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Effects of Some Chemicals on Vase Life of Some Cut Flowers I. Effect of 8-Hydroxyquinoline Sulfate, Silver Nitrate, Silver Nano Particles and Chitosan on Vase Life and Quality of Cut Rose Flowers (*Rosa hybrida*. Cv. "Black Magic")

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ABSTRACT

This investigation was carried out during 2015 and 2016 seasons to determine whether the selected chemical agents could be used to improve the postharvest quality of *Rosa hybrida* L.cv. "Black Magic" cut flowers. Freshly cut flowers were placed in glass cylinders containing 100 ml of preservative solutions [8-hydroxyquinoline sulfate (8-HQS) at 200 ppm, silver nitrate ($AgNO_3$) at 10 ppm, Chitosan at 50, 75 or 100 ppm silver nano-particles (SN) at 5, 10 or 15 ppm, and distilled water as a control treatment].All preservative solutions and the control treatment included sucrose at 20 g/l. Compared to the control, treatments which contained of ($AgNO_3$) or (SN) improved the quality and vase life of the flowers. They were more effective in promoting water uptake, increasing fresh weight of the flower and water balance so that the vase life of cut flowers were extended to 13.67 and 12 days during the first and second seasons, respectively. The results of bacterial count in the vase solution and the Scanning Electron Microscopy (SEM) pictures of the cut base of the stem indicated that ($AgNO_3$) or (SN) strongly reduced bacterial population in both the vase solution and the cut stem base. The results indicated that using ($AgNO_3$) or (SN) combined with 20 g/l sucrose could be used as a commercial cut flower preservative solution for prolonging the vase life and enhancing post-harvest quality of selected rose cut flowers.

Keywords: Cut flowers, Rose, Silver nano-particles, Silver nitrate, Chitosan, Preservative solution, Vase life.

INTRODUCTION

Roses are considered the most important cut flower in the floricultural industry of the world. One of the reasons of early wilting of cut flowers is the blockage of vascular system, due to bacterial growth, which inhibit water supply to flowers and results in water stress (Van Meetern *et al*., 2001). Besidesvascular blockage, bacteria produce pectinases and toxic compounds and produce ethylene, thereby, accelerate senescence. Stem blockage could take place also by macromolecules, extra cellular polysaccharides and degradation products of dead cells. In order to reduce microbial growth we used many antimicrobial compounds namely, silver nitrate, 8-hydroxyquinoline sulfate (8-HQS), silver nano-particles and chitosan.

The wide antimicrobial effect ofsilver nitrate is well-known, since Ag^+ions replaces the hydrogen cations (H^+) of sulfhydryl or thiol groups (^-SH) on surface proteins in cell membranes of bacteria, which leads to loss of membrane integrity and causing cell death (Feng *et al*., 2000). The problem with silver nitrate that it might causes toxicity to human and many other organisms (Ratte, 1999), so it is not used in commercial vase solutions recently. Silver nano-particles have higher surface area to volume ratio compared with other silver forms which may make it more effective as a biocide (Jiang *et al.,* 2004) and it have lowest toxicity effect (Foldbjerg *et al.*,2009). 8-HQS is one of the very important preservatives used as a germicide in floral industry, it acts as an antimicrobial and antifungal agent (Ketsa *et al*., 1995) and also increases water uptake by reducing physiological stem blockage (Reddy *et al.,* 1996). Many studies carried out onthe antifungal activity of Chitosan, and it was observed that the effect of Chitosan may be related to its effect on fungal cell wall and cell membrane (Zakrzewska *et al.* 2005).

The aim of this study was to investigate the effect of new antimicrobial compounds (SN and Chitosan) compared with traditional compounds such as $AgNO_3$and 8-HQS on postharvest quality of cut rose flowers.

MATERIALS AND METHODS

The present research was conducted in the laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the two successive seasons of 2015 and 2016. This investigation aimed to study the effect of different preservative solutions on postharvest quality of cut Rose flowers.

Plant material:*Rosa hybrida*.cv. "Black Magic", Fam. Rosacea.

Rose flowers were cut early in the morning at the commercial stage of cutting (when the sepals start to reflex), immediately transported to the laboratory of Department, where they were graded according to flower size and length of the stem. The lower leaves on the stems were removed leaving the top 4 leaves, then, stems were re-cut at about 2.5 cm from the bases, and the original fresh weights of flowers before treatment were recorded. Flowers were placed individually in 100 ml glass cylinders filled with prepared preservative solutions under laboratory conditions (24 hrs illumination with fluorescent light, temperature of 24 ºC ± 2 and a relative humidity between 60 – 70%). In addition, three jars filled with similar preservative solution without flower stem were added to each treatment and placed in the laboratory under the same conditions in order to measure the average daily evaporation value.

Substances used in the preservative solutions: Distilled water - 8-hydroxyquinoline sulfate 8-HQS at 200 ppm - silver nitrate $AgNO_3$ at 10 ppm - Chitosan at 50 or 75 or 100 ppm - silver nano-particles SN at 5 or 10 or 15 ppm. All treatments combined with sucrose at 20 g/l.

Silver nano-particles used in this experiment was a transparent colorless complex nano-silver solution with a particle diameter less than 1nm; (a product of Shanghai Ehoo Biotechnology Co., Ltd, Shanghai, China).

Experimental design: The experiment was arranged in a randomized complete block design, with contained five replicates, each replicate consisted of five glass cylinders (100 ml capacity, and each cylinder contained one cut flower stem.

Statistical analysis: Data collected from the current research were statistically analyzed and comparison between means was done according to Duncan Multiple Range Test (Little and Hills, 1978).

Collected data:

Post-harvest characters: The following data were recorded in both seasons:

1. **Vase life (days) :** Number of days from the beginning of the treatment till the end of longevity of the flowers (when petals wilt, necks (peduncle) were bent, petals abscised or showed discoloration, or when the flower stem lost 10% of its fresh weight, whatever took place first).
2. **Maximum increase of fresh weight of flower stems during the longevity period.**
3. **Water uptake:** was measured as ml of daily solution uptake per 10 g fresh weight of the flower.
4. **Water balance** was calculated by subtracting water loss from water uptake as follows

   Water balance = water uptake – water loss.
5. **Average bacterial counts (C.F.U/ml):** Solutions (0.1 mL) were spread on general medium (nutrient agar), incubated for 24 h at 37 ºC and were evaluated by serial dilutions. Number of colonies per petri dish was counted accurately. All bacteria counting was replicated three times (Balestra *et al*., 2005).
6. **Scanning electron microscopy (SEM):**

Rose stem bases were examined by SEM at the Electron Microscopy Unit, Mansoura University. On the sixth day of the vase life, stem base samples (0.5 mm) were taken from the cut roses, immediately placed into a fixative mixture of 2.5 % buffered glutaraldehyde + 2 % paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4. Dehydration was done through graded ethanol series. Samples were dried by CO2 critical point drying and coated with gold. Stem base surfaces were examined at 20 kV accelerating voltage using a JSM-6510LV SEM (JEOL, Japan) and photographed.

RESULTS AND DISCUSSION

It is obvious from Table (1) that the lowest colonies forming units of bacteria were those when Silver nano-particlesSN treatments were used, followed by the treatment of $AgNO_3$.The control treatment had significantly higher colonies forming units of bacteria compared with all other treatments.Silver nitrate (10 to 50 ppm) is one of the most common forms of silver salts used as a strong antimicrobial agent in flower preservative solutions (Halevy and Mayak, 1981). Silver nano-particles has large surface area-to-volume ratios, and thus has a great efficacy against several bacterial species (Jiang *et al*., 2004). Similar results were reported by (Liu, 2009) on cut Gerbera and (AbdelKader, 2012) on cut roses.

Illustrated data in Figures (1&2) shows that the highest values of solution uptake (7.68 and 7.83 ml/10g flower fresh weight in the first and second seasons, respectively, were recorded by flowers placed in 15 ppmSN + 20 g/l sucrosesolution. Moreover, treatments included 8-HQS, $AgNO_3$orSN had the best values of solution uptake during the longevity period in the two seasons compared with control treatment which had a sudden decrease in uptake after the 2nd day of the vase life. Similar results were reported by (Lu, 2010) who reported that pulsing cut roses with silver nano particles reduced number of bacteria in and improved water uptake the vase solution and the cut stem end in the first two days of the flower vase life. Improved water uptake by cut roses as a result of either silver nitrate or silver nano-particles is a result of their role to inhibit bacterial growth which is the main cause for vascular blockage in the cut stem ends. On the other hand, the least values of solution uptake were when treatments containing Chitosan were used.Although Chitosan reduced number of bacteria in the vase solution (Table, 1), these figures showed that it did not improve water uptake of cut roses. Probably this is due to the large size of Chitosan molecule which might impair water movement through the vascular system, which suggest using micro or nano particles of Chitosanin future experiments.

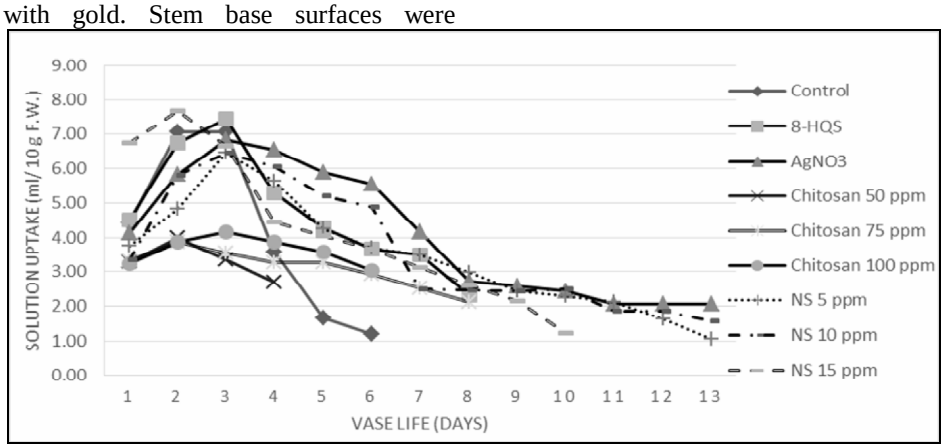

Fig. 1. Effect of preservative solutions on solution uptake (ml/ 10 g fresh weight) by Rosa *hybrida*cv. "Black Magic" cut flowers during 2015 season

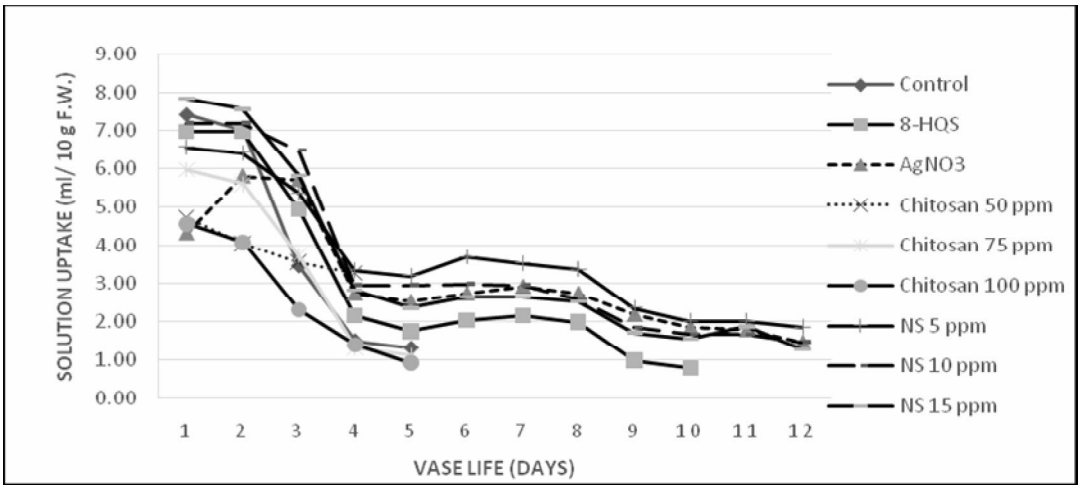

Fig. 2. Effect of preservative solutions on solution uptake (ml/ 10 g fresh weight) by Rosa *hybrida*cv. "Black Magic" cut flowers during 2016 season

It could be observed from data in Figures (3&4)that, in the two seasons, rose cut flowers treated with 8-HQS, $AgNO_3$ and SN had a positive values of water balance until the 3rd day then decreased gradually. Moreover, the previously mentioned treatments had better values of water balance throughout the longevity period during the two seasons when compared with the control treatment which had a sharp decrease in water balance after the 1st day of the vase life in the first season and after the 2nd day in the second season. Otherwise, the treatments included Chitosan showed the least values of water balance in the two seasons.

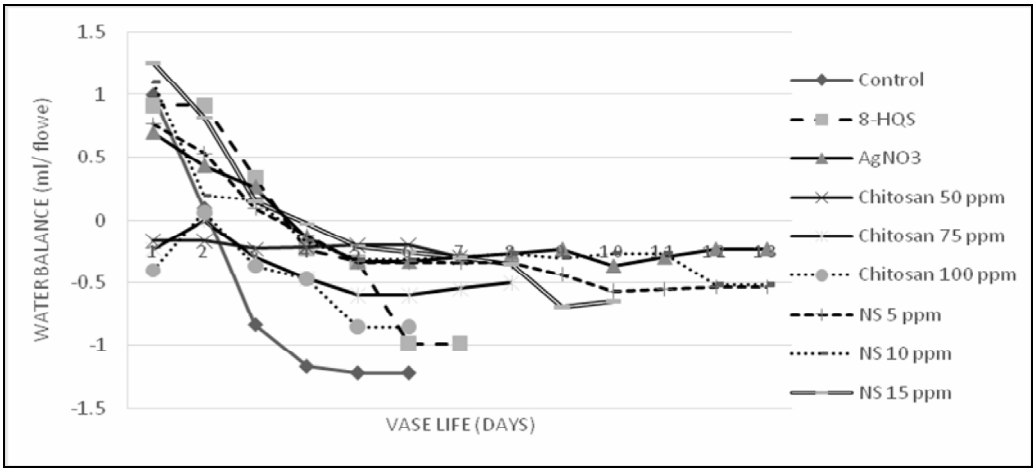

Fig. 3. Effect of preservative solutions on water balance (ml/ flower) of Rosa *hybrida*cv. "Black Magic" cut flowers during 2015 season

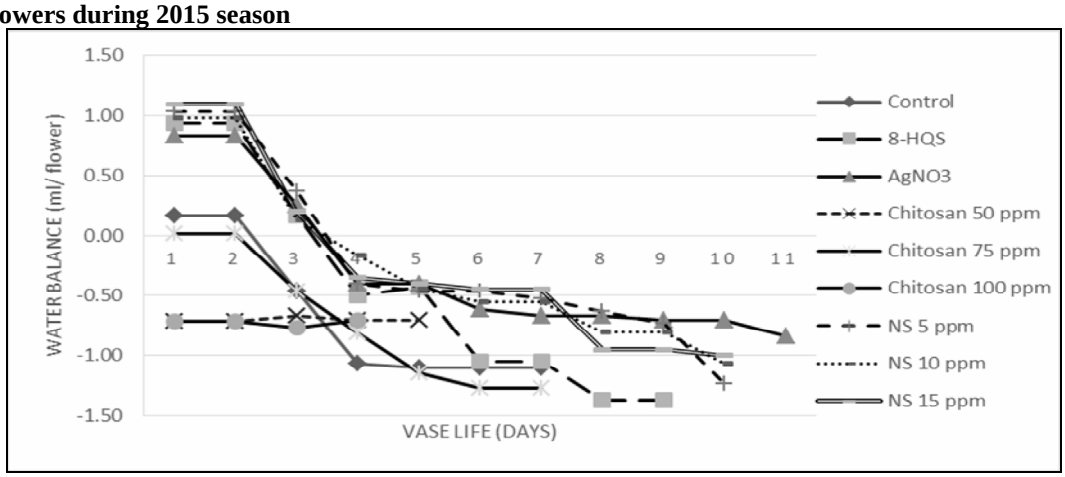

Fig. 4. Effect of preservative solutions on water balance (ml/ flower) of Rosa *hybrida*cv."Black Magic" cut flowers during 2016 season

Data presented in Table (1) also showed that the longest vase life in the first season (13.67 days) was when $AgNO_3$ at 10 ppm and SN at 10 ppm were used, and in the second season, the longest vase life a 12 days

was achieved when $AgNO_3$ at 10 ppm and SN at either 5, 10, or 15 ppm were used as antimicrobial agents. Thus, the solutions of $AgNO_3$ at 10 ppm + 20 g/l sucrose and SN at either 5, 10, or 15 ppm + 20 g/l sucrose caused a significant increase in the longevity of rose cut flowers compared with the other treatments in both seasons.Resultspresented in Table (1) also indicate that using 10 ppm $AgNO_3$ + 20 g/l sucrose scored the highest significant value of maximum increase in fresh weight (22.90 and 21.05 % in the first and second season, respectively) when compared with the other treatments except those of SN and 8-HQS. Sucrose act as a source of energy required for the continuation of the vase life of the cut flowers (Halevy and mayak, 1981), and may also act as osmotically active molecule, thereby lead to the promoting of subsequent water relations and lengthening their vase life (Elgimabi and Sliai, 2013). In addition to sucrose, the presence of strong antimicrobial agent ($AgNO_3$ or silver nano particles) would increase water uptake and improve water relations, thereby increase fresh weight and the vase life of the flower. Similar results were reported by (Lu *et al*., 2010) and (AbdelKader, 2012) on cut roses.

Table 1. Effect of preservative solutions on vase life (days), percentage of maximum increase in fresh weight and bacterial counts of *Rosa hybrida*cv. "Black Magic" cut flowers during the two seasons

Preservative solution	Vase life (days)		Maximum increase in fresh weight (%)		Bacterial counts
	2015	2016	2015	2016	(CFU/L) × 10^9
D.W. + 20 g/l sucrose (Control)	6.33 c	6.00 cd	19.08 b	15.11 bc	1066 a
8-HQS at 200 ppm + 20 g/l sucrose	8.67 bc	10.00 b	19.96 ab	18.41 ab	77 b
$AgNO_3$ at 10 ppm + 20 g/l sucrose	13.67 a	12.00 a	22.90 a	21.05 a	0.029 c
Chitosan at 50 ppm + 20 g/l sucrose	8.00 c	6.00 cd	1.89 c	0.00 d	0.66 c
Chitosan at 75 ppm + 20 g/l sucrose	7.33 c	7.67 c	0.00 c	2.57 d	0.33 c
Chitosan at 100 ppm + 20 g/l sucrose	7.00 c	5.00 d	1.91 c	0.00 d	0.043 c
SN at 5 ppm + 20 g/l sucrose	13.33 a	12.00 a	19.47 ab	20.47 ab	0.012 c
SN at 10 ppm + 20 g/l sucrose	13.67 a	12.00 a	22.08 ab	19.26 ab	0.007 c
SN at 15 ppm + 20 g/l sucrose	11.00 a	12.00 a	21.21 ab	16.88 abc	0.002 c

***Means with the same letter within each column are not significantly different at 0.05 probabilityaccording to Duncan Multiple Range Test.**

Scanning electron microscopy (SEM):

On the sixth day of the vase life, rose stem bases were examined by SEM. Fig. (5) shows the cross section of the surface of bases of cut rose stems cv. "Black Magic" treated with silver nano-particles at 5 ppm (A) compared with the control treatment (B).It is evident that most of the xylem vessels of cut rose stem bases treated with silver nano-particles at 5 ppm were clean and open for solution uptake, while most of the xylem vessels of those of the control treatments were closed and filled with microorganisms and debris. This evidence supports the view that silver nano particles have a strong antibacterial activity. Previous reports support the results of this experiment, since (Lu *et al*., 2010) reported that 1h pulse with SN at 100 mg/l inhibited bacterial growth in the vase solution and at the cut stem ends of cut roses during the first 2 d ofthe postharvest period, and (AbdelKader, 2012)showed that a holding solution of 5 mg/l reduced bacterial growth in solutions of cut roses.

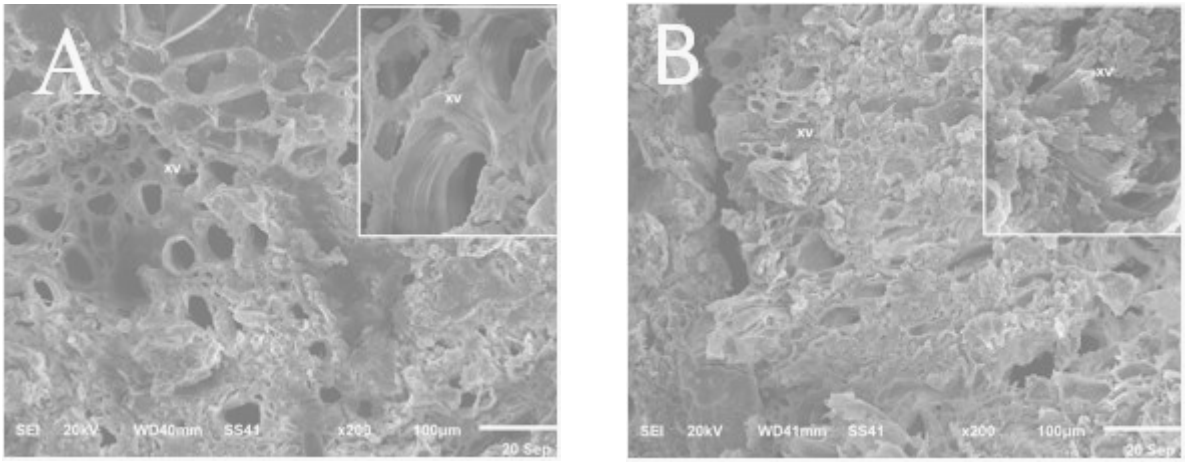

Fig. 5. Cross sections of the surface of bases of cut rose stems cv. "Black Magic" treated with Silver nanoparticles at 5 ppm (A) compared with the control treatment (B). Scale bars in panels = 100 µm, magnification 1200×. Scale barsin insets = 10 µm, magnification 200×. xv = xylem vessels.

CONCLUSION

From the previous results, it can be generally concluded that adding ($AgNO_3$) at 10 ppm or Silver nano-particles (SN) at 5 or 10 or 15 ppmto the vase solution were highly efficient in reducing bacterial growth in the vase solution and the cut stem ends of cut rose flowers which led to increasing water uptake, and thus improvement water relations of the flower. These compounds combined with sucrose at 20g/l to the vase solution led to the improvement of the keeping quality value of *Rosa hybrida* L.cv. "Black Magic" cut flowers. This work highly recommend the use of silver nano particles as a strong inhibitor of bacterial growth in the preservative solutions of cut flowers.

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تأثير بعض المركبات الكيميائية على عمر بعض الزهور المقطوفة

أ. تأثير ٨- هيدروكسي كينولين سلفيت ونترات الفضة والنانو فضة والشيتوزان على عمر وجودة زهور الورد المقطوفة

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أجري هذا البحث خلال موسمي ٢٠١٥و٢٠١٦ لدراسة تأثير استخدام بعض الكيماويات المختلفة على جودة ومدة بقاء أزهار الورد المقطوفة.تم وضع الزهور المقطوفة حديثاً في أوعية زجاجية يحتوي كل منها على ١٠٠ مل من محاليل الحفظ٨- هيدروكسي كينولين سلفيت (٢٠٠ جزء في المليون)، نترات الفضة (١٠ جزء في المليون)، شيتوزان بتركيزات ٥٠و ٧٥و ١٠٠ جزء في المليون، نانو فضة بتركيزات ٥و ١٠و ١٥ جزء في المليون) بالإضافة إلى الماء المقطر كمعاملة مقارنة. كل محاليل الحفظ بالإضافة لمعاملة المقارنةاحتوت على ٢٠ جم/لتر من السكروز. بالمقارنة مع الكونترول فإن المعاملات التي احتوت على نترات الفضة أو النانو فضة أدت لتحسين جودة وعمر الزهور، حيث كانوا أكثر تأثيراً في تشجيع امتصاص الماء، وزيادة الوزن الطازج للزهور والاتزان المائي حيث أدى ذلك إلى زيادة عمر الزهور إلى ١٣,٦٧ و١٢ يوم خلال الموسم الأول والثاني على الترتيب. وقد دلت نتائج العد البكتيري في محلول الزهرية وصور الميكروسكوب الإلكتروني لقاعدة الساق على أن نترات الفضة أوالنانو فضة قللت بدرجة كبيرة عدد البكتيريا في كل من محلول الزهرية وقاعدة الساق. ولقد دلت نتائج العد البكتيري في محلول الزهرية وصور الميكروسكوب الإلكتروني لقاعدة الساق على أن نترات الفضة والنانو فضة قللت بشدة النمو البكتيري في كل من محلول الزهرية وقاعدة الساق المقطوعة. هذه النتائج دلت على أن نترات الفضة أو النانو فضة بالإضافة إلى ٢٠ جم/لتر من السكروزيمكن استخدامها كمحلول حفظ زهور تجاري لزيادة عمر زهور الورد وتحسين جودتها.