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Combination Effect of 1-Methylcyclopropene (1-MCP) with Ajowan Essential Oil and Silver Nanoparticles on Postharvest Life of Gerbera (*Gerbera jamesonii*) Cut Flowers

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***Abstract*. Gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) is one of the most important cut flowers in floriculture industry with high economic value. Short postharvest life is the main barrier for gerbera marketing which is related to water relation disruption resulting from microbial population in preservative solution and exposure to ethylene. Therefore, an experiment was conducted to study the combination effect of an ethylene inhibitor, 1-methylcyclopropene (1-MCP) (0 and 1 $\mu L \cdot L^{-1}$), and two antimicrobial agents, ajowan [*Carum copticum* (L.) C. B. Clarke] essential oil (EO) (0 and 15 $\mu L \cdot L^{-1}$) and silver nanoparticles (AgNPs) (0 and 40 $\mu L \cdot L^{-1}$), on postharvest life of 'Pink Power' gerbera cut flowers. The results showed that stem diameter reduced in both cut stem-end (CSE) and below the flower head (BFH) but the amount of decrease in CSE was more than BFH, and applied treatments had no significant effect on the decrease rate. However, both ajowan EO and AgNPs treatments reduced the number of bacteria at the CSE; ajowan EO was more effective than AgNPs and induced more vase solution uptake. Stem-end electrolyte leakage (EL) was unaffected by 1-MCP treatment, whereas petal EL was lower in 1-MCP-treated flowers. The least wilting percentage and the longest vase life were observed in combination treatment of 1-MCP and either ajowan EO or AgNPs. It could be concluded that 1-MCP combined with ajowan EO or AgNPs, potentially can be used as a preservative solution to enhance postharvest life, delay flower senescence, and prolong vase life of gerbera cut flowers.**

Gerbera plant is from Asteraceae family and can be grown as a cut flower, pot flower, or garden plant (Reinten et al., 2011). This cut flower ranks fourth after roses, chrysanthemum, and lily in the FloraHolland auction in 2014 (Azadi et al., 2016). Postharvest quality is one of the most important characteristics that determines consumer preference and satisfaction and is a key factor in the commercial value of cut flower (Scariot et al., 2014). In addition, the longevity of cut flowers is crucial to persuade the consumer to repurchase them (Reid and Jiang, 2012). Therefore, one of the main problems in gerbera export and marketing is postharvest losses, and short postharvest life attribute to the water relation disruptions resulting from an increase in microbial population in preservative solution and ethylene action.

Synthetic chemicals are extensively used in floriculture industry to improve yield and quality of cut flowers. Several treatments have been applied to extend gerbera postharvest life. Silver nitrate, thiosulfate, or nanoparticles (AgNPs) in preservative solutions could extend postharvest life of cut flowers (Bahrehmand et al., 2014; Geshnizjany et al., 2014; Liu et al., 2009; Solgi et al., 2009). In the past decades, awareness increased among people regarding side effects of chemicals used in agriculture, and some researches have been conducted to find alternative treatments or materials to preserve product quality or to increase storage life.

Therefore, recently most researchers and producers tend to use environmental and health-friendly materials, such as EOs, which are organic natural substances extracted from different parts of medicinal plants to improve postharvest life of cut or pot flowers (Tabassum and Vidyasagar, 2013). EOs have strong antimicrobial characteristics against some pathogens that ascribe to high levels of phenolic compounds, such as carvacrol, thymol, and eugenol (Perricone et al., 2015). It has been reported that EOs could extend the vase life of gerbera (Solgi et al., 2009) and alstroemeria (Fazlalizadeh et al., 2013; Madadzadeh et al., 2013).

Ethylene concentration in ambient atmosphere affects the vase life of cut flowers (Jalili Marandi et al., 2011). Many flowers perish rapidly in response to ethylene; thus, it is important to avoid pollution with ethylene via removing ethylene from storage rooms by ventilation or by treating the flowers with ethylene action inhibitors. During the past two decades, ethylene biosynthesis inhibitor 1-MCP as a nontoxic to humans has been demonstrated to extend the storage life of a range of cut flowers (Scariot et al., 2014). Hassanpour Asil et al. (2013) reported that the application of 1-MCP extends the vase life of cut spray carnation 'Optima' flowers by retarding fresh weight (FW) loss, ethylene production, and chlorophyll and anthocyanin degradation. Celikel et al. (2002) also showed that pretreatment of 'Monalisa' and 'Stargazer' lilies with 1-MCP completely inhibited the ethylene response, but did not prevent normal senescence, wilting, and abscission of open flowers.

To the best of our knowledge, the combination effect of 1-MCP with antimicrobial agents on postharvest life quality of gerbera has not been studied. Considering the role of 1-MCP (as an ethylene inhibitor),ajowan EO, and AgNPs (as antimicrobial agents), the purpose of the current study was to investigate the possible effects of 1-MCP, ajowan EO, and AgNPs and their combination on improving the postharvest life of gerbera cut flowers cv. Pink Power.

Materials and Methods

Plant, chemical, and EO materials. Cut gerbera 'Pink Power' flowers were harvested when the two outer row disc florets were open and pollen grains were visible (Dole and Wilkins, 2005) from a commercial greenhouse and immediately transferred to postharvest laboratory of the University of Kurdistan, Sanandaj, Iran. Flowers were selected based on their uniformity in shape and color without signs of mechanical damage and disease. Then, to eliminate air blockage in the stem, flower stems were cut submerged in deionized water, leaving the stems without leaves ≈40 cm long. The EOs of ajowan seed were extracted using a Clevenger apparatus, and AgNPs were purchased from Nanocid Company (http://www.nanocid.com) as liquid solution.

Experimental design and treatments. Experiment was conducted in a factorial based on completely randomized design with three replications, with four flowers in each replication at 20 ± 2 °C with 65% ± 5% relative humidity and 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity (fluorescent lamps) under a daily light period

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of 12 h. Factors included 1-MCP (EthylBloc; Agrofresh, Inc., Spring House, PA), vase solution additive, and sampling times except for the vase life parameter. Factors for the vase life evaluation were 1-MCP and vase solution additives. Half of the cut flowers were placed with their bases in water in gas-tight 200-L glass aquarium chambers at 20 °C and treated with 1-μL·L^{-1} 1-MCP for 18 h. The other half of the flowers were sealed in air in identical chambers without 1-MCP treatment. The 1-MCP treated and nontreated flowers were then placed in the vase solution [distilled water containing 1.5% (w/v) sucrose] containing 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} of AgNPs. The vase solution in the control was distilled water containing 1.5% (w/v) sucrose solely.

Stem diameter. Diameter of CSE and BFH was measured at 0, 1, 3, 5, 7, and 9 d after treatments by a digital Vernier caliper.

Vase solution uptake. The weights of vases without their cut flowers were daily recorded during the vase life evaluation period using a balance. Vases were sealed by polyethylene film to minimize evaporation and inhibit contamination. Average daily vase solution uptake was calculated by the formula: vase solution uptake rate (mg·g^{-1} stem FW) = (St_{-1} – St); where, St is the weight of vase solution (g) at t = day 1, 3, 5, 7, 9, and 12, St_{-1} is the weight of the vase solution (g) on the previous day (He et al., 2006).

Relative fresh weight (RFW). The FWs of the cut flower were registered daily during the vase period. The RFW of stems was measured by the following formula: RFW (%) = (Wt/Wt_0) × 100; where, Wt was the weight of the stem (g) at t = day 1, 3, 5, 7, 9, and 12, and also Wt_0 was the weight of the same stem (g) at t = day 0 (He et al., 2006).

Electrolyte leakage (EL). EL of petal or stem-end was determined (at 0, 5, and 12 vase periods) using Sairam et al. (1997) method at which 0.2 g of sample was placed in 10 mL of distilled water and then was maintained at 40 °C for 30 min in a warm bath, and its EC was read after being cooled at 25 °C by using the conductivity meter kit (C_1). Then, the sample was placed in the water bath at 100 °C for 20 min, and its electrical conductivity was read for the second time EC (C_2) after being cooled. Based on the following equations, the desired indices were calculated in terms of percentage (C_2).

$$EL = (C_1/C_2) \times 100$$

C_1 = electrical conductivity after exposure to 40 °C

C_2 = electrical conductivity after exposure to 100 °C

Bacteria population. The samples from the flower stem-end were taken at 0, 5, and 12 vase periods, homogenized and diluted with sterile peptone water to obtain the microbial count. Serial dilutions were performed in triplicate. Total aerobic mesophilic bacteria counts were enumerated using the pour plate method on the plate count agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain) after incubation at 30 °C for 2 d (Balestra et al., 2005; Liu et al., 2009).

Wilting percentage and vase life. The gerbera cut flowers were assessed for visual appeal during the vase life evaluation period. The vase life was defined as the period from the beginning of the experiment to the time when 50% of floret petal wilted or bent neck was observed (Joyce et al., 2000).

Statistical analysis. Data were subjected to analysis of variance with SAS 8.0 software (SAS Institute Inc., Cary, NC). Sources of variation were vase period (days) and treatments. Mean values were calculated and reported as the mean ± SE (n = 3). The least significant difference test at P = 0.05 was used to compare means among treatments.

Results and Discussion

Stem diameter. Cut stem-end and BFH diameters were stable during the first five days and decreased thereafter during the vase period. Generally, the decreasing rate of

Table 1. Below the flower head (BFH) diameter changes in gerbera cut flowers treated or untreated by 1-μL·L^{-1} 1-MCP plus 15-μL·L^{-1} ajowan EO or 40-μL·L^{-1} silver nanoparticles (AgNPs) treatments. Flowers were stored at 20 °C for up to 9 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

		BFH diam (mm)					
		Vase period (days)					
Treatments		0	1	3	5	7	9
– 1-MCP	Control	5.20 ± 0.16	5.32 ± 0.16	5.32 ± 0.16	5.18 ± 0.16	5.20 ± 0.17	5.09 ± 0.12
	Ajowan	5.22 ± 0.05	5.33 ± 0.05	5.29 ± 0.03	5.20 ± 0.05	5.21 ± 0.05	5.08 ± 0.07
	AgNPs	5.18 ± 0.05	5.31 ± 0.05	5.26 ± 0.08	5.20 ± 0.06	5.12 ± 0.06	5.01 ± 0.07
+ 1-MCP	Control	5.25 ± 0.16	5.39 ± 0.15	5.39 ± 0.15	5.26 ± 0.16	5.27 ± 0.17	5.15 ± 0.12
	Ajowan	5.27 ± 0.05	5.40 ± 0.04	5.37 ± 0.03	5.28 ± 0.04	5.28 ± 0.04	5.16 ± 0.06
	AgNPs	5.25 ± 0.05	5.39 ± 0.06	5.33 ± 0.08	5.27 ± 0.07	5.17 ± 0.07	5.08 ± 0.07
		$LSD_{0.05}$ = 0.28					

Table 2. Cut stem-end (CSE) diameter changes in gerbera cut flowers treated or untreated by 1 μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs) treatments. Flowers were stored at 20 °C for up to 9 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

		CSE diam (mm)					
		Vase period (days)					
Treatments		0	1	3	5	7	9
– 1-MCP	Control	7.45 ± 0.37	7.64 ± 0.37	7.21 ± 0.35	7.13 ± 0.31	7.31 ± 0.49	6.72 ± 0.31
	Ajowan	7.37 ± 0.53	7.57 ± 0.53	7.38 ± 0.22	7.19 ± 0.28	7.26 ± 0.77	7.47 ± 0.11
	AgNPs	7.44 ± 0.35	7.63 ± 0.35	7.76 ± 0.17	7.57 ± 0.25	7.33 ± 0.23	7.26 ± 0.14
+ 1-MCP	Control	7.52 ± 0.37	7.74 ± 0.37	7.31 ± 0.35	7.05 ± 0.49	7.22 ± 0.69	6.82 ± 0.31
	Ajowan	7.44 ± 0.53	7.67 ± 0.52	7.49 ± 0.21	7.29 ± 0.27	7.36 ± 0.78	7.58 ± 0.10
	AgNPs	7.51 ± 0.35	7.73 ± 0.37	7.88 ± 0.18	7.67 ± 0.27	7.44 ± 0.25	7.36 ± 0.15
		$LSD_{0.05}$ = 1.10					

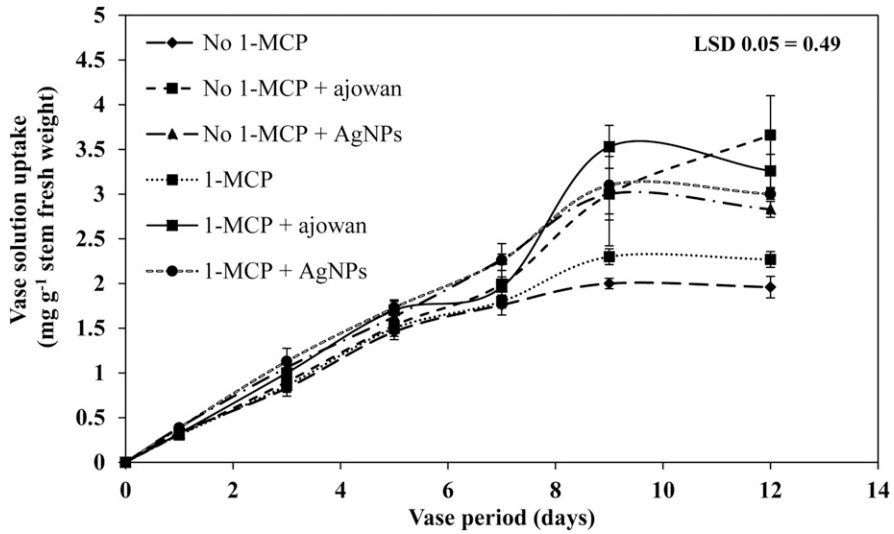

Fig. 1. Vase solution uptake of gerbera cut flower either untreated or treated with 1·μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs). Flowers were stored at 20 °C for up to 12 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

Table 3. Relative fresh weight (RFW) changes in gerbera cut flowers treated or untreated by 1 μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs) treatments. Flowers were stored at 20 °C for up to 12 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

Treatments		RFW (%)					
		Vase period (days)					
		1	3	5	7	9	12
– 1-MCP	Control	115.05 ± 1.14	106.68 ± 3.07	99.30 ± 0.53	92.52 ± 3.07	84.75 ± 2.31	72.96 ± 2.14
	Ajowan	112.93 ± 0.89	111.13 ± 0.31	106.35 ± 1.34	102.00 ± 1.91	91.45 ± 1.01	75.55 ± 2.82
	AgNPs	116.51 ± 2.06	117.09 ± 1.51	110.61 ± 2.92	95.39 ± 1.87	88.44 ± 2.51	75.03 ± 2.38
+ 1-MCP	Control	121.39 ± 0.67	119.21 ± 2.16	115.02 ± 0.93	98.59 ± 3.18	90.48 ± 3.13	73.49 ± 1.55
	Ajowan	128.33 ± 0.79	125.62 ± 0.35	133.00 ± 3.19	104.05 ± 3.16	96.74 ± 3.88	81.81 ± 2.42
	AgNPs	132.36 ± 1.70	131.71 ± 2.33	125.98 ± 3.65	105.02 ± 2.27	94.10 ± 3.42	78.05 ± 2.69
				$LSD_{0.05}$ = 6.00			

diameter was higher at BFH than at CSE. Although there were no significant differences between treated and untreated flowers but the decrease in two measured traits of treated cut flowers was less than untreated controls (Tables 1 and 2).

Stem diameter is an important parameter in keeping gerbera flower quality and is an index used to determine its marketability (Ansari et al., 2011). Also, stem diameter affects stem bending of gerbera cut flowers. The present results are in agreement with Ansari et al. (2011) and Jalili Marandi et al. (2011) who reported treatment of gerbera cut flower with AgNPs and cut rose with ajowan, and summer savory (*Satureja hortensis*) EOs had no significant effect on stem diameter, respectively. By contrast, Bahrehmand et al. (2014) reported that application of AgNPs increased stem diameter of tuberose (*Polianthes tuberosa*) cut flower. It may also be argued that the reduction in the diameter of CSE end rather than the BFH end of stem flower may be due to the higher bacterial numbers/or increase in water loss and decrease in water uptake. It may be concluded that higher reduction of CSE than BFH may be related to high bacterial population which reduced water uptake.

Vase solution uptake and RFW. Vase solution uptake increased during the vase life regardless of treatments, and there was no significant difference among treatments during the first 5 days. The highest uptake was observed in AgNPs + 1-MCP treatment on the seventh day and thereafter. The solution uptake of all treated flowers was higher than the control in either 1-MCP treated or untreated samples (Fig. 1).

The RFW of all treatments except of 1-MCP + ajowan EO exhibited similar trends and was decreased from day 1 to 12 of experiment. However, the RFW of 1-MCP + ajowan EO was slightly increased until day 5 and then decreased (Table 3). RFW in 1-MCP + ajowan EO or + AgNPs treatments were higher than 1-MCP solely or without 1-MCP treatments at the last data point (day 12) (Table 3).

The vase solution uptake has significant effect on the RFW of cut flowers. Therefore, it could be considered that one reason for high effectiveness of EOs and AgNPs in RFW improvement may be related to their bactericidal properties and inhibition of microbial vascular occlusion and then have an improved vase solution uptake (Balestra et al., 2005; Solgi et al., 2009).

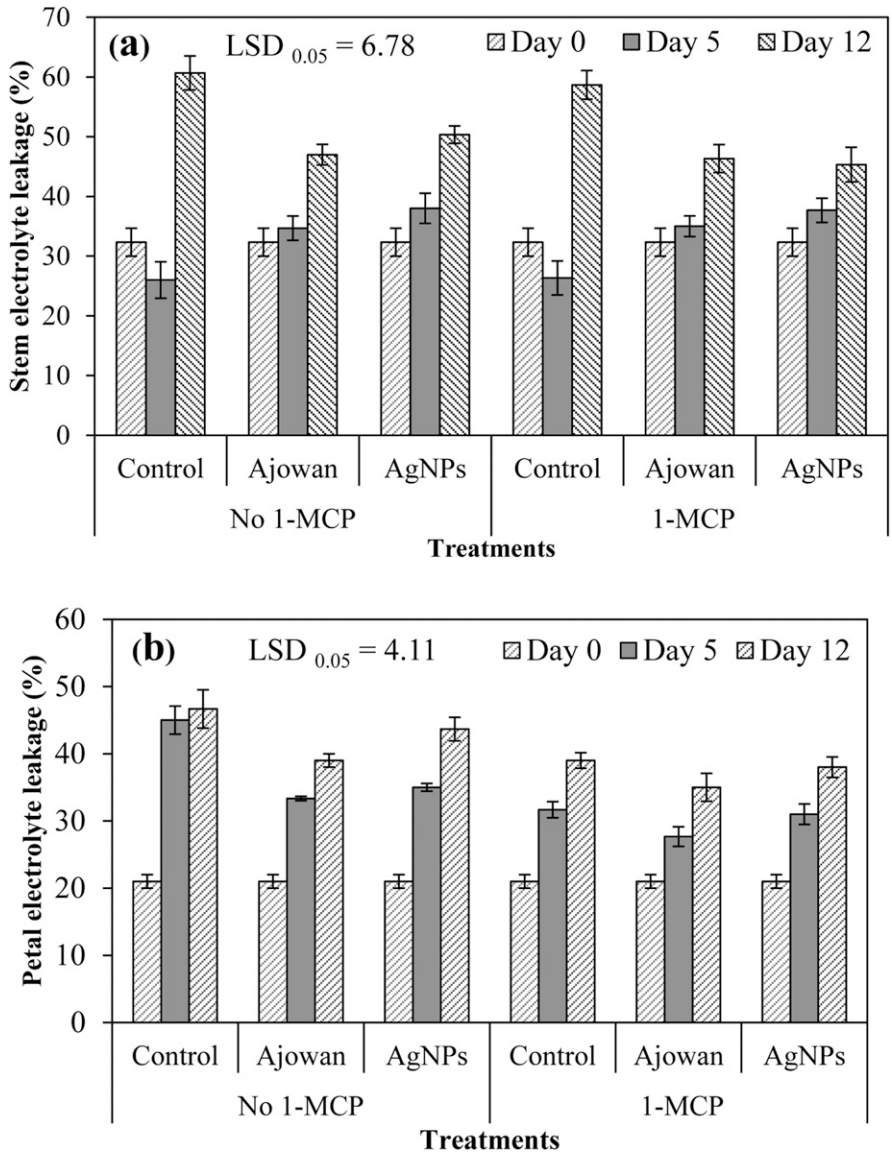

Fig. 2. Stem (**A**) and petal (**B**) electrolyte leakage in gerbera cut flower either untreated or treated with 1 μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs). Flowers were stored at 20 °C for up to 12 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

Stem and petal EL. Electrolyte leakage of either stem or petal increased during the vase life irrespective of treatments (Fig. 2A and B). Stem EL was suppressed in ajowan EO and AgNPs treatments, whereas there was no significant difference in 1-MCP treated or untreated flowers (Fig. 2A). Petal EL was also suppressed in ajowan EO and AgNPs treatments rather than control (Fig. 2B). In contrast to stem EL, petal EL of 1-MCP-treated flowers was lower than untreated flowers regardless of antibacterial treatments.

Changes in membrane properties of senescing petals are associated with loss of semipermeability leading to increased ion and water leakage and ending in desiccation of the tissue (van Doorn and Woltering, 2008). It has been shown that the loss of membrane integrity increases the permeability and leakage during senescence in various flowers, including *Arum*, *Ipomoea*, *Dianthus*, *Iris*, *Hemerocallis*, *Rosa*, *Gerbera*, and *Nerine* (Gul et al., 2012; Gulzar et al., 2005; Kazemi et al., 2011). The decrease in petal and stem EL in ajowan EO or AgNPs treatments might be due to the reduction of membrane disruption and the leakage of some cellular components through higher solution uptake and keeping cell turgidity. Sun et al. (2009) also reported that there is a close relationship between water content and EL in petals of mini *Phalaenopsis* cultivars. Along with the decrease in water content, the EL was increased.

It has been reported that AgNPs-treated plants showed an efficient cellular electron exchange mechanism, which arrest electron leakage, reducing the reactive oxygen species (ROS) production and hence lipid peroxidation (Hatami and Ghorbanpour, 2013; Lu et al., 2002). Petal EL of 1-MCP treated flowers was lower than untreated ones, which might be due to suppressing ethylene production. It is generally accepted that ethylene is responsible for deteriorations related to oxidative stress and also ethylene mediates the production of ROS.

Bacteria population. The bacteria growth at CSE was increased during the vase life in the control flowers. Ajowan EO treatment significantly decreased bacteria populations while AgNPs treatment was effective in restricting bacterial growth. Bacterial population was unaffected by 1-MCP treatment (Fig. 3).

Microorganisms were considered to be one of the main causes of reduced vase solution uptake by cut flowers. It has been reported that the main cause of the blockage in gerbera is bacterial growth. The microorganism proliferation in the vase solution also causes water relation interruption as a result of occlusion in the basal end of cut flower stem (Hassan et al., 2014). It has also been observed that xylem occlusion by bacteria is one of the main reasons in reducing water uptake (Balestra et al., 2005) and causing the microbiological vascular blockage of stem. Therefore, the use of materials with antimicrobial properties can help to improve water uptake and the water balance of the cut flowers (Kwon and Kim, 2000).

The results indicated that ajowan EO and AgNPs treatments decreased the number of bacteria at CSE of gerbera. Interestingly, on the ninth day of the vase period ajowan EO treatments were more effective than silver treatment to inhibit the growth of bacteria. Increase in water uptake could be attributed to inhibition of microbial growth in the end of the stem and vase solution. Similarly, it has been reported that AgNPs treatments increased vase solution uptake in rose flower

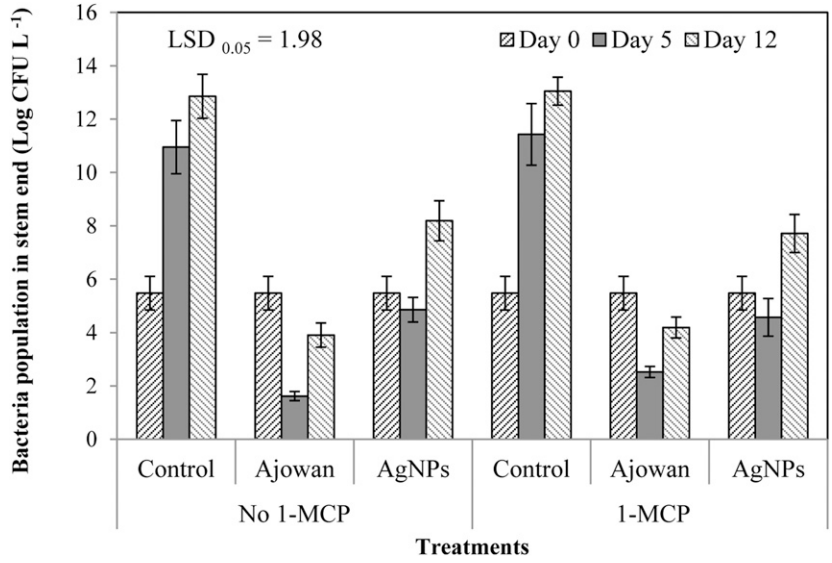

Fig. 3. Bactria populations in gerbera cut flower either untreated or treated with 1 μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs). Flowers were stored at 20 °C for up to 12 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

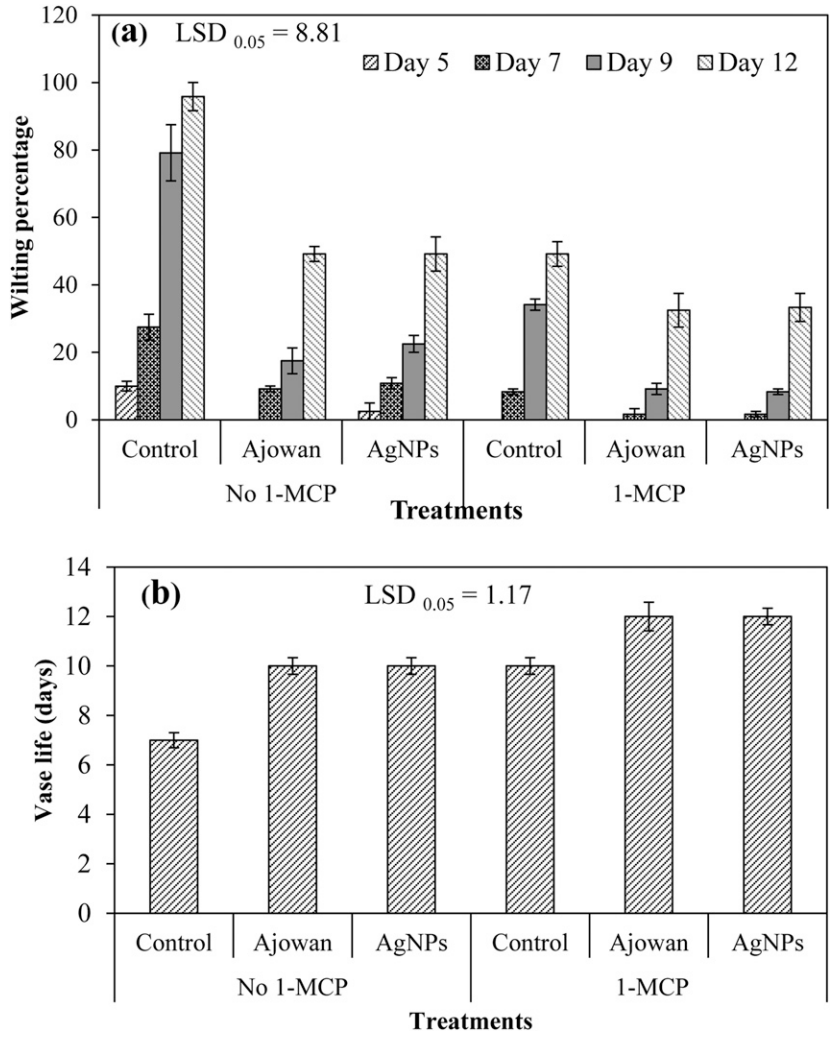

Fig. 4. Wilting percentage (**A**) and vase life (**B**) of gerbera cut flower either untreated or treated with 1 μL·L^{-1} 1-MCP plus 15 μL·L^{-1} ajowan EO or 40 μL·L^{-1} silver nanoparticles (AgNPs). Flowers were stored at 20 °C for up to 12 d. Values represent means ± SE (n = 3). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

(Lü et al., 2010; Nazemi Rafi and Ramezanian, 2013), gerbera (Kazemi and Ameri, 2012), and tuberose (Bahrehmand et al., 2014). Significant effect of EOs on vase solution uptake by suppressing the growth of microbial agents in postharvest life of cut flowers has already been reported (Solgi et al., 2009).

Wilting percentage and vase life. The first sign of wilting was observed in 1-MCP untreated flowers without antibacterial agent in vase solution and increased progressively up to 100% before 12 d after harvest, whereas the wilting percentage of 1-MCP-treated flowers was significantly lower than those observed in untreated flowers (Fig. 4A and B). The wilting percentage of 1-MCP-treated flower on the ninth day was lower than those of 1-MCP untreated on the 12th day. The lowest wilting percentage and the highest vase life were observed in antibacterial agent + 1-MCP treatments (Fig. 4B).

Our findings are in correspondence with the prolonging effects of AgNPs on rose (Hassan et al., 2014) and also EOs in gerbera (Solgi et al., 2009), gladiolus (Hegazi and Gan, 2009), rose (Jalili Marandi et al., 2011), and cloves (Kazemi and Ameri, 2012). EOs, such as thymol, eugenol, and carvacrol, exhibit antimicrobial and antioxidant properties (Lambert et al., 2001). The hydrophobic EOs by partitioning in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to a leakage of cell contents (Burt, 2004), and thus might affect the flower vase life.

In general, the reasons for positive effects of AgNPs treatment in increasing the vase life of some cut flowers, including carnations, gerberas, acacias, and roses are 1) because AgNPs have a high surface area to volume ratio and are more effective at preventing the growth of bacteria and other microorganisms at the CSEs (Furno et al., 2004), 2) Because of physiological activity of Ag^+, AgNPs have positive effects on plant stem hydraulic conductivity, 3) Ag^+ is considered to be a general inhibitor of aquaporins, improving water relations (Lü et al., 2010; Niemietz and Tyerman, 2002), 4) they could act as anti-ethylene agents, and 5) AgNPs release Ag^+ (Lok et al., 2007), which has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability (Park et al., 2005).

In our study, treatment with 1-MCP extended the vase life of gerebera up to 3 d compared with the control. Similar results have also been reported that the vase life of various cut flowers, such as carnation, delphinium, snapdragon, rose, and begonia, can be extended by exposure to 1-MCP (Ichimura et al., 2002). Ethylene receptors to which 1-MCP are bound, may be replaced by newly synthesized receptors which can bind ethylene, may lower the effectiveness of 1-MCP on the gerbera vase life.

Conclusion

Based on the results of this study, it could be concluded that all applied treatments improve the vase life of gerbera cut flowers. The combinations of 1-MCP with EO or AgNPs, significantly extended the vase life of gerberas in comparison with 1-MCP or AgNPs treatments solely. The long vase life of flowers treated with 1-MCP + or 1-MCP + AgNPs was accompanied by reducing the EL in the stem and petal, prevented vase solution microbial proliferation in the end of the cut stem, improved solution uptake, and reduced wilting percentage.

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