Full Length Research Paper

Pulsing of Carbohydrates, biocides, and ethylene action inhibitor on vase life of cut rose (Rosa hybrida L.) flowers

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Accepted 21st January, 2014

Pulsing solutions comprised of ethylene action inhibitor-sliverthio-sulphate (STS), carbohydrates (CHOs) and biocides (BIOs) on the vase life of rose flower cultivar ‘Red Calypso’ was evaluated experimentally in a laboratory. The treatments were arranged in a 2×4×3 factorial combinations that included STS (0 or 0.225 mM), CHOs (0, D+ Sucrose (Suc) (20 g l⁻¹), D- Glucose (Glu) or D- Fructose (Fru) (10 g l⁻¹) each) and BIOs (0, Aluminium sulphate-Al₂(SO₄)₃ (1 g l⁻¹) or 8-Hydroxyquinoline sulphate-HQS (0.4 ml l⁻¹), in a Completely Randomized Design (CRD), with three replications. To simulate the common practice exercised by Ethiopian flower growers, each combinations were dissolved in tap water and the flower stalks were pulsed for 24 h in a cold room (5 ± 1°C) and maintained in 500 ml vase of flower food (10 g l⁻¹). The vase life was significantly (P<0.001) promoted by the pulsing solutions comprising of STS, Glu and HQS. The three way interaction effect resulted in maximum flower head diameter (MFHD), lowest vase solution absorbance (VSAbS), and increase in the stem hydraulic conductance (HC) at three positions. However, in the presence or absence of STS the effect of Al₂(SO₄)₃ and/or Suc was lowest in maintaining vase life of the cut flower stalks. In addition, the lowest MFHD and HC were observed in this treatment, while higher VSAbs were recorded. The pulsing treatment (STS + Glu + HQS) also significantly (P<0.001) increased vase solution uptake during vase life. Whereas, relative fresh weight (RFW) showed insignificant (P>0.05) differences among the pulsing solutions throughout the vase life. At the late days of vase life, the total soluble solid (TSS) was significantly (P<0.001) different among the treatments. Hence, combination of STS, Glu and HQS for pulsing ‘Red Calypso™’ cut flower stalks is highly recommended.

Key words: Rose, pulsing, vase life, carbohydrates, biocides, ethylene action inhibitor.

INTRODUCTION

Rose (Rosa hybrida L.) is one of the most important ornamental plants that belong to the family Rosaceae (Ritchie, 1997). It is a luxury commodity grown for cut flower production, and undoubtedly remain the queen of cut flowers. The historical association of this flower with romance and beauty ensures that roses will continue to be highly desired cut flowers in the future. Therefore, the floriculture business greatly deals with rose production (Butt, 2003) as compared with other ornamental crops.

Decades have elapsed since cut flowers began to be produced in Ethiopia for commercial purposes (Lemma and Edward, 1992). The majority of flower growers, with an estimated 80% of the production area, in the country cultivate roses (EHPEA, 2008), while some of the production area is under cultivation of cuttings and bouquet fillers, primarily Hypericum, Carnation, Gypsophila, Allium and Carthamus. Ethiopia is the second largest flower exporter in Africa next to Kenya. It produces large budded and long stemmed roses with
vibrant colors. The country is blessed with a favorable climate and vast land, water and labor resources which together make it an incredible investment hub. There is also a positive trend in the country’s export statistics of floriculture produce; conversely, the foreign currency earnings from the floriculture sector are still very low.

In flower industry, consumer and florists’ always demands and interests for new cut flower species, so does the quality and longevity of these precious produces (Teixeira da Silva, 2003).

To meet such objectives and satisfy the need of an evolving cut flower market, constant adoption of regulatory and evaluation parameters are inevitable. Delaying the rate at which the quality of such perishable commodities deteriorate and/or extending their natural appearance during vase life (Chapman and Austin-brown, 2007), may provide additional time for harvesting and delivering the crop to wholesalers and retailers, and ultimately to the consumers.

Generally, maximum vase life is essential to assure both buyer and consumer confidence in the cut flower industry. However, about 20% of the total fresh produce is known to be lost in between the time of leaving the farm and reaching the consumer (Panhwar, 2006). The losses could result in complete wastage of the produce or lower prices due to reduced quality. Since the value of the produce loss increases several fold from the farm gate to the final consumer, postharvest losses are even more significant. Many commercial cultivars of roses are also quite sensitive to postharvest ethylene problem (Reid, 2004). Thus, incorporation of different chemical preservatives to the holding (vase) solution is recommended to prolong the vase life of cut flowers (Ichimura et al., 2006). Commercial postharvest solutions are products used in bulk by growers, wholesalers, exporters and importers and ‘flower foods’ are those products that are made for use in vases, by retailers and customers (Faragher et al., 2002). They provide an easy, accurate, sometimes well-proven and often economical treatment for flowers. Nowadays, a wide range of commercial products are available, from general purpose solutions to special purpose solutions such as bud opening, anti-ethylene treatments, hydrating solutions and solutions for specific flowers.

Floral preservative/holding solutions are mainly comprised of sugar/carbohydrates and biocides sources (Ichimura et al., 2006). The sugars provide a respiratory substrate, while the biocides control harmful bacteria and prevent plugging of the conducting tissues. Longevity of flowers has been associated with the sugar content of the flower. Thus, the quality and vase life of many cut flowers can be improved when pulsed immediately after harvest with a sugar solution, for a short period, and often at low temperature (Ichimura and Suto, 1999). This beneficial effect of sugars on flower senescence was attributed to the supply of substrates for respiration, structural materials and osmoticum (Pun and Ichimura, 2003). Biocides also improve water uptake and balance, and prevent the plugging of stems caused by microbial growth. According to Ichimura et al. (1999), treatment with sucrose and 8-hydroxyquinoline sulphate (HQS) extended the vase life of cut roses more than HQS alone. Thus, the prominent influence of a biocide is vital when it complements with carbohydrates. Furthermore, Ichimura et al. (2005) noted that treatments with some biocides, such as silver nitrate, HQS and sodium dichloro-isocyanurate, maintain the hydraulic conductance of the stems of cut roses and extended their vase life. Similarly, germicides like silver nitrate, and Al_{2}(SO_4)_{3}, have been shown to inhibit bacterial growth in cut rose stems (van Doorn, 1997). However, different compounds can result in different effect on the vase life of different cut flowers. For example, the biocide sodium benzoate was effective both in improving water uptake and preventing bacterial growth (Knee, 2000), but did not greatly extend flower longevity.

Similarly, the adverse effects of ethylene on the vase life of cut flowers can also be prevented by pre-treating ethylene sensitive cut flowers with chemical inhibitors of ethylene biosynthesis or perception (Sherman, 1985). Thus, treatments that inhibit ethylene biosynthesis or action can be used to protect rose cut flowers against exposure to ethylene (Redman et al., 2002). Roses should be pre-treated with STS or 1-MCP to prevent the effects of ethylene, especially if they would be sold through a supermarket (Reid, 2004). A delay in fresh weight loss by STS and 1-MCP pre-treatment in ethylene-treated flowers has also been reported for *Boronia hetrophylia* (Macnish et al., 1999) to be effective in maintaining freshness of the flower.

Cuttings from ornamental plants, particularly rose cut flowers, have a limited shelf life. Methods of maintaining the quality of these fresh products over time, such that the consumer may be able to still enjoy them after harvest have improved dramatically. The appearance, quality, and longevity of cut flowers depend upon the conditions of cultivation, proper harvest time, product transport conditions and postharvest handling, which are dependent on the stresses imposed upon them: decrease in water uptake, transpiration, stem hydraulic conductivity, fresh weight, and water content of the cut flowers. Once flowers are purchased, the longer they last in a vase solution or flower arrangement, the longer the purchaser would have to enjoy the aesthetic qualities, fragrance, and appearance of cut flowers. The cut flower industry has a vital stake in overcoming the poor postharvest reputation of cut roses. Thus, this research is initiated with the objectives of determining the effect of pulsing solutions on the physiological characteristics of fresh cut rose during vase period.
MATERIALS AND METHODS

Cut flower stalks of ‘Red calypso™’, a bright red rose cultivar, were obtained from a local greenhouse owned by the Top Rose Flower P.L.C., Ethiopia. The chemicals used in the study, STS™,75 Improved, HQS (T.O.G-30) and long life were obtained from Milchan Bros Ltd., Israel, while [D- Glucose AR], [D- Fructose, extra pure], and [D+ Sucrose GR6] were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai. The pulsing treatments were arranged in 2×4×3 factorial combinations that included 2 levels STS (0 control and 0.225 mM STS), 4 levels of CHO's (0 control, D-Glucose (10 g l⁻¹), D- Fructose (10 g l⁻¹) and D+ Sucrose (20 g l⁻¹)) and 3 levels of BIOs (0 control, Al₂(SO₄)₃ (1 g l⁻¹) and HQS (0.4 ml l⁻¹), in CRD (completely randomized design) with 3 replications. The pulsing solutions were prepared from tap water and the pH was adjusted to 3.5–4.5 with citric acid, except that of Al₂(SO₄)₃ which was adjusted to a pH of 3.5, with KOH. The cut flowers were harvested early in the morning at normal harvest maturity stage 0, as described by Capdeville et al. (2005). The lower leaves from all of the stems were trimmed off to a height of 20 cm from the base. The stem ends were re-cut under water to prevent embolism and each stem used in the different experiments had a length of 45 cm. During the initial 24 h of the pulsing treatment, flower stalks were placed in 1 liter capacity glass vases that contained 500 ml of each pulsing solution.

To simulate the practice exercised by the growers, the flower stalks were kept in a cold room (5±1°C). Following the pulsing treatments the flower stalks were removed from the cold room and the pulsing solutions were replaced with 500 ml vase solution that contained flower food at a concentration of 10 g l⁻¹. Evaluations were made for the duration of 16 days of vase life, by keeping the flower stalks in vase evaluation room, at ambient condition with 12 h of photoperiod with cool-white fluorescent lamps. The flower stocks were maintained in this solution until completion of the experiment. In studying the effect of the different pulsing solutions on the vase life of the cut flower stalks, the experiments were divided into two groups. The first group contained none destructive measurements with 4 flowers per treatment; and a total of 288 cut flower stalks were used. While the second group contained destructive measurements with 5 flowers per treatment; and a total 360 cut flower stalks were used. In the destructive experiment, the total soluble solid was evaluated.

Significance tests were made by analysis of variance (ANOVA) for Complete Randomized Design in factorial arrangement according to Gomez and Gomez (1984). The ANOVA was carried out using SAS procedure of version 9 (SAS institute, 2002) and Crop Stat 7.2. Mean comparisons were made using least significance difference (LSD).

Data collected

Flower longevity (FLNG) were recorded as described by Mayak and Halevy (1974) and Liao et al. (2000), as the number of days elapsed after harvest (day 0) until the flowers showed symptoms of bent neck or advanced signs of fading on all petals.

Maximum flower head diameter (MFHD)

The rate of flower opening was measured from the time of petal unrolling to the time when the outer most petals reach 90° with respect to the vertical.

Solution turbidity of microbial count assessment (VS Abs)

Solution turbidity attributable to microbial growth was assessed at the end of the experiment by measuring absorbance's at 400, 500 and 600 nm with a spectrophotometer, and calculating the mean of these values (Knee, 2000). Distilled water was used as a blank.

Hydraulic conductance of stem segment

Hydraulic conductance (HC) was determined as described previously by van Doorn et al. (1989) with some modification, in stem sections excised at 3 positions (0 to 5, 10 to 15, 20 to 25 cm) from the base at the end of the experiment. Stems were cut under water into 5 cm long segments, and were inserted into tygon tubes to which 130 cm head pressure of water (130 KPA) was connected. The tubes were connected to the segments after two hours of equilibration and water flowing through the segments was collected in plastic containers. The flow rate was determined by measuring the amount of water passed through the segments after thirty minutes.

Solution uptake (S)

The volume of solution 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 formula
Table 1. The three way interaction effect of STS, CHOs, and BIOs on FLNG, MFHD, and VSAbs of rose cultivar ‘Red Calypso™’ cut flower stalks. Data represent means of 3 replicates (4 flowers each) per treatment ± SE.

\[ S = \frac{(S_2 - S_1) - (S_4 - S_3)}{Wt_0} \]

Where:
- \( S \) is solution uptake (ml / day / g fresh weight)
- \( S_t \) is solution weight (g) at \( t \) = days 1, 2, 3, etc.
- \( S_{t-1} \) = Solution weight (g) on the previous day, and
- \( Wt_0 \) = Fresh weight of the stem (g) on day 0.

**Relative fresh weight (RFW)**

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 a time as possible, 20-30 s. 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). The RFW was expressed as a percentage of initial fresh weight.

\[ \text{RFW} (%) = \left( \frac{Wt}{Wt_0} \right) 100 \]

Where:
- \( Wt \) = Weight of stems (g) at different days (t)
- \( Wt_0 \) = Weight of the same stalk (g) on day 0.

**Determination of total soluble solids (TSS)**

Tissue sap was extracted by squashing the petals. The TSS in the sap was measured with a table refractometer and expressed as % sugar within four days interval.

**RESULT AND DISCUSSION**

The results depicted that FLNG, MFHD, and VSAbs were significantly \((P<0.001)\) different for the pulsing treatments that comprised STS, CHOs, BIOs, and their interaction. The results depicted that a combined pulsing treatment of STS, Glu and HQS extended the vase life up to 17 days, largest MFHD, while lower VSAbs values were recorded (Figure 1A, B and C, respectively).

The presence/absence of STS pulsing treatments that comprised (HQS), (Fru + HQS) and (Suc + HQS) also comparatively extended the vase life of the cut flower stalks. On the other hand, the inclusion \(\text{Al}_2(\text{SO}_4)_3\) in the pulsing solutions significantly \((P<0.001)\) lowered the vase life of the cut flower stalks. Addition of STS in the pulsing solution did not improve the effect of \(\text{Al}_2(\text{SO}_4)_3\), except in the presence of Suc±STS, which was not significantly lower than that of the control treatment; addition of either Glu or Fru in the pulsing solutions of \(\text{Al}_2(\text{SO}_4)_3\) had little effect in promoting the vase life of the cut flower stalks (Figure 1A).
In comparing the MFHD of 'Red Calypso' cut flower stalks, significant differences were observed among pulsing solutions that comprised CHO$_2$s (P<0.01), BIO$_2$s (P<0.001) and their interaction (P<0.001). The smallest flower head diameter (5.78 cm) was recorded in cut flowers pulsed with (STS + Glu + Al$_2$(SO$_4$)$_3$). Interestingly, the MFHD recorded 9.73 cm in the cut flowers pulsed with Glu with zero levels of STS (Figure 1B).

The VS Abs is the spectrophotometric value of the vase solution which is the indirect way of determining the degree of cleanliness of the vase and has direct relationship with the solution microbial concentrations and other impurities. The smallest VS Abs value (0.009) was recorded in the presence of HQS but with zero levels of both STS and CHO$_2$s (Figure 1C). On the other hand, higher VS Abs values were recorded in pulsing treatments that contained (STS + 0 CHO$_2$s + Al$_2$(SO$_4$)$_3$), (STS + Glu + Al$_2$(SO$_4$)$_3$) and (Suc + Al$_2$(SO$_4$)$_3$) + STS ).

High values of VS Abs were also recorded in cut flowers pulsed with tap water (control), ([0 BIO + Suc] + STS). In general, adding HQS in the pulsing solution significantly lowered the VS Abs value of the vase solution.

Promotion of the vase life of Red Calypso by STS shows the importance of this compound. The results of this study confirmed with the outcome obtained by Liao et al. (2000) who showed an increase in the longevity of cut rose flowers, cultivar Diana, pulsed for 2 h with 0.2 mM STS. In addition, Mor and Halevy (1984) indicated that a pulse treatment with STS or (STS + 4% Suc) was markedly effective in extending the vase life of cut sweet pea flowers, which is in agreement with the results observed in this experiment. Although the amount of ethylene evolved was not measured in this experiment, STS might have been slightly effective in...
Figure 3. The effect of CHOs and biocide (HQS) on the aesthetic quality of the cut flower stalks.

reducing the action of ethylene. Regarding the type of sugars used, the results obtained were in agreement with the study of Ichimura et al. (2006) who reported extension of the vase life of rose cultivar ‘Rote Rose’ pulsed with solution containing sugars. The authors reported that Glu followed by Fru and Suc was the most effective treatment in prolonging the vase life of rose cultivar ‘Rote Rose’ flowers. However, Figure 1A showed that the lowest vase life was recorded in pulsing solutions that consisted Al$_2$(SO$_4$)$_3$. Lack of improvement in the longevity of the cut flower stalks pulsed with solutions that comprised Al$_2$(SO$_4$)$_3$ even in the presence of STS might be as a result of Al$_2$(SO$_4$)$_3$ toxicity to the cut flower stalks, which was also witnessed from scorching of petals and abscission of leaves.

Although the type of sugar significantly influenced the MFHD, flower stalks pulsed with water but returned to long life (control) were able to fully open during vase life, suggesting that long life was able to provide the respiration energy required by the cut flowers for head opening. Ichimura et al. (2002) showed an increase in flower diameter when 20 g of sucrose l$^{-1}$ + 200 mg of HQS l$^{-1}$ were used in the pulsing solution, which of course varied among the varieties tested, Bridal Pink (124 mm) and Calibra (75 mm). Similarly, an increase in flower head diameter was observed in rose cultivars ‘Sonia’ and ‘Delilah’ (Ichimura et al., 2005) with identical treatments. The lowest MFHD in response to Al$_2$(SO$_4$)$_3$ pulsing seems to be due to the adverse effects of the chemical for this particular rose cultivar. Either rose cultivar ‘Red Calypso’ is sensitive to Al$_2$(SO$_4$)$_3$ l$^{-1}$ or the dosage could be high for this particular rose cultivar. In addition, there are possibilities for complex formation between Al$_2$(SO$_4$)$_3$ and other compounds present in the pulsing and/or vase solutions which are detrimental to the flower stalks vase life. Moreover, lower flower head opening could also be associated with reduced antimicrobial activity of Al$_2$(SO$_4$)$_3$ as compared with HQS or may be due to some other reasons. These phenomena evidently need further investigation.

The inability of STS to lower VSAbs might be due to its failure in controlling microbial activity while that of CHOs could be associated with proliferation of microbes in the vase solutions. A significant increase in VSAbs due to Suc pulsing could be attributed to its enhanced simulative effect on microbial proliferation as compared with that of Glu and Fru. In addition to the proliferation of bacteria due to exogenously applied CHOs in the vase solutions, endogenous metabolites such as CHOs, amino acids and amides, could also leach into the vase solution to become substrates for microbial growth (Halevy et al., 1974). The reduction in VSAbs for the pulsing treatments that comprised HQS could be due to the antimicrobial activity of this biocide. The HQS is a well-known antimicrobial compound (Ichimura et al., 2006). The presence of HQS in the pulsing solutions was the most effective treatment in reducing the VSAbs increasing clearness of the vase solution, which could be due to its negative effect on
Figure 4. The effect of STS, CHO, and biocide (HQS) on the aesthetic quality of the cut flower stalks.

Figure 5. The effect of Al$_2$(SO$_4$)$_3$, STS, as well as CHO on the aesthetic quality of the cut flower stalks.

microbial proliferation. On the other hand, the higher VSAbs values recorded in cut flower stalks pulsed in the presence of Al$_2$(SO$_4$)$_3$ could be associated with its failure to clarify the vase solution. Knee (2000) reported
High values of VSAbs, in the absence of BIOs and/or in solutions that contained Al₂(SO₄)₃.

Hydraulic conductance

A significant ($P<0.001$) difference in the HC of stem sections excised from different positions of the stem were recorded due to pulsing of the flower stalks with either STS, CHObs, BIOs, and their interactions. In cut flower stalks pulsed with STS per se, significant ($P<0.01$), ($P<0.01$) and ($P<0.05$) differences in HC of the respective stem sections were recorded. Generally, the lowest HC value was recorded in stem sections excised at 0 to 5 cm from the base, while the highest HC value was recorded in stem sections excised at 20 to 25 cm from the base of the flower stalks. The result showed that the pulsing of the cut flower stalks with inclusion of (HQS ± STS) significantly improved the stem HC of the cut flower stalks (Figure 3). The maximum stem HC was observed in flower stalks pulsed with solutions that contained (0 STS + Glu + HQS), (0 STS + 0 CHO + HQS) or (STS + 0 CHO + 0 BIO). Whereas, inclusion of Al₂(SO₄)₃ in the pulsing solution, the stem HC was the lowest; even if Al₂(SO₄)₃ was combined with (STS ± CHObs). In the pulsing solution that contained Al₂(SO₄)₃ the presence of STS slightly increased the stem HC in sections excised at different stem positions, except in the control treatment devoid of CHObs.

The lowest stem HC of rose cultivar was found in the basal parts of the stem, this is also in accordance with the study of van Doorn et al. (1989), who reported a positive correlation between the number of bacteria in lower parts of the stems and development of xylem blockage. Ohkawa et al. (1999) also observed inhibition of bacterial proliferation and maintenance of the HC of the stem following pulsing treatment of cut flower stalks with silver nitrate.

Yet, the mechanism by which STS pulsing has increased HC of the cut flower stems is not well understood and there are limited studies on the antimicrobial activity of STS. According to van Doorn et al. (1989) STS pretreatment had effect neither on the number of bacteria nor on HC. The controversy between the results obtained in the present study and that of the above author could be due to the effect of...
long life, which might have an antimicrobial effect and thus reduced bacterial proliferation xylem blockage during vase life of the cut flower stalks. The significant \( P<0.001 \) increase in HC of the three stem sections in response to pulsing of 'Red Calypso™' cut flower stalks with CHOs, suggests that Glu was able to enhance water flow as compared with other CHOs. This might be ascribed from high uptake of Glu than Fru and Suc, as it was suggested by Ichimura et al. (2006). Accordingly, the higher the uptake of Glu by the flower stalks as compared with Suc, the lesser will be the amount of Glu in the vase solution to support bacterial proliferation and increase HC. In this study, it appears that inclusion of HQS in the pulsing solution water relation of the cut flower stalks was maintained and FLNG was promoted subsequently. Whereas, vase life was impeded in pulsing solutions that had high VSAbs and low stem HC, which are indications of bacterial proliferation and xylem blockage. Similar result was also predicted by Ichimura et al. (2005) that vascular occlusion is not associated with longer vase life of cut 'Delilah' vs. 'Sonia' flowers. According to Halevy and Mayak (1981) pulsing of flowers with \( \text{Al}_2(\text{SO}_4)_3 \) has been used as a microbial inhibitor in commercial preservatives. However, it is clearly observed in Figure 4 that the presence of \( \text{Al}_2(\text{SO}_4)_3 \) in the pulsing treatment resulted in the lowest HC.

The inhibitory activity of Al ions in bacterial proliferation may not be associated with high VSAbs and low stem HC, which are indications of bacterial proliferation and xylem blockage. Similar result was also predicted by Ichimura et al. (2005) that vascular occlusion is not associated with longer vase life of cut 'Delilah' flowers. According to Halevy and Mayak (1981) pulsing of flowers with \( \text{Al}_2(\text{SO}_4)_3 \) has been used as a microbial inhibitor in commercial preservatives. However, it is clearly observed in Figure 4 that the presence of \( \text{Al}_2(\text{SO}_4)_3 \) in the pulsing treatment resulted in the lowest HC. This indicates that ineffectiveness of \( \text{Al}_2(\text{SO}_4)_3 \) may not be associated with the inability of the BIO in inhibiting stem vascular occlusion due to bacterial proliferation.

Table 2. Effects of STS, CHOs, BIOs, and their interaction on the relative fresh weight (RFW) and Total soluble solid (TSS) of rose cultivar 'Red Calypso™' cut flower stalks, at 5 ± 1°C and their subsequent vase life in long life, at ambient conditions.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Treatments</th>
<th>Relative fresh weight (RFW) in (%) on day</th>
<th>Total soluble solid (TSS) in (%) on day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene action inhibitor</td>
<td>Control</td>
<td>102.49</td>
<td>108.89</td>
</tr>
<tr>
<td></td>
<td>STS</td>
<td>102.41</td>
<td>107.66</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LSD (_{(0.05)})</td>
<td>0.492</td>
<td>1.94</td>
</tr>
<tr>
<td>CHOs</td>
<td>Control</td>
<td>102.45</td>
<td>107.38</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>102.64</td>
<td>109.89</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>102.58</td>
<td>107.75</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>102.13</td>
<td>108.08</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LSD (_{(0.05)})</td>
<td>0.696</td>
<td>2.742</td>
</tr>
<tr>
<td>BIos</td>
<td>Control</td>
<td>102.35(^b)</td>
<td>107.67(^c)</td>
</tr>
<tr>
<td></td>
<td>( \text{Al}_2(\text{SO}_4)_3 )</td>
<td>101.92(^c)</td>
<td>106.19(^b)</td>
</tr>
<tr>
<td></td>
<td>HQS</td>
<td>103.08(^b)</td>
<td>110.97(^b)</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>LSD (_{(0.05)})</td>
<td>0.603</td>
<td>2.375</td>
</tr>
<tr>
<td>Interaction significance</td>
<td>STS;CHOs</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>STS BIOs</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>CHO BIOs</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>STS CHOs BIOs</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Where NS, *, **, *** indicates nonsignificant or significant difference at p < 0.05, 0.01, or 0.001 respectively. LSD \(_{(0.05)}\) = Least Significant Difference at 5% level of significance. Means within a column followed by the same letter(s) are not significantly different in LSD test; Data represent means of 3 replicates.
It seems \( \text{Al}_2(\text{SO}_4)_3 \) may be toxic to the rose cultivar ‘Red Calypso™’ and caused a reduction in its vase life. In contrast, maintenance of S uptake of the cut flower stalks with HQS suggests that HQS might have inhibited the development of vascular occlusion. This result agrees with previous reports by Ichimura et al. (2006) that HC of stem segments in stalks pulsed with Glu + HQS remained nearly constant throughout the experimental period. These findings appear to support the view that vascular occlusion is due to mainly bacterial proliferation. van Doorn et al. (1989) showed that the development of vascular occlusion was correlated with growth of bacteria at the cut surface and inside the stem. Thus, inhibition of vascular occlusion with HQS might be attributed to its antimicrobial action, which could also be the reason for the significant correlation observed between HC and FLNG, S, and RFW (Table 2).

Solution uptake

Termination of vase life for many cut flowers is characterized by wilting. Generally, wilting is caused by an imbalance between S uptake by the flowering stems and water loss via transpiration from their leaves and/or other organs despite of their stem being held in water continuously (Halevy and Mayak, 1981; van Doorn, 1997). The results in Table 1 show an increase in S uptake of the flower stalks during the first 9 days of vase life, independent of the composition of the pulsing treatments except 0 CHO and \( \text{Al}_2(\text{SO}_4)_3 \). The rate of S uptake by the cut flower stalks was significant (\( P < 0.001 \)) in those flowers that were pulsed with STS and BIoS throughout the study period, except that some inconsistencies were observed in CHO pulsing treatment at the later period of vase life. Pulsing the cut flower stalks with HQS significantly (\( P < 0.001 \)) increased S uptake during the entire period of vase life, as compared with \( \text{Al}_2(\text{SO}_4)_3 \) and (0 BIo) the control. The interaction effects of (STS + CHO) were significant after day 3 of pulsing, whereas that of (STS + BIo) was inconsistent (Table 1). On the other hand, the interaction effect of (CHO + BIo) and the three way interaction effect of (STS + CHO) + BIo gave a significant (\( P < 0.001 \)) difference in S uptake (Figure 2).

The rates of vase S uptake by Gerbera ‘Monarch’, Gypsophila ‘Crystal’ and Matthiola ‘Ruby Red’ stems were highly variable but generally decreased over time (Knee, 2000). The difference in S uptake of the cut flower stalks between STS pulsing and the control suggests that STS may have lower effect in promoting S uptake. A pulsing treatment of ‘First Red’ rose flower stalks with STS maintained S uptake throughout the vase life (Chamani et al., 2005). The authors suggested that the response of the cut flowers to STS pulsing appears to be the result of its effect on stem water relations rather than an anti-ethylene response. The discrepancies between the results obtained in this study and that of the above author could be due to the difference in the usage of long life as vase solution.

The lower rate of S uptake following pulsing treatment of the cut flower stalks with CHO as compared with that of the control might be due vascular blockage as a result of bacterial proliferation and in the presence of the CHOs. In addition, S uptake in cut flower stalks pulsed with Glu was greater than that of Fru and Suc (Table 1). Differences in solution uptake among these CHOs could be ascribed due to their differences in favouring bacterial proliferation or their mechanism of transport/uptake. In many plants, the uptake rates of sugars to tissue or protoplasts vary for various sugars (van Doorn et al., 1989). Since transporters of sugars have been found to be involved in the uptake of sugars Ichimura et al. (2006), the activities of sugar transporters may be responsible for differences in S uptake in roses.

Among the BIoS used the HQS increased S uptake as compared to \( \text{Al}_2(\text{SO}_4)_3 \) and the control. In a preliminary observation, cut rose flower stalks placed in a vase solution without HQS often showed incipient wilting after a few days of vase life while those stalks placed in a vase solution with HQS remained fresh (data not shown). This confirms the influential effects of HQS on S uptake, which might be due to its effect on bacterial control. The lower rate of S uptake in cut flower stalks pulsed in the presence of \( \text{Al}_2(\text{SO}_4)_3 \) could be due to its ineffectiveness in controlling bacterial growth or its adverse effect on xylem HC. Phytotoxicity of \( \text{Al}_2(\text{SO}_4)_3 \) on ‘Red Calypso™’ cut flower stalks might be the major cause for leaf and petal damage (Figure 5) so that the transpiration pool could not maintained and S uptake diminished. Son et al. (1994) noted that \( \text{Al}_2(\text{SO}_4)_3 \) 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.

The significant (\( P < 0.001 \)) increase of S uptake due to the interaction effect of (CHO + BIo) as well as (STS + CHO) + BIo are in agreement with previously reported results by Liao et al. (2000), that the vase life of cut rose flowers was prolonged slightly more by a pulse treatment of STS followed by Suc than by STS alone. Therefore, it seems that STS plays a key role in maintaining the vase life of cut rose flowers by delaying the reduction in S uptake. In the present study, regardless of the type of CHO used, addition of HQS in the pulsing solution increased S uptake of ‘Red Calypso™’ cut flower stalks and extended their vase life. The combined effect of (STS + CHO) + BIo resulted in an increased S uptake by the cut flower stalks (Figure 4). In addition, the effect of STS in
delaying flower senescence might be due to its inhibitory effect on ethylene action, while that of the CHOs particularly (Glu) might be improving the energy supply to the cut flowers, and the BIOs particularly (HQS) could have maintained the antimicrobial activity in the vase solution and resulted in an increased water and sugar uptake. Vascular occlusion and depletion of soluble respiral substrate are considered to be primarily responsible for shortened vase life in cut roses (Ichimura et al., 2003). Addition of BIOs in pulsing/vasel solutions was found to control microbial proliferation, reduce vascular occlusion, and increase S uptake. Knee (2000) reported a longer flower life and increased S uptake when most effective BIOs (HQS) was used in vase solutions, plus it is associated with low resistance to water flow and VSAbs.

Relative fresh weight

The Fresh weight (FW) of each flower stalk relative to its initial FW was compared to determine the water status of ‘Red Calypso™’ cut flower stalks. The relative fresh weight (RFW) value of the cut flower stalks pulsed with the different solutions increased to above 100% till day 6 of vase life (Table 2). Thereafter, a decline in the RFW was observed until the end of the experiment. In most of the days, pulsing of ‘Red Calypso™’ cut flower stalks either with STS or CHOs resulted in non significant (P>0.05) difference in the RFW. In contrast, significant differences in the RFW of the cut flower stalks were recorded among the BIOs tested in all days of vase life. Pulsing of the cut flower with HQS significantly (P<0.001) increased the RFW as compared with Al₂(SO₄)₃ and the control/0 BIO (Table 2 and Figure 5). In most of the cases, the interaction effects of (STS + CHOs), (STS + BIOs), (CHOs + BIOs), and (STS + CHOs + BIOs) were insignificant (P>0.05) or showed inconsistent results.

Fresh weight loss was maintained near equilibrium in most of the cases, which could be due to a relatively lower rate of transpiration and S uptake under the ambient condition. Mayak et al. (2001) reported retardation of fresh weight loss by STS treatment during the first few days after harvest. The author suggested that although it is likely that the effect of STS is related to its anti-ethylene properties, the Ag⁺ might reduce microbial activity in the vase and thereby increase water uptake, or Ag⁺ could be improving water conductivity of the xylem vessels.

The non significant difference observed in the RFW of the cut flower stalks pulsed with CHOs is in agreement with that of Ichimura et al. (2002), who reported delayed FW loss in two cut flower rose cultivars, ‘Noblesse’ and ‘Sonia’ treated with Suc and an increase in the maximum FW in other cultivars. On the other hand, the significant increase in RFW gain of cut flower stalks pulsed with HQS is in agreement with the findings of Ichimura et al. (1999, 2002) who pointed out delay in FW loss of cut flowers pulsed with HQS. The authors also reported a maximum increase in the FW of the cut flowers pulsed with (Suc + HQS), which was much greater than that of HQS alone. Thus, changes in RFW and FLNG are expectedly related to S uptake and vase life of the cut flowers stalk.

Total soluble solid

The total soluble solid (TSS) in the petals was measured by squeezing the sap from the petals. Pulsing treatments that consisted of STS or CHO significantly (P<0.05) different in the petal TSS at the 4th day of vase life, while that of BIOs was significantly (P<0.001) different when compared with the control (Table 2). Generally, an increase in the TSS content of the petals was observed on the 4 days of vase life following pulsing of the flower stalks with STS, CHO, and BIO. However, the TSS of the petals was significantly (P<0.001) different on the 8th and 12th days of vase life. A significant (P<0.01) different in the TSS of petals was also observed as a result of the interaction effect of (STS + CHO + BIO) on the 12th day of vase life.

One of the important factors that affect longevity of cut flowers during vase life is diminishing of respiration substrates, 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). The decrease in the TSS value at the late period of vase life appears to be due to increased respiration and progression of senescence. However, it is inevitable that during the first few days of vase life, the petal TSS values could increase in due to increased S uptake and accumulation of CHO in the petals. In the later days of vase life, S uptake could be reduced due to reduced stem HC and hence the amount of CHO that reach the petals may not be sufficient to replenish the amount to be lost by respiration. The CHO are important reserve compounds, being Suc the most abundant soluble CHO, sometimes the only one in the phloem sap. Furthermore, a decrease of macromolecular components such as starch occurs through the course of petal senescence. Thus, the pulsing treatment that consisted of (STS + CHO + BIO) was able to maintain the water and sugar status of the cut flower stalks for longer period of time and enhance the vase life of the cut flower stalks.
Summary

It was generally observed that FLNG, MFHD, VSABS, and HC were significantly influenced by the pulsing treatments that comprised STS, Glu, HQS, and their interaction. The results depicted the combined pulsing treatment extended the vase life, largest MFHD and higher HC at three stem segments, while lower VSABS values were recorded. The TSS in petal sap showed a slight increase in the first 4 days of the study, followed by decrease. In this study, longevity of cut flowers during vase life is a function of several factors including chemicals added to either pulsing/vase solutions and/or the inherent characteristics of the flowers themselves. Therefore, extension of the vase life of ‘Red Calypso™’ cut flower stalks by pulsing them with solutions that contained (STS + Glu + HQS) and long life as vase solution, could be due to the antibacterial effects of HQS and long life plus energy supply from Glu (as pulse) and long life (as vase) solution, and STS as being antagonistic to ethylene.

Besides the Ethiopian cut flower industry has a vital stake in overcoming the poor postharvest reputation of cut roses in order to exploit a maximum export potential. The biocide Al₂(SO₄)₃ has been the most commonly used by most cut flower growers in country, while it was found to be ineffective in this study. This might be due to the fact that the compounds might be phytotoxic for the cultivar, and other compounds have not been commonly used by most of the growers in the country. 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 assessments and internode extended the vase life of the cut flower stalks. At this moment it is not possible to describe the detailed composition of long life due to the manufacturing company secrets. However, it is expected that long life, as other commercial flower food formulations, could at least contain sugar and biocide.

On the contrary, STS has been used to promote the vase life of many species of cut flowers, it is currently abandoned in some parts of the world, due to the damage it causes both to the environment and animal health. Therefore, it is high time to look for other alternatives in order to promote the vase life of cut flowers and meet consumer demand. To this end, directing the research in looking for another form of ethylene action inhibitor other than STS possibly to reduce the rate of flower senescence is inevitable. However, it can be seen from the results that use of HQS and Glu is comparatively preferable than using Al₂(SO₄)₃ and Suc which are being used by most of the flower growers in the country. Thus, use of HQS and Glu as pulsing solution followed by long life as vase solution is recommended for the Ethiopian flower growers and/or retailers.

REFERENCES

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