INFLUENCE OF MALIC ACID ON THE LONGEVITY OF CUT
Eustoma grandiflorum L. FLOWERS

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ABSTRACT
Cut Lisianthus (Eustoma grandiflorum L.) flowers are sensitive to microbial contamination and have relatively short vase life. Increasing the vase life of cut flowers is important from economical point of view. Malic acid is an organic acid with some roles in plants such as osmotic balance, pH regulation and energy source. An experiment was conducted to determine the effect of different concentrations of malic acid to extending the vase life and post harvest quality of Lisianthus cut flowers. Cut Lisianthus flowers were kept in solutions containing 0, 50, 100 and 200 mg l⁻¹ malic acid. The maximum vase life (13.50 days) was observed in flowers held in solution containing 100 mg l⁻¹ malic acid. The minimum vase life (8.50 days) was observed for the flowers kept in control solution. The maximum solution uptake (4.832 ml g⁻¹ F.W.) was calculated in 100 mg l⁻¹ malic acid, too. The highest dry matter (22.41%) was obtained in 200 mg l⁻¹ malic acid. The highest total chlorophyll content (9.973 mg g⁻¹ F.W.) was obtained in 50 mg l⁻¹ malic acid. Our results revealed that malic acid has the potential to extending vase life of cut Lisianthus flowers.

Keywords: Cut Lisianthus (Eustoma grandiflorum L.), vase solutions, flower longevity, ornamental plants, organic acids.

INTRODUCTION
Lisianthus (Eustoma grandiflorum) (Gentianaceae), a relatively new floral crop to the international market, quickly ranked in the top ten cut flowers worldwide due to its rose-like flowers, excellent post-harvest life, and being available in various colors (Kunitake et al., 1995). The cut inflorescences typically have a vase life of 3 to 6 weeks (Dennis et al., 1989). Lisianthus is a moderately cold-tolerant annual or biennial plant native to the southern part of the United States and Mexico (Roh and Lawson, 1988). This plant grows to 50-75 cm in height with 20-40 flowers. By nature, Lisianthus initially forms a rosette and grows very slowly during the winter, stems elongate in the spring, and it flowers in summer (Roh et al., 1989).

Microbial contamination due to bacterial growth in preservative solutions reduces the vase life of cut flowers (van Doorn, 1997; He et al., 2006). Stem end blockage is a major factor in the imbalance between water uptake and water loss of cut flowers (van Doorn, 1997; da Silva, 2003; He et al., 2006). Water balance is a major factor that determines the quality and longevity of cut flowers (Lü et al., 2010). There are three types of stem end blockage in cut flowers: physical air emboli (van Meeteren et al., 2006), microbial (Liu et al., 2009) and physiological wound-induced (He et al., 2006). Microorganisms are the most common cause of stem end blockage (van Doorn, 1997). Microbes can also produce ethylene, secrete toxic compounds and accelerate senescence (Williamson et al., 2002). Some compounds improve water uptake in cut flowers and extend vase life.

Malic acid is an intermediate organic acids in TCA cycle which produces cellular energy by oxidative phosphorylation. Malic acid plays an important role in osmotic balance of vacuole, pH regulation and energy source (Casati et al., 1999). Some studies have been performed on the effect of malic acid on vase life of several cut flowers (Darandeh and Hadavi, 2012; Kazemi et al., 2010). Germicide compounds in the vase solution reduce the number of microorganisms. Malic acid can reduce the population of bacteria in the vase solutions and decrease ethylene production and sensitivity (Kazemi et al., 2010).

MATERIALS AND METHODS
Cut Lisianthus (Eustoma grandiflorum) flowers were obtained from an economic producer in Tehran, Iran at their optimum developmental stage. They were immediately stood in buckets and transported to the postharvest laboratory. Buckets containing the flower stems were covered with a plastic film shroud to minimize moisture loss during transportation. At the laboratory, stems were re-cut under deionized water. The flowers were selected for uniformity of size, color and freedom from any defects.

The experimental design was a randomized completely blocks design (RCBD) with four treatments containing 0, 50, 100 and 200 mg l⁻¹ of ascorbic acid and four replications. In each experiment, five cut Lisianthus flowers were placed into 1000 mL vase filled with 250 mL of preservative solutions including the aforementioned concentrations of citric acid and 500 mL of 3% sucrose. Distilled water was used as a control. The mouths of the pots were covered with a sheet of low density polyethylene film to minimize evaporation and to prevent contamination. The flowers were kept in a vase life room under the following conditions: 20 ± 2°C, relative humidity of 60-70%, 12 µmol m⁻²s⁻¹ light intensity (cool white florescent tubes) and a daily light period of 12 h.

Criterion for the end of vase life was the time that flowers were showing symptoms of petals wilting or curling. Vase life was the period from the time of putting the cut flowers into the second preservative solution until flowers were showing symptoms of petals wilting or curling. Vase life was the period from the time of putting the cut flowers into the second preservative solution until the end of vase life.

Solution uptake was calculated as following formula:

\[\text{Solution uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100\%\]
Solution uptake = 500 - (volume of rest solution at the end day + mean of evaporation of 4 vase at that time

Fresh weight was measured with a digital balance. The first measurement was calculated exactly after the treatment and the last one was calculated at the last day of vase life. Loss of fresh weight (g) was obtained by following formula: fresh weight in first day of experiment - fresh weight at the end of vase life. To determine the dry matter, cut flowers were exposed at 70°C for 48 h in an oven and calculated by a digital balance. Dry matter percentage was obtained by following formula:

Dry matter percentage = Dry weight / fresh weight × 100

Chlorophyll contents of leaves were measured using spectrophotometry after five days. Chlorophyll was extracted by acetone 80% and measured by following formula:

Chlorophyll a = 9.93 (A660) – 0.777 (A643)
Chlorophyll b = 17.6 (A643) – 2.81 (A660)
Total chlorophyll = 7.12 (A660) – 16.8 (A643)

Data were subject to analysis of variance (ANOVA) and means were compared by the least significant difference (LSD) test at p≤0.05 using SPSS statistical software.

RESULTS AND DISCUSSIONS

Current study showed that malic acid prolonged the post-harvest life of cut Lisianthus (*Eustoma grandiflorum* L.) flowers. Differences of vase life in cut flowers held under different concentrations of malic acid was significant (p≤0.05) (Table-1). The minimum vase life (8.60 days) of Lisianthus was obtained in cut flowers kept in control vase solution (Table-2). Cut flowers kept in vase solution containing 100 mg l⁻¹malic acid had the longest vase life (13.50 days) (Table-2). There was no any positive effect between increasing vase life of cut flowers and increasing malic acid concentration. Positive effect of ascorbic acid on post-harvest of several cut flowers has been demonstrated (Eidyan, 2010; Kazemi *et al*., 2010; Jamshidi *et al*., 2012). Contrary to our findings, Darandeh and Hadavi (2012) did not show direct effect of malic acid on postharvest vase life of *Lilium*. The interaction effect between malic acid and citric acid on vase life proved significant. Study of Kazemi *et al*., (2010) on the effect of malic acid on vase life of cut carnation flowers demonstrated that malic acid at 150 mg l⁻¹ increased vase life. These researchers showed that the vase solution containing 150 mg l⁻¹ malic acid significantly decrease the number of bacteria on vase solution and increased vase life compared to the control. Malic acid prevents vascular blockage by reducing the number of bacteria in vase solution. Bacteria in vase solution block vessels on the cut surface. Stem occlusion reduced the water uptake (van Meeteren, 1978a). Some bacteria in vase solution produce ethylene. Ethylene induces vascular blockage and senescence. The use of other organic compounds such as salicylic acid, citric acid and ascorbic acid for increasing the vase life of cut flowers has been reported by some researchers (Darandeh and Hadavi, 2012; Jamshidi *et al*., 2012).

Table-1. Analysis of variance (ANOVA) of the effect of different concentrations of malic acid on various traits in cut Lisianthus (*Eustoma grandiflorum* L.) flowers.

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>df</th>
<th>Vase life (day)</th>
<th>Solution uptake (ml g⁻¹ F.W.)</th>
<th>Total chlorophyll (mg g⁻¹ F.W.)</th>
<th>Dry matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>16.88*</td>
<td>1.53*</td>
<td>7.47**</td>
<td>14.44**</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>6.97</td>
<td>0.43</td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>22.13</td>
<td>11.44</td>
<td>2.66</td>
<td>12.78</td>
</tr>
</tbody>
</table>

*: significance level at 1%, #: significance level at 5%

Table-2. Mean comparison of the effect of different concentrations of malic acid on various traits in cut Lisianthus (*Eustoma grandiflorum* L.) flowers*.

<table>
<thead>
<tr>
<th>Malic acid (mg l⁻¹)</th>
<th>Vase life (day)</th>
<th>Solution uptake (ml g⁻¹ F.W.)</th>
<th>Total chlorophyll (mg g⁻¹ F.W.)</th>
<th>Dry matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.60</td>
<td>2.55</td>
<td>7.66</td>
<td>16.42</td>
</tr>
<tr>
<td>50</td>
<td>13.25</td>
<td>3.61</td>
<td>9.97</td>
<td>20.13</td>
</tr>
<tr>
<td>100</td>
<td>13.50</td>
<td>4.83</td>
<td>8.19</td>
<td>21.85</td>
</tr>
<tr>
<td>200</td>
<td>12.12</td>
<td>3.36</td>
<td>6.79</td>
<td>22.41</td>
</tr>
</tbody>
</table>

*Values in each row that are followed by the same letter are not significantly different by LSD test.

Solution uptake was affected by malic acid (p≤0.01) (Table-1). Cut flowers in the solution containing 100 mg l⁻¹malic acid showed the maximum solution uptake rate (4.83 ml g⁻¹ F.W.) (Table-2). All concentrations of malic acid improved solution uptake than the control (2.55 ml g⁻¹ F.W.) (Table-2). Preferential solution uptake of cut flowers incubated in malic acid suggesting a possible decrease in xylem blockage due to reduced microbial growth and ethylene production. Positive effect of malic acid may be attributed to its antimicrobial activity that reduce bacterial population and resulted in increase the vessels conductivity and water uptake. Low water uptake by cut flowers is often due to occlusions located mainly in the basal stem end (He *et al*.,...
2006) and microorganisms and their decay products are a common cause of stem end blockage (van Doorn, 1997; Williamson et al., 2002). In many cut flowers, suppression of microbial growth in the vase solution results in delayed wilting (van Doorn, 1997).

Based on the present results, the chlorophyll content was affected by malic acid (p≤0.01) (Table-1). The maximum total chlorophyll content (9.97 mg g⁻¹ F.W.) was observed in cut flowers treated with 50 mg l⁻¹ malic acid (Table-2). The minimum total chlorophyll content (6.79 mg g⁻¹ F.W.) was observed in cut Lisianthus flowers treated with 200 mg l⁻¹ malic acid. Darandeh and Hadavi (2012) studies on Lilium revealed that malic acid increased the content of chlorophyll significantly than the control. These workers demonstrated that chlorophyll content was highest in cut flowers treated with 0.15%. Study of Kazemi et al. (2010) on the effect of malic acid on cut carnation flowers revealed that the total chlorophyll of flowers treated with 150 mg l⁻¹ malic acid was the maximum compared to the other concentrations and control. The differences of chlorophyll content between treatments could be attributed to a various amount of malic acid taken up by cut flowers (Kazemi et al., 2010).

Data analysis showed that the effect of malic acid was significant on dry matter (Table-1). Results showed that malic acid in proper concentration increased dry matter of cut Lisianthus flowers. Dry weight increased in case of 200 mg l⁻¹ ascorbic acid (22.41%) compared to the control (16.42%) (Table-2). Positive effect of malic acid on dry matter is probably due to its antimicrobial properties. The present results are in agreement with those reported by Jamshidiet al. (2012). These workers showed that malic acid decreased microbe's population and increased dry weight.

CONCLUSIONS
Cut Lisianthus (Eustoma grandiflorum L.) flowers have relatively short vase life. Enhancing the vase life of cut flowers is important. Malic acid has some roles in plants. Malic acid extends the vase life and postharvest quality of Lisianthus cut flowers if apply in proper concentration. In the present study, the maximum vase life was observed in flowers held in solution containing 100 mg l⁻¹ malic acid. The highest solution uptake was obtained in 100 mg l⁻¹ malic acid.

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