

EFFECT OF ACCEL, SUCROSE AND SILVER THIOSULPHATE ON THE WATER RELATIONS AND POST HARVEST PHYSIOLOGY OF CUT TUBEROSE FLOWERS

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ABSTRACT

This study investigated the influence of cytokinins, gibberellins, sucrose and silver thiosulphate on water relations and post-harvest physiology of cut tuberose (*Polianthes tuberosa* L) flowers. Tuberose flowers held in de-ionised water (DIW) had a vase life of 13 days with 63% floret opening. Addition of gibberellins (GA₄₊₇) in the vase solution had no effect on vase life or floret opening along the spike. Pulsing of the cut flowers with 10% sucrose for 24 hr before transfer to DIW improved their vase life by 4 days and improved the floret opening by 21% above DIW controls. Addition of Benzylaminopurine (BA) at low concentrations (25-50 mg L⁻¹) improved vase life of the cut tuberose stems while higher concentrations (75-100 mg L⁻¹) gave no improvement. A 24 hr pulse in 10% sucrose improved the vase-life by 3.6 days and floret opening by 13%. Pulsed stems transferred to holding solutions containing various concentrations of BA improved vase life by an extra 3 days at BA concentration of 25 mg L⁻¹. Higher BA concentrations gave no significant (P>0.05) improvement over the pulsed stems. However, floret opening was greater at 25 and 50 mg L⁻¹ BA (P<0.05). Of all treatments, STS gave the greatest improvement of vase life at 7 days longer than the DIW control and 3.5 days longer than the sucrose-pulsed solutions. Very high (88%) floret opening was observed in cut stems held in STS. There was a general decrease in water uptake by tuberose stems over time. Lowest rates of water uptake were noted in all treatments after 8 days. Among the treatments, the lowest water uptake was recorded in the DIW control and in GA₄₊₇ treatments. Greatest uptake was in 10% sucrose + 25 mg L⁻¹ BA. Transpiration losses were greatest for 25 mg L⁻¹ BA and least for 10% sucrose. Differences among treatments in transpiration losses were noted only in the first 10 days. In general, water deficit was noted in cut flowers held in DIW and in GA₄₊₇ after day 6, while stems in BA treatments manifested symptoms from day 8. The cut flowers pulsed in 10% sucrose and held in 25 and 50 mg L⁻¹ BA and in 2 mM STS only showed water deficit status from day 12 of their vase life. Overall results suggest that STS, BA and sucrose can help improve tuberose vase life and floret opening through improvement of the water balance.

Key Words: Benzyladenine, Benzylaminopurine, *chrysanthemum*, gibberellins, Kenya, *Polianthes tuberosa*

RÉSUMÉ

Cette étude a évalué l'influence de cytokinines, de gibberellines, du saccharose et le thiosulphate d'argent sur les relations entre l'eau et la physiologie après récolte de fleurs de tube rose. Les fleurs de tube rose maintenues dans l'eau de-ionisée (EDI) avaient une vie de vase de 13 jours avec 63% d'ouverture de fleuron. L'addition de gibbérellines (GA 4+7) dans le vase n'a pas eu d'effet sur la vie du vase et l'ouverture du fleuron aux alentours du spike. L'impulsion de 10% de saccharose dans des fleurs coupées pour 24 h avant le transfert de EDI avait amélioré la vie du vase par 4 jours et améliora l'ouverture du fleuron par 21% au delà du contrôle de l'EDI. L'addition du benzylaminopurine (BA) à des faibles concentrations (25-50 mg L⁻¹) améliora la vie du vase et la tige de tube rose coupée alors que les concentrations élevées (75-100 mg L⁻¹) ne donna aucune amélioration. Un

pulse de solution de 10% pendant 24 h améliora la vie du vase de 3.6 jours et celle de l'ouverture du fleuron de 13%. Des tiges injectées transférées à des solutions contenant des concentrations variées de BA améliorèrent la vie du vase par un extra 3 jours pour la concentration de 25 mg L⁻¹. Des concentrations élevées de BA donnèrent aucune amélioration significative sur la tige injectée ($P < 0.05$). Cependant l'ouverture du fleuron était grande à 25 et 50 mg L⁻¹ de BA ($P < 0.05$). Pour tous les traitements, STS donna la plus grande augmentation de la vie du vase de 7 jours par rapport au contrôle EDI et de 3.5 jours par rapport au injection de la solution de saccharose. Des ouvertures du fleuron très grandes (88%) étaient observées dans des tiges coupées maintenues dans STS. Il était observé une décroissance dans la quantité d'eau absorbée par les tiges dans les temps. Les plus faibles taux d'absorption d'eau ont été observés pour tous les traitements après 8 jours. Les plus faibles étaient observées pour l'EDI et le GA4+7. Le taux le plus élevé était observé pour 10% de saccharose + 25 mg L⁻¹ de BA. Les pertes par transpiration étaient les plus élevées pour 25 mg L⁻¹ de BA et plus faible pour 10% saccharose. Les différences dues aux pertes liées à la transpiration étaient observées uniquement pour les 10 premiers jours. En général, les déficiences en eau étaient observées dans les fleurs coupées et maintenues dans l'EDI et après 6 jours, alors que les tiges traitées au BA montrèrent des symptômes dès le 8ème jour. Les fleurs coupées injectées de 10% saccharose et maintenues dans 25 et 50 mg L⁻¹ de BA et dans 2mM STS montrèrent des déficits en eau dès le 12ème jour de leur vie en vase. Les résultats suggèrent que STS, BA et le saccharose peuvent aider à améliorer la vie en vase du tube rose et les ouvertures de fleurons par l'amélioration du bilan hydrique.

Mots clés: Benzyladamine, Benzylaminopurine, *chrysanthemum*, gibberellins, Kenya, *Polianthes tuberosa*

INTRODUCTION

The short vase-life of many cut flowers continues to pose a challenge to the florist industry in general. Tuberose is grown in Kenya for the export market. Its commercial production has increased in recent years, especially among small-scale farmers because it can be grown outdoors under minimal management (HCDA, 1998). The most important quality attribute of cut flowers in general, besides their intrinsic aesthetic value, is their vase life. For spiked flowers such as tuberose, floret opening along the spikes is also important. Further expansion of production of tuberose cut flower is hampered by premature abscission and/or abortion of the distal florets, and by the short vase-life of the remaining florets (Watako, 1992; HCDA, 1998). Postharvest losses in many cut flowers are estimated to be as high as 40% in the absence of floral preservatives. Short vase life of tuberose can be associated with, among other factors, unfavorable water balance, i.e., the difference between water uptake and water loss. Proper water balance in plant tissues enables cut stem cells to remain turgid, thus, prolonging vase life and delaying the onset of senescence.

Empirical studies are still underway to assess the efficacy of different treatments to reduce post-harvest losses. This and other studies will enable the understanding of factors that predispose cut flowers to earlier aging and will contribute to the

formulation of better strategies for handling cut flowers to reduce postharvest losses.

Many post-harvest procedures for cut flowers involve use of various compounds and technologies that inhibit the effects of ethylene, reduce respiration or maintain better water relations. Most of these procedures address metabolic stresses faced by cut stems.

Water uptake and water loss by harvested cut flowers in vases may fluctuate cyclically with an overall declining trend (Carpenter and Rasmussen, 1973; Mayak *et al.*, 1974; Halevy, 1976; De Stigter, 1980). Whenever transpiration exceeds water uptake, resistance to water flow develops in the stems leading to water deficit. This resistance can be attributed to microbial occlusions (Accatti, *et al.*, 1981), physiological vascular blockage (Halevy and Mayak, 1979, 1981; Marousky, 1971) or air embolism (Crafts, 1968). Development of water deficit is caused by a reduction in the water holding capacity of the flower tissue due to physiological changes associated with senescence at the cellular level (Van Meeteren, 1978; 1979). Various chemicals such as sucrose have been added to vase solutions to improve water relations.

Sucrose is useful as a respiratory substrate and as an osmolyte that helps in the maintenance of a favorable water balance. Sucrose at 2-4% in the holding solution reduced stomatal aperture in rose cut flower leaves, thus reducing water loss and improving water retention and solute uptake

capacity (Marousky, 1969; De Stigter, 1980). A high sucrose concentration in the holding solution of up to 16% was reported to delay the onset of autocatalytic ethylene production in carnation cut stems (Dilley and Carpenter, 1975; Mayak and Dilley, 1976) thereby delaying the onset of senescence and extending vase life. Sucrose at 2% was slightly improved the vase life of tuberose cut stems (Watako, 1992). However, the mode of action was not investigated.

Various ethylene antagonists have been reported to improve vase life through improved water relations in cut stems, in addition to reducing the effects of ethylene. Silver nitrate was found to reduce the rate of decline of water uptake in *Anthurium* (Paull and Goo, 1985), maidenhair fern (Fujino and Reid, 1983) and narcissus (Piskornik, 1981; 1985). The silver ions could be acting as a biocide thus eliminating microbial blockage of the xylem vessels (Aarts, 1957; Kofranek and Paul, 1974). Silver ions also interfere with binding sites of wound ethylene (Paull and Goo, 1985; Sisler, 1982), thus preventing physiological blockage of cut stems.

Cytokinins, at low concentrations, have been thought to act as ethylene antagonists while they elicit ethylene at high concentrations (Yang and Hoffman, 1984; Yip and Yang, 1986). Cytokinins have not been widely studied as regulators of water balance in cut stems. However, in roses, kinetin enhanced fresh weight in all flower parts, delayed the subsequent reduction in fresh weight by 1 day, promoted growth and expansion of petals, and maintained petal turgidity for an extended period of time (Mayak and Halevy, 1974). Application of BA (Benzylaminopurine) to *chrysanthemum* flower buds at an early stage of development also increased fresh weight (Jeffcoat, 1977). Carnation flowers treated with BA did not show a change in fresh weight over a 10-day period, while control flowers lost over 60% of the initial weight (Cook *et al.*, 1985). The maintenance of fresh weight in carnations upon cytokinin treatment was attributed to maintenance of cell integrity through membrane stabilisation. Likewise, BA retarded the decrease in the petal water content and the increase in ion leakage in gerbera, thus delaying senescence (Van Meeteren, 1978). Cytokinins were also thought to promote favorable water balance by directly preventing

ethylene-induced vascular occlusions in stems of carnations (Mor *et al.*, 1983; Cook *et al.*, 1985; Paulin and Muloway, 1985) and *Ipomoea tricolor* (Kende and Hanson, 1976). Our previous work with *Alstroemeria* indicated an improvement of vase life and post harvest handling by Accel (Muthui *et al.*, 2001). To our knowledge, there are no reports on the effects of cytokinins on the vase life or water relations of cut tuberose flowers. The objective of this study was to evaluate the effects of various plant growth regulators and sucrose on vase life and floret opening of cut tuberose (*Polianthes tuberosa* L.) stems.

MATERIALS AND METHODS

The tuberose flowers used were obtained from Cianda Flowers Ltd., commercial flower growers in Kiambu in Kenya. Kiambu is situated at 2300 m above sea level and around 10° South of the Equator. Tuberose flowers were harvested at the commercial cut stage with one floret open, and brought to the laboratory within 2 hr. Flowers were re-cut under water to 60 cm long and the lower leaves removed. Experiments were carried out under cool white fluorescent light (4160 J/sec) at a temperature of $23 \pm 1^\circ\text{C}$ and a RH of $70 \pm 10\%$. De-ionised water (DIW) was used as control treatment. One set of 10 flowers was placed straight away in 25, 50, 75 100 mg BAL⁻¹ equivalent of Accel™ (Abbott Laboratories, North Chicago, USA). Accel is a liquid concentrate containing 20 g a.i BA L⁻¹ and 2.0 g a.i GA L⁻¹₄₊₇ (Abbott Laboratories, North Chicago, USA).

Another set was first pulsed in 10% sucrose for 24 hr before being transferred to holding solutions containing Accel at the same concentrations of BA equivalent. A further set of cut stems was placed in 2.5, 5.0, 7.5 and 10 mg GA L⁻¹₄₊₇. Additional stems were pulsed for 1 hr in 2.0 mM silver thiosulphate (STS) anionic complex and then transferred in DIW. Silver thiosulphate complex was prepared according to Gorin *et al.* (1985).

The vase life of cut tuberose flowers was considered terminated when the last open floret wilted and lost decorative value (Woodson, 1987; Watako, 1992). At that point, numbers of unopened flowers were recorded and the percentage of those that opened was determined.

Rates of water uptake and transpiration loss were determined using the procedure outlined by Van Meeteren (1978) and Mayak and Halevy (1974). The cut stems were placed in boiling tubes holding 60 ml of the test solutions. The top of the boiling tube was sealed with a piece of polythene to prevent any evaporation; thus water loss was only *via* the cut flower. At the start of the experiments, and after every 48 hr, the weights of the boiling tube with and without the flower were recorded. The rate of water uptake and transpiration loss (g hr^{-1}) were determined from the changes in weight between two successive measurements i.e. without the cut stem and with the cut stems, respectively, divided by the number of hours during the interval.

Treatments were replicated 4 times and experiments repeated twice. The data presented are pooled. Experiments were arranged in a completely randomised design (Steel and Torrie, 1981). Analysis of variance was by the General Linear Model Procedure of SAS (SAS Institute Inc. 1995). Means were compared using Tukey's method at 5% level of probability.

RESULTS

Vase-life and floret opening. Tuberose cut flowers held in de-ionised water lasted for 13 days with floret opening of about 63% along the spike (Table 1). Addition of various concentrations of GA_{4+7} to the vase solution had no significant ($P>0.05$) effect on either the vase life or florets opening along the spike (data not shown). Benzyladenine at low concentrations (25 and 50

mg L^{-1}) improved vase life by 2-3 days and floret opening by 9-11%. Higher concentrations of BA of 75 and 100 mg L^{-1} had no significant ($P>0.05$) effect on vase-life or floret opening along the spike (Table 1). Pulsing tuberose flowers for 24 hr in 10% sucrose improved vase life by 4 days and floret opening by 13%. The presence BA in the vase solutions increased vase life and floret opening of sucrose-pulsed cut stems (Table 1). This effect was more pronounced at low concentrations (25 mg L^{-1}) than at higher concentrations ($>50 \text{ mg L}^{-1}$). Benzyladenine concentrations greater than 75 mg L^{-1} actually suppressed vase life and floret opening of the 10% sucrose pulse treatment (Table 1). Longest vase life and highest floret opening was achieved for stems pulsed in STS. Silver thiosulphate gave an improvement of 7 days in vase life and 25% more florets opening along the spike compared with DIW control.

Water relations. Rates of water uptake and transpiration losses in cut tuberose stems, decreased over time (Fig. 1). During the first 6 days, cut stems pulsed in 10% sucrose and then held in either DIW or 25 mg BA L^{-1} , exhibited the highest rate of water uptake and the lowest transpiration losses (Fig. 2). From the 6-10th day, flowers pulsed in STS exceeded those pulsed in 10% sucrose and those held in DIW in water uptake. Those pulsed in 10% sucrose and subsequently held in 25 mg L BA^{-1} still showed the greatest rate of water uptake. Cut stems held in DIW and GA_{4+7} had the lowest water uptake and highest transpiration losses when compared to

TABLE 1. Effect of plant growth regulators and sucrose on vase life and floret opening of cut tuberose (*Polianthes tuberosa* L.) flowers

Vase solution	Vase life (days)	Floret opening (%)
Deionised Water (DIW)	13.2 ^d	62.8 ^d
Accel (25 mg L BA^{-1} equivalent)	15.8 ^c	73.9 ^b
Accel (50 mg L BA^{-1} equivalent)	15.2 ^c	71.9 ^{bc}
Accel (75 mg L BA^{-1} equivalent)	14.7 ^{cd}	68.7 ^c
Accel (100 mg L BA^{-1} equivalent)	14.2 ^d	66.6 ^{cd}
10% sucrose + DIW	16.8 ^{bc}	75.5 ^b
10% sucrose + Accel (25 mg L BA^{-1} equivalent)	19.8 ^a	84.6 ^a
10% sucrose + Accel (50 mg L BA^{-1} equivalent)	18.8 ^{ab}	82.4 ^a
10% sucrose + Accel (75 mg L BA^{-1} equivalent)	17.5 ^b	78.5 ^b
10% sucrose + Accel (100 mg L BA^{-1} equivalent)	15.6 ^c	73.2 ^b
Silver thiosulphate (STS)	20.3 ^a	88.0 ^a

those held in STS, 10% sucrose+BA or in 10% sucrose pulse. Transpiration losses were greatest for cut stems held in 25 mg BA L⁻¹ and least for those pulsed in 10% sucrose. Flowers held in DIW controls and those held in GA₄₊₇ exhibited water deficits after only 6 days in the vase solution, while those in 25 mg BA L⁻¹, pulsed in 10% sucrose and those pulsed in STS showed water deficit status after 8 and 10 days, respectively.

DISCUSSION

The fact that GA₄₊₇ added to the vase solution had no effect on either vase life or floret opening along the spike of tuberose cut stems is consistent with earlier reports on *Chrysanthemum* (Garibaldi and Deamborgia, 1988), iris (Swart, 1986), *Lilium*

longiflorum (De Hertogh and Blakely, 1972), *Liatrix spicata* (Perez *et al.*, 1985), gladiolus (Nunes, 1989), and hybrid *Limonium* (Doi and Reid, 1995). In most biological systems, polar GAs (e.g. GA₁ and GA₃) are thought to be more active biologically (Noma *et al.*, 1982) than their non-polar counterparts (e.g., GA₄₊₇).

Benzyladenine at low concentrations (25 mg L⁻¹), improved vase-life and floret opening of the tuberose cut stems, while high concentrations (100 mg L⁻¹) were ineffective. Benzyladenine has similarly been reported to improve the vase life and flower opening in *Alstroemeria* (Dai and Paull, 1991; Muthui *et al.*, 2001), *Anthurium* (Paull and Goo, 1985; Shirakawa *et al.*, 1964), carnations (Cook *et al.*, 1985) and *Chrysanthemum* (Maclean and Dedolph, 1962; Heide and Oydvin,

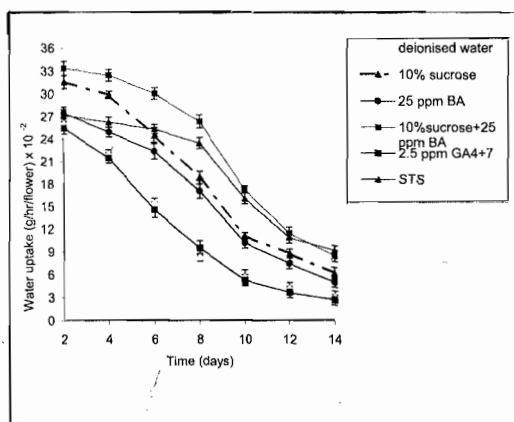


Figure 1. Effect of plant growth substances on water uptake (g/hr/flower x 10⁻²) of cut tuberose flowers.

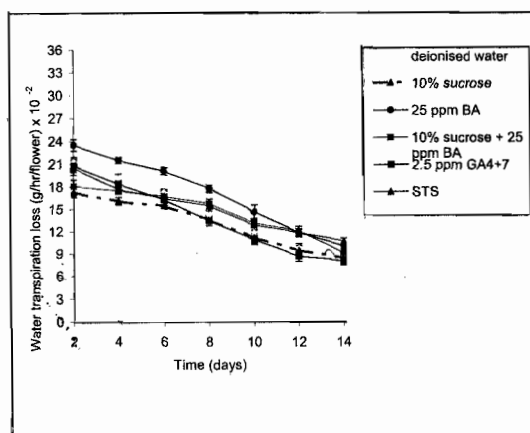


Figure 2. Effect of plant growth substances on transpiration loss (g/hr/flower x 10⁻²) of cut tuberose flowers.

1969) similar results were also obtained for daffodils (Ballantyne, 1966), gerbera (Van Meeteren and Van Gelder, 1980), iris (DeMunk and Gijzenberg, 1977; Vonk *et al.*, 1986; Wang and Baker, 1979), *Leucospermum* (Napier *et al.*, 1986) and tulips (Systema, 1981). The lack of positive effect on vase life and floret opening at higher BA concentrations could be due to increased ethylene production (Hutchinson *et al.*, 1997), which may accelerate tuberose senescence.

In terms of water balance, BA-supplemented vase solutions gave a better water balance than was recorded for cut tuberose stems held in DIW. Kinetin has similarly been reported to enhance fresh weight increase in all rose flower parts, to promote growth and expansion of the petals, to maintain petal turgidity for an extended period and to enhance stomatal opening (Mayak and Halevy, 1974), thereby increasing transpiration. The higher rate of water uptake more than compensated for the increased transpiration losses in the cut rose stems, with an overall delay in senescence. In other studies, cut stems held in BA-supplemented vase solutions, for example carnation (Cook *et al.*, 1985), *Anthurium* (Paull and Goo, 1985) and gerbera (Van Meeteren, 1979), maintained their fresh weight over a longer time during which control flowers lost > 60% of their initial weight. Although the mode of action of Accel is not clear, improved water balance of cut stems could be linked to protective effects of cytokinins on cell turgidity probably through decreased ion leakage from the cells (Van Meeteren, 1979). In addition, BA at low concentrations, may prevent ethylene-induced vascular occlusions (physiological blockage) at the base of the cut stem (Eisinger, 1982; Halevy and Mayak, 1981; Mor *et al.*, 1983), thus promoting the rate of water uptake.

Vase life and floret opening of tuberose flowers were improved significantly ($P < 0.05$) by a 24-hr pulse treatment in 10% sucrose applied before holding them in either DIW or vase solution supplemented with BA. Sucrose pulse treatments have been reported to improve vase lives of *Alstroemeria* (Chepkairor and Waithaka, 1988), *Anthurium* (Paull and Goo, 1982), brodiae (Han *et al.*, 1990), carnation (Nichols, 1973a), freesia (Woodson, 1987), gladiolus (Bravdo *et al.*, 1974), gypsophila (Farnharm, 1975; Downs *et al.*, 1988),

lily-of-the-Nile (Mor *et al.*, 1983), limonium (Shillo and Halevy, 1980) and strelitzia (Halevy and Mayak, 1979). Several modes of action of sucrose in promoting vase life have been proposed. Sucrose could act as a respiratory substrate or as an osmolyte (Nichols, 1973; 1975; Sacalis, 1973). In the present study, pulsing the tuberose cut flowers for 24 hr in sucrose improved their water relations. Similar results of improved water relations by sucrose have been reported in gladiolus (Bravdo *et al.*, 1974), carnation (Aarts, 1957), roses (Marousky, 1969; 1971) and tuberose (Naidu and Reid, 1989). The improvement of water balance by sucrose in tuberose cut stems could be as a result of improved osmotic concentration of petal tissues (Halevy, 1976; Halevy and Mayak, 1979), sugars' ability to maintain mitochondrial structure (Kalteler and Steponkus, 1974) or improved membrane integrity (Aarts, 1957; Sacalis, 1973), thus maintaining the turgidity of the tissues and resulting in delayed wilting and senescence.

Inclusion of the ethylene antagonist, STS, in the vase solution resulted in the greatest improvement of vase life and floret opening for cut tuberose stems. A brief STS pulse has been reported to improve the vase life of *Alstroemeria* (Chepkairor and Waithaka, 1988), *Anthurium* (Paull and Goo, 1982), gladiolus (Mor *et al.*, 1983) and gypsophila (Downs *et al.*, 1988) cut flowers among many others. STS has been thought to act primarily as an ethylene antagonist. However, silver ions from the STS complex may also be biocidal, thus reducing or eliminating bacterial buildup in the vascular tissue of cut stems as reported for carnation (Kofranek and Paul, 1974; Halevy and Mayak, 1981) and *Anthurium* (Paull and Goo, 1985). The Ag^+ could also be interfering with the wound ethylene binding sites at the stem base (Sisler, 1982). Pulse treatment of cut *Anthurium* stems with Ag^+ , inhibited ethylene-induced vascular occlusions (Paull and Goo, 1985). A similar effect could have occurred in tuberose flowers. Inhibition of ethylene-induced vascular occlusion could have resulted in increased water uptake enabling more water to reach the distal buds, thereby promoting cell enlargement and floret opening in tuberose. Increased water uptake could also help in the re-distribution of absorbed substrates (Hutchinson *et al.*, unpublished data)

thus strengthening the capability of the young floret sinks resulting in more florets opening.

In summary, low concentrations of BA (<25 mg L⁻¹), a 24-hr 10% sucrose pulse and a 24-hr pulse of 2 mM STS each improved the vase life and floret opening of cut tuberose flowers. These compounds all improved the water balance of the cut stems, resulting in delayed wilting and senescence. Continued use of STS is being challenged because of environmental considerations of disposing silver, a heavy metal. Therefore, BA at low concentrations, has potential for use as a commercial cut flower preservative for prolonging vase life and post harvest quality of cut tuberose stems.

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