Journal of Chemical Health Risks

Journal of Chemical Health Risks (2016) 6(4), 281–289

ORIGINAL ARTICLE

Investigating the Potential of Increasing of the Vase Life and some Qualitative Traits of *Tuberose* Cut Flowers by Non-Harmful Compounds Environmentally

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(Received: 24 February 2016 Accepted: 9 May 2016)

KEYWORDS

Environment;

Natural compounds;

Tuberose

ABSTRACT: In order to investigate the use of natural and healthy treatments and reduction of the risks of using chemicals at the preservative solutions of cut flowers, *Eucalyptus* extracts (12,25, 50%), rosemary (12, 25, 50%), sour orange (4.5 ml l^{-1}) combined with 4% sucrose were used in vase solution of tuberose. This experiment was performed in a completely randomized design with factorial arrangement in three replications. Traits of vase life, the percentage of opening buds, total soluble solids, solution uptake, relative fresh weight, chlorophyll a, b and total chlorophyll were evaluated. The maximum and minimum vase life were obtained for the flowers treated with *Eucalyptus* extract of 50% + sour orange fruit extract (11.33 days) and control group (6.33 days), respectively. The treatment of rosemary extract of 50% + sour orange extract had the highest percentage of the opening buds and relative fresh weight. The highest total soluble solids and solution uptake were related to treatments of *Eucalyptus* extract of 25% + sour orange extract and *Eucalyptus* extract of 50% + sour orange extract, respectively. Flowers treated with *Eucalyptus* extract of 12% + rosemary extract had the maximum chlorophyll a, b and total chlorophyll. The combination of the extracts of rosemary and *Eucalyptus* with sour orange fruit extract improves the vase life and some physiological traits of it. Since the environment is faced with serious risks, the use of harmful chemicals to extend the life of horticultural products is not explainable. Thus, the use of natural, safe, inexpensive and available for preservative solutions of the cut flowers is an appropriate way to minimize harm to the environment.

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INTRODUCTION

Tuberose with the scientific name of *Polianthes tuberose* L belonging to the Agavaceae family is herbaceous, perennial plant belonging to the monocotyledons, native of tropical and subtropical regions and one type of flower bulbs that are used as a cut flower. This flower cultivation in Iran is suitable due to climatic conditions and global markets is increasing for exporting [1, 2].

Nowadays, the use of flowers and ornamental plants in the residence places and work places seems to be necessary due to the human condition including urbanization, less access to nature and a reduction in per capita green space. Cut flowers are particularly important in this context. Before cutting flowers, they provide their needs, which are often water and food, through their mother plant and since they are alive and active after picking, need to water and sugars such as sucrose, but the growth of microbial population results in vascular obstruction and solution [3]. Thus, the addition of anti-bacterial materials to preservative solutions has application [4]. There are many reports about the use of chemicals in preservative solutions of flowers. The use of compounds containing silver ions has been suggested to control ethylene production in cut flowers as well as improve water relations [5, 6]. However, compounds containing silver used in the past has no justification for eco friends to use. The use of chemicals despite to control microbial contamination of preservative solutions, has a bad impact on the environment as well as health of consumers. Thus, using natural ingredients such as essential oils and herbal extracts can be one of the main strategies used in postharvest physiology of cut flowers [7].

Positive results of some extracts of medicinal plant and fruits in preservative solutions of cut flowers as antimicrobial and healthy compound have been reported and proved [8, 9].

Using stevia and thyme extracts increases the vase life of rose cut flower [10]. The essential oil of geranium increased the vase life of chrysanthemum cut flowers [11].

*Eucalyptus*is one of the medicinal plant that antibacterial and anti-fungal properties of itsessence have been known [12]. The essential oil of *Eucalyptus* increased vase life of ornamental sunflower and improved its physiological traits [13].

Sour orange (*Citrus aurantium* L.) from Rutaceae family has acidic materials such as citricacid, malic acid and the flavonoid compounds and has high potential for using in different fields [14]. Treatment of 200 mg l^{-1} of malic acid caused reduction of the number of bacteria in the vase solution of cut *Gerbera* and increased the vase life [15].

Apple fruit extracts and rosemary essential oils, individually and in combination with each other, raised vase life of *Alstroemeria* cut flowers and lead to improveits qualitative traits [16].

The aim of this study was to evaluate the use of Eucalyptus and rosemary extracts combined with sour orange fruit extract as antimicrobial, healthy and environmentally friendly compounds to apply in preservative solutions of *Tuberose* cut flower in order to increase its vase life and physiological traits.

MATERIALSAND METHODS

Tuberose cut flowers were harvested from commercial greenhouses in the city of Varamin at the stage that the lowest floret pair was opened and were properly transferred to the Physiology Laboratory of Horticulture Department of Gorgan University of Agricultural Sciences and Natural Resources, Northern Iran. The flowers were recut at the length of 55 cm and were placed in a solution prepared previously. The treatments included extracts of rosemary (12, 25 and 50%), *Eucalyptus* (12, 25 and 50%) and sour orange (4.5 ml). Four percent sucrose was used in all treatments. Distilled water and 4%

sucrose was used as a control. To produce herbal extracts, 1:10 ratio of plant material to water with distiller's device was used. To use the sour orange fruit extract, several sour oranges were harvested from sour orange trees at the Campus of Agricultural Sciences and Natural Resources of Gorgan University, and their extract was taken out with a hand juicer. Then, the concentration of the extracts was determined by a pH meter. Concentration of 4.5 ml L^{-1} was obtained for pH 5. Since orange juice is an acidic substance, with the change in value of the extract can be obtained pH desired

The flowers were under condition of temperature of 20 ± 2 °C, humidity of 60 ± 5%, light of at 600 lux and 12 h photoperiod.

Measured characteristics included vase life, opening of florets, total soluble solids, solution absorption, relative fresh weight, chlorophyll a, chlorophyll b and total chlorophyll. Vase life was evaluated on a daily basis by Reid (1996)'s method in which the changes in flower color, falling of florets, and the opening of flowers are considered [17]. The percentage of flower opening was determined by counting the total number of florets on the first day and daily counting of the open florets until the last days of each flower. For the measurement of soluble solids of petals, every 3 days 0.5 g of petals was separated and pulverized in a mortar, and its extract was obtained after it was crushed. The Brix degrees of the obtained extract were read by using a manual refractometer device [16].

Relative fresh weight was measured every 3 days by using a digital scale and was calculated by the following formula.

wt /wt=0 ×100= relative percentage of fresh weight (RFW)

Wt: Stem fresh weight in the same day and days 3, 6, ...

Wt=0 = Weight of the stem in day zero

Water absorption was measured every 3 days by using a graduated cylinder and was calculated by the following formula.

WA= (St-1)-St /wt=0

WA: The amount of absorbed solution

St: Solution weight (g) in days zero, 3 and …

St-1: Solution weight (g) in the previous day

Wt=0: Stem fresh weight in day zero

Arnon (1967)'s method was used to measure chlorophyll [18]. After pulverizing the leaves in a porcelain mortar and using acetone to volume it in this liquid, the amount of chlorophyll determined based on mg/fresh weight gram using a spectrophotometer. This was conducted on the first, fourth, seventh, and tenth days of the experiment.

The study was arranged in a factorial experiment based on completely randomized design with 3 replications that each replication included 5 flowers. Data were analyzed using SAS software and mean comparisons were done according to the LSD test.

RESULTS

Vase life

Analysis of variance showed that the effects of treatment were significant at the 1% level (Table 1). The results of the means comparison showed that the maximum and minimum vase life were related to *Eucalyptus* extract (50%) + Sour orange extract and control, respectively (Figure 1).

Table 1. ANOVA of effect of treatments on vase life and bud opening of tuberose cut flowers.

S.O.V	df	Vase life	bud opening
Treatment	6	8.41^{*}	351.03^{ns}
Error		1.62	146.92
CV (%)	-	14.88	19.52

** Significant differences at 1%, ^{ns} no significant differences.

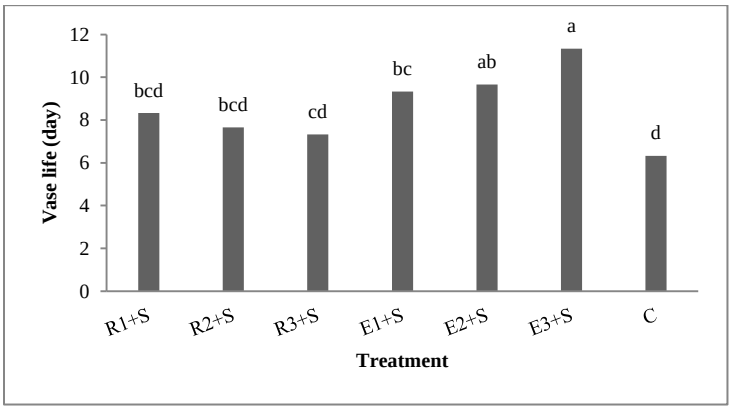

Figure 1. The effect of preservative solutions on vase life of tuberose cut flowers.
R1: Rosemary extract (12%),**R2**: Rosemary extract (25%), **R_3**: Rosemary extract (50%)
E1: *Eucalyptus* extract (12%),**E2**: *Eucalyptus* extract (25%), **E3**: *Eucalyptus* extract (50%)
S: Sour orange extract, **C**: Control.

Percentage of bud opening

Analysis of variance showed that the effects of treatment were not significant on percentage of bud opening (Table 1). The results of the mean comparison of data showed that the maximum and minimum percentage of bud opening were observed in rosemary extract (50%) + sour orange extract and control, respectively (Table 2).

Table 2. Mean comparison of effect of preservative solutions on measured traits after harvesting tuberose cut flowers.

Treatment	Bud opening	Total soluble solids	Solution uptake	Fresh weight	Chlorophyll a	Chlorophyll b	Total chlorophyll
R1+S	60^{ab}	6.38^{ab}	0.58^{a}	76.54^{c}	0.19^{c}	0.16^{d}	0.35^{c}
R2+S	64.18^{a}	5.70^{bc}	0.47^{b}	81.45^{bc}	0.22^{bc}	0.17^{cd}	0.39^{b}
R3+S	74.86^{a}	4.93^{c}	0.46^{b}	112.77^{a}	0.21^{bc}	0.18^{b}	0.38^{b}
E1+S	61.9^{a}	6.18^{ab}	0.34^{c}	$84.51b^{c}$	0.25^{a}	0.23^{a}	0.49^{a}
E2+S	66.23^{a}	6.80^{a}	0.40^{bc}	96.25^{ab}	0.23^{ab}	0.18^{bc}	0.41^{a}
E3+S	67.36^{a}	5.68^{bc}	0.66^{a}	98.38^{ab}	0.14^{d}	0.16^{d}	0.30^{c}
C	40.06^{b}	3.58^{d}	0.27^{d}	74.31^{c}	0.14^{d}	0.09^{e}	0.23^{d}

In each column, means with the similar letters are not significantly different at 1% level of probability using LSD test.

Total soluble solids

The results of analysis of variance of data showed that the effect of treatment, time and interaction between treatment and time were significant at the 1% level on total soluble solids (Table 3). All treatments in this experiment increased total soluble solids compared to control, maximum increase was related to Eucalyptus extract (25%) + sour orange extract, and the minimum was related to the control (Table 2). The solution uptake

changes showed that it was increasing until 7th day and after that, it started to decrease (Figure 2).

Table 3. ANOVA of effects of treatment and time on measured traits of Tuberose cut flowers.

S.O.V	df	Total soluble solids	Solution up-take	Fresh weight	Chlorophyll a	Chlorophyll b	Total chlorophyll
Treatment	6	13.84**	0.21**	2307.51**	0.20**	0.019**	0.14**
Time	3	97.45**	1.85**	11568.72**	0.33**	0.22**	1.29**
Treatment*Time	18	6.35**	0.12**	680.10ns	0.013**	0.008**	0.09**
Error	56	1.62	0.08	431.96	0.001	0.0002	0.003
CV (%)	-	22.7	19.22	23.3	16.31	10.009	16.92

** Significant differences at 1%, ns no significant differences.

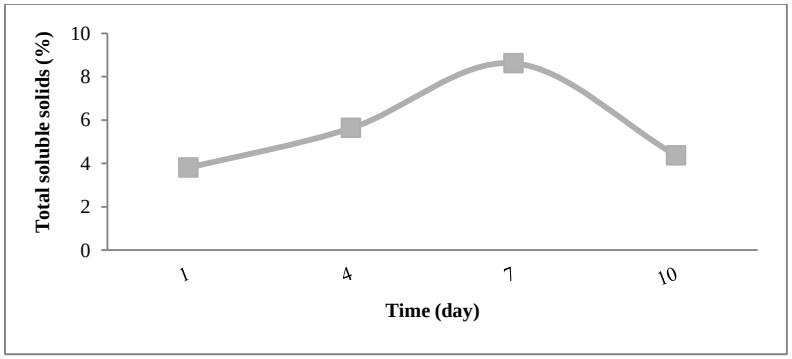

Figure 2. Changing process of total soluble solid of tuberose cut flowers during experiment.

Solution uptake

The results of analysis of variance of data showed that the effect of treatment, time and interaction between treatment and time were significant at the 1% level on solution uptake (Table 3). All treatments in this experiment increased solution uptake compared to control, maximum increase was related to Eucalyptus extract (50%) + sour orange extract, and the minimum was related to the control (Table 2). The solution uptake changes showed that it was increasing until 7th day and after that, it started to decrease (Figure 3).

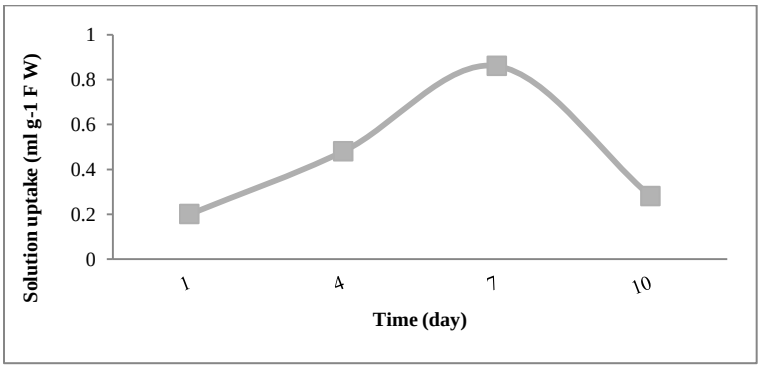

Figure 3. Changing process of solution uptake of tuberose cut flowers during experiment.

Relative fresh weight

The results of analysis of variance of data showed that the effect of treatment and time were significant at the 1% level on relative fresh weight, but interaction between treatment and time were not significant (Table 3). Mean comparison showed that the maximum increase in relative fresh weight was associated with the rosemary extract (50%) + sour orange extract and the minimum was related to the control (Table 2). The relative fresh weight changes showed that it was increasing until the 4th day and decreased until 10th day (Figure 4).

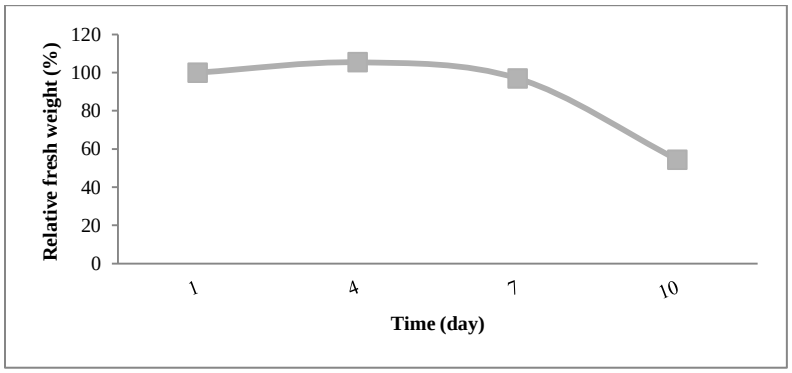

Figure 4. Changing process of relative fresh weight of tuberose cut flowers during experiment.

Chlorophyll a, chlorophyll b, total chlorophyll

The results of analysis of variance of data showed that the effect of treatment, time and interaction between treatment and time were significant at the 1% level on chlorophyll a, b and total chlorophyll (Table 3). The maximum and minimum amount of chlorophyll a, b and total were obtained in rosemary extract (12%) + sour orange extract and control, respectively (Table 2). The chlorophyll changes showed that it was increasing until the 7th day and after that it was decreased (Figure 5).

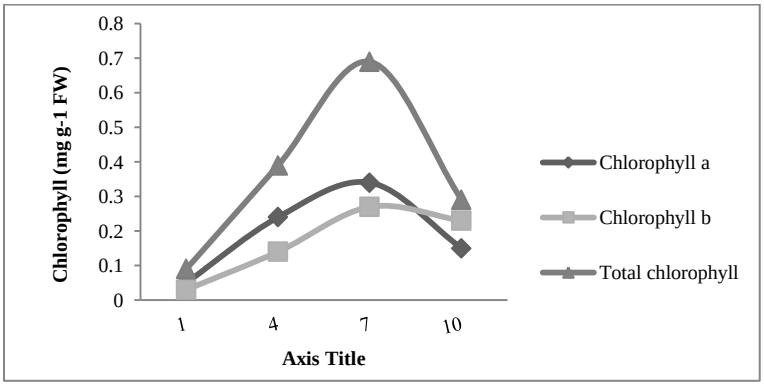

Figure 5. Changing process of chlorophyll of tuberose cut flowers during experiment.

DISCUSSION

The vase life of cut flowers is evaluated based on morphological characters like petals and leaves wilting and steam bending [19]. The most important effect of studied treatments on the increasing the vase life is due to their bactericidal properties. The use of sucrose lonely has no positive effect on the vase life of the flower and this is likely because of providing the appropriate condition for growth of bacteria and fungi [19]. The negative

effects of microorganisms in reducing the vase life of cut flowers are attributed to steam blocker bacteria, producing toxic compounds and endogenous ethylene production that has a very important role in reducing the durability and quality of cut flowers [20]. In current study, it seems that the increased vase life of flowers is resulted by antimicrobial activity and the compounds in the extracts of *Eucalyptus* and sour orange. The vase life of gerbera cut flowers was increased by using *Eucalyptus* essential oils that is in accordance with the results of this study [21]. The sour orange extract increased the vase life of *Narcissus* cut flower [8].

"Since the opening flower are required to use ATP and providing the required ATP is needed to break down sugar molecules during the process of respiration, so anything that reduces the amount of plant respiration can delay the opening of flowers and simulating the flower ageing" [22]. In this study, although there was no significant effect of treatments on the percentage of buds opening, treatments had a significant difference compared to control by increasing the concentration of the extracts and the percentage of buds opening was increased. Therefore, perhaps the used treatments have improved conditions of sucrose absorption with increasing concentration and sucrose could increase the percentage of buds opening by contribution of extracts. Extracts of rosemary and thyme improve the development of rose flower buds that is consistent with our results [23].

One of the most important factors in delaying postharvest senescence of cut flowers is to increase the carbohydrate content of flowers [11]. Sugar (total soluble solids) is one of the most important factors in determining the vase life of cut flowers. Therefore, carbohydrates increase the vase life of flowers [24]. The antimicrobial compounds increased the carbohydrate content of stem of rose cut flowers and since the treatments used in our study increased the total soluble solids [25], it is in accordance with their results.

Water balance is one of the main factors in determining the quality and vase life of cut flowers [26] and lack of water normally causes the stem vessels blockage [27]. The formation of air bubble inside the stem vascular prevents transformation of water through the stem and thus, the hydraulic resistance is increased and it causes severe water stress. Preventing water absorption is attributed to other factors such as vascular blockage by microorganisms [28]. Antimicrobial compounds in the vase solution protect the vessel by preventing microbial growth and unblocked the vessel and therefore, the water absorption can be done without interrupting [29-31]. On the other hand, the fresh weight of the flowers is also increased. In the present study, the used extracts improve water absorption by reducing vascular occlusion and following that, they have increased the fresh weight of the flowers. The relative weight and water absorption of carnation cut flowers were increased by rosemary extract [32] in accordance with our results. Sucrose and lemon extracts increased the amount of fresh weight of marigold cut flowers [33].

Leaf senescence is associated with a decrease in chlorophyll. Reduction of leaf chlorophyll of cut flowers, simultaneous with senescence, can be resulted from chlorophyll degradation, internal plant hormone deficiency or imbalance between them, as well as excessive accumulation of sugar in the leaves. Water stress resulted by separation of cut flower from the mother plant and the vessel blockage by bacteria causes an increase in oxygen free radicals of the chloroplast and it is followed by the degradation of chlorophyll molecule and the chloroplast membrane that leads to a decrease in photosynthesis and growth [19]. In this study, since the lowest chlorophyll level is related to the control, it is perhaps due to the lack of antioxidants and the flowers treated with extracts have more chlorophyll because of antioxidant properties of extracts. The most important effect of the essential oils in maintenance of chlorophyll is due to its antioxidant properties [19]. Extracts of rosemary, *Euca-*

lyptus and sour orange delayed decreasing the chlorophyll content of tuberose cut flower [9].

CONCLUSIONS

Combination of extracts of rosemary and Eucalyptus with sour orange fruit extract improves the vase life and some physiological traits of it. Since the environment is faced with serious risks, the use of harmful chemicals to extend the life of horticultural products is not explainable. Therefore, the use of natural, safe, inexpensive and available substance in preservative solutions of cut flowers is an appropriate solution to reduce damage to the environment through this way. According to the results of this research, the effect of this compound on other cut flowers is appropriate and explainable.

ACKOWLEDGEMENTS

The authors declare that there is no conflict of interests.

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