EFFECT OF POST HARVESTTREATMENTS OF SUCROSE, 8 – HQ AND NANOSILVER ON THE PHYSIOLOGICAL AND BIOCHEMICAL CHANGES OF CUT LILIUMFLOWERS DURING VASE LIFE PERIOD

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Abstract: An investigation was undertaken on the effect of certain preservatives *viz*., sucrose, 8 – HQ and Nanosilver on the physiological and biochemical changes of cut Liliumflowers during vase life period. The result of the investigation proved that the use of these preservatives in combination is a must to improve the quality, flower opening and vase life. Further, the use of sucrose, 8 – HQ and Nanosilver (NS) in the vase solution significantly reduced the number of microbial colonies with the passing of time, there by the water conductance through the xylem vessels increased. Among all the treatments imposed, treatmentof NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent was found to be the best by registering less PLW (12.92 per cent), less membrane integrity (17.30 per cent), less POD (0.022, 0.025 and 0.03) and increase in the flower diameter (17.82 cm) , vase life (17.84 days) and number of opened flowers. Microscopic examination of cut stem ends on the end of vase life period was also studied and reported.

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

Introduction: Among various cut flowers, lilium has just opened its way in floriculture industry of our country due to its immense potential as cut flower. Lilium ranks 6th among the top ten cut flowers of the world. Large volume of cut flowers *i.e.,* around 28-32% are lost annually due to poor post harvest handling measures because of its perishable nature (Dadlani, 1997). Keeping quality of flower is decided by its hereditary factor. However, it can be manipulated to certain extent by using novel preservative treatments. Keeping of cut flowers in various preservatives has effectively been used form long time to improve their longevity (Khan *et al*., 2007).

Nanosilver particles (NS) has been widely used as a preservative due to its anti bacterial property. It also has the additional benefit of high durability, simple and easy to use and lack of side effect than other anti-bacterial agents (Van Doorm, 1997). With the above background the present study has been investigated on the effects of NS solution treatments on extending vase life of cut lilium flowers.

However, the diminishing keeping quality of cut lilium badly affects the growers as well as the traders. Lower status of water, carbohydrates, proteins and fats in the floral tissue, poor handling and marketing methods badly impair the physiology and biochemistry of flower petal leading to shortened vase life of cut flowers. Keeping this in view, the present investigation was also aimed towards discerning the events leading to senescence of cut lilium by supplying, 8 – HQ and sucrose through the vase solution at different concentrations.

Materials And Methods: Lilium var. Pollyanna flowers were procured (lower first bud turns from green to original colour of the variety, but has not yet opened) from the M/s. Balaji Flowers, Devashola Estate, The Nilgiris during spring season (March – April) of 2012. Thereafter, they were kept under precooling (7^{o}C) 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: H_1 –Nanosilver (NS) 50 ppm, H_2 –NS 75 ppm,

H_3 – 8-HQ 150 ppm, H_4 – 8-HQ 200ppm , H_5 – H_1 + 2% Sucrose, H_6 – H_2 + 2% Sucrose , H_7 – H_3 + 2% Sucrose, H_8 – H_4 + 2% Sucrose, H_9 – H_3 + H_5, H_{10} – H_3 + H_6, H_{11} – H_4 + H_5, H_{12} – H_4 + H_6, 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 loss in weight (PLW): The initial and final weight of the flower stalk was taken and it 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. (100^{o}C) and the absorbance was measured again.

The percentage of solute leakage was calculated using the following formula,

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

Extraction of the sample for the assay of enzyme activity: One gram of fresh petal tissue was homogenized in a pre-chilled mortar and pestle with 0.1M phosphate buffer (pH 7.0) and 0.1% ploy vinyl pyrrolidone (PVP) and centrifuged at 27, 000 rpm for 20 minutes. The clear supernatant enzyme extract was used for the assay of enzyme activity was estimated at room temperature (i.e. 27°C).

Assay ofPeroxidase: Peroxidase activity was determined by adopting the procedure of Malik and Singh (1980). 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.

Flower diameter (cm) and vase life (days): The diameter of the opened flowers was measured in cm across the centre of the flower at the largest point. The vase life of cut flower was recorded as per the method suggested by Nowak and Mynett(1985). 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.

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). 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.

Results And Discussion: The loss in weight of flowers is due to changes in metabolic activities and it was found that the treatment NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent (H_{10}) recorded the lowest PLW and showed consistently less weight loss than the other treatments (Fig 1).

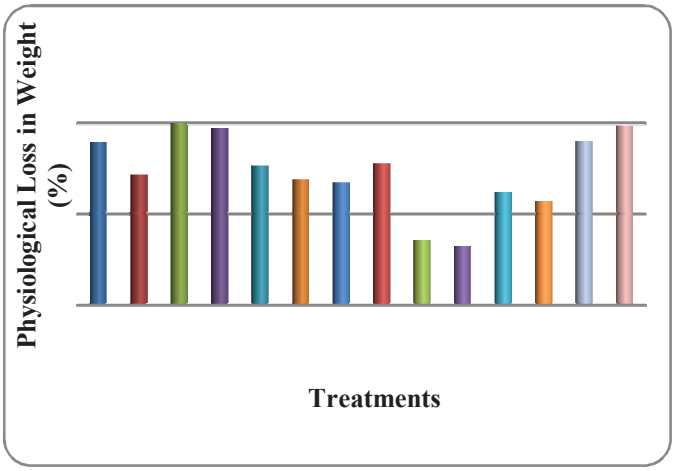

Fig.1. Effect of holding solutions on physiological loss in weight (%) during vase period

The present study revealed that the loss of membrane integrity (Table 1) was lowest in treatment H_{10} (NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent) 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 effectand 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).

Table. 1 Effect of holding solution membrane integrity (per cent)

Treatments	Membrane integrity (per cent)			
	Bud stage	Open flower	Senescence flower	Mean
H_1	25.28	42.63	78.97	**48.96**
H_2	22.43	39.96	72.25	**44.88**
H_3	28.66	45.21	79.75	**51.21**
H_4	22.43	39.96	72.25	**44.88**
H_5	23.33	38.23	75.08	**45.55**
H_6	28.66	45.21	79.75	**51.21**
H_7	21.82	38.00	69.29	**43.04**
H_8	23.33	38.23	75.08	**45.55**
H_9	18.32	33.50	62.55	**38.12**
H_{10}	17.30	31.53	61.05	**36.63**
H_{11}	19.46	35.80	65.00	**40.09**
H_{12}	19.46	35.80	65.00	**40.09**
H_{13}	26.17	48.11	82.48	**52.25**
H_{14}	30.92	52.08	84.62	**55.87**
Mean	**23.40**	**40.30**	**73.08**	
	H	**D**	**H x D**	
S.Ed.	**0.77**	**0.35**	**1.34**	
C.D (P=0.05)	**1.53**	**0.71**	**2.66**	

Peroxidase (Fig 2) 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 NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent (H_{10})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) and Baker *et al.* (1977).

In the present study, the flower diameter (17.82 cm) was the highest in flower stems that are held in the treatment combination NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent (H_{10}). Cheon Young Song *et al.* (1996) reported that carbohydrates are the main source of nutrition for flowers and are a source of energy necessary for maintaining all biochemical and physiological processes fundamental for prolonging vase life by improving water balance and increasing flower diameter.

Among the holding solutions, the treatment NS 75 ppm + 8 – HQ 150 ppm + sucrose 2 per cent (H_{10}) resulted in the longest vase life (17.84 days). This might have been due to cellular disintegration of floret tissues through osmotic injury (Halevy and Mayak, 1981) resulting in early wilting. On the other hand, short vase life of flowers associated with an increase in respiration and hydrolysis of cell components, a decline in water status, starch content,

reduction in cell wall polysaccharides, proteins, nucleic acids and increase in permeability and ion leakage.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 (Goszeynska and Rudnicki, 1988) (Table 2).

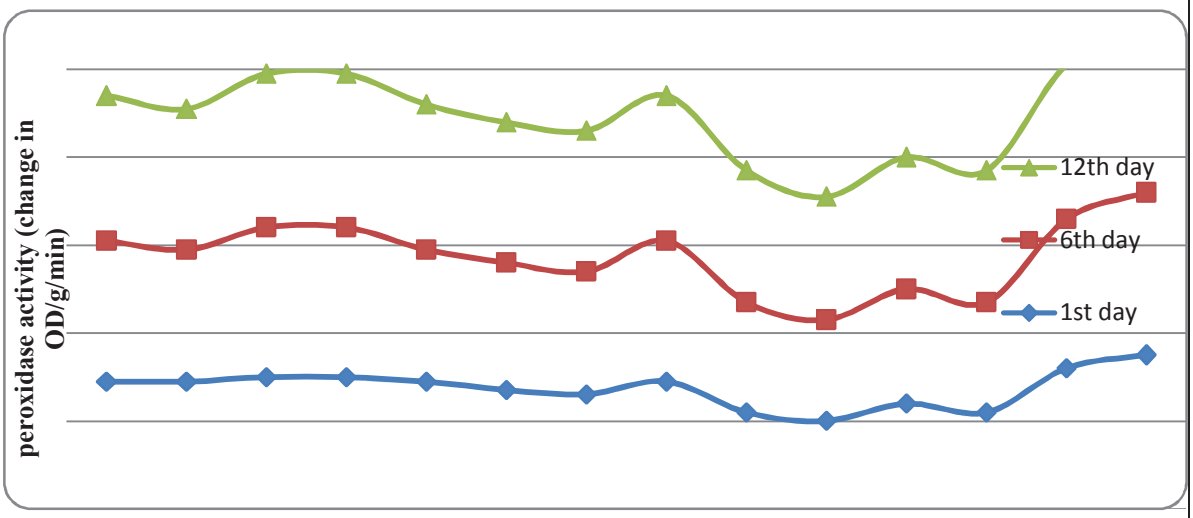

Fig. 2. Effect of holding solutions on peroxidase activity (change in OD/g/min) during vase period

Table. 2 Effect of holding solution on days taken for bud opening and open flower diameter (cm)

Treatments	Flower diameter (cm)	% increase/decrease in diameter over control	Vase life (Days)	% increase/decrease in vase life over control
H_1	15.43^{e}	14.98	$11.38^{f,g}$	36.61
H_2	$15.72^{c,d,e}$	17.14	13.51^{d}	62.18
H_3	$14.48^{f,g}$	7.89	10.23^{h}	22.81
H_4	$15.25^{e,f}$	13.64	$10.86^{g,h}$	30.37
H_5	$15.57^{d,e}$	16.02	12.66^{e}	51.98
H_6	$15.74^{c,d,e}$	17.29	14.32^{c}	71.91
H_7	$16.40b^{c,d,e}$	22.21	14.70^{c}	76.47
H_8	15.45^{e}	15.13	11.81^{f}	41.78
H_9	$16.93^{a,b}$	26.15	16.21^{b}	94.60
H_{10}	17.82^{a}	32.79	17.84^{a}	114.17
H_{11}	$16.50^{b,c}$	22.95	14.70^{c}	76.47

H_{12}	$16.55^{b,c}$	23.32	15.08^{c}	81.03
H_{13}	$14.04^{g,h}$	4.61	9.16^{i}	9.96
H_{14}	13.42^{h}	0.00	8.33^{j}	0.00
Mean	**15.66**	**-**	**12.91**	-
S.Ed.	**0.45**	**-**	**0.38**	-
C.D (P=0.05)	**0.93**	**-**	**0.78**	-

Microscopic observations showed that NS treated spikes have bacterial free xylem vessels at the cut stem end (Fig 3). Whereas, on day 13 after holding treatment, particles (probably bacteria and decay products) were observed in control held with distilled water and also in sucrose treatment. In contrast, few particles were evident on day 13 in liliumflowers held with NS particles. The data suggest that anti-bacterial benefits of NS particles in the holding solution are transient. Vascular occlusion has been considered to be mainly due to microbial proliferation. Efficacy of nanometer sized particles bearing Ag+ as antibacterial agent (NS) is well established. Antibacterial activity of NS is partly a function of particle size, with higher surface to volume ratio increasing the proportion of atoms at the grain boundary (Raffi*et al*., 2008).

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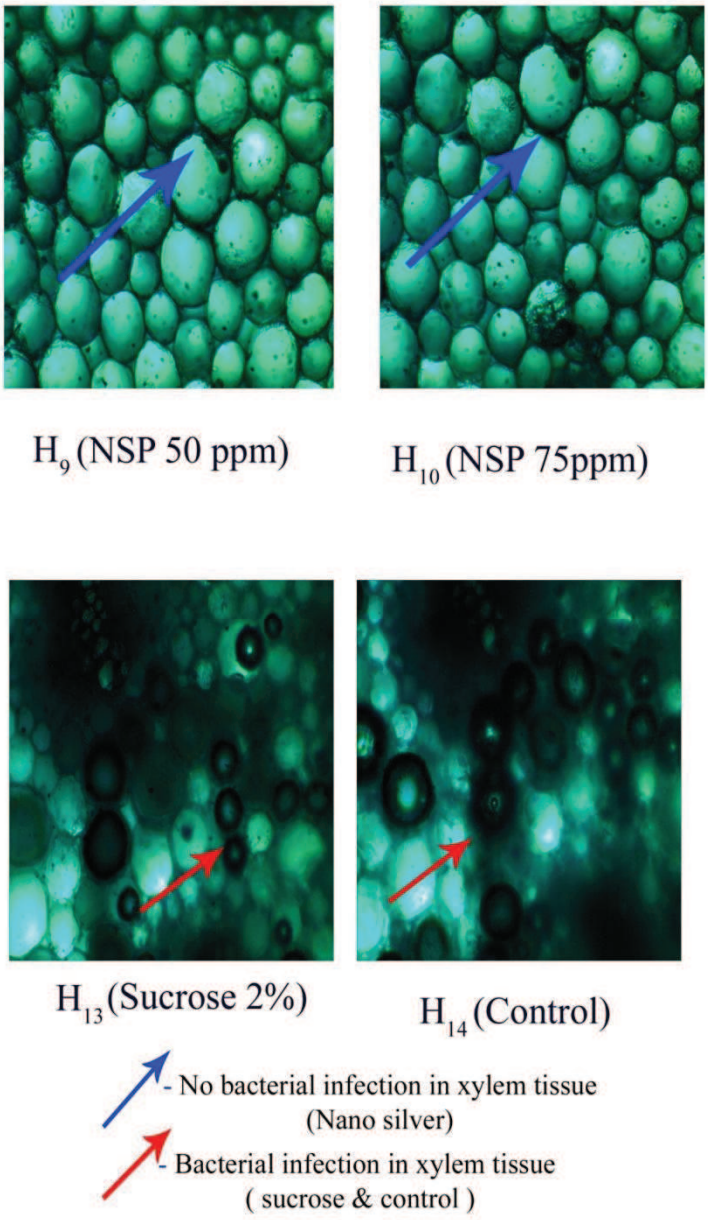

Fig. 3. Comparison of Nanosilver and sucrose on bacterial infection in xylem tissue of Lilium var. 'Pollyanna'

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