

EFFECT OF NANOSILVER ON PHYSIOLOGICAL PERFORMANCE OF *PELARGONIUM* PLANTS EXPOSED TO DARK STORAGE

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ABSTRACT

This investigation was conducted to evaluate the effects of post-harvest application of silver nanoparticles (N-Ag) and dark storage on plastid pigments, petal abscission, lipid peroxidation and activities of ascorbate peroxidase (APX) and guaiacol peroxidase (POD) in pelargonium cultivars 'Blue Wonder' and 'Anthony'. N-Ag was applied as foliar spray in concentrations 0, 20, 40, 60 and 80 mg·cm⁻³ and then the plants were stored for 5 days in a growth chamber in darkness at temperature 20±2 °C and relative humidity 65%. The results revealed that after dark storage the petal abscission of cv. 'Blue Wonder' increased up to 40% but significantly decreased in plants treated with 60 mg·cm⁻³ of N-Ag. Cultivar 'Anthony' showed lesser petal abscission than 'Blue Wonder', namely 25% in control and 4% after treatment with 60 mg·cm⁻³ N-Ag. In both cultivars, treatment with N-Ag resulted in higher contents of leaf chlorophylls and carotenoids in comparison to untreated control. In response to treatments with 20-60 mg·cm⁻³ N-Ag, the activity of APX and POD was higher at the end of 5 days storage period than in the control plants, which coincided with improved post-harvest performance of both pelargonium cultivars. The enhancement of enzyme activities indicated possible beneficial effect of the applied treatment on alleviation of dark storage-induced oxidative stress. Also, in both cultivars, MDA content decreased significantly with the increase of N-Ag concentration up to 60 mg·cm⁻³ and then a rapid increase at 80 mg·cm⁻³ followed. It is concluded that treatment with silver nanoparticles is effective for preventing dark storage-induced petal abscission of pelargonium.

Key words: *Pelargonium zonale*, nanosilver, leaf senescence, lipid peroxidation

INTRODUCTION

Pelargonium zonale (*Pelargonium x hortum*) is grown as potted plant for its colourful, showy flowers and scented foliage. Leaf senescence and petal abscission is a common problem in pelargonium which leads to high post-harvest losses during transport and storage. Dark storage-induced leaf yellowing decreases visual quality and may have a physiological effect by reducing photosynthesis that is crucial for normal flower development and shelf life (Reid *et al.* 2002). Phytohormones and environmental stresses are important factors in controlling the abscission process (Taylor and Whitelaw 2001). It was demonstrated that

ethylene has an important role in initiating formation of abscission layer in different plants (Macnish *et al.* 2005). Changes in plastid pigments such as chlorophyll are widely used to characterise the senescence syndrome (Matile *et al.* 1996). One of the most important changes observed during plant senescence is also the decrease of antioxidant enzyme activity and the increase of lipid peroxidation and increase of the level malondialdehyde (MDA), the end product of lipid peroxidation.

Plant cells are normally protected against oxidative damage by a broad spectrum of radical scavenger systems, including antioxidative enzymes such as ascorbate peroxidase, glutathione reductase and superoxide dismutase, as well as non-enzyme

matic compounds like glutathione and carotenoids (Cameron and Reid 2001). Therefore, any treatment that can help to diminish the ROS level would be advantageous in improving plant performance and longevity. For improving quality and longevity of ethylene-sensitive flowers like pelargonium, various treatments with ethylene antagonists have been proposed over the years (Serek and Trolle 2000). Foliar sprays with silver thiosulphate (STS) prevented petal abscission of seed-propagated geraniums (*Pelargonium × hortorum* Bailey) and Regal Pelargoniums (Deneke *et al.* 1999). The use of silver salts, a heavy metal, on potted crops has been criticized as an environmental hazard (Nell 1992). The shortcomings of the commonly used STS have created a need for novel approaches to control the negative effects of ethylene on floral longevity. In the field of floriculture, the use of nanomaterials is relatively new and needs more researches. N-Ag is a colloidal suspension of silver particles in the size range of 10 to 100 nm and it is more stable in comparison to silver salts solutions. N-Ag has high total surface area due to its small size. Thus, the adhesion of silver nanoparticles to the cell surface is strong, which leads to a high efficacy (Shah and Belozero-va 2008). The effect of N-Ag on extending maintenance period of asparagus leaves (from 2 to 21 days) has been reported. It also increased the content of ascorbate, chlorophyll and fibre in the treated leaves (An *et al.* 2008). N-Ag is one of the potential candidates for modulating the redox status of plants because of its ability to support electron exchange with Fe^{2+} and Co^{3+} (Mukherjee and Mahapatra, 2009). Several studies have demonstrated that spraying with silver ions decrease the flowers and flower buds abscission in orchid plant (Uthaichay *et al.* 2007). Additionally, Wagstaff *et al.* (2005) reported that silver ions decreased by 100% flower abscission in alstroemeria as compared to untreated control plants. A literature survey revealed that no work has been done on the effect of N-Ag foliar sprays during dark storage on post-harvest longevity of pelargonium plant. Therefore, this research was carried out to introduce the alternative substance for ethylene antagonists in for improving the post-production quality

of potted pelargonium. Physiological response of the potted pelargonium plants stored in the dark to N-Ag pre-treatment was evaluated by measuring the activities of APX and POX, content of MDA and content of plastid pigments.

MATERIALS AND METHODS

Plant materials

Two cultivars of *Pelargonium zonale*, ‘Anthony’ and ‘Blue Wonder’, were raised in the greenhouse at the University of Guilan, Rasht, Iran, under normal environmental conditions: 25 °C day/17 °C night temperatures, natural light (16 h light/8 h dark), 75% relative humidity (RH) to produce stock plants. Then, two month-old uniform plants (at blooming stage) were stored in dark chamber at 20±2 °C and 65% RH for 5 days to simulate sub-optimal transport conditions.

N-Ag treatment and dark storage

N-Ag (Nanosid co. Iran), was prepared in deionized water to final concentrations of 20, 40, 60 and 80 mg·cm⁻³. Whole plants were sprayed with equal amounts of 50 cm³ aqueous suspension of N-Ag at blooming stage. The plants were then transferred to the growth chamber for simulated transport for 5 days, in darkness at temperature 20±2 °C and 65% relative humidity. Control plants were treated with deionized water.

Pigment measurements

Leaf material (0.5 g) was homogenized and pigments extracted in 0.5 cm³ 80% acetone. The samples were shaken vigorously for 15 second at room temperature. After centrifugation at 14 000 rpm for 20 min at room temperature, total chlorophyll content in the supernatant was measured and calculated as described by Lichtenthaler *et al.* (1987). The absorbance of the extract was read at 645 nm (Chlorophyll *a*), 663 nm (Chlorophyll *b*) and 470 nm (Carotenoids) in an UV/VIS spectrophotometer (PG Instruments Ltd.). Measurements were performed on twenty leaves per treatment (n=20). Pigments content were calculated using the equations as below:

$$\text{Chl } a \text{ (mg}\cdot\text{g}^{-1} \text{ FW)} = 11.75 \times A_{663} - 2.35 \times A_{645}$$

$$\text{Chl } b \text{ (mg}\cdot\text{g}^{-1} \text{ FW)} = 18.61 \times A_{645} - 3.96 \times A_{663}$$

$$\text{Carotenoids (mg}\cdot\text{g}^{-1} \text{ FW)} = 4.69 \times A_{470} - 0.268 \times (20.2 \times A_{645} + 8.02 \times A_{663})$$

Lipid Peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in leaves according to Heath and Packer (1968). 0.5 g of fresh leaf tissues were homogenized with 10% solution of trichloroacetic acid (TCA) and centrifuged at 10 000 rpm for 10 min at 4 °C. Two cm³ of the supernatant was blended with 2 cm³ of 0.6% thiobarbituric acid (TBA) and the mixture was heated at 95 °C for 30 min, then cooled on ice and centrifuged at 4 000 rpm for 20 min. MDA content in the resultant supernatant was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity) by using an extinction coefficient of 155 mM·cm⁻¹.

Extraction and determination of enzyme activities

Sampling for the analysis of APX and POD activities was carried out at the end of fifth day of the dark storage. Leaf tissues (0.5 g FW) were placed into liquid nitrogen and then homogenized with a pre-chilled mortar and pestle under ice-cold conditions in 4 cm³ of 50 mM potassium phosphate buffer, pH 7.0, with addition of 1 mM EDTA. The homogenate was centrifuged at 15 000 rpm at 4 °C for 20 min. The supernatant was stored at -20 °C and used for determination of enzyme activities.

Ascorbate peroxidase (APX, EC; 1.11.1.11)

The activity of APX was determined by the method of Gerbling *et al.* (1984). The reaction mixture consisted of 2 cm³ of 50 mM sodium phosphate buffer (pH 7.0) with 0.2 mM EDTA, 0.5 mM ascorbic acid, 250 mM H₂O₂ and 50 mm³ of enzyme extract.

Guaiacol peroxidase (POD, EC; 1.11.1.7)

Peroxidase activity with guaiacol as substrate was assayed by a modified procedure of Lee and Kim (1994). The assay mixture contained 40 mM phosphate buffer, 15 mM guaiacol, 5 mM H₂O₂ and 50 mm³ of enzyme preparation in a total volume of 1 cm³. The reaction was initiated by the addition of H₂O₂ and the change in absorbance at 470 nm was measured for 1 min.

Petal Abscission

The abscission rate was calculated as the number of petals that have been shed at the end of 5 days of darkness divided by the total number of petals at

the start of the dark storage period, and expressed as percentage (Ascough *et al.* 2008).

Statistical analysis

Data analyses were performed based on a completely randomized design (CRD) with factorial experiments, which is replicated three times. Data were subjected to analysis of variance (ANOVA) using the general linear model (Proc GLM) of the statistical analysis system (SAS 9.1). Multiple comparisons among treatment means were done using Tukey's test at $\alpha=0.05$

RESULTS AND DISCUSSION

Morphological observations

Flowers that were not treated with N-Ag showed senescence after about 24 h whereas flowers treated with silver nanoparticles detained senescence and petal abscission until the third and the fourth day (Fig. 1). This indicated a rapid progression of senescence-related events triggered by dark storage. Petal abscission during simulated transport varied among the two studied genotypes and was lower in 'Anthony' than in 'Blue Wonder'. Likewise, distinct variations were observed between the two cultivars in longevity of flowers during daily observations after darkness. It is proven that one of the reasons for plant organ abscission is imbalance between phytohormones. Ethylene is playing an important role in this process. It has been shown that silver ions inhibit ethylene action by preventing its binding to the receptors in plant cells (Mishra *et al.* 2008). Application of N-Ag can displace copper from the receptor protein and consequently, block ethylene perception since copper has a critical role in ethylene binding (Khan 2006). In effect, N-Ag treatment could decrease petal abscission rate in both cultivars with increasing concentration up to 60 mg·cm⁻³ and then increase it because of toxicity effect of higher concentration of nanosilver.

Plastid pigments

The retention of chlorophylls in the presence of N-Ag reflects the delay of senescence. Control leaves started to lose chlorophyll (*a*, *b*) and carotenoids at the 4th day whereas these treated with N-Ag leaves at the 5th day. Laves of 'Anthony' and 'Blue Wonder

cultivars treated with $60 \text{ mg}\cdot\text{cm}^{-3}$ N-Ag retained 87% and 84% of chlorophyll (a) as compared to control, which retained only 52% and 22%, respectively (Table 1). Dark storage accelerated senescence in the leaves of pelargonium and lead to chlorophyll degradation. Purvis (1980) reported that activity of chlorophyllase induced by ethylene lead to destruction of internal membrane of chloroplast. Results of this experiment showed that application of $60 \text{ mg}\cdot\text{cm}^{-3}$ N-Ag caused a decrease of ethylene action and prevented the destruction of chlorophyll. In contrast, $80 \text{ mg}\cdot\text{cm}^{-3}$ of N-Ag had negative effect on plastid pigments content.

Antioxidants enzymes

After 5 days of treatment, leaves from control plants of both cultivars showed significantly reduced POD activity in comparison with the leaves from treated plants, especially in response to $60 \text{ mg}\cdot\text{cm}^{-3}$ N-Ag. However, there is no significant difference in POD activity between control and plants treated with $80 \text{ mg}\cdot\text{cm}^{-3}$ of N-Ag (Fig. 2). Leaves treated with N-Ag showed significantly higher APX activity after 5 d of treatment in relation to the control (Fig. 3).

Lipid peroxidation

Lipid peroxidation level in leaves of the both pelargonium cultivars was determined indirectly by analysing the content of its and product - malondialdehyde (MDA). MDA content in cultivar 'Anthony' leaves was significantly lower than that in the leaves of 'Blue Wonder' in all the treatments (Fig. 4). In both cultivars, the MDA content decreased significantly with the increase of N-Ag concentration up to $60 \text{ mg}\cdot\text{cm}^{-3}$ and then rapidly increased at $80 \text{ mg}\cdot\text{cm}^{-3}$.

MDA, a product of lipid peroxidation, has been proposed as a suitable marker of lipid peroxidation (Bailly *et al.* 1996). The level of this compound in plant cells increase under dark-induced senescence and stress situations. It is reported that N-Ag treated plants showed an efficient cellular electron exchange mechanism, which arrest electron leakage, reducing the ROS production and MDA levels (Lu *et al.* 2002).

Dark storage accelerates leaf senescence. When leaves are induced to senesce or exposed to stress, several deteriorative processes such as deg-

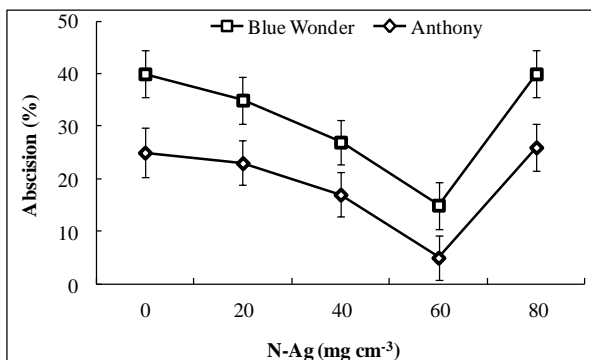


Figure 1. Effect of different concentrations of nanosilver (N-Ag) on petal abscission in two pelargonium cultivars after 5 days dark storage. The error bars indicate the standard deviation

Table 1. Effect of different concentrations of nanosilver (N-Ag) on plastid pigments (chlorophyll *a*, *b* and carotenoids) in two pelargonium cultivars

N-Ag (mg·dm ⁻³)	Plastid pigments (mg·g ⁻¹ FW)	Anthony	Blue Wonder
0	Chlorophyll <i>a</i>	0.032±0.0014	0.012±0.0021
	Chlorophyll <i>b</i>	0.020±0.0023	0.010±0.0024
	Carotenoids	1.32±0.033	1.028±0.0054
20	Chlorophyll <i>a</i>	0.038±0.0024	0.029±0.0043
	Chlorophyll <i>b</i>	0.033±0.0027	0.031±0.0021
	Carotenoids	1.57±0.050	1.23±0.1021
40	Chlorophyll <i>a</i>	0.039±0.0028	0.035±0.0038
	Chlorophyll <i>b</i>	0.029±0.0023	0.015±0.0042
	Carotenoids	1.77±0.076	1.21±0.1029
60	Chlorophyll <i>a</i>	0.048±0.0037	0.044±0.0028
	Chlorophyll <i>b</i>	0.039±0.0021	0.040±0.0030
	Carotenoids	1.82±0.018	1.32±0.1097
80	Chlorophyll <i>a</i>	0.036±0.0076	0.016±0.0012
	Chlorophyll <i>b</i>	0.019±0.0023	0.010±0.0011
	Carotenoids	1.28±0.014	1.021±0.0051
LSD _{0.05} for N-Ag x C	Chlorophyll <i>a</i>		0.0032
	Chlorophyll <i>b</i>		0.0031
	Carotenoids		0.134

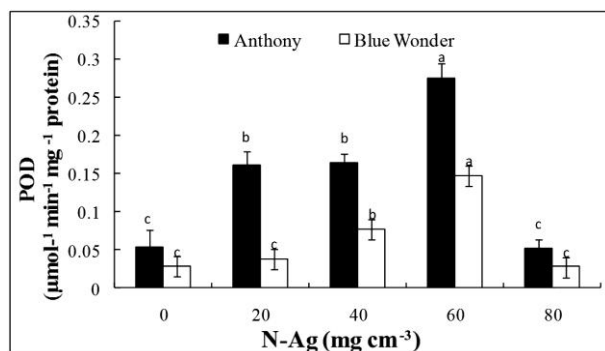


Figure 2. Effect of different concentrations of nanosilver (N-Ag) on peroxidase (POD) activity in two pelargonium cultivars after 5 days dark storage. The error bars indicate the standard deviation. The means for each cultivar indicating by the same letter do not differ significantly at $\alpha=0.05$ according to Tukey's test

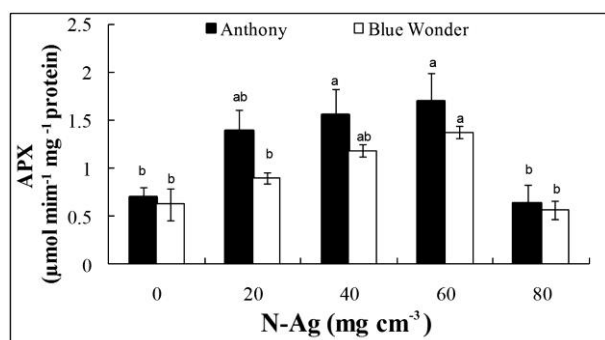


Figure 3. Effect of different concentrations of nanosilver (N-Ag) on ascorbate peroxidase (APX) activity in two pelargonium cultivars after 5 days dark storage. The error bars indicate the standard deviation. The means for each cultivar indicating by the same letter do not differ significantly at $\alpha=0.05$ according to Tukey's test

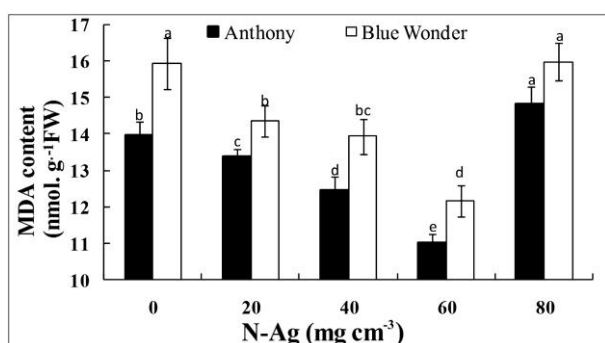


Figure 4. Effect of different concentrations of nanosilver (N-Ag) on lipid peroxidation in two pelargonium cultivars after 5 days dark storage. The error bars indicate the standard deviation. The means for each cultivar indicating by the same letter do not differ significantly at $\alpha=0.05$ according to Tukey's test

radiation of chlorophyll and proteins, modifications in membrane permeability and the activity of antioxidants like catalase and peroxidase, are triggered. Costa *et al.* (2006) suggested that POD catalyzes the oxidation of phenolic compounds in the presence of H₂O₂ and generates the phenolic radicals that mediate chlorophyll degradation. A delay in chlorophyll degradation in broccoli was closely related to lower POD activity, which is in agreement with our results. Some reports showed that activities of antioxidant enzymes under different stress conditions were relatively higher in tolerant species than in the sensitive ones (Bor *et al.* 2003). Current findings clearly indicate that post-storage performance of pelargonium pot plants were both positively and negatively affected by N-Ag treatments *via* their biochemical and enzymatic variations. It can be concluded that the 'Anthony' could be recommended as a better cultivar than 'Blue Wonder' for transport and cultivation under low light intensity.

CONCLUSION

The results of the experiment indicate that application of N-Ag at appropriate concentration decrease degradation of plastid pigments, reduce petal abscission rate and retain antioxidative enzymes activity under dark storage conditions. It is proposed that nanosilver treatment could provide a new strategy for improving longevity of pelargonium pot plants during shipment.

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