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Effects of Silver Nanoparticles (SNPs) Pulsing Treatment and Sucrose Holding on Flower and Leaf Senescence of Cut Rose

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Abstract

This experiment was carried out to evaluate the effect of silver nanoparticles pulsing treatment (0, 25, 75 and 125 mg L^{-1}) on vase life and some postharvest physiological parameters of cut rose flower 'High & Magic' in sucrose solution (0, 2 and 3%). This research performed as a factorial experiment based on a completely randomized design with three replications under 23 ± 2 °C, 60 ± 5% RH and 12 µmol m^{-2} s^{-1} light intensity (cool white florescent tubes) under a daily light period of 12 h. Results showed that 125 mg L^{-1} silver nanoparticles without sucrose or with 2% sucrose had the lowest stem end bacteria. Pulse treatments with silver nanoparticles at all concentrations improved solution uptake, maintenance of the relative fresh weight, flower diameter and chlorophyll fluorescence ratio (Fv/Fm) as compared to control. The highest amount of flower opening with the largest flower diameter was observed in 75 mg L^{-1} pulsed silver nanoparticles with 2% sucrose solution on day 9 of vase period. Control flowers (deionized water) without or with 2% sucrose exhibited the shortest vase life. The concentrations of 75 and 125 mg L^{-1} with 2% sucrose or without it had the longest vase life as compared to other treatments.

Keywords: Antimicrobial agents, Pulse treatment, Silver nanoparticles, Vascular occlusion, Vase life.

INTRODUCTION

The end of vase life for many cut flowers is characterized by wilting (He *et al.*, 2006). Stem end blockage is an important factor in the imbalance between water uptake and water loss in cut flowers (van Doorn, 1997a; da Silva, 2003). Development of vascular occlusion is caused by various factors such as bacteria (Bleeksma and van Doorn, 2003), air emboli (van Doorn, 1990) and physiological responses of stem to cutting (Marousky, 1969) but the main factor is the growth of bacteria in the vase solution and inside the lowermost, opened xylem conduits (van Doorn, 1997b). Many studies have reported that Bacillus, Pseudomonas and Acinetobacter are three main bacteria which were identified in a few cut flowers vase solution (van Doorn and de Witte, 1991; Balestra *et al.*, 2005).

Silver nanoparticles represent one of the most extensively studied nanomaterials, which have fascinated scientists due to their unique optical, catalytic, sensing and antimicrobial properties (Wu *et al*., 2012). Silver nanoparticles are especially attractive for their antimicrobial sterilization features among the nanoparticles (Solgi, 2014). Because of their high surface area to volume ratio, nanometer-sized silver (Ag^{+}) particles (NS) are considered to inhibit bacteria and other microorganisms more strongly than Ag in various oxidation states; Ag, Ag^{+}, Ag^{2+}, Ag^{3+} (Furno *et al.*, 2004). It is believed that silver ions interact with bacterial cell walls, plasma membranes, bacterial DNA and proteins, as well as ribosomes, resulting in bactericidal effects (Guo *et al*., 2013). Numerous receptors and enzymes responsible for cell respiration locate in the peptidoglycan layer. Since silver ions can bind to negatively charged peptidoglycan, they could easily attach to the thiol group (SH) of receptors and enzymes along the peptidoglycan membrane resulting in misfolding of proteins, further disabling the bacteria oxygen metabolizing enzymes and leading to bacterial death (Guo *et al.*, 2013). In a comparative study on silver nanoparticles and silver ions, it was observed that silver nanoparticles showed a higher antibacterial potency than silver ions, which suggested that silver nanoparticles might possess intrinsic antibacterial capability besides the elution of silver ions (Choi *et al.*, 2008). Lü *et al*. (2010) reported vase life extension for Rosa hybrida cv. 'Movie Star' by pulse treatments at 50 and 100 mg L^{-1} for 1 h. Liu *et al.* (2009) reported vase life extension for cut gerbera cv. Ruikou flowers following pulsing with 5 mg L^{-1} silver nanoparticles solution for 24 h.

On the other hand, the sugar content is another factor controlling vase life because the carbon supply is cut (Halvey and Mayak, 1979). The vase life of individual flowers is limited by a decrease in available sugars in the cells. Exogenous sugars increase petal life period, possibly by preventing the early decrease in available sugars. Floral bud opening can also be limited by sugar levels (van Doorn and Han, 2011). This view is supported by the finding that sugars, such as sucrose, added to vase water extend the vase life of cut roses (Marousky, 1969; Kaltaler and Steponkus, 1976; Clark *et al*., 2010). In addition, many studies have reported on the application of sucrose in extending the vase life of cut flowers. For example, Paulin and Jamain (1982) and Kaltaler and Steponkus (1976) demonstrated that the vase life of cut carnations and roses were increased following sucrose treatment. Sucrose is useful as a respiratory substrate and as an osmolite that helps in the maintenance of a favorable water balance (Marousky, 1969). Sucrose at concentration of 2% improved the vase life of tuberose cut stem (Watako, 1992). Sucrose serves as a substrate for respiration, and is widely used to postpone the deterioration of cut flowers (Clark *et al.*, 2010). In addition, sucrose is an energy source for bacteria, and the use of sucrose alone in preservative solutions promotes microbial growth (Nair *et al.,* 2003). Then, antimicrobial agents prevent the blockage of xylem vessels (Nair *et al.*, 2003; Meman and Dabhi, 2006). So, the aim of this study was the investigation on the interaction effects of different concentrations of silver nanoparticles and sucrose on the numbers of stem end bacteria. We also investigated the effects of silver nanoparticles and sucrose at different concentrations on vase life, water uptake, relative fresh weight (RFW), flower diameter, and chlorophyll fluorescence of cut rose. The objective of this study was to evaluate the effect of silver nanoparticles pulsing on cut rose flower and leaf senescence during sucrose holding treatment in cv. 'High & Magic'.

MATERIALS AND METHODS

Plant materials

Cut rose (*Rosa hybrida* cv. 'High & Magic') flowers were obtained from a commercial greenhouse based on hydroponic rose growing system, near Tabriz in January, 2014. Flowering stems were harvested at commercial bud stage when the color was fully developed and the petals had not yet started unfolding. They were immediately transported within 1h to the postharvest biology laboratory of the Department of Horticulture Sciences, University of Tabriz where they were re-cut under deionized water (DI). Re-cutting was to ensure no air blockage of the stem end. The flowers were selected for uniformity of size, color and free from any defects. The upper two leaves were kept on each stem. The experiments were carried out at 23 ± 2°C, 60 ± 5% RH and 12 µmol m^{-2} s^{-1} light intensity (cool white florescent tubes) under a daily light period of 12 h.

Experimental design and treatments

In this experiment, 180 cut rose stems were placed individually into 750 mL glass vases. Flower containers were covered with a sheet of low density Al foil to minimize evaporation and prevent contamination. Vases were arranged on benches in a factorial experiment based on a completely randomized design with 3 replications. All solutions were freshly prepared at the beginning of the experiments and were not renewed in the course of the experiment.

Treatments

Four concentrations (0, 25, 75 and 125 mg L^{-1}) of silver nanoparticles (Nanocid Company Tehran, Iran) as pulse treatments were used in experiments. Control stems were pulsed with deionized water (0 mg L^{-1} silver nanoparticles). All pulse treatments were applied for 2 h in a dark room. The treated stems were immediately stood in vases with three different concentrations of sucrose (0, 2 and 3%).

Measurements

Determination of microbial population

To determine the number of bacterial colonies in the stems of the different treatments, 2 cm length (~1 g) segments were cut from the stem ends at the end of vase life. These explants were washed three times with sterile deionized water to reduce the surface load of microbes. They were subsequently ground and diluted with 0.85% sterile normal saline (NaCl). Liquid extract (0.1 mL) was spread on nutrient agar plates and bacterial colonies were enumerated after incubation for 24 h at 37°C (Balestra et al., 2005). Colony counts were expressed as colony forming units per ml (Log_{10} CFU mL^{-1}) (Jedrzejuk *et al.,* 2016), and then they were tested by Gram's staining for positive or negative gram bacteria (Gregersen, 1978).

Solution uptake

Solution uptake was recorded daily by measuring weights of vases without flowers. Average daily solution uptake was calculated as: water uptake (g $stem^{-1}$ d^{-1}) = (S_{t-1} −S_t); where, St is the weight of vase solution (g) at t = days 2, 5, 8, etc., and S_{t-1} is the weight of vase solution (g) on the previous day (He *et al.*, 2006).

Relative fresh weight

Fresh weights of cut stems were monitored daily during vase life. RFW was calculated using the formula: RFW (%) = (W_t/W_t=0)×100; where, W_t is the weight of stem (g) at t = day 2, 5, 8, etc., and W_t=0 is the weight of the same stem (g) at t=day 0 (He *et al*., 2006).

Chlorophyll fluorescence measurement

The leaves were adapted for 20 min. in dark room. Afterwards, the leaf clip was oriented

with the side containing the shutter plate. When light shine was applied onto the leaf, the fluorescence signal was continued for 3 seconds and observed fluorescence yield or photosynthetic yield (Fv/Fm). Chlorophyll fluorescence was measured by Plant Efficiency Analyser Hanstech (Maxwell and Johnson, 2000).

Flower diameter

Flower bud diameter was measured by a caliper (mm), and maximum flower diameter was used to evaluate the bud size difference between the treatments during 2, 6, 10 and 14 days of vase life.

Vase life

Cut stems were inspected daily for visual wilting during vase life evaluation. Vase life was the period from the harvest time to the time when the petals lost turgor (Fig. 1) (Lü *et al*., 2010).

Fig. 1. Different stages of flower opening of cut rose flower cv. 'High & Magic' during vase life.

RESULTS AND DISCUSSION

Determination of microbial population

The use of silver nanoparticles as a bactericide in this study resulted in a significant reduction of microbial population. The control had significantly ($P< 0.01$) higher numbers of bacteria than silver nanoparticles pulse treatments. Moreover, our experiment indicated a significant difference ($P<0.01$) between bacteria population in various concentrations of silver nanoparticles. The lowest bacterial population was obtained with 125 mg L^{-1} silver nanoparticles. So, the best results were obtained at the concentration of 125 mg L^{-1} silver nanoparticles (Fig. 2), which is in agreement with the results of Solgi *et al.* (2009) on cut gerbera. This concentration does not have any toxic effect on cut flowers. Using sucrose alone in preservative solutions promotes microbial growth (Nair *et al*., 2003). In all concentrations of silver nanoparticles, without sucrose, the number of bacteria was minimum, while the treatments with the same concentration of silver nanoparticles with sucrose had more microbial population. The result of this study clearly showed that silver nanoparticles can increase the vase life of 'High & Magic' rose flowers. Silver nanoparticles have

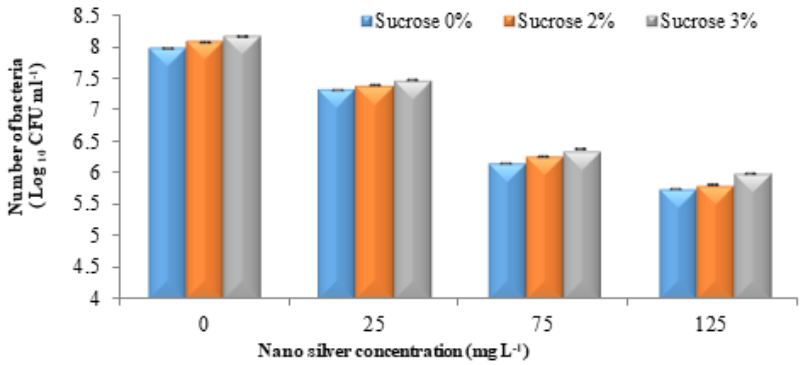

Fig. 2. Number of bacterial colonies in stem end of cut rose cv. 'High & Magic' following silver nanoparticles pulse and sucrose treatments with different concentrations.

antibacterial effects (Sondi and Salopek-Sondi, 2004; Song *et al.,* 2006; Yoon *et al*., 2007). Liu *et al.* (2009) reported that the vase life of cut gerbera cv. 'Ruikou' was extended after pulsing with 5 mg L^{-1} silver nanoparticles for 24 h. Sucrose mainly acts as a food source or for water balance maintenance, and antimicrobial agents prevent the blockage of xylem vessels (Nair *et al.*, 2003; Meman and Dabhi, 2006). Silver nanoparticles can interact with bacterial membranes and this is considered to be the main mechanism for the antimicrobial effect of silver nanoparticles.

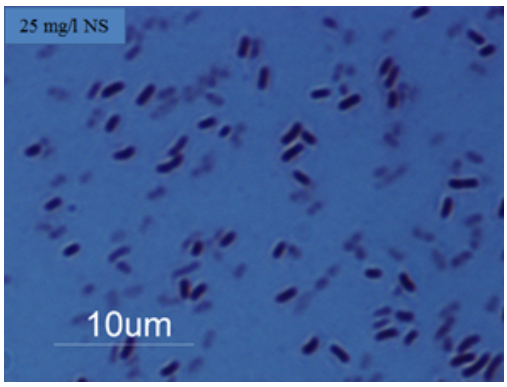

Fig. 3. Gram's staining. Gram-positive *Coccobacillus* was recorded from the stem ends of cut rose flower cv. 'High & Magic'.

Based on gram's staining (Gregersen, 1978), gram-positive Coccobacillus was recorded from the stem ends of cut rose flowers cv. 'High & Magic' (Fig. 3). Gram-positive and gram-negative bacteria have differences in their membrane structure, the most distinctive of which is the thickness of the peptidoglycan layer (Kim *et al.,* 2007). The growth of gram-positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria were inhibited by Ag-NPs (Cho *et al.,* 2005). Grigoreva *et al.* (2013) have found that silver nanoparticles are adsorbed on the outer membrane of gram-negative Salmonella typhimurium and the cell wall of gram-positive Staphylococcus aureus, and penetrate and accumulate in cells without aggregation and damage of neighboring cytoplasm.

Solution uptake

The application of silver nanoparticles as a bactericide in this study resulted in a significant ($P<0.01$) reduction of the microbial population and improved the water uptake of 'High & Magic' flowers. Pulse treatments, particularly 75 and 125 mg L^{-1}, improve water uptake. This suggested that silver nanoparticles reduce the vascular blockage of cut roses. In contrast, 3% sucrose treatments have lower water uptake than 0 and 2% sucrose treatments (Fig. 4 right).

The development of vascular occlusion is correlated with the growth of bacteria at the cut surface and inside the stem (van Doorn *et al.*, 1989). Thus, the inhibition of vascular occlusion by silver nanoparticles improve water uptake and keep water uptake optimum for a longer time. On the other hand, for each vase solution, 2% sucrose had the maximum water uptake (Fig. 4) and when sucrose was added to vase solution, the water uptake was increased (Ichimura *et al.*, 1999). Carbohydrates accumulated in flowers increase their osmotic pressure and improve their water absorbing capacity (Halvey and Mayak, 1973). Results confirmed findings of Marousky (1969) about the role of sugars in improving water balance in plant, involving in the regulation of stomata action, the accumulation of sucrose in plant tissues, increasing osmotic pressure and water absorption capacity and maintaining the cell turgidity.

Relative fresh weight

The fresh weight of cut flowers in pulse treatment was significantly ($P<0.01$) greater than control for all concentrations. During the experimental period, cut stems pulsed at 125 mg L^{-1} or 75 mg L^{-1} silver nanoparticles in 2% sucrose exhibited the highest fresh weight (Fig. 4 left). Control flowers showed the lowest relative fresh weight. Relative fresh weight is important to the evaluation of the vase life (Chamani *et al.*, 2005). While our findings are similar to those of Liu *et al.* (2009), silver nanoparticles pulsing postponed relative fresh weight loss.

Flower diameter

The largest flower diameter was achieved in 75 and 125 mg L^{-1} SNP followed by 2% or 3% sucrose (Fig. 5 left).

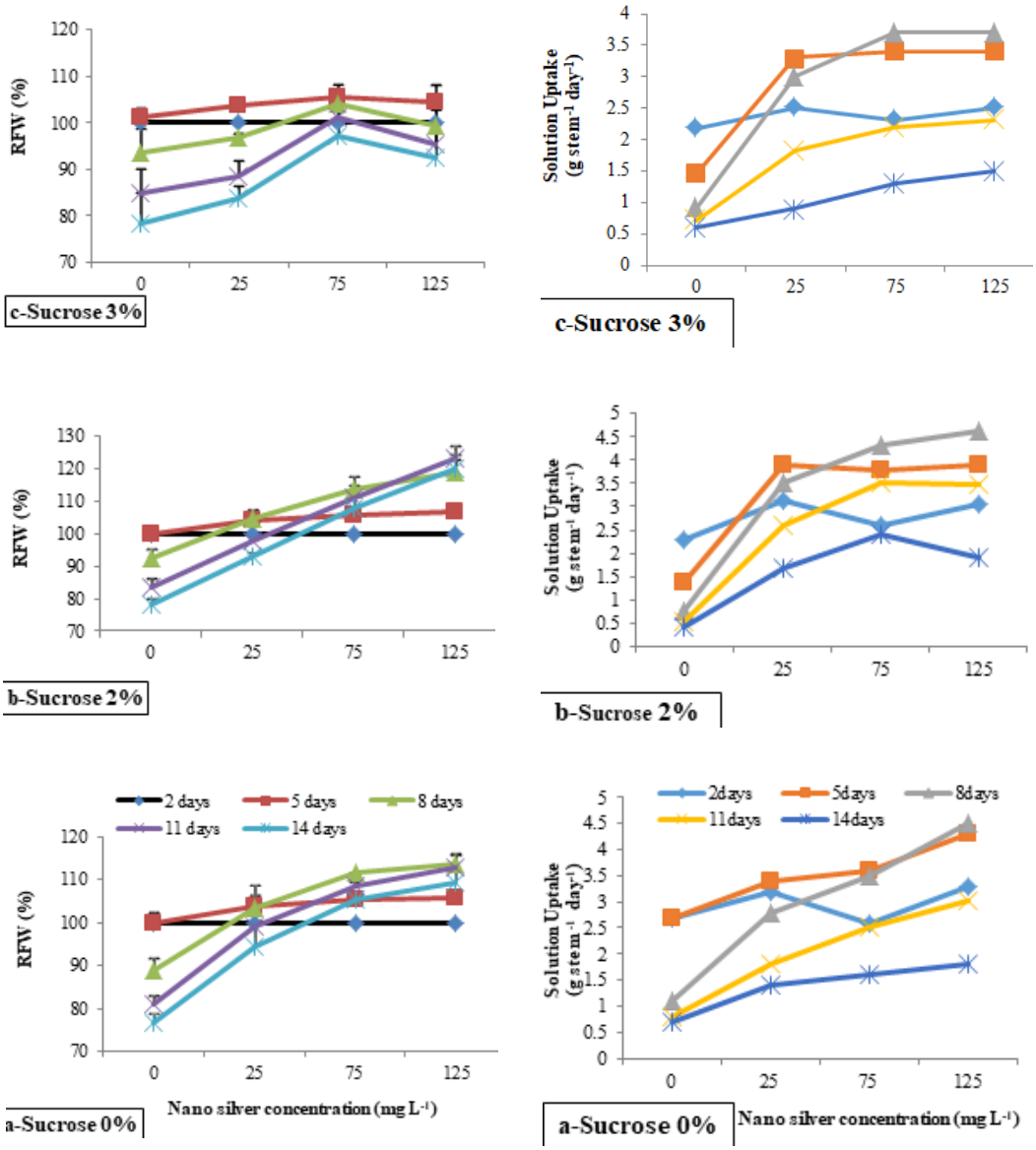

Fig. 4. Relative fresh weight percent (left) and solution uptake (right) of cut rose cv. 'High & Magic' following silver nanoparticles pulse and sucrose treatments with different concentrations.

Similar results were reported by Ichimura *et al.* (1999), which sucrose adding to antibacterial agent such as 8-HQS showed maximum flower diameter. It appears that treatment with 2% sucrose had a larger diameter than the control or 3% sucrose treatments. Soluble carbohydrates increase the osmotic pressure and enhance the petal cell expansion processes (Halvey and Mayak, 1979; Mayak *et al.*, 1999). Ichimura *et al.* (1999) suggested that methyl glucoside was also involved in flower opening of roses. It is taken into the vacuole of petal cells to lower the osmotic water potential and thus promote flower opening. These results show that it is necessary to apply antibacterial agents with sucrose in order to obtain an optimum diameter.

Chlorophyll fluorescence

For all sucrose concentrations, Fv/Fm was significantly ($P<0.05$) higher at 75 and 125 mg L^{-1} silver nanoparticles than in other treatments (Fig. 5 right). Results show importance of silver nanoparticles as an antibacterial agent for the inhibition of vascular occlusion at the end of the cut stems. This inhibitory effect of silver nanoparticles delayed water stress occurrence. Senescence of cut flowers was induced by several factors, such as water stress (Sankat and Mujaffar, 1993),

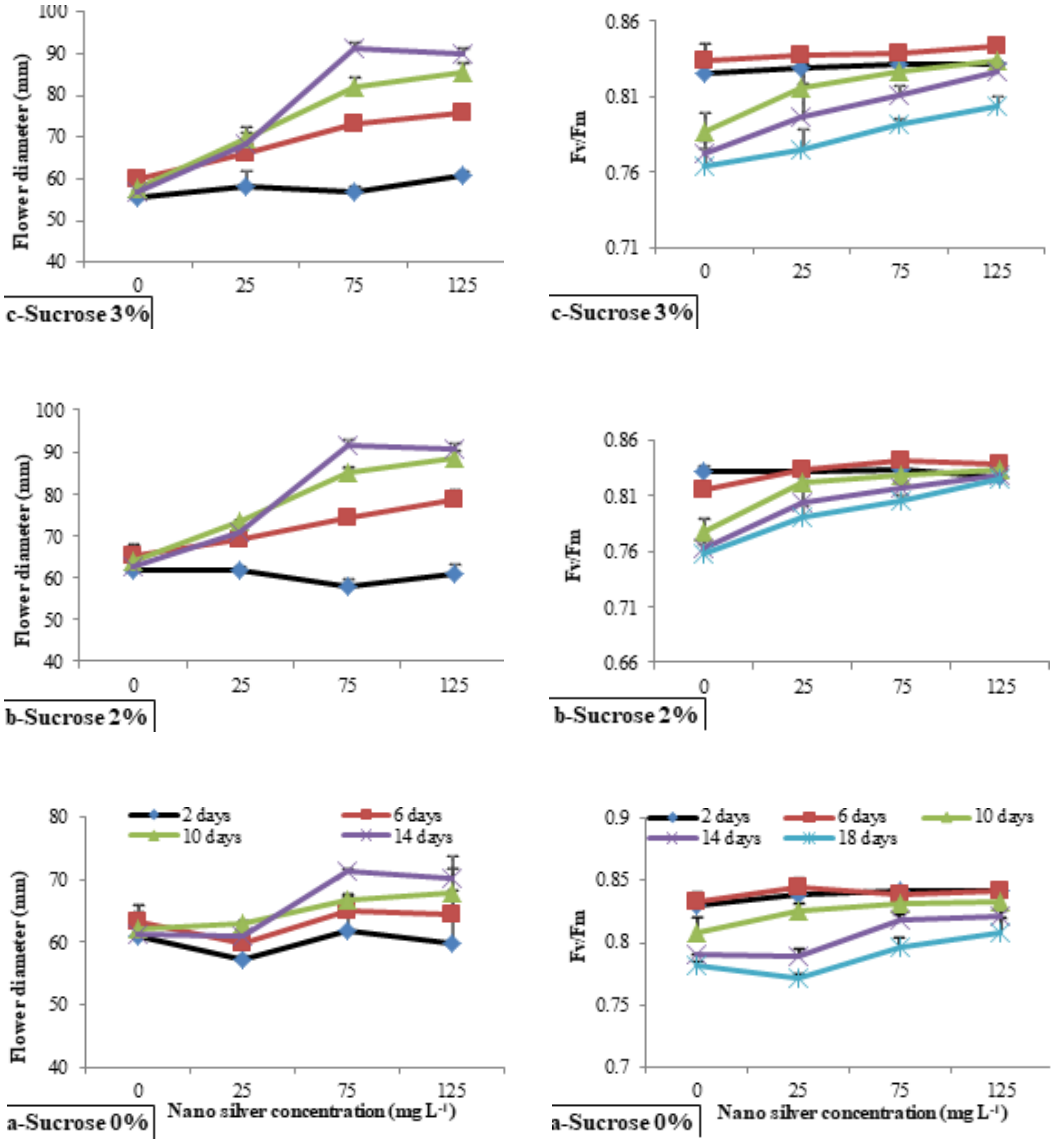

Fig. 5. Flower diameter (left) and chlorophyll fluorescence ratio (Fv/Fm) of cut rose cv. 'High & Magic' following silver nanoparticles pulse and sucrose treatments with different concentrations.

carbohydrate depletion (Ketsa and Narkbua, 1999), micro-organisms (Witte and van Doorn, 1991), and ethylene (Wu *et al*., 1991). Environmental stresses that affect PS II efficiency lead to a characteristic decrease in Fv/Fm (Ma *et al*., 1995).

Vase life

During the experimental period, cut stems pulsed at 125 mg L^{-1} silver nanoparticles and then held at 2% sucrose exhibited the significantly ($P<0.001$) longest vase life. Control flowers showed the shortest vase life period. All silver nanoparticles pulse treatments markedly extended cut rose vase life as compared to the control (Fig. 6). Silver nanoparticles as an anti-bactericidal agent improved vase life of cut flowers (Alt *et al*., 2004; Morones *et al.*, 2005). Ohkawa *et al.* (1999) reported that silver-containing compounds extended the vase life of cut roses. The positive effect of silver nanoparticles pulse treatment is attributed to the inhibition of bacterial growth in the vase solution and at the end of cut stems during the first two days of the postharvest period which was confirmed with current study. The result of this study clearly showed that silver nanoparticles can increase the vase life of 'High & Magic' flower. It is consistent with reports of silver

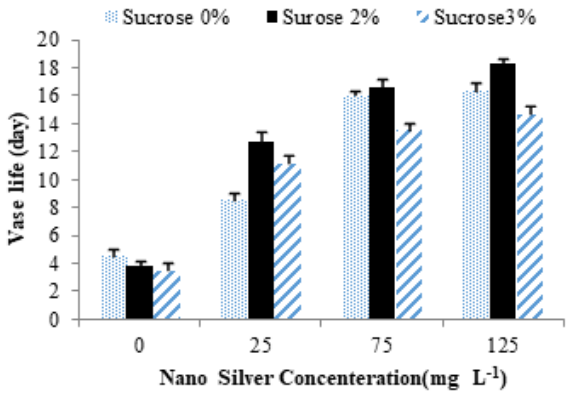

Fig. 6. Vase life of cut rose cv. 'High & Magic' following silver nanoparticles pulse and sucrose treatments with different concentrations.

being able to increase the vase life of Anthurium (Paull and Goo, 1982), carnations (Mayak *et al.*, 1977), gerbera (van Meeteren, 1978), and sweet peas (Mor *et al*., 1984). Silver can reduces ethylene production in cut carnation and cut rose (Veen, 1979; Faragher *et al.*, 1987).

CONCLUSION

In conclusion, pulse treatment with nano silver at 75 and 125 mg L^{-1} for 2h significantly extended the vase life of cut rose cv. 'High & Magic' flowers. Along with the inhibition of microbial growth at stem ends, nano silver treatments tended to both keep water balance of cut rose and suppress the decrease in fluorescence ratio of stem leaves during vase life.

So, this may positively influence water uptake in another way by keeping fresh leaves which could be beneficial for their effect on transpiration process and the improvement of the solution uptake, besides its anti-bacterial effect.

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