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Nanosilver, salicylic acid and essential oils effects on water relations of gerbera 'Rosalin' cut flowers

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Key words: clove, hydraulic conductivity, peppermint, thyme, vase life.

Abstract: **The effects of pulse and permanent treatments were studied on vase life, water content and hydraulic conductivity of gerbera cut flower cv. Rosalin. This study was conducted as a factorial experiment based on completely randomized design with three replications. The first factor was pulse treatments, using nanosilver (NS) 5 and 10 mg/L, salicylic acid (SA) 50 and 100 mg/L and distilled water as control, and the second factor was permanent treatments applying distilled water, sucrose, peppermint, thyme and clove essential oils (EO). The results showed that NS 10 mg/L + peppermint EO 100 mg/L and NS 10 mg/L + thyme EO 100 mg/L treatments had the best effect on longevity and maintaining the water content and hydraulic conductance of Rosalin cut flower, compare to other treatments. These solutions enhanced life of gerbera cut flowers to about 14 days. Flower water content was high (about 90%) except in 4% sucrose permanent treatment flowers which decreased more rapidly during the vase life. The effective hydraulic conductivity was observed in NS 10 mg/L + peppermint EO 100 mg/L (0.16 cm/min) and NS 10 mg/L + thyme EO 100 mg/L (0.21 cm/min) solutions and had nearly stable trend even in day 8 after pulsing.**

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1. Introduction

Gerbera (*Gerbera jamesonii* Bolus, Asteraceae) commonly known as Transvaal Daisy, Barberton Daisy or African Daisy, is one of the ten most popular and important commercial cut flowers grown in a wide range of climatic conditions. Gerbera is a perennial, tropical, herbaceous plant with colorful and attractive flowers that are widely used as a decorative garden plant or cut flowers. Cut gerbera flowers consist of a terminal composite floral head (inflorescence), called the capitulum, and a stem, which called scape and has no leaves (Dole and Wilkins, 2006). Gerbera has the fourth place in the international cut flower market. The flowers are hardy and resist against transport conditions. However, the most important problem of the gerbera cut flowers is short vase life. The end of vase life of cut gerbera flowers is often due to bending of the scape, which precedes wilting of the ray florets (Nair *et al*., 2003; Van Son, 2007; Ansari *et al*., 2011; Perik *et al*., 2012; Kilic and Cetin, 2014; Aghajani and Jafarpour, 2016). However, postharvest life of cut flowers could be affected by the application of various chemicals as preservatives (Nair *et al*., 2003; Prashanth *et al*., 2010).

Insufficient water uptake is one of the main reasons for water deficit and wilting during the vase life (Knee, 2000; Van Ieperen *et al*., 2002). Stem end blockage is a main factor in the imbalance between water uptake and water loss from cut flowers (He *et al*., 2009). Researches showed that bacteria (microbes) (Van Meeteren, 1978; He *et al.*, 2006) or bacteria and decay products (Liu *et al.*, 2009) cause the blockage of cut gerbera flower. Bacteria in vase water can block the vessels in the surface of cut stems (Ferrante *et al.*, 2007). Bacterial inhibitors such as silver nanoparticles or salicylic acid could extend vase life of cut flowers (Loubaud and Van Doorn, 2004; Solgi *et al*., 2009; Vahdati Mashhadian *et al*., 2012). Physiological substances such as lignin, mucilage or gum (Van Doorn and Cruz, 2000; Loubaud and Van Doorn, 2004, Wang *et al*., 2014) and cavitation (Van Meeteren *et al*., 2006) decrease the vase life of some cut flowers. The air emboli or cavitation would be reduced if cut flowers were put into water after cutting immediately (Van Ieperen *et al*., 2002). Cut flowers are sensitive to microbial contamination at the stem end and this determines a reduction of their vase life (Van Meeteren, 1978, Van Doorn and De Witte 1994, Balestra *et al*., 2005).

Nano technology is based on engineered particle of 1-100 nm (diameter). Nanosilver (NS) is included in this technology and can have more chemical and biological activities in order to reduce size. In recent years, NS is being used as a new antiseptic for many industrial processes like medical industry, water purification and vegetable disinfection (Rai *et al*., 2009). In addition, NS treatment has been proposed for improving the postharvest life of cut flowers (Liu *et al*., 2009; Solgi *et al*., 2009; Ansari *et al*., 2011; Danaee *et al*., 2013).

The role of salicylic acid (SA), as an internal growth regulator and a natural phenolic compound, has been completely proved in multiple physiological processes like ethylene biosynthesis, stomatal conductance, respiration, senescence and the activation of defense systems against different pathogens. By activating antioxidant enzymes, SA delays the process of senescence in flowers. In addition, SA inhibits ethylene synthesis and action (Raskin, 1992; Hayat *et al*., 2010; An and Mou, 2011; Jamshidi *et al*., 2012).

Exogenous supply of carbohydrate can play an important role in lengthening the vase life and postharvest conditions of cut flowers. The gerbera cut flowers have short postharvest life. Sucrose effect on enhancing the vase life of cut flowers is associated with water balance. The application of sucrose treatment and sugars accumulated in the flowers increase the sugar and osmotic concentration, improve water absorption and flower turgidity (Reddy and Singh, 1996; Prashant *et al*., 2010; Bhanusree *et al*., 2015). Researches also showed that the combined use of NS 5 mg/L with 4% sucrose and 2.5 mg/L gibberellic acid increase postharvest life of gerbera (Ansari *et al.*, 2011).

Many chemicals have been used in cut flowers vase solutions for inhibiting microorganisms' growth and extending the vase life by improving water uptake. These chemicals include silver nitrate, 8-hydroxyquinoline sulfate and 8-hydroxyquinoline citrate, which are expensive and harmful for the environment and human health (Nowak *et al*., 1990; Ichimura *et al*., 1999; Nair *et al*., 2003; Motaghayer and Esna-Ashari, 2009; Solgi *et al*., 2009; Ansari *et al.*, 2011). It is crucial to use natural, safe and inexpensive compounds for the large-scale application of preservatives improving cut flower vase life (Kilic and Cetin, 2014). Essential oils (EO) are organic, natural, safe and eco-friendly substances that have strong anti-inflammatory, antibacterial, antifungal, antioxidant and anticarcinogenic effects. These properties are attributed to the high levels of phenolic compounds (Solgi *et al*., 2009; Bayat *et al*., 2011; Raut and Karuppayil, 2014).

The application of different medicinal plants EOs on increasing the vase life of cut flowers have been studied by many researches. The effect of peppermint (*Mentha pipperita* L.) EO has increased freshness and quality of flower color and prevented the discoloration in alstroemeria (Babarabie *et al*., 2016), flower's quality and delay of leaf and flower senescence of tuberose cv. Pearl (Hoseini and Korehpaz, 2015) and vase life of 'Utopia' rose cut flowers (Saghazadeh *et al*., 2014). Thyme (*Thymus vulgaris* L.) EO (Solgi *et al*., 2009) and water extract of thyme (Amini *et al*., 2014) was added to the preserving solution for extending the vase life of gerbera 'Dune' cut flower and essence containing *Thymus vulgaris* and *Cuminum cyminum* increased solution uptake and quality of gerbera 'Sorbet' cut flowers (Dareini *et al.*, 2014). Clove (*Eugenia caryophyllata* Thunb.) EO increased lisianthus cut flower vase life (Kazemi *et al*., 2014) and clove EO and water extract increased gerbera 'Ecco' vase life (Ziyaei Movahed *et al*., 2010).

There are two different ways for treating cut flowers; pulse and permanent treatment. Pulsing is a short-term treatment that can be done by producers and it helps postharvest vase life and flowering after storage period. Permanent treatment mostly is a long-term treatment, which can be done by consumers for enhancing cut flower vase life (Abdel-Kader and Rogers 1986; Nowak *et al.*, 1990; Arora and Singh, 2002).

Sucrose can maintain the cell's turgor pressure and provide energy for cellular respiration, also is an important nutrient for microorganisms. Therefore, it should not be used without anti-microbial agents in preservatives (Nowak *et al.*, 1990). The effect of NS (Liu *et al.*, 2009) and SA (Jamshidi *et al.*, 2012) treatments alone on extending cut flowers vase life was assessed in different researches. Since the effects of different concentrations of various preservative solutions on the postharvest life of cut flowers are altering depend on plant species, the applied chemicals and interaction of their compounds in vase solution and the method of treatment, the determination of the effective preservatives as well as the method of application is very important. Therefore the aim of this study was to screen the effects of NS and SA as pulse treatment and sucrose and thyme, clove and peppermint EOs as permanent treatment on vase life and hydraulic conductivity of gerbera 'Rosalin' cut flowers.

2. Materials and Methods

Plant growth conditions and treatments

Gerbera (*G. jamesonii* cv. 'Rosalin') flowers were grown in standard hydroponic greenhouse conditions in Ferdowsi University of Mashhad, Iran. The flowers were harvested during morning by pulling out the stems from the plants when 2-3 rows of stamens of the bisexual disc florets were mature. Stems were pulled, not cut and the base of stem was removed before hydration (Dole and Wilkins, 2006). The stems were taken immediately to the laboratory and recut under water to 35 cm length. The cut flowers were immediately immersed individually into 500 ml vase solutions. In order to simulate the domestic use, the vase solutions were not changed and the stems were not recut during the experiment. The end of gerbera cut flower vase life was considered as the time in which more than one third of the outer petals of inflorescence start to be brown or wilted or curled or stem bending (≥90°) or breaking was occurred (Dole and Wilkins, 2006). This study was conducted as a factorial experiment based on completely randomized design with three replications and four stems in each replicate. The first factor was pulse treatments: distilled water (D), salicylic acid (SA) 50 and 100 mg/L (Merck Company), Nanosilver (NS) 5 and 10 mg/L (Nanocid Company, Iran). The second factor was permanent treatments: distilled water, sucrose 4% (Merck Company), peppermint EO 100 mg/L, thyme EO 100 mg/L, clove EO 300 mg/L (Zardband Company, Iran) (Table 1). Pulse treatments were applied for 24 h. Treated stems were then stood into vases containing permanent treatments. Vase solutions were freshly prepared at the beginning of the experiment and not renewed during of the study.

The EOs constituents were determined by Zardband Company (Iran) using GC-MS analysis

Table 1 - Pulse and permanent treatments used in the experiment

Pulse treatment/Permanent treatment
Distilled water/Distilled water
Distilled water/Sucrose
Distilled water/Peppermint EO
Distilled water/Thyme EO
Distilled water/Clove EO
SA 50 $mg.L^{-1}$/Distilled water
SA 50 $mg.L^{-1}$/Sucrose
SA 50 $mg.L^{-1}$/Peppermint EO
SA 50 $mg.L^{-1}$/Thyme EO
SA 50 $mg.L^{-1}$/ Clove EO
SA 100 $mg.L^{-1}$/Distilled water
SA 100 $mg.L^{-1}$/Sucrose
SA 100 $mg.L^{-1}$/Peppermint EO
SA 100 $mg.L^{-1}$/Thyme EO
SA 100 $mg.L^{-1}$/ Clove EO
NS 5 $mg.L^{-1}$/Distilled water
NS 5 $mg.L^{-1}$/Sucrose
NS 5 $mg.L^{-1}$/Peppermint EO
NS 5 $mg.L^{-1}$/Thyme EO
NS 5 $mg.L^{-1}$/ Clove EO
NS 10 $mg.L^{-1}$/Distilled water
NS 10 $mg.L^{-1}$/Sucrose
NS 10 $mg.L^{-1}$/Peppermint EO
NS 10 $mg.L^{-1}$/Thyme EO
NS 10 $mg.L^{-1}$/ Clove EO

(Table 2). GC-MS analysis revealed that the major constituents of the EOs were: thymol (53.5%) in thyme EO; 1-menthol (41.22%) and menthone (24.01%) in peppermint EO and eugenol (62.4%) in clove EO.

Table 2 - Major chemical constituents of the EOs

Peppermint EO		Thyme EO		Clove EO	
GCMS Analysis (%)		GCMS Analysis (%)		GCMS Analysis (%)	
Limonene	2.25	Terpinene gamma	7.20	Alpha Copaene	0.04
Cineole	4.59	Para-Cymene	27.4	Beta Caryophyllene	3.79
Menthone	24.01	Thymol	53.50	Alpha Humulene	0.45
Isomenthone	3.83			Oxyde De Caryophyllene	0.29
1-Methyl acetate	4.38			Eugenol	81.83
Neomenthol	2.84			Isoeugenol	0.13
1-Menthol	41.22			Acetate De Eugenyle	12.50
Pulegone	1.56			Methyl Eugenol	0.01
Menthofuran	2.98				
Density (20°C)	0.9036	0.923		10.636	
Refractive Index(20°C)	14.605	1.502		15.335	
Optical Rotation (°)	-23.68	-1.0		-0.35	
Batch	13/47/23	45109		68382	

Measuring hydraulic conductivity

Hydraulic conductivity was measured by a slight modification in the method of Melcher *et al*. (2012) (Fig. 1). A piece of 15 cm of flower stem end was cut with a sharp blade under distilled water. The upper part of the stem (part 1 in Fig. 1) was inserted into a silicon tube (part 2 in Fig. 1) (internal diameter 4 mm) filled with degas distilled water and the basal part of the stem (part 3 in Fig. 1) was kept in the degas distilled water. Using a three-way glass valve (part 4 in Fig. 1), the silicon tube was connected from one side to the degassed distilled water tank (part 5 in Fig. 1) and from the other side was attached into a U-shaped pipe (part 6 in Fig. 1) below the stems end. The whole set (stem, three-way glass valve, degas distilled water tank and U-shaped pipe) was fixed (Van Ieperen *et al*., 2002). The stem vase was placed on a digital scale (part 7 in Fig. 1) connected to the computer and the stem and degas distilled water weight changes were recorded at time regular intervals (30 minutes). Fifty cm head pressure of water (*h*; which made 5 kPa pressure) was applied, so that water had passed through the segments. The flow rate was then determined by measuring the volume of the passed water. Three stem segments were used for each treatment (Ichimura *et al*., 2005).

Measurements of stem hydraulic conductivity involves measuring the flux for a given driving force ($Q/\Delta P$; where ΔP is the pressure drop across the segment), normalized by the length of the stem segment and referenced either to the cross-sectional area of the stem (Melcher *et al*., 2012). In equation 1, K (cm/min) is hydraulic conductivity, Q (cm^3/min) is the recorded flux (gravimetric or volumetric flow rate), L (cm) is the length of the measured segment, A (cm^2) is the cross-sectional area of the stem segment and h (cm) is head pressure of water height. The data were collected at days 2, 4, 6 and 8 after pulse treatment.

$$K = QL/Ah) \qquad (1)$$

To observe the microscopic effects of chemicals and EOs on stem closure and hydraulic conductivity during the vase life, 2 cm of treated stem was used for histological study. The cut stem segments (3-5 cm in length) were stored in a solution of FAA (formalin (40%): glacial acetic acid (50%): ethyl alcohol (70%):

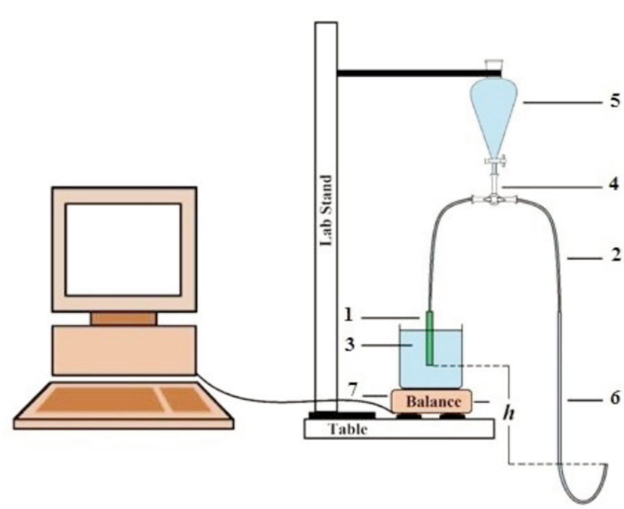

Fig. 1 - Hydraulic conductivity system scheme; the upper part of the stem (1); silicon tube (2); the basal part of the stem (3); the three-way glass valve (4); the degas distilled water tank (5); the U-shaped pipe (6); the digital scale (7); h: water height.

[13:5:200]) to preserve the tissue before sectioning. Stem transverse sections, at 16-µm thickness, were made using a manual rotary microtome (Leitz 1512, Germany) after fixing in FAA and permanent mounts were prepared in paraffin wax. Cross sections were stained with Safranin O/Fast Green Stain method and embedded on microscope slides. Digital images were made at 10X magnification with a digital camera (Olympus DP71, Japan) attached to a light microscope (Olympus BH2, Japan) and computer.

Flower water content

Flower water content (*WC*) was measured as mentioned in equation 2. Flower fresh weights (*FW*) were assessed at the beginning of the experiment and flower dry weights (*DW*) were recorded after drying to constant weight in an oven for at least 48 h at 85°C. Water content was calculated for three replicates (He *et al*., 2006; Lu *et al*., 2012).

$$WC = (FW-DW)/DW * 100 \qquad (2)$$

The experiment was conducted in the laboratory at 20-22°C, 40-50% RH, and 15 µmol/m^{2}s light intensity (cool white florescent tubes) under a daily light period of 12 hours. The obtained data were analyzed using MSTAT-C program and mean comparison was done using LSD range test.

3. Results

Flower vase life

Results of this study showed that in single effect of applied treatments, all NS pulse treatments markedly (P<0.01) extended vase life of gerbera 'Rosalin' cut flowers. The 10 mg/L NS pulse treatment gave the longest vase life (12.20 days) as compared to the other treatments (Table 3). SA 100 mg/L (9.08 days) pulse treatment significantly increased flower vase life compared to the control (8.13 days). However, there was no significant difference between NS 5 mg/L and SA 100 mg/L.

The single effect of applied treatments indicated that peppermint and thyme EOs (100 mg/L) application in preservative solutions as permanent treatment (Fig. 2) could extend the vase life of gerbera cut flowers to 9.98 and 10.35 days respectively (Table 3). However, there was no significant difference among these two treatments and control in extending vase life. Flowers placed in sucrose 4% and clove EO 300 mg/L had the least (8.83 days) vase life (Table 3).

The results showed that the interaction of pulse and permanent solutions had remarkable effect on flower longevity. The best treatments were NS 10 mg/L + thyme EO 100 mg/L (14.25 days) and NS 10 mg/L + peppermint EO 100 mg/L (14 days) which had significant difference with other treatments (Table 4). It has been observed that SA 50 mg/L + 4% sucrose was less effective than control on flower postharvest life and sucrose had negative effect on gerbera flowers life (Table 4).

Flower water content

Flower water contents for the pulse treatments with distilled water and 50 mg/L SA were a little higher than other treatments (Table 5). In 4% sucrose per-

Table 3 - The simple effect of pulse and permanent treatments on 'Rosalin' gerbera cut flower vase life (day)

Treatments	Vase life (Day)
Pulse treatment	
Distilled water	8.13 d
SA 50 mg.L^{-1}	8.30 cd
SA 100 mg.L^{-1}	9.08 bc
NS 5 mg.L^{-1}	10.02 b
NS 10 mg.L^{-1}	12.20 a
Permanent treatment	
Distilled water	10.02 a
Sucrose	8.55 b
Peppermint EO	9.98 a
Thyme EO	10.35 a
Clove EO	8.83 b

The means showing similar letters in each column have no significant difference according to the LSD range test (P<0.01).

Fig. 2 - Gerbera 'Rosalin' cut flowers treatment by NS (10 mg/L) and peppermint EO (100 mg/L). During pulse treatment, vase solution was covered by dark plastic coverage to prevent undesirable light reaction in NS.

manent treatment flowers, water content declined more rapidly during the vase life period and was significantly different from the others. Generally, the interaction of pulse and permanent treatment showed that 4% sucrose had negative effect especially after 10 mg/L NS application as pulse treatment (Table 4).

Hydraulic conductivity

The hydraulic conductance of the stem end segments did not change over the first 2 days after pulsing and had very low rates. Thereafter it changed over time and increased slightly on day 4. The rate of stem flower hydraulic conductivity sharply increased at day 6 and 8 after pulse treatment application. Hydraulic conductivity of stems treated with 5 and 10 mg/L NS pulse markedly showed lower rate during the experiment compared to other treatments (Table 6).

In addition, permanent treatments had significant effect on hydraulic conductance throughout assessment. Result showed that peppermint EO 100 mg/L had lower look rate in hydraulic conductance than other solutions (Table 7).

However, hydraulic conductance of the stem segment was nearly the same at the initial day of the experiment. The interaction of pulse and permanent treatment determined that NS 10 mg/L + peppermint EO 100 mg/L and NS 10 mg/L + thyme EO 100 mg/L had the lowest rate even in day 8 after pulsing (Table 8). In the NS 10 mg/L + peppermint EO 100 mg/L flowers, the hydraulic conductance of the stem segments slightly increased thereafter. Hydraulic con-

Table 4 - The effect of different treatments' interactions on 'Rosalin' gerbera cut flower vase life (day) and water content (%)

Treatment	Vase life (Day)	Water content (%)
Distilled water/Distilled water	9.58 efg	90.95 ab
Distilled water/Sucrose	8.08 i	86.15 c
Distilled water/Peppermint EO	7.83 ij	91.29 a
Distilled water/Thyme EO	8 i	91.39 a
Distilled water/Clove EO	7.17 jk	91.21 a
SA 50 mg.L^{-1}/Distilled water	9.08 fgh	91.31 a
SA 50 mg.L^{-1}/Sucrose	7 k	85.02 c
SA 50 mg.L^{-1}/Peppermint EO	8.08 i	91.09 a
SA 50 mg.L^{-1}/Thyme EO	8.92 gh	90.97 a
SA 50 mg.L^{-1}/ Clove EO	8.42 hi	91.15 a
SA 100 mg.L^{-1}/Distilled water	9.83 def	90.54 ab
SA 100 mg.L^{-1}/Sucrose	8.58 hi	83.44 d
SA 100 mg.L^{-1}/Peppermint EO	9.17 fgh	90.45 ab
SA 100 mg.L^{-1}/Thyme EO	9.83 def	90.73 ab
SA 100 mg.L^{-1}/ Clove EO	8 i	89.46 b
NS 5 mg.L^{-1}/Distilled water	9.67 efg	90.55 ab
NS 5 mg.L^{-1}/Sucrose	8.58 hi	82.68 d
NS 5 mg.L^{-1}/Peppermint EO	10.83 c	90.81 ab
NS 5 mg.L^{-1}/Thyme EO	10.75 c	90.56 ab
NS 5 mg.L^{-1}/ Clove EO	10.25 cde	90.42 ab
NS 10 mg.L^{-1}/Distilled water	11.92 b	90.52 ab
NS 10 mg.L^{-1}/Sucrose	10.50 cd	81.1 e
NS 10 mg.L^{-1}/Peppermint EO	14 a	91.36 a
NS 10 mg.L^{-1}/Thyme EO	14.25 a	91.22 a
NS 10 mg.L^{-1}/ Clove EO	10.33 cde	90.99 a

The means showing similar letters in each column have no significant difference according to the LSD range test (P<0.01).

Table 5 - The effect of pulse and permanent treatments on 'Rosalin' gerbera cut flower water content (%)

Treatment	Water content (%)
Pulse Treatment	
Distilled water	90.20 a
SA 50 mg.L^{-1}	89.91 a
SA 100 mg.L^{-1}	88.93 b
NS 5 mg.L^{-1}	89.00 b
NS 10 mg.L^{-1}	89.04 b
Permanent treatment	
Distilled water	90.77 a
Sucrose	83.68 b
Peppermint EO	91.00 a
Thyme EO	90.97 a
Clove EO	90.65 a

The means showing similar letters have no significant difference according to the LSD range test (P<0.01).

Table 6 - The effect of pulse treatments on trends of hydraulic conductivity of gerbera cut flower stem on day 2, 4, 6 and 8 of the experiment

Pulse treatment	K (cm/min)			
	Day 2	Day 4	Day 6	Day 8
Distilled water	0.57 d	5.42 a	7.58 b	8.95 c
SA 50 mg.L^{-1}	1.04 b	5.35 a	12.96 a	9.17 c
SA 100 mg.L^{-1}	1.72 a	6.44 a	10.42 a	15.47 a
NS 5 mg.L^{-1}	0.84 c	2.50 b	4.45 c	13.08 b
NS 10 mg.L^{-1}	0.57 d	0.91 c	2.05 d	4.97 d

The means showing similar letters in each column have no significant difference according to the LSD range test (P<0.01).

ductivity of other treated flowers increased sharply after day 6 during the rest of the vase life (Table 8).

The survey of slope trend of each treatment during the experiment's period, showed that the lowest slope was observed in NS 10 mg/L + peppermint EO 100 mg/L and NS 10 mg/L + thyme EO 100 mg/L respectively. While in other treatments, slope trend was enhanced so that the most slope trend was considered in 50 mg/L SA + 4% sucrose.

In addition, histological study showed that in the NS 10 mg/L + peppermint and thyme 100 mg/L EOs treated flowers stem remained healthy for longer period while the other stems became hollow after a few days (Fig. 3 and 4).

Fig. 3 - The gerbera cut flower healthy stem.

Table 7 - The effect of permanent treatments on trends of hydraulic conductivity of gerbera cut flower stem on day 2, 4, 6 and 8 of the experiment

Permanent treatment	K (cm/min)			
	Day 2	Day 4	Day 6	Day 8
Distilled water	0.81 c	5.14 a	8.66 b	14.62 a
Sucrose	1.46 a	4.93 ab	13.50 a	12.76 a
Peppermint EO	0.84 c	2.74 c	3.61 d	6.25 c
Thyme EO	0.69 d	3.65 bc	6.00 c	8.77 b
Clove EO	0.94 b	3.52 bc	5.07 cd	9.25 b

The means showing similar letters in each column have no significant difference according to the LSD range test (P<0.01).

Table 8 - The trends of hydraulic conductivity of gerbera cut flower stem in all treatment on day 2, 4, 6 and 8 of the experiment

Treatment	K (cm/min)				Slope trend
	Day 2	Day 4	Day 6	Day 8	
Distilled water/Distilled water	0.42 ghi	5.60 bcd	9.41 cde	17.04 cd	53.664
Distilled water/Sucrose	0.64 fg	5.96 abcd	8.88 def	14.04 ef	43.117
Distilled water/Peppermint EO	0.21 hi	4.89 cde	4.62 ghi	0.00 j	5.16
Distilled water/Thyme EO	0.62 fg	4.52 de	6.41 fg	0.00 j	52.384
Distilled water/Clove EO	0.96 de	6.14 abcd	9.23 cde	13.69 f	41.263
SA 50 mg.L^{-1}/Distilled water	0.76 ef	7.91 a	11.28 cd	14.95 def	45.931
SA 50 mg.L^{-1}/Sucrose	1.14 d	7.58 ab	43.58 a	0.00 j	16.337
SA 50 mg.L^{-1}/Peppermint EO	1.51 bc	1.94 fgh	2.74 ij	0.00 j	12.728
SA 50 mg.L^{-1}/Thyme EO	0.57 fg	4.39 de	9.54 cd	14.21 ef	46.081
SA 50 mg.L^{-1}/ Clove EO	1.19 cd	5.67 bcd	12.34 c	16.71 cde	53.215
SA 100 mg.L^{-1}/Distilled water	1.53 b	7.30 ab	11.33 cd	14.69 def	43.499
SA 100 mg.L^{-1}/Sucrose	1.67 ab	6.87 abc	21.41 b	21.51 a	74.065
SA 100 mg.L^{-1}/Peppermint EO	1.83 ab	5.17 cd	6.60 efg	20.43 ab	57.224
SA 100 mg.L^{-1}/Thyme EO	1.67 ab	7.95 a	10.99 cd	20.72 ab	60.198
SA 100 mg.L^{-1}/ Clove EO	1.94 a	5.10 cd	5.31 gh	0.00 j	5.769
NS 5 mg.L^{-1}/Distilled water	1.04 de	4.22 de	8.54 def	18.74 bc	5.741
NS 5 mg.L^{-1}/Sucrose	1.91 a	3.18 ef	5.28 gh	22.59 a	64.126
NS 5 mg.L^{-1}/Peppermint EO	0.52 fgh	2.27 fg	4.69 ghi	10.40 g	32.056
NS 5 mg.L^{-1}/Thyme EO	0.45 ghi	1.97 fgh	4.26 ghi	7.48 hi	23.375
NS 5 mg.L^{-1}/ Clove EO	0.40 ghi	1.07 ghi	0.67 k	6.19 i	16.963
NS 10 mg.L^{-1}/Distilled water	0.40 ghi	1.51 ghi	3.93 hi	7.68 ghi	24.278
NS 10 mg.L^{-1}/Sucrose	1.91 a	1.86 fgh	4.79 ghi	5.65 i	14.158
NS 10 mg.L^{-1}/Peppermint EO	0.16 i	0.12 i	0.28 k	0.41 j	0.0904
NS 10 mg.L^{-1}/Thyme EO	0.21 i	0.57 hi	0.94 jk	1.42 j	0.4012
NS 10 mg.L^{-1}/ Clove EO	0.23 hi	0.67 hi	1.02 jk	9.65 gh	28.601

The means showing similar letters in each column (Day) have no significant difference according to the LSD range test (P < 0.01).

Fig. 4 - The gerbera cut flower hollow stem.

3. Discussion and Conclusions

Flower vase life

NS particles enter into cell, tissue and organs, so they can replace with silver salts (such as silver nitrate or silver thiosulfate) in preservative solutions. NS inhibits the respiration and electron transfer system and material transfer in microbial cell membrane (Paull and Lyons, 2008). Various researches indicated that flowers treated with NS solution, had more vase life. Silver ions, because of small size, have more contact with outer space and influence more on their environment. NS, in comparison with silver ions, showed antimicrobial property at inferior concentration (Solgi *et al*., 2009; Ansari *et al*., 2011)

Different studies determined antimicrobial effects of main components of thyme (Nikolić *et al.*, 2014 a), peppermint (Kazem Alvandi *et al.,* 2011; Nikolić *et al.*, 2014 b) and clove EO (Boukaew *et al.,* 2017). In addition, Amini *et al*. (2014) reported that thyme EO in pulsing with distilled water treatment showed the best results for extending cut gerbera flower vase life and preventing more weight loss. Hydrophobicity is an important characteristic of thyme and peppermint EOs. This enables them to separate the lipid components of the bacterial cell membrane and mitochondria, binding to membrane proteins and releasing lipopolysaccharides, which results in disturbing cell wall structures (Solgi *et al*., 2009).

Researches indicated also that SA pulse treatment, followed by NS as permanent solution (Danaee *et al*., 2013), SA utilization as permanent treatment (Jamshidi *et al*., 2012) significantly promoted the vase life of gerbera cut flowers. However, in this study, SA had no special effect on flower longevity. Although Ziyaei Movahed *et al*. (2010) reported that clove EO increased gerbera vase life. In this study, it had the least effect on postharvest life of gerbera cut flowers. Despite of sucrose important role in extending the vase life of cut flowers, Ansari *et al.* (2011) reported a negative effect on 'Rosalin' gerbera cut flowers. The main reason could be severe bacterial growth in vase solution.

Flower water content

Cut flowers and foliage can have limited commercial value because they dehydrate during vase life because of water uptake decrease. Water deficit could develop even when cut flowers are placed in water (Nazari Deljou, *et al*., 2012). Gerbera cut flowers stem break of is mainly caused by water shortage in the flowers due to the increased difficulty of water flow from the water source to petals. It is also supposed to be a competition for available water between flower heads and stems. The increase in flow resistance leads to stem break as a result of microbial activity in the vase water (Balestra *et al*., 2005). It could be concluded from the results of this study that in all treatments with antimicrobial agents such as NS, SA and thyme, peppermint and clove EOs flower water contents were high and had no harmful decrease.

Hydraulic conductivity

Many experiments were performed to find out the cause of stem bend in gerbera cultivars. Research showed that removal of the floral head prevented stem bending, indicating that bending is physically due to the gravitational pull on the floral head. Stem bending in cut gerbera can be due to lack of mechanical support. Bending might relate to lack of wall thickening, particularly in the xylem. At least two other factors might contribute to mechanical stem strength. The first factor is gerbera stems elongation during vase life. Elongation zones usually have weakly developed xylem and sclerenchyma. Since the stems are usually placed in water under an angle, stem elongation will increase the gravitational pull of the floral head result in earlier stem bending. The second factor is the presence of a cavity in the center of the gerbera stem. Observations showed the cavity at the time of harvest, in several cultivars (Perik *et al*., 2012). Other factors affecting stem bending could be adverse water relations such as lack of turgor. After a few days of vase life, there are many bacteria in the vase solution. Stem bending can be due to xylem blockage by bacteria, which results in low water uptake. As transpiration is not inhibited as much as water uptake, net water loss occurs followed by the loss of turgor and stem bending (Van Meeteren, 1978).

Generally, in cut flowers fresh weight decreased before stem bent occurred, and this is accompanied by a decline in absorption of water by the flowers. Stem break could be prevented by pretreatment of the stems with NS by adding to the vase water. Van Meeteren (1978) suggested that there are two different pathways for water uptake: a direct one through the xylem vessels at the cut surface and an indirect one through the cavity in the stem. Only the direct water uptake is strongly inhibited by growth of bacteria in the vase water. Stem bend occurs when the direct water uptake is inhibited by bacterial activity. Van Meeteren (1978) suggested that the minimum concentration of silver nitrate could avoid stem bending and inhibit bacterial growth in the water. There is an association between a high population density of bacteria in the water and scape bending (Van Doorn and De Witte, 1994). The results of this experiment also showed that bacteria would block the main water pathway (xylem vessels) over the time and the stem would become hollow (Fig. 3 and 4). So that active water uptake is effectively prevented. Bacterial activity could be significantly inhibited by adding NS (as pulse treatment), peppermint (1-menthol and menthone) and thyme (thymol) EOs (as permanent treatment) to vase solution.

Based on the results of this study, new antimicrobial agents such as NS, thyme and peppermint EOs had a positive effect on flower vase life and water content. It might be due to this fact that these are very effective antimicrobial agents, which inhibited the microbial growth and prevented bacterial plugging in conducting tissues. However, exogenous supply of sugars can increase water balance and osmotic concentration and plays an important role in lengthening the vase life of cut flowers, but in this research, sucrose had negative effect on flower water contents and gerbera 'Rosalin' vase life period. This issue can be related to the negative effect of sucrose on microbial growth. Although, there was no available data about the effect of EOs on stem hydraulic conductivity changes in cut flowers, this research showed that EOs could improve hydraulic conductance and keep it in a normal and stable condition for longer period.

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