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RESEARCH ARTICLE

Effect of different vase solutions on postharvest longevity of cut foliage *Ophiopogon japonicus*

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Abstract:*Ophiopogon japonicus* (L.f.) Ker-Gawl (family Liliaceae) is an export-oriented foliage plant with attractive white-green strips. The vase life of this cut foliage ends when the leaves loose turgidity and/ or bright green colour. Thus, to enhance the vase life of *O. japonicus*, effect of 0.25, 0.5, and 1.0 mM $CuSO_4.5H_2O$, 10, 20, and 30 g/L sucrose and combination of sucrose with 0.5 mM $CuSO_4.5H_2O$ as holding treatments, 20 g/L and 30 g/L sucrose solutions as 24 h pulsing treatments and 6-Benzylaminopurine 5, 10, 20 mg/L BAP were tested. Relative fresh weight of leaves, solution uptake rate and vase life were assessed. Vase solution bacterial enumerations were carried out to evaluate the effectiveness of $CuSO_4.5H_2O$ as a biocide. To investigate the function of BAP, amount of chlorophyll was quantified using leaf pigment extracts. The vase life of *O. japonicus* could be extended from 5.1 days to 12.5 days by giving BAP based vase solutions. Chlorophyll contents of leaves dipped in BAP solutions were significantly higher than the control (distilled water). $CuSO_4$ solutions did not show any significant antibacterial effects compared to the control. According to vase life data, the most effective vase solutions were $CuSO_4.5H_2O$ (0.5 mM), sucrose 20 g/L with $CuSO_4.5H_2O$ (0.5 mM) as holding treatments, sucrose 20 g/L and 30 g/L as pulsing treatments and BAP treatments (5, 10, 20 mg/L).

Keywords: vase life, BAP, $CuSO_4$, sucrose, *Ophiopogon*, foliage.

INTRODUCTION

Ophiopogon japonicus is a perennial, ornamental foliage plant belongs to the family Liliaceae (USDA, 2013). At present, this plant is grown commercially in Sri Lanka. *O. japonicus* has a high demand in the foliage export industry due to its attractive white-green stripped lanceolate leaves. The ageing process of cut ornamental products is accelerated following the detachment from mother plants at harvest (Tsegaw *et al.,* 2011). Furthermore, the vase life of cut flowers and foliage is shortened by vascular occlusions that are caused by microbial blockages, physiological plugging and physical factors (Edrisi *et al.,* 2012). Two major factors affecting the rate of water uptake of cut flowers are xylem hydraulic conductance and water potential difference between the plant tissue and vase solution. Water potential of plant tissue may vary due to transpiration and cell enlargement (van Meeteren and van Gelder, 1999). Further, hydraulic conductance of xylem conduits of stem can decrease by air embolism due to the process of xylem cavitation (Singh and Moore, 1992). Similarly, cut stem end can be blocked by large amounts of microorganisms and their by-products (Jones and Hill, 1993) leading to decline of water levels in tissues (van Meeteren and van Gelder, 1999). Xylem conduits can also be blocked by different pectic substances, polysaccharides or proteins (Singh and Moore, 1992). Thus, postharvest treatments are very crucial to delay the rate of deterioration and to prolong the vase life of cut flowers and foliage (Tsegaw *et al.,* 2011).

Different types of preservatives or vase solution ingredients are currently used to extend the vase life of flowers and foliage products (Thambugala *et al.,* 2010). Copper is a multifunctional biocide, which is involved in enzymatic reactions related to biosynthesis and action of ethylene (Hojjati *et al.,* 2007), reduces bacterial growth and multiplication, and inhibits enzymes involved in physiological stem occlusion (Damunupola and Joyce, 2008). Among many chemical preservatives, sucrose is the most commonly used vase solution ingredient as it improves the water balance and osmotic potential of cut flowers (Lal *et al.,* 1990). Incorporation of antimicrobial agents into vase solution can decrease the bacterial population (van Doorn *et al.,* 1990). Cytokinins have proven to increase the vase life of cut foliage by maintaining the integrity of the tonoplast membrane and preventing leakage of proteases which hydrolyses the soluble proteins including those in the chloroplast and mitochondrial membranes (Subhashini *et al.,* 2011). Moreover, cytokinin can delay the senescence process, via maintaining chloroplast activity and declining chlorophyll degradation of plants (Siddiqui *et al.,* 2011).

Cut foliage of *O. japonicus* has a decorative value in floral arrangements and is also marketed as an export ornamental potted plant. The vase life of *O. japonicus* ends when the leaves lose their turgidity and bright green colour. Thus, objective of this study was to determine the efficacy

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of different vase solution ingredients to prolong the vase life of *O. japonicus* cut foliage.

MATERIALS AND METHODS

Healthy leaves of *O. japonicus* with same maturity level were collected from the Department of Botany, University of Peradeniya, Sri Lanka (7° 15' 47" N, 80° 36' 10" E) at 08.00 - 09.00 hours. The cut ends were immediately immersed in distilled water (DW) and kept under shade. After transporting to the laboratory, leaves were recut under DW using a disinfected sharp secateurs. Different concentrations of treatment solutions were prepared and filled into autoclaved 2.5 cm diameter boiling tubes and sealed with parafilms (Penchiney, Chicago). Individual leaves of 40 cm long were dipped in 50 mL of vase solutions (treatments) separately. Further, the initial fresh weight of cut leaves and initial weight of vase solutions were measured using an electronic balance (OHAUS, USA). The measurements were taken daily, until 80% of the leaves reached the end of their vase life. Experiments on vase life evaluation were conducted in an air-conditioned laboratory under relative humidity of 80 ± 2 % and room temperature of 26 ± 2 ^{0}C with 12 h light on-off cycles using fluorescent lamps (light intensity of 15 µmol m^{-2} s^{-1}). All four experiments were arranged in a Completely Randomized Design (CRD) with 08 replicates per treatment. In Experiment 1, different concentrations (0.25, 0.5 and 1.0 mM) of $CuSO_4.5H_2O$ (Essex, England) solutions were used as the vase solution treatments. Miles and Misra (1938) method was used for the enumeration of microorganism in vase solutions. In Experiment 2, different concentrations (10, 20 and 30 g/L) of sucrose solutions were used as the treatments alone (dissolved in a liter of DW) and in combination with $CuSO_4.5H_2O$ (one liter of 0.5 mM solution), (sucrose + best concentration of the biocide treatment) separately. In Experiment 3, leaves were subjected to 24 hours pulsing treatments of two different concentrations of sucrose solutions (20 g/L and 30 g/L) before transferring to 0.5 mM $CuSO_4.5H_2O$. In Experiment 4, different concentrations (5, 10, 20 mg/L) of 6-*Benzylaminopurine* (PARK, UK) solutions were used as vase solutions.

Chlorophyll extraction of leaves, dipped in DW and BAP (5, 10, 20 mg/L) treatments were carried out according to the method described by Yang *et al.* (1998). Relative fresh weight and solution uptake rate were calculated as described by He *et al. (*2006) and Jiping *et al.* (2012) where "end of vase life" was assessed using a self-prepared index, based on leaf bending percentages. According to the index where, 0% = straight leaves (without rolled tip or leaf), 5% = slightly rolled tip, 20% = slightly rolled leaf, 50% = moderately rolled leaf and 90% = severely rolled leaf.

The vase life was considered to be terminated when leaf rolled (20%) and or phytotoxic effects such as yellow-brown spots on leaf blade were visible. Data were analyzed using nested ANOVA procedure in Statistical Analysis Software version 9.13 (SAS Institute Inc., Cary, NC, USA) and the number of bacterial colony forming units were analyzed using Kruskal Wallis rank sum test, using Minitab (Release 16) software.

RESULTS AND DISCUSSION

Relative fresh weight and vase life of leaves dipped in all vase solutions were significantly ($p < 0.05$) different from the control (DW). However, there was no significant difference in solution uptake rate in all treatments except sucrose pulsing treatments (Table 1). In the Experiment 1, there was a significant ($p < 0.05$) difference between the DW and 0.5 mM $CuSO_4$ 0.5 mM solution on vase life of *O. japonicus* cut foliage. The longest vase life (~7 days) was obtained in 0.5 mM $CuSO_4$ whereas the shortest

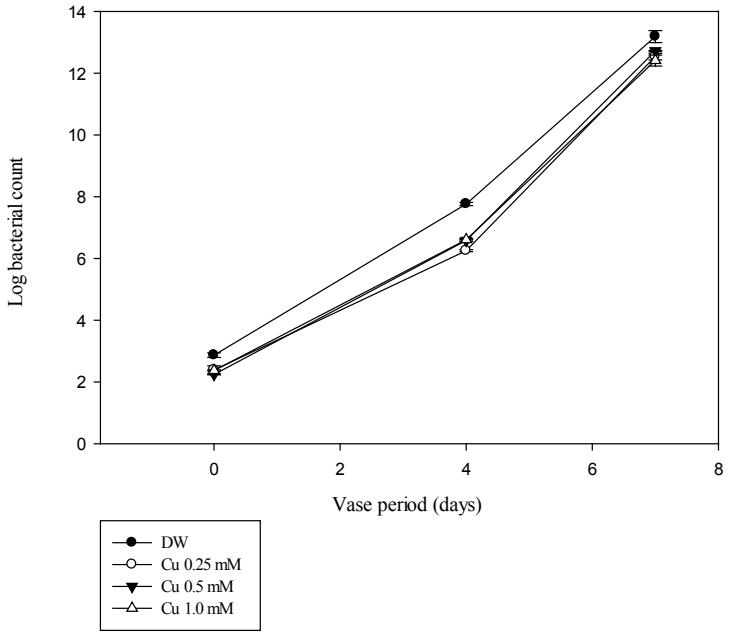

Figure 1: Log bacterial count with different $CuSO_4$ treatments during the vase period.

Table 1: Relative fresh weight, vase solution uptake rate and vase life of cut foliage of *O. japonicus* leaves subjected to different treatments.

Experiment and main factors	Relative fresh weight	Vase Solution uptake rate (g g^{-1} of initial fresh weight)	Vase life/days
Experiment 1			
Distilled water	105.34^{c}	0.43	3.75^{bc}
Deionized water	106.99^{bc}	0.49	4.75^{abc}
Tap water	106.37^{bc}	0.48	2.88^{c}
$CuSO_4$ (0.25 mM)	108.39^{ab}	0.38	4.50^{abc}
$CuSO_4$ (0.5 mM)	108.67^{ab}	0.38	6.75^{a}
$CuSO_4$ (1.0 mM)	109.99^{a}	0.36	5.88^{ab}
$LSD_{0.05}$ (n=8)	2.871	ns	2.26
Experiment 2			
Distilled water	103.29^{dc}	0.21	4.73^{b}
Sucrose (10 g/L)	102.24^{d}	0.24	4.14^{b}
Sucrose (20 g/L)	102.73^{d}	0.18	3.99^{b}
Sucrose (30 g/L)	102.27^{d}	0.22	3.69^{b}
Sucrose (10 g/L) + $CuSO_4$	104.67^{ab}	0.28	6.35^{b}
Sucrose (20 g/L) + $CuSO_4$	104.00^{bc}	0.21	10.05^{a}
Sucrose (30 g/L) + $CuSO_4$	105.34^{a}	0.19	6.80^{b}
$LSD_{0.05}$ (n=8)	1.20	ns	3.16
Experiment 3			
Distilled water	96.78^{b}	0.48^{a}	4.47^{b}
Sucrose 20 g/L (pulsing)	93.99^{b}	0.32^{ab}	9.60a
Sucrose 30 g/L (pulsing)	101.26^{a}	0.28^{b}	10.39^{a}
$LSD_{0.05}$ (n=8)	3.58	0.10	4.03
Experiment 4			
Distilled water	100.60^{b}	0.28	5.13^{b}
BAP (5 mg/L)	102.87^{a}	0.24	9.88^{a}
BAP (10 mg/L)	103.63^{a}	0.25	12.50^{a}
BAP (20 mg/L)	102.78^{a}	0.26	12.25^{a}
$LSD_{0.05}$ (n=8)	1.46	ns	4.35

Each data point represents the mean of 08 replicates. Means in each column followed by the same letter are not significantly different.

vase life (~3 days) was recorded in tap water (Table 1). Most ions at high concentrations reduce water uptake rate due to its toxic effects (van Meeteren *et al*., 1999). In agreement, in the present study too the leaves dipped in a higher concentrations of $CuSO_4$ (1.0 mM) solution showed various toxic symptoms such as yellow-brown colour patches on the leaf lamina and tip burning symptoms at five days of vase life. There was no significant difference ($p = 0.6803$) between the bacterial growth in DW and the $CuSO_4$ treatments. However, the bacterial population in the vase solutions increased along with the vase life period of *O. japonicus*, irrespective of the treatment (Figure 1). When considering the vase life data, there was no significant difference between DW and $CuSO_4$ (0.25 mM), $CuSO_4$ (1.0 mM) treatments. However, $CuSO_4$ (0.5 mM) and DW had a significant difference on vase life and this may be due to the action of copper involved in enzymatic reactions related to biosynthesis and action of ethylene (Hojjati *et al.,* 2007) and/or its involvement in stem end wound reaction inhibition. Furthermore, as a multifunctional biocide, copper may inhibits the enzymes involved in physiological stem occlusion (Damunupola and Joyce, 2008), thus enhancing the vase life of *O. japonicus* cut foliage.

According to the Experiment 2, the longest vase life (~10 days) was recorded in sucrose (20 g/L) + $CuSO_4$ (0.5 mM) solution while the shortest vase life (~4 days) in 30 g/L sucrose treatments (Table 1). Further, there was a significant ($p<0.05$) positive effect on solution uptake rate with sucrose pulsing treatments. There was no significant difference between DW and 20 g/L sucrose treatment although there was a difference with 30 g/L sucrose treatment. No

significant difference was observed between two different concentrations of sucrose. However, the longest vase life (~10 days) was recorded in sucrose (30 g/L) pulsing treatment while shortest vase life (~4 days) was recorded in DW treatment (Table 1). The results indicated that sucrose as a vase solution enhances the osmotic balance of cell and maintains turgidity. Further, sucrose also contributes to cell metabolism and supplies energy. However, sucrose has a positive influence on the microbial population (Put and Klop, 1990). Microorganisms and their byproducts such as toxins, extra polysaccharides and enzymes can create microbial plugging in xylem vessels. Ultimately, longevity of cut flowers is reduced.

This constraint can be overcome by adding a biocide to the vase solution that contains sucrose (Jones and Hill, 1993). Cu^{2+} as a biocide may suppress bacterial growth (van Meeteren *et al.,* 1999). The results of the Experiment 2 agreed with van Meeteren *et al.* (1999) that the sucrose alone treatments showed the shortest vase life compared to sucrose with $CuSO_4$ treatments. Results were in agreement with Wirthensohn *et al*. (1996) who reported that 10 g/L or 20 g/L sucrose with 8-HQC (200 mg/L) which acts as a biocide significantly enhance the vase life of *Eucalyptus* sp. foliage that is used as a filler in floral arrangements. Pulsing treatment with high sugar concentration 200 g/L for 20 hours at 20 °C has also been effective in opening of florets per spike in cut flower *Gladiolus* cv. "American Beauty" against the control (0 g sugar) treatment (Babaji *et al.*, 2014).

When considering the vase life of *O. japonicus* treated with BAP (Experiment 4), the longest vase life (~12days) was recorded in 10 mg/L BAP treatment while the shortest (~5 days) was recorded in DW. One of the major constraints of cut foliage of many tropical plant species is leaf yellowing leading to leaf senescence (Subhashini *et al.,* 2011). The exogenous application of cytokinin, have an impact on the reduction of the production of ethylene and delay leaf senescence (Setyadjit *et al.,* 2004). Based on our results and former reported literature, application of BAP can be recommended to increase the postharvest longevity of cut foliage of *O. japonicus*. Moreover, results obtained from this study were in agreement with Subhashini *et al.* (2011) who reported that 5 mg/L BAP as a pulse treatment for 24 h was the most effective treatment to enhance the marketability of cut leaves of *Dracaena marginata* 'Bi colour', *D. sanderiana* 'White' and *D. deremensis.* According to Janowska *et al*. (2013) different concentrations (25, 50 and 75 mg/L) of benzyladenine significantly increased the vase life of *Limonium latifolium.*

During leaf senescence process, chlorophyll breakdown is initiated with the removal of the phytol tail. This reaction is catalyzed by the chlorophyllase enzyme (Gupta *et al*., 2011). There was a significant difference ($p<0.05$) between the DW and three BAP treatments on chlorophyll *a+b* contents of cut foliage of *O. japonicus,* as found in Expt. 4. The highest total chlorophyll contents (chlorophyll *a+b*) 61.41µg/mL was recorded in BAP 20 mg/L while the lowest (35.32 µg/mL) was recorded in DW at the end of vase life of cut leaves, which indicated a 1.7 fold increment of chlorophyll content. However, there was no significant difference of total chlorophyll contents, among all BAP treatments (Table 2).

Results of the BAP treatments agreed with the findings of Janowska *et al*. (2013) in that the application of benzyladenine enhanced the leaf greenness and its quality. Furthermore, vase life of *O. japonicus* treated with BAP was significantly higher than DW (Table 1) and it is in agreement with the ability of enhancement of chlorophyll content by BAP treatments. According to Gupta *et al.* (2011) during the period of senescence, chlorophyll *b* is converted to chlorophyll *a*.

CONCLUSION

Among the tested vase solutions, the most effective vase solutions for *O. japonicus* were 0.5 mM $CuSO_4$, sucrose 20 g/L with 0.5 mM $CuSO_4$, sucrose 20 or 30 g/L pulsing treatments for 24 h and BAP (5 - 20 mg/L) solutions. The vase life of *O. japonicus* could be extended from ~5 days to ~12days by giving BAP based vase solutions. However, in the present study, no significant antibacterial effects among the $CuSO_4$ solutions were discerned. Thus, enhancement of vase life may be due to the action of copper involved in enzymatic reactions related to biosynthesis and action of ethylene. BAP treatments increased the total chlorophyll content of *O. japonicus* leaves thus enhancing the vase life. Further research is needed to examine the possibility of combining sucrose pulsing followed by combined copper salt and BAP treatments for further extensions in vase life.

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Table 2: Average chlorophyll a+b contents with different BAP treatments during the vase period.

	Days				
Treatment	**0**	**3**	**6**	**9**	**12**
DW	48.98±3.55	53.52±3.05	50.76±8.64	45.91±0.74	35.32±2.52
BAP 5	47.89±2.81	61.25±3.95	46.18±3.83	58.11±5.64	59.78±2.27
BAP 10	47.48±4.70	72.51±0.32	56.27±4.18	62.29±4.80	50.38±3.56
BAP 20	50.35±5.59	71.77±0.31	53.04±1.57	52.58±7.99	61.41±2.15

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