The Longevity and Quality of Tuberose (*Polianthes tuberosa* L.) Cut Flowers as Affected by Ethanol, Methanol, Citric Acid and Silver Thiosulphate

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ABSTRACT: This investigation was conducted at Antoniades Research Branch, Horticulture Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons of 2018 and 2019 to elucidate the effects of ethanol, methanol, citric acid and silver thiosulphate on the longevity and quality of Tuberose (*Polianthes tuberosa* L.) cut flowers. Nine treatments were used in this investigation (control, ethanol at both 2 and 4 %, methanol at both 2 and 4%, citric acid at both 0.2 and 0.4 g / L were used as pulsing treatments for 24 hours and silver thiosulphate (STS) at 0.1 and 0.2 mg/L were used as pulsing treatment for one hour). The gained results exhibited that all treatments ; caused significant ($P \leq 0.05$) increase in the vase life *of P. tuberosa* cut spikes except STS treatments as compared to control treatment. The application of any ethanol treatments caused the highest significant ($P \leq 0.05$) increase in vase life with no significant difference between them. Moreover, methanol and ethanol treatments caused observed increase in final water uptake, floret's opening and reducing sugars and decrease in floret's welting percentage.

Key words: vase life , *Polianthes tuberosa* L, ethanol, methanol, citric acid , silver thiosulphate

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is a bulbous flowering plant belongs to the family Asparagaceae (De Hertogh and Le Nard, 1993). It is used in flower arrangement and individual florets give fragrance to bouquets and boutonnieres (Reid,1996). Although tuberose spikes have a high economic value and is exported to Arabian countries .They are highly perishable in nature and need to be treated to improve their vase life and postharvest quality.

Ethanol and methanol increase the vase life of flowers by inhibiting ethylene biosynthesis and act, also, as an antimicrobial compound to prolong vase life of some cut flowers (Wu, *et al.* , 1992).

Citric acid is a weak organic acid, also, has an effect on the flower longevity of cut flowers. Organic acid supplies the cells with carbon and energy, which are used in the respiratory cycle and some other biochemical pathways (Da Silva, 2003). Citric acid minimizes the microorganism's growth in vase solution and increases water conductance in the cut flower xylem (Hell and Stephan, 2003).

Silver thiosulphate (STS) $[Ag(S_2O_3)_2]^{3-}$ is an inorganic biocide used in floriculture industry as a dip, spray or pulse (Altman and Solomos, 1995; Joyce and Beal, 1999). It is used as ethylene inhibitor. The flower treatment by Ag^+ as STS substantially reduces binding activity by substitution for Cu^{++} (Beyer, 1976). Copper

is a part of enzymatic reactions related to biosynthesis and the action of ethylene (Himelblau and Amasino, 2000).

The aim of the present investigation is to explore the effects of ethanol, methanol citric acid and silver thiosulphate, on the longevity and quality of Tuberose (*Polianthes tuberosa* L.) cut flowers.

MATERIALS AND METHODS

This investigation was carried out at Antoniades Research Branch, Horticulture Research Institute, A.R.C. Alexandria, Egypt during two successive seasons of 2018 and 2019.

A-Source of the cut flowers:

Cut flower spikes were obtained from a well-known commercial nursery in Alexandria.

B-Cut flowers preparations:

Flower spike were harvested in the phase of 2-3 opened florets.

C-Chemicals used in the experiment

- Control (distilled water).
- Ethanol at 2 and 4 %.
- Methanol at 2 and 4%.
- Citric acid at 0.2 and 0.4 g / L.
- Silver thiosulphate (STS) at 0.1 and 0.2 mg / L.

Cut flower treatments

On the 27th of August 2018 and 2019 (in the first and second seasons, respectively) cut flowers were transplanted to the laboratory under dry conditions, whereas they were recut before treatments to the length of 60 cm. The flower spikes were pulsed in glass jars containing (sucrose 2% + control, sucrose 2%+ ethanol, sucrose 2%+ methanol and sucrose 2%+ citric acid) for 24 hours while STS treatments were pulsed for one hour then the pulsing treatments were continued to 24 hours in jars containing 2% sucrose.

latter, the flowers were transferred to glass jars containing 400 cm^3 distilled water (three spikes per vase) to supplement their shelf-life period. The experiment was carried out with no spike re-cutting and no changing of vase solution.

The lab conditions: The flowers were remained in the lab at the average temperature of (29.5^{o}C), average humidity (70%-75%) and 24 hours fluorescent light (about 450- 500 lux).

D-Experimental layout and statistical analysis

The experimental layout was completely randomized design (CRD). It consists of nine treatments with three replicates each replicate contains three spikes. The means of the individual factors were compared by L.S.D test at 5% level of probability. The data were statistically analyzed according to the method described by Snedecor and Cochran (1989).

Data were recorded as follows:

1-The postharvest characters

a-Vase life (days) it was defined as the number of days from starting the experiment to the fading stage. The fading stage was set at the wilting of 50% florets of the total florets number (Dole *et al.*, 2005).

b- Loss of spike fresh weight percentage (L.S.F.W. %), it was determined at the fading stage as the flowing formula

$$\text{L.S.F.W.} = \frac{\textit{Initial fresh weight} - \textit{Final fresh weight}}{\textit{Initial fresh weight}} \times 100$$

c- Final water uptake (g), it was calculated at the end of the experiment as the following formula.

Water uptake (g)

=*The* amount of solution at the beginning of the experiment −the amount of the solution remaining at the end of the experiment.

d- Flower fresh weight / flower dry weight ratio (FWR), at the fading stage the flowers were oven dried at 75° C for 48 hours to get the flower dry weight (F.D.W.) Then the fresh weight was divided by the dry weight as below.

$$\text{FWR}=\frac{\text{Fresh weight per flower (g)}}{\text{Dry weight per flower (g)}}$$

e- Vase solution uptake rate (VSU rate), the VSU rate was measured according to the formula below.

$$\text{VSU rate} = \frac{(\text{St}-1) - \text{St}}{\text{IFW of stem}} \times 100$$

Where (St) is weight of vase solution (g) at 29th of August (3 days after the experiment start), 1st of September (5 days after the beginning of experiment) and 2nd of September (6 days after the beginning of the experiment),(St-1) is weight of the vase solution (g) on the previous day and (IFW) is the initial fresh weight (g) .

f- Relative fresh weight (RFW), fresh weight of the spikes was determined just before the immersion of the spikes into the solutions and collected on the 3rd, 5th and 6th day from the experiment start. The fresh weight of each spike was expressed relative to the initial weight to represent the water status of the flower.

$$\text{RFW} = \frac{Wt}{W0} \times 100$$

Where *Wt* is the weight of spike (g) at 29th of August (3days) and 1st of September (5 days) and 2nd of September (6 days) and *W0* is the initial fresh weight of the same spike (g)

2- Florets characters

The percentage of floret's opening, floret's welting and the diameter of opened florets was calculated after the third day of the experiment start.

3- Chemical analysis

- **Chlorophyll a and b content (mg / g fresh weight)** was determined in the 1st and 2nd leaves under inflorescence at the end of the control vase life according to **Moran (1982).**
- **Reducing sugars content (mg/g dry weight)** was determined according to **Miller (1959)** at the end of vase life
- **Total soluble solids (TSS %),** at the 5th day of the experiment start, nine florets of the spike were taken , the sap of the florets was obtained by squashing. A drop of the sap was put on the prism surface and the TSS was measured by a digital Refractometer (model ATAGO).

RESULTS

1-The postharvest characters

Results in Table (1) show that there was no significant different between pulsing the flower spikes by ethanol 2 or 4% and methanol 2 or 4%. All of these treatments; led to the highest significant increase in vase life as compared to control treatment during both seasons. The longest vase life was obtained after pulsing the flower spikes by ethanol 2% which recorded (7.17 and 7.10 days) in first and second seasons, respectively while control treatment recorded (4.33 and 4.00 days) in both seasons . Also, citric treatments gave moderate significant increase in vase life. On the other hand, the highest significant decrease in vase life was obtained after application of STS at 0.1 and 0.2 mg / L during both seasons.

Also, results in Table (1) clear that all used chemical concentrations except STS treatments caused significant decrease in LSFW as compare to control treatment. The highest decrease in LSFW which recorded (11.58 and 14.77 %) during the first and second season, respectively was obtained after application of Citric acid at 0.4 g/l Results of Table (1), also, demonstrate that application of any used concentration of ethanol or methanol treatment resulted in the highest significant increase in final water uptake during both seasons. Moreover, the treatment of ethanol 2% caused the highest increase in final water uptake which recorded (71.63 and 71.31 g/ spike) in the seasons of 2018 and 2019, respectively. On the other hand, applying of STS treatments at any of both concentrations, resulted in the highest significant decrease in final water uptake during both seasons.

Results of Table (1) reveal that all used chemical concentrations except STS treatments caused significant increase in FWR as compared to control treatment. The highest increase in FWR which were (8.38 and 9.2) in the first and second season, respectively was obtained after application of ethanol at 2%.

Table (1). Means of vase life (days), loss of spike fresh weight (LSFW) (%), final water uptake (g) and flower fresh weight/ flower dry weight ratio (FWR) of *Polianthes tuberosa* cut spikes as affected by pulsing treatments with ethanol, methanol, citric acid and STS in the seasons of 2018 and 2019

Treatment	Vase life (days)		Loss of spike fresh weight LSFW (%)		Final water uptake (g)		Flower fresh weight/flower dry weight ratio (FWR)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	4.33c	4.00d	27.29a	26.79a	45.12c	41.64d	6.34b	6.60c
Ethanol at 2%	7.17a	7.10a	15.28c	15.33ed	71.63a	71.31a	8.38a	9.20a
Ethanol at 4%	6.87a	6.64ab	17.90c	14.79e	67.26a	68.85ab	8.29a	8.70ab
Methanol at 2%	6.64a	6.53ab	18.06c	18.28cde	70.29a	61.79abc	8.31a	8.70ab
Methanol at 4%	6.34a	6.30b	16.47c	16.66de	70.53a	66.62ab	8.13a	8.40ab
Citric acid at 0.2g/L	5.33b	5.10c	17.38c	19.90bc	55.30b	55.09c	8.33a	8.30b
Citric acid at 0.4g/L	5.22b	5.00c	11.58d	14.77e	61.00b	60.39b	8.30a	8.20b
STS at 0.1 mg/L	3.00d	3.00e	22.31b	21.89abc	6.28d	6.27e	6.19b	5.70d
STS at 0.2 mg/L	3.00d	3.00e	25.07a	23.94ab	8.47d	8.10e	4.50c	4.90d
L.S.D.at 0.05	0.84	0.64	3.34	4.96	5.80	10.82	1.51	0.82

Means of treatments in the each column that have the same letters, are not significantly different at 5% level

Vase solution uptake rate (VSU %)

Figure (1) clear that on the 3rd day from starting the experimentation in the first and second seasons, STS treatments recorded the lowest VSU value as compared to other treatments , by the end of this day the vase life of these treatments were terminated. Also, Figure (1) show that , before the 5th day, the vase life of control plant was terminated. Moreover, Citric acid treatments recorded the highest VSU values and the highest value was obtained after application of citric acid at 0.4 g/L on the 3rdday from the experiment start. This value decreased sharply on the 5th day and by the end of this day citric acid treatments were terminated. On the other hand, the VSU value of ethanol and methanol treatments recorded slight decrease on the 5th day of the vase life, this value decreased sharply on the 6th day from the experimentation start.

Relative fresh weight RFW (%)

Figure (2) showed that on the 3rd day from the beginning of experimentation the RFW values were more than 100 % for all treatments except STS treatments and control plant .The highest RFW was obtained from application citric acid at 0.4 g/L. Moreover, on the 5th day from initiation the experimentation these values decreased and they were more than 80% for all treatments. On the 6th day from the experimentation stat, application of ethanol at 2% recorded the highest value of RFW.

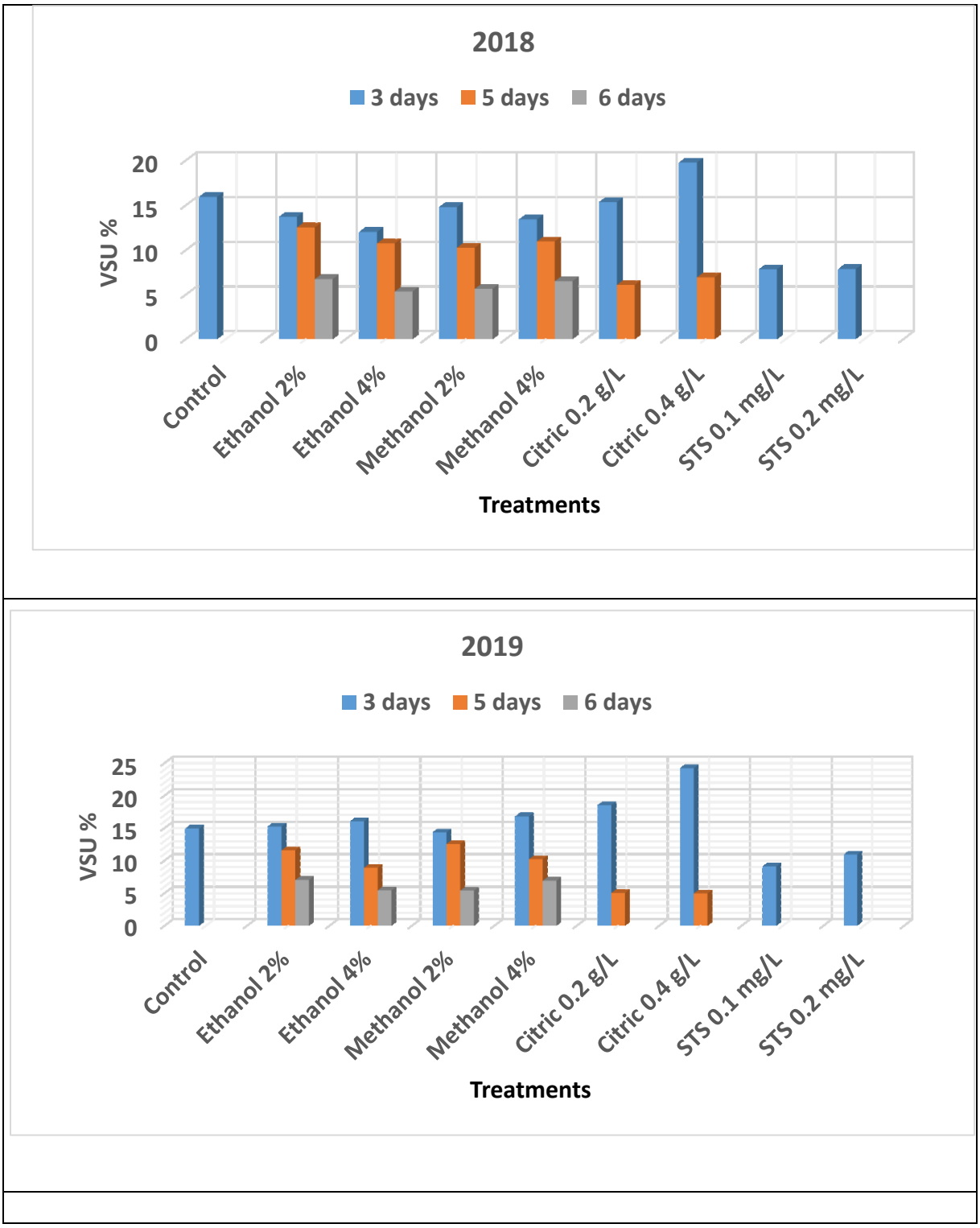

Figure (1). Effect of ethanol, methanol, citric acid and STS concentrations on vase solution uptake rate VSU (%) during the shelf life of *Polianthes tuberosa* cut spikes in the seasons of 2018-2019

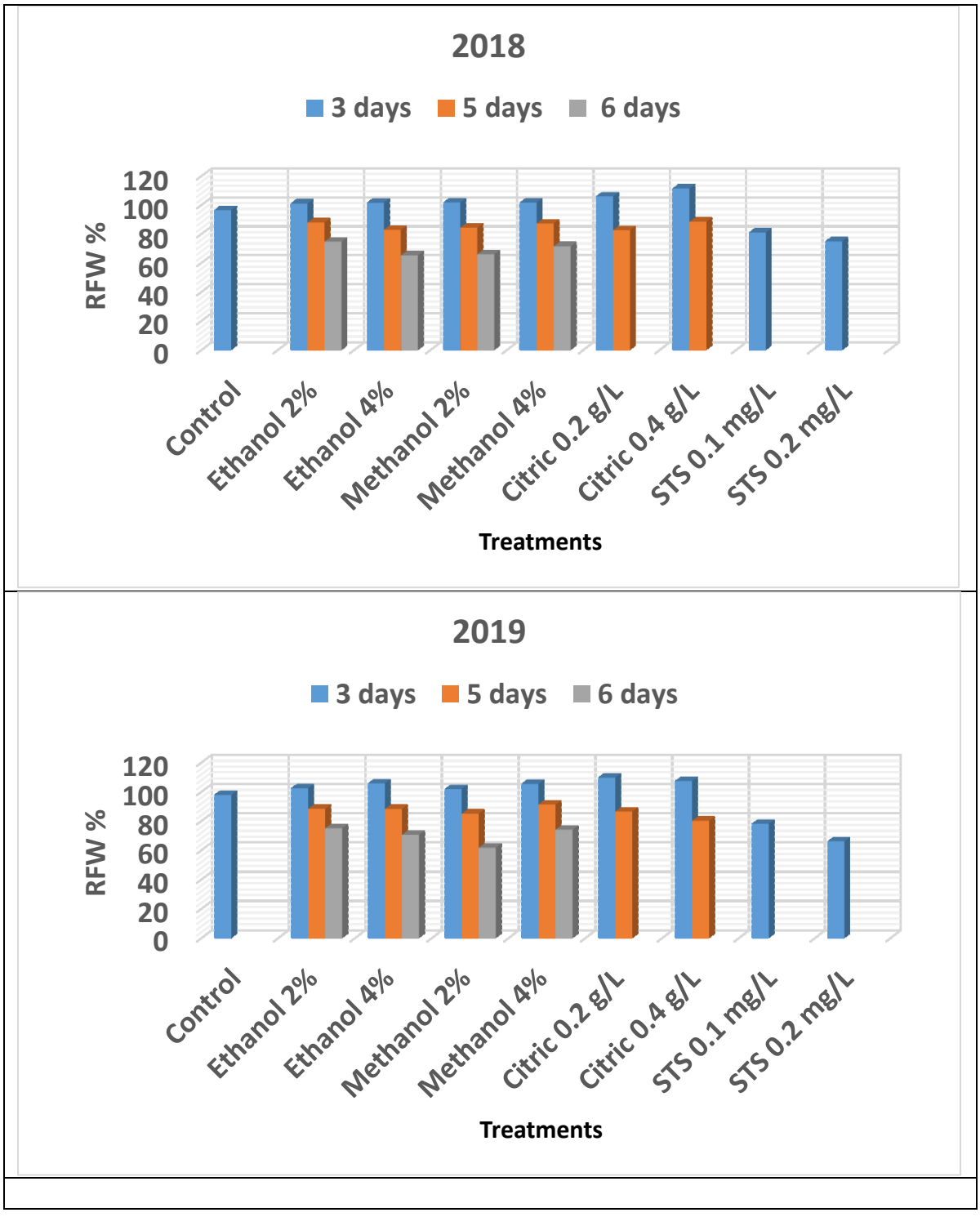

Figure (2). Effect of ethanol, methanol, citric acid and STS concentrations on relative fresh weight RFW (%) during the shelf life of *Polianthes tuberosa* cut spikes in the seasons of 2018-2019

2- Florets characters

Results of Table (2) exhibits that the lowest significant floret's opening percentage after three days of initiation the experimentation was obtained by applying STS at 0.1 and 0.2 mg /L as recorded (0.0 %) during both seasons , while the highest percentage of floret's opening was recorded after application of citric acid at any concentration and using citric acid at 0.4g/L resulted in the highest florets opening percentage whish was (36.96 and 37.35%) during the first and second season respectively. For the floret's diameter all treatments caused significant increase in floret's diameter expect STS treatments, the highest significant values were obtained after pulsing the flower spikes by citric acid at 0.2 and 0.4 g /L in both seasons. For the floret's welting percentage, it is observed that STS application caused toxicity to the flower spike and all the florets were welt. Moreover, Table (2) clear that all the treatments decreased the floret's welting percentage significantly compared to control treatment during both seasons. Also, the lowest decrease in florets welting percentage was recorded after application of ethanol at 2% which recorded (1.3 and 1.5 %) during both seasons.

Table (2). Means of florets characters of *Polianthes tuberosa* cut spikes as affected by pulsing treatments after 3 days of experiment start in the seasons of 2018 and 2019

Treatment	Floret's opening (%)		Floret's diameter (cm)		Floret's welting (%)	
	2018	2019	2018	2019	2018	2019
Control	33.02a	31.01bc	1.90c	1.80c	25.7b	24.33b
Ethanol at 2%	27.96b	28.05c	4.07a	3.62a	1.30c	1.50c
Ethanol at 4%	23.51bc	27.62c	4.00a	3.83a	2.10c	1.73c
Methanol at 2%	24.63bc	27.55c	3.93a	3.73a	4.50c	4.50c
Methanol at 4%	20.07c	26.38c	2.83b	2.67b	7.10c	6.33c
Citric acid at 0.2 g/L	34.01a	36.84ab	4.83a	4.17a	11.90c	10.11c
Citric acid at 0.4 g/L	36.96a	37.35a	4.67a	4.00a	12.90c	11.84c
STS at 0.1 mg/L	0.00	0.00	0.00d	0.00e	100.0a	100.0a
STS at 0.2 mg/L	0.00	0.00	0.00d	0.00e	100.0a	100.0a
L.S.D.at 0.05	5.05	6.22	0.92	0.56	11.66	10.99

Means of treatments in the each column that have the same letters, are not significantly different at 5% level

3- Chemical analysis

As for the effect of different treatments on chlorophyll a and b contents, Table (3) elucidate that at the end of the vase life of the control plant, all treatments were higher than the control plants except citric acid at 0.4 g/L during both seasons and the highest significant chlorophyll a and b values were obtained after pulsing flower spikes in ethanol at 2% in both seasons.

Results of Table (3) demonstrate that the lowest significant reducing sugar content and T.S.S. percentage was obtained from control plant and applying any

treatment resulted in significant increase in reducing sugar content and T.S.S. percentage value with no significant different between them.

Table (3). Means of Chlorophyll a and b content (mg/ g fresh weight), reducing sugars (mg/g dry weight) and T.S.S. (%) of *Polianthes tuberosa* cut spikes as affected by pulsing treatments with ethanol, methanol and citric acid in the seasons of 2018 and 2019

Treatment	Chlorophyll a (mg/ g fresh weight)		Chlorophyll b (mg/ g fresh weight)		Reducing sugars (% dry weight)		T.S.S (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	0.51b	0.49b	0.15b	0.16b	0.04b	0.03b	7.97b	6.60b
Ethanol at 2%	0.67a	0.65a	0.21a	0.22a	0.11a	0.11a	13.13a	14.87a
Ethanol at 4%	0.56b	0.52b	0.16b	0.16b	0.10a	0.09a	11.90a	12.87a
Methanol at 2%	0.59a	0.59ab	0.19ab	0.19ab	0.12a	0.13a	12.40a	13.63a
Methanol at 4%	0.56b	0.55ab	0.16b	0.16b	0.10a	0.12a	12.17a	12.83a
Citric acid at 0.2 g/L	0.60ab	0.62ab	0.19ab	0.18ab	0.12a	0.16a	14.17a	13.47a
Citric acid at 0.4 g/L	0.49b	0.45b	0.15b	0.14b	0.10a	0.14a	12.83a	10.77a
L.S.D.at 0.05	0.10	0.13	0.03	0.04	0.02	0.08	2.62	4.07

Means of treatments in the each column that have the same letters, are not significantly different at 5% level

DISCUSSION

The obtained results elicited that all treatments caused significant increase in the vase life *of Polianthes tuberosa* cut spikes except for STS treatments.

Application of ethanol and methanol treatments caused increase in final water uptake, vase solution uptake rate, relative fresh weight, chlorophyll a and b content and decrease in loss of spike fresh weight percentage, which resulted in increase in vase life. Moreover, for florets characters although ethanol and methanol treatments caused increase in florets dimeter, they caused decrease in florets opening percentage and florets welting percentage after three days from the experiment start which could be explained by the highest VSU value on the second measuring time. For reducing sugar content and T.S.S. percentage the results showed that these treatments caused significant increase which may be due to the increase in vase life in these treatments. The positive effect of flower charters after ethanol and methanol treatments may be due to antimicrobial effects of methanol and ethanol (Heins and Blakely 1980 and Wu *et al*., 1992), besides that ethanol has the ability to reduce 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, ACC oxidase, which results in loss of protein which ultimately decreases the formation of ethylene. (Podd and Staden 2004). These results are in agreement with those obtained by Ola (2017) on chrysanthemum, whereas application of ethanol at 3% ; increased vase life, decreased water loss, enhanced relative fresh weight and diameter of flower heads. Hossain *et al.,* (2007) reported that bougainvillea flowers longevity increased due applying 4, 8, and 10% of ethanol, Farokhzad *et al.* (2005) reported that use of ethanol inhibited ethylene

production and increased water uptake in cut lisianthus and Petridou *et al.* (2001) mentioned that ethanol and methanol improve vase life of cut chrysanthemum.

Using citric acid treatments caused a moderate significant increase in most studied characters and accelerate the end of the vase life of tuberose plants compared to methanol or ethanol treatments as it recorded the highest significant increase in florets opening percentage, florets diameter and florets welting percentage after three days of the experiment start. This results may be due to the ability of citric acid in reducing the water pH, resulting in decrement in bacterial growth, which block the xylem vessels in the cut region of stem (Nowak and Rudniki, 1990). These results are in agreement with those obtained by Motaghayer and Esna-Ashari (2009) who mentioned that applying citric acid at 0.45 g/L resulted in a little effect on flower longevity of *Polianthes tuberosa* spikes and with those obtained by Jowkar and Salehi (2005) who indicated that 0.45 g/L citric acid increased the vase life of 69.4 % more than control of *Polianthes tuberosa* spikes.

On the other hand, the negative effects of STS treatments may be due to the sensitivity of the tuberose spikes to silver which resulted in toxicity symptoms like decrease in final water uptake, severe burning of the floret and drops in all studied florets characters (0.0% of floret's opening and 100 % of floret's welting) these results are in harmony with those obtained by Abbasi and Hassanpour (2011) and Jowkar and Salehi (2006) on *Polianthes tuberosa*.

CONCLUSION

Based on the previous study, it is obvious that pulsing tuberose spikes in a solution of sucrose 2% + ethanol 2% for 24 hours ; resulted in the highest significant increase in vase from (32-65%) more than the control , increase in final water uptake, chlorophyll a & b content and decrease the loss of flower fresh weight & floret's drop percentage .

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الملخص العربي

القدرة الحفظية والجودة لأزهار نبات التيبروز بعد القطف تأثرا بالإيثانول والميثانول وحمض الستريك وثيوسلفات الفضة

أسماء محمد أحمد طه

فرع بحوث نباتات الزينة بأنطونيادس – الإسكندرية

قسم بحوث الزينة وتنسيق الحدائق– معهد بحوث البساتين – مركز البحوث الزراعية

أجريت هذه التجربة خلال موسمي (٢٠١٨ و٢٠١٩) في قسم بحوث الزينة وتنسيق الحدائق– حديقة أنطونيادس ، معهد بحوث البساتين، الاسكندرية، جمهورية مصر العربية لدراسة تأثير الإيثانول والميثانول و ستريك اسيد وثيوسلفات الفضة على القدرة الحفظية والجودة لأزهار نبات التبروز بعد القطف . أستخدمت تسعة معاملات في هذه التجربة هي (الكنترول (ماء مقطر) ، أيثانول بتركيز ٢ و٤% ، ميثانول بتركيز ٢ و٤% ، حمض الستريك بتركيز ٠.٢ و ٠.٤ جرام / لتر كمعاملات إنباض لمدة ٢٤ ساعة) و ثيوسلفات الفضة بتركيز ٠.١ و ٠.٢ ملجم / لتر كمعاملات إنباض لمدة ساعة.

اظهرت النتائج ان جميع المعاملات أدت إلى زيادة معنوية في عمر الأزهار ما عدا معاملات ثيو سلفات الفضة مقارنة بالكنترول .أعلى زيادة معنوية في عمر الأزهار تم الحصول عليها من استخدام اي من معاملات الأيثانول. بالإضافة لذلك معاملات الايثانول والميثانول أدت إلى زيادة ملحوظة في امتصاص الماء ، نسبة تفتح الزهيرات، السكريات المختزلة ، وادت إلى نقص في نسبة ذبول الزهيرات .