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Improving Physiological Quality of Cut Tuberose (*Polianthes tuberosa* cv. Single) Flowers by Continues Treatment with Humic Acid and Nano-Silver Particles

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Abstract

Tuberose (*Polianthes tuberosa* L.) is an important commercial cut flower that has a short vase life. An experiment was carried out by using five different levels of humic acid (HA) (0, 25, 50, 75, and 100 mg L^{-1}) and seven different levels of silver nano-particles (SNP) (0, 0.5, 1, 2, 3, 4 and 5 mg L^{-1}) mixed with 1.5% sucrose on cut tuberosa cv. Single flower. The loss of fresh weight on third and sixth days in 25 mg L^{-1} HA + 1.5% sucrose, 50 mg L^{-1} HA + 1.5% sucrose and 75 mg L^{-1} HA+ 1.5% sucrose was less compared to other treatments. Also HA decreased lipid peroxidation. Silver nano-particles increased the water uptake, fresh weight, total protein, and declined lipid peroxidation compared to the control. Results showed that suitable levels of HA and SNP lead to better morphological and physiological properties and increase the vase life of cut Tuberose flower.

Keywords: Humic acid, Lipid peroxidation, Silver nano-particles, Total Protein, Tuberose flowers, Vase life.

INTRODUCTION

Tuberose (*Polianthes tuberosa* cv. Single) from Agavaceae family is a perennial commercial and important cut flower (Feng *et al.*, 2000). Microbial contaminations due to bacteria growth in preservative solution cause stem end blockage and reduce the vase life of cut flowers (van Meeteren, 1978; van Doorn and Dewitte, 1994; Balestra *et al*., 2005; Solgi *et al*., 2009). Water balance is a main factor determining quality and longevity of cut flowers (Lü *et al*., 2010). One of the disorders of cut Tuberose is bending of florets and buds after keeping in preservative solutions (Naidu and Reid, 1986). The use of antimicrobial compounds such as silver nano-silver particles (SNP) as a novel antimicrobial compound, and antiseptic compound is important in floriculture, micropropagation, and pharmaceutics industries (Sondi and Salopek-Sondi, 2004; Navarro *et al*., 2008; Kim *et al.*, 2009; Lu *et al*., 2010). SNP releases Ag+ (Lok *et al*., 2007), which interacts with cytoplasm components and nucleic acids and inactivates respiratory chain enzymes and changes membrane permeability (Russel and Hugo, 1994; Park *et al.,* 2005). Although, many compounds such as hydroxy quinoline, aluminum sulphate, and silver thiosulphate (STS) were applied in vase solution (Nair *et al.*, 2003; Damunpola and Joyce, 2006; Ichimura and Shimizu-Yumuto, 2007). Solgi *et al.* (2009) found that the use of concentrations of 1 and 2 mg L^{-1} SNP extend the vase life of (*Gerbera jamesonii* cv. 'Dune'). Basiri *et al*. (2011) investigated the effect of different concentrations of SNP on cut carnation and found that SNP with 5, 10, 20, 40 and 80 mg L^{-1} extend the vase life of cv. White Liberty flowers.

Humic acid (HA) is one of the major components of humus that can be used as a growth regulator, which improves plant growth and development and enhances stress tolerance and recently has improved the vase life of Gerbera cut flowers (Nikbakht *et al.*, 2008). HA increases vase life of gerbera (*Gerbera jamesonii* cv. Malibu) (Nikbakht *et al.*, 2008).

Cut Tuberose has a short vase life and is sensitive to microbial contamination at the stem base and bacterial population decreases the vase life of this flower. Therefore, the aim of this study was to evaluate the effect of different concentrations of SNP and HA on the vase life and other traits of Polianthes tuberosa cv. Single.

MATERIALS AND METHODS

Plant material

Cut Tuberose cv. Single was harvested in optimum developmental stage in the morning (Dole *et al.*, 2005) and transported to the laboratory of Guilan University, immediately. Then, the stems were cut to length of 60 cm and taken to the laboratory.

Experiment design and treatments

The effect of SNP (0, 0.5, 1, 2, 3, 4 and 5 mg L^{-1}) and HA (0, 25, 50,75 and 100 mg L^{-1}) was compared to the control (distillated water and 1.5% sucrose) in a split plot experiment based on completely randomized block design with four replications. After pulsing with SNP and HA, cut flowers were put in a 400 ml vase solution. Each plot included 8 flowers. Environmental condition in the vase life room included 12 h photoperiod, 12 µmol s^{-1} m^{-2} light intensity, provided by fluorescent lamps, temperature of 25°C, and RH of 70%.

Vase life

The end of vase life was defined as the time during which more than 50% of florets were wilted (Dole *et al.*, 2005).

Lipid peroxidation

Measuring of lipid peroxidation was carried out by MDA concentration as peroxidative action product of fatty acid was used (Zhang, 1992).

Total protein content

Total protein content was obtained by Bradford test (Bradford, 1976).

Solution uptake and loss of fresh weight in 3rd and 6th days

Water uptake was measured by water absorption at the end of vase life. Relative fresh weight was calculated by the following formula:

Water uptake (ml) = (S_{t-1} - St), where; St is amount of vase solution (ml) at t = days 1, 2, 3, 4, and S_{t-1} is amount of vase solution (ml) on previous day (He *et al.*, 2006).

Statistical analysis

Mean comparison was carried out with LSD statistical test and analysis of variance was carried out by SAS software.

RESULTS

Vase life

Mean comparisons showed that there was a significant difference between different concentrations of HA and SNP ($p \leq 0.05$). The best HA treatment was 25 mg L^{-1} which increased the vase life about 1.5 days more than the control. The next effective treatment was 50 mg L^{-1} HA. Results showed that the most effective SNP treatments were 0.5 and 1 mg L^{-1} which increased the vase life up to 6.5 and 7 days, respectively, compared to control flowers (4.5 days) (Figs. 1, 2) .

Petal lipid peroxidation

The 3rd and 6th lipid peroxidation varied significantly between different concentrations of HA and SNP (Figs. 3, 4). Adding 25 mg L^{-1} HA into vase solution decreased lipid peroxidation about 40% compared to control (17 and 30 units, respectively) (Fig. 3). Moreover, comparing different levels of SNP showed that petal lipid peroxidation decreased significantly (about 8 units) with 0.5 mg L^{-1} SNP. 1 mg L^{-1} SNP had the second priority in decreasing petal lipid peroxidation (Fig. 4).

Total protein content

Our observations showed that the result of adding different levels of HA and SNP into vase solution varied significantly ($p \leq 0.05$). Adding 25 mg L^{-1} HA into vase solution increased total protein content from 470 to 550 µg g^{-1} in 3th day. Moreover, adding 1 mg L^{-1} SNP enhanced total protein content about 20% in same day (575 µg g^{-1} compared to 470 µg g^{-1} in control flowers) (Figs. 5, 6).

Vase solution uptake at 3rd and 6th days

Vase solution uptake varied significantly among different concentrations of HA and SNP. Results showed that at 6th day, the control cut flowers with 105 and 106.25 ml had more efficiency than HA (Table 1); while, water uptake in different concentrations of SNP (exempt 5 mg L^{-1}) in the 6th day was more than the control. The most effective treatments were 1 and 0.5 mg L^{-1} with 133.75 and 123.75 ml, respectively (Table 1). In 3rd day, vase solution uptake improved in 1 mg L^{-1} SNP comparing to 50 mg L-¹ HA (96.75 and 59.25, respectively) (Table 1).

Fresh weight loss at 3th and 6th days

Our results showed that there was a significant difference between different levels of HA and SNP ($p \leq 0.05$). Mean comparisons at 6th day showed that 1 mg L^{-1} SNP and 25 mg L^{-1} HA (with 13.96 and 14.27 g, respectively) had significant priority to other levels and 5 mg l^{-1} SNP was not efficient (Table 2). (Mean comparisons at 3rd day showed that 0.5 mg L^{-1} SNP and 25 mg L^{-1} HA prevented fresh weight loss about 50% more than the control (9.09 and 8.67 g, respectively).

DISCUSSION

Our observations showed that adding 25 mg L^{-1} HA (6 days) and 1 mg L^{-1} SNP (7 days) into preservative solution, were the most effective treatments for extending vase life in Tuberose (*Polianthes tuberosa* cv. Single) (Figs. 1, 2). It seems that the main reasons which caused longer vase life of Tuberose were respiration control, reduction of stem-end bacterial activity, and improvement of water relations (Esfahani *et al.*, 2011; Shabani *et al*., 2011). Hatami *et al.* (2011) found that pulse treatment with SNP and sucrose extended vase life in rose (*Rosa hybrida* 'Red Bion'). Kim *et al*. (2005) reported that integrated use of SNP, silver colloids, and hydrogen peroxide (H_2O_2) improved vase life in *Lilium* 'Dream Land' and 'Siberya'. Nikbakht *et al*. (2008) found that adding 1000 mg L^{-1} HA into preservative solution extended vase life of gerbera (*Gerbera jamesonii* cv. Malibu). Our results confirm the above-mentioned observations.

Our observations proved that adding 25 mg L^{-1} HA and 0.5 mg L^{-1} SNP into the vase solution are the best treatments which prevent increasing petal lipid peroxidation (Figs. 3, 4). Main reason for there results is positive effect of HA and it's specific cytokinin-like characteristics on cellular respiratory system and inhibitory effects of SNP on stem-end bacteria (with interrupting respiratory chain and respiratory system) (Nikbakht *et al.*, 2008). Talebi *et al.* (2011) showed that applying lipid peroxidation inhibitors extend vase life of cut rose cv. Sansiro. These observations confirm studies of Zhu *et al.* (2004), Talebi *et al*. (2011), and Zheng *et al*. (2011) about flower longevity and reducing lipid peroxidation with applying vase life extending compounds.

Among all treatments, 25 and 50 mg L^{-1} HA and 1 mg L^{-1} SNP are the most effective compounds which can increase total protein content (Figs. 5, 6). It must have been for the positive effects of HA and its specific cytokinine-like characteristics and prevention of interrupting enzyme activities (Nikbakht *et al.*, 2008). Also, SNP and HA with their antimicrobial and hormone-like characteristics increased total protein content. These results are similar to Gulzar *et al.* (2005). Calatejari *et al*. (2008) found that adding plant growth regulators, especially benzyl adenine (BA), extended vase life and increased total protein content in rose cv. Red Giant.

Mean comparison showed that vase solution uptake at 6th day was better with SNP. Also, vase solution uptake at 3rd day was better with 0.5 and 1 mg L^{-1} SNP (Table 1). Ag+ is an antibacterial compound that prevents stem end blockage and increases vase solution uptake (Liu *et al.*, 2009). Basiri *et al*. (2011) recognized that adding the 5 mg L^{-1} SNP into vase solution increased water uptake in carnation (*Dianthus caryophyllus*) more than the control.

Our results showed that the best inhibitors for fresh weight loss preventing, were 0.5 mg L^{-1} SNP and 25 mg L^{-1} HA (Table 2). Antibacterial effect of these compounds prevents over-perspiration and improving water relations which ultimately inhibit fresh weight loss (Morones et al., 2005; Nikbakht *et al.,* 2008; Solgi *et al.*, 2009). Hatami *et al*. (2011) found that pulse treatment with SNP extended vase life of cut rose cv. Red Bion and prevented fresh weight loss during the vase life. Nikbakht *et al*. (2008) reported that adding 1000 mg L^{-1} HA into vase solution prevented fresh weight loss significantly (32.62 g). Our results confirm Nikbakht *et al.* (2008) and Hatami *et al*. (2011).

In conclusion, this study was carried out to investigate the effects of SNP and HA on vase life of cut Tuberose (*Polianthes tuberosa*). Our results showed that these treatments cause longer vase life, prevent petal lipid peroxidation and fresh weight loss, improve water relations and increase total protein content. Therefore, using these compounds is recommended.

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Tables

Table 1. Effect of HA and SNP on solution uptake of cut *Polianthes tuberosa* cv. Single flowers.

Treatments	Solution uptake on 3th day (ml)	Solution uptake on 6th day (ml)
Control	51.50 d	106.25 c
1.5% sucrose	59.25 d	105.00 c
25 mg L^{-1} HA	58.50 d	104.30 c
50 mg L^{-1} HA	59.25 d	101.00 c
75 mg L^{-1} HA	56.50 d	97.50 c
100 mg L^{-1} HA	53.75 d	97.40 c
0.5 mg L^{-1} SNP	93.25 a	123.75 ab
1 mg L^{-1} SNP	96.75 a	133.75 a
2 mg L^{-1} SNP	75.87 bc	122.50 ab
3 mg L^{-1} SNP	83.00 ab	110.05 b
4 mg L^{-1} SNP	77.25 bc	115.00 ab
5 mg L^{-1} SNP	60.00 d	63.75 d

*Means in the same column followed by the same letter are not significantly different by LSD test (α=5%).

Table 2. Effect of HA on the weight loss of cut Polianthes tuberosa cv. Single flowers.

Treatments	Weight loss on 3th day (gr)	Weight loss on 6th day (gr)
Control	16.55 a	20.64 a
1.5% sucrose	15.01 a	21.46 a
25 mg L^{-1} HA	8.67 b	14.27 b
50 mg L^{-1} HA	13.25 ab	16.29 ab
75 mg L^{-1} HA	13.20 ab	16.21 ab
100 mg L^{-1} HA	15.01 a	22.33 a
0.5 mg L^{-1} SNP	9.09 b	18.81 ab
1 mg L^{-1} SNP	9.17 b	13.96 b
2 mg L^{-1} SNP	11.65 ab	18.9 ab
3 mg L^{-1} SNP	10.65 ab	19.96 ab
4 mg L^{-1} SNP	13.71 ab	19.12 ab
5 mg L^{-1} SNP	12.84 ab	23.78 a

*Means in the same column followed by the same letter are not significantly different by LSD test (α=5%).

Figures

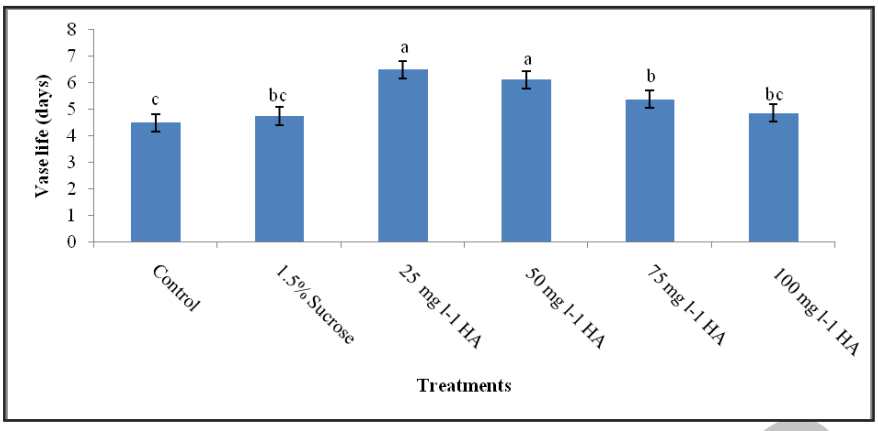

Fig. 1. Effect of HA on vase life of cut *Polianthes tuberosa* cv. Single flowers.

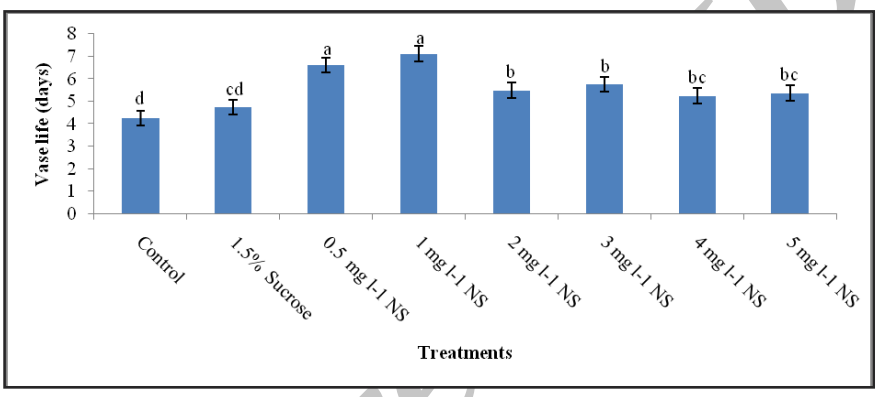

Fig. 2. Effect of SNP on the vase life of cut *Polianthes tuberosa* cv. Single flowers.

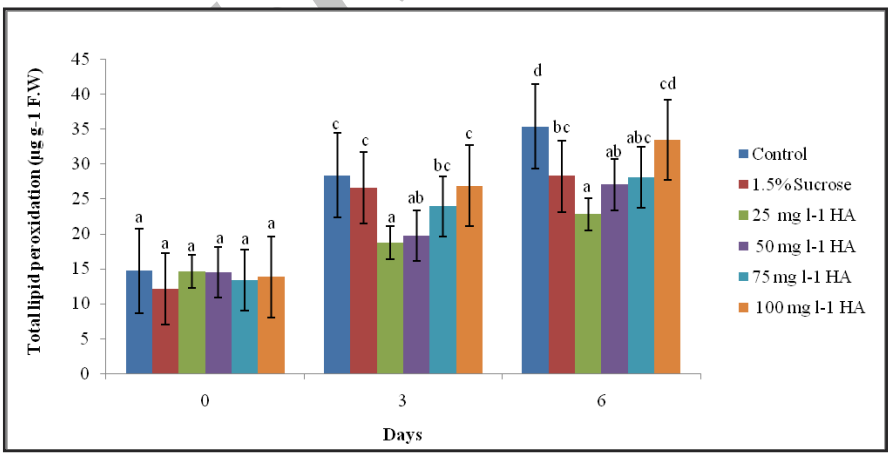

Fig. 3. Effect of HA on total lipid peroxidation of petals, in cut *Polianthes tuberosa* cv. Single flowers.

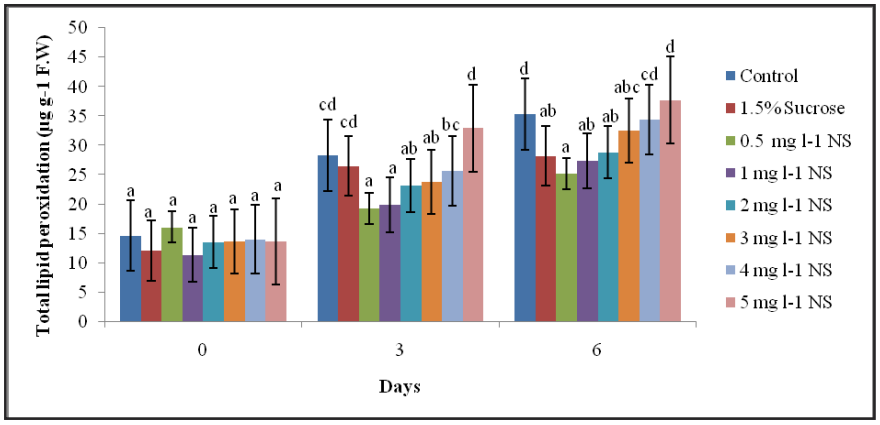

Fig. 4. Effect of SNP on total lipid peroxidation of petals in cut *Polianthes tuberosa* cv. Single flowers.

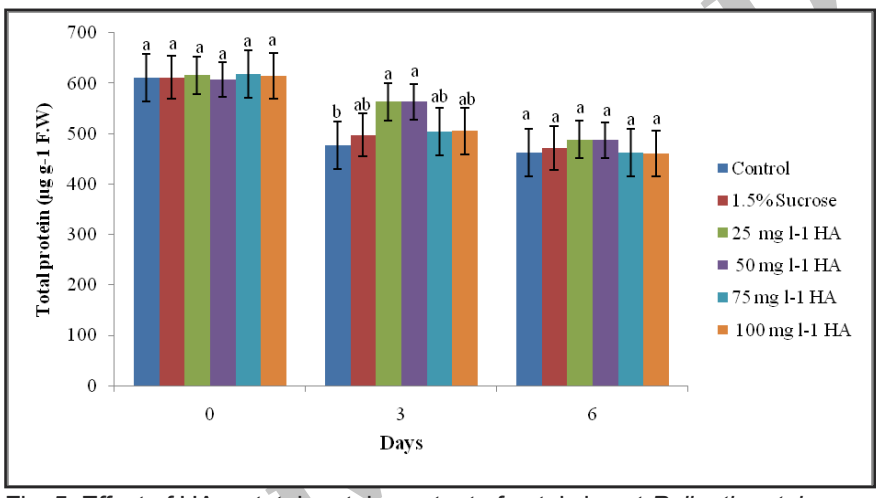

Fig. 5. Effect of HA on total protein content of petals in cut *Polianthes tuberosa* cv. Single flowers.

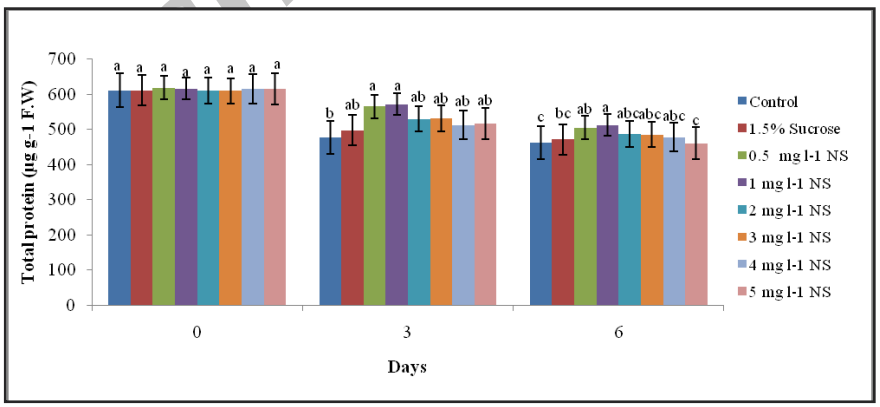

Fig. 6. Effect of SNP on total protein content of petals in cut *Polianthes tuberosa* cv. Single flowers.