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The Effects of Nanosilver, Nanosil and Hydrogen Peroxide on Vase Life Cut Rose (*Rosa hybrida*) 'Grand Press Angela'

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Abstract

To evaluate the effect of nanosilver (5, 10 and 20 mg l^{-1}), hydrogen peroxide (200, 400 and 600 µM) and Nanosil[1] (200, 400 and 600 µM) as antimicrobial compounds in vase solution of cut rose cv. 'Grand Press Angela' flowers, an experiment with 10 treatments and 3 replications was conducted in a completely randomized design. Cut rose flowers were continuously treated with these compounds. The results indicated a significant and positive impact of Nanosil on the measured traits. The highest vase life (13.16 days), maximum solution uptake (0.943 ml g^{-1} F.W.), the lowest fresh weight loss (6.31g), the lowest bacterial population in stem end (87.67 Log_{10} CFU ml^{-1}) and the highest petals carotenoid (17.61 µg g^{-1} F.W.) were related to the 400 µM Nanosil treatment. Most POD enzyme activities were recorded for the treatment of nanosilver 5 mg l^{-1} (3.94 nmol g^{-1} F.W.) and 400 µM Nanosil (3.86 nmol g^{-1} F.W.). Different concentrations of nanosilver were not successful in keeping vase life and its related traits because of the continuous use of these compounds. Different concentrations of hydrogen peroxide of evaluated characteristics were superior to the control. Overall, treatment with 400 µM Nanosil is recommended as an effective treatment for long-term preservation of cut flowers of roses as it was the best treatment and also had the longest vase life.

Keywords: Antimicrobial solutions, Prolonged treatment, Vascular obstruction, Vase solution.

[1] As compound of hydrogen peroxide and silver salt, nanosil is one of the most recent products used as antimicrobial particularly antiseptic or disinfectant and sterilant. Nanosil is an extraordinary and innovative compound to form a sustainable solution from hydrogen peroxide and silver ion by applying a new method. The product is a strong, rapid, sustainable, colorless and environment friendly sterilant and disinfectant.

INTRODUCTION

Rose (*Rosa hybrida*) is one of the most popular cut flowers of Rosaceae family and enjoys a high economic importance in the flower industry (Hossini Darvishani and Chamani, 2013; Solgi and Ghorbanpour, 2015; Jin *et al*., 2006). But the premature wilting of this flower which is mainly caused by water stress and sensitivity to ethylene, establishes many restrictions for exporting this cut flower to target markets. In this regard, the use of methods that can increase the shelf life of cut flowers of roses is very important (Hatami *et al*., 2012; Zaky, 2013; Solgi and Ghorbanpour, 2015). Given the short vase life of most varieties of roses is related to the adverse hydraulic conductivity of xylem mainly caused by microbial blockage, poor vascular connection of stem to peduncle at abscission layer and transpiration, use of preserving solutions containing microbicides can maintain water balance and increase the shelf life of cut flowers by reducing the activity and growth of microorganisms (Solgi and Ghorbanpour, 2015; Abri *et al*., 2014). In the previous years, many chemical and natural disinfectants were used in the vase of cut roses solution and the positive effect of these substances have been reported on maintaining water balance and sustainability of the cut flowers (Figueroa *et al*., 2005; Liao *et al*., 2000; Solgi and Ghorbanpour, 2015., Oraee *et al*., 2011). Silver nanoparticles have antibacterial properties and its antimicrobial features against various microorganisms have been reported (Maneerung *et al*., 2008; Navarro *et al*., 2008). The silver ions form complexes with organic compounds existing in the body of bacteria containing oxygen, hydrogen and sulfur, leading to the destruction of the walls and cell membranes of bacteria and these damages finally lead to death of the bacterial cell. In addition, the silver ions prevents reproduction and multiplication of microorganisms by reacting with DNA of bacteria, and thus prevent the activity and growth of microorganisms (Navarro *et al*., 2008; Solgi and Ghorbanpour, 2015; Solgi *et al*., 2009). The positive effects of silver nanoparticles have been reported on the microbial content control, maintaining water absorption and retention cut gerbera (Solgi *et al*., 2009) and Susan (Kim *et al*., 2005). Hydrogen peroxide is a strong oxidizing and disinfectant agent and its use in a solution of cut rose flower vase 'Candy' value has been positive (Hamdollahi *et al*., 2014). Nanosil is a complex of silver ions - hydrogen peroxide which is used in the disinfection and decontamination of microbial water (Khazaei *et al*., 2012); but no report has been provided on the effect of Nanosil on the vase life of cut flowers.

The purpose of this study was to investigate the effects of silver Nanoparticles, Hydrogen peroxides and Nanosil (silver complex × hydrogen peroxide) on the vase life of cut rose.

MATERIALS AND METHODS

This study used a Completely Randomized Design with 10 treatments, 30 plot, 5 flowers in per plot and 3 replications on the cut rose flower of "Grand Press Angela". The used flowers were purchased from a commercial hydroponic greenhouse in Tehran and were immediately transferred to the laboratory of Horticultural Sciences of Islamic Azad University in Rasht. The treatments were: control (distilled water), nanosilver (5, 10 and 20 mg l^{-1}), Nanosil (as compound of hydrogen peroxide and silver salt) and hydrogen peroxide each in three concentrations (200, 400 and 600 µM) that were used continuously. From start to finish, the flowers were kept under the controlled conditions of temperature 2 ± 22, humidity of 70-75 % and a photoperiod of 12 hours of light with a luminous intensity of 12 µmol $m^{-2}s^{-1}$.

The examined features

Vase life

To measure the vase life, flowers were daily visited. Finally, vase life was calculated by counting the days since the treatments begin until they wilt and the color of the 5 flower petals and leaves are changed (Abdolmaleki *et al*., 2015).

Solution uptake

Solution uptake of flowers were calculated using the formula:

$$\text{Solution uptake} = \frac{Vt0 - (Et + Vt1)}{FW}$$

Vt0: Initial volume of vase solution (500 cc); Et: Average evaporation of the solution; Vt1: the volume of solution remaining on the last day F.W.: fresh weight of flowers on the first day.

Fresh weight loss

Fresh weight of the cut rose was measured at the end of the vase life using a digital scale. Then, reduced fresh weight loss was calculated using the following formula:

Fresh weight loss = initial weight- (weight of falls+ weight of re-cut+ final fresh weight)

Electrolyte leakage

Sampling for measuring electrolyte leakage from healthy petals was conducted on the fifth day and then, Kaya *et al*. (2001) method was used to measure electrolyte leakage.

Bacterial population in stem end

24 hours after the treatments, sampling of the end of the stem was done and counting bacteria colony was done using Liu *et al*. (2009) method.

Petals anthocyanin

Anthocyanin was measured by spectrophotometry at 535 nm. Finally, anthocyanin was obtained from the following formula in terms of mg 100 g^{-1} F.W.:

$$\text{Anthocyanin} = \frac{e \times b \times c}{d \times a} \times 100$$

e= sample weight; b= sample volume to measure; c = the total solution made; d= the volume of samples; a = read number.

Petals carotenoid

On the fifth day of the test, sampling was done from petals to measure carotenoids. Petals carotenoid pigments were read by spectrophotometry at the wavelengths of 440, 645 and 663 nm and pigment concentration was calculated using the following formula:

Petals carotenoid: $4.69 \times A_{440} - 0.268 \times (20.2)A_{645} + (8.02)A_{663}$

Ethylene

Sampling to measure the released ethylene was conducted 24 hours after applying the treatments. A flower was select from each pot and the amount of ethylene was measured by Hashemabadi (2014) method.

Antioxidant enzymes (POD and CAT)

Petals sampling was done on the fifth day and then In *et al*. (2007) was used to measure the peroxidase activity and Chance and Maehly (1995) method was used to measure catalase activity.

At the end of the study, data analysis was done by the MSTATC statistical software and mean comparisons were performed using LSD test.

RESULTS

Analysis of variance of the effects of different treatments on vase life of rose cut flowers showed no significant difference between treatments at 1% level (Table 1). A comparison of the data showed that the highest vase life was related to 400 µM Nanosil with 13.16 days which statistically showed no significant differences with treatments of 200 and 600 µM Nanosil (12.66 days) and 200 and 400 µM hydrogen peroxide (10.83 day). The vase life (8 days) belonged to 5 mg L^{-1} of nanosilver (Table 2).

Solution uptake

The effects of different treatments on the absorbance of cut rose flowers were significant level of 5% (Table 1). A comparison of the averages showed that the use of Nanosil could maintain the solution uptake better than other treatments, so that the maximum absorbance (0.943 ml g^{-1} F.W.) was that of 400 µM Nanosil treatment which showed no statistically significant difference with 200 and 600 µM Nanosil for each three hydrogen peroxide concentrations. The lowest solution uptake was related to two treatments of 5 and 20 mg l^{-1} of nanosilver, 0.653 and 0.655, respectively. There was no statistically significant difference between the groups (Table 2).

Fresh weight loss

The results showed that the effects of different treatments on the fresh weight loss of cut rose flower was significant at 1% level (Table 1). Fresh weight loss at 400 µM Nanosil treatment (6.31 g) was than the other treatments. Nano silver and hydrogen peroxide treatments were not successful in maintaining fresh weight, had more fresh weight loss than Nanosil and control (Table 2).

Bacterial population in stem end

The results showed that the efficacy of different treatments on end of the stem bacteria was significant at the 1% level (Table 1). According to the results of mean comparisons, by the use of detergent compounds, the number of end of the stem bacteria decreased significantly compared to control group as the highest number of bacteria was recorded for the control (390 Log_{10} CFU ml^{-1}) group. There was no statistically significant difference between 3 Nanosil concentrations and 3 hydrogen peroxide concentrations. These treatments had less stem bacterial compared to different concentrations of nanosilver and control. The lowest number of the stem bacteria (87.67 Log_{10} CFU ml^{-1}) was related to Nanosil 400 µM treatment (Table 2).

Petal's carotenoids

Based on the analysis of variance results, the values of petal carotenoids were significant in different treatments at the 1% level (Table 1). Comparisons of means showed that the highest amount of petals carotenoids were belonging to two treatments of 400 µM Nanosil (17.61 µg g^{-1} F.W.) and 20 mg l^{-1} of nanosilver (16.9 µg g^{-1} F.W.), respectively. There were no statistically significant differences between the treatments of 10 mg l-1 of nanosilver (16.15 µg g^{-1} F.W.) and 400 µM hydrogen peroxide (15.75 µg g^{-1} F.W.). The least amount of petals carotenoids belonged to two groups of 200 µM Nanosil (12.4 µg g^{-1} F.W.) and 5 mg l^{-1} of nanosilver (13.33 µg g^{-1} F.W.), which was no statistically significant difference between them (Table 2).

Table 1. Analysis of variance of the effects of different concentrations of H_2O_2, Nanosil and Nano silve on the measured traits.

SoV	df	Vase life (day)	Solution uptake (ml g^{-1} F.W.)	Fresh weight loss (g)	Bacterial population in stem end (Log_{10} CFU ml^{-1})	Petals carotenoid (µg g^{-1} F.W.)	Petals anthocyanin (mg g^{-1} F.W.)	Ethylene (nl^{-1} h^{-1} g^{-1} F.W.)	Electrolyte leakage (%)	POD (nmol g^{-1} F.W.)	CAT (IU/g F.W.)
Treatments	9	9.18**	0.031*	6.10**	28098**	8.25**	11.87*	0.162**	144.8**	0.445**	0.762**
Erorr	20	2.61	0.011	1.60	3585	1.64	4.03	0.016	19.01	0.100	0.100
CV (%)		15.21	13.37	13.89	34.41	8.6	5.42	36.29	16.74	9.11	9.53

**, *, ns: Significant at 1% and 5% and non-significant.

Table 2. Mean comparison of measured traits affected by different concentrations of H_2O_2, Nanosil and Nano silver on the.

Treatments	Vase life (day)	Solution uptake (ml g^{-1} F.W.)	Fresh weight loss (g)	Bacterial population in stem end (Log_{10} CFU ml^{-1})	Petals carotenoid (µg g^{-1} F.W.)	Petals anthocyanin (mg g^{-1} F.W.)	Ethylene (nl^{-1} h^{-1} g^{-1} F.W.)	Electrolyte leakage (%)	POD (nmol g^{-1} F.W.)	CAT (IU/g F.W.)
Control	9.16^{cd}	0.726bc	8.07^{cd}	390^{a}	14.56^{bcd}	36.01^{bc}	0.960 ^{a}	33.6 ^{ab}	3.31 ^{bcd}	2.61^{f}
200 µM H_2O_2	10.83^{abc}	0.818abc	10.47^{ab}	143.3^{c}	14.46^{bcd}	39.56^{a}	0.240 ^{bc}	23.69^{cde}	3.21 ^{cde}	3.15 ^{cde}
400 µM H_2O_2	10.83^{abc}	0.874ab	9.18^{abc}	105^{c}	15.75 ^{abc}	36.5^{abc}	0.336 ^{bc}	31.57 ^{ab}	2.70 ^{e}	2.78 ^{ef}
600 µM H_2O_2	9.66^{cd}	0.814abc	9.22^{abc}	101^{c}	14.12^{bcd}	38.66^{ab}	0.221^{bc}	20.01^{ef}	3.13 ^{de}	2.89 ^{def}
200 µM Nanosil	12.66^{ab}	0.885ab	8.76 ^{bc}	123.3^{c}	12.40^{d}	39.50^{a}	0.404^{b}	15.13^{f}	3.54 $^{a-d}$	3.62 ^{abc}
400 µM Nanosil	13.16^{a}	0.943a	6.31 ^{d}	87.67^{c}	17.61^{a}	38.53^{ab}	0.195 ^{bc}	22.34^{def}	3.86 ^{a}	3.33 ^{bcd}
600 µM Nanosil	12.66^{ab}	0.906ab	7.95 ^{cd}	120^{c}	13.70^{cd}	33.40^{c}	0.125 ^{c}	19.53^{ef}	3.68 ^{abc}	3.05^{def}
5 mg L^{-1} Nano silver	8.00^{d}	0.653c	10.46^{ab}	260 ^{b}	13.33^{d}	36.44^{abc}	0.401^{b}	36.56^{a}	3.94^{a}	4.11^{a}
10 mg L^{-1} Nano silver	9.00^{cd}	0.754bc	9.49^{abc}	158^{bc}	16.15^{ab}	35.82^{bc}	0.289^{bc}	29.95^{abc}	3.78 ^{ab}	3.82^{ab}
20 mg L^{-1} Nano silver	10.33^{bcd}	0.655c	11.14^{a}	251 ^{b}	16.90^{a}	35.63^{bc}	0.314 ^{bc}	28.04 ^{bcd}	3.60^{a-d}	3.81 ^{ab}

*Similar letters in *each column indicate not significant difference at 5% probability level (LSD test).

Petal's anthocyanin

The results of the data analysis showed that the effects of disinfectants on the petal anthocyanins were significant at 5% (Table 1). As can be seen in Table 2, the lowest amount of petals anthocyanin belonged to 600 µM Nanosil (33.4 mg 100 g^{-1} F.W.) treatment. There was no statistically significant difference between the control treatments and 10 and 20 mg l^{-1} of nanosilver treatments. The most amount of petal anthocyanin was for 200 µM hydrogen peroxide treatments (39.56 mg 100 g^{-1} F.W.) and 200 µM Nanosil (39.5 mg 100 g^{-1} F.W.) was had statistical difference with 400 and 600 µM hydrogen peroxide, 400 µM Nanosil and 5 mg l^{-1} of nanosilver (Table 2).

Ethylene

The effects of different treatments on the ethylene of cut tose flowers were significant at the 1% level (Table 1). Mean comparisons showed that by the use of disinfectants, the amount of ethylene produced is significantly reduced compared to control. The control with 0.96 nl^{-1} h^{-1} g^{-1} F.W. the highest rate of ethylene production. 600 µM Nanosil treatment (0.125 nl^{-1} h^{-1} g^{-1} F.W.) was the most successful treatment to inhibit the production of ethylene and had the lowest ethylene production among the treatments (Table 2).

Electrolyte leakage

Analysis of variance results showed that electrolyte leakage of cut rose flowers produced significant difference between the different treatments at the 1% level (Table 1). Investigating the comparison of the effects of different treatments on electrolyte leakage showed that treatment of cut rose flowers with different nanosil levels significantly reduced electrolyte leakage; The 2 concentrations of 200 and 600 µM hydrogen peroxide were more successful compared to the control and nanosilver in the reduction of electrolyte leakage. Overall, among all treatments, the most electrolyte leakage was related to the 5 mg l^{-1} of nanosilver (36.56 %) which had no significant difference with the control (33.6 %), 400 µM hydrogen peroxide (31.57 %) and 10 mg l^{-1} of nanosilver (29.95 %). The least electrolyte leakage was related to 200 µM Nanosil treatment with 15.13 % (Table 2).

Peroxidase enzyme (POD)

Analysis of variance of data showed that the effects of different treatments on peroxidase activity was significant at the 1% level (Table 1). Comparison of means showed that the highest POD activity were related to different concentrations of nanosilver and Nanosil. POD activity at three concentration of hydrogen peroxide were less than other treatments. The least POD enzyme activity was related to 400 µM hydrogen peroxide treatment with 2.7 nmol g^{-1} F.W. (Table 2).

Catalase enzyme (CAT)

The effects of different treatments on catalase activity was significant at the 1% level (Table 1). CAT enzyme activity increased in all treatments compared to the control. The control (2.61 IU g^{-1} F.W.) had the lowest amount of CAT enzyme activity. Most CAT activity was related to the 5 mg l^{-1} nanosilver (4.11 IU g^{-1} F.W.) which had no significant difference with 10 and 20 mg l^{-1} of nanosilver and 200 µM Nanosil (Table 2).

DISCUSSION

The most important trait in the study of postharvest of cut flowers is vase life which has a direct relationship with the solution uptake by the cut flower. In fact, as the situation of cut flower is more favorable to absorb solutions, the flower life will be longer. So, in the context of post-harvest physiology of cut flower, removing water absorption obstacles, including the removal of microorganisms from the vase is very important. The most common way to remove microorganisms from the vase solution is the use of antimicrobial agents (Figueroa *et al*., 2005; Abri *et al*., 2014; Liao *et al*., 2000).

In the present study, the treated flowers with different nanosil concentrations, especially 400 µM Nanosil treatment, had the highest solution absorbance and vase life. According to the antimicrobial effects Nanosil, it can be concluded that by reducing the microbial content (Table 2), nanosil prevents blockage of the xylem of cut rose flowers and as a result, by continuing to absorb water, increase the life of this flower. This treatment also had the least reduced fresh weight and was among the best treatments to reduce the produced ethylene and increase peroxidase activity. Given that silver ion is a component of the Nanosil and its anti-microbial and anti-ethylene effects have been proven (Mirdehghan *et al*., 2013), we can relate the effect of Nanosil on controlling ethylene to silver ion.

Hydrogen peroxide in the study was better in most traits compared to control. Researchers reported that 5 µM hydrogen peroxide spray increases antioxidant activities in plants (Gecheva *et al*., 2002; Chylinski *et al*., 2007). Goldani and Kamali (2011) argued that hydrogen peroxide reduces ethylene production which is in line with the results of this study. Also, It had a powerful effect on controlling bacteria of the end of the stems. So, we can say that, the antimicrobial properties of hydrogen peroxide causes solution uptake and fresh weight control and prevent water stress and the production of ethylene and increase the cut flower life. These results are in line with the results of Hamdollahi *et al*. (2014) and Sadeghi *et al*. (2014) studies. The researchers believe that nano silver has antimicrobial activity and by reducing microbial content of preservative solution increases the shelf life of cut flower (Liu *et al*., 2009). But in this study, although by using different concentrations of nano silver, the number of bacteria of the end of the stem was reduced compared to control, the other traits did not change by the use of nano silver which may be due to high concentration of nano silver and longtime use of these compounds in this study. The researchers believe that the use of high concentrations of nano silver and long-term use of these compounds in vase solution, not only do not increase the vase life but also It has a negative impact on the rose life and flower quality (Solgi and Ghorbanpour, 2015). In other studies, the negative effects of prolonged use of high concentrations of nano silver has been reported on the shelf life of cut flowers of gerbera (Solgi *et al*., 2009; 2011), which agrees with these results.

Enzymatic activities in different plants and in different situations of a certain plant do not comply with a specific trend. Indeed, specific enzymes in different plants are responsible for neutralizing oxygen radicals. Even, some studies show that enzymatic activities are high in optimum treatments whilst their activities are low in optimum treatments in other studies. Interestingly, scientific and compelling explanations can be found for both states in literature. For example, when enzymatic activities are high in optimum treatment, one can say that high enzymatic activity has extended vase life of the flower, or when they are low in optimum treatment, one can say that since the plant has had optimal activity in this treatment, there has been no need for high enzymatic activity, reducing the activity of the certain enzyme. Hydrogen peroxide activates some antioxidant enzymes (Chylinski *et al*., 2007). It has been reported that the application of hydrogen peroxide enhances peroxidase enzyme activity (Rahmani *et al*., 2013). According to Yamane *et al*. (1999), the activity of peroxidase enzyme (POD) was enhanced in the petals of carnations and lilies as they aged. As antioxidant enzymes become more active, antimicrobial compounds put a barrier against membrane permeability and induces cell death, thereby it extends the vase of cut carnations (Hashemabadi, 2014).

Chang Li and Guo Quan (2011) reported the positive impact of vase life extension compounds on the activity of antioxidant enzymes in carnation, which resulted in membrane stability and longer vase life. It has been reported that higher hydrogen peroxide in stressful conditions was associated with higher activity of CAT, APX, and SOD enzymes (Tanoua *et al*., 2009), which is consistent with our findings.

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