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ORIGINAL ARTICLE

Influence of Pulsing Biocides and Preservative Solution Treatment on the Vase Life of Cut Rose (*Rosa hybrida* L.) Varieties

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ABSTRACT

Currently, the cut flower industry in Ethiopia is facing an increasing total fresh loss of about 20% due to poor postharvest handling. Cut flowers need to last longer in a vase or flower arrangement with their aesthetic qualities, fragrance and appearance maintained in order to get consumer's acceptance. Therefore, this study was conducted to develop possibilities of extending vase life of cut rose flowers in Ethiopia. Pulsing solutions comprising biocides (1g $Al_2(SO4)_3l^{-1}$, 0.4 ml $HQSl^{-1}$, 0.6 ml $NaOCll^{-1}$, 0.6g $Ca(ClO)_2l^{-1}$), 10 g l^{-1} long life, distilled water and tap water as control on the vase life of rose (*Rosa hybrid* L.) cut flower varieties - Red Calipso, Akito, and Viva were evaluated under laboratory conditions at Ziway Sher Ethiopia. Treatments were arranged in 7x3 factorial combinations of pulsing solutions and varieties in a completely randomized design (CRD) with four replications. Results showed that maximum vase life of 15.50 and 15.08 days were obtained from cut flower stems pulsed in $NaOCL1^{-1}$ and HQS, respectively. The minimum vase life of 13.25 and 13 days were obtained from cut flower stems pulsed in $AL_2(SO4)_3$ and tap water, respectively. $AL_2(SO4)_3$ which has been the most commonly used biocide by most Ethiopian cut flower growers was found not effective as compared to the other biocides in the present study. $NaOCL1^{-1}$ and HQS as pulsing biocides on extending the vase life of cut flowers could be considered as alternatives to $AL_2(SO4)_3$ currently on use in most of the cut flower industry in Ethiopia.

Keywords: biocide, flower food, rose varieties, vase life

INTRODUCTION

After harvest flowers are cut of from their mother plants. As they get detached, their ageing processes accelerate. To delay their ageing processes and subsequently increase their vase lives, post harvest treatment is crucial. This is because flowers take up about 80% of their water requirement within the first two hours after harvest (Roskam, 2010). Delaying the rate of deterioration extends the quality and maintains the natural appearances of cut flowers to attract wholesalers, retailers, and finally consumers (Chapman and Austin-brown, 2007). Fresh flower foods have effects on the postharvest vase life of flowers. They extend flower longevity by providing three essential ingredients, namely biocide, sugar and acidifier. Growth regulators or hormones

may be incorporated to the fresh flower foods to reduce leaf yellowing. Wetting agents and detergents may also be added to reduce the surface tension of water to promote water uptake in cut stems during dry transport (Michael, 2000).

Under normal conditions, cut flowers could last only for a few days maintaining their beauty and attractiveness. However, most of the people like to enjoy cut flowers in their natural beauty and appearances for a longer period of time having the socioeconomic value of flowers intact. Hence there is a dire need to explore possibilities of extending vase life by using different biocides and preservative solutions. Once flowers are purchased, the longer they last in a vase or flower arrangement, the better the purchasers would enjoy the aesthetic qualities, fragrance, and appearance of cut flowers, and will be encouraged to buy them again. As long as they are properly handled, most of the commercial cut roses will last in the vase for 10 or more days (Chapman and Austin-brown, 2007).

Hybrid Tea, Intermediate and Sweetheart roses are currently produced in Ethiopia. In fact, Ethiopia is the largest rose exporter in Africa following Kenya to the Dutch Auction Markets (CBI, 2008). However, about 20% of the total fresh produce is lost in between the time of leaving the farm and reaching the consumer due to physiological and pathological problems during the postharvest handling (Panhwar, 2006). Though the volume of export is growing and the country is benefiting from the export of floriculture produce, yet, there is lack of research on postharvest handling of fresh cut flowers.

Therefore, the study was initiated to evaluate the effect of pulsing biocides and preservative solution on the physiological characteristics of fresh cut roses and identify the best pulsing biocides and preservative solution combination in extending the vase life of cut rose varieties.

MATERIALS AND METHODS

Site Description

The experiment was conducted at AQ Roses PLC. Farm (Ammerlaan Quality Roses) within the premises of Ziway Sher Ethiopia (7^{o} 56'N, 38^{o} 43'E with elevation 1646 m). Ziway is located in the rift valley about 163 km South of Addis Ababa, the capital city of Ethiopia. The area receives mean annual rainfall of 750-850 mm; with about 70 % during the main rainy season from June to September. The Mean annual maximum and minimum temperatures range from 28.4 °C to 14.0 °C, respectively (BTA, 2001). Experimental plants of Red Calypso, Akito and Viva were grown under greenhouse condition with an average day and night temperatures of 20 °C.

Treatments and Design

The treatments were arranged in a 3 × 7 factorial combination of varieties (Red Calypso, Akito and Viva), and biocides (1g $Al_2(SO_4)_3$ l^{-1}, 0.4 ml HQS l^{-1}, 0.6 ml NaOCl l^{-1}, 0.6 g $Ca(ClO)_2$ l^{-1}), 10 g l^{-1}long life, distilled water and tap water as control with four replications in a completely randomized design (CRD). Each experimental unit consisted of six flower stems. In studying the effect of the different pulsing solutions on the vase life of the cut flower stems, the experiments were divided into two groups. The first group contained explicitly none destructive experiment while the second group contained destructive measurements. Evaluations were made by keeping the flower stems in an evaluation room of mean temperature of 21^{o}C and 60% RH with 12 h of photoperiod. The sources of irradiance for the room were cool-white fluorescent lamps.

Experimental Materials and Procedures

Plant material: Flower stems of three varieties of Rose *(Rosa hybrida* L.*)* intermediate - Red Calypso, Akito and Viva with red, white and yellow colors respectively were used. The cut flowers were harvested at normal harvest maturity (stage

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I) when the buds were tight and the sepals enclosed in the floral bud (Capdeville *et al*., 2005).

Harvesting was done early in the morning and the stems were kept in buckets partially filled with tap water in upright position. To simulate the practice exercised by the growers, they were kept in pre-cooling room (8-10 ^{o}C) before grading to remove greenhouse heat. Sorting and grading were done after two hours to choose flowers that are fit for export purpose. Sorted and graded flowers were kept in cooling room (2-4 ^{o}C) immediately after bunches were put in buckets partially filled with pulsing biocides (1g $Al_2(SO_4)_3$ l^{-1}, 0.4 ml HQS1^{-1}, 0.6 ml NaOCll^{-1}, 0.6g $Ca(ClO)_2$ l^{-1}), 10 g l^{-1}long life, distilled water and tap water as a control.

The pulsing biocide solutions were prepared in water obtained from a bore hole for all of the treatments. The pH of pulsing solutions were adjusted to 3.5–4.5 with citric acid and for Aluminum sulphate with KOH. The postharvest physiological characteristics of the flower stems were studied throughout the vase life period following the initial pulse treatment of 24 hours. The flower stems were taken out from the cold room with all the pulsing solutions replaced by 500 ml vase solution that contained flower food (long life) at a concentration of 10 g l^{-1}.

Following 24 hours of pulse treatment, the lower most leaves from all flower stems were trimmed off to the height of 20 cm. The stem ends were re-cut slanted under water to get stem lengths of 45 cm. The vase life evaluation was continued with readymade flower preservative (long life) until the completion of the experiment.

Data Collected

Flower longevity or degree of bent neck: were recorded as described by Liao *et al.* (2000), as the number of days after harvest (day 0) to until the flowers show symptoms of bent neck or advanced signs of fading on all petals.

Maximum flower head diameter: Flower bud diameters were measured daily with Vernier Caliper (cm) and maximum flower diameter was used to evaluate the bud size difference between the treatments. The maximum diameter of the flower buds was recorded by using the rating scale of Capdeville *et al.* (2005).

Hydraulic conductance of stem segment: Hydraulic conductance was determined using the procedure described by van Doorn *et al*. (1989) in stem sections excised at 3 positions (0 to 5, 10 to 15, 20 to 25 cm) from the base.

Solution uptake: The volume of water uptake was calculated by subtracting the volume of water evaporated from a flask of the same volume without cut flowers. The water loss volume was calculated by subtracting the increase in fresh weight from the water uptake volume. Vase solution usage was determined using the formulae described by Chamani *et al*. (2005).

Relative fresh weight (%): The flowers were weighed at noon time during several days of vase life. For that purpose, flowers were taken out of water for as short time as possible (20–30 seconds). The fresh weight of each flower was expressed relative to the initial weight to represent the water status of the flower (Joyce and Jones, 1992).

Water content (g/g): A dry weight of four outer petals was recorded after drying the petals to constant weight in an oven at 70°C. Water content was calculated as described by Rana (2010).

Freshness of flowers: Freshness of the flowers was determined by wilting score (Macnish *et al*., 1999) within three days interval. Stems were scored on a 1-5 scale for the freshness of the flowers (1 = fresh flower, 2 = very slight petal enrolling, 3 = noticeable in-rolling, 4=petal shriveling, 5 = maximum petal shriveling).

Total soluble solids (TSS) (%): Sap was obtained by squashing the petals. The TSS in the sap was measured with a hand Refractometer (model RCZ and serial number SN 00850) during the 3rd, 7th and 10th day.

Petal discoloration score: Flower petal color change or discoloration (fading) was assessed according to procedures described by Macnish *et al*. (1999) with a rating scale of 1 = none/slight, 2 = moderate, 3 = advanced within three days interval.

Solution turbidity of microbial count assessment: Solution turbidity attributable to microbial growth was assessed at the end of this experiment by measuring absorbances at 400, 500 and 600 nm with a spectrophotometer (model Uv-7804c, ultra violet visible), and calculating the mean of these values (Knee, 2000). Distilled water was used as a blank.

Statistical Analysis

Significance tests were made by analysis of variance (ANOVA) using SAS procedure of version 9 (SAS institute, 2004). Mean comparisons were made using least significance difference (LSD).

RESULTS AND DISCUSSION

Flower Longevity

The results presented in Table 1 showed that longevity of cut flower was significantly ($P < 0.001$) increased by pulsing treatment of biocides and preservative solution. The maximum vase life was observed in cut flower stems pulsed with 600mg NaOCl l^{-1} (almost 16 days) followed by 0.4 ml HQS l^{-1} (almost 15 days), and the lowest (almost 13 days) was observed in $Al_2(SO_4)_3$ pulsed and tap water (control) treated flower stems. $Al_2(SO_4)_3$, which is a common biocide used in most Ethiopian cut flower growers was not able to extend the vase life of cut flower stems better than the control, treated with tap water. This clearly indicated that the addition of $Al_2(SO_4)_3$ as a pulsing biocide with the preservative solution was not superior to the ordinary tap water. In agreement to the current findings, Reid *et al*. (2001) reported that addition of sucrose greatly improved opening and vase life of opened flowers while $Al_2(SO_4)_3$ provided no significant additional benefits. Similarly, Son *et al*. (1994) noted that $Al_2(SO_4)_3$ resulted in an increased respiration rate, reduced chlorophyll content of the leaves, lower rate of photosynthesis, and damage of both flowers and leaves in 'Sonia' rose. Longer flower vase life was not necessarily associated with low resistance to water flow in the stem or low microbial accumulation, phytotoxic effects of some biocides can also shorten vase life of roses (Knee, 2000).

Results showed that the response of the biocides and preservative solution differ from variety to variety (Table 1). Akito and Viva stayed significantly longer (14.57 and 14.43 days), respectively compared to Red Calypso (13.57 days). Vase life can vary not only from one species of plant to another but also from one variety to another within the same species (Timmerman and Kroon, 2009). It has been reported that short vase life of cut flowers is mainly attributed to vascular occlusion which constricts the water supply to the flowers (De Stigter, 1980), mainly caused by blockage of xylem vessels by microorganisms that accumulate in the vase solution.

Table 1. Effects of pulsing solutions and varieties on flower longevity and maximum flower head diameter of cut flower stems evaluated at room conditions

Factors	Treatments	Flower longevity (days)	Maximum flower head diameter (cm)
Pulsing solutions	Tap Water (Control)	13.00^{d}	5.87^{a}
	$Al_2(SO4)_3$	13.25^{d}	5.91^{a}
	HQS	15.08^{ab}	6.32^{a}
	$Ca(ClO)_2$	14.58^{b}	6.22^{a}
	NaOCl	15.50^{a}	6.43^{a}
	long life	14.25^{bc}	6.12^{a}
	Distilled water	13.67^{cd}	5.81^{a}
	F-test	***	Ns
	SEM±	0.31	0.19
Varieties	Red calypso	13.57^{b}	6.43^{a}
	Akito	14.57^{a}	6.16^{a}
	Viva	14.43^{a}	5.70^{b}
	F-test	***	***
	SEM ±	0.20	0.12
	CV (%)	7.56	10.76

SEM= standard error of the mean. Ns and *** indicate non significant at $P > 0.05$ and significant at $P < 0.001$ probability level, respectively. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P = 0.001$ probability level.

Van Doorn *et al.* (1990) also reported a positive correlation between abundance of bacteria and the decrease in hydraulic conductance of the stem. Louband and van Doorn (2004) reported stem blockage in rose (*Rosa hybrida* cv. Red One) was mainly due to living bacteria and their decay products. Many germicides such as HQS, $AgNO_3$ and $(Al_2(SO_4)_3$ have been shown to inhibit bacterial growth in cut rose stems (van Doorn, 1997). Therefore, extension of cut flower stems vase life with pulsing/vase solutions that contained calcium hypo chloride ($Ca(ClO)_2$), sodium hypo chloride (NaOCl), 8-Hydroquinoline sulphate (HQS) and flower preservative (flora life) could be due to the antibacterial effects and energy supply from flower preservative (flora life). Pun and Ichimura (2003) indicated that cut roses after detachment from the mother plant are devoid of food, hormones and water supply and depend exclusively on stored food at and after the time of harvest and/or on the application of exogenous sugars.

Maximum Flower Head Diameter

In comparing the difference in the maximum flower head diameter of the three cut rose varieties, non significant differences were observed due to differences in the pulsing treatments. On the other hand, there exist significant ($P < 0.001$) differences between varieties (Table 1). Red calypso (6.43 cm) and Akito (6.16 cm) exhibited significantly higher flower head diameter than Viva (5.70 cm) and that could be attributed to their genetic makeup. Ichimura *et al*. (2002) reported that the way of flower opening varied among 25 cultivars. 'Bridal Pink' flowers opened the fastest, followed by 'Black Tea'. 'Noblesse' flowers reached stage 5 the latest, but most flowers did not completely open. In addition, most of flowers of some cultivars, such as 'Grand Gala' and 'Jerfalrei', wilted without complete flower opening. Several findings showed that flowers' petal growth is associated with flower bud opening which results from cell expansion (Kenis *et al*., 1985) that requires the influx of water and osmolytes such as glucose into petal cells (Evans and Reid, 1988; van Doorn *et al*., 1991).

According to Chamani *et al*. (2005) shortened vase life was associated with abnormal opening and inhibition of flower opening. Van Doorn *et al*. (1991) also reported that the decrease in water potential was correlated with inhibition of corolla growth and flower opening.

Solution Absorbance

Vase solution absorbance was significantly (P< 0.001) influenced by the interaction between pulse treatment of the cut flower stems and varieties (Table 2). The highest vase solution absorbance values were recorded from the three varieties when treated with tap water (control) while the lowest vase solution absorbance values were recorded for the three varieties in NaOCl and for Red calypso and viva in HQS.

The significant reduction in vase solution absorbance in pulsing treatments of NaOCl and HQS could be due to the antimicrobial effect of these biocides. HQS is a well-known germicide although it is toxic to some rose cultivars leading to shortening of their vase life (Ichimura *et al*., 2006). Knee (2000) also reported a reduction in solution absorbance (0.057) of 'Classy' roses treated with HQC. These observations confirm that pulsing treatments with NaOCl and HQS were the most effective in reducing microbial proliferation on all varieties which also resulted in clearer vase solution. On the other hand, the higher vase solution absorbance values recorded in rose cut flower pulsed with long life could be associated to its lower effect in clarifying the vase solution or in suppressing microbial population. Knee (2000) also reported high values of vase solution absorbance in the absence of biocide and in $Al_2(SO_4)_3$ solutions.

Stems of some plants exude sap containing various cations, anions and amino and organic acids (van Meeteren *et al*., 2000) or chemicals like phenols, which could increase vase solution absorbance, and also that can be harmful to other cut flowers or to themselves. In the present study, it was observed that addition of biocides: NaOCl, HQS and $Ca(ClO)_2$ into the pulsing solution was associated with longer flower life and low vase solution absorbance. Hence, it appears that increased solution turbidity is an indication of reduced vase life and reduced solution usage.

Table 2. The Interaction effect of pulsing solutions with varieties on vase solution absorbance

	Vase solution absorbance		
Treatments	Red calypso	Akito	Viva
Tap water (Control)	0.0625^{a}	0.0642^{a}	0.0657^{a}
$Al_2(SO4)_3$	0.0542^{c}	0.0470^{d}	0.0532^{b}
HQS	0.0352^{f}	0.0415^{e}	0.0340^{f}
$Ca(ClO)_2$	0.0450^{d}	0.0440^{e}	0.0472^{c}
NaOCl	0.0357^{f}	0.0355^{f}	0.0307^{f}
long life	0.0557^{b}	0.0610^{b}	0.0552^{b}
Distilled water	0.0582^{b}	0.0545^{c}	0.0540^{b}
F-test	***		
SEM±	0.001		
CV (%)	4.36		

SEM= standard error of the mean. *** indicate significant at P < 0.001probability level. Means followed by the same letter are not significantly different at P < 0.001 probability level.

Hydraulic Conductance

The result presented in Table 3 showed a significant ($P < 0.001$) difference in the hydraulic conductance of the stem sections excised at 10-15cm and 20-25cm from the base of the cut flower stems in pulsing treatments with biocides. However, no significant difference was recorded in the stem sections excised from 0-5cm from the base. In stem sections excised at 10 to 15 cm, the highest hydraulic conductance was recorded from $Ca(ClO)_2$ treated stems followed by $Al_2(SO4)_3$ and NaOCl while the lowest was from tap water and distilled water treated stems. In the same manner, in stem sections excised at 20 to 25 cm; NaOCl, long life and $Ca(ClO)_2$ treated stems gave significantly higher hydraulic conductance than the other treatments.

The significant increase in hydraulic conductance of the stem sections in response to pulsing of cut flower stems with biocides suggests that biocides were able to enhance water flow as compared to distilled water and tap water. The results showed the advantage of using biocides in pulsing solutions to improve the stem hydraulic conductance which is critical in improving vase life of the cut flower stems. This finding appears to support the view that vascular occlusion is due to mainly bacterial proliferation. Pulsing of cut flower stems with germicides such as HQS inhibited bacterial proliferation and maintained the hydraulic conductance of the stem segments (Ichimura *et al*., 2003). Treatments with antimicrobial activities inhibit bacterial proliferation and suppress the decrease in hydraulic conductance of cut roses (van Doorn *et al.,* 1989). The hydraulic conductance of cut rose flower stems decreases with time after harvest, and this decrease is associated with bacterial proliferation (van Doorn *et al*., 1989; Ichimura *et al*., 2003). According to Van Doorn *et al.* (1989), the development of vascular occlusion was correlated with the growth of bacteria at the cut surface and inside the stem. Thus, inhibition of vascular occlusion by biocides attributed to their germistatic action.

In this study, the lowest hydraulic conductance was observed in 0-5cm excised stem and there is an increase in hydraulic conductance as the height of stem cut increases. This could be attributed to the blockage due to bacterial growth at the stem end. The finding was supported by van Doorn *et al.* (1989) who reported the lowest hydraulic conductance value in the basal stem segments for Sonia, Ilona, Motrea, Jack Frost, and Mercedes rose cultivars. Similar results were also reported by Durkins (1980).

Aluminum sulphate has been used as a microbial inhibitor in commercial preservatives (Halevy and Mayak, 1981). However, it is clearly observed in Table 3 that $Al_2(SO_4)_3$ did not respond as effective as $Ca(ClO)_2$, NaOCl and long life for hydraulic conductance on these cultivars; it responded the same as HQS which prolonged vase life of cut flowers; HQS may maintain the balance between water lost from leaves and solution gained from the stems. According to Knee (2000), different compounds can result differently in the vase life of different cut flowers; For example, the biocide sodium benzoate was effective both in improving water uptake and preventing bacterial growth but did not greatly extend flower vase life. Thus, the ineffectiveness of $Al_2(SO_4)_3$ on vase life may not be associated with the inability of the biocide for inhibition of vascular occlusion due to bacterial proliferation. It seems due to the phytotoxic effect of $Al_2(SO_4)_3$ when reacted with the fluoride of tap water obtained from rift valley area of Ethiopia (Ziway) (tap water contains 0.16 mg F^- per liter of water) and may have caused a reduction of vase life. In contrast, $Ca(ClO)_2$ and NaOCl maintained water uptake of the cut roses and suppressed the decrease of hydraulic conductance in the flower stem suggesting that they inhibits development of vascular occlusions. Thus, they might have improved the stem water relationship leading to extension of the vase life.

Table 3. Effects of pulsing solutions and varieties on vase hydraulic conductance of cut flower stems at different distances from the base (cm) evaluated at room conditions

Factors	Treatments	Hydraulic conductance (ml) at a distance from the base (cm)		
		0 to 5	10 to 15	20 to 25
Pulsing solutions	Tap Water (Control)	0.41^{a}	0.57^{c}	1.28^{b}
	$Al_2(SO4)_3$	0.48^{a}	0.82^{ab}	1.30^{b}
	HQS	0.51^{a}	0.80^{b}	1.30^{b}
	$Ca(ClO)_2$	0.47^{a}	0.94^{a}	1.67^{a}
	NaOCl	0.46^{a}	0.81^{ab}	1.77^{a}
	long life	0.41^{a}	0.79^{b}	1.69^{a}
	Distilled water	0.41^{a}	0.68^{bc}	1.37^{b}
	F-test	Ns	***	***
	SEM ±	0.03	0.04	0.07
Varieties	Red calypso	0.43 ^{a}	0.82^{a}	1.47^{a}
	Akito	0.49^{b}	0.81^{a}	1.46^{a}
	Viva	0.41^{a}	0.68^{b}	1.52^{a}
	F-test	***	***	Ns
	SEM ±	0.02	0.03	0.04
	CV (%)	22.25	19.81	15.90

SEM= standard error of the mean. Ns and *** indicate non significant $P > 0.05$ and significant at $P < 0.001$ probability level, respectively. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$.

Solution Uptake

The rate of solution uptake significantly ($P < 0.001$) varied among biocides and preservative solution throughout the study period (Table 4). Among the biocides, pulsing of the cut flower stems with $Al_2(SO_4)_3$ and HQS significantly ($P < 0.001$) and consistently increased solution uptake during the sampling periods as compared with the other treatments. An enhanced solution uptake in $Al_2(SO_4)_3$ and HQS pulsed flower stems could be attributed to reduced microbial proliferation in response to the The results indicated significant ($P < 0.001$) variation in solution uptake of Red calypso, Akito and Viva cut flower stems pulsed with biocides and vase solution (Table 4). Red calypso exhibited the highest solution uptake throughout the sampling periods. During 1, 4 and 10 days in the vase, Akito showed the lowest solution uptake while Viva was intermediate between Red calypso and Akito. During 7 and 13 days, however, there was no significant difference between Akito and Viva with respect to solution uptake.

biocides and a significant reduction in vascular occlusion. The biocides could have maintained the antimicrobial activity in the vase solution and resulted in an increased water and sugar uptake, thus improving the energy supply to the cut flowers. Vaslier and van Doorn (2003) indicated that there may be absence of vascular blockage in $Al_2(SO_4)_3$ treated flowers. Flowers placed in water without antimicrobial compounds had a low water potential as a result of vascular blockage in the lower most segment of the stem (van Doorn *et al*., 1991).

Variability among cultivars as to the effect of solution treatment of water uptake may be due to differences in xylem anatomy, which has been shown to greatly influence hydraulic conductivity (Nijsse *et al*., 2001). Van Doorn and Stead (1997) did not find significant differences in xylem anatomy among rose cultivars 'Frisco', 'Sonia', 'Madelon', and 'Cara Mia'; however, Twumasi *et al.* (2005) found that water availability during preharvest environment can affect xylem vessel diameter. In this

study, since all cultivars were tested at the same time and produced under the same management, it is not possible that some cultivars experienced more water stress than others due to environmental conditions during their growth. Previous study also demonstrated such difference in solution uptake by cut flower stems of different species (Knee, 2000). Burge *et al*. (1996), rated vase water uptake by *Gerbera* 'Monarch', *Gypsophila* 'Crystal' and *Matthiola* 'Ruby Red' stems which were highly variable but generally decreased over time.

Table 4. Solution uptake of cut flower stems as affected by pulsing solutions and varieties

Factors	Treatments	Solution uptake in ml/day/g fresh weight				
		Vase life in days				
		1	4	7	10	13
Pulsing solutions	Control	0.4358^{b}	0.2525^{bc}	0.2642^{b}	0.2233^{bc}	0.2175^{bc}
	$Al_2(SO4)_3$	0.5592^{a}	0.3258^{a}	0.3058^{a}	0.2667^{a}	0.2475^{ab}
	HQS	0.4625^{ab}	0.2867^{ab}	0.3117^{a}	0.2433^{ab}	0.2592^{a}
	$Ca(ClO)_2$	0.4417^{b}	0.2792^{b}	0.2500^{bc}	0.2192^{bc}	0.2025^{c}
	NaOCl	0.4158^{bc}	0.2750^{b}	0.2875^{ab}	0.2192^{bc}	0.2433^{b}
	long life	0.3942^{bc}	0.2475^{bc}	0.2525^{bc}	0.2033^{c}	0.2117^{bc}
	Distilled water	0.3566^{c}	0.2217^{c}	0.2242^{c}	0.1883^{c}	0.1875^{c}
	F-test	***	***	***	***	***
	SEM ±	0.0243	0.0150	0.0127	0.0122	0.0117
Varieties	Red calypso	0.5296^{a}	0.3100^{a}	0.3229^{a}	0.2718^{a}	0.2682^{a}
	Akito	0.3454^{c}	0.2211^{c}	0.2386^{b}	0.1861^{c}	0.1961^{b}
	Viva	0.4389^{b}	0.2782^{b}	0.2511^{b}	0.2121^{b}	0.2082^{b}
	F-test	***	***	***	***	***
	SEM ±	0.0159	0.0098	0.0083	0.0080	0.0082
	CV (%)	19.40	20.05	19.60	16.50	18.36

evaluated at different vase life stages and room conditions
SEM= standard error of the mean. *** indicate significant at $P < 0.001$ probability level. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$ probability level.

In general, solution uptakes by flower stems treated with long life, distilled water and tap water (control) were statistically at par (Table 4). The lower rate of solution uptake by cut flower stems pulsed with long life might be associated with bacterial proliferation using the carbohydrates as a food source that is added in long life and the concomitant vascular blockage. Vascular occlusion and depletion of soluble carbohydrate are considered to be primarily responsible for shortened vase life in cut roses (De stiger, 1980; Ichimura *et al.*, 2003).

Addition of biocides in the pulsing/vase solution controls microbial proliferation, reduces vascular occlusion, and increases solution uptake. Knee (2000) observed that for most of the effective biocides added in vase solutions, longer flower life and increased solution uptake were associated with low resistance to water flow and vase solution absorbance. In the present study too, increased solution uptake by the three varieties cut flower stems was associated with extension of their vase life except $Al_2(SO4)_3$. The observations might be as a result of $Al_2(SO_4)_3$ being toxic to the cut flower stems which was also witnessed from scorching of petals and abscission of leaves.

Relative Fresh Weight

The effects of biocides and preservative solution in pulsing of the cut flower stems fresh weight were not significantly ($P > 0.05$)

different on the first day (Table 5). However, after the fifth day the effects of biocides and preservative solution in pulsing of the cut flower stems fresh weight were significantly ($P < 0.001$) different. During the 5th, 9th and 13th days of evaluation, flower stems pulsed with HQS and tap water (control) exhibited the highest and lowest fresh weight values, respectively. The result is in agreement with the findings of Ichimura *et al*. (1999, 2002) who observed a delay in fresh weight loss of cut flowers pulsed with HQS. The relative fresh weight value of the cut flower stems pulsed with the different solutions increased above 100% up to day five of vase life and declined after wards. It could be due to a decline in solution uptake as indicated in Table 4, which is attributed to microbial proliferation and a significant increase in vascular occlusion. The finding is in agreement with the report of Chamani *et al.* (2005) on rose 'First Red'.

Table 5. Effects of pulsing solutions and varieties on relative fresh weight of cut flower stalks evaluated at room conditions

Factors	Treatments	Relative fresh weight (%)			
		Vase life in days			
		1	5	9	13
Pulsing solutions	Tap water (Control)	102.19^{a}	106.19^{c}	85.22^{c}	70.51^{d}
	$Al_2(SO4)_3$	102.42^{a}	107.75^{a}	90.30^{bc}	73.75^{bc}
	HQS	102.58^{a}	107.87^{a}	103.78^{a}	83.12^{a}
	$Ca(ClO)_2$	102.67^{a}	107.56^{ab}	92.20^{bc}	74.33^{b}
	NaOCl	102.30^{a}	107.22^{b}	96.22^{ab}	81.32^{a}
	long life	102.69^{a}	107.44^{ab}	88.91^{bc}	71.66^{cd}
	Distilled water	102.53^{a}	107.12^{b}	88.94^{bc}	72.77^{cd}
	F-test	Ns	***	***	***
	SEM±	0.15	0.16	3.08	0.84
Varieties	Red calypso	102.45^{a}	107.271^{a}	91.55^{a}	75.54^{a}
	Akito	102.61^{a}	107.296^{a}	94.30^{a}	75.23^{a}
	Viva	102.40^{a}	107.357^{a}	90.82^{a}	75.28^{a}
	F-test	Ns	Ns	Ns	Ns
	SEM ±	0.10	0.10	2.01	0.55
	CV (%)	0.52	0.50	11.50	3.99

SEM= standard error of the mean. Ns and *** indicate non significant $P > 0.05$ and significant at $P < 0.001$ probability level, respectively, Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$ probability level.

In the present study, the significant positive correlation between flower longevity and relative fresh weight on day 13 suggests that measuring relative fresh weight of roses could be potentially taken as a useful indicator of flower longevity. Similarly, Pompodakis and Joyce (2003) showed a significant linear correlation between fresh weight and vase solution usage by cut flowers on day 8 and between flower life and foliage life from days 6 to 8. Thus, changes in relative fresh weight and flower longevity are, expectedly, related to solution usage and flower longevity by the rose flowers.

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Water Status of the Three Cut Rose Varieties

Fresh weight of petals

Fresh weight of the outer rose petals, in all pulsing treatment, significantly ($P < 0.01$) varied on the 3rd day while on the 7th and 11th day it became non significant ($P > 0.05$) (Table 6). The overall result showed that fresh weight of most treatments declined as the vase life day proceeded. This study has confirmed earlier work showing that the short vase life of cut rose flowers to be the result of a rapid decline in water uptake and drying out of stems, as also reported by Ichimura *et al*. (2002) on cut flower (shoot) basis.

Sucrose at lower concentrations prolonged the vase life of gladiolus florets by increasing their water uptake but at higher concentrations seemed to impede the uptake, no significant differences were observed among pulsing treatments that comprised long life or the control (Bravdo *et al*., 1974). They also noted that sucrose decrease water loss from the gladiolus leaves or rose petals, thereby maintaining positive water balance in the flower (Bravdo *et al*., 1974). Pun and Ichimura (2003) also suggested that the increase in the water uptake by sucrose treatments could be due to the increase in the osmotic concentration of the florets and leaves. The authors also suggested that the uptake of solution with sucrose was not increased in rose except at day one of the experiment which was in agreement with the present study. There was no significance difference ($P > 0.05$) among varieties in fresh weight petals. It may be attributed to the fact that all the three varieties are categorized under intermediate roses.

Table 6. Effects of pulsing solutions and varieties on fresh and dry weight of petals recorded at different period of the vase life and at room conditions

Factors	Treatments	Fresh weight of petals (g)			Dry weight of petals (mg)		
		Vase life in days			Vase life in days		
		3	7	11	3	7	11
Pulsing solutions	Control	1.00^{c}	1.13^{a}	0.92^{a}	155.60^{a}	144.40^{a}	133.30^{a}
	$Al_2(SO4)_3$	1.08^{bc}	1.00^{a}	0.88^{a}	166.70^{a}	155.60^{a}	155.60^{a}
	HQS	1.20^{ab}	1.16^{a}	0.96^{a}	166.70^{a}	166.70^{a}	166.70^{a}
	$Ca(ClO)_2$	1.27^{a}	1.19^{a}	0.97^{a}	166.70^{a}	144.40^{a}	144.40^{a}
	NaOCl	1.09^{bc}	1.10^{a}	1.01^{a}	155.60^{a}	166.70^{a}	144.40^{a}
	long life	1.13^{abc}	1.01^{a}	0.90^{a}	155.60^{a}	144.40^{a}	155.60^{a}
	Distilled water	1.11^{bc}	1.08^{a}	1.01^{a}	166.70^{a}	144.40^{a}	155.60^{a}
	F-test	***	Ns	Ns	Ns	Ns	Ns
	SEM±	0.05	0.05	0.04	7.30	9.40	9.40
Varieties	Red calypso	1.09^{a}	1.10^{a}	0.92^{a}	200.00^{a}	200.00^{a}	200.00^{a}
	Akito	1.15^{a}	1.08^{a}	0.97^{a}	185.70^{b}	157.10^{b}	152.40^{b}
	Viva	1.14+	1.10^{a}	0.96^{a}	100.00^{c}	100.00^{c}	100.00^{c}
	F-test	Ns	Ns	Ns	***	***	***
	SEM ±	0.03	0.03	0.02	4.80	6.10	6.10
	CV (%)	13.30	12.86	14.50	13.47	18.48	18.68

SEM= standard error of the mean. Ns and ** indicate non significant at $P > 0.05$ and significant at $P < 0.001$ probability level, respectively. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$ probability level.

Dry Weight of Petals

Significant ($P < 0.001$) differences in petals dry weight was recorded between varieties and Red Calypso and Akito have got significantly bigger head diameter than Viva. However, non significant ($P > 0.05$) differences were observed in stems pulsed with biocides and preservative solution (Table 6). Petal growth is the result of cell expansion which requires the influx of water and osmolytes into the vacuole (Van Doorn, 2001). Starch is hydrolysed during petal growth and it is important for the maintenance of cell size (Evans and Reid, 1988). Carbohydrates are substrates for the synthesis of cell wall components during petal cell enlargement (Mayak *et al*., 2001). Reducing sugars, such as glucose and fructose, are the main constituents of the sugars in mature petals (Kaltaler and Steponkus, 1974). Fructose has been reported as a predominant carbohydrate during opening of flowers in roses and in *Campanula* (Ichimura *et al.*, 1999; Vergauwen *et al*., 2000).

The non significant difference between biocides and preservative solution in petals dry weight could be due to the same source of carbohydrate for all treatments (long life). Floral buds are the primary sinks of accumulation, so that the addition of exogenous sugars to the already existing stored food might have contribution for keeping the petals dry weight. It seems that respiration can be compensated by exogenous application of carbohydrate from long life and photosynthesis due to exposure of the cut flowers to 12 h supplementary lights.

Water Content

During the 3rd, 7th and 11th sampling days, the water content ratio was not significantly ($P > 0.05$) influenced by pulsing treatments (Table 7). On the other hand, significant ($P < 0.001$) difference was observed between varieties. The interaction between pulsing treatments and varieties was found to be not significant for this parameter. Throughout the sampling periods, Viva contains more petal moisture followed by Akito and Red calypso, respectively. Water is continually being lost in cut flowers through transpiration and because of a decrease in water conductance in the stem, which results in dropping of flowers and the premature wilting of both flowers and leaves (Teixeira da Silva, 2003). *Zinnia elegans* L. and *Dianthus caryophyllus* L. stems harvested from plants that received a water stress treatment took up more water and were less likely to wilt than stems harvested from well-watered plants (Twumasi *et al*., 2005). Cut *Dendranthema grandiflorum* Tzvelev flowers grown at 20% substrate water content were able to rehydrate after experiencing dry storage, but flowers grown at 70% substrate water content could not (van Meeteren *et al*., 2005). So, it is necessary to link irrigation condition to post harvest handling as it affects rehydration. Work is needed to determine if short-term water stress immediately prior to harvest increases water uptake and vase life. Symptoms of water deficit as a result of transpiration, a decrease in water uptake, low hydraulic conductance and loss in fresh weight make cut flowers stems unacceptable by consumers.

Total Soluble Solids (TSS)

Significant differences were observed in terms of petal TSS values among the different pulsing treatments and varieties throughout the sampling periods (Table 7). Pulsing treatments of biocides and vase solution was significantly ($P < 0.001$) different on the 3rd and 7th day and significantly ($P < 0.001$) different on 11th day when compared with the control. In general, there was no consistency of TSS values due to the pulsing treatments during the three sampling periods. It was observed that Red calypso exhibited the highest TSS value while Akito the lowest and Viva was found to be intermediate between the two throughout the sampling period. One of the important factors that affect longevity of cut flowers during vase life is diminishing of respiratory substrates (Rogers, 1973), whose speed of change depend, at least in part, on the amount of reserves that are present in the flower when they are cut and on the exogenous sugar application to the vase solution (Pun and Ichimura, 2003). Carbohydrates are important reserve compounds, being sucrose the most abundant soluble carbohydrate, sometimes the only one in the phloem sap.

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Table 7. Effects of pulsing solutions and varieties on water content ratio and total soluble solid percent of petals evaluated at room conditions

Factors	Treatments	Water content ratio (g/g)			Total soluble solid (%)		
		Vase life in days			Vase life in days		
		3	7	11	3	7	11
Pulsing solutions	Control	5.43^{a}	6.83^{a}	5.90^{a}	7.28^{ab}	7.78^{c}	10.50^{ab}
	$Al_2(SO4)_3$	5.48^{a}	5.43^{a}	4.66^{a}	7.06^{ab}	8.50^{ab}	11.44^{a}
	HQS	6.20^{a}	5.96^{a}	4.76^{a}	6.11^{c}	8.94^{a}	11.50^{a}
	$Ca(ClO)_2$	6.62^{a}	7.24^{a}	5.65^{a}	6.06^{c}	8.44^{ab}	11.33^{a}
	NaOCl	6.01^{a}	5.60^{a}	5.99^{a}	6.56b^{c}	7.44^{c}	10.33^{b}
	long life	6.26^{a}	6.00^{a}	4.78^{a}	7.44^{a}	8.89^{a}	10.78^{ab}
	Distilled water	5.66^{a}	6.48^{a}	5.49^{a}	7.11^{ab}	8.33^{b}	10.56^{ab}
	F-test	Ns	Ns	Ns	***	***	***
	SEM±	0.58	0.54	0.53	0.26	0.17	0.31
Varieties	Red calypso	4.45^{c}	4.50^{c}	3.60^{c}	7.12^{a}	8.64^{a}	12.14^{a}
	Akito	5.19^{b}	5.87^{b}	5.36^{b}	6.50^{b}	7.81^{c}	9.90^{c}
	Viva	10.4^{a}	10.00^{a}	8.60^{a}	6.78^{ab}	8.55^{b}	10.71^{b}
	F-test	***	***	***	***	***	***
	SEM ±	0.40	0.35	0.34	0.17	0.11	0.20
	CV (%)	26.82	24.48	26.79	12.76	6.68	8.5

SEM= standard error of the mean. Ns and *** indicate non significant $P > 0.05$ and significant at $P < 0.001$ probability level, respectively. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$ probability level.

The result showed that, as the vase life days proceed up to 11th day, the TSS% is increasing on all the treatments. This is in agreement with the finding of Van Doorn (2001) who observed that the level of fructose and glucose increase rapidly in petals at the time of flower opening and continues to increase until the petals are about to drop.

Freshness of Flowers

The effect of pulsing with biocides and preservative solution on freshness of flowers during the 7th, 10th and 13th days in the vase is presented in (Table 8). In the early seven days of vase life, sign of wilting was not observed in all of the pulsing treatments. However, during the 10th and 13th days of evaluations there were distinct differences among the treatments in such a way that the control flowers showed a sign of wilting and those treated with NaOCl remained fresh. The cut flower stems pulsed with $Al_2(SO_4)_3$ reached more than the maximum score of loss in freshness during the 13th days of evaluation. The varieties and their interaction with pulsing treatments did not show significant differences with respect to flower freshness throughout the sampling periods.

The effect of long life pulsing in keeping the flower stems fresh seems to be mainly associated with its effect on ethylene action since the ready foods of flowers contain anti ethylene compounds, which is beneficial for maintenance of flowers as fresh as possible for a longer period of time. In this connection, Joyce and Jones (1992) and Mayers *et al.* (1997) have shown that most of the cut flower species pulsed with silver thio sulphate (STS) are able to maintain their freshness as compared with untreated controls. Premature flower fall or abscission and flower senescence (wilting) are ethylene-related postharvest problems for a number of cut flowers (Mayers *et al.*, 1997). Reid (1985) also noted that unintentional exposure to ethylene can reduce postharvest life of cut flowers by eliciting abscission and/or accelerating senescence. Such effects of

ethylene can however be prevented by pre-treating sensitive cut flowers with chemical inhibitors of ethylene biosynthesis or perception (Sherman, 1985).

Table 8. Effect of pulsing solutions and varieties on freshness of flowers and petal discoloration evaluated at room conditions

Factors	Treatments	Freshness of flower on 1-5 scale			Petals discoloration on 1-3 scale		
		Vase life in days			Vase life in days		
		7	10	13	7	10	13
Pulsing solutions	Control	1.08^{a}	2.83^{a}	4.33^{a}	1.08^{a}	2.50^{a}	3.00^{a}
	$Al_2(SO4)_3$	1.00^{a}	2.33^{b}	3.50^{b}	1.00^{a}	1.75^{c}	2.92^{a}
	HQS	1.00^{a}	1.67^{d}	3.00^{c}	1.00^{a}	1.75^{c}	2.08^{b}
	$Ca(ClO)_2$	1.00^{a}	1.83^{cd}	3.00^{c}	1.00^{a}	1.75^{c}	2.08^{b}
	NaOCl	1.00^{a}	1.58^{d}	2.25^{e}	1.00^{a}	1.58^{c}	2.00^{b}
	long life	1.08^{a}	2.00^{c}	2.92^{d}	1.08^{a}	1.91^{bc}	2.08^{b}
	Distilled water	1.08^{a}	2.58^{ab}	2.92^{d}	1.08^{a}	2.17^{ab}	2.92^{a}
	F-test	Ns	***	***	Ns	***	***
	SEM±	0.05	0.12	0.15	0.05	0.13	0.07
Varieties	Red calypso	1.00^{a}	2.14^{a}	3.25^{a}	1.00^{a}	1.86^{a}	2.46^{a}
	Akito	1.03^{a}	2.03^{a}	3.28^{a}	1.03^{a}	1.89^{a}	2.39^{a}
	Viva	1.07^{a}	2.18^{a}	3.28^{a}	1.07^{a}	2.00^{a}	2.46^{a}
	F-test	Ns	Ns	Ns	Ns	Ns	Ns
	SEM ±	0.03	0.08	0.08	0.03	0.09	0.05
	CV (%)	18.24	19.26	15.5	18.24	14.37	9.99

SEM= standard error of the mean. Ns and *** indicate non significant at $P > 0.05$ and significant at $P < 0.01$ probability level, respectively. Means of the same main effect within a column followed by the same letter(s) are not significantly different at $P < 0.001$ probability level.

Furthermore, the effects of sugars on the maintenance of flower freshness have also been reported by Liao *et al*. (2000) and Pun and Ichimura (2003). However, Pun and Ichimura (2003) said that the role of sucrose in the senescence of cut flower species is not fully understood. While Liao *et al*. (2000) suggested that the beneficial effects of sugars in prolonging flower vase life in rose have been attributed to the suppression of ethylene biosynthesis or sensitivity to ethylene. In this connection, Joyce *et al*. (1996) reported that the vase life of cut *Grevillea* flowers is particularly limited due to rapid senescence and associated wilting, abscission and color fading. Hammer *et al*. (1990) also reported that provision of sucrose in the vase solution raised the steady state respiration level of *Grevillea* 'Sylvia'. Apparently, effectiveness of long life pulsing in prolonging the vase life of cut flowers could also be through providing additional substrates for respiration, improving the water balance, delaying ethylene production and decreasing the sensitivity to ethylene.

Petals Discoloration

In all of the treatments, petal color was not affected by the pulsing treatments, varieties and their interaction on the first seven days of vase life (Table 8). However, on the 10th and 13th day after pulsing, petal discoloration was much higher in cut flower stems pulsed with the control (tap water). The best treatments that maintained typical varietal petal color of the flower stems were pulsing with NaOCl, $Ca(ClO)_2$, HQS and long life (Table 8). On the other hand, cut flower stems pulsed with $Al_2(SO_4)_3$, distilled water and control significantly lost their varietal

color during the final sampling period, 13th day. In the present study, the reduction of petal discoloration due to biocides and preservative solution could be attributed to the availability of substrate for respiration and reduction of microbial proliferation that xylem blockage to keep the cut flowers fresh for extended period of time, without losing their aesthetic value.

In general, the results of the present study are in agreement with that of Macnish *et al*. (1999) who did not observe flower discoloration in the early stages of *B. heterophylla* treatment. Presumably, it might be the stored food which was sufficient for respiration that retarded biochemical changes. Lower physiological alteration during the early periods of vase life might have also resulted in minimal discoloration of petals.

Biocides had a fundamental benefit in delaying the discoloration rate whereas fading of the petals color was most intensive in $Al_2(SO4)_3$ treatment. This confirms that $Al_2(SO4)_3$ is phytotoxic to the petals too, which resulted in discoloration scale of (2.92) which is the nearest to advanced discoloration in control (score of 3). Ageing of petals come with change due to deterioration in a biochemical characteristic which includes increase in activity of hydrolytic enzymes, degradation of starch and chlorophyll and loss of cellular compartmentation (Nooden, 1988; and van Altvorst and Bovy, 1995). In addition, Halevy et al. (1996) reported that physiological changes result in surge of respiration and ethylene biosynthesis which are accompanied by senescence.

CONCLUSION

The Ethiopian cut flower industry has a vital stake in overcoming the poor postharvest handling reputation of cut roses in order to exploit their maximum export potential. The biocide $Al_2(SO_4)_3$ which has been the most common biocide used in extending the vase life of cut flower stems by most of the Ethiopian cut flower growers was not found effective as compared to the other biocides-NaOCL1[1] and HQS in the present study. It was also observed that the quality of water to be used in the vase determines the effectiveness of the biocides to be used. In addition, the use of long life (flower food) as a vase/holding solution was found to be interestingly effective in maintenance of physiological characteristics, subjective to assessments in terms of extending the vase life of the cut flower stems of the three rose varieties.

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