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AN INVESTIGATION INTO THE POTENTIAL ENHANCEMENT OF VASE LIFE AND PHYSIOLOGICAL CHARACTERISTICS OF GERBERA CUT FLOWERS BY APPLE FRUIT EXTRACT AND ROSEMARY ESSENTIAL OILS

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ABSTRACT

Gerbera (*Gerbera jamesonii*) from the *Asteraceae* family with scientific name is one of the world's best cut flowers. Post-harvest senescence is a main limiting factor of many cut flowers' marketability. This research was conducted to investigate the effects of the treatments with rosemary essential oils (200 mg L^{-1}) and apple extract (30, 45 and 60 ml L^{-1}) separately and in combination on the vase life and some physiological characteristics of gerbera cut flowers with a factorial arrangement in a completely randomized design with three replications. The results showed that all treatments significantly increased vase life, total soluble solids of petal, flower diameter, relative fresh weight, solution absorption, and petal carotenoids and decreased solution pH. The longest and shortest vase lives(20.33 and 9 days) were obtained in the apple extract treatment (60 ml L^{-1}) and control, respectively.Overall, it was found that the rosemary essential oil as a safe and natural antimicrobial compound and apple extract having sugary and acid materials and being affordable and safe could be used in gerbera cut flower's preservative solution.

Keywords: Apple extract, Gerbera, Physiological Response, Rosemary essential oils.

INTRODUCTION

Post-harvest senescence is a major limiting factor of many cut flowers' marketability and extensive effort has been made to improve it (Bowyer and Wills, 2003). When cut flowers are placed in a glass vase, petals and leaves start to wilt after a while and stem bends to which that the main cause is water shortage (Solgi *et al*., 2009). Peduncle resists against water flow that is called *vascular occlusion* and the most important reason, bacteria and physiological factors (Damunupola and Joyce, 2008). The most important compounds that have been recommended are bactericides (Halevy and Mayak, 1981). Using natural compounds such as extracts of some fruits, essences and herbal pharmaceutical extracts have been investigated in the last few years. Research and commercial applications have shown that natural compounds can be viable alternatives to conventional chemical compounds (Solgi *et al*., 2009).

Plant essential oils are natural, environment-friendly organic compounds. They have strong antimicrobial properties against some disease-causing factors because of their high levels of phenolic compounds (Bounatirou *et al*., 2007)cvvvqq. Phenolic compounds affect the permeability of cell membranes and can break the outer membrane of gram-negative bacteria, release lipopolysaccharides and increase permeability of the cell membrane to ATP (Burt, 2004). Rosemary is an herb that is rich in phenolic compounds and has antimicrobial activity against gram-positive and gram-negative bacteria (Ouattara *et al*., 1997).

Among natural compounds, extracts of some fruits can be studied in floral preservative solution too. There are a few reports about these compounds. Agampodi and Jayawardena (2009) reported that the treatment with 50% coconut milk +0.23% sodium hypochlorite increased solution absorption and vase life of anthurium until 21th. Apple fruit extract is one of the compounds and is worth testing as a vase solution of cut flowers because of its some components. Apples contain many organic acids and volatile compounds. They also have fructose (Mahajan, 1994). The application of carbohydrates in preserving solutions improves the water balance in cut flowers by influencing the stomatal closure and reducing the water loss. This is a way to protect quality and extend vase life of cut flowers (Halevy and Mayak, 1981). Positive effects of organic acids on carnation have been reported too (Kazemi *et al*., 2010).

The aim of this study was to investigate the physiological response of gerbera cut flowers to apple fruit extract and rosemary essential oils and the feasibility of the application of the compounds in preservative solution of gerbera in order to extend the vase life and improve some of its characteristics.

MATERIALS AND METHODS

In this experiment, gerbera cut flowers were purchased at the commercial stage from Isfahan Veshareh Greenhouse. They were submitted to the laboratory of Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources in appropriate conditions (in a special cardboard and cellophane). The flowers were recut 30 cm below the water. They were put in pre-prepared dishes containing vase solutions inside cabinet refrigerator (temperature of 9±2°C, 400 lux of light with 12/12 hours of lightness/darkness and relative humidity of 65±5%). The treatments included rosemary essential oils (200 mg L^{-1}), apple extract (30, 45 and 60 ml L^{-1}) and rosemary essential oils (200 mg L^{-1}) plus apple extract (30, 45 and 60 ml L^{-1}). Distilled water was used as control.

Apple extracts were taken from Golden delicious cultivar by a juicer. The Brix of the extract was measured by a refractometer (model HRN32) that gave 9.2. Fruit organic acids rate (citric and malic acid) was measured by titration as follows. Ten ml of filtered fruit extract was diluted with water to 100 ml. Then, titration was performed by 0.1N NaOH in the presence of phenolphthalein. As soon as red light was formed in the solution, titration was stopped. Finally, the amount of titratable acid was calculated in terms of dominant malic acid by Equation (1).

$$C = (V_b \times E) + V_j \qquad (1)$$

where,
C= Total acid in terms of ml/100ml
N= Normality of used NaOH
E= Equivalent weight of citric acid and equivalent gram of malic acid
V_j= Volume of fruit extract sample
V_b= Volume of used NaOH

The amount of citric acid and malic acid in 100 grams of apple samples were 0.26±0.07 and 0.28±0.05, respectively. Fehling method was used for the measurement of the total sugar (Arlington, 1990) that was 6.91 (g/100g). Rosemary essential oils were purchased from Isfahan Nature Extract Company, whose composition is shown in Table 1. The measured parameters included vase life, total soluble solids, flower diameter, fresh weight, solution absorption, petal carotenoid, and pH of solutions. To measure the flowers' vase life, some items including petal wilting after 50%, bent necks and brown stems were considered (Mutui et al., 2001).

To measure the amount of total soluble solids in petals, at each stage 0.5 g of fresh petals were weighed with a digital scale, powdered in porcelain mortar and total soluble solids read by a hand refractometer. Digital caliper was used to measure the diameter of flowers. Fresh weight and solution absorption were measured by a digital scale and a graduated cylinder and were calculated by Equations (2) and (3) (Golshadi Ghale-Shahi *et al*., 2015).

$$FW=\frac{(S_{t-1})-S_t}{w_{t=0}} \qquad (2)$$

where,
Fw =The amount of absorbed solution
S_t = Solution weight (g) in days zero, 3 and …
S_{t-1} = Solution weight (g) in the previous day
W_t = 0 = Stem fresh weight in day zero

$\frac{w_t}{w_{t=0}} \times 100$ =percentage of relative fresh weight(RFW) (3)

where,
W_t = Stem fresh weight in the same day and days 3, 6, …
W_t = 0 = Weight of the stem in day zero

To measure the carotenoid, Arnon (1965)'s method was followed. After pulverizing the flower petal in a porcelain mortar and using acetone to volume it in this liquid, the amount of carotenoid was determined on the basis of mg/fresh weight gram using a spectrophotometer at 480 and 510 nm. The pH of the solution was measured with a pH meter.

This experiment was conducted with factorial arrangement in a completely randomized design with three replications in which each replication included three flowers. SAS Software Package was employed to analyze the data through analysis of variance. The means were compared by LSD test.

Table 1. The constituents of the rosemary essential oils procured from Isfahan Nature Extract Company

Compounds	Percent
Alpha- Pinene	1.72
Beta- Pinene	2.44
1,8 Cineole	6.82
Limonene	5.91
Alpha- Terpinene	0.43
Subinene	1.53
Neoiso- Menthol	1.32
Menthol	20.48
Cis- Dihydro carvone	2.32
Pipertone	1.6
Carvone	13.86
Menthyl acetate	2.12
Beta- Caryophyllene	3.2
Germacrene- D	3.47
Viridiflorol	1.39
Menthone	12.75
Trancs- Sabinehydrate	2.84
Neodihydro Carveol	4.47
Iso- Menthylacetate	0.28
Carvacrol	0.41
Neomenthol	7.63
Para- Cymene	0.98
Other compounds	2.03

RESULTS AND DISCUSSION

The results of analysis of variance of data showed that the effect of treatment, time and interaction between treatment were significant for all studied characters ($p<0.01$) (Tables 2, 3 and4).

In some studies, carbohydrates, organic acids and essential oils have been reported to influence the vase life of cut flowers (Kazemi *et al*., 2012). It seems that acidic compounds and carbohydrates in apple extract result in the extended vase life. In this experiment, rosemary essential oils also increased vase life, but its combination with apple extract had a greater impact.

Vase life: The results showed that maximum vase life (20.33 days) was obtained from the treatment of apple extract (60 ml L^{-1}) and that control treatment had the shortest vase life of 9 days (Fig. 1).

Total soluble solids: The results showed that the highest and lowest total soluble solids were obtained from the apple extract (30 ml L^{-1}) plus rosemary essential oils treatment and the control, respectively (Table 5). The amount of total soluble solids was increased until the ninth day and then it started to decreased (Table 6). Soluble carbohydrates in petals reduced water potential and consequently increased the solution uptake (Ho and Nicholas, 1977). It seems that apple extract with high percentage of carbohydrates such as fructose and sucrose increases the amount of total soluble solids in flower petals. On the other hand, rosemary, both individually and in combination with apple extract, increased total soluble solids.

Table 2. ANOVA of the effect of the treatment on vase life of gerbera cut flowers..

S.O.V	df	Vase life
Treatment	7	42.56**
Error	14	0.6
CV(%)	-	4.88

** Significant 1%

Table 3. ANOVA of the effect of treatment and time on measured characteristics of gerbera cut flowers.

S.O.V	df	Total soluble solids	Flower diameter	Fresh weight	Solution uptake	Carotenoid
Treatment	7	54.66**	3431.061**	4813.71**	1.97**	1.55**
Time	4	424.84**	32280.155**	24400.53**	20.33**	7.65**
Treatment* Time	28	13.96**	1476.46**	2294.7**	1.03**	0.49**
Error	80	0.01	1.05	6.7	0.01	0.002
CV (%)	-	1.65	1.09	2.7	10.82	4.6

** Significant 1%

Table 4. ANOVA of the effect of treatment and time on pH of the solutions of gerbera cut flowers.

S.O.V	Df	pH of solutions
Treatment	7	3.52**
Error	14	6.21
CV(%)		0.26

** Significant 1%

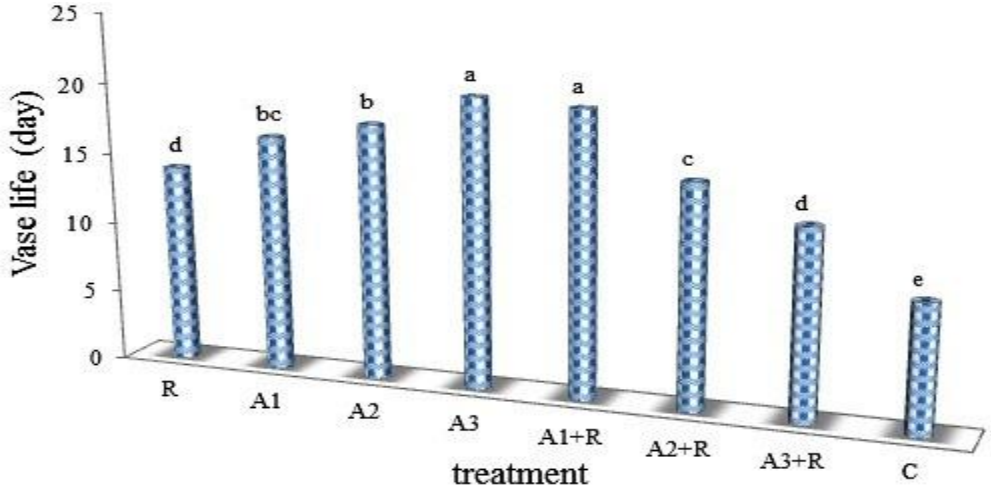


Fig 1. The effect of preservative solutions on vase life of gerbera cut flowers.

Table 5. The effect of preservative solutions on measured characteristics of gerbera cut flowers..

Treatment	Total soluble solids	Flower diameter	Fresh weight	Solution uptake	Carotenoid	pH
R	8.12e	83.04f	86.08e	1.35c	0.84d	5.52b
A1	8.89c	94.21e	89.73d	1.30c	1.28b	4.31de
A2	8.90c	97.76d	100.38c	1.34c	1.26b	4.24e
A3	9.36b	113.72a	103.29b	1.49b	1.15c	4.12f
A1+R	9.80a	110.59b	128.71a	1.81a	1.27b	4.63c
A2+R	9.3b	99.90c	102.30b	1.39bc	1.40a	4.32d
A3+R	8.26d	82.70f	88.09d	1.16d	1.25b	4.16f
C	3.79f	68.70g	66.58f	0.53e	0.42e	6.21a

R: Rosemary essential oils (200 mg L^{-1}). A1: Apple extract (30 ml L^{-1}). A2: Apple extract (45 ml L^{-1}). A3:Apple extract (60 ml L^{-1}). In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Flower diameter: The results of means comparison showed that the highest and lowest flower diameters were related to the apple extract (60 ml L^{-1}) and control, respectively (Table5). The flower diameter variations caused by time showed that the flower diameter increased up to the fifth day and then decreased (Table6). The growth of the petals associated with flower opening is the result of cell development (Knee, 2000). Cell development needs water flow and osmolyte materials such as carbohydrates into petal cells (Evans and Reid, 1988). Talukdar and Barooah (2011) stated that citric acid and sucrose treatment increased the floret diameter of *Polianthes tuberosa*. It can be said that apple extracts combined with organic acids and carbohydrates increased the flower diameter.

Table 6. The effect of time on measured characteristics of gerbera cut flowers..

Time	Total soluble solids	Flower diameter	Fresh weight	Solution uptake	Carotenoid
1	4.40e	117.44c	100c	0.60d	0.29e
5	6.75c	119.71a	114.91b	1.30b	1.50b
9	13.23a	118.09b	123.77a	2.88a	1.76a
13	12.36b	77.50d	97.58d	0.88c	1.07c
17	4.78d	36.40e	41.96e	0.83c	0.95d

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

Relative fresh weight: The results revealed that maximum relative fresh weight was related to the treatment of apple extract (30 ml L^{-1}) plus rosemary essential oils (Table 5). The minimum relative fresh weight was observed in control. The relative fresh weight of flowers was increased until the the ninth day and afterwards, it started to decrease (Table 6).The ability of flowers to absorb water declined by aging process and eventually reduced cell turgor (Ichimura *et al.*, 2002). Rezvanypour and Osfoory (2011) reported that citric acid with sucrose treatments increased fresh weight of cut flowers as compared to silver thiosulfate and aluminum sulfate and since apple extract contains citric acid and sugar compounds, it may increase the fresh weight of cut flowers.

Solution absorption: The results of the means comparison of showed that the maximum and minimum solution uptakes were obtained from apple extract (30 ml L^{-1}) plus rosemary essential oils and control (Table 5), respectively. The variation trend of solution uptake showed that it was increased until the fifth day and then, it exhibited a descending trend (Table 6). The water absorption depends on respiration, temperature and dissolved compounds (Khosravi Nahrabadi *et al*., 2015). One of the most important factors involved in reducing water uptake by peduncle after harvest is bacterial contamination (Van Doornand Dhort, 1994). It seems that the antimicrobial effect of rosemary essential oils and apple extract which is associated with their acidic compounds led to relieve obstruction of vessels and increased solution absorption.

Carotenoids: According to means comparison, the highest and lowest petal carotenoid rates were observed in flowers treated with apple extract (45 ml L^{-1}) plus rosemary essential oils and control, respectively (Table5). The carotenoid changes caused by time showed that the amount of carotenoid increased up to the ninth day and then decreased (Table6). Petal's discoloring is one of the post-harvest problems of the cut flowers which reduces the flowers quality and has significant effect on their senescence (Amarjit, 2000). Antimicrobial compounds prevent the breakdown of flavonoids and make the

flowers fresh by improving the water absorption (Hashemabadi and Bagheri, 2013). In present study, all treatments caused a delay in the reduction of carotenoids. But, it seems that apple extract (45 ml l^{-1}) + rosemary essential oil is more suitable treatment for keeping carotenoids.

pH of solutions: Results of means comparison of data showed that the highest and lowest pHs of the solution were obtained from control and apple extract (60 mlL^{-1}) + essential oil of rosemary, respectively (Table 5). It was indicated that the pH of the solution decreased on the first day as compared to the final day of test (Table 7).

Table 7. The effect of time on pH of solution of gerbera cut flowers.

Time	pH
1	5.05a
17	4.34b

Acidic solutions move in stem faster than alkaline or neutral solutions and thus, they improve solution uptake by vessels. Since apple extract has acidic compounds, it individually and in combination with rosemary essential oil reduced the pH of the solution. The effect of copticum and savory in preservative solutions the pH loss was reported by Jalili Marandi *et al*. (2011), which is consistent with our results for the effect of rosemary essential oils on solution pH loss.

Conclusions: The results showed that rosemary essential oils and apple extract separately and in combination with each other can extend the vase life and some physiological characteristics of gerbera cut flower. Rosemary essential oils as a safe and natural antimicrobial compound and apple extract having sugary and acid materials and being affordable and safe could be used in gerbera cut flowers' preservative solution.

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