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ORIGINAL ARTICLE

Physiological Response of Tuberose Cut Flowers to the Benzyladenine and Plant Extracts

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ABSTRACT
To assess the increasing vase life and some qualitative traits of tuberose cut flowers (Polianthes tuberosa L.), the cut flowers were treated with Benzyladenine (50 mg L^{-1}) and with combination of rosemary and eucalyptus extracts (12, 25, and 50%) with sucrose 4%. The experiment was conducted based on completely randomized design with three replications as factorial. Vase life, percentage of open florets, relative fresh weight, solution uptake, chlorophyll a, b and total chlorophyll were studied. The results showed that the effect of treatments on measured traits was significant at 1% level. In eucalyptus 12% + BA 50 mg L^{-1}treatment the most vase life, relative fresh weight, total soluble solids, chlorophyll a, b and total chlorophyll were achieved. The highest percentage of floret opening was observed in flowers treated with eucalyptus of 25% + BA. The highest solution uptake was observed in eucalyptus extract 50% + BA. The results showed that combination of rosemary and eucalyptus extracts together was less effective than the combination of each of them with BA, although it makes a significant difference compared to control in most traits. In general, results showed that using rosemary and eucalyptus extracts with BA is proper combination for increasing vase life and some qualitative traits of tuberose cut flowers.
***Key words**: Benzyladenine, Tuberose, Rosemary and Eucalyptus extracts, Vase life*

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INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is the perennial plant from Agavaceae family and one of the most important fragrant cut flowers in tropical and subtropical which is native in Mexico [1]. It is an important commercial cut flower in most parts of the world [2]. This flower in the post-harvest stage will wither rapidly [3]. The Inflorescences of tuberose has up to 20 pairs of florets that open bottom-up. Usually about 50% of the buds are opened after harvesting, and florets and buds shed few days after harvesting [4]. Tuberose cut flowers have two fundamental problems in their post harvested life, sensitivity to ethylene and obstruction of vessels [5]. Obstruction of the end of peduncle as a result of bacteria buildup is most important factor for imbalance water uptake, water loss and early senescence of cut flowers [6]. Therefore, the use of anti-ethylene and antibacterial compounds in preservative solution of tuberoses is essential.

Cytokinins were very effective on delay senescence in carnation cut flowers by preventing ethylene biosynthesis [7]. Using thidiazuron as a preservative solution of Alstroemeria cut flowers increased vase life of it [8]. Cytokinins, especially 6-BA, prevent ethylene activity, a harmful gas for cut flowers, and this hormone is most important factor for delaying senescence of petals and leaves of carnation, tuberose and matthiola [9, 10].

In recent years, using natural compounds such as essential oils is the new idea in control of bacterial and fungal infections and reduces postharvest losses of horticultural products including fruits, vegetables and flowers, and great efforts made in field of identification and discovery of natural compounds and their application in postharvest technology of horticultural crops [11]. Oraee *et al*. (2011) reported that thymol increases vase life and decreases the amount of bacteria in the vase solution of gerbera cut flowers [12].
Rosemary is one of the medicinal plants from Lamiaceae family that its culture is common in many parts of Iran [13]. The main compounds of leaves and flowering shoots of rosemary are essential oils that mainly make up from 1,8- cineole, borneol, bornyl acetate and camphor. Babarabie *et al*. (2015) in their study for evaluating the increase of vase life and some quality traits of Alstroemeria cut flowers used rosemary extract [14]. The results of Hosseini darvishani and Chamani study (2013) showed that the use of extracts of peppermint, rosemary and thyme increases vase life of rose cut flowers [15].
Eucalyptus is one of the plants that antibacterial and antifungal properties of its essential oils are known Ebadian *et al* (2011) used in their study from essential oils of eucalyptus and caraway. They reported that these essential oils increased vase life of gerbera cut flowers and reduced bent neck of flowers [16].
The aim of this study is investigate of using combination treatment of BA and essential oils of rosemary and eucalyptus in preservative solution in order to increase vase life and some quantitative traits of this flower.

MATERIALS AND METHODS

This study was conducted in 2014 in a completely randomized design with three replications in the laboratory of Horticultural Sciences Department, Gorgan University of Agricultural Sciences and Natural Resources of Gorgan at temperature of 20 ± 2 °C, humidity of 60 ± 5%, the light of 600 lux with 12-hour lighting period. Tuberose cut flowers were prepared from a greenhouse at the Pakdasht city and were transferred to Gorgan after packaging in suitable condition. The applied treatments included BA (100 mg L^{-1}) and with combination of rosemary and eucalyptus extract (12, 25, and 50%) with sucrose 4%. To produce herbal extracts, 1:10 ratio of plant material to water with distiller's device was used. Measured characteristics included vase life, opening of florets, relative fresh weight, solution uptake, total soluble solids, chlorophyll a, chlorophyll b and total chlorophyll. Vase life was evaluated 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 [18].
Relative fresh weight was measured by using a digital scale and was calculated by the following formula.
$w_t / w_{t=0} \times 100$= relative percentage of fresh weight (RFW)
W_t: Stem fresh weight in the same day and days 3, 6, ...
$W_{t=0}$ = Weight of the stem in day zero
Water absorption was measured by using a graduated cylinder and was calculated by the following formula.
$WA= (S_{t-1})-S_t / w_{t=0}$
WA: 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
For the measurement of soluble solids of petals, 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. Arnon method was used to measure chlorophyll [19].
The data were analyzed by SAS Software and the means were compared by using LSD test.

RESULTS

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 ($p<0.01$) (Table 1 and 2).

Table 1. ANOVA of the effect of the treatment on vase life and florets opening of tuberose cut flowers.

S.O.V	df	Vase life	bud opening
Treatment	9	8.67**	466.03**
Error	18	0.22	0.79
CV (%)		5.22	1.63

** Significant 1%

Table 2. ANOVA of the effect of treatment and time on measured characteristics of tuberose cut flowers.

S.O.V	df	Fresh weight	Solution uptake	Total soluble solids	Chlorophyll a	Chlorophyll b	Total chlorophyll
Treatment	9	1767.13**	.204**	25.43**	.021**	.027**	.101**
Time	3	17319.76	3.29**	186.47**	.550**	.131**	1.12**
Treatment*Time	27	798.9	.12**	12.11**	.008**	.011**	.033**
Error	80	1.33	0.001	0.01	0.0003	0.000001	0.000001
CV (%)		1.29	7.47	2.16	10.4	0.98	0.35

** Significant 1%

Vase life

The results of the means comparison showed that the maximum and minimum vase life were related to eucalyptus extract (12%) plus benzyladenine and the control, respectively (Figure 1).

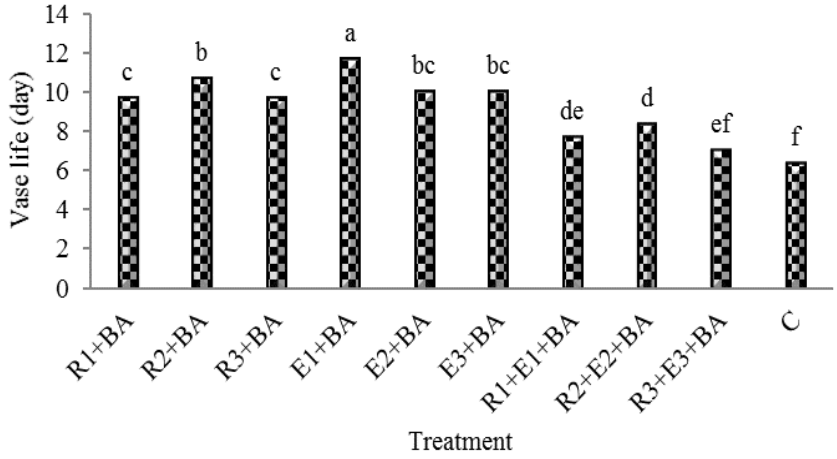

Figure 1. The effect of preservatives solutions on the vase life of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R3**: Rosemary extract (50%)
E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

Percentage of florets opening

The results showed that the highest and lowest percentage of florets opening were related to the treatment of eucalyptus extract (25%) plus benzyladenine and the control, respectively (Figure 2).

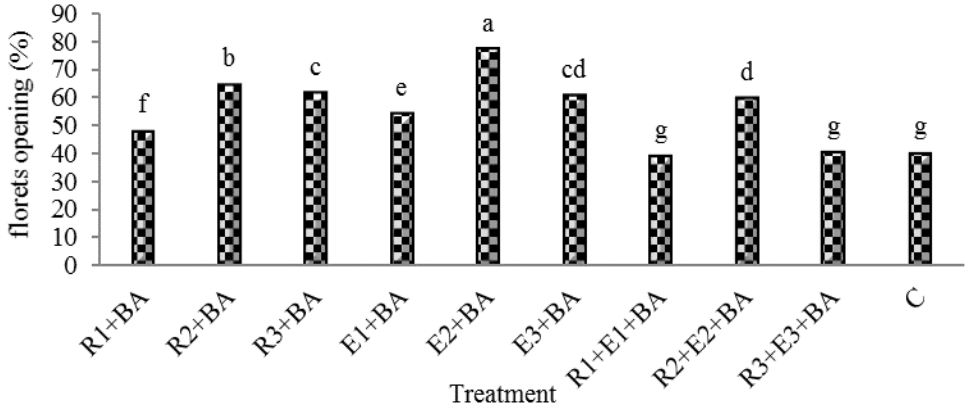

Figure 2. The effect of preservatives solutions on the florets opening of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R3**: Rosemary extract (50%)
E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

Relative fresh weight

The results showed that the highest and lowest relative fresh weight related to treatment with 12% eucalyptus extract plus benzyladenine and the control, respectively (Figure 3). The relative fresh weight changes showed that it was increasing until the fourth day and decreased until 10th day (Figure 4).

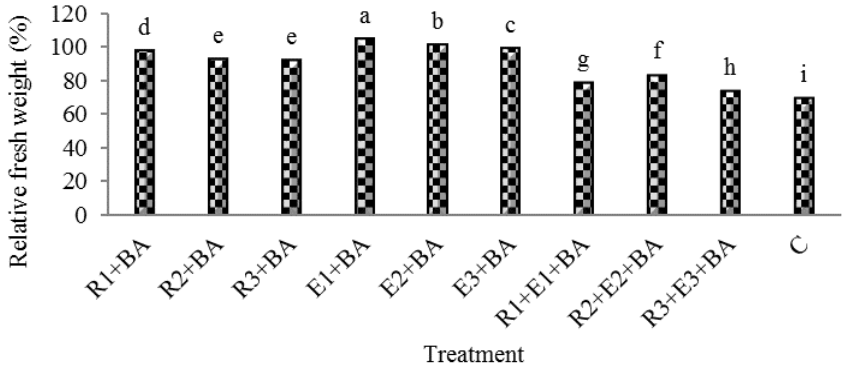

Figure 3. The effect of preservatives solutions on relative fresh weight of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R3**: Rosemary extract (50%)
E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

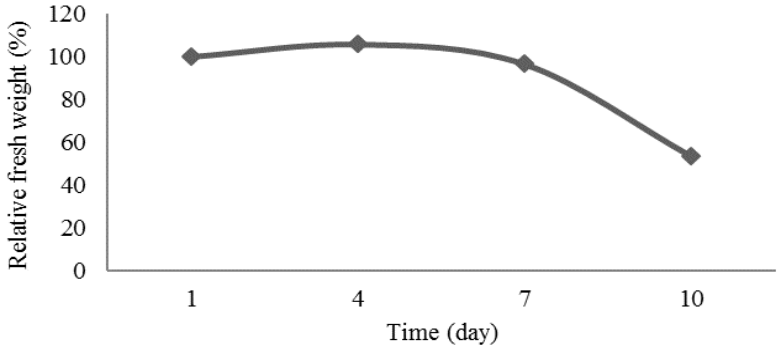

Figure 4. Changing process of relative fresh weight of tuberose cut flowers during the experiment.

Solution uptake

The results of the means comparison showed that highest and lowest solution uptake were related to the treatment of eucalyptus extract (50%) plus benzyladenine and the control, respectively (Figure 5). The solution uptake changes showed that it was increasing until 7th day and after that, it started to decrease (Figure 6).

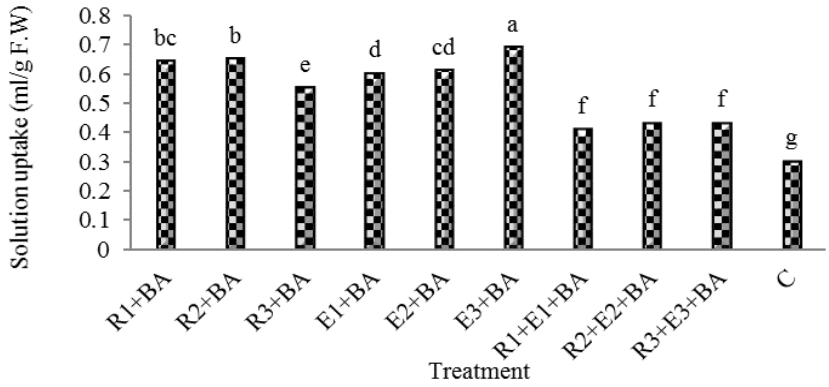

Figure 5. The effect of preservatives solutions on solution uptake of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R3**: Rosemary extract (50%)

E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

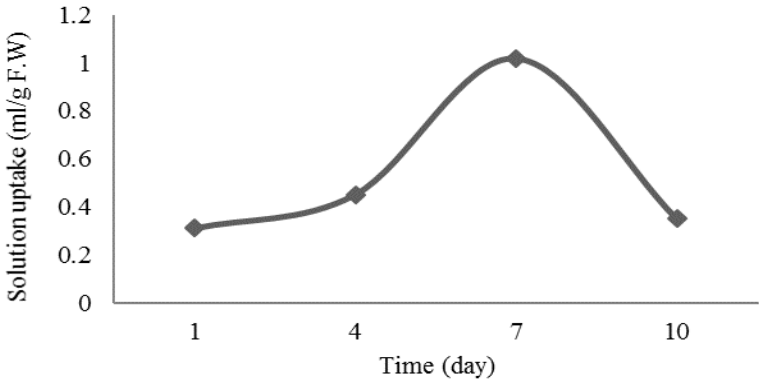

Figure 6. Changing process of solution uptake of tuberose cut flowers during experiment.

Total soluble solids

The results showed that the maximum amount of soluble solids of petal was obtained in treatment with 12% eucalyptus extract plus benzyladenine and the control, respectively (Figure 7). Changing the amount of soluble solids showed that it was increasing until 7th day and after that, it started to decrease (Figure 8).

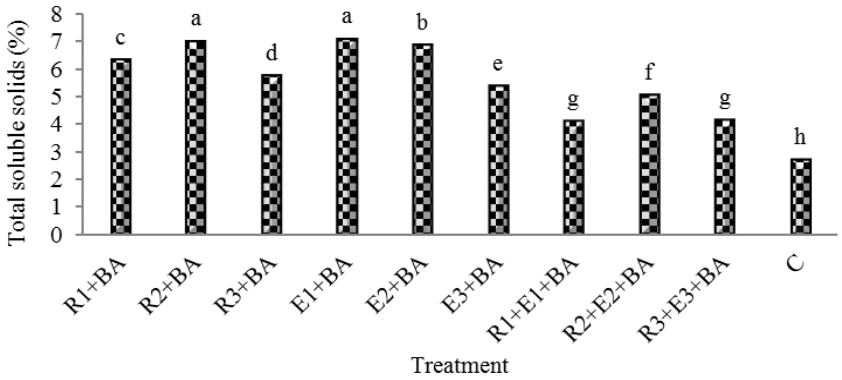

Figure 7. The effect of preservatives solutions on total soluble solids of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R_3**: Rosemary extract (50%)
E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

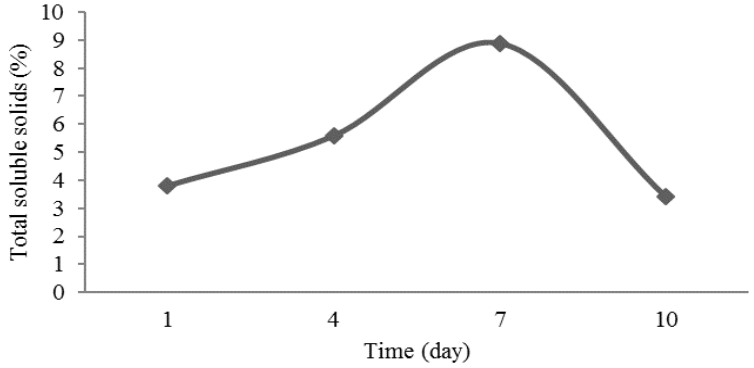

Figure 8. Changing process of total soluble solids of tuberose cut flowers during experiment.

Chlorophyll a, chlorophyll b, total chlorophyll

Results showed that the highest and lowest amount of chlorophyll a, b and total were obtained in 12% eucalyptus extract plus benzyladenine and the control, respectively (Figure 9). Amount of leaf chlorophyll was increased until 7th day of experiment and after that it was decreased (Figure 10).

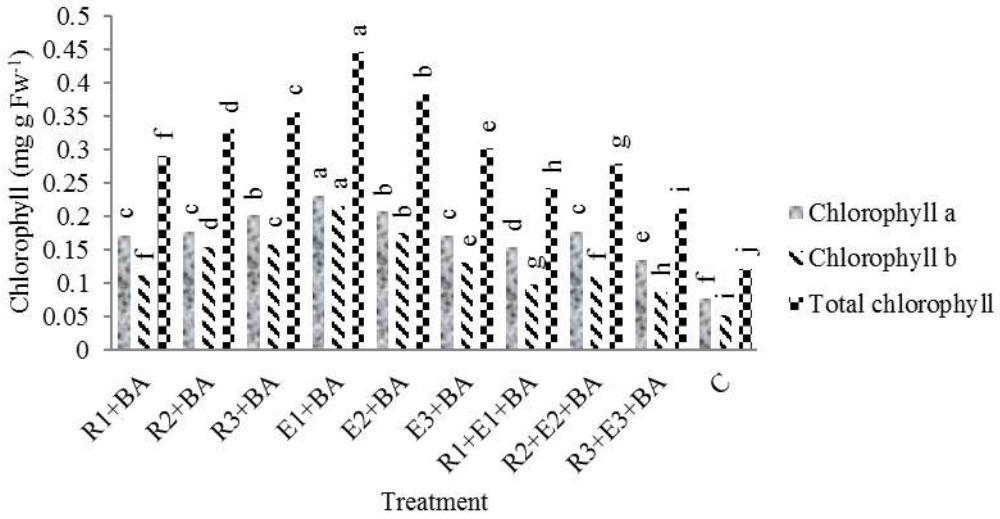

Figure 9. The effect of preservatives solutions on chlorophyll of tuberose cut flowers.
R1: Rosemary extract (12%), **R2**: Rosemary extract (25%), **R3**: Rosemary extract (50%)
E1: Eucalyptus extract (12%), **E2**: Eucalyptus extract (25%), **E3**: Eucalyptus extract (50%)
B: Benzyladenine (50 mg l^{-1}), **C**: Control.

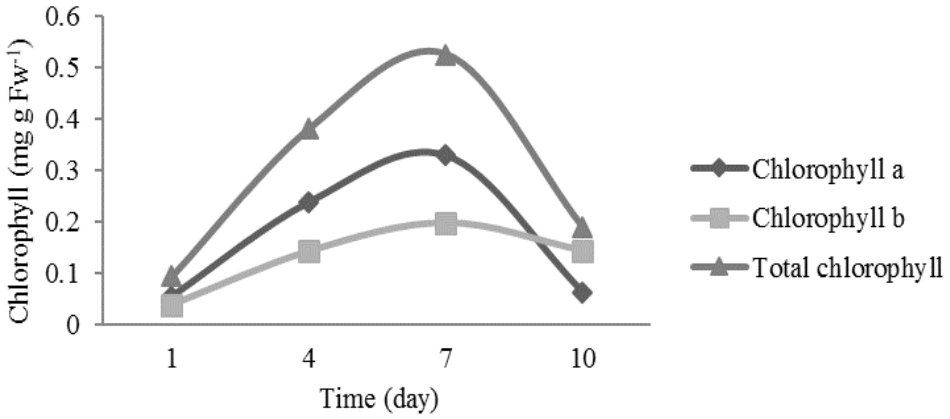

Figure 10. Changing process of chlorophyll of tuberose cut flowers during experiment.

DISCUSSION

One of the largest problems in postharvest physiology of flowers is vascular obstruction and it may be as a result of bubble creation or bacterial growth (they present in vase or in vessels) and another reason of vascular obstruction is plant cutting reaction [20]. Since the peduncles are cutting from the maternal plant, specific enzymes induced in response to cutting and redirected to cutting site in order to obstruct it [21]. Another factor also reduces the vase life of cut flower is sensitive to ethylene [22]. Cytokinins involve in controlling senescence process. Synthetic cytokinins are usually more effective in delaying senescence and BA and kinetin are widely used among them [23]. Vase life stability of its plasma membrane of gladiolus increased dramatically with BA (24). Mohammadi *et al*. (2011) used rosemary extract (in 6, 12, 25 and 50% concentrations) in preservative solution of gladiolus flower and expressed that this compound resulted in increased vase life and some qualitative traits of it [25]. In present study, the best compound for increasing vase life was combination of eucalyptus + BA. Although other treatments increased the vase life, but it seems eucalyptus and rosemary extracts, by removing vascular

obstruction and BA as an anti-ethylene compound, by delaying in senescence, increased vase life of tuberose cut flowers.

As mentioned, one the tuberose cut flowers is opening not and shed of buds. BA is one of compounds that can reduce shedding of florets. Using 25 and 25 mg L^{-1} of thidiazuron was increased the percentage of open florets of tuberose [26]. Since thidiazuron is an alternative phenyl urea with same activity of cytokinin [27], we can say that our results are consistent with Research of Ebtehaj *et al*. [26]. Due to the limited amount of carbohydrate in cut flowers, usually the competition for up-taking them occurred among developing inflorescence buds. It is possible, decreased carbohydrate storage of inflorescence results in not opening the buds that it starts from smaller buds and this problem occur also in other bulb-type species such as freesia [28] and gladiolus [29]. The extracts of eucalyptus and rosemary may provide uptake condition of existed sucrose in vase solution for florets and then the percentage of opening florets increase compared with control group.

In present study, fresh weight and solution uptake increased in treated flowers compared to control. The relative fresh weight is one of indexes that show proper water relations in cut flowers. The gradual reduction of this factor during postharvest time is predictable [30]. In order to prevent deterioration and senescence flower and to increase the vase life of them, it should be prevent from reduce of water uptake [31]. Application of cytokinins on cut flowers reduces damages of water stress and improves water uptake as well as keeps turgescence of petals [8]. Petridou *et al*. (2002) reported that BA limits loss of fresh weight in chrysanthemum cut flowers [32]. Basiri *et al*. (2011) stated that use of rosemary extract increases fresh weight of carnations cut flowers [33].

In this study, the amount of total soluble solids increased by treatments and until seventh day it had increasingly trend. Danaee *et al*., (2011) reported that 50 and 100 mg L^{-1} of BA resulted in increased total soluble solids in gerbera petals and this increasing continued until third day [34] that it is consistent with our results. In another study application of rosemary extract increased total soluble solids in petals of Alstroemeria flower (14).

Cytokines through effect on activities of chlorophyllase enzymes prevent from chlorophyll degradation and delay senescence process [35]. BA increases chloroplast development and chlorophyll synthesis after harvesting [36]. Hassanpour Asil and Karimi (2010) stated that using BA at 50 and 75 mg L^{-1} in preservative solution of lisianthus increased chlorophyll of leaves [37] that is consistent with our results. Impact of herbal essential oils and extracts on delay of reduced chlorophyll of leaves was reported in some cut flowers. Kazemi *et al*. (2014) stated that treating the lisianthus cut flowers with rosemary and thyme essential oils, delayed reduced chlorophyll. They suggested that most important impact of herbal essential oils on maintaining chlorophyll is their antioxidant properties [11]. Therefore, it can suggest that impact of plant extracts on delaying reduced chlorophyll is related to their antibacterial and antioxidant properties. Also, results of Basiri *et al*, (2011) showed that application of rosemary extraction in preservative solution of carnation cut flowers increased chlorophyll of treated flowers compared with control group [33]; it is consistent with our results.

CONCLUSION

According to the results, the best treatment for improving vase life and increasing measured traits was eucalyptus extract + BA and among different concentrations of eucalyptus extract, 12% had best results. Results showed that combination of rosemary and eucalyptus extracts resulted in significant increase in most traits compared to control group, but they had less effect when used separately in combination with BA. Overall, results of current study showed that rosemary and eucalyptus extracts, because of their good properties such as antibacterial property, and BA can use as anti-senescence in preservative solution of tuberose cut flowers.

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The authors declare that there is no conflict of interests.

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