



Article

Effect of PGRs on Antioxidant Activity and Phytochemical in Delay Senescence of Lily Cut Flowers

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Abstract: The short vase life is the major problem in the cut flower industry. This study was conducted to evaluate the role of different vase solutions and oils in enhancing the quality and vase life of lily cut flowers. Salicylic acid (SA; 300 mg L⁻¹), citric acid (CA; 300 mg L⁻¹), gibberellic acid (GA; 100 mg L⁻¹), and clove oil (200 mg L⁻¹) were used as vase solutions. These treatments were applied after pulsing with preoptimized sucrose 5%. It was found that SA (300 mg L⁻¹) + sucrose (5%) improved the performance of cut flowers, which further increased the longevity of all tested lily cultivars up to eight days and the longest vase life by 17.6 days. The maximum change in fresh weight (5.60 g), increase in chlorophyll contents (3.2 SPAD value), highest protein content (6.1 mg g⁻¹ FW), and increase in the activities of superoxide dismutase (SOD) (51.0 U g⁻¹ protein), catalase (CAT) (36.3 U g⁻¹ protein), and peroxidase (POD) (41.6 U g⁻¹ protein), were recorded with the CA (300 mg L⁻¹) + sucrose 5%. Among the cultivars, “Zambesi” performed best compared to “Sorbonne” and “Caesars”. The maximum anthocyanin contents (198%) were recorded in “Caesars”. In conclusion, among the different preservative solutions, SA performed best to prolong the vase life and quality of lily cut flowers.

Keywords: salicylic acid; citric acid; clove oil; vase life; gibberellic acid

1. Introduction

Lilies are used as wonderful cut flowers, and due to a wide range of flower shapes and colors, these attain a remarkable place in landscape designing. Oriental hybrids, Asiatic hybrids, and Longiflorum hybrids are the major groups of lilies mainly used as cut flowers [1]. In Pakistan, exotic cultivars of lilies are highly demanded in the market due to their magnificent long, slender, fragrant, and showy flowers. These are imported in large quantities, because the temperate, cool winters of Pakistan offer potential for the cultivation of fabulous exotic cultivars in large areas [2]. After harvesting, the quality of cut lily flowers depends on many factors, like the cultivar, conditions of preharvest, harvest stage, handling, and environment postharvest.

The longevity of inflorescence and foliage are important parameters of the quality of cut lilies, whereas postharvest physiological disorders that limit the vase life in lily cut stems include vascular blockage and tissue browning followed by petal wilting, flower bud blasting, and leaf chlorosis [3]. The development of browning or yellowing of leaves is the most significant disorder postharvest and starts from the lower leaves and gradually moves upwards [4]. There are two reasons for vascular blockage: one is related to air embolism, and the other is microorganisms whose growth in the vase solution causes a physical blockage of xylem, reduces the uptake of water, and causes senescence [5].

In many countries, different studies have been conducted to increase the cut flower's vase life by treating with various chemicals or preservatives, such as boric acid, salicylic acid, sucrose, and citric

acids, and a certain level of success has been achieved [6]. However, little research has been conducted in Pakistan by using these types of preservative solutions to enhance the quality and vase life of cut lily flowers. This research is of great interest and importance for flower merchants, growers, and scientists in Pakistan. Traditionally used preservative chemicals are silver nitrate and silver thiosulfate, which have negative impacts on the environment due to their nonbiodegradable characteristics [7].

Biodegradable substances such as gibberellic acid, citric acid, sucrose, and salicylic acid do not affect the environment. Under normal environmental conditions, these cut flowers hardly maintain their level of quality and life for two to three days. There is a need to enhance lifespan of cut flower's vase lives, because people want to enjoy the beauty of these flowers for a longer time. This study was conducted with the objective to enhance the vase life and quality of lily cut flowers through pulsing and vase solutions. It was hypothesized that the application of different vase solutions (salicylic acid, citric acid, gibberellic acid, and clove oil) would enhance the quality and vase life of cut lily flowers.

2. Materials and Methods

2.1. Plant Material

The three cut flower cultivars ("Sorbonne" (Oriental), "Zambesi" (Oriental), and "Caesars" (Longiflorum Asiatic hybrids) of uniform size were obtained from a commercial grower of Islamabad, Pakistan via air-conditioned vehicle. Cut flowers were harvested at the green bud stage (three flower buds in inflorescence, and one bud near to open) early in the morning and transported to the postharvest laboratory (20 ± 2 °C), Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Relative humidity of the evaluation room was maintained at $60\% \pm 10\%$ [3]. On arrival, stems were rehydrated with distilled water for 2 h at room temperature (25 °C) and trimmed to 40-cm lengths, followed by tagging according to the experiment.

2.2. Treatments

The treatments were imposed after pulsing (for 24 h); the stems were cut to get the stem length of 35 cm and shifted to the vases containing 500 mL of vase solutions according to treatments. In each glass jars, 3 stems were placed in one replication and with three replications per treatment. The preoptimized pulse of sucrose (5% or 5 g 100 mL⁻¹) and vase solutions: control (distilled water), salicylic acid (SA) (300 mg L⁻¹), citric acid (CA) (300 mg L⁻¹), gibberellic acid (GA₃) (100 mg L⁻¹), and essential oil (clove oil 200 mg L⁻¹) were used.

2.3. Observations and Measurements

2.3.1. Postharvest Traits

Data on the postharvest qualities was recorded by observing the vase lives on a daily basis by daily visiting the flower stems (by individual flower; on the half-wilting/necroses of flowers, the vase life was considered ended) and the volume of solution uptaken by flowers (from day 0 to 5 days of vase life). Fresh weight (g) (initial weight) was recorded on day 0 before placing into vase solutions, and final weight (g) after keeping in vase solutions (on the half-wilting of flowers, the vase life was considered ended) and the change in the fresh weight (g) were recorded. Dry weight (g) was recorded by drying flower stems (in the stage of the half-wilting of flowers) first under sunlight and then dried in an oven at 80 °C. The changes in electrical conductivity (EC) (dS m⁻¹) and pH of the vase solutions were observed by recording the first weight (initial weight) before placing in vase solutions and after keeping in vase solutions with pH and an EC meter (MW802; pH, EC, and total dissolve solids (TDS) meter were made by Milwaukee, Wisconsin, USA). The total soluble solids (TSS) (Brix) of petals were measured on the start and on the half-wilting of flower petals by obtaining sap from each replication. The TSS in the petal sap was measured by a hand refractometer (ATAGO.CO., LTD., Tokyo, Japan) with 0 to 30⁰⁰ Brix range by following the method described by Bayleyegn [8]. Ion leakage (%) of lily

flowers was measured on the last day of the experiment of each replicate by silica powder, and the percentage was determined. The percentage of termination of each symptom was recorded as present or not [3].

2.3.2. Biochemical Traits

The total phenolic content (TPC) (mg GAE g^{-1} FW) was measured by using the Folin–Ciocalteu colorimetric method, and gallic acid was used as the standard [9]. The antioxidant activities of some enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were determined at the end of the vase life of the stems. The activity of CAT (U g^{-1} protein) and POD (U g^{-1} protein) were measured by using the process of Liu et al. [10] with changing the quantity of the enzyme extract, and the activity assay of SOD (U g^{-1} protein) was done by the method of Stagner and Popovic [11]. The total soluble sugar content (mg g^{-1} FW) of the flowers was measured by the anthrone method [12]. Quantification of the total carbohydrate content (mg g^{-1} FW) was done by the Somogy method, with little alterations in amount of sample (flower) and concentration of iodine reagent and adaptations performed by Schopfer [13]. The total sugars (reducing and nonreducing sugars) (mg g^{-1} FW), such as glucose and sucrose, respectively, were measured with little alterations in the amount of sugar extracts by the Arrom and Munné-Bosch method [14]. The chlorophyll content (SPAD value) was determined by the method of Mangave [15]. The Bradford method with some modifications (by increasing the amount of protein reagent) was used for the determination of soluble protein (mg g^{-1} FW) [16]. All the biochemical traits were determined at the end of the vase life of the stems of each treatment with three biological replicates.

2.4. Statistical Analysis

Experiments were arranged according to factorial arrangement under completely randomized design (CRD) with three replications having three stems each [17], followed by Tukey's test. Values of $p \leq 0.05$ were taken as significant. The principal component analysis (PCA) was done thorough XL Stat 2020 (Addinsoft-2020. XLSTAT statistical and data analysis solution, New York, NY, USA).

3. Results

3.1. Comparative Analysis of Vase Solutions

All the vase solutions enhanced the vase life of lily flowers almost double as compared to the control; however, the longest vase life (17.6 days) was recorded with SA 300 mg L^{-1} with 5% pulsing solution in “Zambesi”, which was statistically similar with “Sorbonne”. The change in the fresh weight of flowers was increased with all the vase solutions compared to the control, and the maximum change in fresh weight (5.6 and 5.0 g) was recorded with SA 300 mg L^{-1} with 5% pulsing solution in “Zambesi” and “Caesars”, respectively, while the minimum change (2.1 g) was recorded with CA 300 mg L^{-1} with 5% pulsing solution in “Caesars”, except for the control. The dry weight of all the cut flowers was increased, and the maximum increase in dry weight was recorded with SA 300 mg L^{-1} with 5% pulsing solution in “Zambesi”. The maximum solution uptake was recorded with SA 300 mg L^{-1} with 5% pulsing solution, which was statistically similar with GA₃ 100 mg L^{-1} for “Zambesi” (Table 1).

The vase solution of SA 300 mg L^{-1} with 5% pulsing increased the flower diameters by 21, 14, and 18 cm for “Zambesi”, “Sorbonne”, and “Caesars” cut flowers, respectively. The entire vase solutions reduced the ion leakage in all the cut flowers compared to the control; the minimum ion leakage was recorded with SA 300 mg L^{-1} , GA₃, and clove oil 200 mg L^{-1} with 5% pulsing solution, and the maximum ion leakage was observed in distilled water (control). The vase solution of SA 300 mg L^{-1} with 5% pulsing caused a minimum petal death in all the lily cultivars. The control solution had the maximum (100%) necrosis rate (Table 2).

Table 1. Comparative analysis of the effects of different vase solutions on the vase life, stem fresh weight, stem dry weight, and solution uptake of three cultivars of cut lily flowers.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"	Means	"Zambesi"	"Sorbonne"	"Caesars"	Means
Vase Life (Days)					Change in Fresh Weight (g)			
Control (distilled water)	7.0 f	5.3 g	5.0 g	5.7 D	1.8 f	1.0 g	0.5 h	1.1 D
GA ₃ 100 mg L ⁻¹	14.0 d	14.1 d	14.0 d	14.0 C	4.3 b	3.6 c	3.0 d	3.5 B
CA 300 mg L ⁻¹	14.3 c	15.0 c	12.6 e	14.0 C	2.5 e	2.4 e	2.1 f	2.3 C
SA 300 mg L ⁻¹	17.6 a	16.6 a	15.6 b	16.6 A	5.6 a	5.0 a	4.6 b	5.0 A
Clove oil 200 mg L ⁻¹	15.6 b	15.1 c	14.1 d	15.0 B	4.5 b	3.6 b	3.5 c	3.8 B
Means	13.7 A	13.2 A	12.3 B		3.7 A	3.1 A	2.7 B	
Dry weight (g)					Solution uptake (mL)			
Control (distilled water)	9.0 d	7.0 g	5.0 h	7.0 D	87.0 f	85.6 f	90.0 f	87.7 D
GA ₃ 100 mg L ⁻¹	11.0 b	9.0 d	7.0 g	9.0 C	251.6 a	227.0 d	230.0 d	239.3 B
CA 300 mg L ⁻¹	11.0 b	9.8 c	8.0 f	9.6 B	243.0 d	190.3 e	185.0 e	236.6 C
SA 300 mg L ⁻¹	14.0 a	12.0 b	10.0 c	12.0 A	255.3 a	247.0 b	243.0 c	248.4 A
Clove oil 200 mg L ⁻¹	12.3 b	10.3 c	8.6 e	10.4 B	247.5 b	243.0 c	228.1 d	239.0 B
Means	11.5 A	9.6 B	7.7 C		216.8 A	198.5 B	195.5 B	
Significance	Vase life		Change in Fresh weight		Dry weight		Solution uptake	
Treatment (T)	<0.0001		<0.0001		<0.0001		<0.0001	
Cultivars (C)	<0.0001		<0.0001		<0.0001		<0.0001	
T vs. C	<0.0001		<0.0001		<0.0001		<0.0001	

Capital letters showed main effects, while small letters showed interaction effects.

Table 2. Comparative analysis of the effects of different vase solutions on the flower diameter, ion leakage of petals, termination symptoms of flowers, and total soluble solid of petals of three cultivars of cut lily flowers.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"	Means	"Zambesi"	"Sorbonne"	"Caesars"	Means
Flower Diameter (cm)					Ion Leakage of Petals (%)			
Control (distilled water)	17.3 c	10.0 i	11.3 h	12.8 D	176.3 a	175.3 a	173.6 a	175.1A
GA ₃ 100 mg L ⁻¹	15.3 e	12.8 g	15.0 e	14.3 C	138.6 b	136.5 c	136.1 c	137.1 B
CA 300 mg L ⁻¹	17.5 b	13.0 g	16.5 d	15.6 B	141.3 b	139.3 b	139.0 b	139.8 B
SA 300 mg L ⁻¹	21.1 a	14.5 e	18.5 b	18.0 A	135.3 c	134.0 c	130.6 c	133.3 C
Clove oil 200 mg L ⁻¹	17.6 b	13.5 f	16.7 c	15.9 B	135.5 c	135.1 c	135.6 c	135.4 C
Means	17.8 A	15.6 B	12.7 C		145.4 A	144.0 A	142.9 B	
Termination symptoms of flowers (%)					Total soluble solid of petals (Brix)			
Control (distilled water)	100 a	100 a	100.0 a	100 A	0.4 f	0.4 f	0.4 f	0.40 D
GA ₃ 100 mg L ⁻¹	55.3 g	67.3 b	65.0 b	62.5 B	1.8 b	1.3 e	1.5 d	1.53 C
CA 300 mg L ⁻¹	44.0 h	63.0 c	59.6 e	55.5 C	1.8 a	1.3 e	1.6 c	1.56 B
SA 300 mg L ⁻¹	37.6 i	60.0 e	54.3 g	50.6 D	1.9 a	1.3 e	1.6 c	1.60 A
Clove oil 200 mg L ⁻¹	42.0 h	62.0 d	58.6 f	54.2 C	1.8 a	1.3 e	1.6 c	1.56 B
Means	55.8 C	70.4 A	67.5 B		1.6 A	1.1 C	1.3 B	
Significance	Flower diameter	Ion leakage of petals		Termination symptoms of flowers		Total soluble solid of petals		
Treatment (T)	<0.0001	<0.0001		<0.0001		<0.0001		
Cultivars (C)	<0.0001	<0.0001		<0.0001		<0.0001		
T vs. C	<0.0001	<0.0001		<0.0001		<0.0001		

Capital letters showed main effects, while small letters showed interaction effects.

All the tested vase solutions enhanced the TSS level in all the lily cultivars, but the maximum enhancement was observed in SA 300 mg L⁻¹ with 5% pulsing solution. Among the cultivars, the maximum TSS was recorded in “Zambesi” compared to the other cultivars (Table 2). Similarly, the change in EC was same with all the vase solutions, except the control. In the case of the cultivars, the change in EC was in the order of 0.2, 0.3, and 0.3 dS m⁻¹ for “Zambesi”, “Sorbonne”, and “Caesars”, respectively. The maximum change in pH was recorded with SA 300 mg L⁻¹ with 5% pulsing solution compared to the other treatments. The cultivars did not differ regarding pH. All the vase solutions were very effective in reducing the bacterial count compared to the control, and the minimum bacterial count was recorded with SA 300 mg L⁻¹ with 5% pulsing solution. Clove oil 200 mg L⁻¹ with 5% pulsing vase solution increased the chlorophyll content in all the lily cultivars viz. 3.2 in “Zambesi”, 2.7 in “Sorbonne”, and 2.3 in “Caesars” (Table 3). The GA₃ with 5% pulsing had the least chlorophyll content in cut lily flowers.

The highest protein content (5.8 mg g⁻¹ FW) was recorded in all the cut flowers treated with clove oil 200 mg L⁻¹ with 5% pulsing solution, followed by SA 300 mg L⁻¹ and GA₃ 100 mg L⁻¹ with 5% pulse solutions. The vase solution with SA 300 mg L⁻¹ with 5% pulsing enhanced the peroxidase assay (51.0-U g⁻¹ protein) compared to the other vase solutions. Among the cultivars, the maximum peroxidase activity was recorded in “Zambesi” (Table 4). The lowest peroxidase activity was observed with GA₃ 100 mg L⁻¹ with 5% pulsing solution in all the tested cultivars. The maximum catalase activity was recorded with SA 300 mg L⁻¹ with 5% pulse solution compared to all other vase solutions. Among the tested cultivars, the maximum catalase activity was in “Zambesi”. Similarly, the maximum superoxide dismutase activity (41.6-U g⁻¹ protein) was recorded with SA 300 mg L⁻¹ with 5% pulse solution in all the cultivars. Among the cultivars, “Zambesi” had the maximum superoxide dismutase activity (Table 4).

The different vase solutions had similar effects on the total sugar contents compared to the control. Among the cultivars, “Zambesi” and “Sorbonne” had more total sugar contents than “Caesars”. Similarly, the different vase solutions had the same effects on the reducing and nonreducing sugars compared to the control. The maximum total phenolic contents were recorded with SA 300 mg L⁻¹ with 5% pulse solution compared to the other treatments (Table 5). All the vase solutions had the same effects on the total carbohydrate contents in petals. Among the cultivars, the maximum total carbohydrate contents were recorded in “Zambesi” compared to the other cultivars. The vase solution with SA 300 mg L⁻¹ with 5% pulsing had the maximum improvement in anthocyanin contents. Among the cultivars, the maximum anthocyanin contents were recorded in “Sorbonne” and “Caesars” (Table 6).

Table 3. Comparative analysis of the effects of different vase solutions on the change in electrical conductivity of the solutions, change in pH of the solutions, bacterial counts in the vase solutions, and chlorophyll contents of the petals of three cultivars of cut lily flowers.

Treatments	“Zambesi”	“Sorbonne”	“Caesars”		“Zambesi”	“Sorbonne”	“Caesars”	
	Change in Electrical Conductivity of Solutions (dS m ⁻¹)			Means	Change in pH of Solutions			Means
Control (distilled water)	−0.8 c	−0.7 c	−0.6 c	−0.7 B	0.8 b	0.8 c	0.6 c	0.7 B
GA ₃ 100 mg L ⁻¹	0.5 a	0.6 a	0.5 a	0.5 A	0.7 c	0.6 c	0.7 c	0.7 B
CA 300 mg L ⁻¹	0.5 a	0.6 a	0.5 a	0.5 A	0.7 c	0.7 c	0.6 c	0.7 B
SA 300 mg L ⁻¹	0.4 b	0.5 a	0.5 a	0.5 A	1.8 a	1.7 a	1.3 a	1.6 A
Clove oil 200 mg L ⁻¹	0.5 a	0.6 a	0.5 a	0.5 A	0.8 c	0.8 b	0.8 b	0.8 B
Means	0.2 B	0.3 A	0.3 A		1.0 A	0.9 A	0.8 A	
Bacterial counts in the vase solutions					Chlorophyll contents (SPAD reading)			
Control (distilled water)	2.41 × 10 ⁸ b	2.44 × 10 ⁸ a	2.47 × 10 ⁸ a	2.45 × 10 ⁸ A	1.4 c	1.3 c	1.1 c	1.2 D
GA ₃ 100 mg L ⁻¹	2.05 × 10 ⁸ e	2.15 × 10 ⁸ d	2.13 × 10 ⁸ d	2.11 × 10 ⁸ B	2.2 c	1.8 cd	1.5 c	1.8 C
CA 300 mg L ⁻¹	2.05 × 10 ⁸ e	2.05 × 10 ⁸ e	1.99 × 10 ⁸ f	2.02 × 10 ⁸ C	2.6 b	2.2 c	1.9 cd	2.1 B
SA 300 mg L ⁻¹	1.95 × 10 ⁸ f	2.01 × 10 ⁸ e	2.05 × 10 ⁸ e	1.99 × 10 ⁸ D	2.8 b	2.4 b,c	2.1 c	2.3 B
Clove oil 200 mg L ⁻¹	2.05 × 10 ⁸ e	2.25 × 10 ⁸ c	2.05 × 10 ⁸ e	2.11 × 10 ⁸ B	3.2 a	2.7 b	2.3 b,c	2.7 A
Means	2.10 × 10 ⁸	2.18 × 10 ⁸	2.13 × 10 ⁸		2.4 A	2.0 B	1.7 C	
Significance	Change in electrical conductivity of solutions			Change in pH of solutions	Bacterial counts in the vase solutions		Chlorophyll contents	
Treatment (T)	<0.0001			<0.0001	<0.0001		<0.0001	
Cultivars (C)	<0.0001			<0.0001	Ns		<0.0001	
T vs. C	<0.0001			<0.0001	<0.0001		<0.0001	

Capital letters showed main effects, while small letters showed interaction effects.

Table 4. Comparative analysis of the effects of different vase solutions on the total soluble proteins, peroxidase assay, catalase assay, and superoxide dismutase assay of three cultivars of cut lily flowers.

Treatments	“Zambesi”	“Sorbonne”	“Caesars”		“Zambesi”	“Sorbonne”	“Caesars”	
	Total Soluble Proteins (mg g ⁻¹ FW)			Means	Peroxidase Assay (U g ⁻¹ Protein)			Means
Control (distilled water)	3.5 d	3.2 d	3.1 d	3.2 D	16.6 i	15.3 i	14.6 j	15.5 E
GA ₃ 100 mg L ⁻¹	4.1 c	4.3 c	4.1 c	5.0 B	53.0 c	38.0 h	44.0 f	45.0 D

Table 4. Cont.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"		"Zambesi"	"Sorbonne"	"Caesars"	
	Total Soluble Proteins (mg g ⁻¹ FW)			Means	Peroxidase Assay (U g ⁻¹ Protein)			Means
CA 300 mg L ⁻¹	5.6 a	5.0 b	4.9 b	4.1 C	56.0 b	41.0 g	47.0 e	48.0 C
SA 300 mg L ⁻¹	5.8 a	5.1 b	5.0 b	5.3 B	59.0 a	44.0 f	50.0 d	51.0 A
Clove oil 200 mg L ⁻¹	6.1 a	5.8 a	5.7 a	5.8 A	57.0 b	42.0 g	48.0 e	49.0 B
Means	5.0 A	4.6 B	4.5 B		48.3 A	36.0 C	40.7 B	
	Catalase assay (U g ⁻¹ protein)				Superoxide dismutase assay (U g ⁻¹ protein)			
Control (distilled water)	10.6 j	9.3 j	8.6 k	9.5 E	12.6 i	12.3 i	12.0 i	12.3 E
GA ₃ 100 mg L ⁻¹	37.0 c	26.0 i	28.0 h	30.3 D	42.0 c	33.0 h	32.0 h	35.6 D
CA 300 mg L ⁻¹	40.0 b	29.0 g	31.0 e	33.3 C	45.0 b	36.0 f	35.0 g	38.6 C
SA 300 mg L ⁻¹	43.0 a	32.0 e	34.0 d	36.3 A	48.0 a	39.0 d	38.0 d	41.6 A
Clove oil 200 mg L ⁻¹	41.0 b	30.0 f	32.0 e	34.3 B	46.0 b	37.0 e	36.0 f	39.6 B
Means	34.3 A	25.2 C	26.7 B		38.7 A	31.4 B	30.6 B	
Significance	Total Soluble Proteins		Peroxidase assay		Catalase assay		Superoxide dismutase assay	
Treatment (T)	<0.0001		<0.0001		<0.0001		<0.0001	
Cultivars (C)	<0.0001		<0.0001		<0.0001		<0.0001	
T vs. C	<0.0001		<0.0001		<0.0001		<0.0001	

Capital letters showed main effects, while small letters showed interaction effects.

Table 5. Comparative analysis of the effects of different vase solutions on the total sugar, reducing sugar, nonreducing sugar, and total phenol of three cultivars of cut lily flowers.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"		"Zambesi"	"Sorbonne"	"Caesars"	
	Total Sugar (mg g ⁻¹ FW)			Means	Reducing Sugar (mg g ⁻¹ FW)			Means
Control (distilled water)	1.74	1.78	1.54	1.69 b	1.09	1.07	0.83	1.00 b
GA ₃ 100 mg L ⁻¹	2.14	2.14	2.04	2.10 a	1.29	1.23	1.12	1.21 a
CA 300 mg L ⁻¹	2.23	2.08	2.01	2.12 a	1.33	1.20	1.20	1.24 a
SA 300 mg L ⁻¹	1.98	1.98	1.88	1.95 a	1.21	1.15	1.14	1.27 a
Clove oil 200 mg L ⁻¹	2.03	1.99	1.83	1.91 a	1.3	1.1	1.12	1.20 a
Means	2.02 a	2.0 a	1.87 b		1.07	1.16	1.23	

Table 5. Cont.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"	Means	"Zambesi"	"Sorbonne"	"Caesars"	Means
	Total Sugar (mg g ⁻¹ FW)				Reducing Sugar (mg g ⁻¹ FW)			
	Nonreducing Sugar (mg g ⁻¹ FW)				Total phenol (mg GAE g ⁻¹ FW)			
Control (distilled water)	0.64	0.70	0.71	0.68 c	0.48 b	0.48 c	0.46 c	0.47 B
GA ₃ 100 mg L ⁻¹	0.84	0.90	0.91	0.88 a	0.76 c	0.56 c	0.66 c	0.56 B
CA 300 mg L ⁻¹	0.89	0.88	0.80	0.86 a	0.77 c	0.78 c	0.68 c	0.7 B
SA 300 mg L ⁻¹	0.77	0.83	0.74	0.78 b,c	0.96 a	0.94 a	0.93 a	0.90 A
Clove oil 200 mg L ⁻¹	0.73	0.89	0.71	0.77 b,c	0.85 b	0.83 b	0.80 b	0.81 B
Means	0.79	0.83	0.79		0.76	0.81	0.79	
Significance	Total sugar		Reducing sugar		Nonreducing Sugar		Total phenol	
Treatment (T)	<0.0001		<0.0001		<0.0001		<0.0001	
Cultivars (C)	<0.0001		Ns		Ns		Ns	
T vs. C	Ns		Ns		Ns		<0.0001	

Capital letters showed main effects, while small letters showed interaction effects.

Table 6. Comparative analysis of the effects of different vase solutions on the total carbohydrate contents in petals and anthocyanin contents (%) of three cultivars of cut lily flowers.

Treatments	"Zambesi"	"Sorbonne"	"Caesars"	Means	"Zambesi"	"Sorbonne"	"Caesars"	Means
	Total Carbohydrate Content (mg g ⁻¹ FW)				Anthocyanin Contents (%)			
Control (distilled water)	0.87	0.71	0.83	0.79 B	55.0 h	75.3 g	77.0 f	5.7 D
GA ₃ 100 mg L ⁻¹	1.45	1.31	1.39	1.34 A	115.0 e	116.1 d	144.0 c	14.0 C
CA 300 mg L ⁻¹	1.49	1.41	1.45	1.43 A	113.6 e	165.0 b	194.3 a	114.0 C
SA 300 mg L ⁻¹	1.5	1.42	1.46	1.42 A	136.0 c	177.0 b	198.0 a	156.6 A
Clove oil 200 mg L ⁻¹	1.46	1.40	1.34	1.39 A	124.1 d	145.1 c	156.0 b	135.0 B
Means	1.4 A	1.32 B	1.36 B		112.3 B	133.2 A	144.0 A	
Significance	Total carbohydrate content				Anthocyanin contents			
Treatment (T)	<0.0001				<0.0001			
Cultivars (C)	<0.0001				<0.0001			
T vs. C	Ns				<0.0001			

Capital letters showed main effects, while small letters showed interaction effects.

3.2. Principal Component Analysis (PCA)

The PCA of different biochemical and physiological traits on the vase lives of cut flowers is presented in Figure 1. As a result of PCA analysis, all physiological parameter variations were accounted in the first two factors, explaining 97.6% of the variation. The first PC had 40.9% variability, with the maximum variation by solution uptake, dry weight, and ion leakage. The change in fresh weight and dry weight positively correlated with principal component 1 (PC1), while the change in pH was negatively correlated. The PCA based on the physiological parameters of lily cut flowers showed that the vase life solution uptakes and flower qualities were closely linked but diverse with the ion leakage of cut flowers (Figure 1a). Regarding the biochemical traits, all the biochemical parameter variations were accounted for in first two factors with 95.3% variation. The first PC had 83.7% variability, with the maximum variation by TSS, SOD, POD, CAT, and Total soluble protein (TSP). The PCA based on the biochemical parameters of lily cut flowers showed that the vase life and antioxidant were closely linked but only a little diverse with the total protein and chlorophyll contents (Figure 1b).

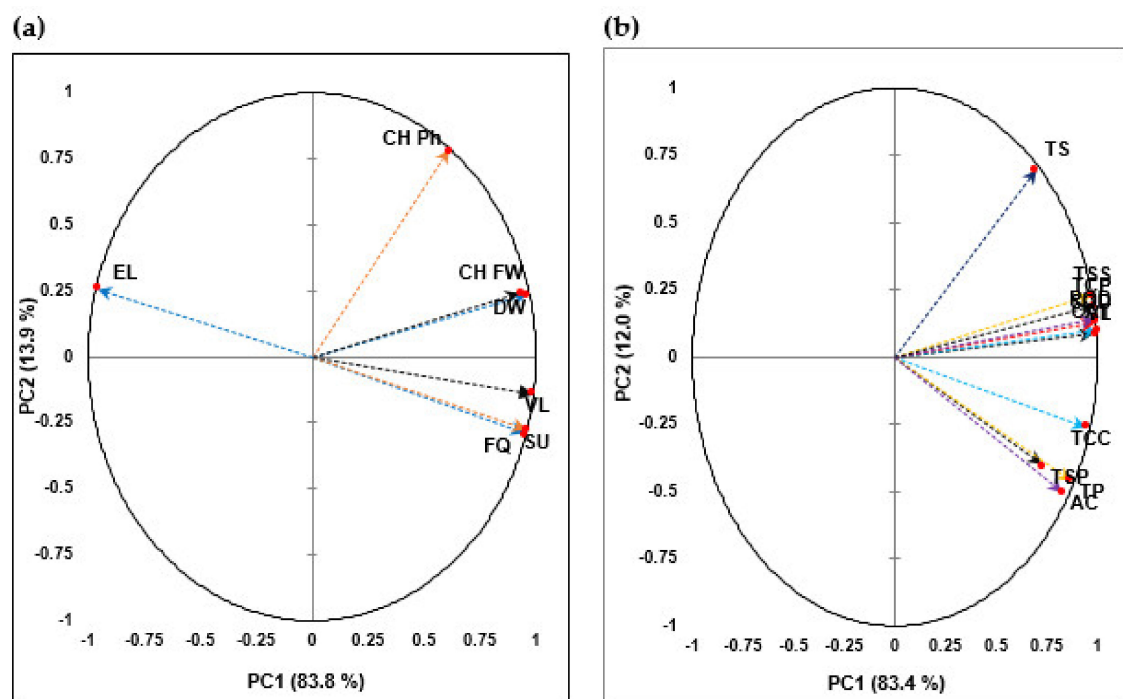


Figure 1. Principal component analysis of (a) the vase life and physiological parameters and (b) vase life and biochemical parameters: (a) (vase life (VL), change in fresh weight (CH FW), dry weight (DW), flower quality (FQ), and solution uptake (SU) and (b) total sugar (TS), total chlorophyll content (TCC), total soluble protein (TSP), total phenolic (TP), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD). PC1 and PC2 mean principal component 1 and 2.

4. Discussion

The findings of the present comparative study showed that the use of different preservative solutions (gibberellic acid, salicylic acid, citric acid, essential oil, and pulsing sucrose) substantially improved the vase life, fresh weight, and dry weight of cut lily flowers. The increase in vase life, quality of flower, and fresh and dry weights attributed to role of SA in maintaining the membrane stability, antioxidant enzymes, and delay in the senescence of petals of the flowers reduced the ion leakage and lipid peroxidation [18]. Moreover, the improvement was owed to the improved water absorption [19,20], as the higher water contents in xylem indicated that the cut flower was alive with a healthier growth, along with cell tissues as functional [21]. Furthermore, the sucrose application, along

with SA, provoked the effect of abscisic acid (which promoted the senescence) and increased the floret longevity, resulting in an increased vase life and fresh weight [21,22].

The improvement in solution uptake at lower concentrations of GA₃ in lily cut flowers was due to the improved water status both by the increased solution uptake/water and with reduced water loss due to a lower transpiration rate [23]. The final symptom, the wilting/dropping of petals of flowers, is due to an imbalance of solution uptake by the flower stem and water lost from the leaves and other organs through transpiration [24]. The use of CA improved the water balance by reducing the stem plugging, increasing the water uptake and longevity of flowers by lowering the pH that inhibits microorganism growth, resulting in a longer vase life, increased fresh weight, and improved solution uptake [25]. The improved growth, vase life, quality of flowers, and fresh weight are attributed to the positive effects of CA on the solution uptake and maintenance of the water balance [25]. Moreover, the application of different preservative solutions decreased the pH (Table 3), as a decrease in pH inhibits the ethylene production, growth, and proliferation of bacteria and pathogens and increases the vase life [26]. The synthesis of ethylene at high concentrations adversely affects the flower longevity and enhances the senescence rate [27].

According to the present results, it was observed that the plant growth regulators increased the activity of the SOD enzyme in lily cut flowers, as the application of SA inhibited the activity of polyphenol oxidase and enhanced the total phenolics, flavonoids, and enzymatic antioxidant activities [28,29]. The gibberellic acid regulated the expression of the genes involved in the activity of SOD [28]. The results of the present experiment showed that, with a lesser amount of gibberellic acid, the activity of POD was higher, while with more gibberellic acid, the activity of POD was less. Maybe this effect is due to peroxidase catalyzation, as discussed by Schopfer [13], and is important in the plant defense system. The higher activity of CAT was also retained by gibberellic acid in comparison to the control in the case of stress in cut flowers. The activity of CAT also increased the vase life of lily cut flowers by reactive oxygen species (ROS) scavenging, as reported by Ezhilmathi [30]. The response induced by gibberellic acid in stress tolerance promoted the activity of antioxidants in plants, which resulted in the scavenging capacity of radicals to deal with oxidative stress.

The GA₃ reduced the biosynthesis of ROS by hindering the processes of cellular membrane degradation and increased the potential of the antioxidants [31]. The GA₃ recommended for senescence delayed and increased the prospective of antioxidants by two ways: one by inhibiting the free radical or excess accumulation of ROS, and other was by ROS-independent pathways that were unidentified as recommended [31]. The activities of POD and CAT showed the steadying effects on the membrane system and lipid bilayers [12,15]. The oxidative stress can be eliminated from cut flowers by scavenging the activity of the ROS and ion leakage, which are reduced by membrane damage through less unsaturated fatty acid oxidation [32]. Similarly, the application of essential oils such as ginger oil enhanced the postharvest quality by decreasing the retardation of chlorophyll (both a and b), lowering the accumulation of malondialdehyde and enhancing the scavenging activities [33].

5. Conclusions

The application of salicylic acid and pulsing sucrose substantially improved the quality and vase lives of cut lily flowers by improving the postharvest and biochemical traits of flowers. The GA₃, citric acid, and clove oil also elongated the vase lives of cut lilies and can be used in prolonging the vase lives of cut flowers for one week. Among the cultivars, “Zambesi” performed best regarding the postharvest and biochemical traits, as compared to “Sorbonne” and “Caesars”.

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References

- Grassotti, A.; Gimelli, F. Bulb and cut flower production in the genus *Lilium*: Current status and the future. *Acta Hort.* **2011**, *900*, 21–35. [\[CrossRef\]](#)
- Neerja, R.; Kumar, R.; Dhatt, K.J.C. Effect of nitrogen level and growing media on growth, flowering and bulb production of *Lilium* cultivars. *J. Ornament. Hort.* **2005**, *8*, 36–40.
- Younis, A.; Zahir, Z.; Riaz, A.; Tariq, U. Microbial inoculation increases the nutrient uptake efficiency for quality production of *Gladiolus grandifloras*. *Pak. J. Agri. Sci.* **2014**, *51*, 875–880.
- Han, S.S. Benzyladenine and Gibberellic acid Improve postharvest quality of cut Asiatic and Oriental lilies. *Hortic. Sci.* **2001**, *36*, 741–745.
- Yonnis, A.; Akhtar, M.S.; Riaz, A.; Zulfiqar, F.; Qasim, M.; Farooq, A.; Tariq, U.; Ahsan, M.; Bhatti, Z.M. Improved cut flower and corm production by exogenous *Moringa* leaf extract application on gladiolus cultivars. *Acta Sci. Pol. Hortorum. Cultus* **2018**, *17*, 25–38. [\[CrossRef\]](#)
- Anjum, M.A.; Naveed, F.; Shakeel, F.; Amin, S. Effect of some chemicals on keeping quality and vase life of tuberose (*Polianthes tuberosa* L.) cut flowers. *J. Res. Sci.* **2001**, *2*, 1–7.
- Ali, S.; Charles, T.C.; Glick, B.R. Delay of carnation flower senescence by bacterial endophytes expressing ACC deaminase. *J. Appl. Microbiol.* **2012**, *113*, 1139–1144. [\[CrossRef\]](#)
- Bayleyegn, A.; Tesfaye, B.; Workneh, T.B. Effects of pulsing solution, packaging material and passive refrigeration storage system on vase life and quality of cut rose flowers. *Afr. J. Biotechnol.* **2012**, *11*, 3800–3809. [\[CrossRef\]](#)
- Liang, Z.X.; Zhang, J.Z.; Sun, M.Y.; Zhang, Y.L.; Zhang, X.H.; Li, H.; Shi, L. Variation of Phenolic Compounds and Antioxidant Capacities in Different Organs of *Lilium pumilum*. *Nat. Prod. Commun.* **2018**, *13*, 1934578X1801300616. [\[CrossRef\]](#)
- Liu, D.; Zou, J.; Meng, Q.; Zou, J.; Jiang, W. Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity. *Ecotoxicology* **2009**, *18*, 134–143. [\[CrossRef\]](#)
- Stagner, D.; Popovic, B.M. Comparative study of antioxidant capacity in organs of different *Allium* species. *Open Life Sci.* **2009**, *4*, 224–228. [\[CrossRef\]](#)
- Dhindsa, R.S.; Plumb-Dhindsa, D.; Thorpe, T.A. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [\[CrossRef\]](#)
- Schopfer, P.; Plachy, C.; Frahry, G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol.* **2001**, *125*, 1591–1602. [\[CrossRef\]](#)
- Arrom, L.; Munné-Bosch, S. Sucrose accelerates flower opening and delays senescence through a hormonal effect in cut lily flowers. *Plant Sci.* **2012**, *188*, 41–47. [\[CrossRef\]](#) [\[PubMed\]](#)
- Mangave, B.D.; Singh, A.; Mahatma, M.K. Effects of different plant growth regulators and chemicals spray on post harvest physiology and vase life of heliconia inflorescence cv. Golden Torch. *Plant Growth Regul.* **2013**, *69*, 259–264. [\[CrossRef\]](#)
- Rabiza-Świder, J.; Skutnik, E.; Jędrzejuk, A.; Ratuszek, M. Effect of postharvest treatments on the longevity of cut inflorescences of ‘Rialto’ oriental lily. *Folia Hort.* **2015**, *27*, 161–168. [\[CrossRef\]](#)
- Steel, R.G.D.; Torrie, J.H.; Dicky, D.A. *Principles and Procedures of Statistics: A Biological Approach*, 3rd ed.; McGraw Hill Book Co.: New York, NY, USA, 1997.
- Hatamzadeh, A.; Hatami, M.; Ghasemnezhad, M. Efficiency of salicylic acid delay petal senescence and extended quality of cut spikes of *Gladiolus grandifloral* cv wing sensation. *Afr. J. Agric. Res.* **2012**, *7*, 540–545.
- Bayat, H.; Aminifard, M.H. Salicylic acid treatment extends the vase life of five commercial cut flowers. *Electron. J. Biol.* **2017**, *13*, 67–72.
- Zamani, S.; Hadavi, E.; Kazemi, M.; Hekmati, J. Effects of some chemical treatments on keeping of chrysanthemum cut flowers. *World Appl. Sci. J.* **2011**, *12*, 1962–1966.

21. Seman, H.H.A.; Rafdi, H.H.M. Effects of salicylic acid and sucrose solution on vase life of cut antigonon leptopus inflorescences and their potential as cut flowers for flower arrangement. *Univ. Malays. Teren. J. Undergrad. Res.* **2019**, *1*, 81–91.
22. Salehi Sardoei, A.; Shahdadneghad, M. Interaction effect of sucrose and salicylic acid on vase life of cut *Gerbera* flowers. *Int. J. Biol. Sci.* **2014**, *1*, 60–65.
23. Goszczynska, D.M.; Zieslin, N.; Mor, Y.; Halevy, A.H. Improvement of postharvest keeping quality of Mercedes roses by gibberellin. *Plant Growth Regul.* **1990**, *9*, 293–303. [[CrossRef](#)]
24. Van Meeteren, U.; Van Gelder, H. Effect of time since harvest and handling condition on rehydration ability of cat chrysanthemum flowers. *Postharvest Biol. Technol.* **1999**, *16*, 169–177. [[CrossRef](#)]
25. Patil, S.R.; Reddy, B.S. Effect of Citric Acid and Sucrose on Post Harvest Water Relations, Fresh Weight and Vase Life of Golden Rod (*Solidago canadensis* L.). *Karnataka J. Agric. Sci.* **2001**, *14*, 427–430.
26. Srivastava, M.K.; Dwivedi, U.N. Delayed Ripening of Banana Fruit By Salicylic Acid. *Plant Sci.* **2000**, *158*, 87–96. [[CrossRef](#)]
27. Marandi, R.J.; Hassani, A.; Abdollahi, A.; Hanafi, S. Improvement of the vase life of cut gladiolus flowers by essential oils, salicylic acid and silver thiosulfate. *J. Med. Plants Res.* **2011**, *5*, 5039–5043.
28. Shabanian, S.; Esfahani, M.N.; Karamian, R.; Tran, L.S.P. Salicylic acid modulates cutting-induced physiological and biochemical responses to delay senescence in two gerbera cultivars. *Plant Growth Regul.* **2019**, *87*, 245–256. [[CrossRef](#)]
29. Zhang, S. Investigations into Senescence and Oxidative Metabolism in Gentian and Petunia Flowers. Ph.D. Thesis, University of Canterbury, Christchurch, New Zealand, 2008; pp. 111–112.
30. Ezhilmathi, E.; Singh, V.P.; Arora, A.; Sairam, R.K. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of gladiolus cut flower. *Plant Growth Regul.* **2007**, *51*, 99–108. [[CrossRef](#)]
31. Rosenwasser, S.; Belausov, E.; Riov, J.; Vered, H.; Friedman, H. Gibberellic acid (GA3) inhibits ROS increase in chloroplasts during dark-induced senescence of pelargonium cuttings. *J. Plant Growth Regul.* **2010**, *29*, 375–384. [[CrossRef](#)]
32. Scandalios, J.G. Oxygen stress and superoxide dismutase. *Plant Physiol.* **1993**, *101*, 7–12. [[CrossRef](#)]
33. Teerarak, M.; Laosinwattana, C. Essential oil from ginger as a novel agent in delaying senescence of cut fronds of the fern (*Davallia solida* (G. Forst.) Sw.). *Postharvest Biol. Technol.* **2019**, *156*, 110927. [[CrossRef](#)]

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