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USE OF CITRIC ACID AND IRON SULFATE IN PROMOTING POST-HARVEST LONGEVITY OF CUT TULIPS (*TULIPA GESNERIANA* L. CV. MARYLIN) IN VASE SOLUTIONS

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SUMMARY

Tulip is one of the most beautiful perennial flowering plants which, based on its brilliance and attraction, is ranked at the top among cut flowers. However, bending of the neck, short display life and senescence of the flowers are the major causes that reduce its market value. These problems can be addressed by using different chemicals to prolong the flower display life. The present research was initiated to prolong the display life of tulips by using citric acid and iron sulfate (50, 100, 150 mgL^{-1}) in distilled water containing 2.5% sucrose. Cut stems of tulips were immersed in the diluted solution of citric acid and iron sulfate. The results showed that all the observed parameters varied among the treatments. The solution containing 100 mgL^{-1} citric acid performed better and resulted in the most extended post-harvest display life (13.34 days), more solution uptake (63.36 mL), maximum flower diameter (57.66 mm), maximum tepal length, width, minimum stem bending and maximum quality of the flower. These characteristics were better also in 150 mgL^{-1} iron sulfate vase solution, but less effective in comparison. From these results, it can be concluded that the use of citric acid and iron sulfate vase solutions helps in prolonging the vase life of cut tulips and enhances the flower quality attributes.

Key words: *citric acid, iron sulfate, tulip, solution uptake, vase life*

INTRODUCTION

Tulip is one of the most adorable flowers, which is ranked third among cut flowers, after *Rose* and *Chrysanthemum* (Ahmad et al., 2014; Manzoor et al., 2001). It is not grown only as a cut flower, but also as a bedding plant, potting plant and border plant (Singh, 2006), mainly distributed in the temperate zones (Bashir et al., 2018). Tulip requires thirteen to eighteen weeks of cool atmosphere for making extra high-quality cut flower. In the winter season, parts of the blooms grow gradually, while in the spring season there is speedy extension of the stalks (De Hertogh & Le Nard, 1993). In tulip development, a short blossoming period and quality knob generation are important issues (Pathania & Seghal, 1997). Bending of the neck, short display life and senescence of the flowers are the major causes that reduce its market value.

Vase life of cut flowers is of vital importance in defining the worth of a plant. Vase life is affected by a number of factors and senescence is one of them. Senescence, as a normal developmental process, affects the cells, tissues and organs or an organization level of a plant. This is an oxidative process involving a general degradation of the cellular structures (Mansouri, 2012), but it could be delayed by applying different acids such as salicylic acid, used in roses and gladiolus flowers (Ezhilmathi et al., 2007; Alaey et al., 2011). Microbial contamination is another factor that causes xylem blockage, imbalance between water uptake and water loss and finally wilting and shortening of the

vase life (van Doorn, 1997; Balestra et al., 2005; Safa et al., 2015). In many cut flower cultivars, stem elongation results in stem bending, which is not appreciated in the cut flower industry. To address the problem of the post-harvest life of cut flowers, various chemical are used. In this paper, we proposed that citric acid and iron sulfate could be used to address this problem in cut tulips.

Citric acid is an organic acid with a broad range of applications in the plant kingdom used to control water pH and microorganism growth (Goszczynska & Rudnicki, 1988; van Doorn, 2010). It is suggested to cut flowers because it lowers the risk of vascular resistance (Bhattacharjee et al., 1993). It increases the vase life of cut flowers because it reduces the bacterial population in vase solutions and increases water flow in xylem (van Doorn, 1997). Citric acid strongly affects longevity of many cut flowers during the post-harvest life (Eidyan, 2010; Darandeh & Hadavi, 2012). Iron sulfate, the second component in this study, is helpful for growth of golf course grasses (Han et al., 2017). Lack of iron sulfate causes chlorosis in green plants (Kong et al., 2020). Iron sulfate is useful for increasing chlorophyll and improving numerous metabolite responses (El-Jendoubi et al., 2014). It is an essential supplement for development of plant tissues (Rout & Sahoo, 2015). The foliar application of iron sulfate on flowers is helpful in increasing the iron content and the display life (Eidyan et al., 2014).

However, the effect of citric acid and iron sulfate on cut tulip flower longevity and post-harvest quality is not well-investigated, and this study has been conducted to fill this gap.

MATERIAL AND METHODS

The experiment was conducted in Post-harvest and Floriculture Lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Cut flowers of a single spike of tulip 'Marylin' were used for this study. The cut flowers grew in an open field. Knobs were planted on raised beds, keeping distance 6 by 6 inches. According to the bulb distributor regulations, the basic dose of NPK (17:17:17) was applied by broadcasting. After the buds emerged, the cutting was transferred to the lab. In the lab, the spikes were treated with water for two hours at room temperature. The lower side leaves were removed.

Solution preparation

Vase solutions containing citric acid and iron sulfate (50, 100, 150 mgL^{-1}) were prepared in distilled water with 2.5% sucrose. The room temperature was 240°C.

Treatment of Chemicals

The following treatments of chemicals were applied:

T_0	Distilled water (Control)
T_1	Sucrose 2.5%
T_2 (Citric acid)	50 mgL^{-1} + 2.5% Sucrose
T_3 (Citric acid)	100 mgL^{-1} + 2.5% Sucrose
T_4 (Citric acid)	150 mgL^{-1} + 2.5% Sucrose
T_5 (Iron sulfate)	50 mgL^{-1} + 2.5% Sucrose
T_6 (Iron sulfate)	100 mgL^{-1} + 2.5% Sucrose
T_7 (Iron sulfate)	150 mgL^{-1} + 2.5% Sucrose

The experiment was arranged according to a complete randomized design (CRD) with three replications and five observational units.

Data Collection

In order to gather information, three new and undiseased stems were collected for every replication and put in the solution. The plants were labelled, and information for the described parameters was gathered for each plant.

Table 1. Data collection details for all studied parameters

Parameters	Data Collection Details
Vase life (days)	Blossom life span was estimated as the days beginning from the time the blooms were put into jars until the end of the bloom vase life. Every individual blossom was checked daily with a specific goal to decide the end of the vase life (Joyce et al., 2000).
Solution uptake (mL)	To determine the amount of solution absorbed during the first five days of the vase life, the measured quantity (250 mL) of vase solution was added into the vase, then the stem was kept in the

	vase. After 5 days, the amount of solution was checked and recorded.
Change in pH	pH of each solution was checked by using pH meter and the values were monitored. After collecting the values, the means of the values were computed.
Chlorophyll contents (SPAD value)	The chlorophyll meter measured the leaf chlorophyll substance of leaf in the field, and the average was processed.
Final length (cm)	Stem length was measured by using a ruler. The initial stem length was recorded before the stem was presented in the arrangement, and the final length was recorded at the end of the stem vase life.
Stem bending (angle)	Stem bending was interpreted as the edges when contrasted with under 90 degrees, appeared by the upper piece of the flower stem to 20 cm downward. Stem bending was estimated in every cut stem by utilizing protector D.
Flower diameter (mm)	At the time of the harvest, the flower diameter for each tulip was measured with a digital caliper. Five stems were selected per replication, and the average was calculated.
Change in EC	Initial EC and final EC of the vase solution were recorded using EC meter (Hanan H19813.6) to calculate pH and EC changes during the study. Change in EC = $(EC_{(Df)} - EC_{(Do)})$
Days to start senescence (days)	Senescence days were observed when the blossom shading changed, tepal dried up and flawed surface or straightforward tepal zone was also observed. Senescence was observed in cut stems, and the days were computed.
Dry weight (g)	Blooming stems after the treatments were put in a paper sack and placed in the oven for 72 hours. After the given time, the sacks were collected from the oven, the weight was measured and the mean was recorded.
Tepal width (mm)	The width of tepals was measured in millimeters by a digital caliper. Tepals from fully developed flowers were selected and measured across the segments where maximum width was observed.
Fresh weight (g)	At the time of harvest, flowering stems were randomly selected from each replication and weighed on an electric weighing balance. Fresh weight was recorded, and the average was computed.

Statistical analysis

The collected data were processed statistically using the computer software Statistix 8.1 and applying Tukey's method. The probability of equating the treatment means the least significant test was utilized at 5% level.

RESULTS

Vase life (Days)

Analysis of variance (Tab. 2) indicated that there are significant effects of different treatments on the display life compared to the control. Among the studied treatments, T_3 performed best and resulted in maximum display life of 13.34 days, followed by T_7 which resulted in display life of 11.63 days. The vase life increment was 97.14 and 71.92, respectively, as compared to the control (Tab. 3). The lowest vase life (6.76 days) was observed in distilled water (Figure 1-a).

Solution uptake (mL)

The data on water uptake of cut tulips are presented in Figure 1-b, while the results for analysis of variance are presented in Table 2. Analysis of variance showed significantly different effects of different treatments as compared to the control. Maximum solution uptake (63.36 mL) was observed in T_3, followed by T_7 with 55.41 mL solution uptake. In contrast, minimum solution uptake was noted in T_0 (26.63 mL).

Change in pH

The results related to the change in pH indicated significantly different variations between the treatments (Tab. 2). In Figure 1-c, the data showed that minimum change in pH was observed in T_3 (0.27), followed by T_4 (0.30) and T_7 (0.31). Maximum change in pH was observed in T_0 (0.65).

Chlorophyll contents (SPAD value)

The data on chlorophyll contents (SPAD value) were statistically analyzed and their means are graphically presented in Figure 1-d. The results for analysis of variance showed highly significant difference, as presented in Table 2. The means indicated superiority regarding chlorophyll content in T_3 (9.86), which was markedly different in percentage (355.38%) as compared to the control (Tab. 3). The value of chlorophyll content in the control (T_0) was 2.17.

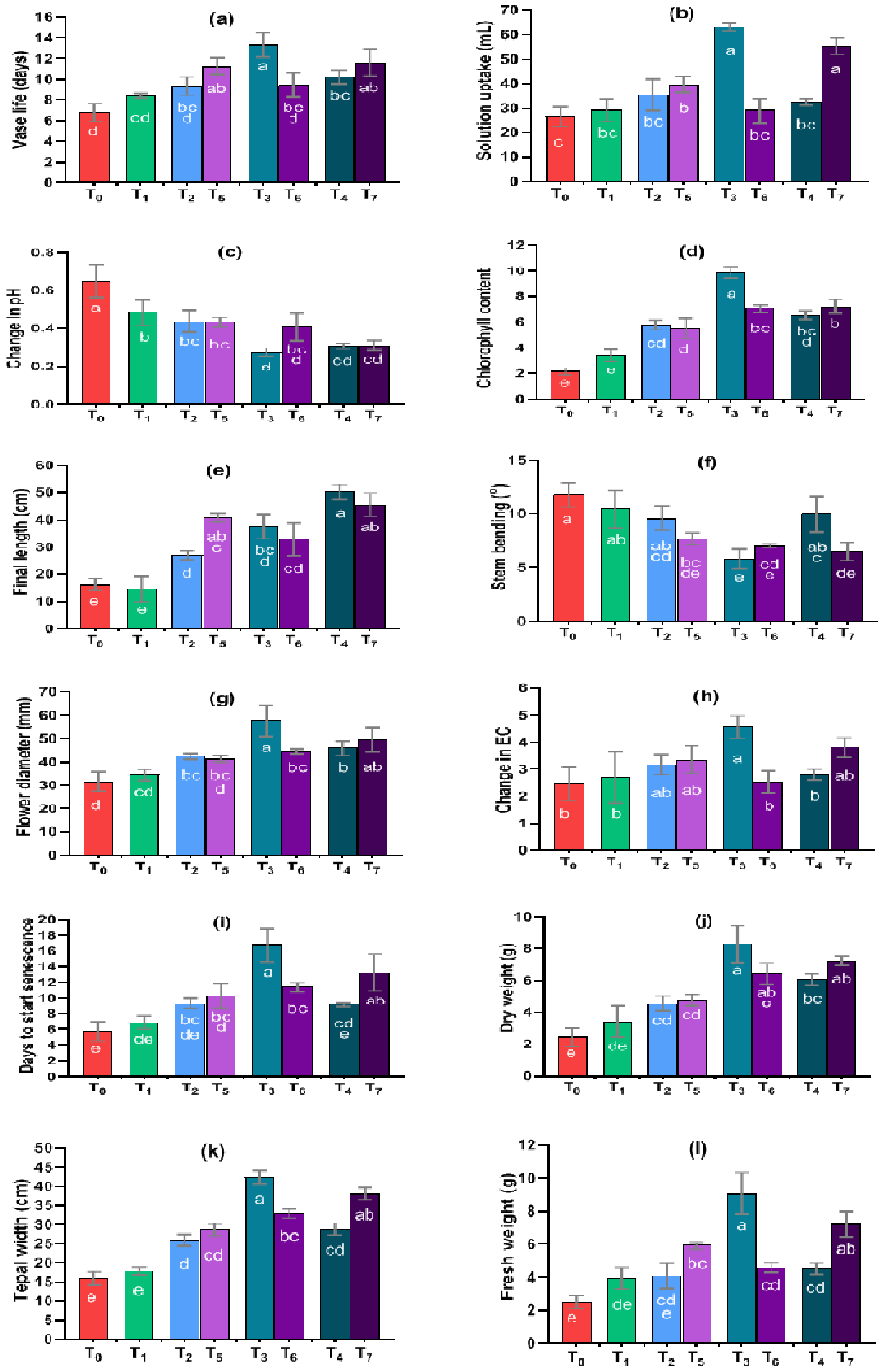


Figure 1. a - Vase life; b - Solution uptake; c - Change in pH; d - Chlorophyll content; e - Final length; f - Stem bending; g - Flower diameter; h - Change in EC; i - Days to start senescence; j - Dry weight; k - Tepal width; l - Fresh weight; these figures present the values of various characteristics of tulip cut flowers treated with different treatments. Bars with the same letters are not significantly different from each other ($p \leq 0.05$); T_0 - Control; T_1 - Sucrose 2.5%; T_2 - Citric acid (50 mgL^{-1}) + 2.5% Sucrose; T_3 - Citric acid (100 mgL^{-1}) + 2.5% Sucrose; T_4 - Citric acid (150 mgL^{-1}) + 2.5% Sucrose; T_5 - Iron sulfate (50 mgL^{-1}) + 2.5% Sucrose; T_6 - Iron sulfate (100 mgL^{-1}) + 2.5% Sucrose; T_7 - Iron sulfate (150 mgL^{-1}) + 2.5% Sucrose)

Table 2. Mean square values indicating the effect of citric acid and iron sulfate in vase solution on different characteristics of tulip cut flowers

S.V	D.F	VL	SU	C. pH	CC	FL	SB
Treatment	7	12.5374**	544.170**	0.04438**	16.9509**	513.064**	13.5605**
Error	16	0.87588	16.605	0.00281	0.2263	14.071	1.2870
S.V	**D.F**	**FD**	**C. EC**	**DSS**	**DWT**	**TW**	**FWT**
Treatment	7	203.040**	1.56057**	36.5779**	11.6361**	248.677**	13.0205**
Error	16	13.716	0.26995	1.9244	0.4510	4.122	0.4549

Legend: ** - Highly significant at $P \leq 0.01$; VL - Vase life; SU - Solution uptake; C. pH - Change in pH; CC - Chlorophyll content; FL - Final length; SB - Stem bending; FD - Flower diameter; C. EC - Change in EC; DSS - Days to start senescence; DWT - Dry weight; TW - Tepal width; FWT - Fresh weight

Table 3. Percentage (%) variations in the values of different parameters under different treatments as compared to the control (T_0)

Parameters	Treatment						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Vase life	24.24	37.78	97.14	51.03	66.60	39.31	71.92
Solution uptake	9.27	32.76	137.89	22.49	48.82	8.57	108.04
Change in pH	-25.64	-32.82	-57.94	-52.82	-33.33	-37.43	-52.31
Chlorophyll content	56.92	166.15	355.38	201.54	153.85	224.61	233.85
Final length	-10.63	66.66	131.69	210.39	151.65	103.01	180.86
Stem bending	-11.33	-18.41	-50.71	-15.27	-34.56	-40.23	-44.47
Flower diameter	8.81	34.32	82.58	45.47	31.40	40.70	56.64
Change in EC	9.46	28.65	85.13	13.65	36.49	2.70	54.05
Days to start senescence	19.07	61.79	189.59	58.38	78.15	97.22	129.48
Dry weight	40.41	87.53	241.37	149.31	95.89	164.25	197.26
Tepal width	12.16	62.68	166.41	81.34	80.29	106.71	139.83
Fresh Weight	56.07	62.27	259.23	79.42	133.51	82.06	186.28

Final length (cm)

The mean values of the final length are graphically presented in Figure 1-e. Analysis of variance indicated significant effects of different chemicals of vase solutions as compared to the control (Tab. 2). Among the studied treatments, T_1 (14.48) had the minimum stem length, followed by T_0 (16.2) and T_2 (27), while the final stem length observed in T_3 was 37.53cm, which was a moderate value as compared to others.

Stem bending (Angle)

The results for analysis of variance on stem bending are presented in Table 2, while the mean values are graphically presented in Figure 1-f. Among the studied treatments, T_3 performed best and resulted in minimum stem bending (5.8˚), followed by T_7 (6.53), while T_0 resulted in maximum stem bending (11.94). The change in stem bending was -50.71 to -44.47% in T_3 and T_7, respectively, as compared to the control (Tab. 3).

Flower diameter (mm)

The results for analysis of variance (Tab. 2) on flower diameter indicated highly significant variations among different treatments. Among the studied treatment, T_3 resulted in maximum floral diameter (57.67), followed by T_7 (49.47), while minimum floral diameter was observed in T_0 (31.58), as presented in Figure 1-g.

Change in EC

The minimum change in EC was observed in T_0, followed by T_6. Results indicated that EC changed as the concentration of citric acid and iron sulfate increased. The mean values are presented graphically in Figure 1-h.

Days to start senescence (days)

The mean values of this trait are graphically presented in Figure 1-i. The results for analysis of variance (Tab. 2) indicated significant effects of different chemical vase solutions compared to the control. Among the studied treatments, T_3 excelled and resulted in maximum days of healthy stems so senescence was delayed and started after 16.7 days, followed by T_7 in which senescence started after 13.23 days, while in the control treatment senescence started after 5.67 days.

Dry weight

The data regarding the effect of different concentrations of iron sulfate and citric acid in sucrose contained in vase solutions indicated significant effects on the dry weight of cut tulips (Tab. 2). The treatments means (Fig. 1-j) indicated that tulips achieved the maximum (8.31 %) dry weight in T_3, which was higher than in all other treatments. On the other hand, the minimum dry weight of cut flowers was noted in T_0.

Tepal width (mm)

Tepal length is one of significant standard qualities of cut tulips. Analysis of variance (Tab. 2) showed significant differences among all the chemicals in vase solutions compared to the control. Among the studied treatments, T_3 showed the highest tepal width, which was statistically similar to T_7. The increase in tepal width in T_3 was 166.41% and in T_7 it was 139.83% as compared to the control, whereas T_0 resulted in the minimum tepal width.

Fresh weight (g)

The results for analysis of variance related to fresh weight indicated significant variations (Tab. 2). Higher fresh weight was observed in T_3 (Figure 1-l), and the increase was 259.23% compared to the control (Tab. 3). The lowest increase in fresh weight (56.07%) was observed in T_1.

DISCUSSION

A number of studies showed that exogenously applied chemicals enhance the vase life of cut flowers by delaying their senescence (Lu et al., 2020). In this study, we found that citric acid and iron sulfate also improved the vase life of tulip cut flowers and delayed the senescence. The antibiotic synthesis in different concentrations is considered as the feature of various chemicals which is most associated with the ability of the bacterium to prevent the proliferation of plant pathogens (Compant et al., 2005). Citric acid is naturally present in fruits and flowers to control enzymatic browning (Jiang et al., 2004). The display life of flowers can be improved by using appropriate levels of citric acid (Darandeh & Hadavi, 2012). The application of citric acid enhanced the durability of the roses and reduced the severity of gray mold (Capdeville et al., 2003). Previous studies suggested that citric acid, as a safe, natural and biodegradable compound, is an appropriate option for conventional chemical applications used to extend the vase life of cut flowers (Mashhadian et al., 2012). Citric acid and sucrose application on cut roses revealed significantly enhanced decorative quality of cut flowers by expanding their vase life. Similarly, gerbera cut flowers vase life was enhanced under the medium concentrations of citric acid (Mehdikhah et al., 2016). Darandeh & Hadavi (2012) studied the effect of foliar application on the lily cut flower vase life, and they also demonstrated that it enhanced the quality of cut flowers by improving their vase life.

In the current study, we found that application of citric acid enhances the solution uptake and this solution uptake means that the xylem blockage is reduced due to decreased microbial growth. The pre-harvest foliar application of citric acid and iron sulfate combined with nitrogen fertigation enhanced the vase life of *Polianthes tuberosa* (Eidyan et al., 2014). Senescence and reactive oxygen species (ROS) production are closely related. If ROS is accumulated in the cell at a critical level, it severely damages cells and they are not detoxified by the antioxidant system (Arora et al., 2002). In most cultivars, the tepal senescence symptoms are followed by tepal abscission (Sosa-Nan, 2007). Citric acid, together with some other chemicals, helps in delaying senescence (Kazemi et al., 2011) and improving the flower quality.

In this study, we found that electric conductivity is maintained by applying citric acid and iron sulfate. The minimum change in electric conductivity occurs by applying citric acid and iron sulfate because they contain organic chemical compounds and covalent bonds (Gul & Tahir, 2013). In contrast, the maximum change of EC occurs in distilled water, which has no solute and is purer than citric acid and iron sulfate. Our results showed a similar trend (Fig. 1-h). Plants show an increase in water use efficiency in case of discontinuity of water in the plant systems. Water retention may be useful for cut flowers after the harvest. In the case of water unavailability, stomatal closure permits plants to reduce transpiration and increase water use efficiency (Nayyar & Gupta, 2006). Citric acid decreases the bacterial population in vase solution and intensifies water conductance in the xylem of cut flowers (van Doorn, 1997). To maximize the productivity and quality, adequate quantities of nutrients must be supplied to plants. Foliar application of iron sulfate is one of the best ways to supply micronutrients to plants (Cabrera et al., 1993).

Application of citric acid slightly affects the solution pH (Ahmad & Dole, 2014) in contrast to distilled water solution in which pH is significantly affected. The change in pH affects the solution uptake, stability and nutrients uptake in cut flowers; these chemicals cause less change in pH more solution and nutrient uptake and increase their

vase life (Fig. 1). Several studies have shown that the use of citric acid in a concentration of 100 mgL^{-1} reduces the pH of vase solution (Kazemi et al., 2011).

Plant growth can be analyzed in terms of an increase in total plant dry mass and its allocation among organs involved in acquiring above-ground or below-ground resources. The dry weight of cut stems indicated the freshness of cut flowers. Our results indicated an increase in dry matter production under the treatments (Fig. 1-j), which is in line with the findings of Bose & Tripathi (1996) and Kazemi et al. (2011), and this increment might be attributed to a more significant accumulation of photosynthate in vegetative parts. Our findings showed that chlorophyll contents increased under the treatments, especially at 100 mgL^{-1} citric acid + 2.5% sucrose treatment, which is in line with the investigations of Darandeh & Hadavi (2012) and Amir & Shahsavar (2009), who reported a significant increase in chlorophyll content under exogenous application of citric acid. In the current study, we found that different concentrations of citric acid and iron sulfate in vase solution affect the qualities of tulip cut flowers in different ways. These results indicated that the selection of an appropriate citric acid or iron sulfate concentration was important for their application in prolonging the vase life of tulip cut flowers.

CONCLUSION

It can be concluded that chemical vase solutions of citric acid and iron sulfate are helpful not only for their beneficial effects while the plants are in the soil, but also when applied on the flowering stem, and hence can be useful for increasing the display life and quality of cut flowers. Citric acid in a concentrated form proved to enhance many quality attributes of cut tulip, and along with other chemicals, can be used for sustainability of various qualities of cut flowers such as better flower size, fresh floral stems and vase life.

Conflict of interest: The authors declare that they have no conflict of interest.

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