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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 21st 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).

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

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

Halevy and Mayak (1979) reported the positive correlation between water uptake and loss after harvesting and water loss will occur at faster rate through leaves during handling of cut stems. The microbial contamination (Louband and van Doorn, 2004) in the vase solution cause stem vessel blockage and various bactericides *viz.*, HQS, $AgNO_3$, $Al_2(SO_4)_3$, $CuSO_4$, $CoCl_2$ (Van Meeteren *et al.*, 1999) utilized for checking the growth of bacteria (Xie *et al.*, 2008; Damunupola *et al.*, 2010) in solution for enhancing the vase life of cut stems. Ethylene is the major factor for reducing the vase life of cut stems as its sensitivity leads to abscission of buds, less bud opening, wilting of petals, yellowing of leaves (Riyaz *et al.*, 2021).

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

Materials and Methods

Plant materials

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

Chemical

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

Experimental design and treatments

The experiments were carried out in a room with a temperature of 23 ± 1 ^{0}C, a relative humidity of 60% and 20 μmol m^{-2} s^{-1} irradiance cool white fluorescent lamps under a diurnal light period of 8 hours. A complete randomized block design was used to study the effect of silver nanoparticles (AgNP) as pulsing and vase solution to extend the post harvest life of *Lilium* cut flowers under controlled climatic conditions in the laboratory. For vase solution, five treatments, *viz.*, T1 (5 ppm NS+ 2% sucrose); T2 (10 ppm NS+ 2% sucrose); T3 (20 ppm NS+ 2% sucrose); T4 (30 ppm NS+ 2% sucrose); T5 (control-distilled water) with three replications were used to assess the effect of different silver nanoparticles treatments on post-harvest life of lilium. For pulsing solution, five treatments *viz.*, T1 (10 ppm NS); T2 (20 ppm NS); T3 (30 ppm NS); T4 (40 ppm NS); T5 (control-distilled water) were formulated for the experiment. In case of pulsing treatment the cut stems were dipped in solution for 24 hrs and after that they were put in the 2% sucrose solution. The freshly prepared solution (400 ml) was put into the glass jars of capacity 500 ml and mouth of jar is closed with parafilm to check the evaporation losses.

Observations

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

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

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

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

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

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

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

$$\mathrm{Re}lative\ \ fresh\,weight\ (\%) = \frac{F_t}{F_0} \times 100;\ where,\ t = 1, 2, 3, \ldots, n\ days\ \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (4)$$

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

Statistical analysis

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

Results and Discussion

Effect of holding treatments

Relative fresh weight and solution uptake

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

Water balance and water loss

The water balance ($F=5.04$, *d. f.* = 4, 4, $p \leq 0.01$) in all the treatments starts declining after 3rd day of harvest in all the treatments. Water balance declined linearly with the time and faster in control flowers than the higher concentration of NS (30 ppm 3 days after harvest; DAH (Fig. 1b). The water loss ($F=2.22$, *d. f.* = 4, 4, $p \leq 0.01$) exceeded the water uptake after 6 days in control and 9 days in NS treated cut flowers. Nano Silver treated cut stems showed water loss steadily until day 12 and no significant difference was observed among the treatments. Among Nano Silver treatment, minimum water loss found with low concentration of Nano Silver 5 ppm (35.06 gr per stem per day) (Fig.1d). The NS treatments have the bactericidal effect in the vase solution, reduce the stem blockage and other factor might be that sucrose at lower concentration act as source of energy, delayed the disintegration of proteins, hence improved the water balance in cut stems Liu *et al.*, (2009). Liu *et al.*, (2012) in *Acacia holosericea* found that lower concentration of Nano Silver (4mgL^{-1}) in holding solution had less number of bacteria than those of higher concentration (40 mgL^{-1}) from 2nd day onwards.

Days to bud opening, days to tepal senescence and days to flower senescence

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

Tepal abscission, days to leaf yellowing and vase life

There was no significant effect observed for tepal abscission (F=4.36, *d. f.* = 4, $p \leq 0.09$), days to leaf yellowing (F=4.95, *d. f.* = 4, $p \leq 0.07$). However, the tepal abscission among all the treatments was observed 2-3 days later than the visible tepal senescence (Fig.2b). The time between bud opening and tepal abscission was increased to 03 days, when cut stems were treated with 30 ppm nanosilver along with 2% sucrose as compare to control flowers (05 days). Delayed tepal abscission in NS treated flower may be due to the inhibitory effect on ethylene as NS binds with the receptor in cell (Mishra *et al.*, 2008) and removing the copper ion from the receptor protein, which ultimately block the ethylene perception (Khan, 2006). Similar trends were also reported by Hatami and Ghorbanpour (2013) with 60 mg cm^{-3} of N-Ag treatment in *Pelargonium*. The leaf yellowing was delayed by 6 days in NS (10 ppm) treated flowers as compare to control flowers (Fig. 2b). This delayed yellowing may be due to as NS promotes the chlorophyll retention in leaves. NS treatment significantly improve the vase life (F=22.43, *d. f.* = 4, $p \leq 0.01$) of flowers after harvest and extend vase life up to 04 days as compare to control (Fig. 2b). Similar trends have been reported by Nemati *et al.*, (2014, 2013), Kim *et al.*, (2005); Liu *et al.*, (2009) and Lu *et al.*, (2010) in cut roses, gerbera and *Lilium.*

Effect of pulsing treatments

Relative fresh weight and solution uptake

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

Water balance and water loss

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

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

Days to bud opening, days to tepal senescence and days to flower senescence

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

Tepal abscission, days to leaf yellowing and vase life

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

Conclusions

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

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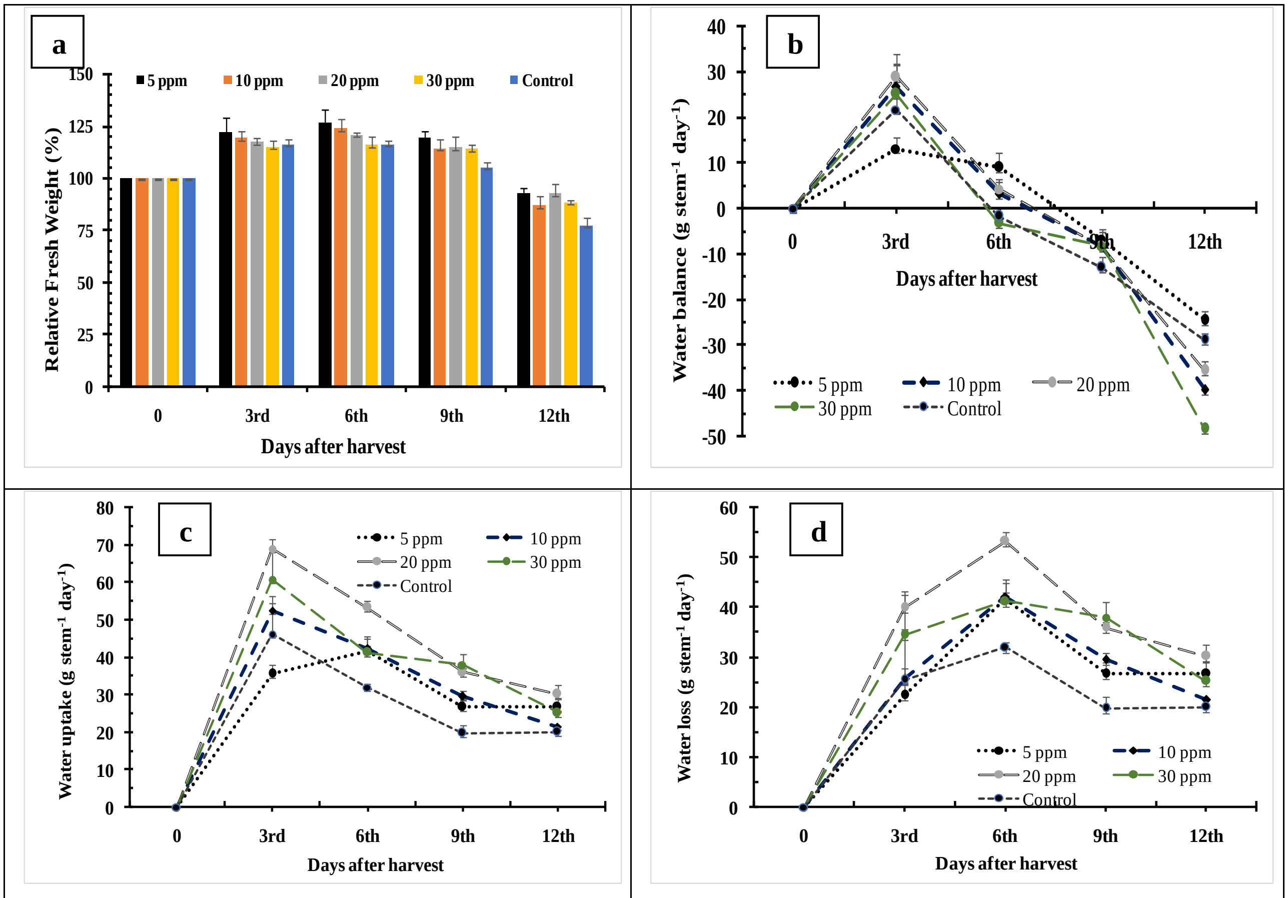

Figure 1. Effects of NS+ sucrose vase solution on Relative Fresh weight (**a**), water uptake (**b**), water balance (**c**) and water loss (**d**) on cut *Lilium cv*. Eyeliner. Bars represent SE. If no bar is visible, it falls within the symbol dimension.

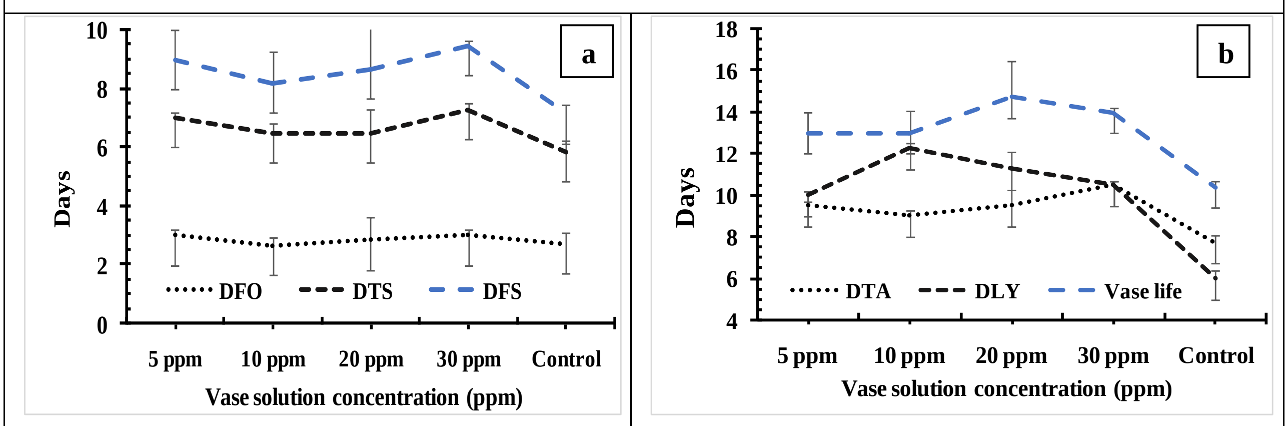

Figure 2. Effects of NS+ sucrose vase solution on days to flower opening (DFO), days to tepal senescence (DTS) and days to flower senescence (DFS) (**a**); days to tepal abscission (DTA), days to leaf yellowing (DLY) and vase life (days) (**b**) on cut *Lilium cv.* Eyeliner. Bars represent SE. If no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

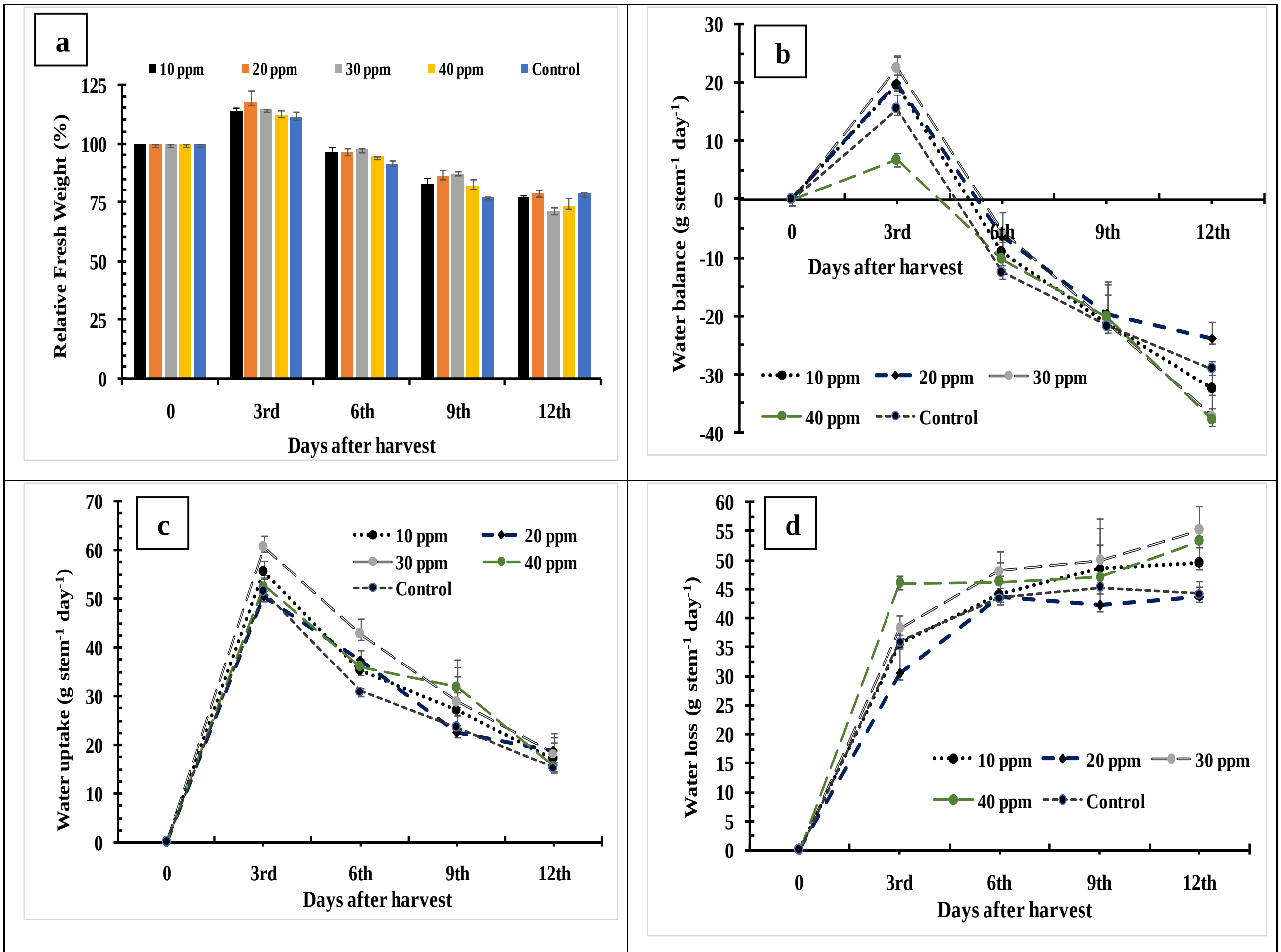

Figure 3. Effects of NS pulse and sucrose treatments on Relative Fresh weight (**a**), water uptake (**b**), water balance (**c**) and water loss (**d**) on cut *Lilium cv*. Eyeliner. Bars represent SE. If no bar is visible, it falls within the symbol dimension.

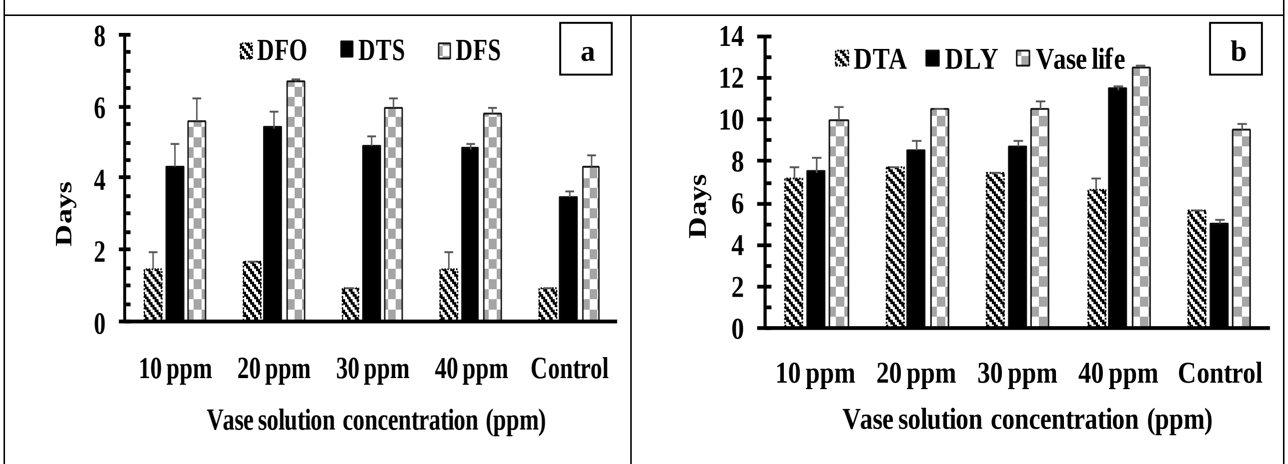

Figure 4. Effects of NS pulse and sucrose treatments on days to flower opening (DFO), days to tepal senescence (DTS) and days to flower senescence (DFS) (**a**); days to tepal abscission (DTA), days to leaf yellowing (DLY) and vase life (days) (**b**) on cut *Lilium cv.* Eyeliner. Bars represent SE. 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.

		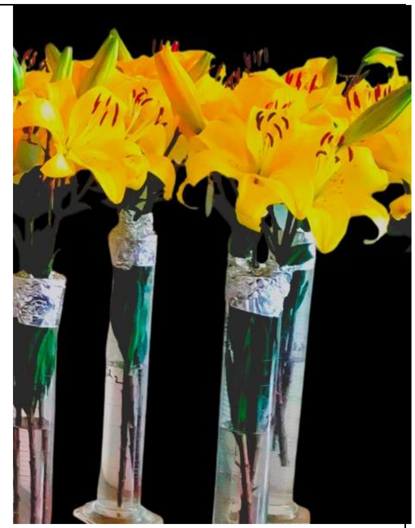
T1-5 ppm NS + 2 % Sucrose	T2-10 ppm NS + 2 % Sucrose	T3-20 ppm NS + 2 % Sucrose
		
T4-30 ppm NS + 2 % Sucrose	T5-Control	

Figure 6. Effect of different concentrations of NS on vase life of *Lilium cv*. Eyeliner flowers.