IMPROVING THE POSTHARVEST QUALITY OF CHRYSANTHEMUM CUT FLOWERS BY NATURAL PRESERVSTIVE SOLUTION

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ABSTRACT: The effect of caraway, anise, lavender, marjoram and thyme essential oils treatments were investigated on the postharvest quality of chrysanthemum cut flowers. The essential oils were used as preservative solutions at 0, 25 and 50 ppm. All used treatments prolonged the vase life of cut flowers. Increasing the concentration of caraway, anise and lavender essential oils to the high rate extended cut flower longevity. The maximum vase life obtained by marjoram and thyme treatments, which minimized the fresh weight changes and reduced the deterioration of total chlorophyll in comparing to control flowers. Total carbohydrate content, RWC and MSI maintained in high level by thyme and marjoram treatments by the end of vase life. Using thyme and marjoram essential oil reduced the blockage of xylem in cut flowers. The effects of thyme and marjoram on flower senescence seemed not entirely limited due to their effects on microbes, but they most likely had a sustainable impact on the above tested physiological parameters. The results suggest that the marjoram and thyme essential oils could be used for improving the postharvest quality of cut chrysanthemum as a promising eco-friendly, non toxic, cheap and natural source as an alternative to chemical sources used in preservative solutions in chrysanthemum flowers.

Key words: Chrysanthemum, Preservative, Essential oils, Caraway, Anise, Lavender, Marjoram, Thyme, RWC, MSI

INTRODUCTION

Chrysanthemum as one of the most popular cut flower in the world (Hashemi et al., 2013), has a long vase life since it insensitive to ethylene and non-climacteric, but petal wilting occurred by change in the carbohydrate content, and water relations. water stress caused by vascular occlusion have been reported as the very important factor in reducing the post harvest quality of chrysanthemum cut flowers (Adachi et al., 1999; Nabigol et al., 2005). The microbial occlusions in xylem not only the main impact on chrysanthemums cut flowers longevity, but also microbial products may decrease the water conductivity and subsequent vase life of cut flowers Conrado et al. (1980).

An increase of bacteria growth in the vase solution cause xylem occlusion by

physical blockage from their cells and products, stimulating emboli in the xylem, causing cellular malfunction through toxic metabolite production and enzyme action with degraded cell walls, and/or by endogenous ethylene production (Ratnayake *et al.*, 2012). To inhibit the microbial growth in the vase life solution, addition of antimicrobial substances to the preservative solution is necessary.

In recent years, there has been an increased interest of using the natural antimicrobial which, safety on human health, environment and are relatively cheap, on contrast with chemical antimicrobial, which have toxicity properties, harmful on human health and environmental polluting (Okigbo and Ikediugwu, 2005).

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Naturally products and natural components derived from plants have applications in controlling microbial growth (Sridhar et al., 2003). Essential oils as a natural compounds and biodegradable have antimicrobial properties. Using these safety and environmentally substances in the vase solution has positive effect on the post harvest quality of cut flowers (Mihajilov Krstev et al., 2010 and Oraee et al. (2011). Antimicrobial activity of essential oils often due to the oxygenated compounds (e.g., alcohols and phenolic terpenes), which possess a higher antibacterial activity potential, especially phenol-type compounds (Soković et al., 2002).

Thyme oil improved the vase life of Gerbera jamesonii and reduced microbial contamination in the stem end (Solgi et al., Mousavi Bazaz and Tehranifar 2009). (2011) prolonged the vase life of alstroemeria cut flower by thyme essential oil. Similar results have been reported for the positive effects of herbal essential oils on the increase of the vase life (Hegazi and Gan, 2009) on gladiolus, (Jalili Marandi et al., 2011) on rose and (Kazemi and Ameri, 2012) on carnation cut flowers. Using the essential oils of lavender and anise in rose vase life solution recorded lowest microbial contamination Shanan (2012). Regarding to item, this research mentioned was conducted to study the effect of caraway, anise, lavender, marjoram and thyme on chrysanthemum cut flower qualitative properties.

MATERIALS AND METHODS Plant materials:

Chrysanthemum morifolium var. Bico Anastasia white were obtained directly from a commercial growers in Giza, harvested in the early morning when the outer petals were fully extended and brought to the laboratory of Horticulture Department, Faculty of Agriculture, Tanta University on 13 February 2016 as soon as possible. Only 3/4 of the cut flower was opened. Lower leaves were removed and the flowering stems were trimmed to a length of 40 cm.

Extraction of essential oils:

The selected essential oils were extracted by hydro-distillation according to Britich Phamacopoeia (1963). The essential oils were extracted from seeds of caraway and anise as well as from the herbs of lavender, marjoram and thyme.

Essential oils treatments:

Three concentrations of each selected oils were prepared at 0, 25 and 50 ppm. Cut flowers were kept in bottles containing 300 ml of preservative solution and 5g/L sucrose w/v at 20 \pm 3 °C , 65 \pm 5 % RH and 10 hrs photoperiod with a light intensity of 64 Lux which maintained using white and cool fluorescent lamps. The flowers were checked once a day for signs of deterioration.

Vase life evaluation:

The longevity evaluated as the number of days in vase life required for 75% of the flowers loosed its ornamental value (lost turgor and wilted).

Relative fresh weight (RFW):

The cut flowers were initially weighed at the beginning of the experiment and weighed again on days 1, 4, 8 and at the vase life of control flowers. Relative fresh weight (RFW) of cut flowers was measured as described by He *et al.*, (2006):

RFW % = $(W_t/W_{t-0}) \times 100$; where, W_t is weight of flowers (g) at t = days 0, 1, 4, etc., and W_{t-0} is weight of the same flowers (g) at t = day 0

Relative water content (RWC):

Leaves RWC was measured as described by Weatherley (1950):

RWC % = $\frac{W \text{ fresh } - W \text{ dry}}{W \text{ turgid} - W \text{ dry}} \times 100.$

Where W _{fresh} is the sample fresh weight, W _{dry} is the oven dry weight of the sample at 70 °C for 48 h and W _{turgid} is the sample turgid weight after being saturated with distilled water for 24 h at 4 °C. The samples were taken from second leaf on the stem base on days 3, 5, 8 and 10 from the beginning of the experiment.

Petals water content (PWC):

Petals water content was determined at the vase life of control flowers according to Kalate *et al.* (2008) by the equation of:

Kalate *et al.* (2008) by the equation of: PWC % = $\frac{FW-DW}{FW} \times 100$

Chlorophyll content (Chlo.):

Total chlorophyll content in the leaves was determined using chlorophyll meter (SPAD-502, Minolta Co., Japan) and represented by SPAD value at the days of 1st, 3rd, 5th, 8th and at the vase life of control.

Total soluble solids (TSS)

Total soluble solids were determined in the extracted flower petals on days 3, 8 and 13 from the beginning of experiment by using digital Refractometer model (HANNA, Romania), given TSS values were recorded in Brix percent.

Total carbohydrate content (TCC)

Leaf total carbohydrate content (%) was estimated at the day of control vase life as a soluble carbohydrate content according to Dubois *et al.*, (1956).

Membrane stability index (MSI)

Leaf samples from each treatment were taken from the second leaf at the stem base on days of 2^{nd} , 5^{th} , 8^{th} and 13^{th} for determining membrane stability according to Sairam *et al.* (1997) and using the following formula:

 $MSI = [1 - (C_1/C_2)] \times 100$

Where

- C_{1:} the electric conductivity was kept at 40 °C for 30 min and
- C_{2:} the electric conductivity was kept at 100 °C in boiling water bath for 15 min.

Scanning electron microscopy (SEM)

Microscopic examination was used to examine xylem occlusion by microorganisms at the base of cut flowers stem. At the end of vase life, sections (0.5 cm) were taken from untreated flowers as well as treated flowers by 50 ppm of thyme and marjoram. The samples were prefixed in mixture of (2.5 % glutaraldehyde and baraformaldehyde) at the room temperature for 24 h. The fixative was washed three times by phosphate buffer solution (PH 7.2-7.4). The specimens were post fixed in osmium Tetroxide (1% w/v in phosphate buffer 0.07 M, PH 7.2) at room temperature for 1.5 h. After washing by phosphate buffer solution 3 times, the samples were hydrated in Ethanol series (30 and 50% for 30 m. and 70% for 24 h.) dried at critical point of Co2 (Balzers CPD-020) and covered with gold (30 nm) in a sputter coater (Balzers SCD-040). The specimens were examined and photographed with TESLA BS- 300 electron microscope.

Statistical analysis

Data were arranged in a completely randomized design with five replicates (five flowers for each) and subjected to the proper statistically analysis as the technique of analysis of variance (ANOVA) as mentioned by Gomez and Gomez (1984). The treatment means were compared by using Least Significant Difference (LSD) test at 5% level of significance as outlined by Waller and Duncan (1969). The data collected were analyzed using MSTAT-C (Nissen, 1989) statistical package.

RESULTS AND DISCUSSION Vase life evaluation

Essential oils treatments and its concentration showed a great variation on chrysanthemum flowers longevity (Table 1). Thyme and marjoram treatments significantly extended the vase life of cut flowers compared to the untreated flowers. flowers treated by thyme oil at 25 ppm resulted in the highest vase life (9 days longer than the control). In addition, treatment of marjoram at 25 and 50 ppm prolonged the vase life by 8 days higher Thymol and untreated flowers, than Carvacrol are phenolic complex that have very strong antibacterial and antifungal effects (Yahyazadeh et al., 2008) acts the main components of thyme and marjoram. Solgi et al., (2009) reported that using the essential oils of Thymus vulgaris as well as their active ingredients in the preservative solution increase the vase life of gerbera cut flowers. Similar results have been obtained by Mousavi Bazaz and Tehranifar (2011) on alstroemeria cut flower, (Hegazi and Gan, 2009) on gladiolus, (Jalili Marandi et al., 2011) on rose and (Kazemi and Ameri, 2012) on carnation cut flowers. The lowest vase life was obtained by 25 ppm caraway which gave the same value of the control, but increasing the concentration increased the longevity of cut flowers. Carvon is the active component in caraway essential oil, which acts as a high inhibitory against fungi (Hartmans et al., 1995). Treating flowers by anise treatments significantly prolonged the vase life compared to control flowers, phenolic complex of Anethole and Estragole are the major component in anise essential oil. Abroad antibacterial spectrum against negative and positive Gram bacteria has been exhibited by the anise essential oil (Ozcan and Chalchat 2006). Treated flowers by lavender treatments recorded significant increase in the vase life of chrysanthemum cut flowers, this increased resulted by Linalool and Linalyl acetate which act the

main components of lavender essential oil and have antimicrobial properties.

Relative Fresh weight (RFW)

A sharp increase was observed of control RFW until the 4th day (Table.1), and then a slight decreased till the 8th day, but a sharp decreased was obtained after that. At any time of the experiment the oil treatments recorded higher RFW compared to the control. The highest value recorded on the 13th day was given by the treatments of 25, 50 ppm marjoram and 50 ppm of thyme.

Relative water content (RWC)

All treatments of essential oils maintained RWC at higher level compared with the control. Control flowers recorded sharp decline in RWC values after the 5th day. The maximum value of RWC was obtained by the treatment of 50 ppm thyme oil (Table 1). All used treatments increased RWC values at all the period of the experiment. Caraway treatments resulted the lowest value in this respect.

Petals water content (PWC)

Thyme and marjoram treatments significantly recorded the highest values of PWC compared to the control (Table 2), since treated flowers by thyme at 25 ppm gave the highest PWC as compared to the control. On the other hand treated flowers by caraway treatment at 50 ppm gave the lowest values of PWC.

Chlorophyll content (CC)

The chlorophyll content of chrysanthemum leaves was slightly increased for 8th day and decreased thereafter during the vase life evaluation period (Table 1). However, the chlorophyll decrease in control leaves was recorded on the 5th day and more critical while all levels of essential oils retarded the chlorophyll reduction, more so with 25 ppm of marjoram

V.I. RPW 1	TΛ	RFW 1	RFW 2	RFW 3	RFW 4	RWC1	RWC 2	RWC3	RWC 4					
Treatments	(p)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	1,000,1	1 1010 ⁻ 7	C 1010' 2	1 DIQ. 4	
						Essential	al oils							
Lavender (L)	14.2	102.91	114.91	116.67	99 .60	3 0.5	87.2	82.1	72.4	60.7	62.5	61.8	60.63	67.9
Caraway (C)	13.0	103.08	105.47	107.24	89.02	8.06	87.6	81.0	70.1	62.2	63.4	61.1	29.06	96.8
Anise (A)	13.3	102.95	105.98	106.82	90.93	91.0	87.2	81.2	71.5	62.3	64.3	63.5	61.03	59.4
Marjoram (M)	17.4	103.29	115.53	117.31	105.21	90.3	87.7	84.6	76.2	63.9	65.7	65.0	62.91	61.0
Thyme (T)	16.4	104.07	11.13	112.89	98.23	90.6	88.0	83.4	75.1	63.1	64.7	64.1	61.95	60.8
L.S.D at 0.05	0.368	0.822	3.66	3.192	1.330	N.S	0.550	0.840	0.969	0.676	0.312	0.543	0.49	0.615
						Concentration	ration							
0 ppm	11.5	101.49	106.58	111.87	81.17	90.6	86.5	79.1	65.2	60.9	62.2	27.0	55.20	53.4
25 ppm	16.5	103.77	113.46	113.46	105.18	6 06	88.2	83.9	76.0	63.9	65.6	66.0	63.79	61.4
50 ppm	16.6	104.52	111.78	111.23	103.44	3 0.5	88.0	84.4	78.0	62.5	64.6	66.3	64.37	62.3
S.D at 0.05	0.285	0.637	2.840	NS	1.030	N.S	0.430	0.653	0.751	0.500	0.241	0.420	0.38	0.476
						Interac	tion							
L 0 ppm	11.5	101.49	106.58	111.87	81.17	90.6 86.	86.5	1.67	65.2	60.9	62.2	57.0	55.20	53.4
L 25 ppm	15.0	102.00	116.83	116.83	107.43	91.2	87.8	82.3	74.0	60.0	61.9	63.1	62.50	58.5
L 50 ppm	16.2	105.25	121.33	121.33	110.19	89.7	87.4	85.0	78.0	61.2	63.6	65.1	64.20	61.7
C Oppm	11.5	101.49	106.58	111.87	81.17	<u> 90.6</u>	86.5	79.1	65.2	60.9	62.2	57.0	55.20	53.4
C 25 ppm	11.5	103.68	101.85	101.87	86.60	<u> 90.8</u>	88.1	80.1	67.8	62.4	63.9	61.0	58.15	55.8
C 50 ppm	16.0	104.07	107.98	107.98	99.29	91.1	88.3	83.9	77.4	63.3	64.0	65.5	63.85	61.2
A 0 ppm	11.5	101.49	106.58	111.87	81.17	90.6	86.5	79.1	65.2	60.9	62.2	57.0	55.20	53.4
A 25 ppm	14.2	103.89	105.62	105.62	96.40	91.1	87.3	82.5	74.1	64.8	65.6	66.7	64.10	63.6
A 50 ppm	14.4	103.48	105.73	102.96	95.22	91.2	87.8	82.1	75.3	61.4	65.3	66.8	63.80	61.2
M Oppm	11.5	101.49	106.58	111.87	81.17	90.6	86.5	79.1	65.2	60.9	62.2	57.0	55.20	53.4
M 25 ppm	20.5	104.82	120.47	120.47	116.62	90.1	87.8	87.0	81.7	66.0	68.5	69.7	67.45	64.5
M 50 ppm	20.3	103.56	119.60	119.60	117.83	90.3	88.8	1.78	81.6	64.8	66.5	68.4	66.10	65.3
T 0 ppm	11.5	101.49	106.58	111.87	81.17	3 0.6	86.5	79.1	65.2	60.9	62.2	57.0	55.20	53.4
T 25 ppm	21.6	104.46	122.51	122.51	118.84	91.1	83.9	1.78	82.4	66.7	68.1	69.4	66.75	64.7
T 50 ppm	16.1	106.25	104.29	104.29	94.69	90.2	87.6	83.6	6.11	61.9	63.8	65.8	63.75	62.1
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				TCC	DWC	MS11	WS12	MSI3	MSIA
Treatments	1351	155.2	E 221	(%)	(%)	(%)	(%)	(%)	(%)
				Essential	al oils				
Lavender (L)	12.14	12.43	11.06	13.00	82.32	81.0	74.8	64.1	58.78
Caraway (C)	12.09	12.36	11.39	12.68	82.48	80.7	71.2	66.2	58.17
Anise (A)	12.19	12.37	11.13	13.10	84.15	79.1	71.0	64.2	59.46
Marjoram (M)	12.32	12.61	11.50	13.37	85.64	80.2	71.4	66.7	63.42
Thyme (T)	12.44	12.54	11.49	12.59	84.69	7.67	74.9	67.4	61.64
L.S.D at 0.05	0.145	0.119	0.249	0.127	0.762	0.419	0.152	0.487	0.695
				Concent	tration				
0 ppm	12.13	12.23	10.67	11.00 81	81.61	8.77	69.1	60.0	52.80
25 ppm	12.37	12.61	11.79	14.36	86.00	82.2	75.6	67.3	63.25
50 ppm	12.21	12.54	11.49	13.48	83.96	80.4	73.3	8.69	64.82
S.D at 0.05	0.113	0.092	0.193	0.098	0.589	0.325	0.342	0.377	0.538
				Interaction	ction				
L 0 ppm	12.13	12.23	10.67	11.00	81.61	8.77	69.1	60.0	52.80
L 25 ppm	12.17	12.56	11.47	13.73	83.09	85.6	82.6	62.3	29.06
L 50 ppm	12.13	12.50	11.03	14.27	82.26	79.4	12.1	0.07	64.46
C 0ppm	12.13	12.23	10.67	11.00	81.61	8.11	69.1	60.0	52.80
C 25 ppm	12.00	12.23	11.73	13.40	84.75	81.5	69.3	68.4	27.00
C 50 ppm	12.13	12.60	11.77	13.63	81.09	82.8	75.4	70.3	64.70
A 0 ppm	12.13	12.23	10.67	11.00	81.61	8.11	69.1	0.09	52.80
A 25 ppm	12.30	12.56	11.53	14.60	85.58	79.6	72.8	65.2	62.26
A 50 ppm	12.13	12.30	11.20	13.70	85.26	80.0	71.2	67.5	63.30
M Oppm	12.13	12.23	10.67	11.00	81.61	8.11	69.1	60.3	52.80
M 25 ppm	12.66	12.87	12.00	14.80	87.17	80.8	21.6	20.6	68.86
M 50 ppm	12.16	12.73	11.83	14.30	88.14	81.9	13.7	69.4	68.60
T 0 ppm	12.13	12.23	10.67	11.00	81.61	8.11	69.1	0.09	52.80
T 25 ppm	12.70	12.83	12.20	15.27	89.45	83.5	82.0	70.2	90.69
T 50 ppm	12.50	12.57	11.60	11.50	83.03	6.11	73.6	72.0	63.06
I C Dato OK	0.252	0 206	U N	0.220	1 3 1 9	0.727	0.765	0.843	1 204

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and thyme. Initial chlorophyll content was reduced in control leaves until 13th day.

Total soluble solids (TSS)

Petals TSS were increased during the vase life evaluation period for 8th day and decreased thereafter (Table 2). The highest reduction occurred by control flowers since, in compared with the treatments of marjoram and thyme.

All treatments of essential oils retarded the TSS reduction, more so thyme at 25 ppm, on the other hand the highest decline value obtained by treated flowers from lavender at 50 ppm.

Total carbohydrate content (TCC)

All used treatments maintained higher values of Total carbohydrate content (TCC) in the leaves compared to the control flowers (Table 2). The highest TCC values obtained by 25 ppm thyme, but increasing the concentration to 50 ppm recorded the lowest TCC value.

Membrane stability index (MSI)

Results of (Table 2) indicated that essential oil treatments significantly retained the values of MSI compared with the control which lost their MSI upon the progression of flower senescence during vase life period.

The highest MSI was recorded at 13th day by thyme 25 ppm followed by 25 and 50 ppm of marjoram. On the other hand MSI for

control flowers recorded the lowest value of MSI.

Scanning electron microscopy (SEM)

The Scanning electron microscopy clearly showed that thyme and marjoram treatments have a positive effect in prolonging the vase life of chrysanthemum cut flowers. The blockage of xylem led to water stress, which recorded the limiting factor of cut flowers longevity and expressed as early wilting of leaves and flowers.

The cross section in Fig. 1 shows the xylem cells in the neighborhood of cut were filled with bacteria and as a result of this blockage the cut flowers would loose its turgidity in the control flowers.

On the other hand, under the treatments of thyme and marjoram a good state of xylem vessels was observed. An important general antimicrobial property of essential and their components oils is their hydrophobicity, which enables them to partition with the lipids of bacterial cell wall, cell membrane and mitochondria, causing increased permeability of these membranes. Leakage of ions from these membranes can lead to bacterial cell death (Solorzano-Santos and Miranda-Novales, 2011). The microbial populations in vase solutions of Lisianthus flowers treated with thyme oil (Thymus vulgaris) were lower than other treatments (Kazemi et al., 2011).

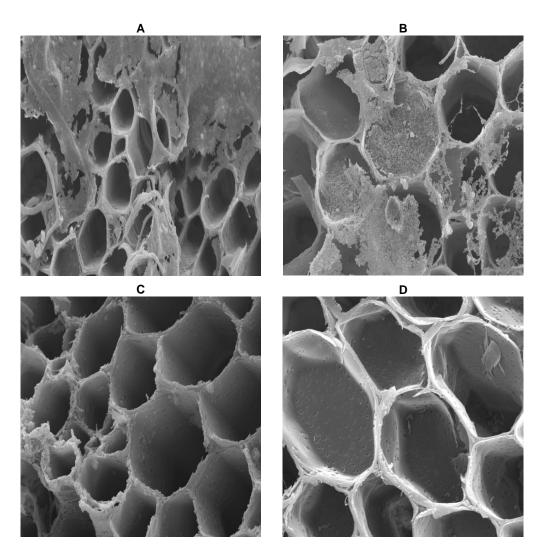


Fig.(1): Chrysanthemum stem cross section at the base of cut flower stem A & B: untreated control flowers, C: the treatment of marjoram at 50 ppm and D: the treatment of thyme at 25 ppm. The section was made at the end of the vase life of each treatment (Magnification:1500x). Note the xylem occlusion in the control flowers and a lot of bacteria appear in the xylem vessels.

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تحسين جودة ما بعد الحصاد لأزهار الأراولا المقطوفة ببعض محاليل الحفظ الطبيعية

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الملخص العربى

أجريت هذه الدراسة فى 13 فبراير 2016 بمعمل البساتين – كلية الزراعة – جامعة طنطا- مصر لدراسة تأثير إستخدام بعض الزيوت الطيارة (كراوية – ينسون- لافندر – بردقوش- زعتر) كمحاليل حفظ على إطالة فترة بقاء أزهار الأراولا (صنف بيكو انستتيا بيضاء) ، واستخدمت التركيزات 0، 25، 50 جزء فى المليون، وقد أستخدم تصميم عشوائي تام فى خمس مكرارت.

وكانت أهم النتائج المتحصل عليها

- أدى إستخدام جميع الزيوت الى إطالة فترة بقاء الأزهار بالفازة مقارنة بالكنترول، وإزداد عمر الأزهار المقطوفة بزيادة التركيز الى 50 جزء فى المليون عند المعاملة بكلا من الكراوية، الينسون واللافندر.
- أدت المعاملة بالبردقوش والزعتر بتركيزاتهما المختلفة إلى زيادة عمر الأزهار المقطوفة وسجلت المعاملة 25 جزء في المليون أفضل النتائج.
 - قللت المعاملة بالبردقوش والزعتر من نسبة الفقد في الوزن الطازج للأزهار معنوياً مقارنة بالكنترول.
- أوضحت النتائج أن المعاملة بالبردقوش والزعتر أدت إلى تثبيط تحلل الكلوروفيل وكذلك الكربوهيدرات الكلية
 بينما حدث تحلل سريع فى الكلوروفيل و الكربوهيدرات فى الأزهار غير المعاملة وكانت أفضل النتائج فى هذا
 الصدد هى زيت البردقوش بتركيز 25 ، 50 جزء فى المليون و الزعتر بتركيز 25 جزء فى المليون.
 - أدى إستخدام البردقوش والزعتر إلى الحفاظ على أعلى قيم لنفاذية الأغشية البلازمية والمحتوى المائي النسبي.
- أظهر الفحص بالميكروسكوب الألكتروني إنسدادت الأوعية الخشبية بالبكتريا للأزهار غير المعاملة وذلك على
 عكس الأزهار المعاملة بالبردقوش والزعتر.
- يمكن التوصية بإستخدام الزيت الطيار للبردقوش والزعتر لإطالة عمر الأزهار بالفازة وتحسين صفات ما بعد الحصاد، حيث تعد هذه المركبات صديقة للبيئة وغير سامة ورخيصة الثمن ومن مصادر طبيعية ويمكن إستخدامها كبدائل واعدة للمركبات الكيماوية في محاليل حفظ أزهار الأراولا.

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