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Response of Vase-life Carnation Cut Flower to Salicylic Acid, Silver Nanoparticles, Glutamine and Essential Oil

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ABSTRACT

The aim of this study was to evaluate the efficacy of silver nanoparticles (SNP), Salicylic acid, Glutamine and essential oils as novel antimicrobial agents in extending the vase-life of carnation (*Dianthus caryophyllus*) flowers. Two separate sets of experiments were conducted in a completely randomized design. In the first trial were applied salicylic acid (1, 1.5 and 2 mM), glutamine (50, 100 and 150 mg L^{-1}) and silver nanoparticles (5, 10 and 15 mg L^{-1}) on carnation cut flowers. The second set included investigating the effect of adding three concentrations of 50, 100 and 150 mg L^{-1} of Lavender essential oil or Thyme essential oil, two concentrations of 12.5 or 17.5 mg L^{-1} silver nanoparticles and two concentrations of 2.5 or 5 mM salicylic acid into preservative solutions containing 200 mg L^{-1} of glutamine. In both experiments distilled water was used as a control. The results of these experiments indicated that both application methods increased vase-life of carnation. In the first trial, the results showed that all salicylic acid, glutamine and silver nanoparticles different concentrations prolonged carnation vase-life, while decreasing malondialdehyde (MDA) content, accumulation of bacteria in vase solution, ACC-oxidase activity and membrane permeability together with total delay of senescence and per oxidation of lipids. In the second trial, all essential oils prolonged carnation vase-life. Lavender essential oil or Thyme essential oil (150 mg L^{-1}), salicylic acid (2.5 mM) and silver nanoparticles (17.5 mg L^{-1}) showed the highest effect (increasing 8.5 days) in comparison to the control.

Key words: Carnation, salicylic acid, essential oils, silver nanoparticles, vase life

INTRODUCTION

Flowers are highly perishable; cut flowers are sensitive to microbial contamination in the vase solution, ethylene effects and water stress, shortening their vase-life (Van Doorn and De Witte, 1997; Sankat and Mujaffar, 1994; Han and Miller, 2003). Ethylene promoted flower senescence (Halevy and Mayak, 1979; Da Silva, 2003), increased respiratory activity and loss of cell membrane fluidity (Halevy and Mayak, 1979). The other consequences include increase in production of oxygen free radicals which is responsible for stress dependent per oxidation of membrane lipids (Kazemi *et al*., 2011a). When the vessels of stems blocked, continuing of the water uptake and transpiration by the leaves of cut flowers caused net loss of water of flower and stem tissue (Hassan, 2005; Kazemi *et al*., 2010; Shanan *et al*., 2010). Zagory and Reid (1986) found that microbial contamination at the stem base or in the vase solution produced ethylene. Ethylene gas, a senescence accelerating agent, with vascular blockage which is caused by accumulation of bacteria in stem base or vase solution reduces vase-life of carnation cut flowers

(Van Doorn *et al*., 1994). Addition of chemical preservatives control microbial activity in the holding solution (Nowak and Rudniki, 1990). One of the preserving agents is SA that inhibits ethylene synthesis and reduces sensitivity of flowers to ethylene (Mahdavian *et al*., 2007; Mba *et al*., 2007; Karlidag *et al*., 2009; Kazemi *et al*., 2011a-d). Application of SA at different concentrations also extended vase-life in *Gerbera* (Kazemi *et al*., 2011a) and lily cut flowers (Kazemi *et al*., 2011b). Essential oils and their derivatives are environmentally friendly and have strong antimicrobial properties. These antibacterial properties are attributed to the high levels of phenolic compounds such as carvacrol, thymol and eugenol (Lambert *et al*., 2001; Solgi *et al*., 2009; Mihajilov-Krstev *et al*., 2010). Solgi *et al*. (2009) and Morones *et al*. (2005) found that the treatment of silver nanoparticles (SNP) reduced bacterial growth in the vase solution during the postharvest period. Glutamine is readily metabolized by plants and cells can metabolize glutamine for energy, so we considered using it as a possible substitute for sucrose (Kazemi *et al*., 2011b). Therefore, the aim of this study was to evaluate the effects of SA, silver nanoparticles, glutamine, Lavender essential oil, Thyme essential oil on extending *Dianthus caryophyllus* cv. 'pink' cut flower vase-life.

MATERIALS AND METHODS

Cut flowers (*Dianthus caryophyllus* L. cv. pink) were harvested before blooming in the morning from a local commercial greenhouse (Pakdasht, Tehran, Iran) and transported with appropriate covers (in plastic packages) immediately to Laboratory. Stems were trimmed to 40 cm length and placed in the glass vials containing 350 mL of the test solutions. Then the flowers were kept in a controlled room under the following conditions: 12 h photoperiod at a photo synthetically activated radiation of 850 lux, provided by fluorescent lamps, constant temperature of 19±2°C and relative humidity of 60±10%. Two separate sets of experiments were conducted in a completely randomized design. In the first trial, SA (1, 1.5 and 2 mM), glutamine (50,100 and 150 mg L^{-1}) and SNP (5, 10 and 15 mg L^{-1}) were applied on carnation cut flowers. The second set included investigating the effect of adding three concentrations of 50, 100 and 150 mg L^{-1} of Lavender essential oil or Thyme essential oil and two concentrations of 12.5 or 17.5 mg L^{-1} SNP and two concentrations of 2.5 or 5 mM SA into preservative solutions containing 200 mg L^{-1} of GLU. In both experiments distilled water was used as a control. Each of the treatments was comprised four flowers and repeated four times. Statistical analysis of recorded data was accomplished using Analysis of Variance (ANOVA) using SPSS statistical software (Version 16, IBM Inc.). The mean values were then compared by least significant differences test at $p \leq 0.05$.

Fresh weight and solution uptake: Fresh weight and solution uptake changes were expressed as relative fresh weight (g g^{-1} initial fresh weight day^{-1}) and relative solution uptake (mL g^{-1} initial fresh weight day^{-1}) over the first five days of the experiments (Van Meeteren, 1978; Pompodakis *et al*., 2004).

Vase life: The end of vase-life was defined as the time that flowers showed symptoms of wilting, loss and discoloration of the petals, whereas the marketability of cut flowers were determined by salability and consumer acceptance (Bayat *et al*., 2011).

Chlorophyll index: Chlorophyll index was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan), which is presented by SPAD value. Average of 3 measurements from different spots of a single leave was considered.

Determination of anthocyanin leakage: Anthocyanin leakage was measured based on the method of Poovaiah (1979). Petal samples were cut into 1*1 cm segments and placed in individual tubes containing 25 mL of deionized water. After two washes with distilled water to remove surface contamination, 10 mL of distilled water was added to samples. After 12 h incubation at 25°C the anthocyanin leakage to liquid was determined at 525 nm using spectrophotometer (Perkin-Elmer-EZ-201).

Determination of ACC-oxidase activity: The extraction and quantification was conducted based on the method described by of Moya-Leon *et al.* (2004) with slight modifications. One gram petal tissue was homogenized by a mortar and pestle with 3 mL extraction buffer consisting of 1% (w/v) polyvinyl polypyrrolidone, 0.1 mM Tricine with (pH adjusted to 7.5), 10% glycerol and 5 mM DTT and 30 mM sodium ascorbate for 2 min. The homogenate was centrifuged at 20000 xg for 20 min and the supernatant was collected for enzyme assays. All procedures were conducted at 4°C.

Determination of ACC oxidase activity: After a 20 min incubation of the enzyme extract with the ACC containing enzyme activation complex [0.1 M Tricine (pH 7.5), 30 mM sodium ascorbate, 0.1 mM ferrous sulfate, 10% (v/v) glycerol, 1 mM ACC, 2.5 mM DTT and 30 mM sodium bicarbonate], the ACO activity was assayed as the amount of evolved ethylene, which was quantified on a GC apparatus.

Assays of MDA content (lipid per oxidation): Malondialdehyde content was measured based on the method of Heath and Packer (1968) with some changes. Fresh petals tissue was homogenized with a mortar and pestle in 5 mL solution of 1% trichloroacetic acid. The homogenate was centrifuged at 10000×g for 5 min. 4.5 mL TCA 20 % solution containing 0.5% TBA acid was added to 1 mL of the supernatant and incubated for 30 min at 95°C water bath. The mixture was cooled immediately in ice and again centrifuged at 10000 xg for 10 min. The absorption was measured with a spectrophotometer at 532 nm.

Microbe population: In the last day of vase life, samples were isolated from vase solutions of carnations in sterile containers. Aliquots of the vase solutions were diluted 100-times and 25 μL aliquots of the diluted solution were spread on sterile nutrient agar in sterile petri plates. The plates were allowed to incubate for 48 h at room temperature and individual colonies of bacteria were counted.

RESULTS AND DISCUSSION

Significant differences ($p \leq 0.05$) were found among various concentrations of SA in extending the vase life and relative fresh weight of carnation flowers (*D. caryophyllus* L.). The longest vase-life and Relative fresh weight was obtained with 2 mM SA (Table 1). Flowers held in 10 or 15 mg L^{-1} SNP had relative fresh weight and longer vase-lives than control. There was no significant difference between vase-lives of these treatments and the best concentration was 15 mg L^{-1} SNP. Application of 50, 100 or 150 mg L^{-1} GLU had positive effects in relative fresh weight and extending vase-life. Interaction SA×GLU×SNP had a significant effect on vase-life. Flowers held in 2 mM SA×150 mg L^{-1} GLU×15 mg L^{-1} SNP had relative fresh weight and longer vase-lives than control. Results of this study showed that application of EOs (Lavender essential oil and Thyme essential oil), significantly increased vase-life of carnation cut flowers as compared

Table 1: Effect of different concentrations of salicylic acid, NSP and glutamine on vase-life and relative fresh weight[a] of carnation flowers

Treatment	Vase-life (days)	Relative fresh weight[b]
SA 1 mM	7.1[d]	1.061[b]
SA 1.5 mM	8[c]	1.034[b]
SA 2 mM	11.8[b]	1[a]
GLU 50 mg L^{-1}	8.1[c]	1.047[b]
GLU 100 mg L^{-1}	10.4[c]	1.035[b]
GLU 150 mg L^{-1}	11.05[b]	1.011[a]
SNP 5 mg L^{-1}	8[c]	1.014[b]
SNP 10 mg L^{-1}	9.9[c]	1.014[b]
SNP 15 mg L -1	10.8[b]	1[a]
SA 2 mM+GLU 150 mg L^{-1}+SNP 15 mg L^{-1}	15.4[a]	0.987[a]
Control	6.3[e]	2.014[c]

Values are the mean of three replication, Mean separation among treatments was done by Tukey test values with different alphabets are significantly different at $p \leq 0.05$. [a]g g^{-1} initial fresh weight day^{-1}, [b]Means of first five days of vase-life for all treatments

Table 2: Effect of different concentrations of essential oils, SNP and SA on vase-life, relative solution uptake and relative fresh weight of carnation flowers

Treatment	Vase-life (days)	Relative solution uptake[a]	Relative fresh weight[b]
Control	7.3[e]	1.162[ab]	1.2[c]
Lavender oil 50 mg L^{-1}	9.1[c]	1.050[b]	1.05[b]
Lavender oil 100 mg L^{-1}	11.4[b]	1.074[c]	1.046[b]
Lavender oil 150 mg L^{-1}	16.4[a]	1[a]	1.021[a]
Thyme oil 50 mg L^{-1}	10[b]	1.081[c]	1.061[b]
Thyme oil 100 mg L^{-1}	12[b]	1.055[b]	1.051[b]
Thyme oil 150 mg L^{-1}	16.2[a]	1.019[a]	1.02[a]
SNP 12.5 mg L^{-1}	10.7[b]	1.054[b]	1.045[b]
SNP 17.5 mg L^{-1}	16[a]	1.012[a]	1.021[a]
SA 2.5 mM	16[a]	1[a]	1.026[a]
SA 5 mM	8.5[c]	1.064[c]	1.054[b]

Values are the mean of four replication, Mean separation among treatments was done by Tukey test values with different alphabets are significantly different at $p \leq 0.05$, [a]mL g^{-1} initial fresh weight day^{-1} during first five days of vase-life, [b]g g^{-1} initial fresh weight day^{-1} during first five days of vase-life

to the control ($p \leq 0.05$). The effect of 150 mg L^{-1} Lavender essential oil or 150 mg L^{-1} Thyme essential oil as continuous in relative fresh weight and extending vase-life was higher than other vase solutions, the difference was statistically significant. Addition of Lavender essential oil, Thyme essential oil and SNP and SA used in the preservative solutions had a significant ($p \leq 0.05$) effect on vase-life, relative fresh weight and relative solution uptake (Table 2). Except for treatments containing 5 mM SA, all other treatments led to significantly longer vase-life. Vase-life was improved from 7.3 days for the control to 16.4 and 16.2 days after keeping flowers in solutions containing Lavender oil and Thyme oil (150 mg L^{-1}) respectively and to 16 day using 17.5 mg L^{-1} of SNP and 2.5 mM SA. All the treatments except 5 mM SA, led to more relative fresh weight than controls ($p \leq 0.05$) (Table 2). Results of these experiments showed that longer vase-life, relative fresh weight and relative solution uptake could be obtained by using solutions containing Lavender oil or Thyme oil (150 mg L^{-1}) and 17.5 mg L^{-1} of SNP and or 2.5 mM SA. The present results are in agreement with those reported by Solgi *et al.* (2009) who used SNP and EOs to prolong the vase-life of *Gerbera* cut flowers. Similarity, Bayat *et al.* (2011) showed that treatment with EOs significantly

Table 3: Effect of different concentrations of salicylic acid, NSP and glutamine on anthocyanin leakage, ACC-oxidase activity, MDA content and colony count

Treatment	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h^{-1} mL^{-1})	Anthocyanin leakage (absorption at 525 nm)	MDA (μmol mg^{-1} protein)	Colony count (CFU mL^{-1})
SA 1 mM	1.2^{c}	200.187^{c}	321.145^{abc}	205.731^{c}	400^{ac}
SA 1.5 mM	2.32^{b}	167.352^{ab}	125.365^{b}	146.239^{b}	192^{b}
SA 2 mM	4.82^{a}	100.245^{a}	110.371^{a}	129.004^{a}	120^{a}
GLU 50 mg L^{-1}	2.41^{b}	174.361^{ab}	160.327^{c}	168.987^{ab}	280^{c}
GLU 100 mg L^{-1}	2.21^{b}	170.369^{ab}	145.365^{ab}	152.364^{b}	264^{ab}
GLU 150 mg L^{-1}	4.8^{a}	121.047^{b}	120.147^{b}	130.784^{a}	180^{b}
SNP 5 mg L^{-1}	2.01^{b}	154.236^{c}	160.365^{c}	150.278^{b}	240^{c}
SNP 10 mg L^{-1}	2.87^{b}	147.691^{c}	164.54^{c}	145.398^{b}	180^{b}
SNP 15 mg L^{-1}	2.94^{b}	111.023^{a}	111.0547^{a}	130.421^{a}	120^{a}
SA 2 mM+GLU 150 mg L^{-1} +SNP 15 mg L^{-1}	4.92^{a}	101.034^{a}	123.451^{b}	132.087^{a}	168^{b}
Control	1.33^{c}	235.147^{c}	325.14^{abc}	217.35^{c}	532^{ac}

Values are the mean of four replication, Mean separation among treatments was done by Tukey test, values with different alphabets are significantly different at $p \leq 0.05$

Table 4: Effect of different concentrations of essential oils, SNP and on anthocyanin leakage, ACC-oxidase activity, MDA content and colony count

Treatment	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h^{-1} mL^{-1})	Anthocyanin leakage (absorption at 525 nm)	MDA (μmol mg^{-1} protein)	Colony count (CFU mL^{-1})
Control	1.88^{c}	238.941^{ab}	280.541^{c}	220.564^{c}	668^{c}
Lavender oil 50 mg L^{-1}	3.33^{b}	114.145^{b}	133.587^{b}	109.654^{b}	140^{b}
Lavender oil 100 mg L^{-1}	3.87^{b}	114.007^{b}	133.002^{b}	109.124^{b}	140^{b}
Lavender oil 150 mg L^{-1}	4^{a}	110^{a}	130.147^{b}	107.247^{a}	120^{a}
Thyme oil 50 mg L^{-1}	3.12^{b}	113.99^{b}	133.874^{b}	109^{b}	136^{b}
Thyme oil 100 mg L^{-1}	3.09^{b}	113.011^{b}	133.654^{b}	109.745^{b}	140^{b}
Thyme oil 150 mg L^{-1}	4.01^{a}	109.983^{a}	130.124^{a}	106.971^{a}	120^{a}
SNP 12.5 mg L^{-1}	3.45^{b}	123.418^{c}	162.354^{ab}	128.74^{ab}	256^{ab}
SNP 17.5 mg L^{-1}	4^{a}	109.941^{a}	131.005^{a}	107.873^{a}	124^{a}
SA 2.5 mM	4.02^{a}	110.236^{a}	130.781^{a}	106.789^{a}	132^{a}
SA 5 mM	1.73^{c}	236.74^{ab}	287.12^{c}	223.547^{c}	132^{a}

Values are the mean of four replication, Mean separation among treatments was done by Tukey test values with different alphabets are significantly different at $p \leq 0.05$

extends the vase life. Significant differences ($p \leq 0.05$) were found among various concentrations of SA, NSP and GLU in chlorophyll content of carnation flowers (*D. caryophyllus*). The treatment by 2 mM SA or 150 mg L^{-1} GLU lead to a considerable delay in degradation of Chlorophyll total in compared to other concentrations and control ($p \leq 0.05$) (Table 3). Chlorophyll Content decreased rapidly in present cut flower in solutions containing 1 mM SA while flowers in the solutions containing 2 mM SA+15 mg L^{-1} GLU+15 mg L^{-1} SNP showed the minimum decrease in chlorophyll content from 15.4 days (Table 3). Flowers held in 50, 100 or 150 mg L^{-1} solutions containing Lavender oil and Thyme oil (150 mg L^{-1}) had a considerable delay in degradation of chlorophyll total than control. There was no significant difference between delay in degradation of chlorophyll total of these treatments and the best concentration was 150 mg L^{-1} Lavender oil and Thyme oil (Table 4). Application of 2.5 mM SA or 17.5 mg L^{-1} SNP had positive effects in delay in degradation of chlorophyll total than control. Except for treatments containing 5 mM SA, all other treatments

led to significantly Chlorophyll content (Table 4). Kazemi *et al.* (2011a-d) observed that chlorophyll biosynthesis increased treatment with SA in the ct flower ($p<0.05$). Canakci (2008) found that Chl a, b increased significantly in SA treated plants in comparison to controls of *Raphanus sativus* plants. Addition of SA, SNP and GLU decreased ACC oxidase activity and anthocyanin leakage of flowers significantly ($p\leq0.05$). Our results showed that decreased ACC oxidase activity and anthocyanin leakage could obtained by using 2 mM SA or 15 mg L^{-1} SNP or 2 mM SA+15 mg L^{-1} GLU+15 mg L^{-1} SNP. Table 3 shows considerable difference between ACC oxidase activity and anthocyanin leakage on different concentrations of SA, GLU and SNP at $p\leq0.05$. This table shows, significant differences ($p\leq0.05$) were found among various concentrations of SA, GLU and SNP on ACC Oxidase activity, anthocyanin leakage, malondialdehyde (MDA) accumulation of flowers (Table 3). Flowers held in 2 mM SA or 150 mg L^{-1} GLU or 150 mg L^{-1} SNP decreased malondialdehyde (MDA) accumulation of flowers significantly than control ($p\leq0.05$). Addition of 1 mM SA to the preservative solution had no significant effect on decreased malondialdehyde (MDA) accumulation of flowers (Table 3). Flowers held in 50,100 or 150 mg L^{-1} solutions containing Lavender oil and Thyme oil (150 mg L^{-1}) decreased ACC oxidase activity and anthocyanin leakage and malondialdehyde (MDA) accumulation of flowers significantly than control ($p\leq0.05$). There was no significant difference in decreased ACC oxidase activity, anthocyanin leakage and malondialdehyde (MDA) accumulation of these treatments and the best concentration was 150 mg L^{-1} Lavender oil and Thyme oil (Table 4). Application of 2.5 mM SA or 17.5 mg L^{-1} SNP had positive effects in decreased ACC oxidase activity and anthocyanin leakage and malondialdehyde (MDA) accumulation of flowers. Except treatments containing 5 mM SA, all other treatments decreased ACC oxidase activity and anthocyanin leakage and malondialdehyde (MDA) accumulation of flowers (Table 4). These results are in agreement with those of Hussein and Orabi (2008), Ahmed *et al.* (2010) who found that the treatment of salicylic acid reduced anthocyanin leakage and ACO activity in cut flowers. Similarity, Liu *et al.* (2006) and Kazemi *et al.* (2011c, d) showed that the treatment of salicylic acid reduced anthocyanin leakage and ACO activity in cut flowers. Ouariachi *et al.* (2011) who found that the treatment of EOs increased antioxidant activity in cut flowers. Moreover these experiments indicated that there was significant difference between bacterial concentration in vase solution and various concentrations of Lavender essential oil or Thyme essential oil at $p\leq0.05$. The lowest bacteria concentration was obtained with 2 mM SA or 150 mg L^{-1} SNP and the highest concentration content was seen with control treatment (Table 3). Among various essential oil treatments, 150 mg L^{-1} Lavender oil and Thyme oil had the strongest effect on decreased and maintenance bacterial population compared to control (Table 4). Bacterial population was significantly decreased by addition of 17.5 mg L^{-1} SNP or 2.5 and 5 mM at levels of 200 mg L^{-1} GLU (Table 4). According to the obtained results, addition of EOs to vase solutions in all concentrations decreased the bacterial population of carnation cut flowers ($p\leq0.05$). Solgi *et al.* (2009) confirm our results as they could increase the vase-life of *Gerbera* cut flowers by adding herbal EOs into preserving solution. It seems that all the treatments statistically increased carnation cut flowers vase-life as compared to the control ($p\leq0.05$). According to the researches, ethylene, as senescence hormone reduces vase-life of cut flowers. During senescence, the oxidative stress increases the per oxidative reactions in membrane lipids which damages the membrane function and causes ions and anthocyanin to leak outward which could be considered as an index for lipid per oxidation and senescence progress (Kazemi *et al.*, 2011b). The effects of senescence can be reduced by inhibitors of ethylene biosynthesis and increase enzyme antioxidant activity (Khan *et al.*, 2003; El-Tayeb *et al.*, 2006;

Shi and Zhu, 2008). SA prevent ACC-oxidase activity that is the direct precursor of ethylene and decrease ROS with increase enzyme antioxidant activity (Ansari and Misra, 2007; Canakci, 2008). The protective function of SA includes the regulation of ROS and antioxidant enzymes (Khan *et al*., 2003; Shi and Zhu, 2008). SA acid with increases the enzyme antioxidant activity cause delay the onset of hydrolysis of structural cell components, decrease ROS production and sensitivity (Canakci, 2008). Other studies have shown that exogenous SA can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress (He *et al*., 2002). Also according to results of these experiments can state that SA could reduced the number of bacteria in the vase solution and with decrease ACC-oxidase activity due to delay the onset of hydrolysis of structural cell components, decrease ACC-oxidase activity and sensitivity also, SA increases chlorophyll content consequently was caused to delay in the onset of hydrolysis of structural cell components and sensitivity. Application of silver ions can displace copper ions from the receptor proteins consequently, block ethylene perception, since copper ions have a critical role in ethylene binding upon receptors. (Means *et al*., 2005; Hedden and Thomas, 2006). This effect of silver ion on ethylene was reported by several researchers (An *et al*., 2008; Eo and Lee, 2009). The vase life of cut flowers increased by addition of various antimicrobial compounds such as SA (Kazemi *et al*., 2011a-d) and silver nanoparticles in vase water for reduction of bacterial population in the vase water. Nano-silver as a pulse and vase solution treatment for cut flowers is relatively new (Solgi *et al*., 2009) and has demonstrated its importance as an anti-bactericidal agent which was improved with current study (Morones *et al*., 2005). Vascular blockage by bacteria causes decreasing water uptake and finally is caused to stem breaking or bending and petal wilting in cut flowers (Van Meeteren, 1978; Van Doorn and De Witte, 1994; Nair and Sharma, 2003; Balestra *et al*., 2005). Carbohydrates are important reserve compounds, being sucrose the most abundant soluble carbohydrate, sometimes the only one in the phloem sap (Figueroa *et al*., 2005). The senescence of cut flowers is closely related to a considerable reduction of the energy needed for synthesis reactions (Kazemi *et al*., 2011b). Addition of sucrose to preservative solutions had positive effects on the vase-life of most flowers, including *Gerbera* (Halevy and Mayak, 1979). Use of sucrose necessitates the addition of biocidal agents, which is not considered an environment friendly method due to the side effects like facilitating the emergence of resistant strains of microorganisms to frequently used biocides. However, using sucrose alone in preservative solutions promotes microbial growth (Nair and Sharma, 2003). As GLU is readily metabolized by plants but not by many microorganisms, so we considered using it as a possible substitute for Suc. Results of current study indicated that, vase life of cut carnation with higher GLU content was longer. It has been approved by current and several other researches that adding GLU to preservative solutions has positive effect on the vase life of most cut flowers, such as lily (Kazemi *et al*., 2011b, c). The GLU probably serves to provide osmotic potential for the expansion of the petal cells and the availability of soluble carbohydrate for that purpose is probably partly responsible for the improved opening of the flowers in preservative solution. EOs has strong antimicrobial properties against some pathogens and bacteria because of high levels of phenolic compounds such as Lavender essential oil and Thyme essential oil. The improved vase-life by using EOs treated preservative solutions might be due to their role in inhibiting the microbial growth and preventing bacterial plugging (Bayat *et al*., 2011). Our results are in agreement with result of Ouariachi *et al*. (2011) who found that the treatment of EOs increased antioxidant activity in cut flowers. Indeed, several studies (Bounatirou *et al*., 2007; Mkaddem *et al*., 2010) have been published on antioxidant activities of phenol rich oils from *Thymus capitatus*.

CONCLUSION

In conclusion, application of SA, NSP, GLU and EOs with different concentrations in preserving solution prolonged the vase-life of carnation cut flowers. Based on the present results, EOs as safe and nature friendly compounds can be appropriate alternative compound in improving of vase-life of carnation cut flowers. The proposed mechanism of action, e.g. inhibition of the growth of bacteria in the vase water or inside the xylem vessels of the flower stem needs further elucidation.

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