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Efficiency of Exogenous Salicylic Acid Application on Leaf Antioxidant Capacity and Vase Life of Cut Flower Lily (*Lilium Longiflorum*)

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Abstract **- In order to study the effect of salicylic acid foliar application on growth parameters and flower quality of Lilium, a factorial experiment in a completely randomized design with tree replication was performed. Aqueous solutions of salicylic acid at 0.0, 50, 100 and 200 µM were sprayed to run-off on each plant and leaf MSI and antioxidant capacity attributes as well as flower vase life and water uptake were evaluated. Results showed that the highest values of measured parameters were found when plants were treated by pre harvest application of salicylic acid at 50 and 100 µM. Results also showed the deteriorative effect of salicylic acid on leaf membrane stability index. Water uptake and vaselife of pretreated flowers after storing in the water was not directly correlated to pre-treatment. Our results showed an increasing trend in MDA and H_2O_2 production during the time after SA spray and but the senescing process in pre-treated flowers with 50ppm SA were lower.**

Key words- **Lilium (*Lilium longiflorum*), senescence, antioxidant, salicylic acid and membrane stability.**

Abbreviations-- **SA- Salicylic acid; EDTA - ethylenediaminetetra acetic acid; H2O2- hydrogen peroxide; POD – Guaiacol peroxidase; PVP - polyvinyl polypyrrolidone.**

I. Introduction

One-third of the value of the global ornamental floriculture market is contributed by cut flowers [10]. Lilies are distinguished by having large and attractive flowers. They are among the six most economically important major geniuses of bulbous plants [11, 12]. Maintaining the freshness and extending the vase life of cut lilies are very important to the success of this export crop. Short postharvest vase life is one of the most important Problems on the cut flowers. The postharvest performance of cut lilies is dependent on many factors such as the cultivar, pre-harvest conditions, stage of harvest, and postharvest environment and handling [30] as well as nutrition, ethylene gas and etc. [23, 25]. The maintenance of vase life is an important quality attribute in these economically significant cut flowers. Generally, the vase life of cut lilies varies between 5 to 14 days depending on the cultivar and vase life of the cut stem ends when flower petals wilt or turn brown [30]. Therefore, a suitable method for vase life extension, which is easy to use, natural, safe and inexpensive compounds are always crucial in this respect for large-scale applications. In this case, a series of experiments were performed to determine the effect of certain pre-treatment substances, on vase life, freshness and physiological characteristics in cut lily such as; Uniconazole [21], Citric Acid and Malic Acid [9], gibberellins (GA_{4+7}) alone or in combination with BA [(30, 37)], STS [36], amino acids mixture [13], Calcium [7], GA_3 and nutrient solution [27].

Salicylic acid (SA), a ubiquitous plant phenolic compound, regulates a number of processes in plants [(32, 16, 3)] and acts as a potential non-enzymatic antioxidant [32]. It is known that SA can enhance disease resistance of a few growing plants or detached plant organs [(26, 29)]. Reference [15] found that different concentrations of salicylic acid can delays senescence and enhances vase life-related properties in gladiolus (*Gladiolus grandiflora*). Findings of reference [1] showed that salicylic acid extends vase life of cut rose (*Rosa hybrida* cv. Black Magic) by disrupting catalase activity and regulation water relations. It has been known that exogenous SA application influences lipid peroxidation, chlorophyll fluorescence, antioxidant enzyme activity [34] and signaling pathogen-induced disease resistance [2]. One of the major roles for this compound is its impact on plant stress [8] reaction and delaying senescence. It has also been shown that SA increases vase life by improving the antioxidant system and reducing oxidative stress damages during rose flower senescence [17]. SA could increase peroxidase activity, raise fresh flower weight, enlarge flower diameter, and improve water balance of cut gerbera flowers [22].

In Our previous study the effect of SA pre-treatment were investigated on the quality of plant and the positive effect of SA on flowering stem length and diameter as well as buds on the inflorescence were observed [33], therefore in the present study we are interested to determine the effectiveness of pre-harvest application of SA in Lilium (*Lilium longiflorum* cv.tresor) cut flower, specially the change in water balance and vase life of cut flowers and antioxidant properties of leaf.

II. Materials And Methods

A. Plant material- The experiment was conducted during 2011 on Lilium at a greenhouse located at university of Maragheh, Iran. Each Lilium (*Lilium longiflorum* cv. tresor) F1 bulb (6cm in diameter) were

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planted in 1.4 L plastic containers with 8:1:1 v/v/v media composition (cocopeat, perlite and sand) and grown under natural light conditions. The temperature conditions were 25 ± 2°C and 16 ± 4°C, during days and nights respectively; with mean relative humidity of 50-70%. They were irrigated daily and were fertilized by 2 different modified 1/2 nutrient solution with the basic formula of Hogland [20] according to the vegetative and reproductive phase each 3 days.

B. Treatments- When average plant height was 20 cm, plants with uniform height were selected for foliar spray of salicylic acid. In this case, 500mL of SA at the rates of 0, 50, 100 and 200ppm were applied as spray on the foliage of each plant at vegetative phase before production of flower buds. The surface of pot was covered with aluminum foil to prevent the adding of SA solution into root medium. Untreated plants were left as a control and sprayed with distilled water in the same volume of salicylic acid.

C. Measurements- The plants were checked daily for signs of deterioration and the following traits were measured on the plant and flower:

Leaf Membrane stability index (MSI)- For determination of membrane stability index (MSI), fresh leaf samples were cut into small discs of uniform size (1 cm^3 each). Then samples were weighed and taken in test tubes containing10 ml of double distilled water. These tubes were incubated at 40°C in a water bath for 30 minutes and electrical conductivity of the samples was measured using conductivity bridge. The samples were transferred to the other test tubes and incubated at 100°C in the boiling water bath for 15 minutes and their electrical conductivity (EC) was measured as above. Membrane stability index was calculated and expressed in percentage using the formula MSI= [1-(C1/ C2)] ×100 [14].

Vase life- Pre-treated Stems were recut to 40-50cm length and store in distilled water. Vase life was considered to be terminated when wilting occurred.

Water uptake- The water uptake was calculated by subtracting the mean volume of water evaporated from three control bottle without cut flowers, from the amount of water decreased in bottles containing flowers in experimental course.

Assays of MDA content (lipid peroxidation)- Lipid peroxidation analysis was performed according to the method of Heath and Packer (1968) using 0.2g of fresh leaf tissue from each treatment. 1 ml MDA extract was added to 4 ml trichloroacetic acid containing 0.5% thiobarbituric acid. The solution was heated at 95 ˚C for 30 min and then quickly cooled in running water. The solution was centrifuged at 10000g for 10 min. The absorbance of the supernatant was measured at 532 and 600 nm. The concentration of MDA was calculated by subtracting absorbance at 600 nm from absorbance at 532 nm, and expressed as mg MDA g fresh weight (= 155mM $^{-1}$ cm).

Enzyme assays- Leaf samples were collected in an ice bucket and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5mM EDTA with pre chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4˚C in Beckman refrigerated centrifuge at 15000 rpm for 15 min. The supernatant was transferred to 30mL tubes and referred to enzyme extract. *Protein Content-* in the enzyme extracts was determined according to Bradford [5] using Bovine Serum Albumin V as a standard**.** *Catalase CAT (EC 1.11.1.6)* activity was measured according to [4], with minor modifications. The reaction mixture (1.5mL) consisted of 100mmol L-1phosphate buffer (pH 7.0), 0.1mmol L^{-1}EDTA, 20mmol $L^{-1}H_2O_2$ and 20 μ L enzyme extract. The reaction was started by addition of the extract. The decrease of $H_2O_2$was monitored at 240 nm and quantified by its molar extinction coefficient (36 $M^{-1}cm^{-1}$) and the results expressed as CAT units mg-1 of protein(U = 1 mM of H_2O_2reduction $min^{-1}mg^{-1}$protein). *Guaiacol peroxidase (POD)* activity was measured spectrophotometrically following the method of Tatiana, et al., (1999) with some modifications. The reaction mixture (3 ml) consisted of 30mM potassium phosphate buffer (pH 7), 6.5mM H_2O_2 and 1.5mM guaiacol. The reaction was started by the addition of 100 μl enzyme extract. The formation of tetraguaiacol was measured at 470 nm. *Hydrogen peroxide (H_2O_2)* concentration was determined according to reference [24] with some modification. Leaf samples of 0.5 g were homogenized in 3mL of 1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm and 4˚C for10 min. Subsequently, 0.75mL of the supernatant was added to 0.75mL of 10mM K-phosphate buffer (pH 7.0) and 1.5mL of 1M KI. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 100 to 1000 μmol mL^{2-1}. H_2O_2 concentration was expressed as μmol g^{-1} DW.

D. Statistical analysis- experiment was arranged in a factorial test with complete randomized design with tree replications. Data were subjected to analysis of variance (ANOVA) MSTATC software to determine treatment effects, and treatment means were compared using Duncan's multiple range test ($p<0.05$).

III. Results And Discussion

a. Results of experiment on vaselife and water uptake in cut flower lilium- According to fig 1 there is a significant difference ($p<0.01$) between treatments in the amount of water uptake during their vase life. It is shown that the flowers which are treated with 100 and 200ppm SA have more water uptake than 50ppm and controls. However the highest vase life is for flowers which are pre-threated with 50ppm SA (Fig. 2). It is clear that SA can absorb through plant and move along floems [28] so, foliar application of SA may modulated in opening of stomata

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[31] (data not shown) as well as more water uptake and photosynthesis. But in this regard the integrity of membranes play a key role and so the higher vase life of 50&100ppm SA-pretreated flowers despite of lower water uptake (maybe adequate) (fig.1) but high membrane stability index of them (Fig.3) is notable.

b. Result of Experiment in leaf

1. Stomata number- The effect of SA foliar application on the flowers were evaluated by counting the number of stomata at the same local of leaves in the all ranges of SA concentrations with a microscope (Olympus, CH-2) the day after spray with(×10) magnification. Results (data not analyzed) showed that the flowers treated with 50 and 100ppm SA have more open stomata about 60 and 67 compare to controls and 200 ppm (54 and 57, respectively).

2. Membrane stability index (MSI)- According to the figure 3 it is clear that the membrane of leaves of flowers treated with 50 and 100ppm SA are more stable that the one treated with 200ppm SA and also controls. So it can be suggested that SA is not only positive but also it can be very deteriorative according to the range of application or may be it has not any effect because of similarity to control. Although there is no difference between membrane integrity in flowers which are treated with 50 and 100ppm SA but, according to the vase life (Fig3) it is suggested that the mentioned dozes for pre application are more effective than the other one. Our findings are in agreement with reference [31].

3. Total protein content- Changes in total protein content are one of the parameters which are related to SA. In the study of the interaction between day and SA on the amount of total protein content, a significant difference ($P<0.01$) is showed in the leaf (Table 1).

Total protein content in the leaf have an increasing trend from the first day after treatment as the highest level of protein is shown at 6 day after SA application and also SA in 50ppm concentration have more effect on protein content in the leaves (Figure 4). Our results are in agreement with reference [1]. Generally at the beginning, the level of total protein in leaf can be less because of not activation of translation system and protein synthesis. This is an event which is illustrated in our results.

4. Lipid peroxidation (MDA)- According to table 1 it is resulted that the effect of day and treatment as well as the interaction between them on the amount of malon dialdehyde which is produced in the leaves is significantly different ($P<0.01$). Our findings showed an increasing trend in MDA production in the leaves and also the effect of SA at 100 and 50ppm concentration in preventing of membrane lipid peroxidation were more than 200ppm and control flowers (figure 5). It seems that the effect of SA in the stability of membranes in low levels is more than high levels such as 200ppm and growing phase in flowers will progress with less stress. Our results are agreed with findings of reference [1]. It seems that SA with decreasing in ethylene production or sensitivity of lilium flowers which are sensitive to ethylene cause to delay in senescence.

5. H_2O_2 production- Our findings imply that the amount of H_2O_2 production in the leaves was significantly different ($p<0.01$) and it is increased during the time in all treatments except for 50ppm SA treatment (figure 6). Increase in hydrogen peroxide in the leaves showing the existence of oxidative stress during the time and also increase in MDA and leaf senescence in consequent but this trend in 50ppm SA during the time are in across with others which are implying on the effectiveness of this treatment in preventing of senescence likely.

Generally the effect of SA on activation of antioxidant enzymes especially catalase can be the reason of less hydrogen peroxide level which is agree with [18] and [6] references. However this is not documented in our experiment in leaves.

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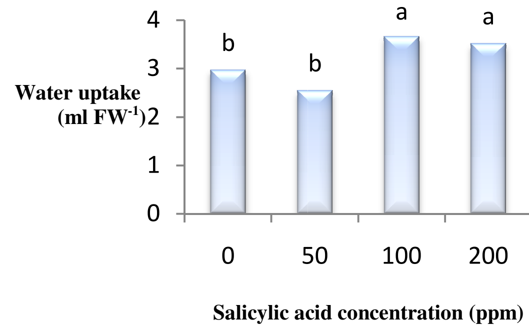

Fig 1. The effect of SA pre application on water uptake in cut flowers

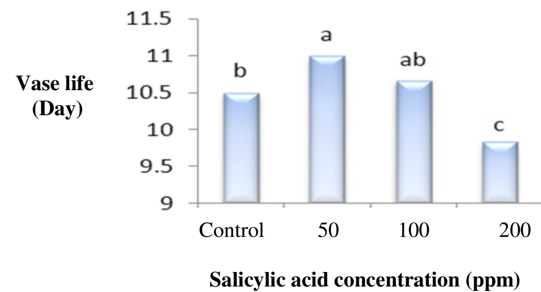

Fig 2. The effect of SA pre application on vaselife of cut flowers

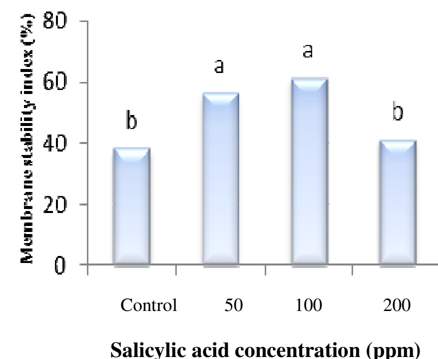

Fig3. The effect of SA pre application on Membrane stability index of cut flowers

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Table 1. Analysis of variance of SA pre-application on leaf biochemical traits

Means of Squares					Source of variation	
Hydrogen Peroxide	**Total protein**	**Malon dialdehide**	**Guayacol peroxidase**	**catalase**	**df**	
0.34	230.67**	68.16**	0.716	0.0797	7	day
0.46	248.51	69.94**	0.719	0.1155	4	SA
0.73**	250.97**	46.88**	0.535	0.1281	28	SA*day
0.17	74.73	16.47	0.596	0.0909	84	E

** significant at $p<0.01$, * significant at $p<0.05$

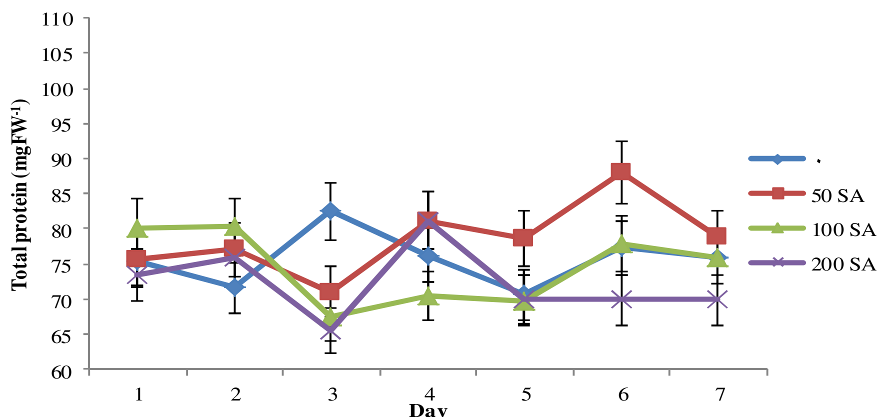

Figure4. The effect of SA pre-treatment on total protein in leaf.

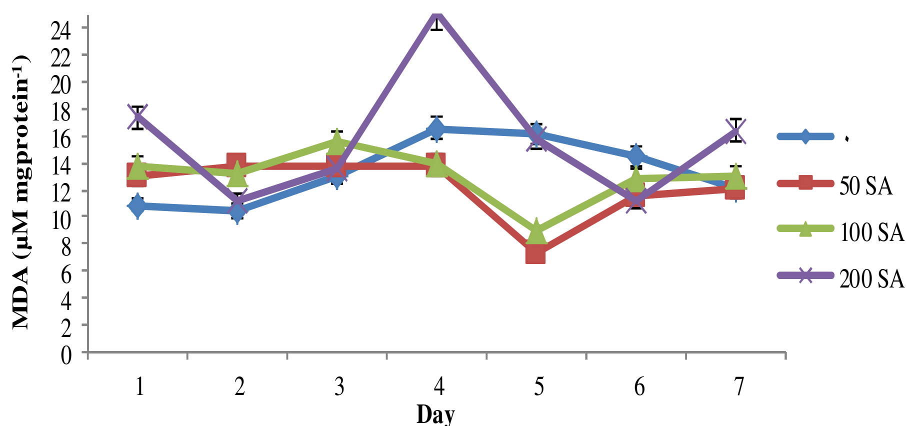

Figure 5. The effect of SA pre-treatment on MDA production in leaf during the time

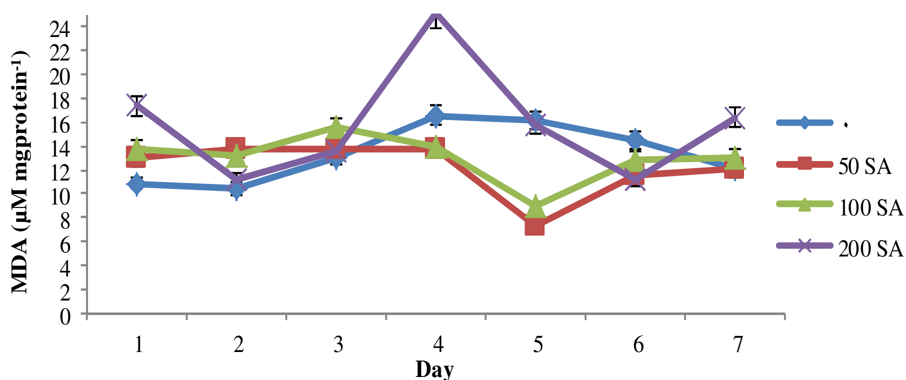

Figure6. The effect of SA pre-treatment on H2O2 production in leaf during the time

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