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The role of silver nano-particles and silver thiosulfate on the longevity of cut carnation (*Dianthus caryophyllus*) flowers

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Abstract

The purpose of this study was to evaluate the efficacy of silver nano-particles (SNP) and silver thiosulfate (STS) in extending the vase life of cut carnation (*Dianthus caryophyllus* L. cv. 'Tempo') flowers. Pulse treatments of SNP @ 0, 5, 10 and 15 mg l^{-1} and STS @ 0, 0.1, 0.2 and 0.3 mM were administered to carnation flowers for 24 hr. The longest vase life (16.1 days) was observed in flowers treated with 15 mg l^{-1} of SNP + 0.2 mM STS. The least chlorophyll was destroyed in flowers treated with 15 mg l^{-1} of SNP + 0.3 mM STS. Our findings showed that the 15 mg l^{-1} SNP treatment inhibited bacterial growth in the preservative solution. The control flowers bloomed faster than the treated flowers. The maximum peroxidase activity and the minimum lipid peroxidation were obtained in cut flowers that were treated with 15 mg l^{-1} of SNP and 0.3 mM STS. Overall, results of the study revealed that SNP and STS treatment extended the longevity of cut carnation 'Tempo' flowers by reducing oxidative stress, improving anti-oxidant system, reducing bacterial populations and delaying flowering.

Key words

Dianthus caryophyllus L., Peroxidase activity, SNP, STS, Vase life

Introduction

Carnation (*Dianthus caryophyllus* L.), which is one of the most popular cut flower in the global flower trade, belongs to Caryophyllaceae family (Galbally and Galbally, 1997; van Doorn, 1997). This species is sensitive to ethylene, and the senescence of carnation flowers is accompanied with increase in the synthesis of ethylene, increased vascular blockage and a concomitant climacteric increase in respiration (Nichols, 1996; Hashemabadi and Mostofi, 2007). Microbial contamination due to bacterial growth in preservative solution also reduces the vase life of cut carnations (He *et al*., 2006; van Doorn, 1997). The termination of vase life for many cut flowers is attributed to wilting (He *et al*., 2006). Stem end blockage is a main factor in the imbalance between water uptake and water loss of cut flowers (van Doorn, 1997; Da Silva, 2003; He *et al*., 2006). Water balance is a main factor that determines the quality and longevity of cut flowers (Lü *et al*., 2010) because water uptake and transpiration must be balanced. There are three types of stem end blockage in cut flowers: physical air emboli (Van Meeteren *et al*., 2006), microbial (Liu *et al*., 2009b) and physiological wound-induced (Williamson *et al*., 2002; He *et al*., 2006; Loubaud and van Doorn, 2004). Microorganisms are the most common cause of stem end blockage (van Doorn, 1997). Microbes can also produce ethylene, secrete toxic compounds and accelerate senescence (Williamson *et al*., 2002). Even when cut flowers are kept in water, stem end blockage leads to wilting (van Doorn, 1997; He *et al*., 2006). Some compounds, such as silver nitrate, 8-hydroxyquinoline citrate (8-HQC), aluminum sulphte, essential oils and nano-silver, improve water uptake in cut flowers and extend vase life (Ichimura *et al*., 1999; Sondi and Salopek-Sondi, 2004; Ichimura and Shimizu-Yumoto, 2007; Bounattirou *et al*., 2007; Navarro *et al*., 2008). Silver nano-particle treatment is a relatively new antimicrobial approach, and the particles are applied as a pulse in a preservative solution for cut flowers; this approach can kill approximately 650 bacterial species in water (Furno *et al*., 2004; Morones *et al*., 2005; Liu *et al*., 2009b; Solgi *et*

al., 2009). Silver nano-particle treatment strongly inhibits microbial activities because of the high surface area to volume ratio of these particles (Furno *et al*., 2004; Jiang *et al*., 2004). Silver ions are generally applied as silver thiosulfate (STS). This substance inhibits ethylene-mediated processes, such as flower senescence and abscission, and aquaporins (Altman and Solomos, 1995; Niemietz and Tyerman, 2002; Ichimura *et al*., 2008). One drawback of STS treatment is the narrow range of concentrations at which STS is not phytotoxic and is effective as an ethylene inhibitor (Nell, 1993). A positive effect of silver nano-particles on the vase life of some commercially important plants, such as rose, carnation and gerbera cut flowers, has been observed (Ohkawa *et al*., 1999; Kim *et al*., 2005; Liu *et al*., 2009b, 2009a). In light of the above, the objective of the present study was to evaluate the effects of silver nano-particle (SNP) in extending the longevity of cut carnation (*Dianthus caryophyllus* L. cv. 'Tempo') flowers.

Materials and Methods

Cut carnation (*Dianthus caryophyllus* L. cv. 'Tempo') flowers were obtained from a hydroponic greenhouse in Mahallat, Iran at their optimum developmental stage. The flowers were immediately placed in buckets in an upright position and transported to the postharvest laboratory. To minimize moisture loss during transportation, buckets containing flower stems were covered with plastic film. In laboratory, stems were re-cut under deionized water to a length of approximately 45 cm. The flowers were selected for uniformity of size, color and lack of defects.

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of treatments consisting of four STS concentrations (S_1:0, S_2:0.1, S_3:0.2, S_4 :0.3)mM STS × four SNP concentrations (N_1:0 , N_2:5 , N_3:10, N_4:15) mg l^{-1} SNP × three replications × sixteen treatments. In each experiment, five cut carnations were placed into a 1000 ml vase that was filled with 250 ml of preservative solution containing the aforementioned concentrations of STS and SNP. These cut carnation stems were then placed into 1000-ml pots filled with 500 ml of preservative solution supplemented with 3% sucrose and 600 mg l^{-1} of hydroxy quinoline sulfate. Distilled water was used as control. To minimize evaporation and prevent contamination, the mouth of the pots were covered with a sheet of low density polyethylene film. The flowers were kept in vase in a room under the following conditions: temperature of 20 ± 2°C, relative humidity of 70-75%, light intensity of 15-20 µmol $m^{-2}s^{-1}$ using cool white florescent tubes and a daily light period of 12 hr.The criterion for the end of vase life was the time point at which the flowers showed symptoms of petal wilting or curling. Vase life was the time period from when the cut flowers were placed into the second preservative solution until the end of vase life.

For determining bacterial concentration in stems after pulsing, 2-cm length (approximately 0.5 g) segments were cut from the stem ends. Explants were washed three times with sterile distilled water to reduce surface microbial loads. The explants were then ground and diluted with 0.9% sterile saline. Aliquots (0.1 ml) of extract were spread on nutrient agar plates, and bacterial colonies were enumerated after incubation for 24 hr at 37°C. All bacterial counts were conducted on triplicate sub-samples (Bleeksma and van Doorn, 2003; Balestra *et al*., 2005).

Using an SPAD-502 chlorophyll meter manufactured by the Minolta Company, chlorophyll index (CI) of cut carnations was estimated at two stages: after treatment with SNP and STS and at the end of vase life of cut carnations. The chlorophyll index loss was calculated by the following formula:

Chlorophyll content after SNP and STS treatment - Chlorophyll content at the end of vase life

To measure the cut carnation blooming (opening) index, the diameter if flowers were measured immediately before and after SNP and STS treatment. For this feature, the length of the largest diameter and the vertical diameter were measured, and mean of 10 resulting numbers were calculated. This process was continued till the cuts reached maximum diameters.

The POD activity of cut carnation flowers was determined after 5 days of STS and SNP treatment. Each extract used for measuring POD activity was prepared by freezing 0.5 g of petal tissue in liquid nitrogen and then grinding the tissue in 10 ml of extraction buffer [50 mM phosphate buffer, pH 7 containing 0.5 mM EDTA and 2% PVPP (w/v)]. The resulting homogenate was centrifuged for 20 min at 15,000 g, and the supernatant was used to determine enzymatic activity. POD activity was assayed by spectrophotometric measurement of guaiacol formation in 1 ml of a reaction mixture consisting of 450 µl of 25 mM guaiacol, 450 µl of 225 mM H_2O_2 and 100 µl of crude enzyme. The activity was expressed as mM mg^{-1} f. wt.

Lipid peroxidation in cut carnation flowers was estimated by following the method of Bates *et al.* (1973). Using SPSS statistical software, data were subjected to analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) test at $p \leq 0.05$ level.

Results and Discussion

The effect of different concentrations of SNP and STS and their interaction were significant at $p \leq 0.01$ on chlorophyll decreasing index (Table 1). Increasing the STS concentration inhibited chlorophyll degradation. The least chlorophyll degradation was observed in cut carnations treated with 0.2 or 0.3 mM STS. SNP treatment also significantly inhibited chlorophyll degradation. The least chlorophyll loss was observed in treatments using 5 or 15 mg l^{-1} SNP. Regarding interaction effects between SNP and STS, the minimum chlorophyll losses were obtained in treatments using S_4N_4 (15 mg l^{-1} SNP + 0.3 mM STS)

(0.64) and S_3N_4 (15 mg l^{-1} SNP + 0.2 mM STS) (0.69) (Table 2). Factors involved in leaf chlorophyll degradation include ethylene and anti-ethylene compounds, such as STS and 1-MCP that decreased the acceleration of leaf chlorophyll degradation. Hashemabadi *et al*. (2009) revealed that the concentrations of 60 and 80 nl l^{-1} of 1-MCP delayed the leaf chlorophyll degradation process in cut carnations (*Dianthus caryophyllus* L. cv. 'Tempo'). A similar result was obtained by Jiang *et al*. (2002) study on coriander that showed a positive effect of 50 nl l^{-1} of 1-MCP on the inhibition of chlorophyll degradation in this species, but no significant differences were seen between the concentrations of 100 and 1,000 nl l^{-1} of 1-MCP. These findings showed that 100 nl l^{-1} of 1-MCP is sufficient to block ethylene receptors in coriander leaves. Basiri *et al*. (2011) showed that nano-silver retains the post-harvest quality of cut carnations flowers and leaves, and the lowest quality was observed in the flowers and leaves of control plants. No significant differences were found in leaf chlorophyll using concentrations of 0 to 80 mg l^{-1} of nano-silver, but later these differences became significant. This latter result is in agreement with the results obtained in our study.

Malondialdehyde (MDA) is produced by lipid peroxidation of the cellular plasma membranes. Increase in STS and SNP concentration caused decrease in MDA content. A minimum MDA content (39.99 nmol g^{-1} f. wt.) was observed in S_3N_4 treatment (Table 2). The effect of different concentrations of SNP and STS and their interaction on MDA were significant at $p \leq 0.01$ level (Table 1). Carnation (*Dianthus caryophyllus* L.) is quite sensitive to ethylene; therefore, appropriate pre-treatment of this sensitive species with ethylene inhibitors, especially if carnation is to be sold in supermarkets or other areas with ethylene, could be helpful (Reid, 2002). In contrast, one reason for the short life span of cut flowers is reduced water uptake, which causes water stress in plants (Reid, 2002).

Petals senescence is commonly accompanied by biochemical, biophysical and morphological deterioration. In membranes, decreasing lipid fluidity is one sign of deterioration (Arora *et al*., 2007). The fatty acids released by this breakdown are then peroxidized, which in turn affects membrane permeability. This membrane deterioration is a prerequisite for ethylene synthesis (van Doorn and Stead, 1994). Ethylene enhances flower senescence and wilting, increases the permeability of petal cellular membranes and accelerates the decrease in cellular membrane fluidity (Zamani *et al*., 2011). A study by Sexton *et al.* (1995) on *Lathyrus odoratus* L. showed that STS (0.2 mM) delayed wilting and prevented abscission. Gerailoo and Ghasemnezhad (2011) demonstrated that protein degradation and MDA accumulation were shown to be suppressed by 150 mg l^{-1} SA during the vase life of *Rosa hybrida*. These researchers showed that the least ethylene was produced in the 150 mg l^{-1} SA treatment. Talebi *et al*. (2011) showed that SNP treatment decreased the peroxidation and permeability of cellular membranes by increasing catalase and peroxidase activity.

The effect of SNP or STS on the vase life of cut carnation flowers was significant ($p \leq 0.01$ or $p \leq 0.05$, respectively) as compared to control (Table 1). The vase life of cut flowers was 3 and 4.2 days longer than the controls under S_4N_2 treatment (Table 2). The combined effect of SNP and STS treatment did not significantly affect the vase life. STS is an ethylene inhibitor and extends the vase life of some cut flowers (Kim *et al*., 2005). Many studies indicate that SNP delayed flower senescence and increased the quality and vase life of cut flowers. Similar to our findings, Liu *et al*. (2009b) and Basiri *et al*. (2011) showed that SNP could extend the vase life of cut carnation. The positive effect of SNP on extending the vase life of cut rose, gerbera and lily has been demonstrated (Ohkawa *et al*., 1999; Kim *et al*., 2005; Liu *et al*., 2009b; Solgi *et al*., 2009). Basiri *et al*. (2011) showed that all concentrations of SNP extended the vase life of cut carnation compared with control, and the longest vase life was observed in N_2 treatment. These researchers indicated that SNP treatment had a positive effect by increasing the quality and vase life of cut flowers and leaves of carnation. The use of SNP as a pulse or continuous treatment for cut flowers is relatively new (Liu *et al*., 2009b) and demonstrated the importance of SNP as anti-microbial agents (Morones *et al*., 2005). Liu *et al*. (2009a) showed that all SNP pulse treatments markedly extended the vase life of cut gerbera compared with control. The pulse treatment using 5 mg l^{-1} SNP had the longest vase life, which was twice that of the control. Kim *et al*. (2005) reported that Ag^+ ion nanoparticles

Table 1 : Analysis of variance (ANOVA) of the effect of different SNP and STS on various traits in cut carnation (*Dianthus caryophyllus*)

S.O.V	df	Mean Square					
		Chlorophyll loss index	Vase life	Bacterial No. in vase solution	Flower opening index	POD activity	MDA content
STS (S)	3	00.771**	1.823*	0398.806ns	0.045*	0.002**	000174.634**
SNP (N)	3	02.010**	3.414**	4908.917**	0.112*	0.001**	000161.111**
S × N	9	00.511**	0.781ns	0388.009**	0.020ns	0.056**	123851.417**
Error	32	00.105	0.841	0025.021	0.033	3.659	000011.961
Total	48	-	-	-	-	-	-
C.V. (%)	-	36.660	6.980	0016.570	13.40	43.83	000011.084

**: significance level at 1%, *: significance level at 5%, ns: not significant

mixed with other compounds extended the vase life of cut lily. Solgi *et al*. (2009) demonstrated that the addition of 5, 10 or 20 mg l^{-1} $AgNO_3$ to preservative solutions extended the vase life of cut gerbera flowers. Flowers held in 5 or 10 mg l^{-1} SNP had a longer vase life than control. There were no significant differences in the vase life of these treatments, and the best concentration used for extending vase life was 5 mg l^{-1} SNP. Ansari *et al*. (2011) showed that treatment of gerbera with 5 mg l^{-1} SNP, 4% sucrose and 2.5 mg l^{-1} of GA resulted in a vase life of 19.5 days, which was greater than the controls (17.67 days). Additionally, Mohammadi Ostad Kalay *et al*. (2011) demonstrated that a maximum vase life (14.33 days) of cut rose was obtained with 2 mg l^{-1} NS; the vase life of the control was 5.79 days.

POD activity increased as SNP and STS concentrations increased. Maximum POD activity was observed in S_3N_4 treatment activity in S_3N_4 treatment was 6 times greater than the activity of control (0.011 mmol g^{-1} f.wt.) (Table 2). The effect of SNP and STS on POD activity was significant ($p \leq 0.01$). The highest POD activity was detected in cut carnation flowers treated with 0.2 and 0.3 mM STS (0.046 mmol g^{-1} f.wt.) or 15 mg l^{-1} SNP (0.044 mmol g^{-1} f.wt.) (Table 1). POD activity increase gradually as floral senescence progresses (Gerailoo and Ghasemnezhad, 2011). An increase in POD activity of petals might strengthen vascular cells, which remain functional during the later stage of senescence (Panavas and Rubinstein, 1998). Previous studies have showed that POD is involved in senescence, because it catalyzes the degradation of H_2O_2. The POD enzyme uses H_2O_2 as a substrate for several reactions, and the specific activity of POD increases in carnation and daylily during senescence (Bartoli *et al*., 1995; Panavas and Rubinstein, 1998). Talebi *et al*. (2011) showed that SNP treatment of cut roses increased peroxidase enzymatic activity. Cytokinin application improved the postharvest quality of cut roses by reducing petal senescence because cytokinin has an anti-ethylene effect (van Doorn and Cruz, 2000). Some anti-ethylene compounds, such as cytokinin and Ag^+, increase the activity of POD and CAT enzymes.

STS had no significant effect on bacterial population in the stem end of cut carnations flowers; however, the effect of SNP on bacterial populations was significant ($p \leq 0.01$). The bacterial concentration in the stem ends decreased significantly with increasing SNP concentrations (Table 1). The interaction between STS and SNP had a significant effect on the bacterial concentration in the stem end ($p \leq 0.01$). The lowest (0 CFU ml^{-1}) and the highest bacterial concentration (78.33 CFU ml^{-1}) were observed in the stems treated with S_1N_4 and control, respectively (Table 2). The presence of SNP in preservative solution, with or without STS, had a significant effect on bacterial concentrations in the stem ends of cut carnations. Low water uptake by cut flowers

Table 2 : Mean comparison of the effects of different treatments on various traits in cut carnation (*Dianthus caryophyllus*)

Treatments	Flower opening index	Bacterial number in vase solution (log_{10} CFU $m l^{-1}$)	Vase life (day)	Chlorophyll loss index	POD (m mol g^{-1} f. wt.)	MDA content (m mol g^{-1} f.wt.)
S_1 (0 mM STS)	1.51^{a}	26.25^{ab}	12.33^{b}	1.81^{a}	0.018^{c}	52.52^{a}
S_2 (0.1 mM STS)	1.43^{b}	16.17^{b}	14.52^{ab}	1.84^{a}	0.025^{b}	55.32^{a}
S_3 (0.2 mM STS)	1.40^{b}	13.33^{b}	15.32^{a}	1.37^{b}	0.046^{a}	47.12^{b}
S_4 (0.3 mM STS)	1.38^{b}	15.42^{b}	14.81^{ab}	1.41^{b}	0.046^{a}	48.22^{b}
N_1 (0 mg l^{-1} SNP)	1.56^{a}	46.75^{a}	11.04^{b}	2.13^{a}	0.027^{b}	55.20^{a}
N_2 (5 mg l^{-1} SNP)	1.33^{b}	16.50^{b}	15.20^{a}	1.26^{b}	0.031^{b}	52.42^{a}
N_3 (10 mg l^{-1} SNP)	1.43^{ab}	05.42^{c}	14.56^{ab}	1.74^{a}	0.033^{b}	47.58^{b}
N_4 (15 mg l^{-1} SNP)	1.39^{b}	02.50^{c}	15.09^{ab}	1.30^{b}	0.044^{a}	47.98^{b}
S_1N_1	1.73^{a}	78.33^{a}	13.64^{ab}	2.33^{a}	0.011^{k}	57.60^{a}
S_1N_2	1.42^{a}	19.33^{cd}	14.80^{ab}	1.42^{bcde}	0.017^{j}	51.17^{bcd}
S_1N_3	1.43^{a}	07.33^{def}	14.52^{ab}	1.71^{abcd}	0.018^{j}	51.33^{abcd}
S_1N_4	1.47^{a}	00.00^{f}	14.36^{ab}	1.79^{abcd}	0.027^{g}	49.99^{bcd}
S_2N_1	1.54^{ab}	38.67^{b}	13.95^{ab}	2.19^{ab}	0.021^{i}	57.60^{a}
S_2N_2	1.27^{ab}	18.00^{cd}	15.26^{ab}	1.19^{de}	0.022^{hi}	55.74^{ab}
S_2N_3	1.42^{a}	07.33^{def}	14.67^{ab}	1.90^{abcd}	0.023^{h}	53.81^{abcd}
S_2N_4	1.49^{a}	00.67^{f}	14.20^{ab}	2.09^{abc}	0.035^{e}	54.15^{abc}
S_3N_1	1.50^{a}	26.67^{c}	14.07^{ab}	2.33^{a}	0.033^{f}	51.41^{abcd}
S_3N_2	1.35^{ab}	16.67^{cde}	15.62^{ab}	1.15^{de}	0.038^{d}	54.54^{abc}
S_3N_3	1.34^{ab}	02.33^{f}	15.10^{ab}	1.31^{cde}	0.044^{c}	42.54^{ef}
S_3N_4	1.33^{ab}	07.67^{def}	16.10^{a}	0.69^{e}	0.069^{a}	39.99^{f}
S_4N_1	1.47^{a}	43.33^{b}	14.50^{ab}	1.67^{abcd}	0.043^{c}	54.19^{abc}
S_4N_2	1.30^{ab}	12.00^{def}	15.10^{ab}	1.28^{cde}	0.048^{b}	48.23^{cde}
S_4N_3	1.52^{a}	04.67^{ef}	13.94^{ab}	2.03^{abc}	0.047^{b}	42.66^{ef}
S_4N_4	1.27^{ab}	01.67^{f}	15.70^{ab}	0.64^{e}	0.043^{c}	47.78^{de}

*Values in each row that are followed by the same letter are not significantly different by Duncan's test.

is often due to occlusions, which are mainly located in the basal stem end (He *et al*., 2006), and microorganisms and their decay products are a common cause of stem end blockage (van Doorn, 1997; Williamson *et al*., 2002). SNP treatment of cut flowers strongly inhibits microbial activities (Jiang *et al*., 2004; Furno *et al*., 2004). Basiri *et al*. (2011) indicated that the lowest bacterial concentration in the stem end of cut carnations was obtained with 5 mg l^{-1} SNP; the highest concentration was observed in the control treatment. Thus, SNP treatment had antibacterial effects and could extend the quality and vase life of cut carnations. Lü *et al*. (2010) showed in cut rose that the bacterial concentration in the stem ends of control was significantly higher than in flowers treated with any concentration of SNP. In many cut flowers, a suppression of microbial growth in the vase solution results in delayed wilting (van Doorn, 1997; Loubaud and van Doorn, 2004). The antibacterial activity of SNP is partly a function of particle size, because a higher surface to volume ratio increases the proportion of atoms at the grain boundary (Raffi *et al*., 2008). Liu *et al*. (2009a) demonstrated that SNP inhibited bacterial growth for the first 2 days of vase life in the stem ends of cut gerbera. Oraee *et al*. (2011) showed that all treatments with NS were effective at decreasing the bacteria in the stem end and preservative solution of cut gerbera. Solgi *et al*. (2009) showed that SNP extended the vase life of cut gerbera. Lü *et al*. (2010) reported that SNP suppressed decreases in the hydraulic conductance of cut rose stems, which were apparently associated with a reduction in the concentration of stem end bacteria. Our results confirm the efficacy of SNP in extending the vase life of cut carnations.

Data analysis of the variance showed that the effect of SNP and STS on the flower blooming index in cut carnation was significant ($p \leq 0.05$) (Table 1). The lowest flower blooming index (1.27) was observed in cut flowers treated with S_2N_2 and S_4N_4. The highest flower blooming index (1.73) was observed in cut flowers treated with S_1N_1 (Table 2). Compared with the control treatments, increase in STS concentration reduced the acceleration of flower blooming, and all STS concentrations delayed flower opening. Additionally, different SNP concentrations delayed flower opening. A delay in flower blooming by the application of anti-ethylene compounds has been reported by some researchers (Hashemabadi and Mostofi, 2007). Some compounds, such as 1-MCP and STS, reduce the respiration rate of cut flowers by delaying the climacteric respiration peak, thus the production of ATP decreases. Mohammadi Ostad Kalay *et al*. (2011) demonstrated that cut rose flowers treated with 250 mg l^{-1} 8-HQS and 2 mg l^{-1} NS had the largest flower diameter and percentage of open flowers.

Maximum vase life (16.1 days) was obtained and this treatment enhanced the cut flower vase life for 2.5 days. Minimum bacterial pollution was seen in S_1N_4 and S_2N_4. Most traits such as chlorophyll index and enzymes activity enhanced after treatment with S_3N_4. Overall, interaction effect of S_3N_4 is proposed for increasing the vase life of cut carnation "Tempo".

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