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Influence of eucalyptus oil, humic acid and chemical preservative solutions on vase life and physiological characteristics of calla (*Zantedeschia aethiopica* R.) cut flowers

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Abstract

The calla lily cut flower (*Zantedeschia aethiopica* cv. 'Romance'), with a white spathe, is a significant ornamental flower. There is a growing interest in developing cost-effective and eco-friendly preservative solutions to extend vase life. The Ornamental Plants and Landscape Gardening Research Department, Horticulture Research Institute, Giza, Egypt, conducted this study in the postharvest laboratory during the 2020 and 2021 to examine the effects of various holding solutions, both individually and in combination, including distilled water (DW) as a control, eucalyptus oil (EO) at concentrations of 1 and 2 mL/L, humic acid (HA) at 25 and 50 mL/L, sucrose (Suc) at 20 g/L, 8-hydroxyquinoline citrate (HQ) at 0.2 g/L, citric acid (CA) at 0.2 g/L, and gibberellic acid (GA3) at 0.05 g/L. The results showed that all holding solutions significantly improved the measured characteristics compared to distilled water. It's worth mentioning that putting cut flowers in a solution with humic acid at 25 mL/L, either alone or with sucrose at 20 g/L and citric acid at 0.2 g/L, made the flowers last longer, look better, and have higher flower fresh weight (IFFW%), relative fresh weight (RFW%), water uptake rate (mL), and total carbohydrate content (%). During both seasons, humic acid (50 or 25 mL/L) as a single treatment yielded the highest phenolic content followed by eucalyptus oil (2 mL/L).

Key words: Zantedeschia aethiopica, eucalyptus oil, cut flowers, humic acid,8-HQC, citric acid,GA3,vase life, water uptake.

Introduction

In recent years, cut flowers have gained significant importance in both foreign and local markets due to their vibrant colors, attractive nature, and role as a source of national income. Maintaining high quality is crucial for assessing the value of cut flowers in both export and domestic markets. The Calla lily (*Zantedeschia aethiopica* L.), belonging to the monocotyledonous flowering plant family Araceae, features spathe and spadix-type inflorescences known for their elegant beauty, vibrant colors, and appealing foliage. These qualities have made it one of the most popular tropical cut flowers, significantly contributing to the floriculture industry.

Calla lilies are native to temperate regions of Africa, cultivated in temperate and tropical areas of Mexico, and widely grown in Brazil. This perennial plant has white, cup-shaped blooms on long, erect, and rigid stems. Floral therapies and toxic cures use *Z. aethiopica*, and its flowers and bulbs are edible. Moreover, gardens or commercial scales can grow calla lily plants for use in high-end floral arrangements (Hlophe *et al.*, 2015; Dias *et al.*, 2022).

Extended vase life is a key factor in assessing the quality of perishable cut flowers. Therefore, it is important to use natural preservatives, such as essential oil extracts from medicinal or aromatic herbs, which are environmentally friendly and have a long-standing history of safety. Eucalyptus essential oils (EEO) are very appealing because they can help heal wounds and make cut flowers last longer by improving their post-harvest properties (Yonsawad and Teerarak, 2019; Maurya et al., 2021; Soliman and El-Sayed, 2023).

Humic-based fertilizers and minerals create ideal conditions for plant growth and development, helping to preserve carbohydrate levels in flowers and stems. This preservation supports respiration substrates and reduces sensitivity to ethylene. Studies have shown that humic acid preservative solutions enhance vase life, flower size, fresh weight, chlorophyll content, and photosynthesis rates in chrysanthemums (Fan *et al.*, 2015; Vehniwal and Abbey, 2019; El-Attar and Sakr, 2022).

Generally, experts recommend adding chemical preservatives to vase solutions to extend the vase life of cut flowers (Khan *et al.*, 2015; Patel *et al.*, 2021). Most preservatives used in flowers consist of carbohydrates, germicides, growth regulators, ethylene inhibitors, and some mineral compounds. Carbohydrates, such as sucrose, are essential for the biochemical and physiological processes in cut flowers post-harvest. The exogenous application of sucrose provides the necessary substrates for respiration and allows cut flowers harvested at the bud stage to open, which might not occur naturally (Nowak and Rudnicki, 1990; Khan *et al.*, 2015).

Storage solutions widely use 8-Hydroxyquinoline (8-HQ) and its derivatives, 8-HQS (sulfate) and 8-HQC (citrate), due to their germicidal properties. They inhibit microorganism growth, prevent vascular occlusion, and promote solution absorption by the plant (Jowkar *et al.*, 2017; Sales *et al.*, 2021). Studies have shown that vase treatments with sucrose and 8-HQC positively affect vase life in cut roses and lilies by delaying senescence, alleviating water stress, and reducing peroxidation damage (Zeng *et al.*, 2023). Citric acid is another chemical that prolongs vase life by maintaining the pH of the solution and preventing microbial growth. Boric acid and aluminum sulfate also help reduce microbial infections (Shaikh *et al.*, 2024). Gibberellic acid (GA₃) is known to delay senescence in plants. Its exogenous application in cut flowers can delay petal abscission and color fading, thereby extending the post-harvest life of calla lilies. Suppressed ethylene production and delayed stalk senescence, possibly due to the maintenance of higher RNA content, may link to the extension of vase life in treated plants (Dehale *et al.*, 1993; Chandel *et al.*, 2023).

The point of this study is to find out how different holding solutions, like Eucalyptus oil, humic acid, and chemical preservatives like citric acid, 8-HQC, and GA₃, affect the quality, chemical makeup, and vase life extension of *Zantedeschia aethiopica* R.

Material and methods

This study was conducted in February during the two successive seasons of 2020 and 2021 at The Ornamental Horticulture Department of Cairo University, Egypt, and the Postharvest Laboratory of Ornamental Plants and Landscape Gardening Research Department, Horticulture Research Institute, Giza, Egypt.

Plant material: We collected cut stalks of *Zantedeschia aethiopica* R. from a commercial growing farm (Flora max) in Giza, Egypt, in the early morning when the inflorescences were at the 1/3 opening stage. We then bunched them in groups of 10, wrapped them in Kraft paper inside carton boxes, and transferred them dry to the laboratory within an hour. Prior to treatment, we re-cut the stalk bases under water and adjusted them to 60 cm long. The stalks were pre-cooled by placing them in cold water at 4 °C for 1 h to remove the effect of high field heat, then placed in vases (500 mL) containing 400 mL of different holding solutions. The experiment was conducted under continuous lighting (fluorescent light at 1000 Lux) at 18 ± 2 °C and 50–60% RH to complete vase life.

Eucalyptus oil and humic acid treatments: essential oil (*Eucalyptus globulus*) was obtained from (Nefertari Pharma Synta Company® at 1 and 2 mL/L. Ten mL of eucalyptus oil was dissolved in three mL of ethyl alcohol and three to five drops of Twine 80 as a surfactant were added. Humic acid from the Central Laboratory for Organic Agriculture at the Agriculture Research Centre was used at concentrations of 25 mL/L and 50 mL/L.

Data recorded

Flower vase life (days): Janowska and Jerzy (2004) and Zaky and Amin (2013) determined the vase life of each flower by counting the number of days from cutting until the spathe showed visible signs of senescence (when one third of the spathe had dried and/ or wilted).

General appearance: The quality of the cut flower was evaluated on a scale of damage in the flower, such as wilting symptoms, by using a scale ranging from 1 to 5, where 1 = bad, 2 = moderate, 3 = good, 4 = very good, and 5 = excellent, according to Sangwanangkul *et al.* (2008); El-Shewaikh *et al.* (2018).

Increase of flower fresh weight (IFFW %):It was determined

at the fading stage as the flowing formula (Rida and El-Gedawey 2022).

IFFW (%) = [(Final fresh weight-initial fresh weight) / (Initial fresh weight) \times 100]

Water uptake (mL/3 flowers): During the vase life evaluation time, the weighting of the vases containing solutions without cut flowers was recorded every three days. *i.e.* 3, 6, 9...etc. Water uptake was calculated using the following formula according to (He *et al.*, 2006; El-Quesni *et al.*, 2012)

WU= (St-1)-(St)

St-1 = Weight of the vase solution from the day before St = Weight of the vase solution on day t. (3, 6, 9 and 12)

Relative fresh weight (RFW %):The fresh weight of cut flowers was recorded at the beginning of the experiment, then every three days in 0 day, 3rd, 6th, 9th and12th. during the vase-life evaluation period in both seasons. Relative fresh weight was calculated using the following formula (He *et al.*, 2006; Gun 2020):

RFW $\% = Wt/W0 \times 100$. Where, Wt is weight of cut flowers (g) at evaluated days, while W0 is the initial weight of cut flowers (g)

Total carbohydrate (%): Estimated according to the methods described by (Dubois *et al.*, 1956; Amin *et al.*, 2020).

Total phenolic content (%): Total phenolic content was estimated as per the method outlined by Singleton *et al.* (1999); Amin *et al.* (2020).

Layout and statistical analysis: The experiment was a complete randomized design with 11 treatments; each treatment contained 3 replicates, and each replicate contained 3 cut calla flowers. The combination of 11 treatments, 3 replicates, and 3 cut calla flowers resulted in a total number of flowers. Analyzed the data using the analysis of variance (Sedecor and Cochran, 1980) and MSTAT-C statistical software (1989). Duncan's Multiple Range Test was applied (P=0.05) to compare the means of treatment.

Results and discussion

Flower vase life (days): The data presented in Table 1 clearly indicate that all holding solutions significantly prolonged the vase life (days) of calla cut stalks compared to untreated cut flowers (control). The longest vase life was markedly extended in response to using humic acid at 25 mL/L, sucrose at 20 g/L + citric acid at 0.2 g/L (15.33, 14.00) days compared to cut flowers held in distilled water (D.W.) which were recorded (6.33, 6.67 days) in the first and second seasons. Humic acid treatment resulted a decrease in ethylene synthesis, which could explain the extended vase life. The results are in agreement with the results obtained by Khan et al. (2020). on Zinnia eleganse, who indicated that humic acid levels and different cultivars significantly affect flower vase life. Similar findings were revealed by El-Baset and Kasem (2022). on Dendranthema grandiflorum, who found that using humic or Fulvic acids improved flowering characteristics and the percentages in the vase life parameter.

General appearance: Table 1 show that holding calla cut flowers in solutions containing humic acid at 25 mL/L alone or with sucrose at 20 g/L and citric acid at 0.2 g/L recorded the highest flower quality. Eucalyptus oil came in the following category compared to the other treatments in the two seasons. Flowers' quality is largely visual and includes an appearance of visual freshness, uniform color, and the lack of defects such as damage

Table 1. Effect of holding solution treatments on vase life (days), general appearance and increase of	
flower fresh weight (%) of Z. aethiopica cut flowers during the 2020 and 2021 seasons	

Treatments	Vase li	fe (days)	General		IFFW(%)	
	2020	2021	2020	$\frac{1}{2021}$	2020	2021
Control (D.W)	6.33g	6.67e-g	1.00g	1.00g	0.69h	0.48i
Eucalyptus oil (1 mL/L)	13.33bc	12.67а-с	4.00a-c	3.33а-с	6.45c	6.57c
Eucalyptus oil (2 mL/L)	12.67c	12.33bc	3.67bc	3.00a-d	4.65e	4.59f
EO (1 mL/L)+Suc. (20 g/L)+CA (0.2 g/L)	7.33fg	7.00ef	1.67fg	1.33fg	1.29g	1.26h
EO (2 mL/L)+Suc. (20 g/L)+CA (0.2 g/L)	7.00fg	6.67e-g	1.33fg	1.33fg	0.87gh	1.59h
humic acid (25 mL/L)	14.00b	13.67ab	4.67ab	3.67ab	8.76b	9.15b
humic acid (50 mL/L)	11.00d	11.67c	3.33cd	2.67b-е	5.16de	5.10ef
HA (25 mL/L)+Suc. (20 g/L)+CA (0.2 g/L)	15.33a	14.00a	5.00a	4.00a	10.95a	12.24a
HA (50 mL/L)+Suc. (20 g/L)+CA (0.2 g/L)	7.00fg	7.00fg	2.00e-g	1.67e-g	2.46f	2.58g
8-HQC (0.2 g/L)+Suc.(20 g/L)+ CA (0.2 g/L)	9.33e	10.00d	2.33d-f	2.00d-g	5.04de	5.52de
GA ₃ (0.05g/L)+Suc.(20 g/L)+CA (0.2 g/L)	8.00f	10.00d	3.00с-е	2.33c-f	5.49d	6.09cd

Means within a column or row having the same letters are not significantly different as per Duncan's Multiple Range Test at 5% level.

and wilting. The positive effects of HA were due to its ability to delay the breakdown of chlorophyll, preserve the color of leaves, and delay the synthesis of ethylene. These results are in accordance with those reported by Bayat *et al.* (2021). On *Achillea millefolium* L. and Ushasri *et al.* (2022). On *Dracaena reflexa*, it was observed that using humic acid in vase solutions at 4 mL/pot resulted in a maximum increase in vase life with a good visual score for cut foliage shoots. The effect of Eucalyptus oil was due to delaying senescence, this could be explained by the antimicrobial properties of EO, which accelerate the absorption process in flowers while maintaining the quality of the cut flower. These results agreed with Almeida *et al.* (2020). It was shown that the application of Eucalyptus essential oil in all doses tested provided better maintenance on the postharvest quality of roses.

Increase of flower fresh weight (IFFW%) : It is clear from the data in Table 1 that, the lowest increase in flower fresh weight percentage was observed in the control (0.69 and 0.48%) for both seasons, respectively. Adding humic acid at 25 mL/L, combined with sucrose at 20 g/L and citric acid at 0.2 g/L, to preservative solutions increased the flower fresh weight percentage (10.95 and 12.24%) in the first and second seasons, respectively. Humic substances have a significant role in plant physiological processes and exhibit auxin-like hormonal action resulting delayed senescence. Azam *et al* (2022) found that humic acid had a positive impact on the yield and flower number of *Tagetes Erecta*.

Water uptake (mL/3flowers): All applied holding solutions had a beneficial effect on the water uptake rate (mL/3 flowers) of calla cv. 'Romance' cut flowers compared to (D.W.) for end longevity, as indicated in Table 2.The holding solution, which contained

(humic acid 50 mL/L) + (Suc 20 g/L) + (CA 0.2 g/L), provided the highest water absorption (43.07 and 42.56 mL/3 flowers) up to the 9th day, while the control provided the lowest values (13.76 and 12.54 mL/3 flowers) up to the 6th day in the two seasons, respectively. Humic acid improves plants' ability to absorb nutrients and increases nutrient availability and absorption by allowing micronutrients to pass through the leaf surface into the vascular system. These findings are consistent with those of Ahmad et al. (2014) for Oriental lily blossoms. Bolagam and Natarajan (2020) reported that the use of humic acid components considerably increased water intake, thus increasing the process in gladiolus.

Relative fresh weight (RFW%): It is clear from the data presented in Table 3 that most of the vase solutions significantly increased the fresh weight of calla cut flowers compared with the control, which had the lowest increment in fresh weight. Different treatments in the first and second seasons showed an increase in the relative fresh weight of cut flowers up to the 9th day, followed by a sharp decrease during the 12th day. Overall, calla cut flowers stored in a vase solution containing humic acid at 25 mL/L, sucrose at 20 g/L, and citric acid (CA) at 0.2 g/L demonstrated a significant increase in fresh weight from the initial up to the 9th day, with humic acid at 25 mL/L leading the way, compared to the control group, which was only observed up to the 6th day. Ali et al. (2014) recorded results similar to

Table 2. Effect of holding solution treatments on water uptake (mL/3 flowers) of Z. aethiopica cut flowers during the 2020 and 2021 seasons

Holding solutions		1st seaso	n (2020)		2 nd season	on (2021)	(2021)	
	3 rd d	6 th d	9 th d	12 th d	3 rd d	6 th d	9 th d	$12^{th} d$
Control (D.W)	5.75i	13.76i	13.68j	7.35h	4.13g	12.54h	12.60j	6.71e
Eucalyptus oil (1 mL/L)	20.54bc	32.02b	47.38c	44.14c	19.35b	31.24b	46.12c	42.83b
Eucalyptus oil (2 mL/L)	18.32cd	29.82c	45.13d	32.09e	16.77c	28.02c	43.11d	31.75c
EO (1 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	8.83h	20.38g	19.51h	8.28h	7.26f	19.76f	17.74h	9.78d
EO (2 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	7.62hi	17.71h	17.58i	7.89h	5.39g	16.64g	15.76i	6.80e
humic acid (25 mL/L)	22.16b	33.13b	50.57b	45.98b	20.44b	31.83ab	49.19b	44.99b
humic acid (50 mL/L)	14.56ef	26.60de	44.16de	31.38e	13.73d	25.77cd	41.14e	32.06c
HA (25 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	26.63a	36.79a	54.17a	51.24a	23.86a	34.26a	53.98a	50.44a
HA (50 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	16.09de	27.76d	43.07e	35.30d	14.31d	26.14cd	42.56de	33.47c
8-HQC (0.2 g/L) + Suc.(20 g/L) + CA (0.2 g/L)	12.01fg	25.59ef	24.57f	13.39f	10.27e	24.32de	22.95f	12.25d
$GA_3 (0.05g/L) + Suc.(20 g/L) + CA (0.2 g/L)$	9.67gh	24.39f	21.86g	11.46g	8.28f	22.32ef	19.82g	10.98d

Means within a column or row having the same letters are not significantly different as per Duncan's Multiple Range Test at 5% level.

Holding solutions		RFW 1 st se	ason (2020)		RFW 2 nd season (2021)				
	3 rd	6 th	9 th	12 th	3 rd d	6 th d	9 th d	$12^{th} d$	
Control (D.W)	100.4d	101.0g	99.90f	97.55g	100.3d	101.1f	100.0f	97.31h	
Eucalyptus oil (1 mL/L)	100.7c	102.3cd	105.4c	106.4c	100.6c	102.2d	105.3c	106.6c	
Eucalyptus oil (2 mL/L)	100.7c	102.1d	103.7d	104.6d	100.6c	102.2d	103.6d	104.6e	
EO (1 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	100.7c	101.7e	101.3e	99.92ef	100.6c	101.6e	101.3e	100.2f	
EO (2 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	100.6c	101.6ef	100.6ef	99.12f	100.6c	101.7e	100.7ef	98.73g	
humic acid (25 mL/L)	101.5a	103.9b	107.1b	108.8b	101.6a	103.9b	107.4b	109.1b	
humic acid (50 mL/L)	101.1b	102.6c	104.5cd	105.2d	101.1b	102.7c	104.4cd	105.1de	
HA (25 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	101.5a	105.0a	109.1a	110.9a	101.5a	105.4a	110.0a	112.2a	
HA (50 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	100.6cd	101.3f	101.0e	100.2e	100.7c	101.5e	101.4e	100.5f	
8-HQC (0.2 g/L) + Suc.(20 g/L) + CA (0.2 g/L)	100.7c	102.3cd	104.3d	105.0d	100.7c	102.5cd	104.8c	105.5с-е	
$GA_3 (0.05g/L) + Suc.(20 g/L) + CA (0.2 g/L)$	100.6c	102.1d	104.3d	105.5cd	100.8c	102.2d	104.8c	106.1cd	

Table 3. Effect of holding solution treatments on relative fresh weight (RFW%) of Z. aethiopica cut flowers during the 2020 and 2021 seasons

Means within a column or row having the same letters are not significantly different as per Duncan's Multiple Range Test at 5% level.

the present findings by revealing that a higher concentration of humic acid led to an increase in fresh weight, indicating its crucial role in tulip fresh weight. Aslam *et al.* (2018) working on *Tagetes erecta* found that the flower fresh weight was significantly increased due to the fact that humic acid improved nutrient uptake, which ultimately increased growth and yield, and Alziyituni (2023), who discovered that humic acid at a high concentration of 200 mL/L recorded a higher number of flowers and fresh weight as compared with control.

Total carbohydrates (%): Table 4 shows a reduction in total carbohydrates in calla cut flowers held in distilled water compared to the other treatments in both seasons. But when the flowers were treated with humic acid (25 mL), either by itself or with sucrose at 20 g/L and citric acid at 0.2 g/L, the total carbohydrates in the petals went up the most, by 32.3 and 39.9% (28.7 and 27.7%) in both seasons. This is due to the flowers' reduced respiration and metabolic rate. Also, (HA) low concentrations (25 mL) had better effect as compared with high concentrations (50 mL) and an increased photosynthetic rate results in higher energy generation. The abundance of food drives the use of this energy for development. According to Khenizy et al. (2013), flowers held in humic acid at 25 mL, followed by humic acid at 50 mL, had the highest total sugar percentage compared to other treatments used on Gerbera cut flowers in both seasons. Also, it is obvious that adding Suc + CA to humic acid (25 mL) affected positively total carbohydrates in both seasons compared to humic acid 25 mL when used alone. Alam et al. (2023) found in Gerbera jamesonii that adding citric acid and sucrose to preservative solutions significantly improved the vase life and quality of cut flowers.

Table 4. Effect of holding solutions treatments on total carbohydrates (%) and total phenols (%) of *Z. aethiopica* cut flowers during the 2020 and 2021 seasons.

Treatments		oohydrates %)	Total phenol content (%)		
	2020	2021	2020	2021	
Control (D.W)	11.3h	10.5i	22.48f	23.34h	
Eucalyptus oil (1 mL/L)	25.3c	24.5c	30.85b	30.83bc	
Eucalyptus oil (2 mL/L)	24.1cd	22.8d	33.01a	31.57b	
EO (1 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	14.6f	13.3g	27.56d	24.21h	
EO (2 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	12.4gh	11.9hi	22.37f	25.51g	
humic acid (25 mL/L)	27.7b	28.7b	33.55a	31.99b	
humic acid (50 mL/L)	22.8d	21.00e	32.67a	33.35a	
HA (25 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	30.9a	32.3a	29.09c	29.29de	
HA (50 mL/L) + Suc. (20 g/L) + CA (0.2 g/L)	13.8fg	13.00gh	31.26b	30.22cd	
8-HQC (0.2 g/L) + Suc. (20 g/L) + CA (0.2 g/L)) 19.6e	18.1f	24.45e	27.80f	
$GA_3(0.05g/L) + Suc.(20 g/L) + CA(0.2 g/L)$	20.2e	19.1f	28.35cd	28.39ef	

Means within a column or row having the same letters are not significantly different as per Duncan's Multiple Range Test at 5% level.

Total phenolic content (%): Data presented in Table 4 indicate that there were positive effects of humic acid and eucalyptus oil on the total phenolic content percentage. Treating calla cut flowers with humic acid in two concentrations (25 and 50 mL) alone yielded the highest value. Increasing the quantity of HA led to a progressive increase in phenolic accumulation, potentially acting as antioxidants to scavenge ROS. Gholami et al. (2018) observed a similar trend in Cichorium intybus L., stating that vermicompost and humic acid enhance the phytochemical properties, including the total flavonoids. These results agree with those of Khosravi et al. (2016), who found that Eucalyptus and Rosa damascene essences improved fresh weight, solution uptake, bent neck reduction in cut gerbera flowers.

The study revealed that a preservative solution containing 25 mL/L of humic acid, 20 g/L of sucrose, and 0.2 g/L of citric acid is the optimal treatment for calla cut flowers. It extended the vase life, increased relative fresh weight, increased the percentage of total carbohydrates, improved water uptake, increased floral fresh weight, and improved overall attractiveness.

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