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Research Article

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Holding solution pH and composition consistently improve vase life of rose, lily and gerbera

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ABSTRACT

We assessed the influence of postharvest pulsing solutions pH and composition on cut flower quality of rose (*Rosa hybrida* cvs. Avalanche and Black Magic), gerbera (*Gerbera jamesonii* cv. Beaudine) and lily (*Lilium* × *elegans* cv. Fangio) under room (20±2 °C) and cold storage (4±1 °C) conditions. Cut flowers were placed in different acidic (pH, 3.5 - 4) or basic (pH, 7.0 - 7.5) preservative solutions containing water, sugar 5% (flower food), 100 mg/L silver nitrate ($AgNO_3$ act as a bactericide), or a commercial product (2% sugar + bactericide and fungicide). Acidic solutions had higher or similar (never lower) vase life at both room and cold storage conditions and across species. In addition, vase life was 3-4 times longer in cold storage when compared to room conditions. Leaf chlorophyll concentrations for rose and lily were inconsistent or not significant across the species at both conditions (room and cold storage). The commercial preservative solution consistently and significantly had higher vase life than water for all tested cut flower species and under both room and cold storage environments. $AgNO_3$ ranked second in terms of vase life enhancement. Overall, the use of only flower food (sugar) or bactericide ($AgNO_3$) had a positive impact on vase life but only the combined use of a preservative substance (specifically at pH, 3.5 - 4.0) consistently guaranteed a high cut flower quality across flower species.

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INTRODUCTION

Cut flowers are a growing industry worldwide. In 2019, over 12.1 billion flowers and plants (value, $5.6 billion) were traded at the Royal FloraHolland, the largest global marketplace for the floriculture industry (RFH, 2019). In the cut flower industry, one should distinguish between two distinct stages, (1) flower bud growth and development to full opening and (2) maturation, senescence, and wilting. Postharvest management mainly focuses on prolonging flower longevity (vase life) and must achieve two contradictory objectives: enhancing growth processes in the first stage and restricting metabolic processes which lead to wilting and senescence in the second stage (Halevy & Mayak, 1979; Nguyen & Lim, 2021). Flower longevity is an important factor for consumer preference. Extending cut flower vase life using cultural practices and postharvest treatments enable floriculturists to accumulate large quantities of flowers and prolong the sale season for cut flowers (Rudnicki *et al.*, 1991; Al-Ajlouni *et al.*, 2017a, 2017b). The essential postharvest problem that significantly impacts vase life is the development of air-emboli in the xylem vessels, especially in those at the basal part of the stem. Air-emboli partially block water transport to flowers and causes wilting (van Doorn *et al.*, 1989; van Meeteren & van Gelder, 1999; Nguyen & Lim, 2021). Floriculturists normally add antimicrobial agents to the preservative solution and recut the flower stem about 2.5 cm from the base to remove air-emboli and induce rehydration of the flower. However, differences in rehydration ability and vase life are present between cultivars as well as preservative solutions (van Doorn *et al.*, 1989; van Meeteren & van Gelder, 1999).

Several pulsing and holding preservative solutions have been used to prolong flower vase life including, sucrose, 8-hydroxyquinoline sulfate (8-HQS), silver thiosulfate (STS), silver nitrate ($AgNO_3$) and gibberellic acid (GA_3) (Liao *et al.*, 2000; Eason, 2002). The vase life of cut hydrangea (*Hydrangea macrophylla*) flowers significantly increased (15.89 days) when placed in holding solution containing 8-HQS 200 ppm (mg/L) compared to water (4.22 days) (Kazaz *et al.*, 2020). Cut spikes of sweet pea (*Lathyrus odoratus*) stood in 200 ppm 8-HQS plus 2%

sucrose preservative solution showed the highest water balance, chlorophyll content and vase life (17 days) compared to pulsing treatment of 200 ppm 8-HQS + 2% sucrose for 12 h as well as the pulsing with 0.2 mM (65 mg/L) STS for 1 h followed by 2% sucrose solution (Elhindi, 2012).

Van Doorn *et al*. (1989) found that ethylene production and other physiological processes following the stem cutting of roses (*Rosa hybrida*) were apparently negligible and not associated with vascular blockage. Instead, blockage of xylem vessels was mainly due to the presence of bacteria. Reid *et al*. (1996) concluded that lowering hydraulic resistance in the xylem can be achieved by recutting the stem under water, pretreating with detergent, using warm water (40 °C) and lowering preservative solution pH. However, the mechanisms that explain the positive rehydration when placing a flower at low pH is still not understood (Reid *et al*., 1996).

Placing rose flowers in a holding solution containing 2% sugar resulted in lower vase life compared to the control (tap water), though sucrose is a well-known carbohydrate source (Lee & Kim, 2018). However, the addition of 2 or 8 µL/L chlorine dioxide to the sucrose solution extended the flower vase life about six days more than the sucrose treatment and four days more than the control (Lee & Kim, 2018). Sucrose has beneficial effects on the supply of substrates for respiration and consequently prolongs vase-life of cut flowers (Pun & Ichimura, 2003). In fact, sugars improve water relations and balance, delay climacteric ethylene production, and lower the sensitivity of cut flowers to ethylene (Pun & Ichimura, 2003).

Rose, gerbera and lily are the main cut flowers currently traded on the floriculture stock markets. These species were among the top five cut flowers sold by Royal FloraHolland in 2019 (RFH, 2019). The number of flowers sold in 2019 (in millions) was 3304 for rose, 1124 for gerbera and 282 for lilies (RFH, 2019). A significant debate and controversy in postharvest research studies are centered on finding a reference holding solution that enhances vase life for roses, lilies, and gerbera (Liao *et al*., 2000; Elhindi, 2012). Reference solutions that have been proposed include ones that contain sugar and germicides, sucrose + HQS, sucrose + STS (Liao *et al*., 2000; Elhindi, 2012). However, we are not aware of any study that assesses different preservative solutions (sucrose, antimicrobial agents), in a basic and acidic solution environment and under room and cold storage conditions. We hypothesized that adjusting the holding solution pH (acidity) that contain antimicrobial agents or/and sugar can significantly prolong flower longevity (vase life). The objective of this study was to assess the influence of preservative substance composition, pH (7.0 - 7.5 and 3.5 - 4.0) on flower quality (vase life, leaf chlorophyll concentration) of rose, lily, and gerbera under room (20±2 °C) cold storage (4±1 °C) conditions.

MATERIELS AND METHODS

Laboratory Setup and Plant Material

The study was conducted at the University of Jordan (lat. 32° 0' 40.4316" N, long. 35° 52' 20.3628" E) in September, 2019. Two experiments were conducted simultaneously, one in a room and the other in a cold storage facility in the same lab. The temperature for the room experiment was 20±2 °C and light intensity ranged from 7 to 10 µmol/m^2/s. The cold storage was conducted under no light and a temperature of 4±1 °C. Cut rose (*Rosa hybrida* cvs. Avalanche and Black Magic), gerbera (*Gerbera jamesonii* cv. Beaudine) and Asiatic lily (*Lilium* × *elegans* cv. Fangio) were harvested from soilless commercial greenhouses. Cut roses and lilies were harvested when the largest flowering bud on each stem was at the stage of showing color to fully colored (Han, 2003). Gerbera harvesting was done when the outer 2 rows of petals were open. Flowers were transported within 2 hours to the experimental lab (20±2 °C) and each stem was trimmed to 45 cm. Then, each stem was recut (3 cm from base) in the tested postharvest solutions to remove air bubbles from the xylem and placed directly (less than 5 seconds) in a 2 L bottle containing the examined preservative solutions. Bottles were covered with aluminium foil to reduce bacterial and fungal development in the solution.

Postharvest Solution Treatment

Four different postharvest (holding) solutions were used. These holding solutions were water, sugar 5%, $AgNO_3$ (100 mg/L), and a commercial (Spring Pro-Florist, Spring From Holland B.V., Sassenheim, Nederland) solution (2% sugar + bactericide and fungicide, pH 3.5 - 4). To assess the impact of pH, water, sugar 5% and $AgNO_3$ (100 mg/L) solutions were evaluated under acidic (pH, 3.5 - 4) and basic (pH, 7 - 7.5) conditions. Citric acid was used to adjust the holding solution pH while tap water (pH, 7 - 7.5, electrical conductivity 0.7 dS/m) was used to prepare the studied solutions. 500 mL of each tested solution was placed in a 2 L bottle and flowers were placed directly inside the holding solution. The first set of bottles having flowers and preservative solutions was left on the lab bench (room experiment, 20±2 °C) and the second set was placed in the fridge (cold storage experiment (4±1 °C).

Postharvest Quality Assessment

The postharvest quality of the flower and leaves was evaluated in both sets (room and cold storage). Five days after harvest, leaf-level chlorophyll concentration was determined using a chlorophyll concentration meter (MC-100; Apogee Instruments, Inc., North Logan, UT, USA). Vase life for rose and gerbera flowers was defined as the number of days from harvesting the stems and placing them in the holding solution to the day when the first 3 petals fell off or when the pedicel bent (bent neck problem). For the lily, vase life was measured from the first day of harvest to when the first lily flower/per stem fell off or wilted (Al-Ajlouni *et al*., 2017b).

Experimental Design Setup and Statistical Analysis

A randomized complete block design (RCBD) with four replicates and two factors (preservative substance and pH) was used for room and cold storage experiments. Analyses were conducted using SAS software (Version 9.4 for Windows; SAS

Institute, Cary, NC). In both experiments, flower quality (vase life and chlorophyll concentration) was statistically analyzed by the analysis of variance ANOVA and means separated by Fisher's LSD test ($P \leq 0.05$).

RESULTS

Postharvest holding solution significantly affected cut flower vase life and chlorophyll concentration. At room temperature, acidic (pH, 3.5 - 4.0) holding solution significantly increased 'Black Magic' rose vase life and lily chlorophyll concentration (Table 1). Roses (cvs. Black Magic and Avalanche), lily and gerbera placed in commercial holding solution had higher vase life than those held in water. Similarly, $AgNO_3$ treatment had higher vase life for rose 'Black Magic', lily and gerbera when compared to water. In addition, chlorophyll concentration for roses (cvs. Black Magic and Avalanche) in $AgNO_3$ holding solution was higher than in water at room temperature (Table 1).

For cut flowers stored in cold conditions, an acidic environment significantly increased vase life for roses (cv. Avalanche) and gerbera (Table 2). Commercial holding solution significantly increased vase life (compared to water) across the test flower species (roses, lily and gerbera) (Table 2). In addition, chlorophyll concentration of 'Avalanche' rose was higher than the other treatments (Table 3). Roses (cvs. Black Magic and Avalanche) and lilies placed in $AgNO_3$ had higher vase life than those placed in water (Table 2). 'Black Magic' rose and lily had a higher vase life when immersed in the sugar solution (5%) than when placed in water.

DISCUSSION

The main differences between cut flowers and other horticultural products, specifically in terms of postharvest and senescence physiology are (1) cut flowers are more complex organs than seeds, fruits, and most vegetables, and (2) most fruits and vegetables are harvested after they have ripened while most cut flowers are harvested before they ripen (Halevy & Mayak, 1979; Othman *et al.*, 2021). However, one advantage of cut flowers is that preservative substances added to holding solutions can enhance cut flower quality (Halevy & Mayak, 1979; Han, 2003). In the cut flower industry, vase life or longevity is essential to high quality flowers (Woodson, 1991; Burchi *et al.*, 2010; Al-Ajlouni & Othman, 2020). Terms commonly used in evaluating the postharvest quality of cut flowers are vase life, longevity and shelf-life, and typically, data are presented in days (Halevy & Mayak, 1979). Interestingly, leaf visual quality such as leaf size and greenness is a key factor in marketing lily flowers (McKenzie, 1989; Al-Ajlouni *et al.*, 2017b). In our study, the addition of preservative substances, pH and their interaction significantly improved the vase life of

Table 1: Vase life and chlorophyll concentration of cut rose (cvs. Black Magic [BM] and Avalanche [A]), lily and gerbera flowers kept at room temperature (20±2 °C) and placed in different postharvest preservative solutions. Leaf chlorophyll concentration was determined five days (week 1) after harvesting

Postharvest preservative substance (P)	Acidity (A)	Vase life (no. of days)				Chlorophyll concentration (mg/L)		
		Rose (A)	Rose (BM)	Lily	Gerbera	Rose (A)	Rose (BM)	Lily
	Acidic (pH, 3.5-4.0)	11.1	13.6 a	11.9	12.1	322	334	541 a
	Basic (pH, 7.0-7.5)	9.1	11.9 b	11.3	11.4	342	364	483 b
Commercial		17.8 a	19.0 a	11.8 a	11.5 b	297 b	353 ab	528 ab
$AgNO_3$		9.5 b	14.5 b	11.8 a	14.3 a	376 a	367 a	568 a
Sugar		8.6 b	10.8 c	12.6 a	12.4 b	342 a	355 ab	470 b
Water		8.9 b	10.4 c	10.5 b	8.9 c	291 b	314 b	505 ab
P-value	A	0.73	0.05	0.15	0.10	0.51	0.15	0.02
	P	0.001	0.001	0.002	0.001	0.001	0.05	0.03
	P × A	0.58	0.05	0.02	0.005	0.45	0.58	0.06

Different letters indicate differences among treatments according to Fisher's LSD test ($P \leq 0.05$).

Table 2: Vase life of cut rose (cvs. Black Magic [BM] and Avalanche [A]), lily and gerbera flowers kept at a cold temperature (4±1 °C) and placed in different postharvest preservative solutions

Postharvest preservative substance (P)	Acidity (A)	Vase life (no. of days)			
		Rose (A)	Rose (BM)	Lily	Gerbera
	Acidic (pH, 3.5-4.0)	38.8 a	48.1	42.2	39.0 a
	Basic (pH, 7.0-7.5)	36.8 b	47.6	42.4	31.7 b
Commercial		47.7 a	56.7 a	43.0 a	53.0 a
$AgNO_3$		38.5 b	46.3 b	45.8 a	28.7 c
Sugar		35.3 c	53.7 a	43.5 a	41.5 b
Water		35.2 c	39.2 c	37.2 b	28.8 c
P-value	A	0.05	0.15	0.72	0.05
	P	0.001	0.001	0.003	0.001
	P × A	0.04	0.01	0.68	0.001

Different letters indicate differences among treatments according to Fisher's LSD test ($P \leq 0.05$).

Table 3: Chlorophyll concentration of cut rose (cvs. Black Magic [BM] and Avalanche [A]), lily and gerbera flowers kept at a cold temperature (4±1 °C) and placed in different postharvest preservative solutions

Postharvest preservative substance (P)	Acidity (A)	Chlorophyll concentration (mg L^{-1})					
		Rose (A)		Rose (BM)		Lily	
		Week 1	Week 3	Week 1	Week 3	Week 1	Week 3
	Acidic (pH, 3.5-4.0)	297	315 a	302	328	546	375
	Basic (pH, 7.0-7.5)	278	282 b	312	310	588	411
Commercial		345 a	354 a	305	313	535	401
$AgNO_3$		297 b	300 bc	296	322	548	389
Sugar		248 c	261 c	289	306	583	419
Water		293 b	315 ab	334	336	576	358
P-value	A	0.82	0.05	0.55	0.27	0.25	0.15
	P	0.001	0.01	0.26	0.64	0.62	0.2
	P × A	0.06	0.05	0.82	0.80	0.05	0.09

Different letters indicate differences among treatments according to Fisher's LSD test ($P \leq 0.05$).

rose, lily and gerbera. However, foliar chlorophyll concentration response showed inconsistent results (Table 1) or was not significant (Table 3).

Postharvest Preservative Solution Acidity

Preservative solution pH, specifically, pH of 3.5 - 4.0 had a positive impact on flower quality (vase life and leaf chlorophyll concentration). In both room and cold storage conditions, the acidic preservative solution (pH, 3.5 - 4.0) had higher or similar (never lower) flower quality compared to basic solution. Acidity level × postharvest preservative substance interaction assessment revealed that acidic (pH, 3.5 - 4.0) × commercial interaction had consistently higher vase life than other interactions for roses cultivars, 'Black Magic' and 'Avalanche' under room and cold storage conditions as well as for gerbera (cv. Beaudine) under cold storage conditions (Tables 1 and 2). High pH (8.0) preservative solution could enhance the development of microbes in the preservative solution (Pompodakis *et al*., 2004). *Gardenia jasminoides* vase life ranged from 2-4 days and therefore has not been considered suitable for use as a cut flower (Çelikel *et al*., 2020). However, *G. jasminoides* vase life can be extended to more than five days, by simply acidifying the preservative solution using citric acid (200 mg/L) (Çelikel *et al*., 2020). Placing roses (*Rosa hybrida* L.) flowers in 10^{-5} M ABA at pH 6 and 8 in the presence and absence of 1 mg/L $AgNO_3$ solution showed that Abscisic acid (ABA) + pH 6 treatment had higher vase life and lower vase solution usage than ABA supplied at pH 8. This indicated that vase solution pH affected the ABA-mediated stomatal closure of cut roses (Pompodakis *et al*., 2004). The induction of stomatal closure may benefit cut flowers by reducing water deficit stress. Although the mechanisms that explain the positive rehydration when placing cut flowers in low pH is still not understood (Reid *et al*., 1996), acidic preservative show potential for improving vase life.

Cold Storage

Cold storage of cut flowers slows down metabolic activities, reduces transpiration and ethylene production, and restricts fungal development (Rudnicki *et al*., 1991). In our study, the vase life of tested flowers (roses, lilies and gerbera) from cold storage was notably higher than those under room temperature; cold storage resulted in about a 3-4-fold increase in vase life though the differences between room and cold storage were not statistically analyzed. A wide range of temperatures was recommended for the assessment of vase life and it was agreed that room temperature (20±2 °C) is suitable for standardized vase life evaluation (Reid & Kofranek, 1980). However, our results showed that the response of cut flower to a preservative solution under room and cold storage was not similar, specifically for sugar and $AgNO_3$.

Postharvest Solution Preservative Substance

The postharvest life of cut flowers (e.g. rose) is mainly related to water relations (difference between transportation and uptake). When water relations are optimum (sufficient water uptake), the flowers are turgid but the stem is normally under negative tension. After a short period of harvesting, basal xylem vessels fill with air but water uptake resumes and the flower gradually rehydrates after the stem is placed in water. If the stem exposure to air is prolonged, the basal xylem will not be able to take up water. Recutting the stem underwater, placing flowers in warm water (40 °C) or a low pH holding solution can remove the air blockage and permits rehydration, especially during the first days of flower cutting. However, the hydrated stems can gradually decline at later stages (e.g. after 7 days in rose) due to physical obstructions in the xylem. This leads to a significant reduction in stem water potential (more negative than -1 MPa) and xylem conductivity and consequently, the termination of vase life (Reid *et al*., 1996).

Early flower wilting and reduction in vase life are associated with the loss of cell turgor and the development of cavitation along the water transport path. The main source of cavitation is an imbalance of water relations inside the cut flower; high transpiration or/and limited and water uptake. High hydraulic resistance in the lower part of the cut flower stem limits water translocation and leads the flower into an unrecoverable situation and the end of its vase life (van Meeteren & van Gelder, 1999). Interestingly, hydraulic resistance in trimmed stems seems to be influenced by the composition of the vase

solution (van Meeteren & van Gelder, 1999). Therefore, the use of an effective postharvest preserving solution is essential to extending flower longevity in the cut flower industry.

Several preservative substances have been used in postharvest holding solutions including sugar, STS, $AgNO_3$, hormones (ABA) and their combinations. Sucrose holding solution (4% w/v) alone resulted in longer vase life (7.1 days) of cut *Rosa hybrida* L. 'Kardinal' flowers than the combined solution of 4% sucrose and 100 mg/L acetylsalicylic acid (5.6 days), 200 mg/L salicylic acid (5.5 days), or 600 mg/L ascorbic acid (5.3 days) (Ahmad *et al.*, 2013). $AgNO_3$ acts as a bactericide and plays a key role in extending cut flower vase life (Pompodakis *et al.*, 2004). Treated gerbera flower with pulse treatments 4% $CaCl_2$ + 3% sucrose for 24 hours followed (or not) by continuous hormonal (GA_3 25, 30 mg/L; Benzyl adenine 150, 250 mg/L, salicylic acid 100, 200 mg/L) or chemical treatment (STS 0.4, 0.8 mM; HQS 400 mg/L; nano-silver particles 5, 10 mg/L) showed that HQS without pulse treatment prevented stem bending (Bent Neck) and significantly improved vase life (Jafarpour *et al.*, 2015).

Commercial holding solution had consistently higher vase life than water across the studied species (roses, lily and gerbera) and in both room and cold storage conditions (Tables 1 and 2). Generally, $AgNO_3$ ranked second in term of extended vase life. the addition of sugars to cut flowers improves water balance (osmolytes effect), provides substrates for respiration and synthetic materials, inhibits ethylene production, improves petal color expression and promotes flower opening (Ichimura, 1998). However, the preservative solution of 2% sugar neither increased the vase life nor the size of Oriental lily flowers but significantly increased anthocyanin content and, consequently, the intensity of petal color (Han, 2003). A postharvest study on the performance of *Eustoma grandiflorum*, *Matthiola incana* and *Zinnia violacea* flowers in homemade floral preservatives showed that preservatives solutions extended vase life compared to water treatment. Among tested preservative recipes, 0.007 mL/L isothiazolinone, 0.5 mL/L quaternary ammonium chloride, 500 mL/L lime soda, or 400 mg/L citric acid+20 g/L sugar (all dissolved in tap water) demonstrated the best postharvest performance. Conversely, 100 mg/L citric acid+20 g/L sugar+200 mg/L aluminum sulfate or 6 mL/L lemon juice+20 g/L sugar had detrimental effects on the vase life of tested flowers and consequently should not be used as preservative solutions (Ahmad & Dole, 2014). Considering the inconsistent results for sugar (5%) treatment results in our study, we do not recommend using sugar (5%) only as a preservative solution for rose, gerbera and lily cut flowers.

$AgNO_3$ acts as a bacteriostatic chemical, inhibits the growth of bacteria in stems, prevents the reduction of hydraulic conductance and inhibits ethylene production (van Doorn *et al.*, 1989). In rose, hydraulic conductance which is responsible for the uptake of water by the stem was significantly associated with a number of endogenous bacteria in the xylem vessels only when the number of bacteria in the basal 5 cm stem segment exceeded 10^6 CFU per gram fresh weight (van Doorn *et al.*, 1989). Whenever the number of bacteria in the rose stem remained at 10^6 CFU per gram fresh weight, hydraulic conductance was the same, even after stems had been held in the water for seven days (van Doorn *et al.*, 1989). Interestingly, bacteria counts in holding solutions after six days of placing the flower was 2.3×10^5 CFU L^{-1} for tap water, 2.4×10^6 CFU L^{-1} for sucrose and no bacteria were detected in chlorine dioxide + sucrose solution (Lee & Kim, 2018). However, using only $AgNO_3$ as a preservative solution did not consistently have the highest vase life across the tested cut flower species compared to commercial treatment. This suggests that the use of flower food (sugar) coupled with bactericide is essential.

Overall, the combination of flower food (sugar), bactericide and fungicide (e.g. $AgNO_3$) coupled with pH adjustment to a value of 3.5 - 4.0 showed potential for extended vase life of rose, lily and gerbera cut flowers.

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