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Evaluation of Cut Carnation Longevity cv. 'Yellow Candy' under Treatment with Chemical and Organic Antimicrobial Compounds

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Abstract

Carnation (*Dianthus caryophyllus* L.) is valuable in terms of production and exports in the world. Increased vase life of cut flowers is an important factor for their market acceptability. For this purpose this study was carried out to examine the effect of plant essential oils as compared to 8-hydroxy-quinoline sulfate on the control of bacteria population and the longevity of carnation cut flowers on the basis of a complete randomized design with 14 treatments, control (distilled water), control + alcohol (2%), 8-HQS at three levels (100, 200 and 400 mg l^{-1}), and the essential oils of dill seeds, caraway seeds and aromatic geranium, each one at three levels (50, 100 and 150 mg l^{-1}) continuously with 3 replications, 42 plots – each plot including 5 flowers. The recorded traits included vase life, vase solution bacteria population, fresh weight loss, increasing the soluble solids, ionic leakage, ethylene production, chlorophyll a and b. The results of this study showed that the longest vase life was obtained from the treatment of alcohol 2% with 15.83 days. Also, among the treatments containing essential oil, dill (100 mg l^{-1}), geranium (50 mg l^{-1}), caraway (100 mg l^{-1}) and 8- hydroxyquinoline sulfate (400 mg l^{-1}) as better treatments are considered.

Keywords: Alcohol, Carnation, Longevity, Plant essential oils, 8-HQS.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) belongs to the Caryophyllaceae family (Ghasemi Ghahsareh and Kafi, 2009) is one of popular cut flowers for producers and consumers due to its high ability to maintain quality, its portability to far distances and its remarkable ability to absorb water after prolonged transportation (Khalighi and Shafiee, 2000). Ethylene has an important role in the regulation of flowers senescence and its production rate is increased with senescence of flowers (Ketsa and Rugkong, 2000). In addition, carnation are very sensitive to accumulation of bacteria at the stem end or in the vase solution that can cause the vascular obstruction and reduction of vase life (van Doorn *et al*., 1991).

Antimicrobial-activity of essential oils depends on their chemical compositions (alcohol, phenols, aldehydes, ketones and ...), so that the higher amount of phenolic and alcoholic compounds increases the antibacterial property (Sivropoulou *et al*., 1996). Hashemabadi *et al*. (2013) reported that the use of 30% *Artemisia* extraction and 200 mg l^{-1} rifampicin had the most efficiency and enhancing impact on postharvest quality of cut chrysanthemum cv. 'White'. Amini *et al.* (2016) showed that the use of thyme essential oil with a concentration of 200 ppm increased longevity in Sensi cultivar by 2.34 days.

Kuiper *et al.* (1995) believed 8-hydroxy-quinoline is a bactericide substance and an environment-acidifying agent that in addition to prevent the growth of bacteria and reduce the environment pH, it also prevents vessel closure in cutting cross caused by sedimentation of various chemical materials. Liao *et al.* (2000) reported that the vase life of cut roses using silver thiosulfate, 8-hydroxy-quinoline and sucrose, as preservative solution increased noticeably. Ethanol is ethylene synthesis inhibitors and the disinfecting effect of preventing the growth of bacteria in the vase solution and increased the vase life (van Doorn, 1998). In one experiment on the carnation, the results showed that the temporary and permanent use of 4% ethanol increases the vase life and the marketability of the flowers (Bayat *et al*., 2012). Considering the importance of the use of non-toxic substances to increase the postharvest life, the use and comparison of essential oils of aromatic geranium, caraway and dill with 8-HQS to control the population of bacteria and postharvest vase life of cut flowers of carnation, are the main objective of this research.

MATERIALS AND METHODS

Cut carnation cv. 'Yellow Candy' was purchased from a commercial producer in Tehran. Harvested flowers inside commercial packaging were transferred to the postharvest laboratory. After uniform flowers together in terms of the size, they were recut from a height of 40 cm to prevent the vascular obstruction. After weighing, they were put inside the vase solution. 5 flowers were placed in plastic vases with the volume of 500 ml. To compare the effectiveness of different essential oils with 8-HQS, an experiment was performed based on completely randomized design with 14 treatments including essential oils of aromatic geranium, caraway and dill at 3 concentrations (50, 100, 150 mg l^{-1}), 8-HQS at 3 concentrations (100, 200 and 400 mg l^{-1}), control (distilled water) and control along with alcohol (2%), for 3 repeats at 42 plots with 210 cut flowers. Experiment conditions were provided at a temperature of 20±2 ◦C, relative humidity of 65±5 %, the light intensity of 12 µ mol s^{-1} m^{-2} from fluorescent white light source and the lighting duration of 12 hours. Traits of vase life, vase solution bacteria population, fresh weight loss, the increase in the soluble solids, ionic leakage, ethylene, chlorophyll a and b were measured.

Preparation and analysis of essential oils

Essential oil distillation was performed for caraway seeds, dill seeds and aerial organs of aromatic geranium using distillation with water by means of Clevenger apparatus and essential oils were analyzed using gas chromatography/mass spectroscopy (GC/MS) (Tables 1, 2 and 3).

Table 1. Caraway seed essential oil analysis using GC/MS.

Compound	Composition (%)	Retention Indices	Compound	Composition (%)	Retention Indices
1-phellandrene	0.27	8.05	Trans sabinene hydrate	0.14	16.18
α-pinene	0.74	8.38	linalool	0.1	16.08
sabinene	0.75	10.06	4-Terpineol	0.86	20.35
2-β-pinene	1.32	10.03	thymol	0.1	20.81
β-myrcene	0.59	10.76	3-Cyclopentyl cyclopentane 1-N	2.2	21.16
α-Terpinene	0.25	12.07	Propanal 2-methyl 3-phenyl	26.05	23.73
P-cymene	7.11	12.49	Phellandral	0.17	25.13
limonene	3.35	12.69	α–thujenal	11.66	25.65
1,8-cineole	0.1	12.82	1-phenyl 1-butanol	20.72	25.92
γ-Terpinene	21.86	14.16	1,4-Cyclohexadiene 1-methanol	0.1	12.72
α-terpinolene	0.38	15.34	-	-	-

Table 2. Aromatic geranium essential oil analysis using GC/MS.

Compound	Composition (%)	Retention Indices	Compound	Composition (%)	Retention Indices
Spathulenol	0.67	1656	GERANIOL	13.03	1293
6-Octen-1-ol, 3,7-dimethyl	0.15	1543	LINALOOL	1.60	1114
ALPHA.-PINENE	0.12	955	Cyclohexanone	5.50	1202
beta.-Citronellol	22.90	1358	Butanoic acid	4.70	2064
1H-Cycloprop[e]azulene	0.12	1459	6-Octen-1-ol	8.50	1455
1H-Cyclopropa[a]naphthalene	0.10	1597	alpha-Amorphene	1.77	1528
BETA-BOURBONENE	0.94	1447	Geranyl tiglate	3.24	1202
beta.-Cubebene	0.78	1534	Isoaromadendrene epoxide	0.19	1743
CADINA-1,4-DIENE	0.15	1590	Caryophyllene oxide	2.32	1668
cis-2,6-Dimethyl-2,6-octadiene	4.81	2019	Geranyl propionate	0.26	1965
GERMACRENE-D	2.87	1549	L-(-)-Methyl	0.10	1222
CIS-ROSE OXIDE	0.81	1128	1,6-Octadien-3-ol, 3,7-dimethyl	0.65	1275
Delta cadinene	0.42	1495	1,6-Octadien-3-ol, 3,7 dimethyl (R)	7.93	1293
Epizonarene	0.84	1583	E-Citral $$ 2,6-Octadienal, 3,7	0.67	1305
6-Octen-1-ol, 3,7-dimethyl- (R)	0.16	1266	alpha.-Copaene	1.10	1427
Cycloundecatriene-4,7,10	1.64	1520	4,7,10-Cycloundecatriene	1.64	1520
gamma.-Elemene	0.17	1647	1,2 Benzenedicarboxylic acid	0.32	2006
delta.-Cadinene $$ Naphthalene	0.44	1563	CITRONELLA	0.51	1167
Citral	0.61	1305	TRANS-ROSE OXIDE	0.3	1149
Naphthalene	1.0	1610	alpha.-Amorphene	1.77	1528
3,7-GUAIADIENE	0.32	1495	-	-	-

Table 3. Dill seed essential oil analysis using GC/MS.

Compound	Composition (%)	Retention Indices
Linalool	52.23	1096
Cuminal	19.96	1210
Limonene	4.83	1032
P-cymene	4.72	1089
γ-Terpinene	4.59	1055
Safranal	4.01	1123
Myristic Acid	1.52	1210
Acetate Granyl	1.1	1099
α-pinene	0.92	938
Thujadein	0.72	1024
Nonanol	0.4	1325
Cyclohexanol	0.35	1245
α-Terpinene	0.27	1015

Traits Measurement

Vase life

Criteria for evaluating the vase life of carnation were petals wilt and their in rolling that was expressed by the number of days (Fig. 1).

Fig. 1. Comparison of flowers status on the first day of the experiment and at the end of the vase life.

Vase solution bacteria population

24 hours after starting the experiment, the vase solutions were sampled and bacteria were counted by Liu *et al.* (2009) method. The vase solutions were sampled and they were diluted with 0.9 % normal saline solution. 0.1 ml of the above solution was put on the agar and the bacterial colonies were recorded after 24 hours incubation at 37 °C.

Fresh weight loss

To measure fresh weight loss of flowers during the evaluation period the flower was weighted using a digital scale, then it was calculated using the following formula:

Fresh weight loss = initial fresh weight - (final fresh weight in final day + recuts weight)

Increasing brix degree

To measure the degrees of brix, one or two drops of extract in recuts of the branches end

was poured on the glass plate of refractometer model N-1α made in ATAGO Japan company and its brix degree was read and the increase in brix degree was calculated by the difference between the brix degree of last day and the first day.

Petal ionic leakage

0.5 g of petals of each plot was placed inside a closed container containing 50 ml of distilled water at room temperature for 24 hours and electrical conductivity (EC1) of solution was calculated using EC meter device. Then, 0.5 g of petals was freeze for 24 hours and petals then placed at room temperature for 24 hours and EC2 of solution was read with EC meter (Kaya *et al.*, 2001; Ben Hamed *et al*., 2007) and ionic leakage percentage was calculated from the following equation:

$$EC\ (\%) = (EC1 \div EC2) \times 100.$$

Ethylene production

To measure ethylene production, a flower was placed in an airtight jar for 24 hours. After 24 hours, the gas in the jar was sampled. Measurement of ethylene was carried out using GC-8 AIT model Shimadzu (Hashemabadi, 2007).

Chlorophyll a and b

Measurement of chlorophyll content was performed using extraction from the leaf with 80% acetone using Mazumdar and Majumdar (2003) method, then it was measured by spectrophotometer at 660 and 643 nm wavelengths and chlorophyll content in milligrams per gram of fresh weight of leaf was calculated using the following formula:

$$Chl\ a = 93.9\ (A_{660}) - 0.777\ (A_{643})$$

$$Chl\ b = 17.6(A_{643}) - 2.81\ (A_{660}).$$

Data analysis

Data analysis was performed using software MSTATC, the mean comparison was performed using LSD test and EXCEL software was used for drawing the diagrams.

RESULTS

Vase life

According to the ANOVA, the effect of treatments on vase life was significant at the 5% statistically level (Table 4). Results of means comparison of different treatments on vase life trait showed that all factors have increasing effect on the vase life of cut flowers of carnation comparing to the control and the greatest impact was related to the treatment of alcohol 2% (15.83 days), however, treatment of 400 mg l^{-1} 8-HQS (14.83 days), 100 mg l-1 of dill essential oils (15.5 days), 50 mg l^{-1} of geranium essential oils (15 days) and 100 mg l^{-1} of caraway essential oils (14.5 days) had no significant difference with the treatment of alcohol (Fig. 2).

Table 4. Analysis of variance for effect of different treatments on evaluated characters in cut carnation.

S.o.V	df	Mean Square (MS)							
		Vase life	Vase solution bacteria	Fresh weight loss	Increasing brix degree	Ionic leakage	Ethylene production	Chlorophyll a	Chlorophyll b
Treatments	13	8.92*	149422**	28.08*	0.353**	128.2**	11.93**	3.441**	2.002**
Error	28	3.52	6154	13.05	0.099	18.99	0.233	0.000086	0.00016
CV (%)		14.0	33.87	18.08	23.59	19.81	8.57	0.195	0.585

*, **: Significant at the 5% and 1% statistically levels, respectively.

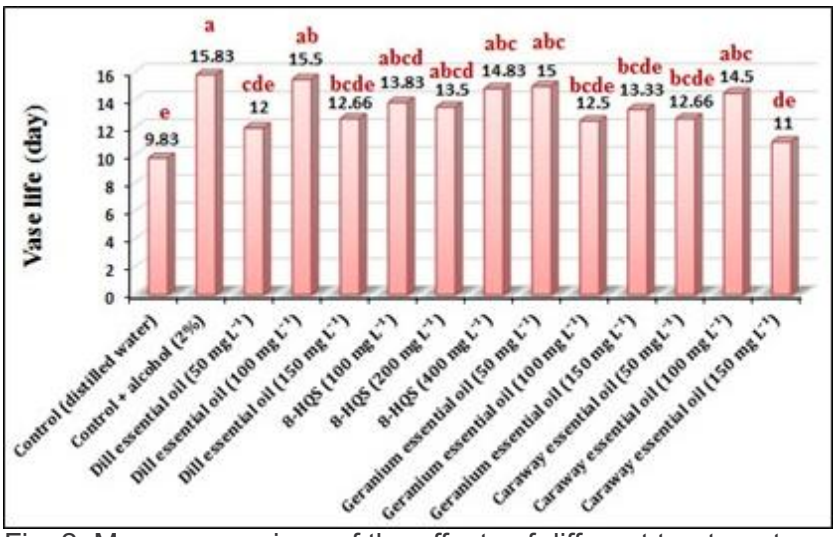

Fig. 2. Mean comparison of the effects of different treatments on the vase life of carnation.

Vase solution bacteria population

According to Table 4, the effect of different treatments on vase solution bacteria populations is significant at the 1% statistically level. Results showed that vase solution bacteria populations was maximum in the control treatment and the lowest bacteria populations was observed in treatment of 200 and 400 mg l-1 of 8-HQS, however, treatments of 100 mg l-1 8-HQS, 50, 100 and 150 mg l-1 of geranium essential oils and 100 mg l-1 of caraway essential oils had no significant difference with them (Fig. 3).

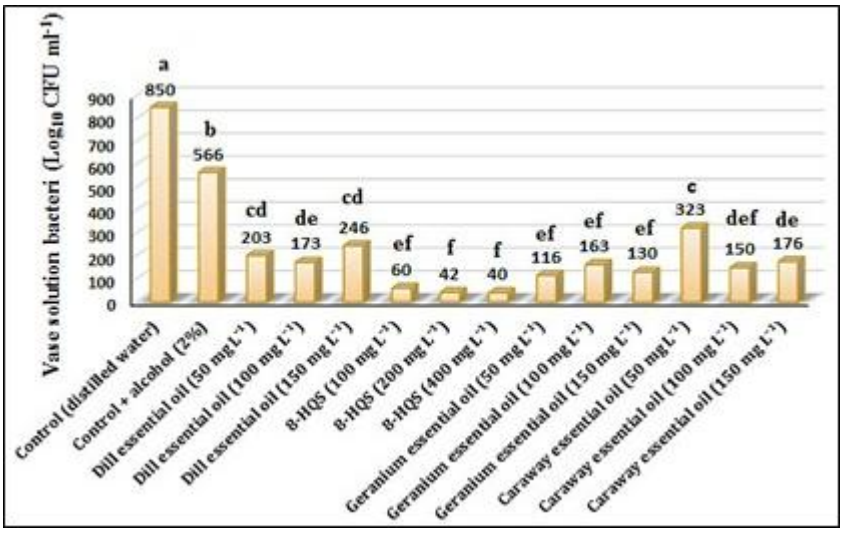

Fig. 3. Mean comparison of the effects of different treatments on the vase solution bacteria populations of carnation.

Fresh weight loss

Based on statistical analysis, carnation cut flowers fresh weight was significant at 5% level (Table 4). The results showed that treatments of alcohol 2%, 50 and 100 mg l^{-1} caraway essential oils had the minimum loss and other treatments had high weight loss (Fig. 4).

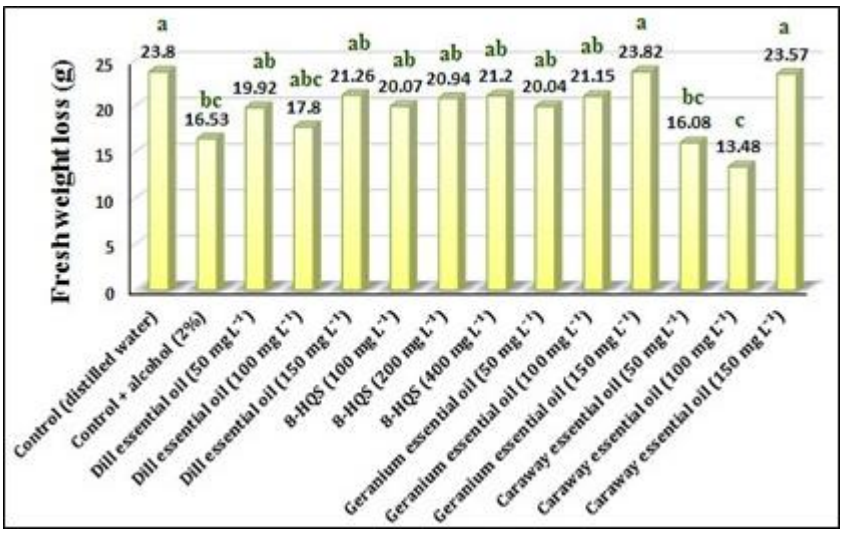

Fig. 4. Mean comparison of the effects of different treatments on fresh weight loss of carnation.

Increasing brix degree

According to the results presented in Table 4, the effect of different treatments on the increase in brix degree was significant at statistical level of 1%. 150 mg l^{-1} of aromatic geranium essential oil treatment had the highest amount of soluble solids and control treatment compared with other treatments had the least amount of total soluble solids (Fig. 5).

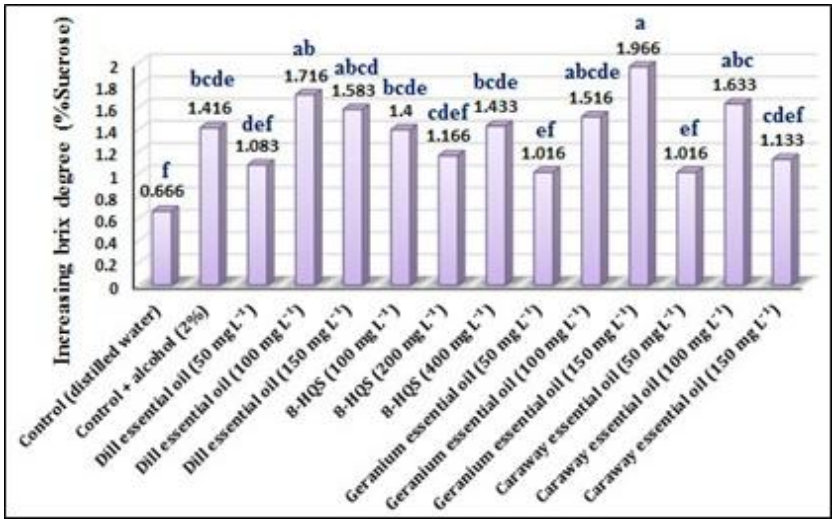

Fig. 5. Mean comparison of the effects of different treatments on increase brix degree of carnation.

Petal ionic leakage

The effect of different treatments on ionic leakage of carnation cut flowers was significant at 1% level (Table 4). The ionic leakage has an increase in treatments of treatment of 150 mg l^{-1} of caraway essential oils, 150 mg l^{-1} of geranium essential oils and 100 mg l^{-1} 8-hydroxy quinoline, but in other treatments, it was dramatically lower than the control treatment (Fig. 6).

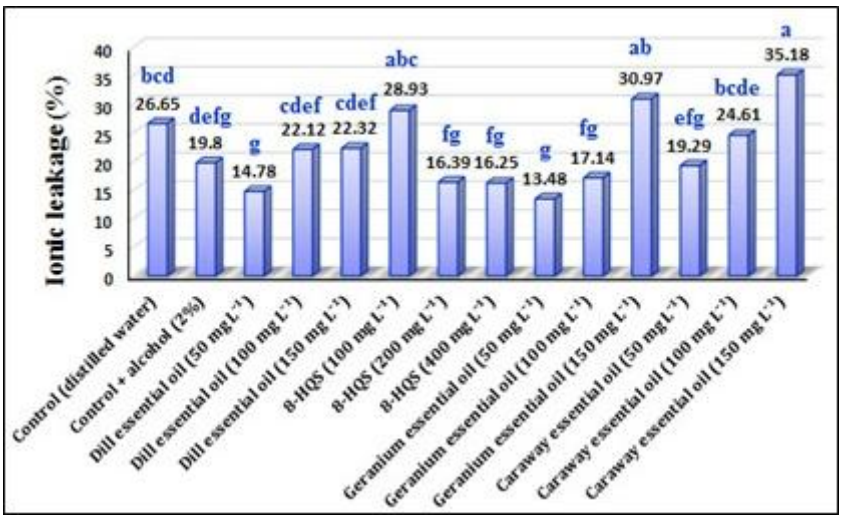

Fig. 6. Mean comparison of the effects of different treatments on ionic leakage carnation petal.

Ethylene production

The effect of different treatments on the amount of ethylene production in cut flowers carnation in 1% level was significant (Table 4). The highest amount of ethylene production was related to the treatment of 400 mg l^{-1} of 8-HQS, however, 100 mg l^{-1} of 8-hydroxy quinoline had no significant difference with it and treatment of dill essential oil with a concentration of 50 mg l^{-1} had the lowest value, but treatment of 150 mg l^{-1} of dill essential oil had not significant difference with it (Fig. 7).

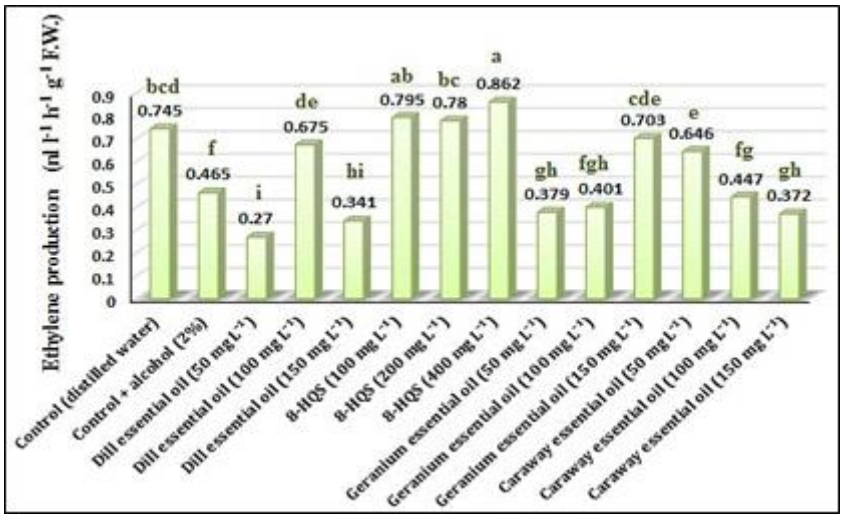

Fig. 7. Mean comparison of the effects of different treatments on ethylene production of carnation.

Chlorophyll a and b

The effect of different treatments on chlorophyll a and b of carnation cut flowers was significant at 1% level (Table 4). Treatment of dill essential oil with a concentration of 150 mg l^{-1} had the lowest value and 400 mg l^{-1} 8-HQS had highest amount of chlorophyll a and b (Figs. 8 and 9).

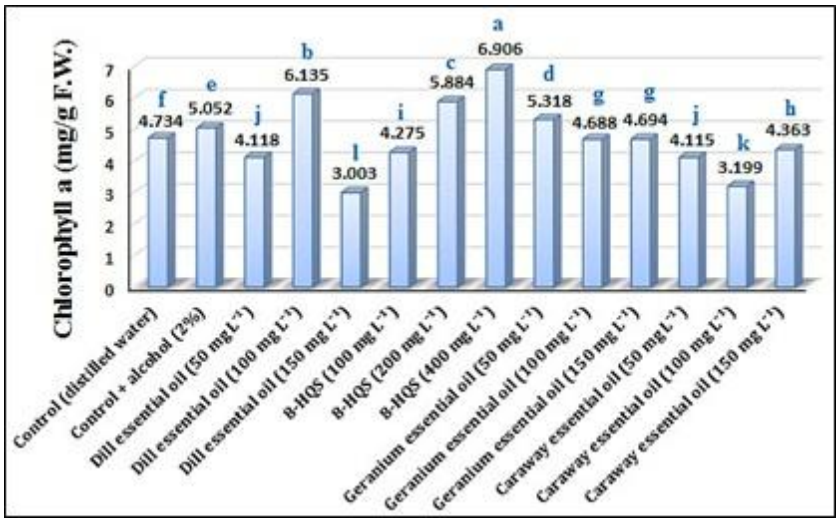

Fig. 8. Mean comparison of the effects of different treatments on chlorophyll a of carnation.

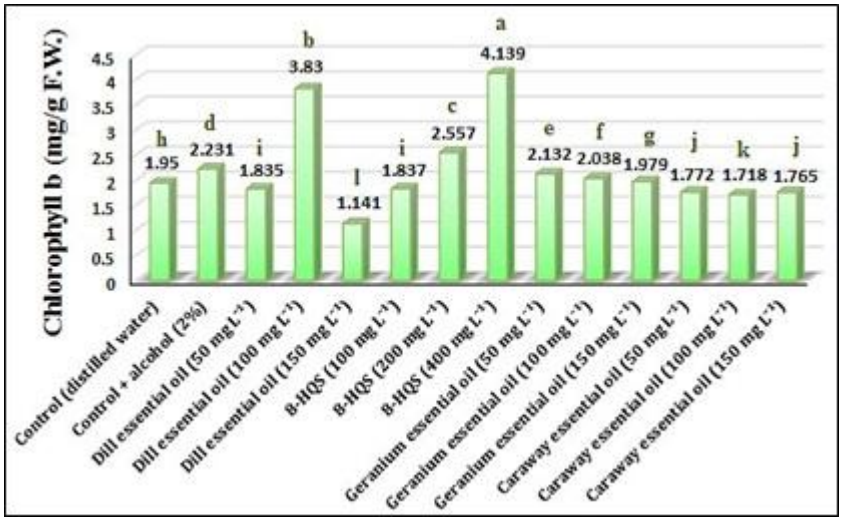

Fig. 9. Mean comparison of the effects of different treatments on chlorophyll b of carnation.

DISCUSSION

Microbial growth in the vase solution caused a loss of hydraulic conductivity in the stem and cut flowers, especially in lower parts (Shanan, 2012). Antimicrobial effect of essential oils on pathogens and their respiratory chain, inhibits microbial activity and causes their death (Solgi *et al.*, 2009). Considering the findings of this study, it can be said that the use of essential oils and 8-hydroxy quinoline in most of treatments has had a positive effect and has reduced the population of bacteria in the vase solution. In treatments with long vase life, the bacteria population decreases dramatically. Antimicrobial activity of essential oils can be related to their phenolic compounds and consequently, the activity of alcohol, aldehydes, ketones, ethers and hydrocarbons (Farrag *et al.*, 1989). Extracts and essential oils of fennel, geranium, lavender and basil in the vase solution reduced the amount of obstruction of xylem vessels and cinnamon essential oils reduced the microbial population (Shanan, 2012). Number of bacteria was rapidly increased in the stem end of roses in the control treatments and those, which were treated with sucrose, but treating with 200 mg l^{-1} 8-hydroxy quinoline sulfate inhibits the increase in the bacteria population that causes

an increase in absorption of water and increases their life significantly (Ichimura *et al.*, 1999; Pearson Mims and Lohr, 1990) that the results of these research are in accordance with our results.

In general, the fresh weight loss depends on two factors: water absorption and transpiration. The results of this study indicate that treatment of alcohol 2% has a very little weight loss. Treatment of 100 mg l^{-1} of caraway essential oils is also one of the treatments that showed the lowest weight loss. Essential oils used in the highest concentration (150 mg l^{-1}) had high fresh weight loss that indicates a negative effect of high concentrations on this trait. Moreover, these treatments had a short vase life. One of the major reasons for fresh weight loss cut flowers, stem end blockage is caused by the growing of microorganisms such as bacteria. Microorganisms release of toxic metabolites and produced ethylene and the use of antimicrobial compounds, inhibits the growth and accumulation of bacteria and improves hydraulic conductivity (Halevy and Mayak, 1981). Mousavi Bazaz and Tehranifar (2011) reported the improvement of fresh weight of *Alstroemeria* cut flowers by using essential oils in the vase solution. The use of essential oils in preservative solution of *Rose* cv. "Grand" cut flowers increased fresh weight by improving water relations (Shanan, 2012). 8-HQS with antibacterial property at high concentrations increases water absorption by reducing the colonization of microorganism and consequently, it increases the fresh weight (Ichimura, 2002). 8-HQC has strong germicidal effects to facilitate water absorption and prevented from stem end blockage (Ichimura *et al.*, 1999) that these results are consistent with our results.

Brix degree improvement could be due to storage and accumulation of carbohydrates of carbon that causes a reduction in cut flowers respiration. Moreover, it is believed that the use of antimicrobial and anti-ethylene compounds increases brix degree (Bartoli *et al.*, 1997). Based on the results obtained in this study, all treatments could increase well the soluble solids content compared to the control treatment. Maintaining or increasing the brix degree in the stems of cut flowers can be related to continues recutting of underwater flower and communicating with other traits, such as water absorption that effects on the amount of carbohydrates of the stem (Hashemabadi, 2014). Adding sugar to the vase solution increases the vase life of gerbera flowers. However, the presence of this compound alone in the preservative solutions leads to accumulation of bacteria and an increase in vascular obstruction. Therefore, sucrose along with antimicrobial compounds such as hydroxy quinoline salts is effective in increasing vase life (Solgi *et al.*, 2009).

An important lipid peroxidation factor is ROS. Electrolyte leakage is a product of membrane lipid peroxidation that shows the destruction of the cell membrane. Electrolyte leakage is considered as an indicator for membrane peroxidation (Gang *et al.*, 2009). The use of disinfectants due to the antioxidant properties neutralizes oxygen free radicals that considering the results of this study, the use of essential oils and 8-hydroxy-quinoline in most of treatments almost had positive effects and reduced electrolyte leakage and improvement of this trait conditions is completely evident with the use of disinfectants. In those treatments with long vase life, electrolyte leakage is nearly decreased.

Ethylene has an important role in the regulation of flowers senescence and its production rate is increased with senescence of flowers (Ketsa and Rugkong, 2000). Considering these results, it is indicated that the use of alcohol and essential oils has a positive impact on most of treatments and reduces the ethylene production. Successful treatments in increasing the longevity of carnation mainly reduced ethylene production. Phenolic compounds in essential oils are able to control ethylene production to some extent (Hashemabadi, 2014) that 8-hydroxy-quinoline does not contain this compound. Zagory and Reid (1986) believe that bacterial pollutant at the stem end and the vase solution causes the production of gas, which simulates aging and thus, reduces the quality and shelf life of cut flowers. The use of ethanol in the vase solution of carnation increases its vase life, this is because ethanol inhibits ethylene production (Podd and van Staden, 1999). Poon *et al.* (2001) reported that acetaldehyde at concentration of 0.05% increases the vase life of carnation cultivars of 'Yellow Candy' and 'Sandrosa' and they stated that acetaldehyde increases the

longevity of these varieties by preventing ethylene production or reducing the sensitivity to ethylene that these results correspond with our results.

Since, leaves of carnation cut flowers have good situations until the end of the vase life and the effects of yellowing and chlorosis are not observed in them, leaves cannot be the index for the end of vase life of cut carnation. According to results, the best condition for plant chlorophyll is observed in the use of germicidal compound of 8-hydroxy-quinoline that was much better and more desirable than essential oils and in the case of treatments of 100 mg l^{-1} of dill essential oil and 50 mg l^{-1} of geranium that had the highest vase life, also showed an increase in chlorophyll content, but chlorophyll content was decreased in 100 mg l^{-1} of caraway essential oil. The most important effect of essential oils in maintenance of chlorophyll is due to their antioxidant properties. The increase in chlorophyll is because of cells activity and increasing the production of sugar, and the increase in sugar decreases chlorophyll loss by regulating the osmotic pressure and respiratory rate (Andersen *et al.*, 2004). The results showed that chlorophyll content of leaves of *Alstroemeria* flowers has been increased using compounds containing sugar and peppermint essential oil (Babarabie *et al.*, 2016). Treatments of essential oils of cinnamon could maintain the chlorophyll index of leaf of cut flower of *Alstroemeria* cultivar "Jamaica" (Fazlalizadeh *et al.*, 2013). Treatments containing antimicrobial compounds increased the chlorophyll content of cut flower carnation cv. "Yellow Liberty". The reason of superiority of the antimicrobial or anti-ethylene compounds can be preventing the activity of chlorophyllase enzyme and not breaking down chlorophyll in old leaves (Basiri and Zarei, 2011). Abdul-Wasea (2012) showed that treatment of 8-HQS with sucrose in delaying chlorophyll degradation compared to control treatment was the most effective treatment that the results of our research are in accordance with the results of these researches.

CONCLUSION

In the present study, treatments of alcohol 2%, dill (100 mg l^{-1}), geranium (50 mg l^{-1}), caraway (100 mg l^{-1}) and 8-HQS (400 mg l^{-1}) had the highest vase life. The results showed that essential oils because of having antimicrobial and antioxidant properties of their phenolic compounds causes improvement of water relation in the vessel, fresh weight loss and prevention of chlorophyll degradation through reducing microorganism growth and preventing vascular obstruction, and were able to increase the life of carnation cut flowers by eliminating the free radicals that reduce electrolyte leakage and ethylene activity. Based on the results of this research, it can be stated that essential oils as eco-friendly and useful alternative to chemical compounds can be considered to increase the life of carnation cut flowers. In general, 100 mg l^{-1} of dill essential oil as the most effective organic treatment can be recommended.

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