

# Improving vase life of carnation cut flowers by silver nanoparticles acting as anti-ethylene agent

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## Abstract

Most of the carnation cultivars are sensitive to exogenous ethylene and their petals exhibit autocatalytic ethylene production during senescence. Compounds containing silver have been shown to act as anti-ethylene agent in improving postharvest characteristics of cut flowers. The experiment was conducted in a completely randomized design with three replications by applying four concentration of silver nano-particles in vase solution of cut carnation cultivar 'Miledy'. ICP-AES analyses revealed that the higher amount of silver was absorbed in various tissues of plants treated with 5 mg L<sup>-1</sup>, compared to other concentrations. Cut stems of 'Miledy' cultivar receiving 5 mg L<sup>-1</sup> of silver nano-particles showed the highest vase life and the lowest rate of ethylene production. The tissue accumulation of Ag element was generally higher in basal stem ends and leaves treated with 5 mg L<sup>-1</sup> than other concentrations. The results of ICP and ethylene measurement showed that silver nano-particles reduced ethylene production as well as bacterial growth in vase solution and resulted improved vase life of carnation.

**Key words:** Chlorophyll, inductively coupled plasma (ICP), vase solution, ethylene

## Introduction

Carnation (*Dianthus caryophyllus*) is one of the most common cut flowers grown around the world (Brickell, 2003). The main limiting factors in postharvest management and handling of this cut flower are premature aging and decreasing the vase life of flowers which mainly resulted from ethylene production and vascular blockage (Van Doorn *et al.*, 1994). Cut carnation flowers are also sensitive to exogenous ethylene (Obsuwan & Uthairatanakij, 2007). Ethylene causes the petals shedding and/or browning which severely reduces the quality in relation to marketability of flowers. In climacteric species, ethylene directly influences the petals senescence and aging is concomitant with the increase in ethylene production and respiration (Serek *et al.*, 2006).

Increasing the ethylene production in the aged petals is concomitant with the continuous increment in both 1- aminocyclopropane 1-carboxylate synthase (ACS) and oxidase (ACO) (Borochoy and Woodson, 1989). Using anti-bacterial agents in the vase preserving solutions cause extending vase life and maintaining display quality of cut flowers by reduction of the bacterial populations in vase solution (Liu *et al.*, 2009). On the other hand, ethylene produced endogenously or applied exogenously has inferior effects on cut flowers as this gas accelerates the aging process in the flower. Compounds containing silver showed double benefit, on the one side acting as anti-microbial agents to control the growth rate of bacterial population, and at the other side acting as inhibitor of ethylene action, reducing ethylene production. In carnation, cut flowers pretreated with STS, the climacteric enhancement in ethylene production was prevented and senescence of flowers was delayed (Reid *et al.*, 1980). Application of STS on Exacum potted plants and miniature roses prevented the undesired effects of ethylene resulted in increasing the display life (Serek, 1993; Serek

and Trolle, 2000). Nano-silver (NS) applications is increasingly becoming widespread in the medical industry, embedded fabrics, water purification, disinfection and various other industrial units (Davies and Etris, 1997; Klaus *et al.*, 1999; Jiang *et al.*, 2004). NS as a pulse and vase solution treatment for cut flowers is relatively new and has demonstrated importance as an antibacterial agent (Alt *et al.*, 2004; Morones *et al.*, 2005). NS releases Ag<sup>+</sup>, which has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability (Park *et al.*, 2005). As with other cations (*e.g.* K<sup>+</sup>, Ca<sup>2+</sup>), positive effects on plant stem hydraulic conductivity of Ag<sup>+</sup> (van Ieperen, 2007) are possible.

Most studies conducted on silver nano-particle in vase solution were focused on its anti-bacterial characteristics resulted in preventing vascular blockage (Liu *et al.*, 2009; Solgi *et al.*, 2009). This is the very first report evaluating the function of silver nano-particles as an anti-ethylene agent.

## Materials and methods

**Plant material:** Cut carnation (*Dianthus caryophyllus* cv. Miledy) flowers were obtained from a commercial greenhouse in Pakdasht, Tehran, Iran. Stems were harvested at the commercial harvest stage in the morning and packaged by plastic film. The bunch of cut flowers were transported immediately to the Laboratory of Postharvest Physiology, at the Department of Horticultural Science, Tarbiat Modares University. After selecting flowers based on their uniformity of size, flower stem ends were placed in buckets partially filled with deionised water (DI). The stem ends were recut approximately 45 cm under DI to avoid air emboli and then all stems were placed in distilled water for one hour.

**Experimental design:** Experiment was conducted at the

Postharvest Laboratory of Department of Horticultural Science, Tarbiat Modares University. The room was operated at  $20 \pm 2$  °C,  $60 \pm 10\%$  relative humidity (R.H.), and  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity, under a daily light period of 12 h. Preparing fresh solutions at the beginning of experiments, three concentrations (5, 10 and  $15 \text{ mg L}^{-1}$ ) of NS (Nanocid Company, Iran) for continuous treatments were used in experiment. Cut carnation stems were placed into glass 1700 mL vases containing 500 mL of either DI (Control) or solutions of NS. The vases were arranged on benches in a completely randomized design (CRD) with three replications.

**Flower vase life and flower diameter:** The vase life of each flower was determined in number of days from harvest until at least two petals showed wilting with in-rolling and/or browning of the petal margins. Flower diameter was measured by digital caliper on different days during experimental period.

**Relative fresh weight (RFW), water uptake, water loss and water balance:** RFW of flowers was calculated as:  $\text{RFW (\%)} = (W_t/W_{t_0}) \times 100$ ; where,  $W_t$  is weight of flower (g) at  $t = \text{days } 0, 2, 4, \text{ etc.}$ , and  $W_{t_0}$  is weight of the same flower (g) at  $t = \text{day } 0$ . Daily water uptake was calculated as:  $\text{water uptake (g stem}^{-1} \text{d}^{-1}) = (S_{t-1} - S_t)$ ; where,  $S_t$  is weight of vase solution (g) at  $t = \text{days } 1, 3, 5, \text{ etc.}$ , and  $S_{t-1}$  is weight of vase solution (g) on the previous day. Daily water loss was calculated as:  $\text{water loss (g stem}^{-1} \text{d}^{-1}) = (C_{t-1} - C_t)$ ; where,  $C_t$  is the combined weights of the flower and vase (g) at  $t = \text{days } 1, 3, 5, \text{ etc.}$ , and  $C_{t-1}$  is the combined weights of the flower and vase (g) on the previous day. Water balance was calculated as water uptake from the vase minus water loss from the stem (He *et al.*, 2006).

The chlorophyll content was measured by SPAD 502 on days 1, 3, 5, etc. To evaluate ethylene production during 24, 48 and 72 hours after treatment, five flowers from each treatment were placed in hermetically sealed glass containers. Ethylene production was measured by gas chromatography via GC Agilent 6890N model at Petrochemical Research & Technology Center. Bacterial populations in vase solution were determined according to Balestra *et al.* (2005) with three replications.

**Ag content in tissues:** To determine the contents of absorbed silver by the cut flower tissues and/or organs during 48 hours of the experiment, 600 mg dried samples from stem end, top of the stem and leaf were obtained with three replicate extractions for each one. After preparing the ash of samples by heating samples in furnace (labtech, Ind & Vac) at 600 °C for 4 h, the ash was dissolved in 3 mL  $\text{HNO}_3$  and diluted to 25 mL with ultrapure water. The solution was filtered through a membrane and subjected to analysis for Ag by ICP-AES (Optima 7300D, Perkin Elmer). The analytical wavelength for Ag detection was 328.068 nm. Reference solutions containing 0, 0.1, 1 and  $10 \text{ mg L}^{-1}$  Ag were used from stock standard solution (Atomic Energy Organization, Iran) for constructing the calibration curve.

**Statistical analysis:** Each treatment was replicated thrice. The vases were arranged on benches in a completely randomized design (CRD). Data were analyzed using one-way ANOVA in SPSS 16 and means were compared by (LSD,  $P=0.05$ )

## Results

The  $5 \text{ mg L}^{-1}$  NS treatment and control had the highest and the lowest vase life, respectively, while this treatment ( $5 \text{ mg L}^{-1}$  NS)

showed significant difference with other NS treatments too. The 10 and  $15 \text{ mg L}^{-1}$  NS treatment decreased vase life in cut flowers to 2.3 and 2.1 days, respectively compared to  $5 \text{ mg L}^{-1}$  (Fig. 1A). In all treatments, no significant difference was observed for flower diameter, although maximum flower diameter was measured on the sixth day. The flower opening was progressing well till the sixth day and afterward the opening rate declined (Fig. 1B).

The changes in relative fresh weight (RFW) of cut carnations showed similar trends for both control and NS treatments. There was no significant difference among all treatments of NS, but the relative fresh weight at different days varied (Fig. 2A). Overall, the treatment containing  $5 \text{ mg L}^{-1}$  of NS showed better vase life than other treatments (Fig. 1A). During the period of experiment, the difference in the water uptake was not observed (Fig. 2B).

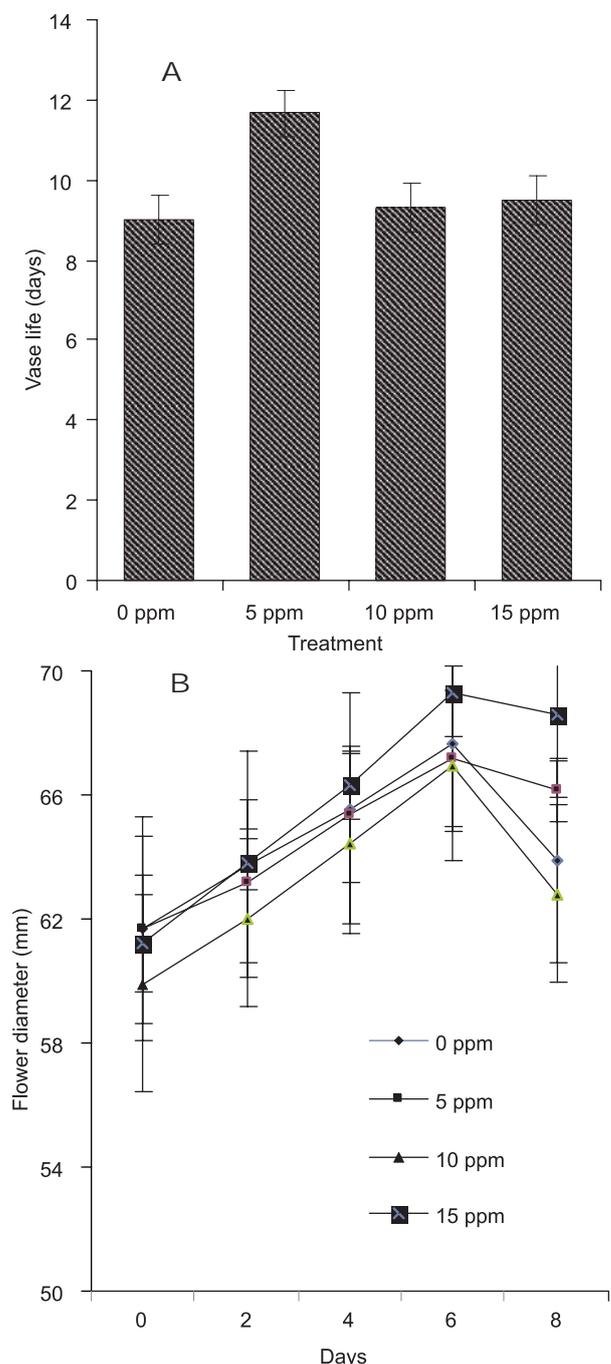


Fig. 1. Vase life (A; values are means at  $P \leq 0.05$ ,  $n = 3$ ), flower diameter (B;  $n = 3$ ). The vertical bars indicate the LSD 0.05 for treatment comparisons.

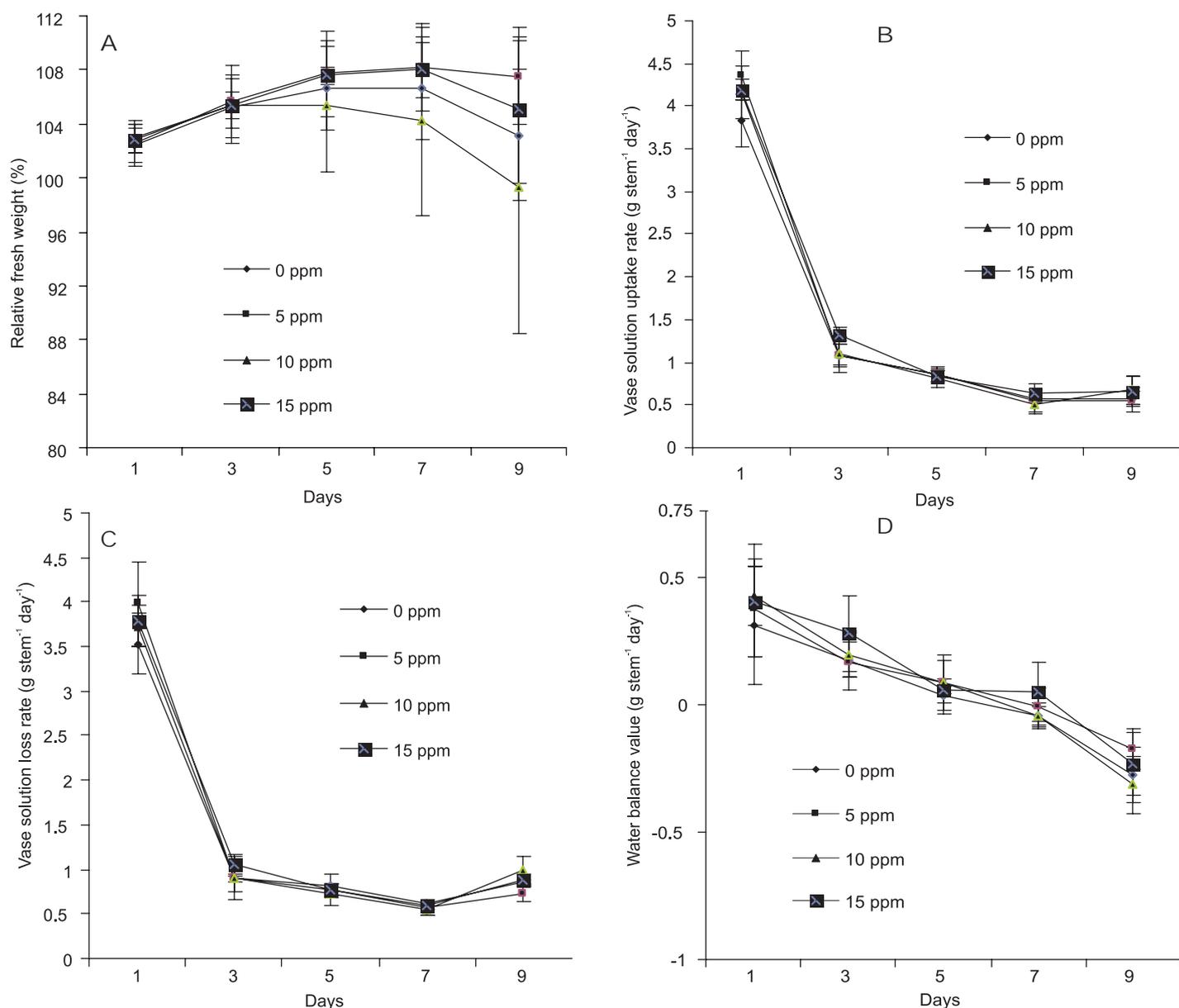


Fig. 2. Relative fresh weight (A; n = 10), relative water uptake (B; n = 3), relative water loss (C; n = 3), water balance value (D; n=3). The vertical bars indicate the LSD 0.05 for treatment comparisons.

There was no significant difference between the NS treatments and control flowers in water loss (Fig. 2C). Water balance declined almost linearly during the experiment, but no significant difference was observed among treatments (Fig. 2D).

No significant difference was recorded for chlorophyll content, among various treatments of NS, but on some days there was a significant difference among treatments in the amount of chlorophyll (Fig. 3A). Data showed that the ethylene production was affected by NS concentrations. The cut flowers of control and the 5 mg L<sup>-1</sup> NS treatment showed the maximum and minimum ethylene production respectively. The ethylene production increased with enhancement in NS concentration that this increment of ethylene can possibly appeared in the response of cut flowers to stress conditions induced by NS toxicity at high concentrations (Fig. 3B).

The population of bacteria in vase solution revealed that the presence of NS inhibited bacterial growth in vase solution. Bacterial colonies were observed only in control (Fig. 3C) not in vase containing NS. The prevention of bacterial growth in

this case was due to the antimicrobial activity of NS in vase solution.

**Ag content in tissues:** Concentration of absorbed Ag in tissues varied under different treatments (Fig. 4). The amount of silver absorbed in the the stem end varied for all treatments too. Control showed the minimum Ag uptake and it was significantly different with other treatments. Higher Ag was absorbed by plant organs treated with 5 mg L<sup>-1</sup> NS. Although in the base of stem, the highest and lowest silver accumulation were found in 5 and 10 mg L<sup>-1</sup> NS, but in the leaf tissue, 5 and 15 mg L<sup>-1</sup> treatment of NS showed the highest and lowest Ag content (Fig. 4).

## Discussion

In the present study, different concentrations of nano-silver had a different effect on various characteristics of carnation cut flowers 'Miledy'. Significant differences were found among various concentrations of NS in improving the vase life of flowers. The longest vase life was attained with 5 mg L<sup>-1</sup> NS (Fig. 1A). The

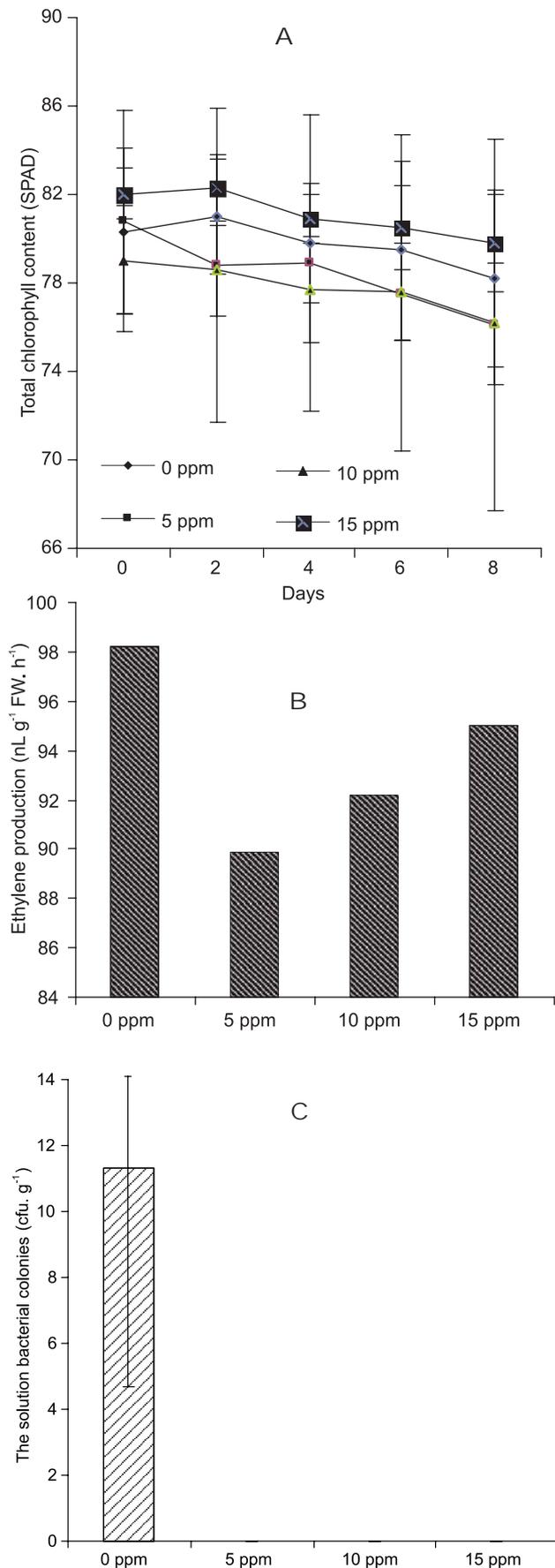


Fig. 3. The total chlorophyll content was measured by SPAD chlorophyll meter (A), Ethylene production ( $\text{nL} \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ ) (B), bacterial colonies in solution ( $\text{cfu} \cdot \text{g}^{-1}$ ) (C). The vertical bars indicate LSD 0.05 for treatment comparisons.

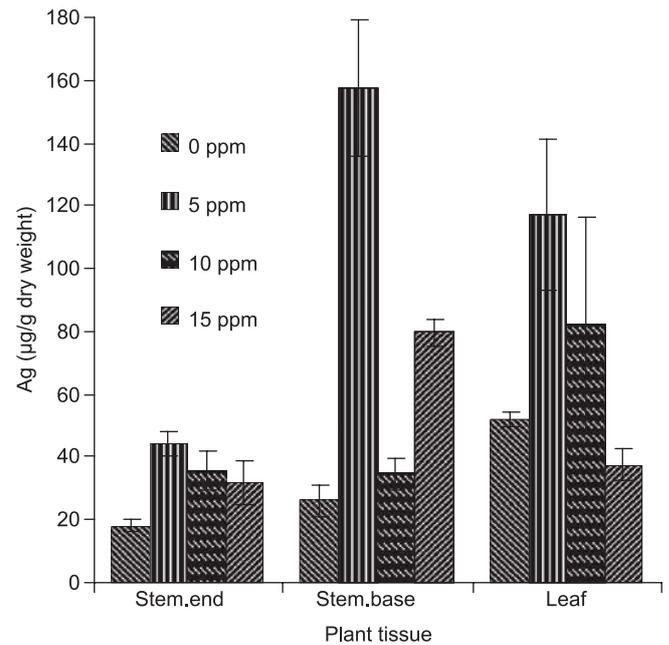


Fig. 4. Concentrations of Ag in cut carnation basal stem end, upper stem end and leaves as determined by ICP-AES ( $n = 3$ ). The vertical bars indicate the LSD (0.05) for treatment comparisons.

continuous treatment with high concentrations of silver ( $15 \text{ mg L}^{-1}$ ) reduced the vase life due to toxicity in plant as also revealed by the studies of Lu *et al.* (2010) on roses cut flower. In some cases,  $15 \text{ mg L}^{-1}$  concentration caused the petal blight. Shortening the vase life of control cut flowers was mainly due to the growth of microorganisms in the vase solution and the increased ethylene production. One of the main factors affecting the fresh weight loss is blockage in the xylem vessels of stem due to microorganism's proliferation such as fungi and bacteria. Due to high surface area to volume ratio of silver nano-particles and strong antibacterial activity, it could suppress the growth of bacterial population in vase solution and in the xylem vessels as well. The results didn't show any significant difference among treatments in relative fresh weight which confirmed the results of Basiri *et al.* (2011) on carnation cut flowers. The results showed the positive effect of silver nano-particles on solution uptake. The rate of solution uptake was the maximum on first day but it decreased with time. One of the most important factor for determining the quality and longevity of cut flowers is water balance. The water balance is affected by two phenomena, water uptake and transpiration (Da Silva, 2003). No difference in the water balance was found among treatments. The experimental results showed that the water balance is more affected by transpiration (water loss), while the uptake of vase solution plays less important role in this characteristic. One of the reasons for the short vase life of control carnation flowers could be related to poor water supply which is in consent with the results of Lu *et al.* (2010) on rose cut flowers. Water deficit and wilting in a cut stem in vase solution will develop when the rate of transpiration exceeds the rate of water uptake (van Doorn, 1997). The total chlorophyll content decreased continuously in NS treatments as well as control treatment during the different times of vase life. The chlorophyll content was high on the early days and declined over time. This was in agreement with results of carnation and *Gerbera* cut flowers subjected to NS (Basiri *et al.*, 2011). Besides

antibacterial effects, NS could act as an anti-ethylene agent.  $Ag^+$  is an effective ethylene action inhibitor (Liu *et al.*, 2012). The silver nano-particles caused reduced ethylene production in *Dianthus caryophyllus* cv. Miledy flowers. Although the 5 mg  $L^{-1}$  NS was the best treatment in terms of reduction of ethylene production, other NS concentrations reduced the production of ethylene too. The beneficial effect of NS for extending the vase life and preventing ethylene production in carnation cut flowers under STS treatment (Wawrzynczak and Goszczyńska, 2003) is in agreement with our results on ethylene production. These data indicated that silver nano-particles could possibly act as an inhibitor of ethylene action and consequently reduce ethylene production. The results showed that nano-silver reduced the ethylene production in carnation flowers and 5 mg  $L^{-1}$  treatment of NS prevented effectively the ethylene production due to the appropriate concentration and lack of toxicity and deleterious effects. The silver nano-particles accumulated in carnation tissues were decreased by enhancement of NS concentration. The concentration of NS in cut carnation basal stem end and leaves was markedly higher than upper stem end (Fig. 4.), same as the results reported for *Acacia holosericea* and cut roses treated by NS (Liu *et al.*, 2012; Lu *et al.*, 2010). In our study, anti-microbial effect of NS confirms the results of previous studies on *Gerbera*, acacia, cut roses and carnation (Liu *et al.*, 2009; Solgi *et al.*, 2009; Liu *et al.*, 2012; Lu *et al.*, 2010; Basiri *et al.*, 2011).

In conclusion, silver nano-particles extended the vase life of cut carnation cv. Miledy mainly by suppression of ethylene production and inhibiting bacterial growth in vase solution. The optimum concentration of nano-particles was 5 mg  $L^{-1}$  NS for cut carnation cv. Miledy.

## Acknowledgement

This research was partially supported by a grant from the Iran National Foundation of Sciences (INFS).

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