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Effect of different chemicals on physiological and biochemical parameters of cut stem *Lilium* cv. Pollyanna during post harvest period

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Abstract
To increase the post harvest shelf life of *Lilium* flowers, experiments were conducted by using Preservative solutions *viz.* Nano Silver (50-ppm), Sucrose (50-ppm), 8-HQ and Distilled water. The experiment was conducted with total 14 treatments. The major reasons for less vase life of cut flowers may be due to nutrient deficiency, bacterial and fungal contaminations, water stress and vascular blockage. Maximum vase life was also found in NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent H10 treatment. Treatment combination of NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent can extend the vase life of harvested *Lilium* flowers by reducing the bacterial infection, improving the physiological and biochemical parameters for harvested flowers.

Keywords: *Lilium*, cut flowers, post harvest, Nano silver, sucrose, vase life

Introduction
Lilies are herbaceous perennial bulbiferous plant. Bulbs are used as food in china (Tiger lily). There are about 400 species among that 100 species are described. Long stem, sturdy and have long vase life. Bulbs are imbricate, fleshy scales without tunic. Leaves are alternate, rarely whorled, and sessile/sub sessile, usually linear to linear- lanceolate. Inflorescence is terminal, raceme or solitary flower. Flowers are often funnel form or campanulate sometimes tubular.
Cut flowers have a limited vase life and have traditionally been cultivated close to marketing centres so that consumers might enjoy maximum utilization of the flowers. People love cut flowers. They not only continue to buy them, but also flower consumption is rapidly increasing in many countries all over the world. So keeping quality is an important parameter for evaluation of cut flower quality, for both domestic and export markets. The techniques of prolonging the vase life of flowers will be a great asset to the growers and users.

Material and Methods
Lilium flowers were procured from the M/s. Balaji Flowers, Devashola Estate, and The Nilgiris during spring season. Thereafter, they were kept under precooling (7 ºC) and then transported with in 3h to the Tamil Nadu Agricultural University. To minimize moisture loss, flowers were covered with plastic film during transportation. At the laboratory, stem ends were re-cut by ≥ 10 cm, and stems with about 50 cm length were used in experiment. The aqueous test solutions were: H1 –Nanosilver (NS) 50 ppm, H 2 –NS 75 ppm, H 3 – 8-HQ 150 ppm, H 4 – 8-HQ 200ppm, H 5 – H1 + 2% Sucrose, H6 – H2 + 2% Sucrose, H 7 – H3 + 2% Sucrose, H 8 – H4 + 2% Sucrose, H 9 – H3 + H5, H 10 – H3 + H6, H 11 – H4 + H5, H 12 – H4 + H6, H 13 – Sucrose 2% and H 14 – Control (Distilled water). The experiment was conducted in a completely randomized design with factorial concept and replicated thrice with holding method of treatment. The observations recorded by adopting the following methods.

Physiological parameters
The observations were recorded at every three days interval from two flowers in each treatment.

Physiological loss in weight (PLW)
The initial weight of the flower stalk was taken and subsequent weights were taken in the following days. The PLW was arrived by using the following formula and expressed in percentage.

$$PLW = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Membrane integrity

Membrane integrity was estimated based on the percentage of solute leakage. Twenty five flower discs were taken in 20 ml of water and kept for two hours. The absorbance was read at 273 nm. The contents of the beaker were boiled for 10 min. and the absorbance was measured again. The percentage of solute leakage was calculated using the following formula,

$$\text{Percentage of solute leakage} = \frac{\text{Final OD at 273 nm - Initial OD at 273 nm}}{\text{Final OD at 273 nm.}} \times 100$$

Biochemical parameters

Total carbohydrate

The total carbohydrate content in the flower was estimated by anthrone method (Hodge and Hofreiter, 1962) [8]. The samples were hydrolysed with HCl and neutralized with solid sodium carbonate and the development of green to dark green colour by the anthrone reagent was measured at 630 nm and expressed as the mg of glucose /g of sample.

Peroxidase activity (POD)

Peroxidase activity was determined by adopting the procedure of Malik and Singh (1980) [10]. One gram sample was extracted with 10 ml of phosphate buffer (pH 7.0). A known volume of extract was added to the experimental cuvette containing 3 ml of pyrogallol and 0.5 ml hydrogen peroxide solution as a substrate to the cuvette and increase in absorbance at 420 nm was recorded. The change in minutes was used to calculate the enzyme activity. The enzyme activity was expressed as units per gram of fresh weight (1 unit = 1m mole/ minute) for every six days interval.

Carotenoid content

Carotenoid was estimated by the procedure outlined by Roy (1973) [14]. One gram sample of fresh petals from all portions of the flower was taken and carotenoid was extracted using 85 per cent acetone and centrifuged. After centrifugation five ml of the supernatant was taken and the volume made to 10 ml of acetone. Then the O.D values were observed using spectrophotometer at 480 nm and 510 nm. The carotenoid content was calculated and expressed as mg/g.

Chlorophyll content

The chlorophyll 'a' and 'b' and total chlorophyll content was estimated in a fully expanded leaf from the top at specified phenophases by adopting the procedure of Yoshida *et al.* (1971) [22] and the contents were expressed as mg g-1 of fresh tissue.

Photomicroscope

Segments of 3 cm length were excised for microscope observation from cut stem ends on the end of vase period. Explants for light microscopy were fixed initially in FAA (Li, 1987) [9]. Paraffin embedding was used to prepare permanent tissue sections. Sections were stained with Ehrlich's haematoxylin solution and examined under a Nikon (AFX – IIA) photomicroscope.

Vase life of flowers

The vase life of cut flower was recorded as per the method suggested by Nowak and Mynett (1985) [12]. The vase life of cut spike was recorded from the day of anthesis of the first flower bud to the senescence of last flower bud.

Result & Discussions

Physiological parameters

The loss in weight of flowers is due to changes in metabolic activities and it was found that the treatment H10 recorded the lowest PLW and showed consistently less weight loss than the other treatments.

The present study revealed that the loss of membrane integrity was lowest in treatment H10 among the treatments at all stages. The loss in selective permeability of the tonoplast is the first step towards membrane breakdown. Destruction of the membrane structure is associated with various factors like water stress, osmotic stress, chilling effect and activation of phospholipases that induce ethylene synthesis. The loss in integrity and an increase in permeability is a sign heralding senescence as reported by Halaba and Rudnicki (1986) [5].

Biochemical parameters

Carbohydrate content was maximum in the treatment combination H10. The carbohydrate source sucrose in the holding solution might have acted as substrate for respiration thereby improving the dry matter of the vegetative parts which in turn are translocated to the petals. These findings are in similarity to the results of Ho and Vidhyasankar (2001) [21].

Peroxidase is apparently related to an increase in peroxides and free radicals which react with cellular constituents and involved in the promotion of senescence. Holding solution treatment with H10 exhibited lowest peroxidase activity (POD) among the treatment combinations. The decrease in POD activity might be due to the effect of benzyl adenine which controls or keeps down the peroxidise activity. Further, the lower peroxidase activity might also be due to the better water relations and cellular integrity with low leakiness from cell membranes. This is in conformity with the reports of Fridovich (1975) [3] and Baker *et al.* (1977) [1].

The carotenoid pigment content in the treatment H10 was found to be maximum among the treatments and recorded decreasing trend from day 1 after treatment till the end of vase life. The enhanced content of carotenes in this treatment might be due to the availability of adequate sucrose in petal cells in the treated stems exhibiting a protective role on chromoplasts (being lipid - protein complex) which are the sites of carotene synthesis. This is in support of the evidence by Yu (1999) [23].

Microscopic observations showed that xylem vessels at the cut stem end clear immediately after cutting. On day 13 after holding treatment, particles (probably bacteria and decay products) were observed in vessels of control treatments held with distilled water. In contrast, few particles were evident on day 13 in vessels of *Lilium* held with NS particles.

NS may positively influence water uptake in another way besides an anti-bacterial effect. Van Meeteren *et al.* (2001) [20] determined that AgNO3 added in deionized water (DI) had positive effect on Bouvardia water status and that use of tap water (containing mixture of ions) had a similar effect to AgNO3 solution. Ions in water, particularly cations, can enhance flow through xylem vessels (Van Ieperen *et al.*, 2000) [19]. Van Doorn *et al.* (1989) [18] and Bleeksma and Van Doorn (2003) [3] found that the wilting of cut roses was related to bacterial infection in the xylem vessels. Van Doorn and De Witte (1994) [17] observed in gerbera an increase in scape curvature with increase in concentration of bacteria in the water, but they concluded that scape curvature was partially

due to the bacterial populations. In gerbera, curvature of the stem appeared to be related to the endophytic bacterial population levels, which also induced higher ethylene levels, resulting in an evident curvature of the younger portion of the stem (Van Dorn and De Witte, 1994; Van Doorn, 1998) [16, 17], though this portion may also have been affected by dry storage (Van Dorn *et al*., 1994) [17]. Various changes (physical, biochemical) have been related to the occurrence of bacteria in the maintenance solutions of cut flowers.

Among the holding solutions, the treatment H10 resulted in the longest vase life. This might have been due to cellular disintegration of floret tissues through osmotic injury resulting in early wilting. On the other hand, short vase life of flowers is associated with an increase in respiration and hydrolysis of cell components, a decline in water status (Van Meeteren *et al*., 2001) [20], starch content (Ho and Nichols, 1977) [7], reduction in cell wall polysaccharides, proteins, nucleic acids (Stead and Moore, 1977) [15] and increase in permeability and ion leakage (Parups and Chan, 1973) [13]. The reduction in vase life has been ascribed to decrease in water content, depletion of carbohydrates, increase of ethylene production and reduction in water uptake of flowers. This is in corroboration with the findings of Goszeynska and Rudnicki (1988) [4].

Table 1: Effect of holding solution on physiological loss in weight (per cent) and membrane integrity (per cent)

Treatments	Physiological loss in weight (per cent)	Membrane integrity (per cent)		
		Day 1	Day 6	Day 12
H_1	35.78	25.28	42.63	78.97
H_2	28.58	22.43	39.96	72.25
H_3	39.94	28.66	45.21	79.75
H_4	38.83	22.43	39.96	72.25
H_5	30.68	23.33	38.23	75.08
H_6	27.63	28.66	45.21	79.75
H_7	26.92	21.82	38.00	69.29
H_8	31.13	23.33	38.23	75.08
H_9	14.14	18.32	33.50	62.55
H_{10}	12.92	17.30	31.53	61.05
H_{11}	24.84	19.46	35.80	65.00
H_{12}	22.77	19.46	35.80	65.00
H_{13}	36.00	26.17	48.11	82.48
H_{14} (Control)	39.38	30.92	52.08	84.62
Mean	29.25	23.40	40.30	73.08
S.Ed.	0.54	0.67	1.17	2.11
C.D (P=0.05)	1.11	1.38	2.39	4.31

Table 2: Effect of holding solution on total carbohydrate content (mg g^{-1})

Treatments	Total carbohydrate content (mg g^{-1})		
	Day 1	Day 6	Day 12
H_1	94.30	102.10	83.21
H_2	100.19	121.40	91.00
H_3	92.10	100.36	82.60
H_4	91.36	91.35	77.60
H_5	98.40	120.00	88.85
H_6	106.00	128.00	96.30
H_7	114.50	128.65	102.65
H_8	96.34	106.42	86.40
H_9	127.60	140.82	117.15
H_{10}	138.00	152.40	127.60
H_{11}	118.00	135.70	104.00
H_{12}	118.25	138.65	108.00
H_{13}	91.08	85.46	72.30
H_{14} (Control)	88.00	79.50	68.25
Mean	105.29	116.49	93.28
S.Ed.	3.06	3.43	2.72
C.D (P=0.05)	6.26	7.02	5.57

Table 3: Effect of holding solution on peroxidase activity (change in OD/g/min)

Treatments	Peroxidase activity (change in OD/g/min)		
	Day 1	Day 6	Day 12
H_1	0.029	0.032	0.033
H_2	0.029	0.030	0.032
H_3	0.030	0.034	0.035
H_4	0.030	0.034	0.035
H_5	0.029	0.030	0.033
H_6	0.027	0.029	0.032
H_7	0.026	0.028	0.032
H_8	0.029	0.032	0.033

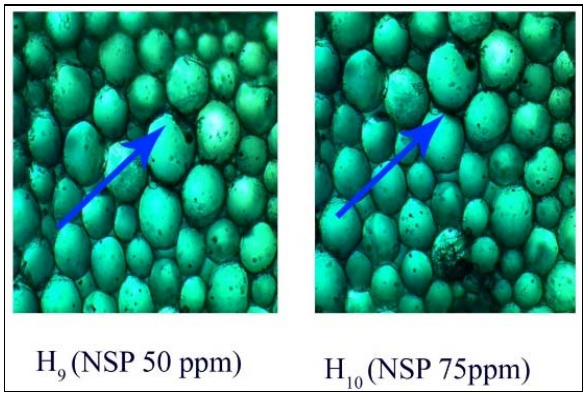
H_9	0.022	0.025	0.030
H_{10}	0.020	0.023	0.028
H_{11}	0.024	0.026	0.030
H_{12}	0.022	0.025	0.030
H_{13}	0.032	0.034	0.035
H_{14} (Control)	0.035	0.037	0.038
Mean	0.027	0.030	0.033
S.Ed.	0.0008	0.0009	0.0009
C.D (P=0.05)	0.0017	0.0018	0.0019

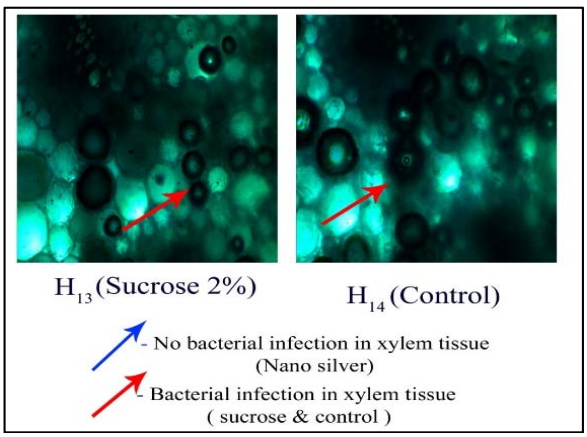
Table 4: Effect of holding solution on carotenoid content (mg g^{-1})

Treatments	Carotenoid content (mg g^{-1})			Vase life (Days)
	Day 1	Day 6	Day 12	
H_1	0.145	0.400	0.115	11.38
H_2	0.149	0.427	0.140	13.51
H_3	0.128	0.360	0.071	10.23
H_4	0.139	0.387	0.098	10.86
H_5	0.149	0.415	0.140	12.66
H_6	0.166	0.427	0.140	14.32
H_7	0.171	0.431	0.157	14.70
H_8	0.147	0.415	0.125	11.81
H_9	0.181	0.453	0.176	16.21
H_{10}	0.184	0.462	0.190	17.84
H_{11}	0.175	0.446	0.168	14.70
H_{12}	0.181	0.446	0.176	15.08
H_{13}	0.110	0.360	0.050	9.16
H_{14} (Control)	0.110	0.274	0.023	8.33
Mean	0.153	0.407	0.126	12.91
S.Ed.	0.004	0.012	0.004	0.38
C.D (P=0.05)	0.009	0.024	0.008	0.78

Table 5: Effect of holding solution on chlorophyll content (mg g^{-1})

Treatments	Chlorophyll content (mg g^{-1})								
	Day 1			Day 6			Day 12		
	Chlo 'a'	Chlo 'b'	Total Chlo	Chlo 'a'	Chlo 'b'	Total Chlo	Chlo 'a'	Chlo 'b'	Total Chlo
H_1	0.320	0.075	0.446	0.304	0.061	0.420	0.297	0.061	0.384
H_2	0.359	0.107	0.547	0.340	0.098	0.524	0.324	0.094	0.501
H_3	0.290	0.055	0.398	0.281	0.037	0.375	0.270	0.041	0.346
H_4	0.303	0.060	0.436	0.296	0.048	0.419	0.284	0.050	0.394
H_5	0.343	0.104	0.482	0.328	0.090	0.461	0.319	0.087	0.437
H_6	0.365	0.111	0.606	0.340	0.103	0.587	0.348	0.092	0.540
H_7	0.417	0.112	0.612	0.402	0.107	0.593	0.406	0.094	0.547
H_8	0.322	0.091	0.474	0.307	0.083	0.451	0.304	0.070	0.432
H_9	0.466	0.211	0.791	0.449	0.204	0.765	0.439	0.197	0.738
H_{10}	0.505	0.295	0.803	0.483	0.273	0.780	0.482	0.264	0.751
H_{11}	0.421	0.129	0.684	0.411	0.117	0.653	0.410	0.109	0.617
H_{12}	0.422	0.194	0.734	0.416	0.174	0.701	0.413	0.167	0.681
H_{13}	0.206	0.036	0.284	0.194	0.021	0.259	0.194	0.018	0.217
H_{14}	0.179	0.031	0.246	0.165	0.018	0.247	0.152	0.014	0.204
Mean	0.351	0.115	0.539	0.337	0.102	0.517	0.332	0.097	0.485
S.Ed.	0.010	0.004	0.016	0.010	0.003	0.016	0.010	0.003	0.015
C.D (P=0.05)	0.021	0.008	0.033	0.021	0.007	0.032	0.020	0.007	0.031





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