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Original Contribution

THE COMPARISON OF ANTIMICROBIAL EFFECTS OF SILVER NANOPARTICLES (SNP) AND SILVER NITRATE ($AgNO_3$) TO EXTEND THE VASE LIFE OF 'RED RIBBON' CUT ROSE FLOWERS

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ABSTRACT
In this research antimicrobial effects of silver nanoparticles (SNP, 33 nm) and silver nitrate ($AgNO_3$) was evaluated on the vase life of Red Ribbon cut *rose* flowers. Pulse treatment was carried out with 50 mg L^{-1} SNP and $AgNO_3$ with and without 5% sucrose and distilled water (DW) was used as a control treatment. After 1 hour, pulse treated flowers were transferred to DW and evaluations were done over 3, 6 and 9 days after treatments**.** Results showed that pulse treatments with 50 mg L^{-1} SNP and 5% sucrose significantly extended the vase life and suppressed reduction in relative fresh weight over the vase period. The water uptake of SNP treated cut flowers was higher during storage periods. *In vitro* microbial analysis and microscope observations of vessels showed that SNP inhibited considerably the growth of microorganisms at the cut end of flower and improving water uptake that followed by extended flowers vase life.

Key words: Anti-microbial, nano-silver cut rose, vase life and vascular occlusion.

INTRODUCTION

Rose flowers are marked either as potted plants or cut flowers. The vase life of cut rose flowers is often short. These cut flowers wilt and the floral axis becomes bent just below the flowers head (bent neck) (1).

Water balance is a major factor determining quality and longevity of cut flowers. It is influenced by water uptake and transpiration, being the balance between these two processes (2). Stem end blockage is regarded as a major cause of imbalance between water uptake and water loss from cut flowers (3). The development of such symptoms is considered to be caused by vascular occlusion, which inhibits water supply to the flowers (3).

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The development of occlusions is thought to be caused by various factors such as bacteria (4), air emboli (5) and physiological responses of stems to cutting. In many cut flowers, suppression of microbial growth in the vase solution results in delayed wilting (6). Many agents have been used in cut flower vase solutions to extend vase life by improving water uptake. These include silver nitrate ($AgNo_3$), aluminium sulphate (7), 8-hydroxyquinoline sulphate in cut rose (1) and silver thiosulphate (STS) in cut sweet pea (8).

The sugar content is another factor controlling vase life, because the carbon supply is limited in cut flowers (9). Usually, sugars such as sucrose was added to vase water to extend the vase life of cut roses that followed by bacteria growth.

Nowadays, $AgNo_3$ is not used in commercial vase solutions, because of the danger for human health and environmental risk (10). Therefore,

developing a new substance as an alternative to these compounds for the floriculture industry is important. Silver nanoparticle (SNP) as a pulse treatment for cut flowers is relatively new (11-12) and has demonstrated importance as an anti bactericidal agent (13-14) that could kill 650 species of bacteria in water (15). SNP releases silver ions Ag^+ (16), which replace the hydrogen cation (H^+) of sulfhydryl or thiol groups (-SH) on surface proteins in bacterial cell membranes, thereby decreasing membrane permeability and eventually causing cell death (17). Liu et al (2009) also reported that vase life of cut gerbera cv. Ruikou flowers extended by pulse treatment of 5 mg L^{-1} SNP solution for 24 hours (11). They also demonstrated that the positive effects of SNP were attributed to inhibition of bacterial growth in the vase solution and in the cut stems end. Ohkawa et al (1999) reported that silver-containing compounds generally extended the vase life of cut roses (18). The objective of our current study was to compare antibacterial effects of SNP and $AgNO_3$ in related to extend the vase life of cut rose flowers. We tested and compared the ability of SNP and $AgNO_3$ alone and followed by sucrose to improved longevity and flower quality in cut rose flowers.

MATERIALS AND METHODS

1. Plant materials and treatments

The cut rose flower (*Rosa hybrida*), 'Red Ribbon' were obtained from a commercial greenhouse in Tehran province, Iran. They were immediately transferred to the postharvest laboratory of horticulture department at university of Guilan, Iran. Buckets containing the flower stems were covered with a plastic film shroud to minimize moisture loss during transportation. The experiments were carried out the same day. The flower stems were re-cut under deionized water to a uniform length of 30 cm. Recutting was to ensure no air blockage of the stem end. Then, they were placed in preservative solutions containing 50 mg L^{-1} SNP (33 nm in diameter) with or without 5% sucrose and 50 mg L^{-1} $AgNO_3$ with or without 5% sucrose for 1 hour in a phytotron operating at 20 ±1 °C, 16:8 h light/dark cycle, 25 $W.m^{-2}$ irradiance and 60±5% RH. The control flowers were kept in distilled water (DW). Each treatment was comprised six flowers and was repeated three times.

2. Measurements

The rose flowers were considered senescent when showing at least one of the following symptoms of senescence: wilting of leaves or flowers, neck bending and incomplete bud opening (1).

Water uptake, water loss and relative fresh weight were recorded daily by measuring weights of vases without flowers and of flowers separately. Average daily water uptake was calculated as:

Water uptake (g $stem^{-1}$ d^{-1}) = (s_{t-1}-st), where s_t is weight of vase solution (g) at *t*= days 1, 2, 3, etc., and s_{t-1} is weight of vase solution (g) on the previous day.

Relative fresh weight (RFW) of stems was calculated as:

RFW (%) = $(w_t/w_{t-0}) \times 100$; where, w_t is weight of stem (g) at *t*= days 0, 1, 2, etc., and w_{t-0} is weight of the same stem (g) at *t*= day 0 (19).

3. *In vitro* antibacterial analysis

The *in vitro* antibacterial analysis was determined as described by Balestra et al (2005) with some modification (20). Five preservative solutions were compared to evaluate the growth of the bacteria. The samples were taken at 1, 3, 5 and 7 days over vase life and the bacteria were counted and examined. For determination of bacteria count in stems of the different treatments, 2 cm length (0.05 g) segments were removed from the end of stems. These explants were washed three times with sterile DI to reduce the surface load of microbes. They were then ground and diluted with 0.9% sterile normal saline. Liquid extract (0.1 mL) was spread on nutrient agar plates and bacterial colonies were enumerated after incubation for 24 h at 37 ◦C. All bacteria counting was replicated three times.

4. Vascular occlusion

Vascular occlusion was determined according to Li (1987) with some modification (21). Segments of 3 cm length were excised for microscope observation from cut stem ends immediately after cutting and on the third day of the vase period. Explants were fixed initially in FAA (formaldehyde acetic acid ethanol). Paraffin embedding was used to prepare permanent tissue sections. Sections were stained with eosin-haematoxylin solution and examined under Olympus photomicroscope.

5. Experimental design and analysis

The experiments were performed with three replications (n=3) according to a completely randomized design (CRD). Statistical significance between mean values was assessed using analysis of variance (ANOVA). Means were compared by the least significant difference (LSD) test at the 0.05 probability level. This procedure was carried out with the aid of the SAS 9.1 software platform.

RESULTS AND DISCUSSION

1. Pulse treatments and vase life

Results showed that in pulsed treatments flowers the vase life significantly increased in compared to control, however flowers treated with 50 mg L^{-1} SNP pulse 5% sucrose for 1 hour significantly extended the vase life as compared to other treatments (**Fig. 1**) Pulse treatment of flowers with 50 mg L^{-1} $AgNO_3$ for 1 hour caused visible damage of leaves and the early abscission, thereby resulting in a lower vase life the same as the control. Also, pulse treatments with 50 mg L^{-1} SNP suppressed water loss of cv. *Red Ribbon* roses and maintained a more favorable water balance than control flowers and lead to extend vase life of flowers (**Fig. 1**). The short vase life of control rose flowers were caused by poor water relations, which is showed in figure 2. The positive effect of SNP pulse treatment was attributed to inhibition of bacterial growth in the vase solution and at the cut stem ends during the first 5 days of the postharvest period. However, physiological activity of Ag^+ from SNP is also a possibility. Ag^+, generally applied as silver thiosulfate, effectively inhibits ethylene-mediated processes, such as flower senescence and abscission (22). As with other cations (e.g. K^+, Ca^{2+}), positive effects on plant stem hydraulic conductivity of Ag^+ (23) are possible. Also, Ag^+ is considered a general inhibitor of Aquaporins (AQPs) (24). AQPs are primary channels of water transport across biological membranes and are abundant in the vacuolar and the plasma membrane (25). AQPs can increase the osmotic hydraulic conductivity of membranes by 10–20-fold (26).

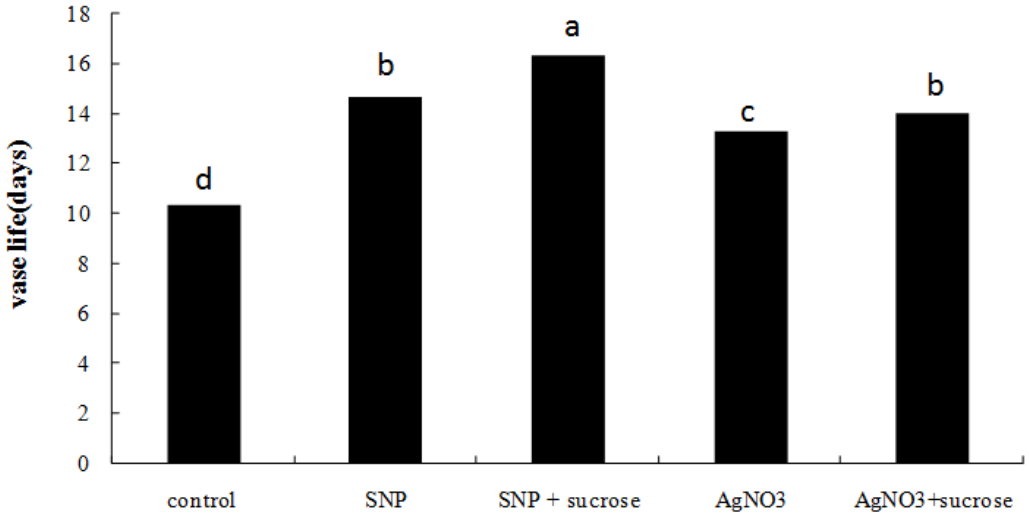

Fig. 1. Effect of Silver nanoparticels (SNP) and silver nitrate ($AgNO_3$) pulse treatments on vase life of cut roses. The similar letters indicate the treatments that are not significantly different from one another ($p<0.05$).

Results also indicated that a pulse treatment with SNP plus 5% sucrose had a pronounced effect on extending the vase life of cut roses flowers compared to other treatments particularly to SNP without that (**Fig. 1**). Kaltaler and Steponkus (1976) reported that exogenous sugars may somehow be maintaining the structural integrity of the cell membranes of rose flowers. Therefore, leakage of these substrates is prevented and/ or reduced by sugar treatment (26). Aarts (1957) also suggested that exogenous sucrose in some way maintains the structure and

semi-permeability of the plasma membrane (27). Furthermore, treatment of cut flowers with sucrose is found to be beneficial in delaying senescence processes (28). In our study, a pulse treatment of sucrose (5%) in combination with SNP extended the vase life of cut rose flowers, suggesting that the sucrose might be required as an osmolyte for flower opening and substrate for cell wall synthesis and respiration. SNP may positively influence water uptake besides an anti-bacterial effect. Silver compound known as antimicrobial matters and among them SNP because of it size and more mobility have great role in prolonging vase life of cut rose flowers. Van Meeteren et al. (2001) reported that $AgNO_3$ had a positive effect on *Bouvardia* water status (29). Ions in water, particularly cations, can enhance water flow through xylem vessels (23).

2. Water uptake and relative fresh weight

SNP and SNP plus 5% sucrose markedly improved the volume of water uptake in cut rose flowers as compared to other treatments (**Fig. 2**). The amount of water uptake by all flowers increased up to 5 days and then decreased with time. In our current study, pulse treatments with SNP plus sucrose significantly extended the vase life of the cut rose flowers in association with a relatively high water content of leaves. This effect attributed to increased hydraulic conductance as well as inhibition of transpiration from leaves. In addition, sucrose mainly acts as a food source or for water balance maintenance, and prevents the blockage of xylem vessels (30-31).

Variations in relative fresh weight (RFW) of cut roses showed the similar trends for control and SNP pulse treatments, such that RFW increased (120% of initial) until day 5 after harvest and declined thereafter (**Fig. 3**). However, RFW of both SNP treatments were higher than that of the control. Water deficit in a cut stem standing in vase solution will develop, when the rate of water uptake is lower than the rate of transpiration (3). Stem occlusion reduced the water uptake and increased the loss of turgidity (32). Liu et al (2009) reported that 20 mg L^{-1} SNP for 24 hours increased vase life of cut gerbera (11). Bacterial plugging of the xylem is an alternative cause of early and rapid cut flower senescence (33). The presence of Ag^+ ions had a profound effect on the water relations of cut rose flowers. Furthermore, SNP have antibacterial effects (34-35) and can extend vase life. Therefore, SNP may have positively influence on water uptake because of antibacterial effects of Ag^+ ions in SNP may affect regulation of water channel activity via inhibition of sulfhydryl-containing proteins (24) and improve solution uptake.

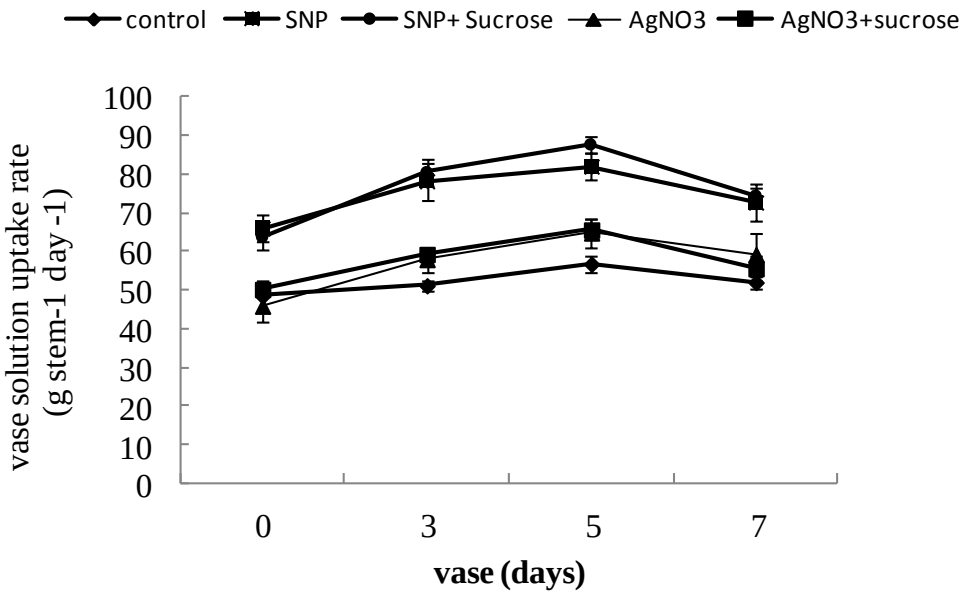

Fig. 2. Effects of Silver nanoparticels (SNP) and silver nitrate ($AgNO_3$) pulse treatments on vase solution uptake in the first seven days of rose flowers vase life. Vertical bars show standard errors of means (n = 3).

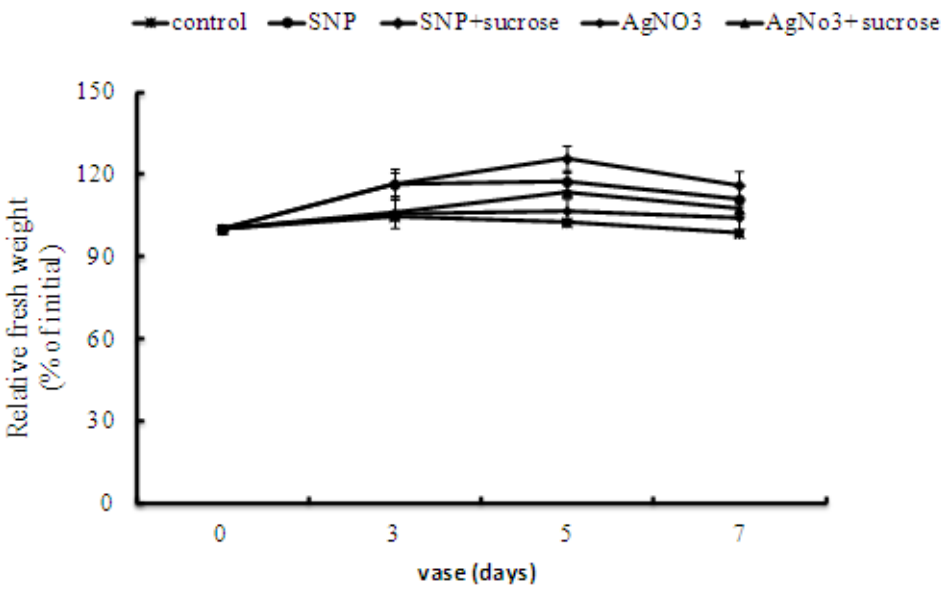

Fig. 3. Relative fresh weight variations during first seven days of rose flowers vase life following SNP and silver nitrate pulse treatments. Vertical bars show standard errors of means (n = 3).

3. Bacterial counts

The number of bacteria in the vase solution increased over the vase life in the SNP and $AgNO_3$ pulse treatments and control flowers (**Fig. 4**). There was a positive relationship between number of bacteria in preservative solution and vase life of cut rose flowers (R^2=0.91). At the first, incorporation SNP and $AgNO_3$ to the vase solution significantly suppressed bacteria number, but after 5 days no significant difference was found in bacteria count in the control and other treatments (**Fig. 4**). The first 3 days, control treatment showed significantly higher bacteria number than others, especially of SNP.

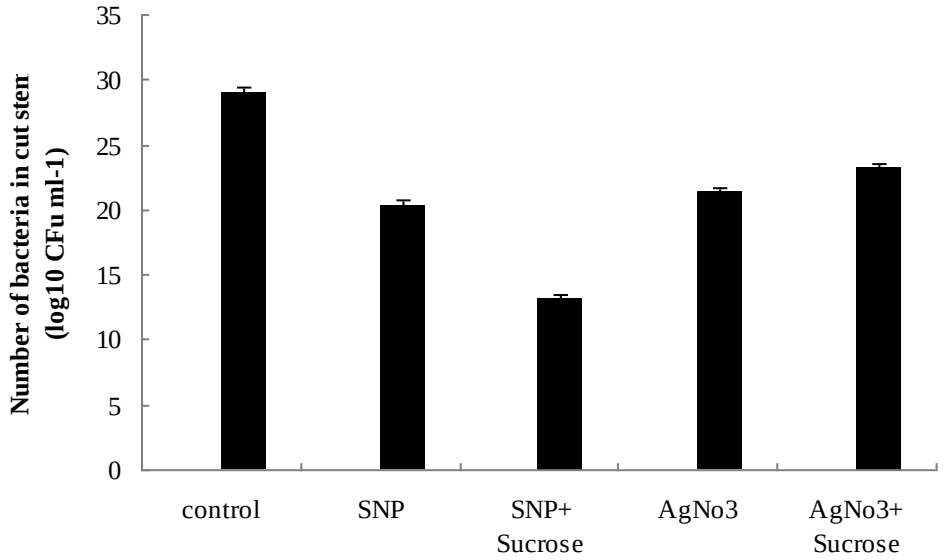

Fig. 4. Changes for SNP and silver nitrate ($AgNO_3$) pulse treatment in number of bacteria in the stem end of cut rose cv. Red ribbon.Vertical bars show standard errors of means (n = 3).

4. Vascular occlusion

Microscope observation showed xylem vessels at the cut stem end during 5 days after pulse treatment (**Fig. 5**). Few particles were evident in vessels pulsed with SNP and then in $AgNO_3$. SNP anchor or penetrate cell wall of bacteria and damage their membrane. Control flowers showed more vascular occlusion than treated ones. Photomicrographs of cross and longitudinal sections of stem ends for cut flowers were shown in fig 5. Panels A and B are stem end sections pulsed in 50 mg L^{-1} SNP for 1 hour; panels C and D are stem end sections of control flowers.

Vascular occlusion has been considered to be mainly due to microbial proliferation (5). Efficacy of nanometer sized particles bearing Ag^{+} as an antibactericidal agent is well established (14). Antibacterial activity of SNP is partly a function of particle size, with higher surface to volume ratio increasing the proportion of atoms at the grain boundary (36). In our study, SNP suppressed the decrease in hydraulic conductance of cut rose flower stems apparently in association with decreased numbers of stem end bacteria (**Fig. 4**).

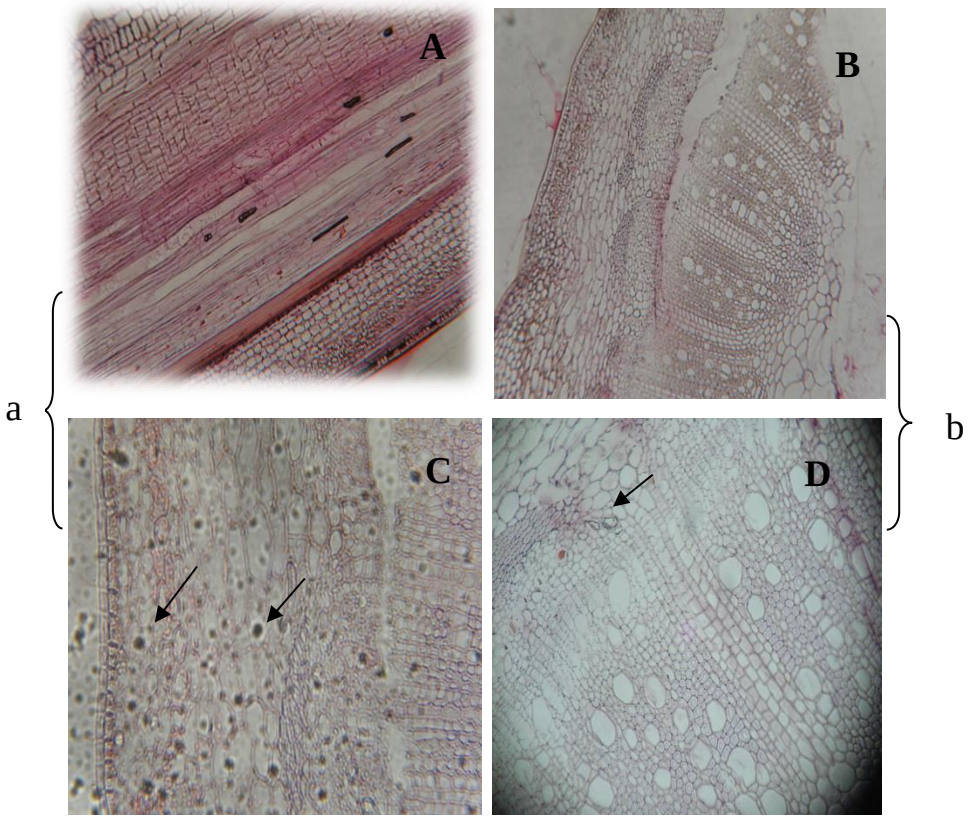

Fig. 5. Effects of different treatments on vascular occlusion in the stem end of cut rose cv Red ribbon. a: Longitudinal section, b: cross section, Panels A and B are stem end sections pulsed in 50 mg SNP/L for 1 h, panels C and D are stem end sections from flowers pulsed in distilled water for 1 h. Blockages in xylem vessels are indicated with arrowheads

CONCLUSION

In conclusion, using pulse treatment with 50 mg L^{-1} SNP for 1 hour significantly extended the vase life of cut cv. *Red ribbon* Rose flowers, along with suppression of microbial growth at stem ends. Mobility of silver ion in stem of rose flowers is very slow. Therefore, application of nano particle with antimicrobial effects can improve speed of that and could prolong cut flower longevity. Although, $AgNo_3$ affect on vase life and maintaining quality of flowers as well as SNP, it's application have to be avoided due to environmental risks. Hence, use of SNP (33 nm in diameter) in compare to other silver compounds in combination with 5% sucrose is suggested to extend the vase life of Red Ribbon cut rose flowers.

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