. The maximum content of chlorophyll b was obtained for treatment of 2 ml of eucalyptus (3.80), 10 ml of eucalyptus (3.72) and 6 ml of myrtus (3.62). The maximum content of total chlorophyll was also related to treatment of 2 ml of eucalyptus (11.39) (Table 2).

Lise *et al*. (2004) believe that the increase of chlorophyll is because of cells activity and increasing sugar. The researchers reported that essential oils are very effective in maintaining achlorophyll due to their antioxidant properties. Kazemi *et al.* (2014) stated that water stress and obstruction of vessels increase oxygen free radicals in chloroplasts, and so result destruction of chlorophyll molecules and membranes of chloroplasts. The results of this research showed that herbal essential oils with anti-radical property maintain and increase the chlorophyll in lisianthus cut flowers that is in accordance with the results of the present research.

Bacterial population on vase solution and stem end

Based on the mean comparison, the number of bacterial colonies of the vase solution and stem end is reduced by using essential oils. As seen in Table 2, the highest amount of bacterial colonies in vase solution was found in the control (98.33 colonies per 10 ml agar) that has no statistically significant difference with treatments of 2 and 10 ml of geranium, 10 ml of myrtus and 6 ml of eucalyptus. The minimum bacterial number of vase solution was recorded for treatments of 2 ml of eucalyptus (55 colonies) and 2 ml of myrtus (58.33 colonies) that there was no statistically significant difference between them. However, the maximum bacterial number of stem end was related to the control treatment with 162 colonies that has no statistically significant difference with treatments of 2 ml of geranium (136 colonies) and 10 ml of geranium (137 colonies). The minimum bacterial number of stem end were recorded for two treatments of 2 ml of eucalyptus (88 colonies) and 2 ml of myrtus (89 colonies) that there was no statistically significant difference between them (Table 2).

Table 2. Mean comparison of effect of various essential oils on the measured traits.

Treatments	Vase life (day)	Dry matter (%)	Water uptake (ml g^{-1} F W)	Carote noid (µg g^{-1} F W)	Petal's protein (%)	Chloro phyll a (mg g^{-1} FW)	Chloro phyll b (mg g^{-1} F W)	Total chlorophyll (mg g^{-1} F W)	Bacteria in vase solution (colonies per 10 ml agar)	Bacteria in the stem end (colonies per 10 ml agar)
Control	9.71 ^{d}	21.39 ^{e}	3.19 ^{c}	4.77 ^{b}	9.07 ^{c}	4.72 ^{j}	2.44 ^{e}	7.16 ^{g}	98.33 ^{a}	162 ^{a}
G_1	13.73 ^{bc}	25.05 ^{bc}	5.40 ^{a}	5.00 ^{a}	13.16 ^{a}	6.02 ^{f}	3.08 ^{bc}	9.10 ^{d}	94.00 ^{a}	136 ^{ab}
G_2	13.42 ^{bc}	24.67 ^{bcd}	5.04 ^{a}	5.02 ^{a}	10.03 ^{bc}	6.35^{d}	3.22 ^{b}	9.57 ^{c}	66.66^{bc}	111 ^{bc}
G_3	14.74^{ab}	25.68 ^{ab}	4.04 ^{bc}	5.10 ^{a}	11.71 ^{ab}	5.72 ^{g}	2.96 ^{cd}	8.73 ^{e}	94.00 ^{a}	137 ^{ab}
M_1	15.73 ^{a}	21.80 ^{e}	4.52 ^{ab}	5.09 ^{a}	11.47 ^{ab}	6.25 ^{e}	3.21 ^{b}	9.46 ^{c}	58.33 ^{c}	89 ^{d}
M_2	14.75 ^{ab}	27.42^{a}	3.80 ^{bc}	5.12 ^{a}	11.24 ^{ab}	7.01 ^{b}	3.62 ^{a}	10.46 ^{b}	72.33 ^{bc}	114 ^{bc}
M_3	12.13 ^{c}	22.73 ^{de}	4.51^{ab}	4.04 ^{a}	11.46^{ab}	5.72^{g}	2.87 ^{d}	8.60 ^{ef}	92.00 ^{a}	126 ^{b}
O_1	15.13 ^{ab}	25.60 ^{ab}	5.39 ^{a}	5.04 ^{a}	12.91 ^{a}	7.59 ^{a}	3.80 ^{a}	11.39 ^{a}	55.00 ^{c}	88 ^{d}
O_2	14.75^{ab}	23.13 ^{cde}	5.14 ^{a}	5.02 ^{a}	10.00 ^{bc}	5.51 ^{h}	2.87 ^{d}	8.51 ^{f}	85.33 ^{ab}	121 ^{bc}
O_3	13.75 ^{bc}	25.04 ^{bc}	3.99 ^{bc}	5.12 ^{a}	10.17 ^{bc}	6.93 ^{c}	3.72 ^{a}	10.66 ^{b}	68.66 ^{bc}	97 ^{cd}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Essential oils of *Geranium*	Essential oils of *Myrtus*	Essential oils of *Eucalyptus*
G_1: 2 ml in 600 ml of distilled water	M_1: 2 ml in 600 ml of distilled water	O_1: 2 ml in 600 ml of distilled water
G_2: 6 ml in 600 ml of distilled water	M_2: 6 ml in 600 ml of distilled water	O_2: 6 ml in 600 ml of distilled water
G_3: 10 ml in 600 ml of distilled water	M_3: 10 ml in 600 ml of distilled water	O_3: 10 ml in 600 ml of distilled water

Anjum *et al*. (2001) reported that addition of a suitable disinfectant to the preservative solution of cut flowers prevents growth of microorganisms and increases water uptake and therefore causes more durability of cut flowers. The positive impact of herbal essential oils on reducing microbial population of solution and stem end of cut flowers of gladiolus (Hegazi and Gan, 2009), cloves (Kazemi and Ameri, 2012) and rose (Shanan, 2012) has been reported is in accordance with the results of the present research.

Malondialdehyde (MDA)

As can be seen in Fig. 1, the concentration of MDA was maximum in the control treatment (14.96 nmol g^{-1} F.W.). However, it has no statistically significant difference with treatments of 2, 6 and 10 ml of geranium and eucalyptus essential oils. The minimum concentration of MDA was related to the treatments of 6 ml of myrtus with 12.65 nmol g^{-1} F.W. and then 10 ml of myrtus (13.09 nmol g^{-1} F.W.) that these treatments were the best ones for this trait (Fig. 1).

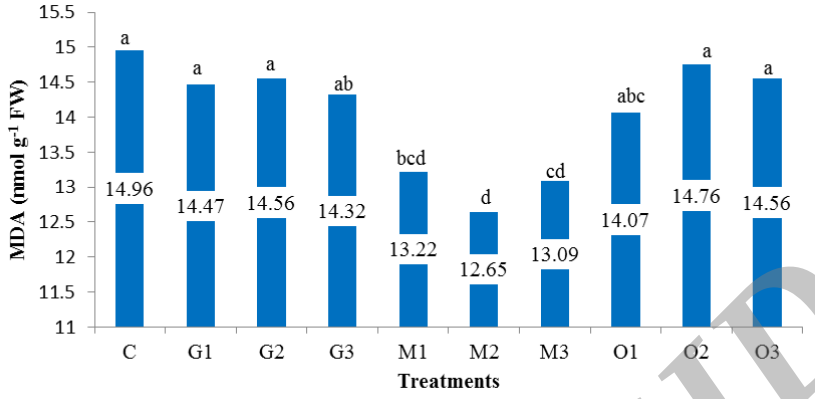

Fig. 1. Effect of various essential oils on the amount of MDA.

Essential oils of *Geranium*	Essential oils of *Myrtus*	Essential oils of *Eucalyptus*
G_1: 2 ml in 600 ml of distilled water	M_1: 2 ml in 600 ml of distilled water	O_1: 2 ml in 600 ml of distilled water
G_2: 6 ml in 600 ml of distilled water	M_2: 6 ml in 600 ml of distilled water	O_2: 6 ml in 600 ml of distilled water
G_3: 10 ml in 600 ml of distilled water	M_3: 10 ml in 600 ml of distilled water	O_3: 10 ml in 600 ml of distilled water

Palma *et al*. (2002) believe that the herbal essential oils by preventing the activity of oxygen species causes reducing the lipid peroxidation in cell membrane and reducing the concentration of MDA. The researchers believe that treatment of cut flowers with sugar and disinfectants by reducing the stresses applied on flowers causes to reduce the accumulation of MDA (Jin *et al*., 2006). Kazemi and Ameri (2012) reported that the positive effect of herbal essential oils of thyme and lavender on the stability of the membrane and reduction of MDA in clove cut flowers that is in accordance with the results of the present research.

Antioxidant enzymes (peroxidase and superoxide dismutase)

Based on the results, the maximum SOD activity was associated with the control treatment with 13.14 IU g^{-1} F.W. By using essential oils, the amount of SOD has been significantly reduced, so that the minimum SOD activity was recorded for treatments of 6 ml of geranium (10.12 IU g^{-1} F.W.) and 10 ml of myrtus (10.09 IU g^{-1} F.W.) that there was no significant difference between them statistically (Fig. 2).

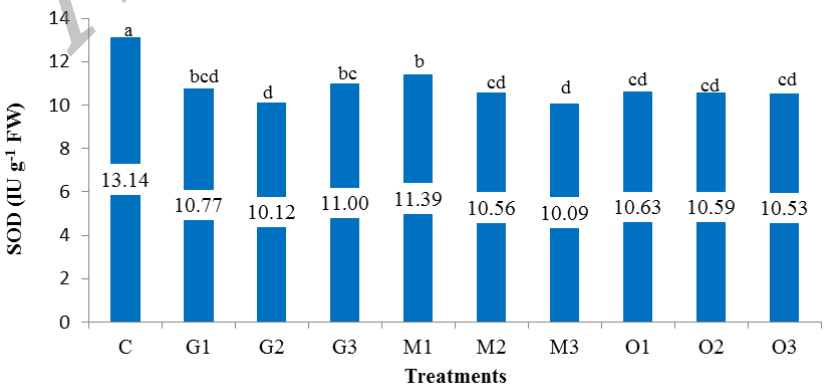

Fig. 2. Effects of various essential oils on the activity of SOD.

Essential oils of *Geranium*	Essential oils of *Myrtus*	Essential oils of *Eucalyptus*
G_1: 2 ml in 600 ml of distilled water	M_1: 2 ml in 600 ml of distilled water	O_1: 2 ml in 600 ml of distilled water
G_2: 6 ml in 600 ml of distilled water	M_2: 6 ml in 600 ml of distilled water	O_2: 6 ml in 600 ml of distilled water
G_3: 10 ml in 600 ml of distilled water	M_3: 10 ml in 600 ml of distilled water	O_3: 10 ml in 600 ml of distilled water

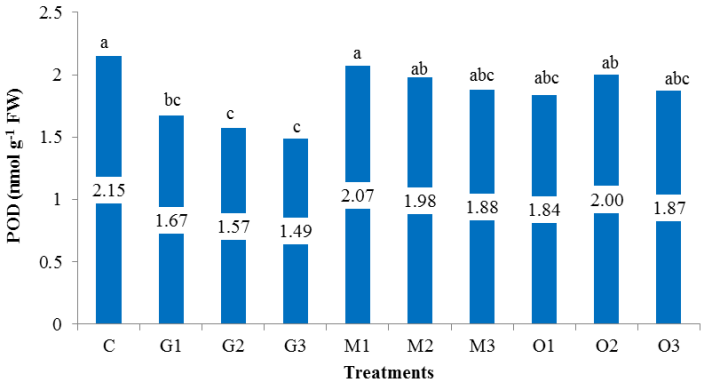

Fig. 3. Effects of various essential oils on the activity of POD.

Essential oils of *Geranium*	Essential oils of *Myrtus*	Essential oils of *Eucalyptus*
G_1: 2 ml in 600 ml of distilled water	M_1: 2 ml in 600 ml of distilled water	O_1: 2 ml in 600 ml of distilled water
G_2: 6 ml in 600 ml of distilled water	M_2: 6 ml in 600 ml of distilled water	O_2: 6 ml in 600 ml of distilled water
G_3: 10 ml in 600 ml of distilled water	M_3: 10 ml in 600 ml of distilled water	O_3: 10 ml in 600 ml of distilled water

Study of the results of POD activity show that among the used essential oils, the concentrations of 2, 6 and 10 ml of geranium has only decreased POD activity significantly. As can be seen in Fig. 3, the maximum POD activity was associated with the control treatment with 2.15 nmol g^{-1} F.W. that has no statistically significant difference with treatments of 2, 6 and 10 ml of myrtus and eucalyptus. The minimum POD activity in the treatment of 10 ml of geranium (1.49 nmol g^{-1} F.W.) and 6 ml of geranium (1.57 nmol g^{-1} F.W.) that there was no significant difference between them statistically (Fig. 3).

Among the various treatments, the control treatment had the highest antioxidant enzymes activity. Xiao Zheng and Huang (2002) reported that after harvesting cut flowers from the stock plant and transferring them to a vase solution, flowers exposed to stress especially water stress. Upadhyaya and Panda (2004) believe that the use of disinfectant in the vase solution reduces the production of oxygen free radicals by reducing water stress and thus they reduce the oxidation of membrane lipids and thereby prevent the wilting of cut flowers.

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افزایش عمر پس از برداشت گل بریده داودی (*Dendranthema grandiflorum L.*) به کمک اسانس‌های ژرانیوم، اکالیپتوس و مورد

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چکیده

این مطالعه با هدف بررسی اثر اسانس‌های گیاهی بر عمر گلجایی و کیفیت پس از برداشت گل بریده داودی بر پایه طرح کاملا تصادفی با ۱۰ تیمار شامل اسانس گیاهی ژرانیوم، مورد و اکالیپتوس در ۳ سطح (۲، ۶ و ۱۰ میلی‌لیتر در ۶۰۰ میلی‌لیتر آب مقطر) همراه با تیمار شاهد (آب مقطر)، در ۳ تکرار و ۳۰ پلات به صورت دائمی انجام شد. صفاتی نظیر عمر گلجایی، جذب آب، درصد ماده خشک، میزان پروتئین گلبرگ، کاروتنوئید گلبرگ، کلروفیل برگ، غلظت MDA، فعالیت آنزیم‌های SOD و POD و جمعیت میکروبی اندازه گیری شدند. نتایج نشان داد که تیمار ۲ میلی‌لیتر اسانس مورد در ۶۰۰ میلی‌لیتر آب مقطر بیشترین عمر گلجایی را داشت که نسبت به شاهد (۹/۷۴ روز) ۵/۹۹ روز عمر گلجایی را افزایش داد. شاهد بیشترین جمعیت میکروبی را داشت. کمترین تجمع MDA نیز برای تیمار ۲ میلی‌لیتر مورد ثبت شد.

کلید واژگان: اکالیپتوس، داودی، ژرانیوم، عمرگلجایی، مورد.