

Extending vase life of cut rose (*Rosa hybrida* L.) cv. Bacara by essential oils

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Abstract: Recent studies showed that some essential oils functions as antibacterial compounds. In this study results showed treatment with essential oils promoted vase life of cut roses via decreasing bacteria number inside the stem. We investigated components in the hydrodistilled essential oils of *Bunium persicum* Bioss, *Mentha spicata* L., *Thymus vulgaris* L. and *Satureja hortensis* L., as hold solutions, and their effects on relative fresh weight, water uptake, vase life, electrolyte leakage, anthocyanin content, soluble sugar content and number of bacteria at stem end of cut flowers of rose. GC-MS analysis of the extracted essential oil of *B. persicum*, *M. spicata*, *Th. vulgaris* and *S. hortensis* L. led to the identification of 14, 20, 13 and 14 major compounds, respectively. In cut rose, the treatment containing the essential oils extended flower opening period longer than the control. The 200 $\mu\text{l l}^{-1}$ essential oil of *M. spicata* treatment almost doubled the vase life of cut roses. Hence these essential oils might be powerful, environmentally friendly substitutes for the chemical compounds currently added to vase waters to control bacterial content.



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

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The authors declare no competing interests.

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1. Introduction

Rose (*Rosa hybrida* L.) cut flowers play an important role in the florist trade (Cairns *et al.*, 2000). The cut flowers can have limited marketable value because they dehydrate during vase life as a result of decreased water uptake and the vase life is often very short. The cut flowers wilt and the floral axis becomes bent just under the flower head (bent neck). The appearance of such symptoms is considered to be caused by various factors such as bacteria, physiological responses of stems to cutting and air emboli. The development of this occlusion is correlated with the growth of bacteria at the cut surface and inside the stem (Van Doorn *et al.*, 1989, 1990) and accumulation of bacteria in vase water shortens the vase life of cut rose flowers (De Witte and Van Doorn, 1988). The addition of chemicals, including some trace elements such as silver nitrate, aluminum sulphate and 8-hydroxyquinoline sulphate into holding solutions has been tried with various accomplishments in efforts to prolong the vase life of cut roses (Van Doorn *et al.*, 1990).

The use of essential oils has recently become a common practice

because of strong antimicrobial activities against several pathogens. Some essential oils of plants have been reported to be effective in extending the vase life of gerbera cut flowers (Solgi *et al.*, 2009). These natural organic substances have high levels of phenolic compounds such as carvacrol (Sharififar *et al.*, 2007). Essential oils indicate a wide range of protective properties against disease states, oxidative stress, and microbial infection (Botelho *et al.*, 2007; Yahyazadeh *et al.*, 2008). And have reportedly been used for controlling plant diseases, particularly on fruits (Yahyazadeh *et al.*, 2008; Ramezani *et al.*, 2016). However, there is little available information (Solgi *et al.*, 2009) on the use of essential oils for the control of microbial contaminations and extending the vase life of cut flowers. Appropriate use of chemical fungicides during the postharvest could help minimize fungal infections; however, the use of chemical compounds is being discouraged for economic aims and because of growing concern about environment safety issues (Wagacha and Muthomi, 2008).

In this study, an attempt has been made to investigate the protective role of carvacrol during vase life in 'Bacara' cut-roses. This information will help to elucidate the best concentrations of these essential oils to be used in holding solutions to obtain remarkable beneficial results.

2. Materials and Methods

Extraction, analysis and antioxidant activity of essential oils

The essential oils from fresh leaves and flowers of *Bunium persicum* Bioss, *Mentha spicata* L., *Thymus vulgaris* L. and *Satureja hortensis* L. were extracted following hydro-distillation method. The leaves and flowers (100 g) were hydrodistilled in a clevenger apparatus with 400 ml of water. The extraction was carried out for 3.5 h. The extracted oils were collected from the graduated receiver. For the deletion of water traces from the oil, the extracts were dried over anhydrous Na₂SO₄ and were stored in sealed ampoule bottle in a refrigerator at 4°C until for analysis.

Gas chromatographic analysis was performed with Agilent 6890A with helium as a carrier gas with a linear velocity of 30 cm/s on HP-5 column (30 m × 0.25 mm i.d, 0.25 μm film thickness). The oven was programmed to rise 50°C (3 min) isotherm, and then to 265°C at a rate of 5°C/min. Injector and detector temperatures were 300°C and 265°C, respectively.

The GC mass analysis was carried out on Agilent 5975 equipped with a HP-5 column with the same characteristics as the one used in GC. Unknown essential oil was recognized by comparing its GC retention time to that of known compounds and by comparison of its mass spectra either with identified compounds or available spectra in the literature.

The antioxidant activity of essential oils was determined based on the DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging capacity by the method described by Brand-Williams *et al.* (1995). The amount of 180 μl DPPH reagent and 20 μl samples were mixed and shocked. Decline of absorbance of tested fusions was monitored for 30 min at 517 nm. For each essential oil, the tests were repeated three times.

Plant material and general processing

Rose flowers (*Rosa hybrida* L.) 'Bacara' grown under standard commercial conditions in a glass covered greenhouse were used as plant material. Flowers were harvested at commercial stage to 0.45 m in length. Flower stems were placed in tap water after harvest and transported by non-refrigerated car to the laboratory within 2 h. The flowers were placed randomly in 3 L glass vases containing 1 L of essential oils solutions of 100, 200 and 400 μl.l⁻¹ concentrations for 24 h. Distilled water was used as control. Sucrose (4%, w/v) was added to all solutions and the concentrations of essential oils were prepared in Tween 20 (0.1%). Then, flowers were individually placed in glass bottles of 25 cm height. Each bottle contained approximately 200 ml of distilled water. Bottles were standing in a controlled room under the following conditions: 12 h photoperiod (06.00-18.00) at photosynthetically activated radiation of 12 μmol m⁻² s⁻¹ provided by fluorescent lamps, 25±1°C and relative humidity of 60-70%. In total, nine floin three replications were placed in Bottles.

Measurements

Relative fresh weight was calculated by the formula:

$$\text{RFW (\% initial fresh weight)} = (\text{FW}_t / \text{FW}_{t=0}) \times 100$$

where RFW was Relative fresh weight, FW_t was the fresh weight of flower (g) at t (in day) = 0, 1, 2, 3, etc., and FW_{t=0} was the fresh weight of the same flower (g) at t (in day) = 0 (He *et al.*, 2006).

Total vase solution uptake. Weight of vases containing vase solution without the cut stems were measured during the experiment period and flower opening was measured daily until the flowers

attained their largest size in the vase and began loss their size.

Cut flower longevity was documented as days vase life from the stage flowers were placed into glass bottles till the end of vase life defined as the time that flowers showed indications of petal wilting or curling, fall of one or more petals (Van der Sman *et al.*, 1996).

Electrolyte leakage was measured using an electrical conductivity meter. Leaves were excised and washed with deionized water. After drying with tissue paper, 1 g fresh weight of leaves were cut into small pieces (about 1 cm²) and then immersed in 20 ml deionized water and incubated at 25°C. After 24 h, electrical conductivity (EC₁) and after 48 h (EC₂) of the bathing solution were recorded for the samples (Lutts *et al.*, 1996).

Determination of anthocyanins. Samples of petal (100 mg) were powdered in a pre-chilled pestle and extracted into methanol: HCl (99:1). Samples were incubated overnight at 4°C in darkness. The content of anthocyanins determined spectrophotometrically at 535 nm using an extinction coefficient (ϵ) of 29,600. The final concentration of anthocyanin was calculated based on weight of sample used and total volume of the extract.

For determination of soluble sugars, fresh petals (0.30 g) were put into test tubes with 10 mL distilled water and sealed. The tubes were incubated in a water bath at 90°C for 30 min, then the tubes were removed and the volume set at 25 ml. The amount of 0.5 ml of supernatant was mixed with 1.5 ml distilled water, 5 ml concentrated sulfuric acid and 0.5 ml anthrone. The mixed solution was read at 620 nm (Frohlich and Kutscherah, 1995).

Ascorbate peroxidase activity. The fresh leaves (1 g) were mixed in 100 mM potassium phosphate buffer (pH 7.8) containing 1% (w:v) PVP (polyvinylpyrrolidone), 0.1 mM EDTA (ethylenediaminetetraacetic acid), and 0.5% (v:v) Triton X-100 at 4°C, except that in the case of APX activity leaves were homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 5 mM ascorbate. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 18000 g for 20 min at 4°C. Peroxidase activity was assayed as per the method of Kar and Mishra (1976).

Determination of bacterial numbers. The lowermost 2 cm (about 0.5 g in weight) of the stems were cut. The samples were washed 3 times with sterile deionized water. They were ground and then dilution

was made with a 0.9% normal salt solution. Liquid extract (0.1 ml) was spread on Plate Count Agar (PCA) plates. Before counting of bacteria, PCA plates were incubated at 38°C for 2 days (Balestra *et al.*, 2005).

Statistical analysis

Completely randomized experiment designs were used. Statistical significance between mean values was assessed using analysis of variance (ANOVA) and a conventional Tukey's test at $p \leq 0.05$ using SAS (9.1) statistical software. For data of maximum flower opening, standard deviation (SD) was calculated and data are expressed in mean \pm SD of three replicates.

3. Results and Discussion

GC-MS analysis of the extracted essential oil of *B. persicum*, *M. spicata*, *Th. vulgaris* and *S. hortensis* L. led to the identification of 14, 20, 13 and 14 major compounds, respectively (Table 1). The results revealed that 5 major components of essential oil from *B. persicum* contained γ -Terpinene, β -Caryophyllene, Cumyl acetate, p-Cymene and β -Pinene. Investigation is limited about the chemical composition of the *B. persicum* essential oil. It has been reported that γ -Terpinene and β -Caryophyllene are main components (Shahsavari *et al.*, 2008). Five major components of *Mentha spicata* L. essential oil were Carvone, 1,8-Cineole, Borneol, Limonene and Pulegone. *Mentha* essential oil was characterized by the dominant presence of carvone (56.9%) in agreement with results from other authors, who reported the presence of 76.65% in samples collected from India (Chauhan *et al.*, 2009) and 64.4% in samples collected in Montenegro (Scherer *et al.*, 2013). Carvacrol (67.3%), Thymol (12.7%), α -Pinene (4.25%), γ -Terpinene (3.53%) and Eucalyptol (3.32%) were most abundant in essential oil of *Th. vulgaris*. Similar results have been obtained by Ben El Hadj Ali *et al.*, (2014) with *Th. numidicus*. The principle compounds identified in essential oil of *Satureja hortensis* L. were Carvacrol (66.4%), p-Cymene (18.1%), Linalool (4.5%), γ -Terpinene (3.95%) and Borneol (1.8%). A comparison of our study with a previous report (Ghasemi-Pirbalouti *et al.*, 2014) suggests that the few differences in the volatile composition of the plant material could be attributed to a series of factors such as the genotype, plant developmental stage, environmental conditions and the methods of extraction.

All assayed essential oils were able to reduce

Table 1 - Major natural volatile components in the hydrodistilled essential oils of *B. persicum*, *M. spicata*, *Th. vulgaris* and *S. hortensis*

No.	Component	Retention indices	Percentage (%)			
			<i>B. persicum</i>	<i>M. spicata</i>	<i>Th. vulgaris</i>	<i>S. hortensis</i>
1	α -Thujene	924	0.05	0.03	0.18	0.3
2	α -Pinene	932	1.75	1.09	4.25	0.5
3	Camphene	946	-	0.56	-	-
4	Sabinene	971	0.8	0.74	-	-
5	β -Pinene	977	3.68	1.59	0.44	0.1
6	Myrcene	989	0.71	0.41	0.86	0.6
7	3-Octanol	922	-	0.21	-	0.1
8	Eucalyptol	1018	-	-	3.32	-
9	p-Cymene	1021	6.91	0.83	-	18.1
10	Limonene	1036	3.28	5.69	0.44	-
11	1,8-Cineole	1039	0.1	13.53	0.2	-
12	γ -Terpinene	1059	27.61	0.58	3.53	3.95
13	Linalool	1086	1.1	-	0.71	4.5
14	Menthone	1154	-	0.26	-	-
15	Borneol	1159	-	8.15	-	1.8
16	α -Terpineol	1169	0.98	3.25	0.54	1.02
17	Pulegone	1231	0.2	3.28	-	-
18	Carvone	1237	-	56.94	-	-
19	Thymol	1287	3.4	0.67	12.79	0.3
20	Carvacrol	1293	0.35	0.47	67.36	66.46
21	β -Caryophyllene	1415	25.1	0.93	-	0.85
22	Cuminyl acetate	1434	16.68	-	-	-
23	Caryophyllene oxide	1580	0.47	0.16	1.23	1.2

DPPH, reaching 50% of reduction with IC₅₀ values ranging 54.19±0.87 mg/mL for *M. spicata*, to 258.16 ± 1.53 mg/mL for *S. hortensis* (Table 2). The variance analysis performed on the DPPH scavenging activity of the essential oils showed significant differences among species ($p \leq 0.05$) and that the essential oil of *M. spicata* was the most potent of all the oils. Therefore, some compounds such as compound Carvone, 1,8-Cineole and Borneol were responsible for the DPPH scavenging effects of *M. spicata* (Mahdavia and Saharkhiz, 2015). The essential oil of *Th. vulgaris* possessed slightly higher DPPH scavenging effects than that of *B. persicum* and *S. hortensis*. This meant that the common compound Thymol and high value of Carvacrol played a leading role (Ghasemi-Pirbalouti and Dadfar, 2013).

Bacterial numbers

Essential oils treatment, particularly 200, 400 μ l l⁻¹ *M. spicata* and 200, 400 μ l.l⁻¹ *Th. vulgaris*, had negative effect on bacterial numbers. Preventing bacterial division in vase water can reduce the occurrence of stem bending (Solgi *et al.*, 2009), suggesting that bacteria are a main cause. A positive correlation between the number of bacteria and water uptake of the flower stem have been reported (Van Doorn *et al.*, 1989). Vascular occlusions in cut rose flower usually develop when the number of bacteria in vase water reach 7-11 Log₁₀ CFU/ml (Van Doorn *et al.*, 1990). Some antimicrobial compounds have been used in tests with cut flowers. These compounds included silver nitrate (Nair *et al.*, 2003), nano-Silver (Nazemi Rafi and Ramezani, 2013), 8-hydrox-

Table 2 - Antioxidant activity of essential oil extracts of *B. persicum*, *M. spicata*, *Th. vulgaris* and *S. hortensis*

DPPH	<i>B. persicum</i>	<i>M. spicata</i>	<i>Th. vulgaris</i>	<i>S. hortensis</i>
IC ₅₀ (mg/mL)	183.5 ± 2.15 b	54.19 ± 0.87 d	153.52 ± 2.66 c	258.16 ± 1.53 a

Mean separation by Tukey Test, $P \leq 0.05$.

yquinoline citrate (Solgi *et al.*, 2009), chlorine bleach, dichloroisocyanuric acid (Jones and Hill, 1993) and essential oils such as carvacrol (Nazemi Rafi and Ramezani, 2013) and thymol (Solgi *et al.*, 2009). The inhibitory effect of some essential oils on bacterial growth has been attributed to alcohols, esters, aldehydes, phenols, carvacrol, thymol, and eugenol (Bassole and Juliani, 2012). Burt (2004) reported that essential oil components including thymol and carvacrol partition in the lipids of the cell membrane, rendering the membrane permeable and leading to leakage of cell contents, thus exerting their antibacterial action.

Relative fresh weight and total water uptake

As expected, relative fresh weight continuously increased early days of experiment (Table 3). Nevertheless, such increases were significantly prolonged by using essential oils in hold solution. The relative fresh weight in 200 $\mu\text{l.l}^{-1}$ *M. spicata* essential oils treated cut flowers was initially about 105.2% (day 1), and increased to 130.1% over 6 days of holding, but in untreated (control) cut flowers, relative fresh weight was initially about 111.3% (day 1), and decreased over 4 days of holding to 105%. Similarly, as with different concentrations of *M. spicata* essential oil, a positive effect of other essential oils on relative fresh weight was confirmed by their impact on duration of increase and percentage of fresh weight. Total water uptake of cut flowers under various treatments from day 1 to 8 in control as well as in all

treated samples (Table 3). The maximum uptake of vase solution was found 1.14-fold increase in the treated flower with 200 $\mu\text{l.l}^{-1}$ *Th. vulgaris* as compared to untreated (control) cut flowers. All treated flowers always took up water more than the control.

Water status is a factor directly correlated with the vase life of rose cut flower. This factor is determined by the balance between water uptake and loss due to transpiration (Fanourakis *et al.*, 2016). Water uptake depends on variation of cultivars (Fanourakis *et al.*, 2016), the viscosity of the vase solution, vascular conductivity and the osmosis gradient between vase solution and stem solute (Alaey *et al.*, 2011). The improved water uptake in this study may be due to possible antibacterial activity of essential oils by inhibiting vascular blockage and/or increasing osmosis gradient. The water lose was affected by treatments and essential oils increased water retaining capacity compared to control treatment (without essential oil). It may be due to positive regulatory role of essential oils on stomatal closure which regulates the rates of transpiration and decreases the water loss of leaves and petals (Solgi *et al.*, 2009).

Flower opening

Generally, the opening of cut flowers kept in essential oils vase solutions was more than those flowers kept in distilled water (control). Also maximum opening of control flowers occurred earlier. Time and diameters of maximum opening flower were different between treatments. However, maxi-

Table 3 - Vase life, total solution uptake, electrolyte leakage, petal anthocyanin content, soluble sugars of petal and bacterial numbers of cut rose (*Rosa hybrida* L.) cv. Bacara treated with *B. persicum*, *M. spicata*, *Th. vulgaris* and *S. hortensis*

Characteristic	Vase life (days)	Total solution uptake (g)	Electrolyte leakage (%)	Petal anthocyanins content (mg g^{-1} FW)	Soluble sugars of petal (mg g^{-1} FW)	Ascorbate peroxidase activity ($\text{Units min}^{-1} \text{mg}^{-1}$ protein)	Bacterial numbers (Log_{10} CFU/ml)	Relative FW in 10 th day (%)
Control	6.70 e	98.350 f	60.6 a	5.24 d	1.41 f	22.6 h	8.2 a	79.41 g
<i>B. persicum</i> 100 $\mu\text{l.l}^{-1}$	8.70 bc	103.25 e	51.4 b	6.52 bc	1.70 f	25.1 g	8.1 a	94.72 cd
<i>B. persicum</i> 200 $\mu\text{l.l}^{-1}$	9.80 b	106.44 b	48.5 bc	7.37 ab	2.29 e	24.6 gh	7.8 ab	100.80 ab
<i>B. persicum</i> 400 $\mu\text{l.l}^{-1}$	7.90 cd	100.23 e	45.7 c	6.07 c	2.14 e	22.9 h	7.3 d	84.22 f
<i>M. spicata</i> 100 $\mu\text{l.l}^{-1}$	11.2 a	110.31 a	19.8 i	7.95 a	4.52 b	54.9 a	6.1 f	104.50 a
<i>M. spicata</i> 200 $\mu\text{l.l}^{-1}$	10.4 ab	108.65 ab	28.3 g	7.68 a	4.05 bc	46.7 b	5.7 g	95.00 c
<i>M. spicata</i> 400 $\mu\text{l.l}^{-1}$	8.70 bc	106.71 b	33.1 f	6.03 c	3.77 c	43.3 bc	5.5 gh	91.48 de
<i>Th. Vulgaris</i> 100 $\mu\text{l.l}^{-1}$	8.30 c	105.94 c	22.4 h	7.19 b	4.35 b	38.6 d	7.5 cd	91.71 de
<i>Th. Vulgaris</i> 200 $\mu\text{l.l}^{-1}$	10.6 ab	112.67 a	17.4 j	7.70 a	5.18 a	40.8 cd	5.5 gh	104.50 a
<i>Th. Vulgaris</i> 400 $\mu\text{l.l}^{-1}$	9.20 b	107.36 b	32.0 f	7.36 ab	3.95 c	41.0 c	5.3 h	98.54 bc
<i>S. hortensis</i> 100 $\mu\text{l.l}^{-1}$	7.80 d	98.670 f	41.5 d	5.83 c	2.35 e	33.7 ef	7.8 bc	83.54 fg
<i>S. hortensis</i> 200 $\mu\text{l.l}^{-1}$	8.60 bc	103.68 de	38.5 e	6.44 bc	3.28 d	32.1 f	7.2 d	87.83 ef
<i>S. hortensis</i> 400 $\mu\text{l.l}^{-1}$	9.10 bc	104.86 d	37.5 e	6.87 b	3.46 cd	35.9 e	6.8 e	97.87 bc

Mean separation for each parameter within rows by Tukey Test, $P \leq 0.05$.

mum diameter was observed with flowers kept in 100 $\mu\text{l.l}^{-1}$ *M. spicata* essential oil vase solution (Fig. 1). Mechanisms of flower opening vary for different flowers and are sensitive to various environmental conditions such as temperature, light, carbohydrate supply (Kumar *et al.*, 2008) and water relations. During the opening, numerous proceedings take place in a well-defined sequence, representing all parts of plant development, such as division, differentiation and elongation of cell (Kumar *et al.*, 2008) or gene expression (Hoeberichts *et al.*, 2005). In an experiment, essential oils at some concentrations had a noticeable effect on promoting flower opening of cut rose flowers. However, the treatments with a relatively higher concentration of essential oils showed a less effect on flower opening. According to Mahdavia and Saharkhiz (2015) the function of essential oils as an effective antioxidant mainly depends on its concentration. It has been demonstrated that a relatively high dose of essential oils may be associated with injury of membranes and nucleic acids and cell death (Ghasemi-Pirbalouti and Dadfar, 2013). The results suggest that essential oils at effective concentrations maintained water uptake and inhibited vascular occlusions development. Thus, essential oils may improve water relations and supplementation of carbon sources, leading to cell expansion and flower opening.

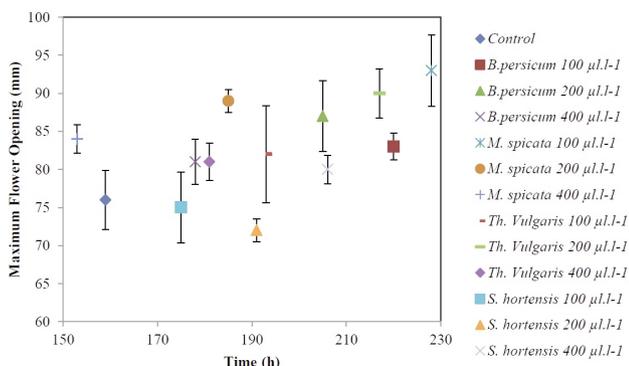


Fig. 1 - Time of maximum flower opening. Values are given as mean \pm SD of three replicates.

Petal anthocyanin content

Petal anthocyanin content in cut flowers was determined. Cut flowers treated with 100 $\mu\text{l.l}^{-1}$ *M. spicata* essential oil in hold solution were observed to accumulate the highest rates of anthocyanin in the petals. Other treatments led to higher amounts of anthocyanins compared to untreated (control) cut flowers. Generally, all cut flowers treated with essential oils showed a similar tendency to maintain anthocyanin Petal, however, essential oils of *Th. vulgaris*

and *M. spicata* were more efficient than *B. persicum* and *S. hortensis*.

One of plant defense system is the presence of endogenous antioxidative compounds such as anthocyanins (Apel and Hirt, 2004). Singlet oxygen produced can be detoxified by anthocyanins and affect the vase life. Accumulation of these pigments in cell vacuoles, their hue and intensity depend on external conditions. Anthocyanin biosynthesis is an essential part of flower development (Dela *et al.*, 2003). In the primary developmental phases of the flower, the anther produces a signal or GA, which promotes petal pigmentation (Kumar *et al.*, 2008). So, in this study, flower opening and development could be effect on anthocyanins accumulation. Additionally, earlier studies have indicated that sugars such as sucrose are required as substrates for anthocyanin biosynthesis (Nagira and Ozeki, 2004). Here, we showed that essential oils increased petal soluble sugar content. It seems that there is an interaction between the soluble sugars concentration and anthocyanins.

Ascorbate peroxidase activity

With cut roses, the highest ascorbate peroxidase activity in petals occurred in those treated with *M. spicata* essential oils at the concentration of 100 $\mu\text{l.l}^{-1}$. As shown in Table 3, the activity of ascorbate peroxidase in control flowers was less than that of the flowers treated with essential oils, however, there were no significant difference between control flowers and flowers treated with different concentrations of *B. persicum* essential oils. Ascorbate peroxidase enzyme is considered to play significant roles in cellular defense against stresses (Sevillano *et al.*, 2009). During the later stages of vase life, petals contain higher levels of reactive oxygen species leading to oxidative damage (Hossain *et al.*, 2006). Generally, treatment with essential oils increased ascorbate peroxidase activity compared the control. Higher levels of the enzymatic activity in treated flowers were likely to counteract the oxidative stress and to scavenge the active oxygen species alleviate to the oxidative stress induced in cut roses and thus delayed flower senescence. These results are in agreement with the findings of Hasan *et al.*, (2014) who mentioned that cut roses were of an efficient antioxidant defense system.

Electrolyte leakage

The ability to increase membrane stability is a characteristic of drought tolerance strategy (Turner, 1986). With control flowers, a significant increase in

electrolyte leakage was detected; however, treatment with any essential oils level significantly inhibited electrolyte leakage increase relative to the control (Table 3). The treatment with 200 $\mu\text{l l}^{-1}$ *Th. vulgaris* essential oils resulted in the lowest electrolyte leakage followed by 100 $\mu\text{l l}^{-1}$ *M. spicata* essential oils. Wilting in cut flower is often accompanied by membrane damage resulting in the leakage of solutes (Ye et al., 2000). With studied cut flower, such as day lily (Panavas et al., 1998), the most changes in the rate of electrolyte leakage were observed during the late stage of vase life. Maintenance of membrane stability in response to essential oils application was most likely due to induced reduction of lipid peroxidation. This is supported by a lower level of electrolyte leakage in essential oil treatments.

Soluble sugars

Another factor controlling vase life of cut rose flower is sugar content, as the carbon supply is cut (Halevy and Mayak, 1979). The essential oils had a strong influence on the soluble sugars concentration in the petal of cut flowers and all treatments led to significantly more soluble sugars. Soluble sugars increased approximately 3.6 fold from 1.41 mg g⁻¹ FW for the control to 5.18 mg g⁻¹ FW for pulsing flowers in solutions containing essential oil of *Th. vulgaris* (200 $\mu\text{l l}^{-1}$) and to 4.52 mg g⁻¹ FW using 100 $\mu\text{l l}^{-1}$ *M. spicata* essential oil. With cut flowers treated with preservative solution, minimum soluble sugar contents were observed in flowers kept in solutions containing 100 $\mu\text{l l}^{-1}$ *B. persicum* essential oils. It seems that essential oils can change the capacity for sugar uptake in petals and stimulate active sucrose uptake. Translocation of soluble sugars is considered as a key factor affecting the vase life (Khayat and Zieslin, 1989). The addition of chemicals including some compounds such as silver nitrate, aluminum sulphate and 8-hydroxyquinoline sulphate to holding solutions has been tried with varied success in efforts to control vascular blockage and prolong the vase life of cut roses (Van Doorn et al., 1990). However, there is no available evidence on the use of essential oils for control of microbial contaminations and holding soluble sugars petal of cut flowers such as rose.

Vase life

Cut flower wilting is a widely reported problem during vase life of rose, particularly when xylem vessels are blocked by microorganisms (Damunupola and Joyce, 2008). In this study, longevity of treated cut flowers was significantly improved compared with the untreated (control) cut flower. Also, signifi-

cant differences were found in longevity conferred by the essential oils of species and concentrations. However, *B. persicum*, *M. spicata* and *Th. vulgaris* decreased vase life at concentrations more than 200 $\mu\text{l l}^{-1}$, 100 $\mu\text{l l}^{-1}$ and 200 $\mu\text{l l}^{-1}$, respectively. The treatment with *M. spicata* 200 $\mu\text{l l}^{-1}$ essential oil almost doubled the vase life of cut roses. Proper doses of some essential oils at proper dose could delay senescence in selected fruits (Ramezani et al., 2016) and cut flowers (Solgi et al., 2009). For example, Solgi et al. (2009) concluded that the vase life of gerbera flowers was extended by the 50 or 100 mg l⁻¹ carvacrol from 8.3 to 16 days. In the present study, similar results were obtained with the vase life of cut rose.

4. Conclusions

The naturally occurring compounds in essential oils, such as thymol, carvone, carvacrol and menthol showed different levels of antibacterial activity. The major qualitative trait of cut roses is their vase life, which mainly depends on the water uptake of the stems after harvest and is hampered by the presence of bacteria. Also, at relatively high concentration of the essential oils tested, early flower opening and senescence were related to an effect other than bacteria. This effect was correlated with low relative fresh weight and water uptake. It cannot be excluded at present that this early senescence might be due to a toxic effect. More information regarding the action of essential oils during vase life is required for better understanding of the mechanism of petal senescence of rose.

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