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Prolonging Vase Life of Carnation Flowers Using Natural Essential Oils and its Impact on Microbial Profile of Vase Solutions

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Abstract: This experiment was conducted during the two summer seasons; 2008 and 2009. Two cultivars of *Dianthus caryphyllus* L. were used; Farida and Madam Collate. Four essential oil treatments were used versus two controls; Tap water and 8-Hydroxyquinoline (8-HQ). These essential oils were extracted from mandarin, coriander, dill and clove. The maximum vase-life over the two seasons was recorded with dill oil followed by coriander in cv. Farida and with 8-HQ in cv. Madam Collate. The essential oils treatments showed accumulative significant reduction percentages in flower fresh weight, which increased by decreasing the used oil concentrations. The accumulative reduction percentage in flower fresh weight and the average flower dry weight showed negative significant correlation coefficients. The relative increased percentages in net water uptake were 10.93 and 7.95% in 8-HQ for both cultivars. The net water uptake had the greatest values with those flowers kept in solution containing dill followed by clove oils in cv. Madam Collate and by mandarin oil in cv. Farida. The highest positive correlation coefficient was recorded between vase life and net water uptake. The highest pH value was observed in vase containing tap water for both cultivars. In comparison, due to different treatments application, the pH values were significantly changed to a fairly acidic. These treatments were ranked, in this respect, at descending order as follows; dill oil, 8-HQ, mandarin, coriander and clove with both cultivars. The highest mean count of total spore-forming bacteria was recorded in the tap water vase solutions, while the lowest counts were with coriander oil. In vase solution containing dill oil, the log count of molds decreased as the dose concentration increased. The same trend was observed in cv. Madam Collate during the seasons. Counts of cellulose decomposing microorganisms were increased by extending the life of the carnation cut flowers in the vase solutions. All the examined preservatives, in particular 8-HQ, and the oils of dill, clove and coriander greatly suppressed proliferation of cellulose decomposers and resulted in flower densities compared with control solutions. The anatomical study indicated that, cv. Farida flower stalks greatly severe from more exposure to microorganisms attack in vase solution, especially cellulose decomposing microorganisms which penetrate tissues as lethal and blockage parasites. These negative influences on cv. Farida could be an evidence for explaining its rapid senescence and the longevity of the flowers of cv. Madam Collate.

Key words: *Dianthus caryphyllus* L., vase life, essential oils, 8-HQ, anatomy and microbiology

INTRODUCTION

Carnation (*Dianthus caryphyllus* L.) belongs to family Caryophyllaceae. The greenhouse carnation originally was native to the Mediterranean region. Carnation is one of the most common cut flowers and of the highest economic importance value in the floricultural industry for decoration and adornment. Cut flowers of carnation are widely used in two types namely; Standard (one flower on the stem) and Spray (multiple flowers on the stem). The vase life is differing among various species and cultivars of carnation, which is one of the most valuable characteristics determining its quality, satisfying consumer preferences and the commercial value (Onozaki *et al*., 2001 and Nukui *et al*., 2004).

Short postharvest vase life is one of the most important problems of the cut flowers. However, longevity of vase life is an important factor for consumer preference (Kader, 2003). Senescence of cut flowers is induced by several factors, *e.g.*, water stress (Sankat and Mujaffar, 1994), micro-organisms (Van Doorn and Witte,

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1997) and ethylene effects (Han and Milller, 2003). In cut flowers, poor water relations have been reported to be the result of increased stem resistance to water flow, resulting from microbial growth and pH of the vase solution. These problems have been overcome in various cut flowers by the use of bactericides and low pH vase solutions (Nowak and Rundnicki, 1990).

Keeping quality is an important parameter for evaluation of cut flower quality, for both domestic and export markets. Addition of chemical preservatives to the holding solution is recommended to prolong the vase-life of cut flowers. All holding solutions must essentially contain a certain germicides. The germicides control harmful bacteria and prevent plugging of the conducting tissues. Therefore, the techniques of prolonging the vase-life of cut flowers will be a great asset to the growers and users.

Recently, most of the studies concentrated on maintaining the longevity of cut flowers by adding some chemical compounds to the vase solution. 8-HQ is broadly use, although it is very expensive and most harmful preservative for human causing irritating to skin, eyes and respiratory tract. The other natural chemical compounds are the essential oils extracted from many aromatic and medicinal plants. These oils are widely used to prevent the microbial proliferation on the vase solution, which in turn will extend the flower vase life (Julia, 1992). Bacterial development in the vase solution has a detrimental effect harming the viability and look characteristics of carnation cut flowers (Henriette and Frank, 1989). Microorganisms grow in vase water; including bacteria, yeasts and molds are harmful to cut flowers through their development in, and their consequent blockage of xylem at cut ends, preventing the water absorption. Suitable germicide application might control microbial activity in the vase water (Nowak and Rundnicki, 1990). The well documented antibacterial traits of the examined essential oils as well as 8-HQ might explain their protective effects on carnation cut flower vase solution (El Hanafi, 2007).

The aim of this study was to distinguish the effect of adding different extractions of essential oils to the vase solution in order to extend the vase life of cut carnation flowers of the two cultivars; Farida and Madam Collate. Moreover, study the correlation relationships between factors that may affect viability of the flower on vases.

MATERIALS AND METHODS

This experiment was conducted during the two summer seasons; 2008 and 2009 at the laboratories of the Ornamental Horticulture, Agricultural Botany and Agricultural Microbiology Departments, Faculty of Agriculture, Cairo University.

The plant materials were two commercial carnation cultivars of *Dianthus caryphyllus* L. namely; Farida (with purple flowers) and Madam Collate (with white flowers). The flowers were secured throughout commercial nursery at the paintbrush stage. The experimental work procedures were as follows; on 1st of May 2008 and 2009, flowers with stem length nearly 50 cm bearing five pairs of leaves and base stem cutting were gathered early in the morning. The flowers were pre-cooled (4°C) and wrapped in Kraft paper in bunches then transported under dry conditions to the laboratories within 2 hrs. Two traditional treatments were chosen as controls to investigate and compare the effects of different used essential oils. These control treatments were; Tap water (T1) and 8-Hydroxyquinoline (8-HQ (T2)). In addition, four different purified natural essential oils of antibacterial and antifungial agents (from T3 to T6) were extracted from mandarin (*Citrus nobilis* var. *deliciosa* (T3)), coriander (*Coriandrum sativum* L. (T4)), dill (*Anethum graveolens* L. (T5)) and clove (*Syzygium aromaticum* L. (T6)).

A stock extract solution of the essential oils prepared by dissolving 10 cm^3 from the raw oil in a container by a solvent of 3 cm^3 Tween 80 + 10 cm ethyl alcohol 10 % and adding tap water to reach 500 ml. Three concentrations; 200(C1), 300(C1) and 400(C1) ppm were prepared from each of the essential oil stock solutions and added to the vase containers of the investigated cut flowers. All the containers were kept in the laboratory at 25 ± 2 °C, 70% relative humidity and 1500 Lux of continuous light (10-14 hrs day/night). Specimens for anatomical study were chosen from the terminal internode and the fifth ones from the top. The micro technique procedures given by Nassar and El-Sahhar (1998) were applied. Slides were examined and the measurements of different tissues were recorded using light microscope and then photomicrographed using Microscope Olympus AX70 made in Japan.

Data were recorded in four days count intervals starting from zero day. The following traits were measured, (1) flowers vase life (days), when the flower petals start to wilt and calculated percentages of flower fresh weight (2) reduction percentage of flower fresh weight (%),calculated as accumulative reduction in flower fresh weight from zero day till flower wilting date . (3) net water uptake per flower (cm3) and (4) flower dry weight (g), in addition to the anatomical characters of the transverse sections.

The microbiological examination of the holding solution was carried out on different periods where 10 ml vase solution samples were taken and diluted in 1% (w/v) water peptone solution. The standard plate count was applied for enumerating the total bacteria on glucose-yeast-extract agar (Postage, 1969) and the same culture-medium was used for counting total spore forming bacteria in pasteurized sample dilutions. Plates were aerobically incubated at 30°C for 48 hrs. The total viable counts of molds (cfu.ml^{-1}) were obtained using Rose-Bengal Chloramphenicol Agar medium (Dixon and Fromtling, 1995) after incubating plates at 30°C for 7 days. Cellulose utilizing microorganisms were enumerated on Alkaline-cellulose agar plates incubated at 30°C for 15 days. Violet-red-bile-glucose agar medium was used for enumerating the total viable counts of *Enterobacteriaceae* after incubation at 37°C for 48 hrs (Atlas, 2004).

After counting the total bacteria, total spore forming, total molds, cellulose utilizing microorganisms and the total viable counts of *Enterobacteriaceae*, the agar plates were used for isolating the different microorganisms colonizing of the examined vase solutions. Fungal isolates were identified according to some of their morphological characteristics and microscopic examination (Gravesen *et al*., 1994). Bacterial isolates were examined for some of their cultural, morphological and biochemical as well as physiological characteristics and identified as described in Barnett and Hunter (1972). Pure isolates of gram-negative, oxidase negative short rods were characterized as candidates belong to the family *Enterobacteiaceae* and were further identified according to Garrity *et al*. (2004) using API 20 Kit (Biomeareux, France).

The experimental layout was complete randomized design (CRD) with three replicates of each treatment. Data were subjected to convenient statistical analysis methods for calculations of means, variance and standard error according to MSTATC software. Mean separations was estimated by calculating LSD values at alpha 5% according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

At the beginning of this experiment, the preliminary study concentrated on using eight different purified natural essential oils as antibacterial and antifungal agents, in addition to 8-HQ as very popular and common germicide. These oils extracted from mandarin (*Citrus nobilis* var. *deliciosa*), blue gum (*Eucalyptus globulus* L.), coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), geranium (*Pelargonium gravulens* L.), clove (*Syzygium aromaticum* L.), sweet basil (*Ocimum basilicum* L.) and manjoram (*Origanum majorana* L.). After analyzing the data obtained in all the values achieved when using the solutions containing oils of blue gum, geranium, clove, sweet basil and manjoram, the authors decided to use only one of them; clove oil as representative of the other, besides mandarin, coriander and dill oils.

Morphological Characters of Carnation Flower:

- Flower Vase Life:

It is evident from the data presented in Table (1) and Figures (1 and 2) that the maximum flower vase-life (16.60 days) over the two seasons and cultivars was recorded with T5 (dill oil). Vase life of carnation cv. Farida showed 14.40 and 13.54 days for the first and second seasons, respectively. The comparing recorded values for cv. Madam Collate were 15.16 and 14.46 days. Relative to T1, 8-HQ (T2) treatment prolonged flower vase life cv.Farida by 5.37 and 54.78% for the 1st and 2nd seasons, respectively. Meanwhile, the flower vase life was prolonged by 14.58 and 73.40% for cv. Madam Collate. The superior effects of dill oil (T5) was followed by coriander (T4) in cv. Farida and by 8-HQ (T2) in cv. Madam Collate.

Statistically, no significant differences existed among these treatments. The minimum vase-life (11.30 and 11.80 days) were noticed with T1 in both seasons for both carnation cultivars, followed by clove oil T6 (12.75 and 13.30) and the values of T1 were lower than that of 8- HQ (T2). It is clear that a positive relationship was found between the doses and the vase life within each treatment, as the dose increased the flower vase life increased. Generally, dill oil (T5) was likely more effective in enhancing flower vase life compared with the other essential oil treatments. Further enhanced could be achieved by increasing the used concentration. It could be concluded that, application of dill, coriander oils and 8-HQ treatments had more important role on prolonging the carnation vase life and could be supported by increasing the dose in both carnation cultivars. The cv. Madam Collate carnation showed significant differences among the three used doses in the 2nd season. While, in case of cv. Farida, it is occurred just in the first season.

Table 1: The effect of different essential oils on the vase life days of two carnation cultivars Farida and Madam Collate during the two seasons, 2008 and 2009

Treatments	First season				second season				
	Concentrations (ppm)								
	200	300	400	Mean	200	300	400	Mean	Average treatment
	cv. Farida								
T1 Tap water	13.6	13.6	13.6	13.60	9.00	9.00	9.00	9.00	11.30
T 2 8-HQ	12.0	15.0	16.0	14.33	12.3	14.5	15.0	13.93	14.13
T 3 Mandarin	13.3	15.0	15.6	14.63	10.0	16.0	17.0	14.33	14.48
T 4 Coriander	15.0	15.6	15.6	15.40	14.0	15.0	15.6	14.87	15.14
T 5 Dill	15.0	16.0	16.0	15.67	14.6	16.6	18.0	16.40	16.04
T 6 Clove	12.3	13.0	13.0	12.77	12.6	12.6	13.0	12.73	12.75
Av. Dose X Cultivar	13.53	14.7	14.97		12.08	13.95	14.6		
Average cultivar				14.40				13.54	13.97
LSD 5%	Treat = 0.880, Dose= 0.420, Cultivar x Season= NS, Treat X Dose= 0.299				Treat = 0.951, Dose= NS, Cultivar x Season= NS, Treat X Dose= 0.805				1.07
Treatments	cv. Madam Collate								
T1 Tap water	14.20	14.20	14.20	14.20	9.40	9.40	9.40	9.40	11.8
T 2 8-HQ	15.60	16.60	16.60	16.27	10.50	18.50	19.90	16.30	16.29
T 3 Mandarin	13.80	15.60	16.20	15.20	10.50	16.70	17.80	15.00	15.1
T 4 Coriander	15.60	16.20	16.20	16.00	14.60	15.70	16.40	15.57	15.79
T 5 Dill	15.60	15.90	16.60	16.03	15.30	17.40	18.80	17.17	16.6
T 6 Clove	12.80	13.50	13.50	13.27	13.20	13.20	13.60	13.33	13.3
Av. Dose X Cultivar	14.60	15.33	15.55		12.25	15.15	15.98		
Average cultivar				15.16				14.46	14.81
LSD 5%	Treat = 0.440, Dose= 0.502, Cultivar x Season= 0.805, Treat X Dose= 0.144				Treat = 0.585, Dose= NS, Cultivar x Season= 0.502, Treat X Dose= 0.237				1.017

Key: 1: Mandarin oil T3 (14.3 days)
2: 8-HQ T2 (14.1 days)
3:Dill oil T5 (16.0 days)
4: Clove oil T6 (12.8 days)
5: Coriander T4 (15.1 days)
6: Tap water T1 (11.3 days)

Fig. 1: Photographs represent the vase life of cv. Farida carnation flowers kept in different essential oil solutions.

Key: 1: Tap water T1 (11.8 days)
2: Mandarin oil T3 (15.1 days)
3: Dill oil T5 (16.6 days)
4: Coriander T4 (15.8 days)
5: 8-HQ T2 (16.3 days)
6: Clove oil T6 (13.3 days)

Fig. 2: Photographs represent the vase life of cv. Madam Collate kept in different essential oil solutions.

These results are in agreement with those of Saini *et al.* (1994), who mentioned that the vase-life of tuberose cut flowers increased when placed in solutions with different concentrations of essential oils. Halevy and Mayak (1981) have been shown that vase life of rose, gypsophila, gerbera, carnation and chrysanthemum was improved significantly with germicide solution. Alternatively, increasing the number of microorganisms in the vase water resulted in poor vase life in many cut flowers (Hoogerwerf and Van-Doorn, 1992).

Reduction Percentage of Flower Fresh Weight:

Data presented in Table (2) showed the highest reduction percentages of the flower fresh weight over the

two seasons for cv. Farida were 10.94 and 11.00 recorded with T2 and T1. The corresponding values of cv. Madam Collate were 11.47% and 11.80%. Relative to 8-HQ (T2), T5 and T6 treatments produced the lower reduction percentages (7.10 and 8.20%) and (7.45 and 8.55%) of the flower fresh weight in cvs. Farida and Madam Collate, respectively. Generally, the two carnation cultivars were diffed in their response to such trait. Regardless the treatment effects, the average accumulated reduction percentages in both carnation cultivars were 9.50% and 9.99%, respectively. It also noticed that most of the essential oil treatments behave similar effects with minuet extents and all these treatments also behaved statistically alike. The treatments causing less reduction percentages of flower fresh weight are considered the favorable treatments because they may show more flourishing appearance.

The present study has indicated that 8-HQ (T2) and dill oil (T5) treatments known to reduce the respiration rate of the flower, so they are very effective in delaying fruit ripening and prolonging the vase life. Fresh weight of roses treated with germicides remained at higher levels and declined slowly by improving the solution uptake of cut rose flowers. While roses kept in tap water declined after two to three days at a rapid rate (Ketsa and Chinprayon, 2007).

Net Water Uptake:

Data presented in Table (3) show the maximum net water uptake values (11.10, 10.45 and 10.35 cm^3) was recorded with cv. Farida flowers which kept in T5, T3 and both (T4 and T6), respectively and significantly differed with flowers kept in T2 (9.15 cm^3). With the other cv. Madam Collate, these values were 12.09, 11.59 and 11.52 cm^3 with T5, T6 and T3, respectively. The two cultivars were significantly differed in their response to net water uptake. While, no significant differences were recorded among seasons. The average recorded net water uptake in cv. Farida were 11.06 and 9.24 cm^3 in first and second seasons, respectively. The corresponding recorded values for cv. Madam Collate were 12.30 and 10.21 cm^3. The relative increase percentages of net water uptake over T2 were 10.93 and 7.95 for the two cultivars, respectively.

Table 2: Reduction percentages of flower fresh weight of two carnation cultivars Farida and Madam Collate as affected by the different essential oils concentrations during the two seasons, 2008 and 2009

Treatments	First season				second season				
	Concentrations (ppm)								
	200	300	400	Mean	200	300	400	Mean	Average treatment
				cv. Farida					
T1 Tap water	11.20	11.20	11.20	11.20	10.80	10.80	10.80	10.80	11.00
T 2 8-HQ	8.70	11.60	12.90	11.07	11.30	10.20	10.90	10.80	10.94
T 3 Mandarin	9.70	10.90	11.30	10.63	7.30	11.60	12.30	10.40	10.52
T 4 Coriander	8.90	9.40	9.40	9.23	9.10	9.10	9.40	9.20	9.22
T 5 Dill	8.00	8.00	8.00	8.00	6.20	6.20	6.20	6.20	7.10
T 6 Clove	9.90	9.90	9.90	9.90	6.50	6.50	6.50	6.50	8.20
Av. Dose X Cultivar	9.40	10.17	10.45		8.53	9.07	9.35		
Average cultivar				10.00				8.98	9.50
LSD 5%	Treat = 0.485, Dose= 0.401, Cultivar x Season= NS, Treat X Dose= 0.355				Treat = 0.253, Dose= 0.420, Cultivar x Season= NS, Treat X Dose= 0.251				0.38
Treatments				cv. Madam Collate					
T1 Tap water	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
T 2 8-HQ	11.3	11.8	11.8	11.63	10.6	11.4	11.9	11.3	11.47
T 3 Mandarin	10.0	11.3	11.8	11.03	7.6	12.7	12.9	11.07	11.05
T 4 Coriander	9.3	9.8	9.8	9.63	9.6	9.6	9.9	9.7	9.67
T 5 Dill	8.3	8.3	8.3	8.3	6.6	6.6	6.6	6.6	7.45
T 6 Clove	10.3	10.3	10.3	10.3	6.8	6.8	6.8	6.8	8.55
Av. Dose X Cultivar	10.17	10.55	10.63		8.83	9.82	9.98		
Average cultivar				10.45				9.55	9.99
LSD 5%	Treat = 0.179, Dose= NS, Cultivar x Season= NS, Treat X Dose= 0.308				Treat =0.196, Dose= NS, Cultivar x Season = NS, Treat X Dose= 0.217				0.951

Despite of the dose effects, it is also notable that T5 treatment showed its dominant effect over either T2 or the other essential oil treatments. Generally, the highest concentrations were likely more effective in increasing net water uptake compared with the lowest one. The above results are in accordance with Reddy *et al*. (1997). They reported that the ideal flower preservative is that allows water absorption through flower tissues. Water absorption from the preservative solution maintains a better water balance and flower freshness which saves from early wilting and reflecting on vase-life improve. Provide antibacterial agents that will keep the water free from bacteria and other microorganisms and can form occlusion inside the stem obstructing the flow of water to the flower (Van Doorn *et al*., 1991).

Table 3: The effect of different used essential oils on net water uptake cm^3 of two carnation cultivars Farida and Madam Collate during the two seasons, 2008-2009.

Treatments	First season				second season				
	Concentrations (ppm)								
	200	300	400	Mean	200	300	400	Mean	Average treatment
	cv. Farida								
T1 Tap water	10.20	10.20	10.20	10.20	8.90	8.90	8.90	8.90	9.55
T 2 8-HQ	8.40	11.00	11.00	10.13	7.60	8.00	8.90	8.17	9.15
T 3 Mandarin	12.00	12.40	12.50	12.30	7.70	8.90	9.20	8.60	10.45
T 4 Coriander	9.20	12.00	13.60	11.60	7.90	8.60	10.80	9.10	10.35
T 5 Dill	11.00	11.00	11.20	11.07	10.40	11.40	11.60	11.13	11.10
T 6 Clove	9.80	10.40	13.00	11.07	7.60	10.40	10.70	9.57	10.32
Av. Dose X Cultivar	10.10	11.17	11.92		8.35	9.37	10.02		
Average cultivar				11.06				9.24	10.15
LSD 5%	Treat = 0.770, Dose= 0.494, Cultivar x Season= NS, Treat X Dose= 0.953				Treat = 0.798, Dose= 0.455, Cultivar x Season= NS, Treat X Dose= 0.8.66				0.547
Treatments	cv. Madam Collate								
T1 Tap water	11.60	11.60	11.60	11.60	10.20	10.20	10.20	10.20	10.90
T 2 8-HQ	9.60	12.50	12.50	11.53	8.60	9.20	10.20	9.33	10.43
T 3 Mandarin	10.40	13.70	15.60	13.23	8.70	10.20	10.50	9.80	11.52
T 4 Coriander	10.80	11.50	12.50	11.60	8.30	11.40	11.70	10.47	11.04
T 5 Dill	13.70	14.20	14.30	14.07	9.00	9.00	12.30	10.10	12.09
T 6 Clove	10.40	12.50	12.50	11.80	10.60	11.10	12.40	11.37	11.59
Av. Dose X Cultivar	11.08	12.67	13.17		9.23	10.18	11.22		
Average cultivar				12.30				10.21	
LSD 5%	Treat = 0.544, Dose= 0.530, Cultivar x Season= NS, Treat X Dose= 0.946				Treat = 0.564, Dose= 0.388, Cultivar x Season= NS, Treat X Dose= 0.801				0.658

Flower Dry Weight:

Data of the flower dry weight Table (4) were recorded at wilting date on flowers placing in different essential oil preservative solutions. The highest values of flower dry weight (3.86 and 4.19 g) were recorded with T1 in cvs. Farida and Madam Collate, respectively. In the first season, the average highest values of the flower dry weight (4.59 and 4.69 g) were recorded with T1 for cvs. Farida and Madam Collate, respectively. While, in the second season, this value (3.73 g) was recorded with T3 for cv. Farida and the corresponding value for cv. Madam Collate was 3.68 g with T1.

Relative to 8-HQ (T2), in both seasons and cultivars, T5 treatment caused an average accumulated increased flower dry weight by 8.67 and 5.51%, respectively. It could be stated that the average loss values of flower dry weight increased by decreasing the used concentration. The treatments causing reduction in the values of flower dry weight are considered the ideal because these treatments may prolonging the vase-life compared to those showing short vase life. The present study indicated that T2 and T5 treatments known to reduce the rate of respiration are considered effective in delaying the wilting of flowers which reflected on prolonging the vase life as mentioned by (Singh *et al.,* 1993. On cut carnation flower, El- Hanafi (2007) stated that there is no significant difference between the essential oils used in the study and 8-HQ concerning this trial which meant that the use of these oils can be an adequate alternative to 8-HQ in maintaining the dry matter of the flowers.

Table 4: The effect of different used essential oils on average flower dry weight (g) of carnation cultivars Farida and Madam Collate during the two seasons, 2008 and 2009.

Treatments	First season				second season				
	Concentrations (ppm)								
	200	300	400	Mean	200	300	400	Mean	Average treatment
	cv. Farida								
T1 Tap water	4.59	4.59	4.59	4.59	3.12	3.12	3.12	3.12	3.86
T 2 8-HQ	2.87	3.25	3.28	3.13	1.92	2.58	5.45	3.32	3.23
T 3 Mandarin	3.02	3.02	3.02	3.02	2.62	2.91	5.65	3.73	3.38
T 4 Coriander	3.00	3.50	3.62	3.37	3.07	3.08	3.30	3.73	3.55
T 5 Dill	3.27	3.85	4.50	3.87	2.72	3.28	4.47	3.15	3.51
T 6 Clove	3.67	3.67	3.67	3.67	3.23	3.23	3.23	3.49	3.58
Av. Dose X Cultivar	3.40	3.65	3.78		2.78	3.03	4.20		
Average cultivar				3.61				3.42	3.52
LSD 5%	Treat = 0.73, Dose= 0.52, Cultivar x Season= NS, Treat X Dose= 0.22				Treat = 0.81, Dose= 0.41, Cultivar x Season= NS, Treat X Dose= 0.61				0.27

Table 4: Continue

Treatments									
				cv. Madam Collate					
T1 Tap water	4.69	4.69	4.69	4.69	3.68	3.68	3.68	3.68	4.19
T 2 8-HQ	3.72	3.80	3.89	3.80	3.15	3.37	3.86	3.46	3.63
T 3 Mandarin	3.18	3.40	3.61	3.40	3.16	3.20	3.72	3.36	3.38
T 4 Coriander	4.00	4.01	4.02	4.01	3.41	3.42	3.66	3.50	3.76
T 5 Dill	4.07	4.07	4.07	4.07	3.59	3.59	3.59	3.59	3.83
T 6 Clove	3.35	3.35	3.35	3.35	2.09	3.09	3.09	2.76	3.06
Av. Dose X Cultivar	3.84	3.89	3.94		3.18	3.39	3.6		
Average cultivar				3.89				3.39	3.64
LSD 5%	Treat = 0.94, Dose= 0.50, Cultivar x Season= NS, Treat X Dose= 0.31				Treat = 0.90, Dose= 0.54, Cultivar x Season= NS, Treat X Dose= 0.55				0.41

Simple Correlation Between Factors That Affect Vase Life:

The correlations between vase life and other studied characters over the two seasons were considered and presented in Table (5). It is clear that, in both cultivars, the highest positive correlation coefficients (+ 0.784 and + 0.827) were recorded between vase life and net water uptake, respectively. With cv. Farida, the other studied traits; reduction percentage of flower fresh weight, average flower dry weight and average flower diameter showed significant negative correlation coefficients (- 0.500, - 0.653 and - 0.160). The same trend with different values was obtained with cv. Madam Collate.

It is also evident that, in both cultivars, reduction percentage of the flower fresh weight was positively correlated with average flower dry weight (+ 0.876 and + 0.777) and average flower diameter (+0.604 and +0.514). While negative correlation coefficient (- 0.466 and -0.432) was recorded between reduction percentage of flower fresh weight and net water uptake in both cultivars, respectively. Net water uptake in relation to vase-life has been used by some workers to determine the flower fresh and dry weights. A few workers also reported a significant positive correlation between the amount of net water uptake and vase-life (Systema 1975 and Buys and Cours, 1981).

Anatomical Studies:

Microscopical measurements of certain characters were recorded to investigate the differences in the internal structure of carnation flower stalks of both cultivars. The amounts of elastic primary walled tissues (collenchyma and parenchyma) forming the cortex and pith, and the rigid secondary walled tissues (sclernchyma and xylem) that give the flower stalks strength were the main studied characters. Measuring the xylem vessel diameter that may affect the total net water uptake was considered. Microphotographs of transverse sections of cvs. Farida and Madam Collate (Figure 3 a and b) showed that, the cortex of the flower stalks have assimilatory tissues (collenchyma with chloroplasts). A ring of sclernchymatous cells was the inner-most layers of cortex just before phloem and xylem continuous cylinder, rays generally absent.

Table 5: Correlation coefficient matrix between the factors affected the vase life of two carnations cultivars; Fariad and Madam Collate as affected by different preservation solutions

Characters	Vase life (days)	Reduced percentages of flower fresh weight	Net water uptake	Av. flower dry weigh	Average flower diameter
		Cv. Farida			
Vase life (days)	1.000				
Reduced F.W percentages	-0.500*	1.000			
Net water uptake	+0.784*	-0.466	1.000		
Average flower dry weight	-0.653*	+0.876	-0.724	1.000	
Average flower diameter	-0.160	+0.604	+0.621	+0.704	1.000
		Cv. Madam Collate			
Vase life (days)	1.000				
Reduced F.W percentages	-0.610*	1.000			
Net water uptake	+0.827*	-0.432*	1.000		
Average flower dry weight	-0.621*	+0.777*	-0.710*	1.000	
Average flower diameter	-0.132	+0.514*	+0.512*	+0.638*	1.000

Data presented in Table (6) and Figure (3 a and b) for the terminal internode cleared that, the stem layout of cv. Farida is quadrangular shape, while the other cultivar tends to be cylindrical in outline shape. Relative to cultivar Madam Collate, the average increase percentage of stem diameter of cv. Farida was 8.1%. Cv. Farida maintained thick transparent outer cuticle layer compared with the other cultivar. The epidermis of the two cultivars showed barrel shaped thin cells. The epidermal cells of cv. Farida extended in the tangential direction and scored high thickness compared with cv. Madam Collate.

Table 6: Different tissue measurements (µ) of flower stalks of the two studied carnation cultivars (average of 5 readings).

	Measurements (µ)	Terminal internode		Fifth internode (from the top)	
		cv.Farida	cv.Madam Collate	cv.Farida	cv.Madam Collate
1	Average stem diameter	3697.50	3419.00	5397.50	4017.50
2	Average cuticle thickness	10.00	8.25	10.00	6.25
3	Average epidermis thickness	31.25	25.00	21.25	27.50
4	Average hypodermis thickness	53.75	------	------	52.50
5	Average angular collenchyma thickness	225.00	165.00	222.50	56.24
6	Average sclernchyma ring thickness	37.50	23.75	106.25	100.00
7	Average parenchyma layers thickness	150.00	181.25	110.00	93.75
8	Average cortex thickness	441.25	370.00	438.75	302.50
9	Average thickness of phloem	66.25	62.50	93.75	66.25
10	Average thickness of xylem	72.50	56.25	110.00	87.50
11	Average vessel diameter	23.75	17.50	21.25	20.00
12	Average pith thickness	2405.00	2375.00	3050.00	3037.50
13	Average pith cell diameter	63.75	56.25	52.50	51.25

The stem epidermis of both cultivars has numerous sunken stomata. The cortex of cv. Farida showed hypodermis with distinct differences from the next cortical layer, since 2-3 rows (approximately 53.75µ) of palisade collenchyma cells, containing numerous chloroplastids, were found just beneath to the epidermis followed by many layers of angular thick walled collenchyma cells. These collenchyma rows were absent in the cultivar Madam Collate, where the cortex showed rather extensive layers of angular collenchyma more homologous in shape. This indicated that, cv. Farida produced thick cortex tissues that comprised more water and food storage cells, elastic cell walls in addition to having sclereids, tannin, mucilage and crystal cells which could be attributed to prolonging vase life compared with the other cv. Madam Collate.

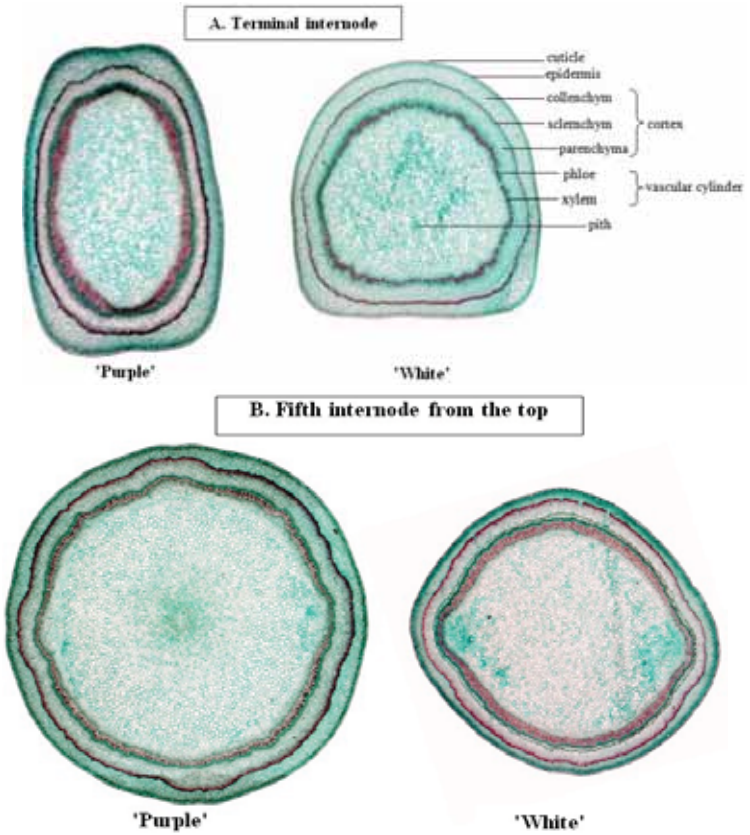

Fig. 3: Transverse sections in flower stalks of the two studied carnation cultivars Farida (Purple) and Madam Collate (White)
A. mid-point of terminal internode (X= 20)
B. mid-point of fifth internode from the top (X= 15)

The innermost cortex layers showed a remarkable thick ring (belt) of sclerenchyma. In respect to cv. Madam Collate, the average increase percentage of sclernchymatous belt thickness was 57.8% compared with cv. Farida. This may confirm the fact that the stalks of cv. Farida seemed to be more rigid when placed in vase. Within stems, cv. Farida showed thicker and wider vascular system as the average xylem and phloem thickness was greater by 28.8 and 6.0% compared with cv. Madam Collate. Moreover, the average vessel diameter and pith thickness and diameter of cv. Farida was wider and greater than that of cv. Madam Collate, this emphasizes the high water uptake and food storage of cv. Farida. The anatomical structure of the fifth internode of the two carnation cultivars was similar, with different values, to that for terminal internodes. The previously mentioned structure of *Dianthus* flower stalks is in agreement with those given by (Metcalfe and Chalk, 1950, Çinbilgel *et al*., 2007 and Yildiz and Minareci, 2008).

pH Values of Vase Solution:

With cv. Farida, the pH values fluctuated within a narrow range between 6.64 and 7.16 (Table 7) with highest values in control vases containing tap water (T1). In comparison, the pH values of the examined solutions were significantly changed to a fairly acidic pH due to essential oil application. On the contrary, 8-HQ (T2) application had no significant effect on the pH value of the vase solution during the two seasons. Madam Collate showed the same trend mentioned with cv. Farida with relatively low magnitude in both studied seasons (Table 8). The above mentioned results are in accordance with those reported by El-Hanafi (2007), who reported that, applying different essential oils to vase solution reduced the pH and cause prolongation of flower vase life. Low pH of the vase solution was related to the acidic pH of petal sap which is associated with better viability of petals. The remarkable acidity of the vase solution in the presence of these oils was attributed to some of their acidic constituents.

Table 7: Average pH value of the vase solution of carnation cut flower cultivars Farida and Madam Collate as affected by different essential oils treatments (in days).

		cv. Farida									
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season 1	T1	6.94	6.94	6.94	7.08	7.08	7.08	7.46	7.46	7.460	7.16
	T2	6.96	7.00	7.03	7.10	7.14	7.17	7.31	6.64	6.900	7.03
	T3	6.94	6.94	6.94	7.08	7.08	7.08	6.09	6.01	6.01	6.69
	T4	6.91	6.93	7.00	7.07	7.07	7.14	7.26	6.02	6.24	6.85
	T5	6.96	6.90	7.06	7.10	7.04	7.20	7.01	6.52	7.15	6.99
	T6	6.58	6.94	6.98	6.71	7.08	7.12	6.51	6.35	6.26	6.73
	Mean	6.88	6.94	6.99	7.02	7.08	7.13	6.94	6.50	6.67	
			$LSD_{0.05}$:		Dose, 0.046;			Treatment 0.26			
Season2	T1	6.90	6.90	6.90	7.04	7.04	7.04	7.42	7.42	7.42	7.12
	T2	6.90	6.96	6.99	7.06	7.10	7.13	7.27	6.78	6.86	7.00
	T3	6.90	6.90	6.90	7.04	7.04	7.04	6.67	6.67	6.67	6.87
	T4	6.90	6.89	6.96	7.05	7.03	7.10	7.22	6.44	6.23	6.87
	T5	6.92	6.86	7.02	7.06	7.00	7.16	6.97	6.84	6.85	6.96
	T6	6.54	6.90	6.94	6.67	7.04	7.08	6.03	6.34	6.25	6.64
	Mean	6.84	6.90	6.95	6.99	7.04	7.09	6.93	6.75	6.71	
			$LSD_{0.05}$ Dose, 0. 38; Treatment , 0.21; Dose*treatment* Season, 0.109								
		cv. Madam Collata									
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	6.79	6.79	6.79	6.92	6.92	6.92	7.29	7.29	7.29	7.00
	T2	6.81	6.85	6.88	6.94	6.98	7.01	7.15	6.67	6.75	6.89
	T3	6.79	6.79	6.79	6.92	6.92	6.92	6.56	6.56	6.56	6.76
	T4	6.78	6.78	6.85	7.89	6.91	6.98	7.10	6.34	6.32	6.88
	T5	6.81	6.75	6.90	6.94	6.88	7.04	6.85	5.74	6.04	6.66
	T6	6.43	6.79	6.83	6.56	6.92	6.96	5.93	6.23	6.15	6.53
	Mean	6.74	6.79	6.84	7.03	6.92	6.97	6.81	6.47	6.52	
			$LSD_{0.05}$ Dose 0.109; Treatment , 0.21								
Season2	T1	6.98	6.98	6.98	6.92	6.92	6.92	7.29	7.29	7.29	7.06
	T2	7.00	7.04	7.07	6.94	6.98	7.01	7.15	6.67	6.74	6.96
	T3	6.98	6.98	6.98	6.92	6.92	6.92	6.56	6.56	6.56	6.82
	T4	6.98	6.97	7.04	7.89	6.91	6.98	7.10	6.34	6.12	6.93
	T5	7.00	6.94	7.10	6.94	6.88	7.04	6.85	5.74	6.04	6.73
	T6	6.63	6.98	7.02	6.56	6.92	6.96	5.93	6.23	6.15	6.60
	Mean	6.93	6.98	7.03	7.03	6.92	6.97	6.81	6.47	6.48	
			$LSD_{0.05}$: Dose 0.38; Treatment, 0.0125; Dose*treatment* Season, 0.183								

The Microbial Profile of Vase Solutions:

Total Bacteria and Spore-former Counts:

Results in Tables (8 - 9) refer to a consistent growth inhibition effect on bacteria exhibited by applying oils during both seasons with the two cultivars. With cv. Farida, the control vases containing tap water (T1) harbored the highest bacterial population densities (6.20) in both seasons compared with those of 8-HQ (5.27 and 5.37) and the other examined oils. The reductions in bacterial population densities in the vase solutions due to all the applied treatments were statistically significant and were dependent upon the type and concentration of the applied preservative. Dill oil (T5), compared to T1,in the first season, appeared a superior antibacterial substance causing 15.48 % reduction in the average log count of total bacteria followed by; 8-HQ (15.00%).

At all sampling periods, increasing oil concentrations in the vase solution up to 400 ppm reduced bacterial log to the minimum levels. However, significantly lower total bacterial counts were enumerated in vases contained (T5) compared with control (T1). For example, the log count of total bacteria in such solution ranged between 3.45 and 6.79 in the 1st season and from 3.52 to 6.86 in the 2nd one. Moreover, the preservative solutions with cv. Madam Collate, showed the same trend with respect to the total bacterial count. Since both T2 and T5 exhibited the lowest counts (5.37 and 5.34), respectively in the first season and 5.53 and 5.50 in the second one.

Total spore former counts scored their highest mean count (3.92) in the vase solutions of T1 up to 400ppm. It is obvious that the two studied cultivars, during the two seasons showed comparable levels of antibacterial activities against spore forming bacteria in the vase solutions. The examined additives exerted their maximal levels of antibacterial activities against spore-forming bacteria at concentrations of 400 ppm.

Table 8: Total bacterial counts in vase solutions of carnation cultivar Farida and Madam Collate as affected by different essential oil treatments (log cfu ml^{-1}).

					cv. Farida						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	4.19	4.19	4.19	6.82	6.82	6.82	7.60	7.60	7.60	6.20
	T2	3.80	3.66	3.26	6.20	5.95	5.30	7.25	6.45	5.52	5.27
	T3	4.00	3.67	3.46	6.52	5.97	5.64	7.23	6.82	6.02	5.48
	T4	4.53	4.43	4.34	6.72	6.07	5.84	6.88	6.24	6.01	5.19
	T5	3.59	3.78	3.45	6.15	5.85	5.62	6.79	6.09	5.85	5.24
	T6	4.18	4.16	3.37	6.8	6.78	5.48	7.06	6.55	5.71	5.57
	Mean	4.05	3.98	2.96	6.54	6.24	5.78	7.14	6.63	6.12	
			$LSD_{0.05}$: Dose, 0.032; Treatment, 0.055								
Season2	T1	4.27	4.27	4.27	6.96	6.96	6.96	7.38	7.38	7.38	6.20
	T2	3.88	3.73	3.33	6.32	6.08	5.41	7.33	6.52	5.58	5.35
	T3	4.09	3.75	3.53	6.65	6.10	5.75	7.31	6.89	6.09	5.57
	T4	4.63	4.52	4.44	6.88	6.22	5.96	6.96	6.31	6.19	5.79
	T5	3.67	3.86	3.52	6.28	5.97	5.73	6.86	6.16	5.91	5.33
	T6	4.27	4.25	3.44	6.95	6.92	5.60	7.14	6.62	5.77	5.66
	Mean	4.14	4.06	3.76	6.67	6.38	5.90	7.16	6.65	6.15	
			$LSD_{0.05}$: Dose 0.0421 ; Treatment, 0.068; Dose*treatment* Season, 0.315								
					cv. Madam Collata						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	4.27	4.27	4.27	6.95	6.95	6.95	7.44	7.44	7.44	6.22
	T2	3.88	3.73	3.32	6.32	6.07	5.41	7.40	6.58	5.63	5.37
	T3	4.08	3.74	3.53	6.64	6.09	5.75	7.38	6.95	6.14	5.59
	T4	4.63	4.52	4.43	6.87	6.21	5.95	7.02	6.37	6.13	5.79
	T5	3.86	3.65	3.52	6.272	5.96	5.73	6.92	6.21	5.97	5.34
	T6	4.27	4.25	3.43	6.94	6.91	5.59	7.20	6.68	5.82	5.68
	Mean	4.17	4.03	3.75	6.67	6.37	5.90	7.23	6.71	6.19	
			$LSD_{0.05}$: Dose, 0. 0.028 ; Treatment, 0.0469								
Season2	T1	4.41	4.41	4.41	7.182	7.182	7.182	7.61	7.61	7.61	6.40
	T2	4.01	3.85	3.43	6.53	6.27	5.59	7.57	6.731	5.76	5.53
	T3	4.22	3.87	3.65	6.86	6.29	5.94	7.54	7.11	6.28	5.75
	T4	4.78	4.67	4.58	7.13	6.45	6.15	7.18	6.51	6.39	5.98
	T5	3.78	3.98	3.63	6.48	6.16	5.92	7.08	6.35	6.10	5.50
	T6	4.41	4.39	3.55	7.17	7.14	5.78	7.36	6.83	5.96	5.84
	Mean	4.27	4.20	3.88	6.89	6.58	6.09	7.39	6.86	6.35	
			$LSD_{0.05}$: Dose 0. 0.044; Treatment, 0.106; Dose*treatment* Season, 0.28								

Table 9: Total counts of spore forming bacteria in vase solutions of carnation cultivars Farida and Madam Collate as affected by different essential oil treatments (log cfu ml^{-1}).

					cv. Farida						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1											
	T1	3.68	3.68	3.68	3.85	3.85	3.85	4.24	4.241	4.24	3.92
	T2	2.76	2.31	2.24	2.89	2.42	2.35	3.18	2.67	2.58	2.60
	T3	2.97	2.67	2.41	3.10	2.80	2.52	2.83	3.15	2.94	2.87
	T4	2.94	2.47	2.16	3.07	2.59	2.26	3.38	2.09	2.00	2.55
	T5	2.86	2.56	2.23	3.00	2.67	2.34	3.30	3.01	2.96	2.77
	T6	2.81	2.53	2.20	2.94	2.65	2.30	3.02	2.92	2.14	2.61
	Mean	3.00	2.70	2.49	3.14	2.83	2.60	3.40	3.01	2.81	
				$LSD_{0.05}$: Dose, 0.015; Treatment, 0.098							
Season2	T1	3.79	3.79	3.79	3.96	3.96	3.97	4.29	4.29	4.29	4.01
	T2	2.84	2.38	2.31	2.97	2.49	2.42	3.21	2.697	2.61	2.66
	T3	3.05	2.75	2.48	3.19	2.88	2.60	3.31	3.190	2.97	2.94
	T4	3.02	2.55	2.22	3.16	2.66	2.33	3.42	2.11	2.02	2.61
	T5	2.95	2.63	2.30	3.08	2.75	2.41	3.33	3.04	2.99	2.83
	T6	2.90	2.61	2.27	3.03	2.73	2.37	3.05	2.95	2.17	2.68
	Mean	3.09	2.79	2.56	3.23	2.91	2.68	3.44	3.05	2.84	
				$LSD_{0.05}$:Dose, ; Treatment, 0.124; Dose*treatment*Season, 0.852							
					cv. Madam Collata						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	4.13	4.13	4.13	4.32	4.32	4.32	4.17	4.17	4.17	4.21
	T2	3.10	2.60	2.52	3.24	2.72	2.63	3.13	2.63	2.54	2.79
	T3	3.33	3.00	2.71	3.48	3.14	2.83	3.23	3.10	2.89	3.08
	T4	3.29	2.78	2.42	3.45	2.90	2.53	3.33	2.06	1.97	2.75
	T5	3.21	2.87	2.51	3.36	3.00	2.62	3.24	2.96	2.91	2.96
	T6	3.16	2.84	2.47	3.30	2.97	2.59	2.97	2.87	2.11	2.81
	Mean	3.37	3.04	2.79	3.53	3.18	2.92	3.35	2.97	2.77	
				$LSD_{0.05}$: Dose, 0.057; Treatment, 0.168							
Season2	T1	4.75	4.75	4.75	4.97	4.97	4.97	4.09	4.09	4.09	4.60
	T2	3.56	2.99	2.90	3.72	3.12	3.03	3.07	2.57	2.49	3.05
	T3	3.83	3.45	3.11	4.00	3.61	3.26	3.16	3.04	2.84	3.37
	T4	3.79	3.19	2.79	3.96	3.34	2.92	3.26	2.02	1.93	3.02
	T5	3.69	3.30	2.88	3.86	3.45	3.01	3.18	2.91	2.85	3.24
	T6	3.63	3.27	2.84	3.80	3.42	2.97	2.91	2.81	2.07	3.08
	Mean	3.88	3.49	3.21	4.05	3.65	3.36	3.28	2.91	2.71	
				$LSD_{0.05}$:Dose, 0.0880; Treatment, 0.211; Dose*treatment*Season, 0.637							

Bacterial development in the vase solution was reported to have a detrimental effect harming the viability and look characteristics of carnation flowers (Henriette and Frank,1989). Bacterial by-products and enzymes are direct effectors on the viability of carnation cut flower (Bergey, 2004). The antibacterial trait of 8-HQ as well as the different essential oils and their protective effects on carnation cut flower in the vase solution were previously documented (Kushal, *et al*., 2003; El Hanafi, 2007). A vast array of available reports on *in vitro* wide spectra antibacterial activities of the examined essential oils indicates possible implications of such natural phytochemicals in vase solution disinfection purposes (Lo Cantore *et al*., 2004 and Saeed and Tariq, 2007).

Total Molds:

Data presented in Tables (10) showed the average total mold counts in all the examined vase solutions along with the three assigned counts in both seasons for the two cultivars. With cv. Farida, molds displayed their maximal mean log counts (2.95 and 3.01) in control vase filled with tap water (T1) in both seasons. Mold counts increased with elapsed time reaching the peaks after 12 days. Adding 8-HQ (T2) or the examined oils to the vase solution significantly suppressed molds development of the solution of both flower cultivars. For instance, and compared to T1, the mean log count of molds in vase solution reduced from 2.95 to 1.70 and 1.64 as a result of incorporating 8-HQ (T2) and dill oil (T5) into the vase solution, respectively.

Dill oil exhibited a striking antifungal activity compared to the commercially applied vase solution disinfectant 8-HQ. Vase colonization with molds was more pronounced at oil concentrations < 300 ppm. With all treatments, as the oil concentration increased the log count of molds decreased. For example, at zero time in vases containing dill oil, the log count of molds decreased from 1.92 to 1.46 then to 1.09 as the oil concentration increased from 200 to 300 then to 400 ppm, respectively

Table 10: Total molds in vase solutions of carnation cultivars Farida and Madam Collate as affected by different essential oil treatments (log cfu ml^{-1})

					cv. Farida						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	2.80	2.80	2.80	2.85	2.85	2.85	3.19	3.19	3.19	2.95
	T2	2.78	1.38	0.85	2.93	1.45	0.9	2.31	1.67	1.03	1.70
	T3	2.00	1.83	0.98	2.11	1.93	1.03	2.38	2.20	1.19	1.74
	T4	2.58	1.59	1.09	2.93	1.68	1.15	3.12	2.72	2.15	2.11
	T5	1.92	1.46	1.09	2.03	1.54	1.15	2.52	1.75	1.31	1.64
	T6	2.13	2	1.77	2.24	2.11	1.87	2.99	2.99	1.14	2.14
	Mean	2.37	1.84	1.43	2.52	1.93	1.49	2.75	2.42	1.67	
			$LSD_{0.05}$: Dose, 0.011; Treatment, 0.022								
Season2	T1	2.87	2.87	2.87	2.91	2.91	2.91	3.241	3.241	3.24	3.01
	T2	2.85	1.41	0.87	2.90	1.49	0.92	2.34	1.69	1.05	1.72
	T3	2.05	1.87	1.00	2.16	1.97	1.06	2.41	2.23	1.21	1.77
	T4	2.65	1.63	1.12	2.90	1.72	1.18	3.17	2.18	2.76	2.15
	T5	1.97	1.50	1.12	2.08	1.58	1.18	2.56	1.78	1.33	1.68
	T6	2.18	2.05	1.82	2.29	2.16	1.91	3.15	3.03	1.16	2.19
	Mean	2.43	1.89	1.47	2.54	1.97	1.53	2.81	2.36	1.79	
			$LSD_{0.05}$: Dose 0.102; Treatment, 0.0273; Dose*treatment* Season, 0.746								
					cv. Madam Collata						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	2.92	2.92	2.92	3.00	3.00	3.00	3.18	3.18	3.18	3.03
	T2	2.84	1.504	0.93	2.99	1.58	0.98	2.37	1.71	1.06	1.77
	T3	2.18	2.00	1.07	2.30	2.10	1.13	2.44	2.26	1.23	1.86
	T4	2.03	1.74	1.191	2.19	1.83	1.26	3.11	2.79	2.21	2.04
	T5	2.10	1.59	1.19	2.21	1.68	1.26	2.59	1.80	1.35	1.75
	T6	2.32	2.18	1.93	2.44	2.30	2.04	2.79	2.07	1.17	2.14
	Mean	2.40	1.99	1.54	2.52	2.08	1.61	2.75	2.30	1.70	
			$LSD_{0.05}$: Dose, 0.175; Treatment, 0.0249								
Season2	T1	2.94	2.94	2.941	3.07	3.07	3.07	3.21	3.21	3.21	3.07
	T2	2.90	1.54	0.95	3.27	1.62	1.00	2.40	1.73	1.07	1.83
	T3	2.23	2.04	1.09	2.355	2.15	1.15	2.47	2.28	1.24	1.89
	T4	2.90	1.78	1.22	3.27	1.87	1.27	3.14	2.23	2.82	2.28
	T5	2.15	1.63	1.22	2.26	1.72	1.29	2.62	1.82	1.36	1.79
	T6	2.37	2.23	1.98	2.50	2.35	2.09	3.13	3.1	1.19	2.33
	Mean	2.58	2.03	1.57	2.79	2.13	1.65	2.83	2.40	1.82	
			$LSD_{0.05}$: Dose, 0.128; Treatment, 0.0411; Dose*treatment* Season, 0.620								

It is worthy to mention that the other carnation cv. Madam Collate in both seasons (Table 10) showed the same trend with relatively same values. All the examined essential oils, in particular dill oil, shown to exhibit *in vitro* wide spectra antimicrobial activities (Omer, 2006 and Basem, 2008).

Cellulose Utilizing Microorganisms and the Enterobacteiaceae:

In all examined vase solutions during the two seasons, counts of cellulose decomposing microorganisms (Tables 11 and 12) increased with increasing the time reaching their peaks after a 12-day period. High population densities of such microbial group were counted in vase solutions containing T1. In comparison with control solutions, all the examined preservatives, in particular 8-HQ, greatly suppressed proliferation of cellulose decomposers and resulted in significantly lower densities. Some bacterial genera belong to the family *Enterobacteriaceae* are known to exhibit different enzymatic activities *i.e.* cellulases and chitinase. By-products and secondary metabolites from these bacterial biotypes directly affect the look and viability of the flowers (Kushal, *et al.*, 2003 and El Hanafi, 2007).

Table 11: Counts of cellulose utilizing microorganisms in vase solutions of carnation cultivars Farida and Madam Collate as affected by different essential oils (log cfu ml^{-1})

					cv. Farida						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	3.24	3.24	3.24	3.423	3.42	3.423	5.42	5.42	5.42	4.03
	T2	2.44	2.2	1.89	2.508	2.28	1.943	4.66	4.25	4.06	2.91
	T3	2.66	2.42	2.27	2.732	2.49	2.331	4.99	4.88	4.62	3.27
	T4	2.82	2.81	2.41	2.893	2.88	2.473	4.88	4.12	4.07	3.26
	T5	2.63	2.58	2.383	2.70	2.65	2.45	4.67	4.45	4.22	3.19
	T6	3.14	2.89	2.762	3.23	2.97	2.837	4.743	4.64	4.28	3.50
	Mean	2.82	2.69	2.49	2.91	2.78	2.58	4.89	4.63	4.45	
		LSD. 0.05: Dose, 0.002; Treatment, 0.245									
Season2	T1	3.30	3.30	3.30	3.49	3.49	3.49	5.49	5.49	5.49	4.09
	T2	2.49	2.27	1.93	2.56	2.33	1.98	4.71	4.30	4.10	2.96
	T3	2.71	2.47	2.31	2.79	2.54	2.38	5.09	4.81	4.10	3.24
	T4	2.87	2.86	2.46	2.95	2.94	2.52	4.98	4.20	4.13	3.32
	T5	2.68	2.63	2.43	2.75	2.70	2.50	4.78	4.56	4.32	3.26
	T6	3.20	2.95	2.82	3.29	3.03	2.89	4.86	4.74	4.38	3.57
	Mean	2.88	2.75	2.54	2.97	2.84	2.63	4.99	4.68	4.42	
		LSD. 0.05: Dose, 0.005 ; Treatment, 0.504; Dose*Treatment*Season, 1.204									
					cv. Madam Collata						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	3.14	3.14	3.14	3.23	3.23	3.23	5.32	5.32	5.32	3.90
	T2	2.37	2.158	1.83	2.43	2.22	1.88	4.58	4.19	4.00	2.85
	T3	2.56	2.55	2.51	2.63	2.62	2.58	4.19	4.055	3.88	3.06
	T4	2.55	2.50	2.31	2.62	2.57	2.35	4.50	4.288	4.06	3.08
	T5	2.66	2.63	2.61	2.73	2.71	2.68	4.68	4.521	3.85	3.23
	T6	2.94	2.87	2.78	3.02	2.949	2.86	5.01	4.82	3.71	3.44
	Mean	2.70	2.64	2.53	2.78	2.72	2.60	4.71	4.53	4.14	
		LSD. 0.05: Dose, 0.004; Treatment, 0.664									
Season2	T1	3.12	3.12	3.12	3.20	3.20	3.20	5.40	5.40	5.40	3.91
	T2	2.43	2.213	1.88	2.49	2.27	1.93	4.65	4.24	4.05	2.91
	T3	2.62	2.614	2.58	2.69	2.68	2.64	4.30	4.16	3.97	3.14
	T4	2.61	2.568	2.37	2.68	2.64	2.43	4.54	4.42	4.19	3.16
	T5	2.73	2.702	2.68	2.80	2.77	2.75	4.82	4.66	3.92	3.31
	T6	3.02	2.944	2.85	3.10	3.02	2.93	5.14	4.94	3.80	3.53
	Mean	2.76	2.69	2.58	2.83	2.76	2.65	4.81	4.64	4.22	
		LSD. 0.05: Dose, 0.005 ; Treatment, 0.723; Dose*Treatment*Season, 0.483									

Table 12: Counts of *Enterobacteriaceae* in vase solutions of carnation cultivars Farida and Madam Collate as affected by different essential oils (log cfu ml^{-1})

					cv. Farida						
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	2.70	2.70	2.70	3.16	3.16	3.16	6.31	6.31	6.31	4.06
	T2	1.44	1.044	0.439	1.559	1.154	0.532	4.836	4.681	4.147	2.20
	T3	1.796	1.777	1.708	1.925	1.906	1.836	5.265	5.186	4.998	2.93
	T4	1.775	1.694	1.332	1.905	1.820	1.449	5.052	4.989	4.592	2.73
	T5	1.985	1.939	1.899	2.119	2.072	2.031	5.338	5.285	4.753	3.05
	T6	2.513	2.38	2.214	2.662	2.526	2.354	5.680	5.206	4.727	3.36
	Mean	2.03	1.92	1.72	2.22	2.11	1.89	5.41	5.28	4.92	
		LSD. 0.05: Dose, 0.09; Treatment,0.135									
Season2	T1	2.98	2.98	2.98	3.33	3.33	3.33	6.50	6.50	6.50	4.27
	T2	1.57	1.17	0.54	1.70	1.28	0.64	4.98	4.82	4.36	2.34
	T3	1.94	1.92	1.85	2.07	2.05	1.98	5.42	4.96	4.75	2.99
	T4	1.92	1.84	1.46	2.05	1.96	1.58	5.25	4.97	4.64	2.85
	T5	2.13	2.09	2.05	2.27	2.22	2.18	5.50	5.37	4.90	3.19
	T6	2.68	2.54	2.37	2.83	2.69	2.52	6.19	5.96	4.87	3.63
	Mean	2.20	2.09	1.87	2.38	2.26	2.04	5.64	5.43	5.00	
		LSD. 0.05: Dose, 0.08; Treatment, 0.132; Dose*Treatment*Season, 0.206									

Table 12: Continue

		cv. Madam Collata									
Period (days)		Zero time			4			12			Mean
Concentration		C1	C2	C3	C1	C2	C3	C1	C2	C3	
Season1	T1	2.72	2.72	2.72	2.97	2.97	2.97	6.29	6.29	6.290	3.99
	T2	1.40	1.01	0.43	1.51	1.12	0.52	4.74	4.59	4.17	2.17
	T3	1.74	1.72	1.66	1.87	1.85	1.78	5.66	4.92	4.72	2.88
	T4	1.72	1.64	1.29	1.85	1.76	1.41	5.41	5.03	4.90	2.78
	T5	1.92	1.88	1.84	2.05	2.01	1.97	5.23	5.18	4.66	2.97
	T6	2.44	2.31	2.15	2.58	2.45	2.28	5.77	5.60	4.63	3.36
	Mean	1.99	1.88	1.68	2.14	2.03	1.82	5.52	5.27	4.90	
		LSD. 0.05: Dose, 0.046 ; Treatment, 0.227									
Season2	T1	2.89	2.89	2.89	3.14	3.14	3.143	6.15	6.15	6.15	4.06
	T2	1.43	1.04	0.44	1.55	1.15	0.53	4.45	4.37	4.09	2.12
	T3	1.79	1.77	1.70	1.91	1.90	1.82	5.67	4.84	4.74	2.90
	T4	1.76	1.68	1.32	1.89	1.81	1.44	5.53	5.45	4.284	2.80
	T5	1.97	1.93	1.89	2.11	2.06	2.02	5.71	5.580	4.41	3.08
	T6	2.50	2.37	2.20	2.65	2.511	2.34	6.08	5.94	4.98	3.51
	Mean	2.06	1.95	1.74	2.21	2.10	1.88	5.60	5.39	4.78	
		LSD. 0.05: Dose, 0.041 ; Treatment, 0.162; Dose*Treatment*Season, 0.221									

Statistically significant differences could be observed in the average total viable counts of *Enterobacteriaceae* in the vase solutions due to the type and concentration of the examined preservative as well as the experimental period period(table 12). Population densities of the *Enterobacteriaceae* did not exceed few hundreds cfu.ml^{-1} in all the examined vase solutions at the beginning of the experiment and reached their maxima of >10^{6} cfu.ml^{-1} after a 12-day period. In most cases, the examined oils as well as 8-HQ reduced the proliferation of *Enterobacteriaceae* in the vase solution. For example, the highest average counts of *Enterobacteriaceae* were enumerated in the T1 vase solutions whereas the lowest were recorded in vases received 8-HQ (T2). Amongst all oils, T3 and T4 exhibited the highest level of antibacterial effect against the *Enterobacteriaceae*.

Biodiversity of microorganisms inhabiting vas solutions., A total of 223 microbial colonies repesenting those developed on the selective media of the *Enterobacteriaceae,* cellulose decomposers and fungi were isollated,purified and identified. Fig. 4 illustrated the number of genus and/or species belong to the aforementioned microbial groups.

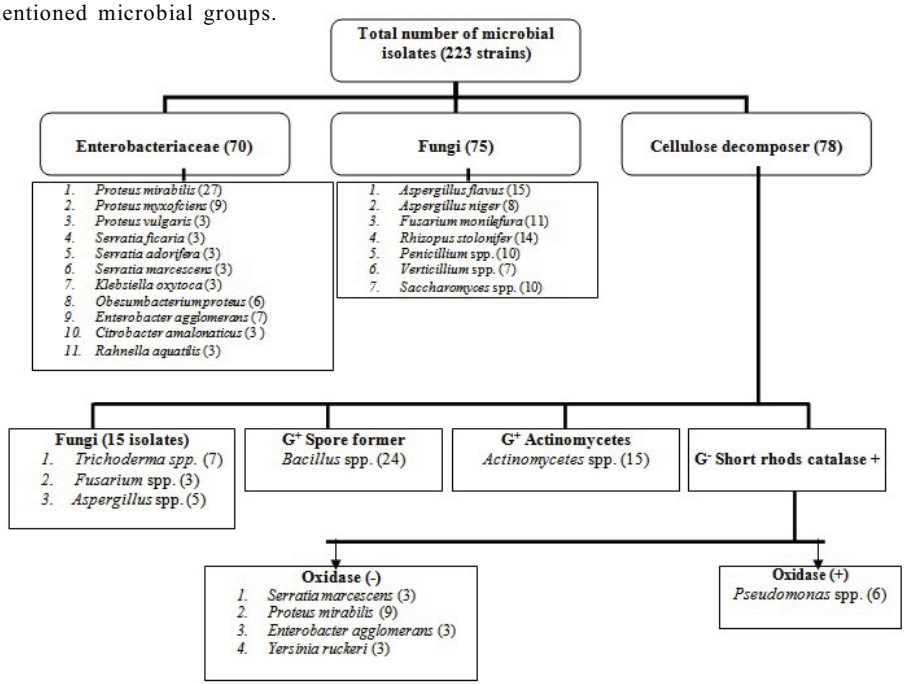

Fig. 4: The description of microbial isolates obtained from the tested vase solutions (number of isolates/strains of species is shown between brackets).

Recommendations:

From all the morphological, anatomical and microbial studies and using different traditional essential oils in order to prolonging the vase life of carnation flowers, it could be recommended that the favorable oils exhibit the best results for extended the vase life and as antimicrobial agents were; dill, coriander and mandarin in addition to the common, harmful and expensive one; 8-HQ.

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