

**Silver nanoparticles ameliorate postharvest quality of *Lilium cv. Eyeliner* cut flowers**

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**Abstract**

*Lilium* is widely known as one of the most important bulbous cut flower internationally. Improper and inadequate post harvest handling results into quality losses for retailed flowers. To address this issue, a study was conducted to evaluate the effects of silver nanoparticles as higher concentration pulse or a lower concentration as vase solution on the postharvest performance of *Lilium* cut flowers. The 20 ppm pulsing of cut stems for 24 hours, followed by retention in 2 per cent sucrose solution substantially improve the relative fresh weight, water uptake, water balance, delayed the leaf yellowing. However, higher concentration (40 ppm) extended the vase life by 03 days as compared to the control flowers. The lower concentration (20 ppm) of nanosilver as holding solution enhanced the physiological parameters and controlled the senescence related processes in leaves and petals. Vase life was enhanced to 4.35 days as compare to flowers placed in distilled water as control. The present findings unequivocally highlight that a 40 ppm nanosilver pulse for 24 hours or a 20 ppm + 2% sucrose vase solution can significantly prolong the vase life and positively influence the physiological parameters of cut *Lilium* flowers.

**Key words:** Cut flower, **Lily**, Postharvest, Sucrose, Vase life.

**Introduction**

The world floriculture industry evolving rapidly likes other industries in the present scenario and further grows in the 21<sup>st</sup> century. The International market of ornamentals expected to grow roughly 6.5% over the next five years from 42.4 billion US dollar to 57.4 billion US dollars (AIPH, 2021). Among the different cut flowers, roses, chrysanthemum, tulip, **lilium** and **gerbera** are the major commodities and contribute more than 70% in the international market. The Netherland is the major player in the cut flower production followed by Colombia, Ecuador, Kenya and major export destinations are European Union (Flora Holland, 2022).

35 The different agro-climatic zones present in India makes it conducive for the production of various  
36 loose and cut flower crops. The commercial cultivation of flowers is done in an area of 322  
37 thousand hectare producing 2152 (000) tones of loose flowers and 828 (000) tones of cut flowers  
38 (APEDA, 2022). The total export of 103.47 million US dollar was made to U.S.A, Netherland,  
39 Germany, U.K, United Arab EMTs, Canada and Italy during 2021-22 (APEDA, 2022). The major  
40 contributors are Kerala, Tamil Nadu, Karnataka, Madhya Pradesh, and Uttar Pradesh; growing  
41 Rose, Liliium, Tuberose, Gladiolus, Anthurium, Carnations, Marigold, etc. as commercial crops  
42 (NHB, 2021).

43 *Lilium* (*Liliaceae*) is the commercially important genus having ornamental, medicinal and edible  
44 values internationally (Zhou *et al.*, 2021). This genus comprises of 115 species having herbaceous  
45 perennial nature distributed in cold regions of Northern hemisphere and currently 50 % identified  
46 species occurs in south west China and Himalayas (Yan *et al.*, 2020; Rong *et al.*, 2011). In most  
47 parts of the world *Lilium* is commercially grown as ornamental plant due to its attractive, showy  
48 and different coloured flowers (Du *et al.*, 2017) and presently ranked fourth in the world trade  
49 (Flora Holland, 2022; Islam and Shimasaki, 2020). Apart this, some of the species are used in  
50 functional food and traditional herbal medicine (Sim *et al.*, 2020). The longevity of *Lilium* cut  
51 flowers postharvest and its ornamental values will depend on variety, growing environment and  
52 conditions *i.e.* water balance during storage and transportation. The most important influencing  
53 factor, water balance influenced by balance of water uptake and transpiration rate. The longevity  
54 and ornamental value can be maintained and improved by increasing water uptake and reducing  
55 transpiration rate. However, lack of pre- and post-harvest standard methods for handling the cut  
56 flowers forces the farmers, traders and retailers to face problems in the international market;  
57 enabling them to distribute the best quality produce to the consumers (Weeraratne *et al.*, 2012).  
58 Hence longer vase life of *Lilium* flowers is identified as the important criteria in the international  
59 flower market. For enhancing the export potential, there is need to standardize the post harvest  
60 handling techniques for *Lilium* to catering the international market. The yellowing and browning  
61 of leaves during storage is the visual sign of senescence, which affect the ornamental values of  
62 flowers.

63 Halevy and Mayak (1979) reported the positive correlation between water uptake and loss after  
64 harvesting and water loss will occur at faster rate through leaves during handling of cut stems. The  
65 microbial contamination (Louband and van Doorn, 2004) in the vase solution cause stem vessel

66 blockage and various bactericides viz., H<sub>2</sub>O<sub>2</sub>, AgNO<sub>3</sub>, Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, CuSO<sub>4</sub>, CoCl<sub>2</sub> (Van Meeteren *et*  
67 *al.*, 1999) utilized for checking the growth of bacteria (Xie *et al.*, 2008; Damunupola *et al.*, 2010)  
68 in solution for enhancing the vase life of cut stems. Ethylene is the major factor for reducing the  
69 vase life of cut stems as its sensitivity leads to abscission of buds, less bud opening, wilting of  
70 petals, yellowing of leaves (Riyaz *et al.*, 2021).

71 The silver nanoparticles have been used commercially in pharmaceutical, cosmetic and textile  
72 industry because of their antimicrobial properties (Navarro *et al.*, 2008; Mousa *et al.*, 2009; Julita  
73 *et al.*, 2020) as they change the structure of bacterial cell membrane, stoppage of DNA replication,  
74 which ultimately leads to cell death (Maneerung *et al.*, 2008; Skutnik *et al.*, 2021). There is  
75 reduction in transpiration, maximize the hydraulic conductance, inhibition of bacterial growth at  
76 stem end and prevention of senescence caused by ethylene. The silver nanoparticles (AgNP) alone  
77 will not enhance the longevity of the flower; however, application of sugar additionally effectively  
78 increases the vase life of the flowers. This can be achieved by application of sucrose in the solution  
79 as it will affect the proteolysis in flower petals, free amino acid aggregation and increasing the cell  
80 sap pH (Han 2003; Julita *et al.*, 2020). The degree of effectiveness of the vase solution is also  
81 depends on the vase solution concentration. Hence, the present study aimed to find out the suitable  
82 Nano Silver based preservative for enhancing the postharvest life of *Lilium cv.* Eyeliner cut  
83 flowers.

## 84 85 **Materials and Methods**

### 86 **Plant materials**

87 This study was conducted at ICAR- Indian Agricultural Research Institute, Regional Station,  
88 Katrain, Kullu, Himachal Pradesh, India during 2021-22. Plants of *Lilium cv.* 'Eyeliner' were  
89 grown at the Research farm under open field conditions. Diseased free, stems with uniform flowers  
90 were harvested and transported immediately in bucket containing water to the laboratory. The  
91 lowermost leaves up to 10-15 cm were trimmed with knife and sharp cut was given at stem end  
92 under distilled water to avoid air embolism.

### 93 94 **Chemical**

95 A colorless Monodisperse Silver Nanoparticles (AG60) from Sissco Research Laboratories Pvt.  
96 Ltd. was used in this study.

97

98 **Experimental design and treatments**

99 The experiments were carried out in a room with a temperature of  $23 \pm 1$  °C, a relative humidity  
100 of 60% and  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$  irradiance cool white fluorescent lamps under a diurnal light period  
101 of 8 hours. A complete randomized block design was used to study the effect of silver nanoparticles  
102 (AgNP) as pulsing and vase solution to extend the post harvest life of *Lilium* cut flowers under  
103 controlled climatic conditions in the laboratory. For vase solution, five treatments, viz., T1 (5 ppm  
104 NS+ 2% sucrose); T2 (10 ppm NS+ 2% sucrose); T3 (20 ppm NS+ 2% sucrose); T4 (30 ppm NS+  
105 2% sucrose); T5 (control-distilled water) with three replications were used to assess the effect of  
106 different silver nanoparticles treatments on post-harvest life of lilium. For pulsing solution, five  
107 treatments viz., T1 (10 ppm NS); T2 (20 ppm NS); T3 (30 ppm NS); T4 (40 ppm NS); T5 (control-  
108 distilled water) were formulated for the experiment. In case of pulsing treatment the cut stems were  
109 dipped in solution for 24 hrs and after that they were put in the 2% sucrose solution. The freshly  
110 prepared solution (400 ml) was put into the glass jars of capacity 500 ml and mouth of jar is closed  
111 with parafilm to check the evaporation losses.

112  
113 **Observations**

114 The vase life of lilium cut stems was measured by visual appearance (ornamental value) on daily  
115 basis until they lost the aesthetic value. Time taken to flower bud opening, tepal senescence, tepal  
116 abscission was observed daily from the lower most flowers on the cut stem (Fig.5). The criterion  
117 for considering a bud as open is lateral movement of more than one; tepal is considered as  
118 senescent when tip showing discoloration; tepal abscission: when more than one petal falls from  
119 the flower and tip of leaf turns yellow it is considered as leaf yellowing (Prisa *et al.*, 2013).

120 The fresh weight of cut stems, amount of water uptake, weight of vases with or without cut stems  
121 were observed daily. The average daily water uptake, water loss as transpiration, water balance  
122 and relative fresh weight were calculated by given formulas below (He *et al.*, 2006).

123  $Daily\ water\ uptake\ (gm) = (W_{t-1} - W_t) \text{ where, } t = 1, 2, 3, \dots, n \text{ days} \dots\dots\dots (1)$

124  $W_t$  is solution weight (gm) at  $t = 1, 2, 3, \dots, n$  days,  $W_{t-1}$  is the weight of solution at the previous  
125 day.

126  $Daily\ water\ loss\ (gm) = (L_{t-1} - L_t) \text{ where, } t = 1, 2, 3, \dots, n \text{ days} \dots\dots\dots (2)$

127  $L_t$  is the gross weight (gm) of vases along cut stems at  $t = 1, 2, 3, \dots, n$  days,  $L_{t-1}$  gross weight at the  
128 previous day.

129  $Water\ balance\ (gm)_t = (Water\ uptake - Water\ loss)_t$ , for day  $t$  ..... (3)

130  $Relative\ fresh\ weight\ (\%) = \frac{F_t}{F_0} \times 100$ ; where,  $t = 1, 2, 3, \dots, n$  days ..... (4)

131  $F_t$  is the weight (gm) of cut stem, and  $F_0$  is the weight of cut stem at 0 day.

132  
133 **Statistical analysis**

134 The randomized complete block design was adopted for conducting the experiment and each  
135 treatment involved three replications consisting three cut stems per replication. Mean and standard  
136 error (SE) values were calculated. Analysis of variance (ANOVA), followed by the LSD-test ( $P <$   
137  $0.05$ ), was used to test the significance of differences between means.

138  
139 **Results and Discussion**

140 **Effect of holding treatments**

141 ***Relative fresh weight and solution uptake***

142 The relative fresh weight ( $F=0.93$ ,  $d. f. = 4, 4$ ,  $p \leq 0.01$ ) and solution uptake ( $F=3.22$ ,  $d. f. = 4, 4$ ,  
143  $p \leq 0.01$ ) by cut flowers increases initially and subsequently decreases (Fig. 1a and 1c). Relative  
144 fresh weight (RFW) and vase solution uptake was higher in all the silver nanoparticle (NS)  
145 treatments as compare to control treatment. The RFW of the control treatment was comparatively  
146 constant for the first 6 days and then rapidly decreased over the period of time (Fig.1a). In contrast,  
147 the NS treated cut stems the uptake of solution was higher up to 06 days after harvest and they  
148 attain the maximum fresh weight as compare to control. The relative fresh weight was observed  
149 maximum (13.30 %) in vase solution containing 5 ppm NS with 2% sucrose up to 09 days after  
150 harvest and higher solution uptake (26.5 %) in 20 ppm NS as compare to control. Similar trends  
151 for nano silver treatments have been reported by Nemat *et al.*, (2013, 2014) and Thakur *et al.*,  
152 (2022) also reported that GO + SNPs at  $1\ \mu L\ L^{-1}$  exhibited better for relative water uptake, relative  
153 fresh weight and extended the vase life of cut flowers by 6 days in bird of paradise cut flowers.

154  
155 ***Water balance and water loss***

156 The water balance ( $F=5.04$ ,  $d. f. = 4, 4$ ,  $p \leq 0.01$ ) in all the treatments starts declining after 3<sup>rd</sup> day  
157 of harvest in all the treatments. Water balance declined linearly with the time and faster in control  
158 flowers than the higher concentration of NS (30 ppm 3 days after harvest; DAH) (Fig. 1b). The  
159 water loss ( $F=2.22$ ,  $d. f. = 4, 4$ ,  $p \leq 0.01$ ) exceeded the water uptake after 6 days in control and 9

160 days in NS treated cut flowers. Nano Silver treated cut stems showed water loss steadily until day  
161 12 and no significant difference was observed among the treatments. Among Nano Silver  
162 treatment, minimum water loss found with low concentration of Nano Silver 5 ppm (35.06 gr per  
163 stem per day) (Fig.1d). The NS treatments have the bactericidal effect in the vase solution, reduce  
164 the stem blockage and other factor might be that sucrose at lower concentration act as source of  
165 energy, delayed the disintegration of proteins, hence improved the water balance in cut stems Liu  
166 *et al.*, (2009). Liu *et al.*, (2012) in *Acacia holosericea* found that lower concentration of Nano  
167 Silver (4mgL<sup>-1</sup>) in holding solution had less number of bacteria than those of higher concentration  
168 (40 mgL<sup>-1</sup>) from 2<sup>nd</sup> day onwards.

169

#### 170 ***Days to bud opening, days to tepal senescence and days to flower senescence***

171 The flower stem of *Lilium* contains 2-5 floral buds, older buds situated at the basal end and younger  
172 one at the apex. The mature older buds will open first then younger ones and symptoms of  
173 senescence appear first on older buds. The harvested closed buds of *Lilium* open 2-3 days after  
174 harvest due to less flow of hormones and sugars. Different treatments did not have significant  
175 effect on days to bud opening ( $F=0.45$ ,  $d. f. = 4$ ,  $p \leq 0.76$ ), days to tepal senescence ( $F=0.55$ ,  $d. f.$   
176  $= 4$ ,  $p \leq 0.70$ ) and days to flower senescence ( $F=2.09$ ,  $d. f. = 4$ ,  $p \leq 0.24$ ) (Fig. 2a).

177

#### 178 ***Tepal abscission, days to leaf yellowing and vase life***

179 There was no significant effect observed for tepal abscission ( $F=4.36$ ,  $d. f. = 4$ ,  $p \leq 0.09$ ), days to  
180 leaf yellowing ( $F=4.95$ ,  $d. f. = 4$ ,  $p \leq 0.07$ ). However, the tepal abscission among all the treatments  
181 was observed 2-3 days later than the visible tepal senescence (Fig.2b). The time between bud  
182 opening and tepal abscission was increased to 03 days, when cut stems were treated with 30 ppm  
183 nanosilver along with 2% sucrose as compare to control flowers (05 days). Delayed tepal  
184 abscission in NS treated flower may be due to the inhibitory effect on ethylene as NS binds with  
185 the receptor in cell (Mishra *et al.*, 2008) and removing the copper ion from the receptor protein,  
186 which ultimately block the ethylene perception (Khan, 2006). Similar trends were also reported by  
187 Hatami and Ghorbanpour (2013) with 60 mg cm<sup>-3</sup> of N-Ag treatment in *Pelargonium*. The leaf  
188 yellowing was delayed by 6 days in NS (10 ppm) treated flowers as compare to control flowers  
189 (Fig. 2b). This delayed yellowing may be due to as NS promotes the chlorophyll retention in  
190 leaves. NS treatment significantly improve the vase life ( $F=22.43$ ,  $d. f. = 4$ ,  $p \leq 0.01$ ) of flowers  
191 after harvest and extend vase life up to 04 days as compare to control (Fig. 2b). Similar trends

192 have been reported by Nemati *et al.*, (2014, 2013), Kim *et al.*, (2005); Liu *et al.*, (2009) and Lu *et*  
193 *al.*, (2010) in cut roses, gerbera and *Lilium*.

194

## 195 **Effect of pulsing treatments**

### 196 ***Relative fresh weight and solution uptake***

197 After the pulsing treatment the relative fresh weight ( $F=2.53$ ,  $d. f. = 4$ ,  $p \leq 0.01$ ) increases in all  
198 the treatments up to 3 days after harvest and thereafter decreases linearly (Fig. 3a). In case of NS  
199 treated flower, the fresh weight start declining after 06 days steadily as compare to control flowers.  
200 This may be due to that higher concentration of NS inhibited the growth of bacteria around the  
201 basal end of cut stems. In the present study, pulsing with 20 ppm NS for 24 hrs and then putting  
202 stems in 2% sucrose solution significantly increased the relative fresh weight and start decreasing  
203 6 days after harvest (DAH) as compare to control. Water uptake was not significantly affected  
204 ( $F=0.77$ ,  $d. f. = 4$ ,  $p \leq 0.07$ ) and higher in the NS treated flowers as compare to the control resulting  
205 into more freshness of cut stems (Fig. 3c). NS treatments increased water uptake rate and relative  
206 fresh weight by limiting the bacterial growth, transpiration rate and stomatal conductance (Rafi  
207 and Ramezani, 2013; Liu *et al.*, 2014; Abdel-Kader *et al.*, 2017) in gerberas, anthuriums and  
208 carnations. Water uptake have positive relation with the relative fresh weight as it start decreasing  
209 due to less water uptake, higher respiration rate (Thakur *et al.*, 2022) Similar finding were reported  
210 by Ezhilmathi *et al.*, (2007) and Ha *et al.*, (2019) in roses, gladiolus, petunia and day lilies.

211

### 212 ***Water balance and water loss***

213 Different NS treatments have no significant effect on water balance ( $F=1.08$ ,  $d. f. = 4$ ,  $p \leq 0.11$ )  
214 and start declining gradually in NS treated from the 6<sup>th</sup> day as in control it starts declining with  
215 faster rate (Fig. 3b). Water loss ( $F=1.08$ ,  $d. f. = 4$ ,  $p \leq 0.11$ ) was observed minimum (55.25  
216 gr/stem/day) in NS (20 ppm) pulsed cut flowers as compare to other treatments (Fig. 3d). This  
217 might be due to the biocidal effect of NS and sugar present in the solution reduces the transpiration  
218 rate by regulating the stomata opening. The water balance is the most critical factor for determining  
219 the quality and post harvest life of cut flowers (Da Silva, 2003). Deterioration of flower quality is  
220 exceeded by disturbance in water balance after harvest. Visible symptoms are the loss of fresh  
221 weight and wilting of petals, when water loss is higher than the water uptake (Ichimura *et al.*,  
222 2003; Julita *et al.*, 2017). The imbalance between water uptake and water loss is majorly due to  
223 xylem blockage caused by developing microorganisms in vase solution (Van Doorn 1999;

224 Ichimura, *et al.*, 1999; Edrisi, *et al.*, 2012). The results are in line with the finding of Julita *et al.*,  
225 (2017) in clematis and Bravdo *et al.*, (1974) in gladiolus.

226

### 227 ***Days to bud opening, days to tepal senescence and days to flower senescence***

228 Days to flower opening ( $F=1.37$ ,  $d. f. = 4$ ,  $p \leq 0.38$ ) tepal senescence ( $F=3.50$ ,  $d. f. = 4$ ,  $p \leq 0.12$ )  
229 and days to flower senescence ( $F=5.40$ ,  $d. f. = 4$ ,  $p \leq 0.06$ ) did not change significantly when  
230 flowers were pulsed with different concentrations of Nano Silver and kept in sucrose (Fig. 4a).  
231 This may be due to longer exposure of high concentration of Nano Silver to the cut stems.  
232 However, among the different treatments of NS, 20 ppm concentration was found best for all the  
233 parameters in the present study (Fig. 4a).

234

### 235 ***Tepal abscission, days to leaf yellowing and vase life***

236 There was no significant effect on tepal abscission ( $F=4.96$ ,  $d. f. = 4$ ,  $p \leq 0.07$ ) among the  
237 treatments (Fig. 4b). Different NS treatments significantly affecting the days to leaf yellowing  
238 ( $F=15.31$ ,  $d. f. = 4$ ,  $p \leq 0.01$ ) and vase life of cut flower ( $F=19.37$ ,  $d. f. = 4$ ,  $p \leq 0.01$ ). There is  
239 enhancement of vase life up to 03 days when flowers were pulsed with 30 ppm of NS solution for  
240 24 hrs (Fig.4b). These results are close **conformity** with the finding of Hatami and Ghorbanpour  
241 (2013), as they reported that application of  $60 \text{ mg cm}^{-3}$  of N-Ag suppress the effect of ethylene  
242 and prevent the chlorophyll destruction in *pelargonium*.

243

## 244 **Conclusions**

245 In conclusion, the use of NS as pulsing and vase solution offers the new opportunities for  
246 enhancing the post harvest life of *Lilium* cut flowers. Pulsing treatment with NS as 20 ppm and 30  
247 ppm for 24 hrs significantly enhances the vase life of cut flowers. Similarly, nanosilver at lower  
248 concentration (5-20 ppm) was found suitable as holding solution for improving the physiological  
249 parameters and extending the vase life of cut flowers.

250

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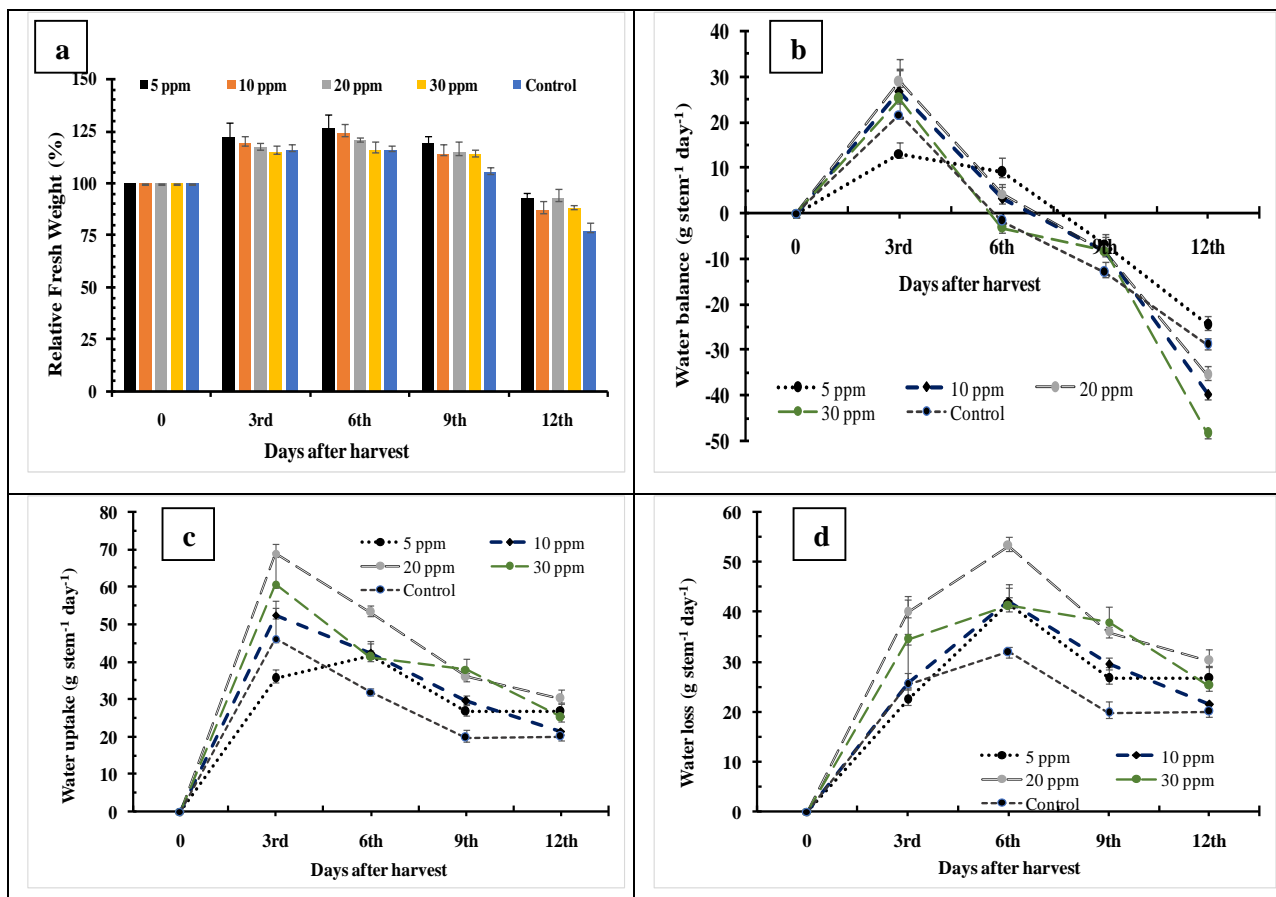
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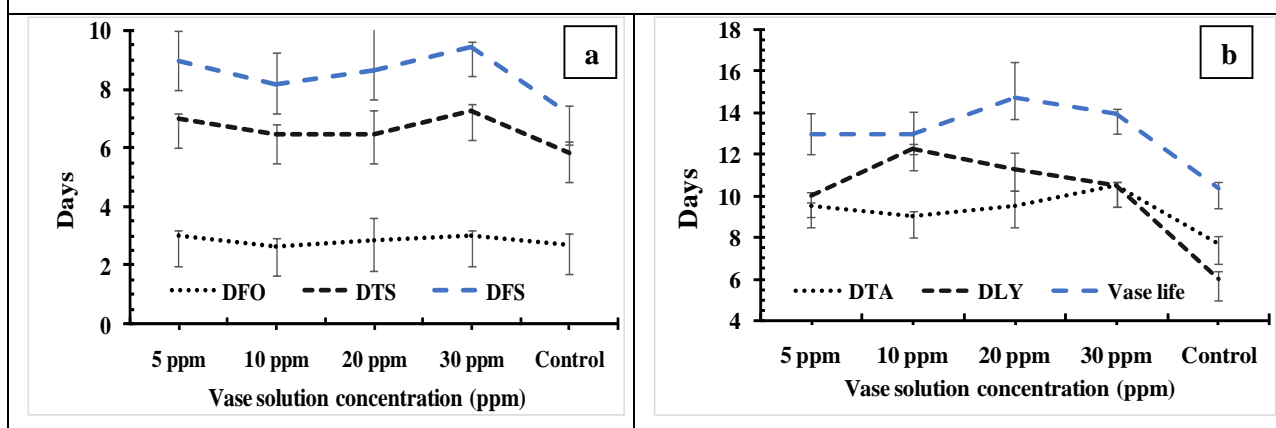
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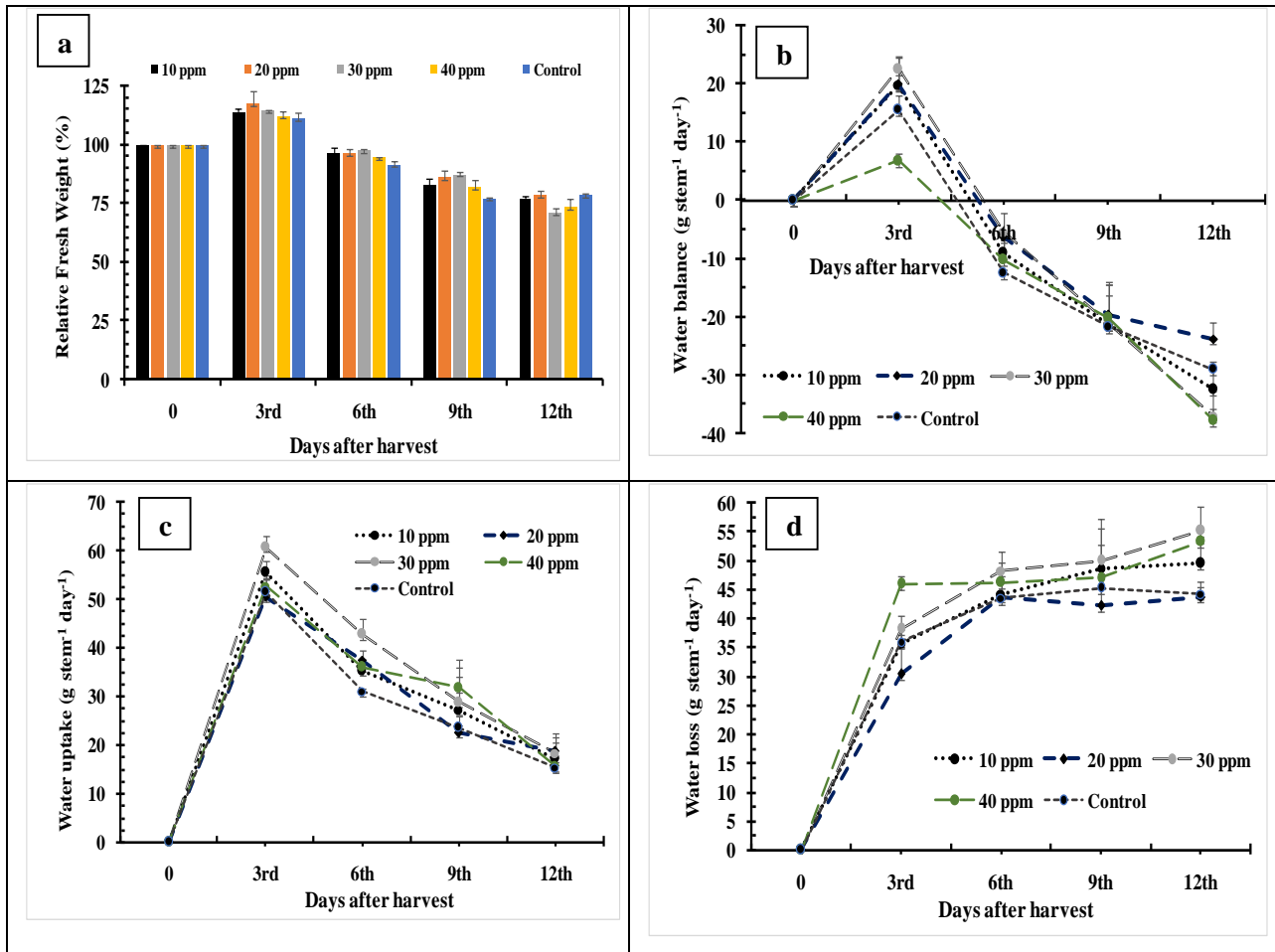
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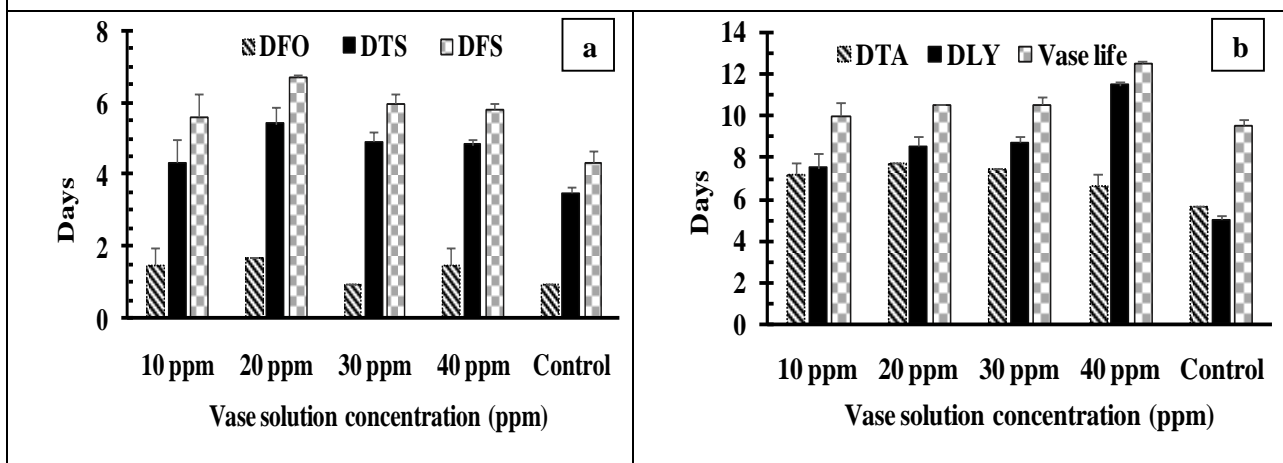
**Figure 1.** Effects of NS+ sucrose vase solution on Relative Fresh weight (a), water uptake (b), water balance (c) and water loss (d) on cut *Lilium cv.* Eyeliner. Bars represent SE. If no bar is visible, it falls within the symbol dimension.



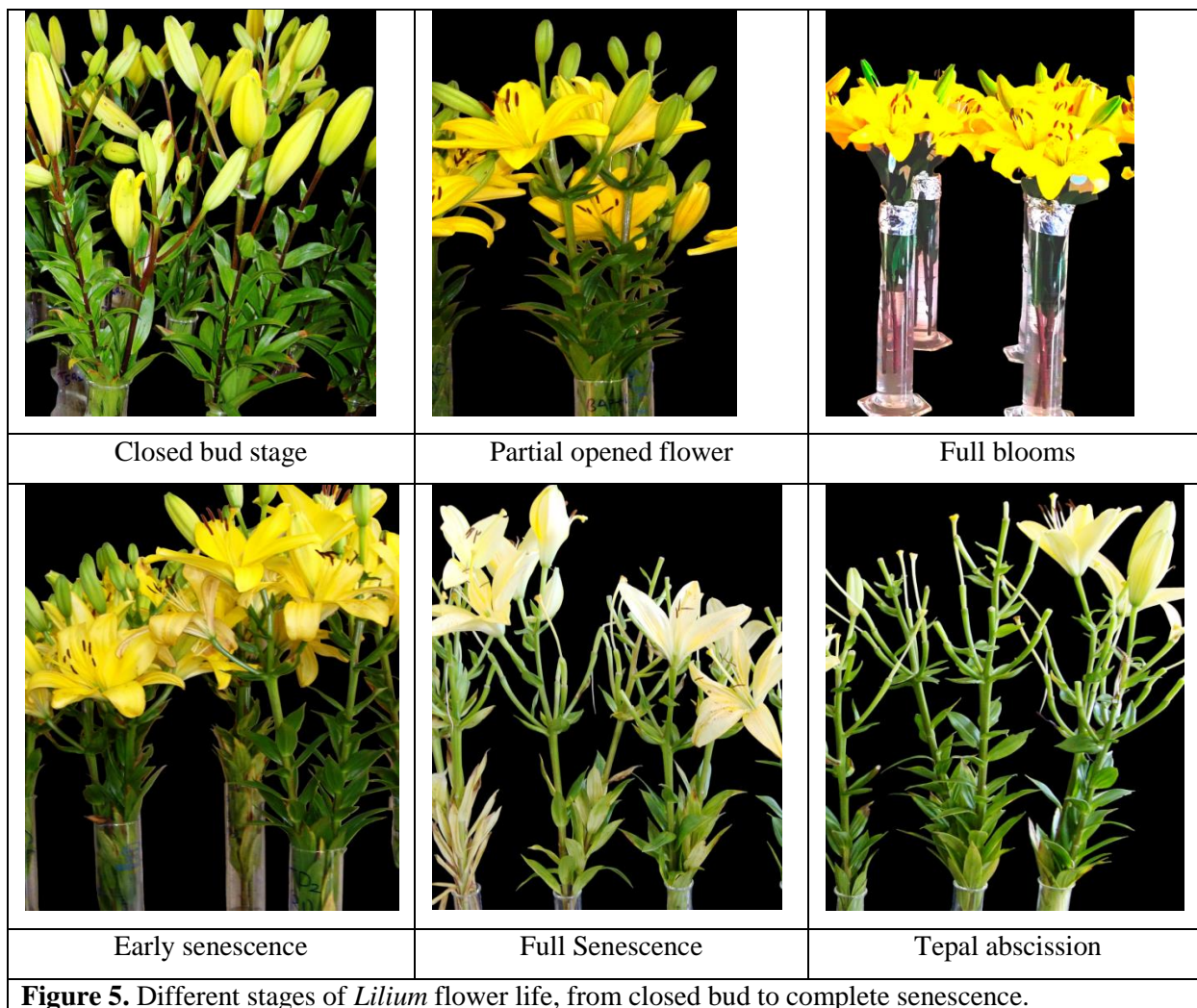
**Figure 2.** Effects of NS+ sucrose vase solution on days to flower opening (DFO), days to tepal senescence (DTS) and days to flower senescence (DFS) (a); days to tepal abscission (DTA), days to leaf yellowing (DLY) and vase life (days) (b) on cut *Lilium cv.* Eyeliner. Bars represent SE. If no bar is visible, it falls within the symbol dimension.



**Figure 3.** Effects of NS pulse and sucrose treatments on Relative Fresh weight (a), water uptake (b), water balance (c) and water loss (d) on cut *Lilium cv. Eyeliner*. Bars represent SE. If no bar is visible, it falls within the symbol dimension.



**Figure 4.** Effects of NS pulse and sucrose treatments on days to flower opening (DFO), days to tepal senescence (DTS) and days to flower senescence (DFS) (a); days to tepal abscission (DTA), days to leaf yellowing (DLY) and vase life (days) (b) on cut *Lilium cv. Eyeliner*. Bars represent SE. If no bar is visible, it falls within the symbol dimension.



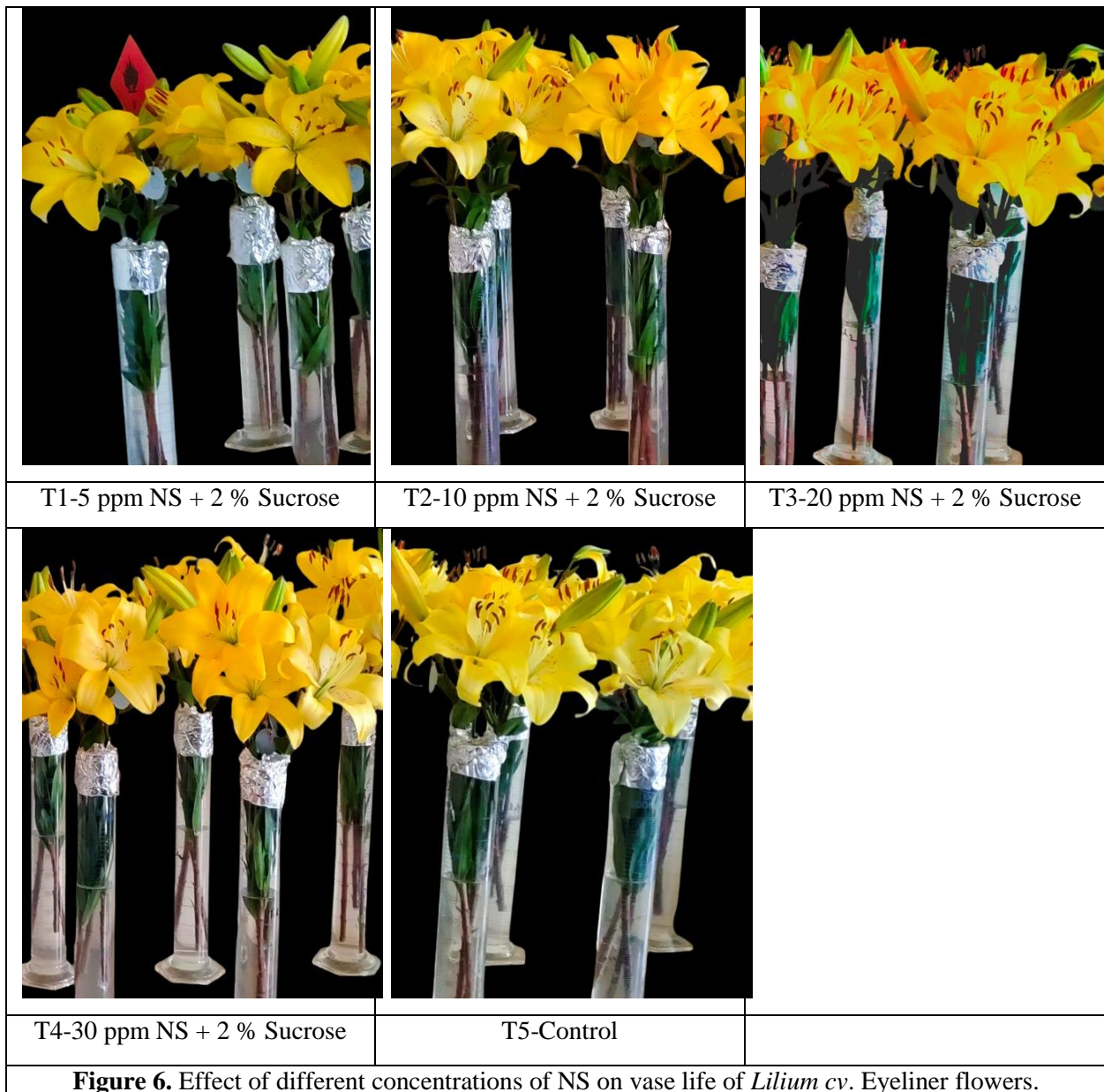
**Figure 5.** Different stages of *Lilium* flower life, from closed bud to complete senescence.

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