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Assessment of vase life and postharvest quality of cut rose (*Rosa hybrida* cv. Angelina) flowers by application of cumin (*Cuminum cyminum* L.) essential oil and 8-hydroxyquinoline sulfate

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Natural preservatives such as herbal essential oils have potential ability for extending postharvest vase life of cut flowers. In this study, application effect of cumin (*Cuminum cyminum* L.) essential oil and 8-hydroxyquinoline sulfate on vase life and postharvest quality of cut rose (*Rosa hybrida* cv. Angelina) flowers were investigated. A factorial experiment with three levels of each in different time after harvesting was conducted. Results showed that usage of different level of cumin essential oil and hydroxyquinoline sulfate had significant effects on rose attributes at the level of 0.05. The results showed that the interaction effect of cumin essential oil and hydroxyquinoline sulfate in measuring time was significant (P<0.05) on all of parameters except for anthocyanin content in rose petals in a way that the highest amount for measured traits was obtained with treatment of 150 mg L^{-1} cumin essential oil and 400 mg L^{-1} 8-hydroxyquinoline sulfate.

1. Introduction

Roses have a critical role in the manufacturing of various medicinal and nutritional products. Rosa, known as the symbol of affection and elegance in Iran, is one of the leading cut flower in global floriculture trade including our country (Butt, 2005; Zamani *et al*., 2011). The genus *Rosa* belongs to the family Rosaceae and includes 200 species and more than 18,000 cultivars (Ahmad *et al*., 2013). Cut flower trading is the prime purpose of rose cultivation, but short vase life is the most crucial problem. Commercially, post-harvest longevity of cut flowers is of importance. Many studies have therefore focused on its quality both in pre and postharvest periods (Mirjalili, 2015). The most common physiological and morphological responses after harvesting are wilting or bent neck caused by pathogens

especially bacteria, resulted in decreasing the vase life of cut rose flowers (Leiv and Hans, 2005; Thwala *et al*., 2013). The development of such symptoms is resulted from vascular occlusion, mainly located in the basal stem end (Lü *et al*., 2010; Farahi *et al*., 2013).

Study on effects of natural plant products, including essential oils as preservatives hasted during last decades (Elgimabi and Ahmed, 2009). In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal and insecticides (Bakkali *et al*., 2008). Cumin (*Cuminum cyminum* L.) is an aromatic plant in the family Apiaceae. Cumin seeds are rich of essential oil especially cumin aldehyde, used as a stimulant as well as carminative and therapeutics (Iacobellis *et al*., 2005; Asghari Marjanloo *et al*., 2009).

There are reports on preservative effects of plant essential oils on other plants pathogens, such as tea essential oil on the Botrytis in grape (Jobling, 2000) and antifungal effect of Persian thyme essential oil on strawberry (Nabigol and Morshedi, 2011). Positive effects of plant essential oils have been reported on longevity of cut flowers' vase life (Deans and Ritchie, 1987; Dudai *et al*., 1999). Thwala *et al*. (2013) used cumin essential oil for decreasing degradation and vessel boring in orchids resulted in delay of senescence.

8-hydroxyquinoline sulphate (8-HQS) as a very important germicide in preservatives is used in floral industry. HQS acts as an anti-microbial agent and increases water uptake (Ali and Hassan, 2014). The positive effect of 8-HQS and calcium chloride alone or in combination with 4% sucrose as chemical preservative solutions to improve postharvest quality of cut gerbera flowers has been shown (Soad *et al*., 2011). It reported that HQS extended the vase life of rose cut flowers, whereas sucrose can promote the effect of HQS (Ichimura *et al*., 1999). It documented that vase life and postharvest quality of different cut flowers were enhanced by 8-HQS treatment through improving water uptake, fresh weight and carbohydrate content (Kim and Lee, 2002; Hassan *et al*., 2003; 2004; Ali and Hassan, 2014). Despite the valuable reports on successful use of various phytochemicals for improving longevity of fresh cut flowers, screening for introducing and developing an exact, cheap and easy-to-use preservative is of importance for floriculture (Wu *et al*., 2016).

The objective of this study was to investigate the effect of different cumin essential oil concentrations and 8-hydroxyquinoline sulphate (8-HQS) on vase life and postharvest quality of cut rose flowers in different measuring time.

2. Materials and Methods

Cut rose (*Rosa hybrida* cv. Angelina) flowers were obtained from the commercial greenhouse around Shiraz. Cut rose flowers were harvested when florets were not opened but sepals were turned back and separated from petals during September 2014 and immediately transported to the laboratory. Prior to insert in solutions, flowering stems of plants were cut under water to prevent air entrance into the xylem conduits that were opened by cutting.

This factorial experiment was conducted in randomized complete blocks design with three replications. Treatments were 8-hydroxyquinoline sulfate (8-HQS) at four level (0, 200, 400 and 600 $mg \cdot L^{-1}$) and cumin essential oil at three level (0, 100, 150 $mg \cdot L^{-1}$) in three measuring time (1st day, 8th day and 16th day) after treatment. After the duration of treatments, the flowers were placed in beakers containing 400 ml distilled water during the vase life evaluation period. The control flowers were kept in distilled water. Replications included five flowers per treatment.

Vase life room conditions was 12 hours day length, 18±2°C, 60±5% RH and 12 µmol s^{-1} m^{-2} light intensity and measured traits were vase life determination (days), petal fresh weight/dry weight rate (%), flower diameter (mm), anthocyanin content (mg 100 g^{-1} F.W.), relative water content (RWC) (%), leakage of ions (%), catalase enzyme activity (CAT) ($Ua \cdot mg^{-1}$ pro), peroxidase enzyme activity (POD) ($Ua \cdot mg^{-1}$ pro), membrane stability index (MSI) (%).

Vase life determination

In this study, vase life was considered as the time during which cut-flower can keep its marketability quality and before senescence symptoms including bending of petal margins and wilting are appeared (Singh, 1994). Cut-flower durability was evaluated from cut flower treatment till their ornamental value has disappeared.

Leakage of ions

Floret samples from each treatment were taken on first day and were repeated on day 7 for determining ions leakage by using the method of Sairam *et al*. (1997). Two florets samples (0.2 g) were taken and placed in 20 ml of double distilled water in two different 50 ml flasks. The first one was kept at 40°C for 30 min while the second one was kept at 100°C in boiling water bath for 15 min. The electric conductivity of the first (C1) and second (C2) samples were measured with a conductivity meter. The leakage of ions was expressed as the membrane stability index according

to the following formula (Ezhilmathi *et al*., 2007):

$$\text{Membrane stability index (MSI)} = [1-(C1/C2)] \times 100 \quad \text{(Eq. 1)}$$

Petal anthocyanin

The amount of 200 mg petal samples was pulverized in 3 ml 99:1 (v/v) methanol and hydrochloric acid and obtained extracts were centrifuged at 12000 rpm for 20 min at 4°C. Supernatants were kept in 4°C and under darkness condition for 24 h. After that, light absorption was estimated by spectrophotometer in 550 nm wavelength and using silence coefficient (ε =33000 mol^2 cm^{-1}) (Krizek *et al*., 1993).

Petal membrane stability index

For determining petal membrane stability, two samples of petals each including 200 mg of each replication were weighted and dipped in 10 ml double distilled water. One of them was placed in 40°C Benmary for 30 min and second one at 100°C Benmary for 15 min. After reaching to the room temperature, electrical conductivity of the solutions was measured with a EC meter and the stability percent of the membrane was determined according Ezhilmathi *et al*. (2007), as equation 1.

Enzymes assays

Peroxidase (POD) enzyme was extracted from 200 mg homogenized samples in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 28 mM Guaiacol, 5 mM hydrogen peroxide and crude extract was prepared and its absorbance at 470 nm was detected during 1 min, using spectrophotometer (BIO-RAD). Enzyme activity was expressed as absorption delta of 470 nm per mg protein (Chance and Maehly, 1995).

Catalase (CAT) enzyme was extracted from 200 mg samples homogenized in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to enzyme extract. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 10 mM hydrogen peroxide and crude extract was prepared and its absorbance at 240 nm was detected using a spectrophotometer (BIO-RAD). Enzyme activity was described by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, as the decrease of absorbance per time per mg of protein (8). Enzyme activity was expressed as absorption delta of 240 nm per mg protein. All steps of enzyme extraction were performed on ice. Cumin essential oil and 8-hydroxyquinoline sulphate (8-HQS) were purchased from Zardband Pharmaceuticals - Medicinal Plants Production Co., Yasuj, Iran and were used.

Statistical analysis

All data were analyzed for significant differences using analysis of variance (ANOVA) using the SAS (Statistical Analysis System) statistical package (SAS Institute, Cary, NC, USA). Data were then subjected to mean separation by the least significant difference test (LSD) at P<0.05.

3. Results

According to results of variance analysis, interaction effects of cumin essential oil (CEO), 8-hydroxyquinoline sulfate (HQS) application and measuring times was significant (P<0.05) on measured traits of vase life, petal fresh/dry weight rate, flower diameter, relative water content (RWC), leakage of ions, catalase enzyme activity (CAT), peroxidase enzyme activity (POD), membrane stability index (MSI) except for anthocyanin content. Interaction effect of HQS and measuring times was insignificant on anthocyanin content too, while main effects of each factor and interaction effects of CEO × HQS and HQS × T were significant (P<0.05) (Table 1).

Table 1 - Analysis of variance for measured traits in cut rose (*Rosa hybrida* cv. Angelina) flowers treated by cumin (*Cuminum cyminum* L.) essential oil and 8-hydroxyquinoline sulfate in different measuring times

S.O.V	DF	Mean squares								
		Vase life	Petal dry weight	Flower diameter	Anthocyanin content	Relative water content	Leakage of ions	CAT	POD	MSI
CEO	2	16.02 *	0.52 *	2.686 *	0.0020 *	17.33 *	37.31*	26.45 *	23.30 *	69.35 *
HQS	3	45.99 *	0.30 *	1.542 *	0.0016 *	48.76 *	44.97*	51.20 *	54.21 *	47.07 *
Time	2	11.33 *	0.78 *	1.033 *	0.0011 *	93.32 *	52.47 NS	21.30 *	23.22 NS	99.33 *
CEO×HQS	6	24.35 *	0.89 *	2.037 *	0.0034 *	58.25 *	41.25 *	35.15 *	35.81 *	49.99 *
CEO×T	4	20.46 *	0.55 *	2.432 *	0.0037 *	48.39 *	39.56 *	28.14 *	38.92 *	58.23 *
HQS×T	6	21.32 *	0.53 *	3.321 *	0.0061 NS	59.41 *	45.81 *	32.18 *	40.25 *	63.28 *
CEO×HQS×T	12	25.41 *	0.24 *	1.421 *	0.0061 NS	39.99 *	21.34 *	45.23 *	39.48 *	48.49 *

*,**, shows significant differences at 5%, 1%, respectively. NS= not significant.

CEO = Cumin essential oil. HQS= 8-hydroxyquinoline sulfate

Concerning the mean comparison, the maximum vase life was obtained by application of 100 mg·L^{-1} cumin essential oil and 600 mg·L^{-1} 8-hydroxyquinoline sulfate. However, the minimum vase life was observed in control treatments (Fig. 1). The greatest petal fresh/dry weight rate was evident in the treatment of 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate and the least with control treatments (Fig. 2). The results indicated that the highest flower diameter was found in 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate and the lowest diameter in control treatments (Fig. 3). Relative water content showed the maximum and minimum value in 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate and control treatment, respectively (Fig. 4). The greatest amount of ions leakage in control treatments and the lowest amount in 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate were found (Fig. 5). According to results of mean comparison, the highest catalase enzyme activity was attained in 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate, while the lowest of that was reported in control treatments (Fig. 6). The greatest peroxidase enzyme activity was observed in 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate and the least activity in control treatments (Fig. 7). According to the obtained results, the highest membrane stability index was obtained in 150 mg·L^{-1} cumin essential oil and 400 mg L^{-1} 8-hydroxyquinoline sulfate (Fig. 8).

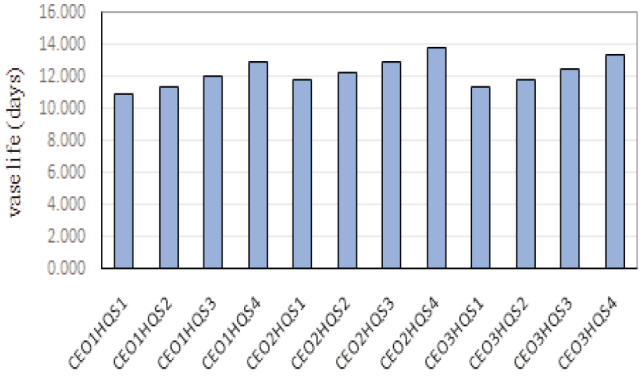

Fig. 1 - Changes of vase life under different levels of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS).

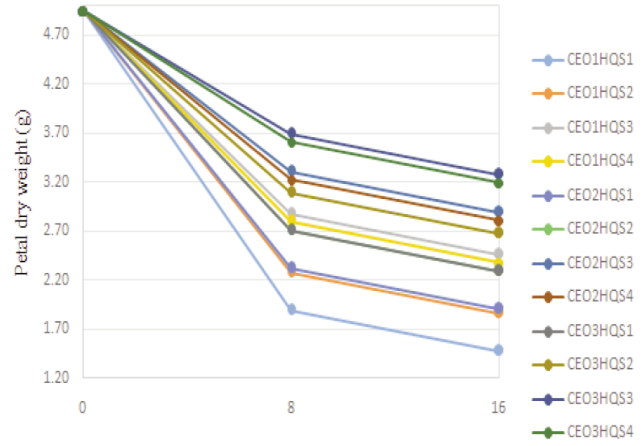

Fig. 2 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on petal dry weight (g) in different measuring times.

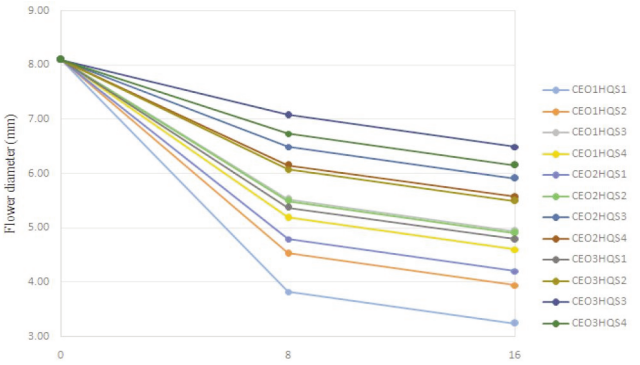

Fig. 3 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on flower diameter (mm) in different measuring times (days).

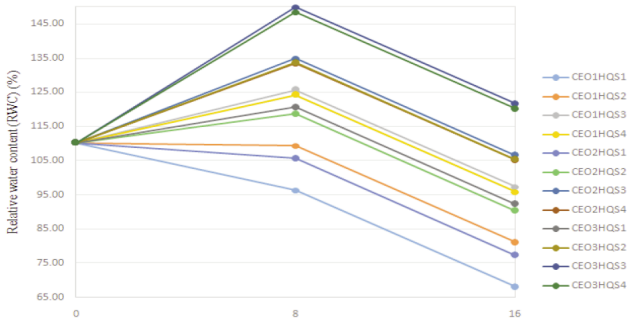

Fig. 4 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on relative water content (RWC) (%) in different measuring times (days).

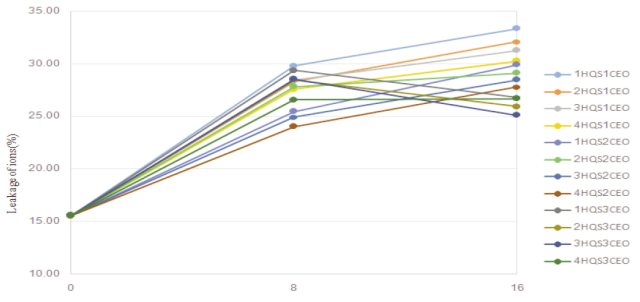

Fig. 5 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on leakage of ions (%) in different measuring times (days).

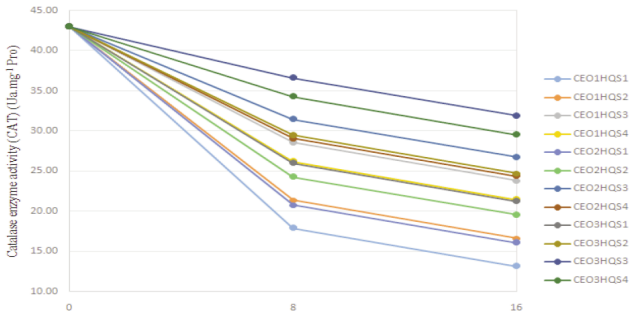

Fig. 6 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on catalase enzyme activity (CAT) (Ua mg^{-1} Pro) in different measuring times (days).

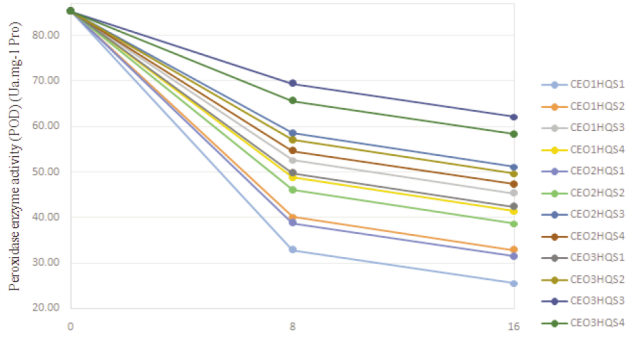

Fig. 7 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on peroxidase enzyme activity (POD) (Ua mg^{-1} Pro) in different measuring times (days).

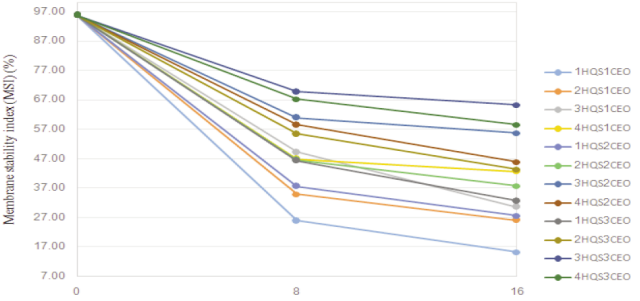

Fig. 8 - Mean comparison for interaction effects of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) different levels on membrane stability index (MSI) (5) in different measuring times (days).

4. Discussion and Conclusions

The results showed that the application of cumin essential oil (CEO) and 8-hydroxyquinoline sulfate (HQS) had positive effect on vase life and postharvest quality of cut rose (*Rosa hybrid* cv. Angelina) flowers in different measuring time. Application of 100 mg·L^{-1} cumin essential oil and 600 mg·L^{-1} 8-hydroxyquinoline sulfate increased vase life of cut rose flowers. This result was in accordance with results of Hussein (1994) and Knee (2000). The application of 8-HQS may prevent the accumulation of microorganisms in xylem vessels and suppressed the xylem occlusion due to its role as anti-microbial agent and hence, it might reduce stem plugging. Essential oils like CEO play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appétit for such plants (Bakkali *et al.*, 2008). Petal fresh/dry weight rate was improved significantly by application of 100 mg·L^{-1} cumin essential oil and 600 mg.L^{-1} 8-hydroxyquinoline sulfate. These results are in line with results of Ali and Hassan (2014) on *strelitzia* cut flowers with application of 8-hydroxyquinoline sulfate and gibberlic acid treatments.

The application of 8-HQS may reduce the plasmolysis of cells which occurred when the rate of cellular water loss is too rapid. The cut rose flowers reached to the highest diameter with application of 100 mg·L^{-1} cumin essential oil and 600 mg·L^{-1} 8-hydroxyquinoline sulfate. These findings are in according to reports of Kim and Lee (2002). HQS not only prevents the vascular obstruction caused by the microorganisms, but also prevents the blockage stimulated by the plant itself. The highest relative water content in cut rose flowers was related to treatment of 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate. These results are similar to Knee (2000) findings on cut carnation flowers. Leakage of ions was occurred in control treatments in the highest amount. Essential oil of cumin mainly conjugated to compounds that have known as phenolic compounds, are responsible for pathogen control in plants (Plotto *et al.*, 2003). These compounds prevent senescence and wilting by their antibacterial property and reducing the pH of the environment (Elgimabi and Ahmed, 2009). Catalase and peroxidase enzymes activities increased significantly by treatments of 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxy quinoline sulfate. These results are consistent with results of Ranjbar *et al.*, 2015. Catalase is an important biological factor with major function in superoxide metabolism and plays an important role in releasing oxygen and hydrogen peroxide free radicals and prevents creation of hydroxyl radicals (Spanou *et al.*, 2012). Peroxidase has different biolog-

ical functions such as detoxification of hydrogen peroxide, lignin biosynthesis, hormonal signaling and response to stress (Gao *et al*., 2010). Maybe the treatment of 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate decreases oxidative stresses in cut rose flowers (Hassan and Ali, 2014). Membrane stability index showed the highest percent in treatment of 150 mg·L^{-1} cumin essential oil and 400 mg·L^{-1} 8-hydroxyquinoline sulfate. These findings are compatible to results of Kazemi and Ameri (2012). They showed the positive effect of herbal essential oils of thyme and lavender on the stability of the membrane and reduction of MDA. The senescence of cut flowers with hormonal regulatory mechanism is involved in changing the physical and biochemical features of cellular membrane (Buchanan Wollaston, 1997). Oxidative membrane injury allows the mixing of the normally separated enzyme (PPO) and oxidizable substrates (polyphenols), which lead to browning (Hodges, 2003). According to Palma *et al*. (2002), the herbal essential oils by preventing the activity of oxygen species reduce the lipid peroxidation in cell membrane and the concentration of MDA. Plant essential oils are bioactive in the vapor phase, and this makes them fumigants for postharvest rotting fungi control in fruits and grains (Paster *et al*., 1995; Hammer *et al*., 1999; Feng and Zheng, 2007). Different studies showed postharvest disease control in different fruit species by using biological agents including essential oils (Bishop and Thompdon, 1997; Feng and Zheng, 2007; Amiri *et al*., 2008).

A limiting factor in cut flower marketing is postharvest senescence. There are many reports used different materials for extending rose cut flower vase life. We studied application of cumin essential oil and 8-hydroxyquinoline sulfate. They had positive effects ($P<0.05$) on vase life and postharvest quality of cut rose (*Rosa hybrida* cv. Angelina) flowers. Results showed they affect some growth and development parameters such as relative fresh weight, flower and stem diameters, anthocyanin and chlorophyll contents as well catalase and peroxidase activities that cause improving vase life of rose cut flowers.

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