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Effect of nano-silver and boric acid on extending the vase life of cut rose (*Rosa hybrida* L.)

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Abstract

Silver nano-particles (2-5 nm diam.), as antimicrobial agent and boric acid, as ethylene production inhibitor are used for enhancing the quality and vase life of cut flowers. In the present study the effects of a preservative solution containing nano-silver and boric acid on some traits of cut rose (*Rosa hybrida* L. cv. Yellow Island) including vase life, ethylene production, dry weight percentage, chlorophyll content, flower opening index, β-carotene of petals and the number of basal stem end bacteria were investigated. The results showed that the effect of nano-silver and boric acid as either solitary or in combination with each other were significant ($p \leq 0.01$) on vase life, ethylene production and β-carotene pigment. The effect of nano-silver on the number of bacteria on the end of stem was significant ($p \leq 0.01$). The highest cut flower longevity (9.69 day) was obtained in pulse-treated flowers with 100 mg l^{-1} boric acid. The least ethylene production (0.59 nl^{-1} g^{-1} h^{-1}) was observed in cut rose treated with 100 mg l^{-1} boric acid along with 5 mg l^{-1} nano-silver. The lowest number of bacteria in the end of stem was calculated in cut flowers treated with the highest concentrations of boric acid (300 mg l^{-1}) and nano-silver (20 mg l^{-1}).

Key words

Basal stem end bacteria, β-Carotene, Ethylene, Flower longevity, *Rosa hybrida* L.

Introduction

Rose (*Rosa hybrida* L.) (Rosaceae) is the major export of cut flowers in the world. Most postharvest problems are stem end blockage, caused by air and microorganisms. Stem end blockage is the main factor in the imbalance between water uptake and water loss from cut flowers (van Doorn, 1997). Water balance is a main factor determining quality and longevity of cut flowers (Lü *et al*., 2010). Water uptake and tranano-silverpiration must be in the balance. Microbes can also produce ethylene and secrete toxic compounds, also pectinase and accelerated senescence (Williamson *et al*., 2002). Ethylene is the major plant growth regulator related to senescence and its external application causes accelerated senescence (Reid and Wu, 1992). Microorganisms are the most common cause of stem end blockage (Williamson *et al*., 2002; van Meeteren, 1978). Despite cut flowers being kept in water, blockage of vessel leads to wilting (van Doorn, 1997; He *et al*., 2006). Some compounds such as silver nitrate, 8-hydroxyquinoline citrate (8-HQC), aluminum sulphate, essential oils and nano-silver improve water uptake in cut flowers and extend vase life (Bounatirou *et al*., 2007; Ichimura *et al*., 1999). Nowadays, some of these compounds, such as silver nitrate, are not applied as it causes black ening of flower stem and is dangerous for humans and environment (Damunupola and Joyce, 2006). Nano-silver is relatively a new antimicrobial compound which is applied as a pulse treatment in preservative solution treatment for cut flowers (Liu *et al*., 2009; Morones *et al*., 2005; Nell, 1992; Solgi *et al*., 2009). Nano-silver strongly inhibits microorganisms' activities because its surface to volume ratio is high (Jiang *et al*., 2004). It releases silver ions (Ag^{+}) (Lok *et al*., 2007) which interact with nucleic acid (lose of DNA replication) and cytoplasm components and prevents respiratory enzymes, dissipates the proton motive force, and also decreases membrane permeability and finally cell death (Feng *et al*., 2000; Maneerung *et al*., 2008; Park *et al*., 2005; Russell and Hugo, 1994). Silver ion is, generally, applied as silver thiosulfate

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(STS) which inhibits ethylene-mediated process, such as flower senescence and abscission and is an inhibitor of aquaporinano-silver (Ichimura *et al*., 2008; Niemietz and Tyerman, 2002). Positive effect of silver nanoparticles on enhancing the vase life of some commercially important cut flowers such as rose, carnation and gerbera has been observed (Kim *et al*., 2005; Ohkawa *et al*., 1999).

Boric acid is another compound which delays senescence of some flowers such as carnation (Serrano *et al*., 2001). Studies about the role of boric acid on vase life of cut flowers are very few. Senescence is associated with a climacteric-like increase in ethylene production (Serrano *et al*., 2001). During climacteric respiration, the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase) and ACC oxidase (Peiser, 1986; Serrano *et al*., 1991) increase, which convert S-adenosyl-methionine (SAM) to ACC and ACC to ethylene, respectively (Serrano *et al*., 2001). Boric acid inhibits ethylene production through reducing ACC synthase and ACC oxidase activities. Boric acid, already as a mixture with chemicals compounds, used to improve vase life of cut flowers (Odom, 1954), may be a good competitor as far as price is concerned (Serrano *et al*., 2001). The present study has investigated the effects of nano-silver and boric acid on improving the quality and extending the vase life of cut rose (*Rosa hybrida* L. cv. 'Yellow Island').

Materials and Methods

Cut roses (*Rosa hybrida* L. cv. 'Yellow Island') were obtained from a greenhouse in Tehran, Iran at their optimum developmental stage. They were immediately kept in buckets and tranano-silverported to the postharvest laboratory. Buckets containing flower stems were covered with a plastic film shroud to minimize evaporation and tranano-silverpiration. At the laboratory, stems were re-cut under deionized water to ~50 cm length. Re-cutting was done to enano-silverure no embolism of stem end. The flowers were selected for uniformity of size, color and freedom from any defects. The upper three leaves were retained on each stem.

The experimental design was a randomized completely blocks design with a factorial arrangement of treatments containing four boric acid concentrationano-silver (0, 100, 200 and 300 mg l^{-1})×four silver nano particles concentrations (0, 5, 10 and 20 mg l^{-1}) × three replicationano-silver × five cut flowers per treatment. In each experiment, cut rose stems were weighed and pulse-treated for 24 hr with 250 ml of preservative solutions including aforementioned compounds. Then, all the cut roses were placed into the vases filled with 500 ml of preservative solution containing 3% sucrose and 600 mg l^{-1} hydroxy quinoline sulfate. Distilled water was used as control. The mouths of vases were covered with a sheet of low denano-silverity polyethylene film to minimize evaporation and to prevent contamination. Re-cutting was carried out every four days. The flowers were kept under controlled room conditionans: 20 ± 2°C, relative humidity of 60-70%, 12 µmol m^{-2} s^{-1} light intensty (cool white fluorescent tubes) and a photo period of 12 hr. Criterion for the end of vase life was the time when flowers were showing symptoms of petal wilting or curling. Vase life was the period from the time of putting cut flowers into the second preservative solution until the end of vase life.

For determination of number of bacteria in stems after pulsing, 2 cm long (~0.5 g) segments were cut from stem ends. Explants were washed three times with sterile distilled water to reduce surface microbial loads. They were then ground and diluted with 0.9% sterile normal saline. Aliquots (0.1 ml) of extract were spread on nutrient agar plates and bacterial colonies were enumerated after incubation for 24 h at 25°C. All bacterial counts were made on triplicate sub-samples (Balestra *et al*., 2005).

In order to make measurement of ethylene production by cut flowers after pulse treatment, a cut rose with 25 cm height was placed into a 1000 ml jar filled with 150 ml of preservative solutions including 600 mg l^{-1} hydroxy quinoline sulfate along with 3% sucrose. The mouths of the jars were sealed to preventing air movement. After 24 hr, gas from inside the jar was sampled and sent to the gas analysis laboratory. Ethylene production was measured by GC-14 A equipment manufactured by Schimadzu Corporation, Japan (Hashemabadi and Mostofi, 2007).

In the end of flower vase life, flowers' fresh weight of each treatment were calculated and then they were dried to conano-silvertant weight in an oven for 24 hr at 72°C. Dry matter percentage of cut flowers was calculated as per the following equation: Dry matter (%) = dry weight/fresh weight × 100.

Frozen petals (0.5 g) were macerated in a mortar with pestle, in 1 ml of 85% methanol (85% methanol + 15% acetic acid) and were kept in a refrigerator for 24 hr. After this time, the macerate was centrifuged at 10 000 rpm, at the temperature of 4°C for 10 min and the supernatant was used to determine its β-Carotene content (Lewinsohn *et al.,* 2005). To determine the β-Carotene content of petals, the supernatant was filtered and 50 µl of it was injected to the HPLC, model: Waters 1525 with column of C18, 250 mm in length and 4.6 nm in particles diameter.

Leaf chlorophyll content was obtained using a manual chlorophyll meter, CL-01 model. For determination of leaf chlorophyll index, leaves were selected from the third node and two measurements were performed. Measurements were made in the beginning and at the end of cut flower vase life. Loss of chlorophyll index was obtained from this formula: Chlorophyll of first day – chlorophyll of last day (Hashemabadi *et al.,* 2009). Flower opening index was calculated in four stages by digital caliper. For this purpose, the biggest flower diameter was calculated and their mean was obtained. Then, flower opening index was calculated from the following formula: (stage 4/stage 3)

Table 1 : Analysis of variance (ANOVA) for the effect of different concentrations of boric acid and nano-silver on the bacterial number in the stem end, dry matter, ethylene production, vase life, chlorophyll index, flower bloom and β-carotene of cut rose (*Rosa hybrida* L. cv. Yellow Island)

Source of variations	df	Vase life	Ethylene production	Chlorophyll index	Dry matter	Flower bloom	β-carotene	Number of bacteria on stem
Boric acid (B)	3	6.04**	0.82**	35.97**	3.98^{ns}	0.05*	5535.86**	0.04^{ns}
Nanosilver (N)	3	3.46**	0.25**	1.40^{ns}	17.40*	0.04ns	3371.79**	3.31**
B × N	9	3.45**	0.59**	9.82**	9.01^{ns}	0.03*	3304.55**	0.02^{ns}
error	32	0.58	0.006	0.59	4.88	0.01	3.63	0.56
total	47	-	-	-	-	-	-	-
cv (%)	-	10.15	0.56	12.53	7.24	6.03	11.08	20.90

**: Significant at α = 1%; *: Significant at α = 5%; ^{ns}= Not signific

+ (stage 3/stage 2) + (stage 2/stage 1) ÷ 3. Data were analyzed by SAS software and meanano-silver were compared by the Tukey's test at p≤0.05.

Results and Discussion

Based on Table 1 (analysis of variance), significant (p≤ 0.01) differences were found among various concentrationano-silver of silver nano particles and boric acid in extending vase life, ethylene production, chlorophyll content and the number of bacteria. Table 1 shows that the addition of silver nano particles and boric acid in the preservative solution had a significant (p≤ 0.05) effect on dry matter percentage and flower opening index. Flower longevity, the time until visible symptoms of senescence, was significantly increased by boric acid. Boric acid at 100 mg l^{-1} significantly extended the vase life of cut rose cv. 'Yellow Island' (Table 2). Silver nano particles and boric acid pulse treatment at highest concentration (20 mg l^{-1} and 300 mg l^{-1}, respectively) caused the shortest vase life and resulted in a 0.8 day vase life less than the control. Decreasing the vase life of cut flowers held in the highest concentration of silver nano particles and boric acid is due to the toxic effects of these materials. It is important to note that in pulse-treated flowers with the preservative solutionano-silver containing silver nano particles and boric acid, vase life was not increased as the boric acid and silver nano particles concentrations increased. These findings are consistent with the results of Lü *et al*. (2010), who reported that the high concentrations of silver nano particles decreased the vase life of cut rose 'Movie Star' and caused to leaf injury and abscission. Serrano *et al*. (2001) revealed that a 24 hr pulse treatment with the preservative solution containing 50, 75 or 100 mM boric acid or continuous treatment with 1 mM boric acid resulted in significantly increasing cut carnation flowers' longevity. These researchers also showed that no additional increase in longevity was found in carnations continuously treated with preservative solution containing 10 mM boric acid, in which an undesirable leaf chlorosis was observed, probably due to boric acid toxicity. Many studies indicate that nano-silver delays flower senescence and increases quality and vase life of cut flowers. Liu *et al*. (2009) and Basiri *et al*. (2011) showed that silver nano particles could extend the vase life of cut carnation. Positive effect of silver nano particles on extending vase life in other flowers such as rose, gerbera and lily has been demonstrated (Liu *et al*., 2009; Solgi *et al*., 2009; Kim *et al*., 2005; Ohkawa *et al*., 1999). Basiri *et al*. (2011) showed that all the concentrations of silver nano particles extended the vase life of cut carnation as compared to control, and the longest vase life was obtained with 5 mg l^{-1} silver nano particles. These workers indicated that silver nano particles had a positive effect on increasing quality and vase life of cut flowers' leaves of carnation. Silver nano particles as a pulse and vase solution treatment for cut flowers was relatively new (Liu *et al*., 2009) and has demonstrated its importance as an anti-microbial agent (Morones *et al*., 2005). Liu *et al*. (2009) showed that all silver nano particles pulse treatments markedly extended cut gerbera vase life compared to control. 5 mg l^{-1} silver nano particles pulse treatment gave longest vase life of over twice that of the control. Kim *et al*. (2005) reported that Ag^{+} ion nanoparticles mixed with other compounds extended vase life for cut lily. Flowers held in 5 or 10 mg l^{-1} silver nano particles had longer vase life than control. There were no significant differences between vase life of these treatments and the best concentration was 5 mg l^{-1} silver nano particles.

All boric acid treatments reduced ethylene production in cut rose flowers as compared to control (Table 2). Least ethylene production (0.59 nl^{-1} g^{-1} h^{-1}) was observed in cut flowers treated with 5 mg l^{-1} silver nano particles and 100 mg l^{-1} boric acid (Table 2). Highest ethylene production (2.42 nl^{-1} g^{-1} h^{-1}) was observed in control cut flowers (Table 2). Maximum ethylene production coincided with the appearance of visible senescence symptoms. Ag^{+} effectively inhibits ethylene-mediated process, such as flower senescence (Ichimura *et al*., 2008). Studies of Kim *et al*. (2005) on the effect of pre-treatment substances including silver nano particles on vase life and physiological characters in *Lilium* spp. revealed that the amount of ethylene produced continuously decreased after treatment of cut *Lilium* flowers with silver nano particles. Serrano *et al*. (2001) revealed that a 24-h pulse

Table 2 : Mean comparison of the effect of different concentrations of boric acid and nano-silver on the bacterial number in the stem end, dry matter, ethylene production, vase life, chlorophyll index, flower bloom and β-carotene of cut rose (*Rosa hybrida* L. cv. Yellow Island)

Treatments (mg l^{-1})	Vase life (day)	Ethylene production (nl $l^{-1}h^{-1}g^{-1}$)	Chlorophyll index	Dry matter (%)	Flower opening index	β- carotene (µg g^{-1} f.wt.)	Number of bacteria on the stem (Log10 CFU ml^{-1})
B_0 (0 mg l^{-1})	7.25^{ab}	1.33^{a}	5.04^{ab}	31.01^{a}	1.09^{b}	47.06^{a}	3.64^{a}
B_1 (100 mg l^{-1})	8.53^{a}	0.85^{bc}	4.29^{b}	30.96^{a}	2.04^{a}	18.41^{b}	3.62^{a}
B_2 (200 mg l^{-1})	7.02^{b}	0.74^{c}	7.60^{a}	30.28^{a}	1.95^{ab}	3.23^{c}	3.53^{a}
B_3 (300 mg l^{-1})	7.10^{ab}	0.88^{b}	7.62^{a}	29.81^{a}	1.94^{ab}	0^{d}	3.54^{a}
N_0 (0 mg l^{-1})	8.17^{a}	1.10^{a}	6.22^{a}	28.83^{b}	2.04^{a}	0.34^{d}	4.26^{a}
N_1 (5 mgl^{-1})	7.10^{b}	0.76^{c}	5.68^{a}	31.48^{a}	1.91a	38.26^{a}	3.67^{ab}
N_2 (10 mg l^{-1})	7.62^{ab}	0.94^{b}	6.16^{a}	31.27^{ab}	1.96^{a}	22.33^{b}	3.38^{b}
N_3 (20 mg l^{-1})	7.01^{b}	1.00^{ab}	6.50^{a}	30.48^{ab}	1.93^{a}	7.78^{c}	3.02^{b}
B_0N_0	5.63^{d}	2.42^{a}	4.11^{c-f}	30.17^{ab}	1.77^{b}	0^{e}	4.42^{a}
B_0N_1	7.78^{a-d}	0.96^{c-e}	2.72^{f}	34.21^{a}	1.92^{ab}	137.02^{a}	3.62^{a}
B_0N_2	8.22^{a-d}	1.03^{b-d}	5.58^{b-e}	28.85^{ab}	2.00^{ab}	36.95^{c}	3.43^{a}
B_0N_3	7.37^{a-d}	0.92^{c-e}	7.72^{ab}	30.80^{ab}	1.90^{ab}	14.27^{d}	3.10
B_1N_0	9.69^{a}	0.74^{ef}	3.58^{ef}	29.64^{ab}	2.23^{a}	1.03^{e}	4.18^{a}
B_1N_1	7.66^{a-d}	0.59^{f}	3.98^{d-f}	32.04^{ab}	1.94^{ab}	3.40^{e}	3.81^{a}
B_1N_2	8.64^{a-c}	0.82^{d-f}	6.47^{a-d}	31.75^{ab}	2.01^{ab}	52.36^{b}	3.43^{a}
B_1N_3	8.12^{a-d}	1.26^{b}	3.12^{ef}	30.40^{ab}	1.99^{ab}	16.85^{d}	3.04^{a}
B_2N_0	8.97^{ab}	0.62^{f}	8.68^{a}	28.86^{ab}	2.04^{ab}	0.32^{e}	4.24^{a}
B_2N_1	6.43^{b-d}	0.73^{ef}	9.16^{a}	30.32^{ab}	1.92^{ab}	12.62^{d}	3.57^{a}
B_2N_2	6.54^{b-d}	0.77^{d-f}	5.36^{b-f}	32.52^{ab}	1.84^{b}	0^{e}	3.31^{a}
B_2N_3	6.11^{cd}	0.84^{d-f}	7.20^{ab}	29.41^{ab}	1.98^{ab}	0^{e}	3.01^{a}
B_3N_0	8.39^{a-c}	0.63^{f}	8.50^{a}	26.63^{b}	2.10^{ab}	0^{e}	4.19^{a}
B_3N_1	6.52^{b-d}	0.75^{d-f}	6.83^{a-c}	29.34^{ab}	1.85^{b}	0^{e}	3.68^{a}
B_3N_2	7.05^{a-d}	1.14^{bc}	7.21^{ab}	31.96^{ab}	1.94^{ab}	0^{e}	3.37^{a}
B_3N_3	6.43^{b-d}	0.99^{b-e}	7.95^{ab}	31.30^{ab}	1.86^{b}	0^{e}	2.91^{a}

*In each column, means with the similar letters are not significantly different at 5% level of probability using Tukey's test

treatment with the preservative solution containing 50, 75 or 100 mM boric acid or continuous treatment with 1 mM boric acid resulted in strong inhibition of the climacteric ethylene production.

Our findings revealed that boric acid has a more important role on the inhibition of chlorophyll degradation than silver nano particles. Least chlorophyll index (2.72) was observed in cut flowers treated with 5 mg l^{-1} silver nano particles without boric acid (Table 2). Maximum chlorophyll index (9.16) was observed in cut flowers treated with 5 mg l^{-1} silver nano particles along with 200 mg l^{-1} boric acid (Table 2). Boric acid delays chlorophyll degradation due to inhibition of ethylene synthesis and reduction of ABA content. Studies of Serrano *et al*. (2001) on the effect of boric acid on the vase life of cut carnationano-silver demonstrated that ABA levels in petals increased during senescence, but much less in boric acid-treated carnationano-silver. Thus, boric acid prevents the early rise in ethylene production. In addition, ABA level reached in boric acid-treated cut flowers were lower than that in control flowers, which may point to an inhibition of ABA biosynthesis by boric acid either directly or by some intermediate effect (Serrano *et al*., 2001).

Minimum dry matter percentage (26.63%) was observed in cut flowers treated with 300 mg l^{-1} boric acid without silver nano particles (Table 2). Maximum dry matter percentage (34.21%) was observed in cut flowers treated with 5 mg l^{-1} silver nano particles without boric acid (Table 2). Increasing boric acid concentration resulted in decreasing the dry matter percentage. Least flower opening index (1.09) was observed in control cut flowers. Most flower opening index (2.23) was observed in cut flowers treated with 100 mg l^{-1} boric acid without silver nano particles (Table 2). Although, effect of silver nano particles was not significant on flower opening, statistically, but was more effective than control. Highest content of β-carotene (38.20 µg g^{-1} f.wt.) was obtained from cut flowers treated with 5 mg l^{-1} silver nano particles without boric acid. Lowest content of β-carotene was obtained from control cut flowers. Increasing boric acid and silver nano particles concentrationano-silver resulted in decrease β-carotene content.

The number of bacteria in stem end decreased significantly with increasing silver nano particles and boric acid concentration. There were significant differences in the number of bacteria in stem end between 20 mg l^{-1} silver nano particles along with 300 mg l^{-1} boric acid pulse treatment and control for the duration of assessment. Least number of bacteria (2.91 colonies) was obtained in the stems treated with 20 mg l^{-1} silver

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nano particles with 300 mg l^{-1} boric acid (highest concentration of silver nano particles and boric acid (Table 2). High concentration of silver nano particles produce a toxic effect on plants. The maximum number of bacterial (3.64 colonies) was obtained in control stems. Low water uptake by cut flowers is often due to occlusions located mainly 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). It strongly inhibits bacteria and other microbe activities (Jiang *et al*., 2004). Basiri *et al*. (2011) indicated that the lowest bacteria concentration in the stem end of cut carnations was obtained with 5 mg l^{-1} silver nano particles, and the highest concentration rate was observed in control plants. So, silver nano particles has antibacterial effects and can extend the quality and vase life of cut carnation. Lü *et al*. (2010) showed that the number of bacteria in the stem ends of control cut roses was significantly higher than that of flowers treated with all concentrations of silver nano particles. These researchers showed that there were significant differences in the numbers of bacteria in stem ends between the 50 and 100 mg l^{-1} silver nano particles pulse treatment and the control for the duration of assessment. Nano-silver suppressed water loss of cut rose and maintained more favourable water balance than control flowers (Lü *et al*., 2010). In many cut flowers, suppression of microbial growth in vase solution results in delayed wilting (van Doorn, 1997). Antibacterial activity of silver nano particles is partly a function of particle size, with higher surface to volume ratio increasing the proportion of atoms at the grain boundary. Liu *et al*. (2009) demonano-silvertrated that silver nano particles inhibited bacterial growth for the first 2 days of vase life in stem ends of cut gerbera. These researchers also revealed that silver nano particles treatment inhibited the growth of microbes from cut carnationano-silver vase solution. Solgi *et al*. (2009) showed that silver nano particles extended the vase life of cut gerbera flowers. Lü *et al*. (2010) reported that silver nano particles suppressed the decrease in hydraulic conductance of cut rose, apparently, in association with decreased number of stem end bacteria. Our results confirm the efficacy of silver nano particles and boric acid in extending vase life of cut roses.

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