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ijhst@ut.ac.ir:ut.ac.ir, Email. ijhst //:Web Page: https

Antimicrobial and Physiological Effects of Silver and Silicon Nanoparticles on Vase Life of Lisianthus (*Eustoma grandiflora* cv. Echo) Flowers

Fereshteh Kamiab[1*], Sadegh Shahmoradzadeh Fahreji[2] and Elahe Zamani Bahramabadi[3]

1. Department of Horticulture, Rafsanjan Branch, Islamic Azad University, Rafsanjan, Iran.
2. Department of Horticulture, Giroft Branch, Islamic Azad University, Giroft, Iran.
3. Department of cellular and developmental botany, Kharazmi University, Tehran, Iran.

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Abstract
Increasing quality and vase life of cut flowers play vital role in flower production industry. . Lisianthus (*Eustoma grandiflora* cv. Echo) has short vase life and it has been revealed that ethylene directly affect the initiation and process of senescence of petals. In this study, the effects of Silver and silicon nanoparticles with four concentrations of 0, 10, 20 and 40 mg L^{-1} with 4% sucrose as a support solution were evaluated on post-harvest life of 'Cinderella Lime' Lisianthus. The morphological and physiological parameters such as microbial population, flower vase life, relative fresh weight, solution uptake, total chlorophyll, ethylene and total dissolved solids were measured. Results revealed that all treatments extended the flower vase life when compared to control. The most effective treatment was the Highest concentration of nanoparticles (40 mg L^{-1}). The average vase life of flowers was about 5 days in control (without any nano particle treatments) however; it reached to 17 days in flowers treated by 40 mg L^{-1} of both nanoparticles. Relative fresh weight, solution uptake, total chlorophyll, and total dissolved solids were also increased in the treated flowers, especially at higher concentrations. Microbial proliferations were not observed by application of both nanoparticles (Silver or Silicon) at 40 mg L^{-1} therefore this concentration was considered as the most effective level for both nanoparticles. Nano silver were more effective than silicon for reducing ethylene content. Overall the results suggest that silicon nanoparticle (40 mg L^{-1}) is applicable as antimicrobial compound in combination with silver nanoparticles (40 mg L^{-1}) as ethylene signaling inhibitor to increase the vase life of Lisianthus flowers commercially.

Keywords: ethylene, Lisianthus, microbial proliferation, nanoparticles, vase life.

Introduction

Lisianthus (*Eustoma grandiflora* L.) is among the top 10 cut flowers in the world, and its sale has increased to more than 50% over the past 10 years. Lisianthus has been originated from North America, *Eustoma grandiflora* L. species is used as cut and potted flower (Kazemi et al., 2012). Several factors shorten the shelf life of the cut flowers, among them reduction of carbohydrates and microorganisms colonization in preservative solution are the majors (Bhattacharjee, 2005).

Silver is one of the most known antibiotics and has been used for its anti-bacterial and anti-fungal properties in the treatment of human diseases in the last

*Corresponding Author, Email: F.kamiab56@gmail.com

century. Beside, silver ion is ethylene signaling inhibitor and is capable of reducing the harmful effects of ethylene. Liao et al. (2000) reported that when flowers of *Rosa hybrida* L. cv. 'Diana' were treated by silver thiosulfate (STS), the longest vase life was achieved. Shahid (2005) showed that when silver nitrate was applied at concentration of 150 ppm, the longest vase life was observed on two varieties of rose cut flowers (*Rosa hybrida* cv. 'Trika' and cv. 'Whisky Mac'). It was also reported that the use of silver nanoparticles in preservative solution increased the shelf life and quality of roses (Ohkawa et al., 1999). Alimoradi et al. (2013) evaluated the effect of STS on the shelf life of Alstroemeria and their results showed that STS increased the durability, diameter and chlorophyll content of Alstroemeria cut flowers.

Silicon is the second most abundant element on the earth's surface (7%) however; its role as an essential element for plants has not been proved so far because most plants are able to complete their life cycle even in the absence of this element (Epstein, 1994). Environmental pollution by silicon is much lower that the silver. Therefore, antimicrobial silver compounds can be replaced by silicon.

During the cell wall deposition, formation of a cellulose-silicon layer and bonding with calcium and pectin, silicon improves the strength of cell walls and protect plant cell against cellular damages cause by pathogens (Mitani and Ma, 2005). Moreover, unlike calcium, silicon has high mobility in the plant. Therefore, it is considered as a complementary element in plant breeding (Ma, 2003).

Silicon also plays a role in growth improvement, increasing the photosynthesis, reducing the evaporation and transpiration, increasing the strength of leaves and increasing the chlorophyll content in leaf area units (Liang et al., 2006). This element reduces the stomatal and circular transpiration, therefore effectively limits water loses (Cook and Leishman, 2011). Furthermore, silicon inhibits fungal mycelium penetration in plant tissues (Yoshida et al., 1962). Several authors have suggested that silicon causes an improvement in water use efficiency and stimulates enzymatic and non-enzymatic and oxidative defense systems (Guneset al., 2007; Lianget al., 2003; Cooke and Leishman, 2011).

Recently, nanoparticles have been applied in agriculture. However, plant cell wall acts as a barrier which prevents easy entry of external factor into the plant cells. Nanoparticles are smaller than the cell wall pores, therefore it easily pass through them. On the leaf surface, nanoparticles enter the plant through the stomata or basis of trichomes and transmit to different tissues (Nair et al., 2010).

Some of the most important research on the effect of nanoparticles on ornamental plants involves the use of silver nanoparticles to increase the vase life of carnation, gerbera and rose that results in improving their postharvest longevity (Liu et al., 2009a; Liu et al., 2009b; Lu et al., 2010).

Lisianthus is now an important commercial cut flower and its short vase life is one of the main concern in its cut flower production. Therefore its post-harvest longevity is a serious challenge for future researches. Water uptake reduces gradually in *Eustoma grandiflora* cut flowers, which possibly could be attributed to the occlusion of xylem vessels (Vn Doorn and Cruz, 2000). In this study , we tried to use antimicrobial compound such as silicon and silver to increase water uptake in Lisianthus flowers. Since researches about the application of nanoparticles especially silicon on the vase life of cut flowers are very limited, the aim of the current research was to investigate the hypothesis that these antimicrobial nanoparticles are involved in occlusion of xylem vessels in Lisianthus stems and to select the optimum concentration for improving vase life and quality of cut Lisianths flower.

Materials and Methods

Plant material and experiments

The experiment was conducted in horticulture laboratory of Birjand University in July 2015. The flowers were kept in a germinator and temperature was set at 20 ± 1˚C, relative humidity was 65 -75%, and light intensity was 15 μ mol m^{-2} s^{-1}. Germinator light was provided by fluorescent lamps and photoperiod was set at 12/12 hours of daylight/darkness. Lisianthus cut flowers were procured from commercial greenhouses in Tehran Province. Flowers were cut in a length of 81 cm by a clipper and were then packed in batches of 51 samples and were transferred immediately to the laboratory. Flowers were immediately removed from the pack and cut diagonally with a length of 40 cm using a clipper and placed in 500 ml erlen containing 400 ml of 4% sucrose solution until the treatments were applied on them. Treatments including silver or silicon nano-articles at four levels (0, 10, 20, 40 mg L^{-1}) were applied to preservative solution. Cut flowers were placed in the solutions, and data were recorded every day. Distilled water was used to prepare solutions and all solutions contained 4%sucrose.

This work was carried out as a factorial experiment based on a randomized complete design with 16 treatments and 48 experimental units with three replications and each replication contained four branches of flowers. Data were analyzed using SAS statistical software at the 5% level, the charts were plotted using MS-Excel software, and comparison of means was done by Duncan's multiple range tests.

Vase Life

The vase life of flowers was considered to be terminated when half of the flowers of each inflorescence were abscised (Mutui et al., 2006).

Relative Fresh Weight

The relative fresh weight was calculated using a digital mg-precision scale by the following formula and was expressed as a percentage (Chamani et al., 2006).

Relative fresh weight (%) = $(W_t/W_0) \times 100$

W_t = stem weight (g) measured on different days (4, 8 and 12)

W_0 = stem weight (g) on the 1st day

Solution Uptake

The solution uptake was calculated using the following equation (Chamani et al., 2006):

Solution uptake (ml g^{-1} d^{-1})= $(S_{t-1}-S_t)/ W_t$

S_t = solution weight (g) measured on different days (4, 8 and 12)

S_{t-1} = solution weight (g) on the day before

W_t = stem fresh weight (g) on the 1^{st} day

Percentage of Soluble Solids in the Stem

Two gram of the stem end was fully pulverized by a mortar, passed through filter paper and then, soluble solids were measured in the resulting aqueous extract by a digital refractometer (Hettiarachchi and Balas, 2005).

Chlorophyll Content

To estimate chlorophyll content, 0.1 g of leaves of each sample was placed in a test tube and 5 ml of 80% acetone was added to it. When all chlorophyll was dissolved in acetone, the tube was centrifuged at 6000 rpm for three minutes. The resulting product was placed in a spectrometer and the absorption of light was read at 663 and 465 nm (Meidner, 1984).

Total Chlorophyll (μm/ml $^{-1}g^{-1}$): $[(17.76 \times OD_{646.6}) + (7.37\times OD_{663.6})] \times V/W$

OD: the read absorbance

V: volume of applied acetone

W: fresh weight of sample (g)

Microbial Count

Soluble microbial counts were measured according to Jowkar (2006) during the experiment on the 5th, 10th and 15th days. Meanwhile, 1 ml samples were taken from the vase solutions by micropipette. The samples were diluted to 10^{-10} using serial dilution with saline. From any dilution, a sample of 1/10 ml was spread on nutrient

agar in Petri dishes size 8. The cultured media were placed in a growth chamber for 48 hours at 37°C. Finally the number of microorganisms was counted based on the number of colony-forming units in millimeters by page counting.

Ethylene measurement

Measurement of ethylene was carried out according to Nabigol, (2013) by gas chromatography. Length and diameter of column (fused silica plot) were 25 and 32 meter respectively. Flow rate of the samples was 2 kg/cm^2 and also isocratic, detector and injection place temperature were 120, 140 and 120°C, respectively. Sampling was conducted 5 day after the start of the experiment. In this case, one flower were picked up from every treatment putted in sealed 300 ml dishes for 3 days and the air of dishes drawn within the vacuum vial. In order to estimate ethylene content, 100 µl of vial air is injected to GC and amount of ethylene (nl/g/h) in injected air was determined based on standard curve (y= 1.652x+1.5).

Results

Microbial Population in Preservative Solution

The highest number of microorganisms was observed in control and the microbial population significantly increased with storage time in preservative solution. The microbial population in third stage was two times higher than that in the first stage of measurement. The most effective treatments in controlling microbial population of preservative solution were silver or silicon nanoparticles with a concentration of 40 mg L^{-1}, both inhibited the growth and proliferation of microorganisms until the day 15 of measurement. However, at concentrations of 0, 10 and 20 mg L^{-1} of silver or silicon nanoparticles, microbial growth was observed in all three phases of microbial population measurement, although it was significantly lower in these treatments when compared to the control plants (Table 1).

Vase life

Figure 1 shows that 40 mg L^{-1}silver nanoparticles in combination with 40 mg L^{-1}silicon nanoparticles was the most effective treatment enable to increase the vase life of Lisianthus cut flowers and exhibited significant differences with other treatments and controls. Flower longevity extended to 25 days in this treatment, while the vase of life control flowers were limited to 5 days.

Table 1. Effect of different concentrations of silver or silicon nanoparticles on microbial population in preservative solution of Lisianthus flowers on the 5th, 10th and 15th days.

Treatments	Microbial population in preservative solution log10 CFU ml^{-1}		
	5th day	10th day	15th day
Ag (0)Si (0)	2.21±0.14 ^{a}	4.28 ±0.09 ^{a}	7.28 ±0.48 ^{a}
Ag (0)Si (10)	0.1±0.09 ^{b}	0.9 ±0.10 ^{c}	1.9 ±0.16 ^{b}
Ag(0)Si (20)	0.1 ±0.01^{b}	0.8±0.11 ^{c}	1.8 ±0.19 ^{b}
Ag(0)Si(40)	0 ±0 ^{c}	0 ±0 ^{d}	0±0.19 ^{c}
Ag (10)+Si (0)	0.12±0.08 ^{b}	1.1 ±0.17 ^{b}	2±0.17 ^{b}
Ag (10)+Si (10)	0.1 ±0.07 ^{b}	1±0.39 ^{b}	1.8 ±0.18 ^{b}
Ag (10)+Si (20)	0.15±0.05 ^{b}	1.2±0.35 ^{b}	2.2 ±0.29 ^{b}
Ag (10)+Si (40)	0±0^{c}	0±0^{d}	0 ±0^{c}
Ag (20)+Si (0)	0.12 ±0.05 ^{b}	1.3±0.19 ^{b}	2.1 ±0.25 ^{b}
Ag (20)+Si (10)	0.11 ±0.04 ^{b}	1.2±0.13 ^{b}	2 ±0.39 ^{b}
Ag (20)+Si (20)	0.1 ±0.09 ^{b}	1±0.05 ^{b}	1.95 ±0.19 ^{b}
Ag (20)+Si (40)	0±0^{c}	0±0^{d}	0 ±0^{c}
Ag (40)+Si (0)	0±0 ^{c}	0±0^{d}	0±0^{c}
Ag (40)+Si (10)	0±0^{c}	0±0^{d}	0 ±0^{c}
Ag (40)+Si (20)	0±0^{c}	0±0^{d}	0 ±0^{c}
Ag (40)+Si (40)	0±0^{c}	0±0^{d}	0 ±0^{c}

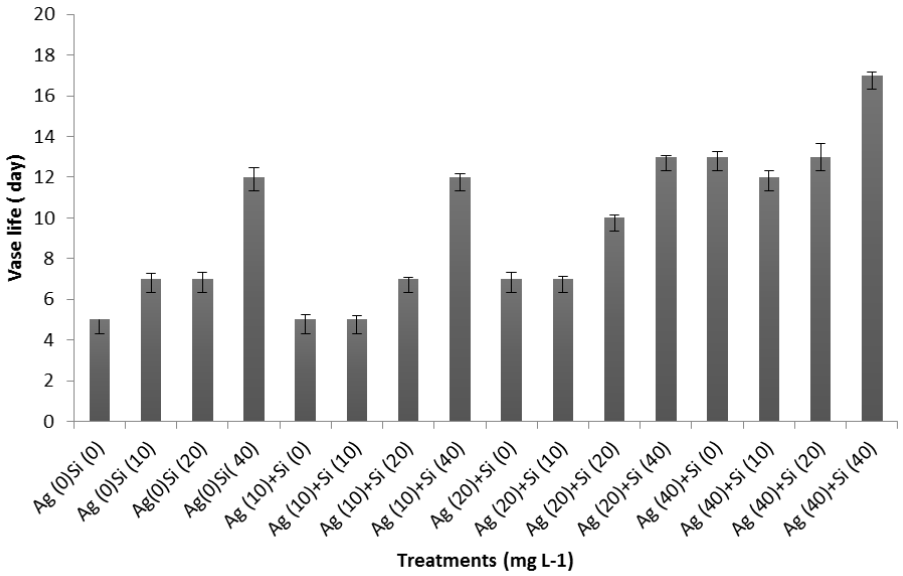

Fig. 1. The effect of silver and silicon nanoparticles on extending the vase life of Lisianthus cut flowers.

Relative Fresh Weight

Figure 2 shows that the highest relative fresh weight measured at the first stage (the 3rd day) was seen in 40 mg L^{-1}silicon nanoparticles treatment which was significantly different from control and other treatments. But in the second and third measurements (6th and 9th day), the highest relative fresh weight was related to treatment with 40 mg L^{-1}silver nanoparticles in combination with 40 mg L^{-1}silicon nanoparticles. In total, the increase in relative fresh weight was observed in all treatments in the second and third stages of measurement and had a significant difference when compared to control plants.

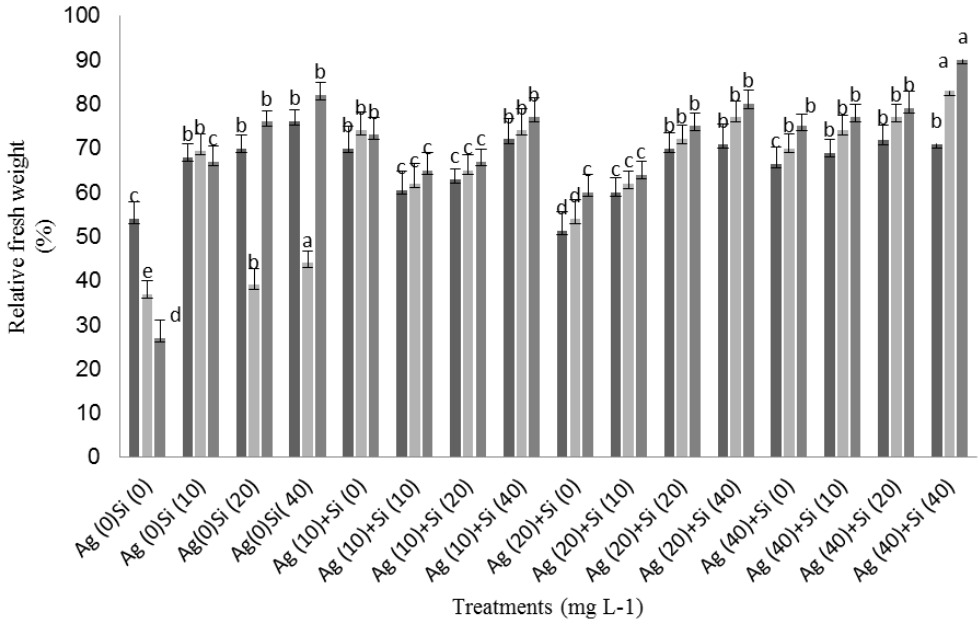

Fig. 2. The effect of different concentrations of silver and silicon nanoparticles on relative fresh weight of Lisianthus cut flowers on the 4th (first column), 8th (second column) and 20th (third column) days.

Solution Uptake

Figure 3 shows that 40 mg L^{-1}silver nanoparticles in combination with 40 mg L^{-1} siliconnanoparticles was the most effective treatment by increasing the solution uptake in Lisianthus cut flower in all three phases of measurement (3rd, 6th and 9th days). The controls and treatments with 10 mg L^{-1} silicon nanoparticles without silver had minimum impact on solution uptake.

Soluble Solids

Figure 4 shows that the highest percentage of soluble solids was observed in treatment of 40 mg L^{-1}silver nanoparticles in combination with 40 mg L^{-1}silicon nanoparticles. The lowest percentage of soluble solids was observed in treatment with 10 or 20 mg L^{-1}silicon nanoparticles which did not show significant differences with control.

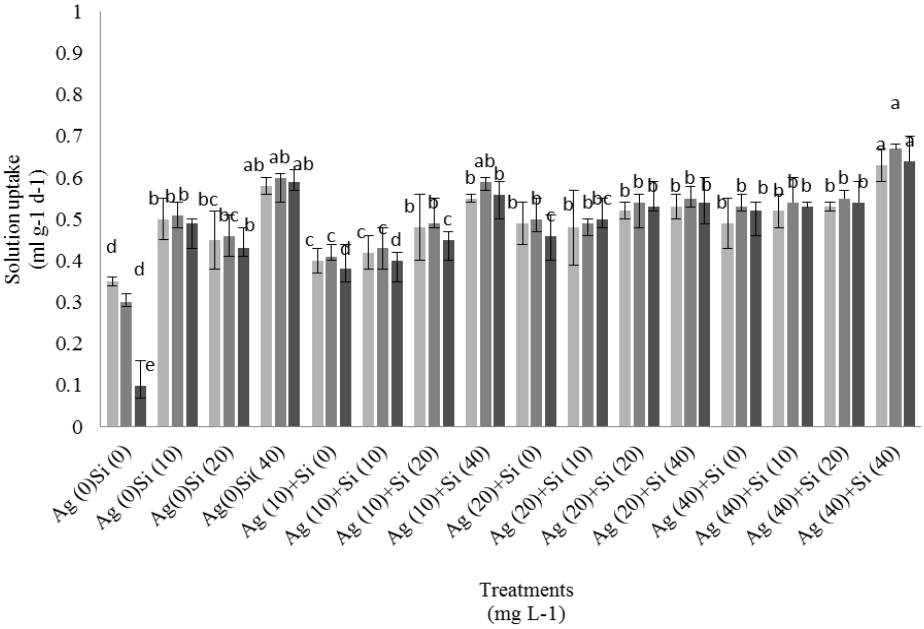

Fig. 3. The effect of different concentrations of silver and silicon nanoparticles on solution uptake of Lisianthus cut flowers on the 4th (first column), 8th (second column) and 20th (third column) days.

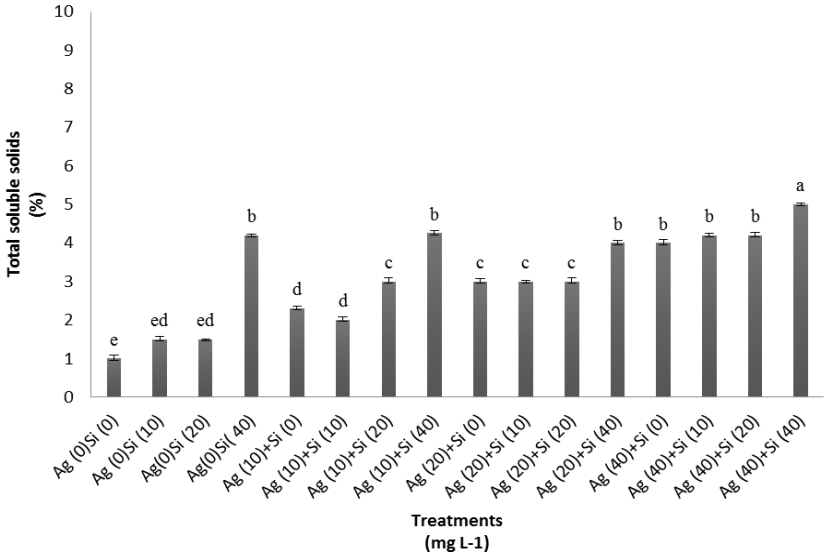

Fig. 4. The effect of different concentrations of silver and silicon nanoparticles on soluble solids percentage of Lisianthus cut flowers.

Chlorophyll

Figure 5 shows that all nanoparticles treatments significantly maintained the chlorophyll content of leaves in comparison with the control. The most effective treatments were 40 mg L^{-1}silver in combination with 40 mg L^{-1}silicon nanoparticles and 40 mg L^{-1} sole silicon nanoparticles or in combination with 10 or 20 mg L^{-1}silver nanoparticles, which represented significant differences with the control. The lowest chlorophyll content was observed in controls.

Ethylene content

Figure 6 shows that all nanoparticles treatments except for 0 and 10 mg L^{-1} silver in combination with s 0 and 10 mg L^{-1} silicon decreased ethylen content significantly when compared to control plants. The lowest ethylene content was observed in 40 mg L^{-1} silver in combination with 40 and 20 mg L^{-1} silicon nanoparticles.

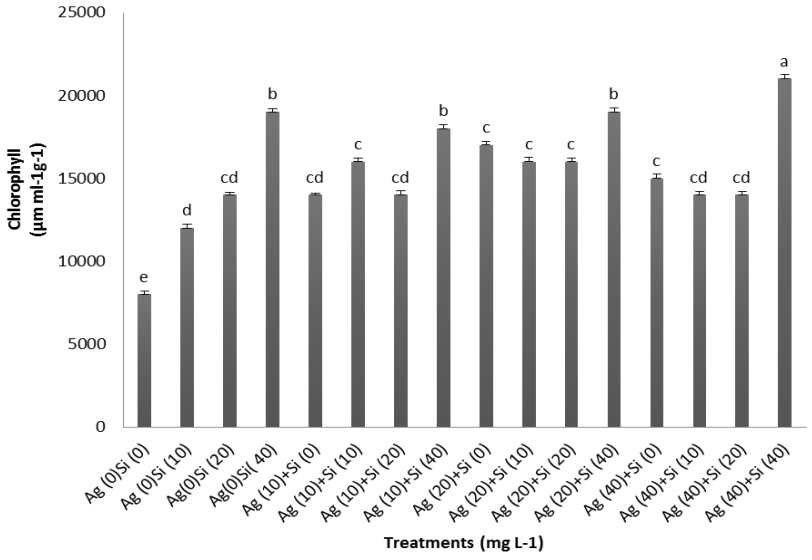

Fig. 5.The effect of different concentrations of silver and silicon nanoparticles on leaf chlorophyll content of Lisianthus cut flowers.

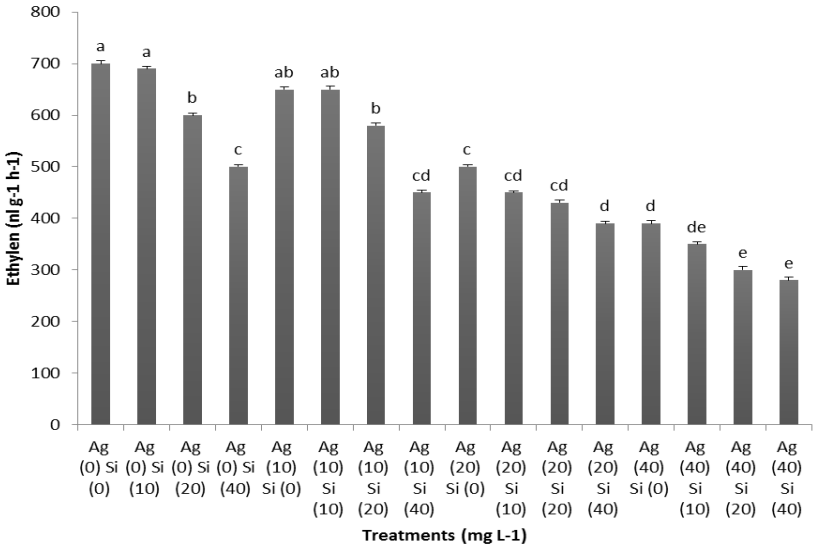

Fig. 6.The effect of different concentrations of silver and silicon nanoparticles on ethylen content of Lisianthus cut flowers.

Discussion

Cut flower breeding is an emerging approach in the industry of modern agriculture however, it is markedly restricted by several defective consequences such as postharvest storage and marketing though a minor damage in postharvest processing may cause severe losses in stake holders. Therefore integrated management approach which protects plants during postharvest stage is unadaptable. Wide ranges of research have been conducted on postharvest physiology of cut flowers in order to increase the vase life and to reduce the wastage of products. Food sources such as carbohydrates are very important for covering the energy needs of the plant. The main source of plant nutrition is removed when flowers are separated from their mother plants. During nutritional limitation, the lack of carbohydrates will limit the vase life. Notably with inappropriate environmental conditions, this process will be accelerated (Monteiro et al., 2002). Moreover, the microbial infection will enhance the destruction of flowers after cutting which increase deposition of the material in the vessels, leads to their closure and lack of water transport and consequently the emergence of the water shortage sings in the plant. Hence, supplying energy and maintaining the plant capability to uptake water are main factors extending the post-harvest life in cut flowers (Silva, 2003).

In this experiment, the simultaneous application of silver and silicon nanoparticles (40 mg L^{-1}) increased the vase life of flowers up to 12 days when compared to control flowers. In all treatments with 40 mg $L^{-1}$of silver or silica, microorganisms were not found in preservative solution. Nanosilver is a microbicide that destroys the pathogenic microbes by invading their cell wall and hence, reduces the bacterial population and prevents vascular obstruction. Silver is also an ethylene biosynthesis inhibitor and when used in preservative solution, reduces the negative effects of ethylene such as aging and wilting of cut flowers (Serek and Reid, 1993). Similar results have been reported about the positive effects of nanoparticles, particularly silver nanoparticles, for increasing the vase life of cut rose and gerbera (Ohkawa et al., 1999; Liu et al., 2009a; 2009b; Hatami et al., 2013).

Moreover, silicon nanoparticle is a powerful antimicrobial substance that inhibits the growth of microorganisms in preservative solution and due to increasing the activity of antioxidant enzymes can reduce free radicals produced under stress condition. The results of the present study are consistent with Kazemi et al. (2012) indicated that silicon usage in combination with malic acid and salicylic acid compounds increased the postharvest life of Lisianthus. Therefore, it may be concluded that silicon nanoparticles are proper alternatives to silver-containing compounds for keeping the vase life. Furthermore the results show that silver and silicon nanoparticles were highly effective in combined form rather than sole application. However, other anti-ethylene substances such as salicylic acid or polyamines can be alternatively used instead of silver.

It is worth noting that anti-ethylene property of silver is effective on increasing vase life of cut flowers. Reduced activity of ACC oxidase enzyme after application of silver has been proved in many experiments (Liao et al., 2000; Liu et al., 2009a; Alimoradiet al., 2013; Hatami et al., 2013). Our findings revealed that the application of silver nanoparticle especially 20 and 40 mg L^{-1} decreased the ethylene content, however ethylene level considerably reduced by application of 40 mg $L^{-1}$of silicon nanoparticle. Delayed senescence and increased resistance of flowers caused by application of these nano particles could be attributed to the ethylene reduction in cut flowers. Moreover, silver and silicon nanoparticle are also powerful antimicrobial substance with capacity to prevent the microbial growth and reduce ethylene biosynthesis.

Therefore by reduction of ethylene level, flowers will stay for longer time.

Fresh weight loss of cut flowers is one of the initial stages of aging. An increase in the membrane permeability in the aging process increases the water loss of petals. Therefore, maintaining the water of flowers by different treatments plays vital role in the inhibition of aging. By increasing the age of cut flowers, the ability to absorb soluble diminishes and finally encounters with a decrease in cell turgor. Thus, measuring the fresh weight of flowers during post-harvest period is another important index to assess the durability of flowers (Farrahi et al., 2012). Nanosilver is a microbicide component that prevents microbe's attack to the cell wall and hence, reduces the number of bacteria that cause vascular obstruction which ultimately increases the water absorption (Singh et al., 2008).

Moreover, the decrease in transpiration is one of the important roles of silicon in plants (Cook and Leishman, 2011) which possibly cause an increase in relative fresh weight of cut flowers in treatments containing nano silicon, especially at a concentration of 40 mg L^{-1}.

Silver and silicon nanoparticles (40 mg L^{-1}) resulted in a significant increase in leaf chlorophyll content. It is known that ethylene causes chlorophyll degradation and that silver nanoparticles have anti-ethylene property. Silicon nanoparticles reduce the chlorophyll degradation by reducing free radicals (Gunes et al., 2007) and also can have a role in increasing the chlorophyll and dissolved solids content through increased photosynthesis in plants (Liang et al., 2003).

Conclusion

According to the results, it was determined that silicon in combination with silver was more effective on the extension of vase life of Lisianthusas compared to their individual application. Silicon and silver nanoparticle both inhibited microbial growth till the 15th day of storage. Moreover, the increased vase life of Lisianthus could be attributed to the antimicrobial property of silicon and anti-ethylene property of silver. Other compounds with inhibitory effects on ethylene biosynthesis like polyamines and salicylic acid can be used instead of silver. Given that these compounds are effective in very low concentrations and the flower vase life can be enhanced up to12 days. Beside their application is economical and affordable. Therefore, silver and silicon nanoparticles are recommended at concentration of 40 mg L^{-1} to enhance the vase life of Lisianthus cut flowers.

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