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# GERANIUM (*Pelargonium graveolens*) EXTRACT AND MECHANICAL TREATMENT IMPROVE WATER RELATION, ENZYME ACTIVITY AND LONGEVITY OF CUT CHRYSANTHEMUM (*Dendranthema grandiflorum* (Ramat.) Kitamura) FLOWERS

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**Abstract.** Application of safe organic compounds for the environment is an important approach to increase the longevity of cut flowers. Essences and herbal extracts are compounds having antimicrobial properties, thereby increasing the post-harvest life of cut flowers. Mechanical treatments such as splitting in the stem end also increase longevity of cut flowers. Essences and extracts of geranium (*Pelargonium graveolens*) are biological compounds with antimicrobial and antioxidant properties that can be used as a vase solution. In this study, different concentrations of rose scented geranium extract and stem end slot were used to improve post-harvest life of cut chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) flowers. The results showed that longevity of cut flowers treated in vase solution containing 100 mg $l^{-1}$ rose scented geranium extract (18.43 days) with a slot of 5 cm at the stem end was significantly more than that of the control (8.11 days). Water status, microbial population in vase solution and stem end, ionic leakage, and enzymes activities were significantly different between treated cut flowers and control.

**Key words:** microbial activity, non-chemical treatments, vascular obstruction, vase life, water absorption

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## INTRODUCTION

Flowers aging is a controlled physiological and biochemical process. This process involves an imbalance of water in the plant, an increase in the activity of degrading enzymes, the destruction of the macromolecules, an increase in respiration and cell membrane damage. Due to the separation of cut flowers from the intact plant, establishing effective water relations in vase solution has an important role in post-harvest physiology of cut flowers [Golias and Kobza 2003].

Among the cut flowers, chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) belongs to Asteraceae (Compositae) family has a very important role in the business of flowers and ornamental plants [Zhang et al. 2013]. Nowadays, it has the second ranking after the rose in the terms of economy and horticultural industry [Teixeira-da-Silva 2003]. Since, visual appeal is the main reason for buying and selling cut flowers, many attempts have been done to increase the vase life of these flowers. Therefore, the use of methods that increase the quality and longevity of cut flowers has a special position in the industry of production, maintenance and business [Anderson et al. 2004, Gul and Tahir 2013].

Water stress and an increase in hydraulic resistance and air embolism within vessels of the stem of flowers such as chrysanthemum causes to prevent the transfer of water in the stem and vascular obstruction. This process can be a major cause of premature wilting of petals, lack of proper flowers opening, drying leaves, leaves chlorophyll degradation and ultimately reducing the longevity [Van Leperen et al. 2001, Damunupola et al. 2010, Ahmad et al. 2011]. Mechanical treatments such as re-cutting the stem end under water and create a split at the stem end are some procedures to solve these problems. Ahmad et al. [2011] reported that creating a split in the stem end and other mechanical treatments increase post-harvest life and improve qualitative features of rose cut flowers. Moreover, Mortazavi et al. [2007] found that applying the mechanical treatments on the cut flowers is effective in most post-harvest traits directly and some of them indirectly. These reports are in accordance with the results of Laird et al. [2003] on rose cut flowers. Other factors such as the population of bacteria at the stem end and vase solution can be effective in lacking proper absorption of water by cut flowers and reducing post-harvest life [Liu et al. 2009a, b, Wittea et al. 2014].

The use of preservative compounds in the vase solution is one of the common methods to extend the vase life of cut flowers. Herbal extracts and essential oils are the examples of preservative compounds. These compounds are friend with the environment, cost effectiveness and having biological origin as well as containing high concentrations of phenolic compounds with antimicrobial and antioxidant properties and can be a proper alternative for high-risk chemical preservative solutions [Solgi et al. 2009]. Therefore, these compounds have been interest and popularity of many researchers around the world [Sivakumar and Bautista-Banos 2014]. Study on gerbera showed that the use of extracts of *Thymus vulgaris* and *Zataria multiflora* as well as the active ingredients in them leads to an increase in the longevity of gerbera cut flowers [Solgi et al. 2009]. Mousavi Bazaz and Tehranifar [2011] reported positive effect of herbal essential oils of peppermint, thyme and caraway in the vase solution to increase the longevity of cut flowers. However, several studies has recently been done on the effect of herbal extracts and essential oils on postharvest quality

of cut flowers, but these studies are very low. There are a few researches on the effects of mechanical treatments at the stem end on postharvest durability and quality of cut flowers. Therefore, the aim of this study was to evaluate the association between the use of geranium (*Pelargonium graveolens*) extract in vase solution, creating split at the stem end and their interactions on microbial activity, improving water relations and activity of antioxidant enzymes to improve some qualitative features and longevity of chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) cut flowers.

## MATERIALS AND METHODS

**Plant material and experiment place.** Chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) cut flowers were prepared from a greenhouse located in Isfahan, Iran. Uniform cut flowers were transferred to the postharvest laboratory of Islamic Azad University, Rasht, Iran, immediately. Experiment was performed as a factorial based on completely randomized design with two factors including the split of the stem end in two levels (non-split and split of the stem end), and the continuous use of geranium (*Pelargonium graveolens*) extract in 6 levels (0, 1, 2, 4, 8, and 10%) with 12 treatments, 3 repetitions, 36 plots, 4 flowers in each plot and a total of 144 cut flowers. Cut flowers were kept in conditions including 12 h photoperiod, light intensity of 12 µmol $m^{-2}$ $s^{-1}$, relative humidity of 60 to 70% and the temperature of 22 ±2°C (tab. 1).

Table 1. Composition the split of the stem end and concentrations of used geranium (*Pelargonium graveolens*) extract

| | |
|---|---|
| $S_0$ | non-split of the stem end (control) |
| $S_1$ | split of the stem end (5 cm) |
| $O_0$ | 500 ml distilled water (control) |
| $O_1$ | 1% geranium extract |
| $O_2$ | 2% geranium extract |
| $O_4$ | 4% geranium extract |
| $O_8$ | 8% geranium extract |
| $O_{10}$ | 10% geranium extract |
| $S_0O_0$ | non-split of the stem end + 500 ml distilled water (control) |
| $S_0O_1$ | non-split of the stem end + 1% geranium extract |
| $S_0O_2$ | non-split of the stem end + 2% geranium extract |
| $S_0O_4$ | non-split of the stem end + 4% geranium extract |
| $S_0O_8$ | non-split of the stem end + 8% geranium extract |
| $S_0O_{10}$ | non-split of the stem end + 10% geranium extract |
| $S_1O_0$ | split of the stem end + 500 ml in distilled water |
| $S_1O_1$ | split of the stem end + 1% geranium extract |
| $S_1O_2$ | split of the stem end + 2% geranium extract |
| $S_1O_4$ | split of the stem end + 4% geranium extract |
| $S_1O_8$ | split of the stem end + 8% geranium extract |
| $S_1O_1$ | split of the stem end + 10% geranium extract |

Table 2. Chemical composition of essential oil of geranium (*Pelargonium graveolens*)

| Number | Compounds | Percentage of total oil | KI index (%) |
|---|---|---|---|
| 1 | spathulenol | 0.67 | 1656 |
| 2 | 6-octen-1-ol, 3,7-dimethyl | 0.15 | 1543 |
| 3 | alpha-pinene | 0.12 | 955 |
| 4 | beta-citronellol | 22.90 | 1385 |
| 5 | 1H-cycloprop[e]azulene | 0.12 | 1459 |
| 6 | 1H-cyclopropa[a]naphthalene | 0.10 | 1597 |
| 7 | beta-bourbonene | 0.94 | 1447 |
| 8 | beta-cubebene | 0.78 | 1534 |
| 9 | cadina-1,4-diene | 0.15 | 1590 |
| 10 | cis-2,6-dimethyl-2,6-octadiene | 4.81 | 2019 |
| 11 | germacrene-d | 2.87 | 1549 |
| 12 | cis-rose oxide | 0.81 | 1128 |
| 13 | delta cadinene | 0.42 | 1495 |
| 14 | epizonarene | 0.84 | 1583 |
| 15 | 6-octen-1-ol, 3,7-dimethyl- (R) | 0.16 | 1266 |
| 16 | cycloundecatriene-4,7,10 | 1.64 | 1520 |
| 17 | gamma-elemene | 0.17 | 1647 |
| 18 | delta-cadinene, 4-diene naphthalene | 0.44 | 1563 |
| 19 | citral | 0.61 | 1305 |
| 20 | naphthalene | 1.20 | 1610 |
| 21 | caryophyllene oxide | 16.11 | 1668 |
| 22 | 3,7-guaiadiene | 0.32 | 1495 |
| 23 | geraniol | 13.03 | 1293 |
| 24 | linalool | 1.60 | 1114 |
| 25 | cyclohexanone | 5.50 | 1202 |
| 26 | butanoic acid | 4.70 | 2064 |
| 27 | 6-octen-1-ol | 8.50 | 1455 |
| 28 | alpha-amorphene | 1.77 | 1528 |
| 29 | geranyl tiglate | 3.24 | 1202 |
| 30 | isoaromadendrene epoxide | 0.19 | 1743 |
| 31 | caryophyllene oxide | 2.32 | 1668 |
| 32 | geranyl propionate | 0.26 | 1965 |
| 33 | L-(-)-methyl | 0.10 | 1222 |
| 34 | 1,6-octadien-3-ol, 3,7-dimethyl | 0.65 | 1275 |
| 35 | 1,6-octadien-3-ol, 3,7-dimethyl (R) | 7.93 | 1293 |
| 36 | e-citral, 3,7-dimethyl-2,6-octadienal | 0.67 | 1305 |
| 37 | alpha-copaene | 1.10 | 1427 |
| 38 | 4,7,10-cycloundecatriene | 1.64 | 1520 |
| 39 | 1,2 benzenedicarboxylic acid | 0.32 | 2006 |
| 40 | citronella | 0.51 | 1167 |
| 41 | trans-rose oxide | 0.30 | 1149 |
| 42 | alpha-amorphene | 1.77 | 1528 |

To create a split at the stem end, at first the flowers were cut at the height of 30 cm, then a 5 cm long split was made at the stem end using a laboratory blade. In order to prepare the pelargonium extract, geranium (*P. graveolens*) cuttings were planted on January 22, 2014 in the beds of peat + perlite + garden soil (1:1:1) in a greenhouse with a relative humidity of 60 to 70% and a temperature of 22 ±2°C. The cuttings were kept in a greenhouse for two months and irrigated using spray once every three days. Then, rooted cuttings in the six-leaf stage were transferred to the main bed. To enhance the growth and development of the plant, Nitroxin bio-fertilizer (liquid Azotobacter) was used. The rooted cuttings were stained with a solution containing diluted bio-fertilizer, then planted in the main bed. To prevent fungal diseases and pests, a fungicide and insecticide were used. Irrigation of plants was performed until the end of the experiment every three days. In order to avoid of reduction in vegetative growth, all buds appeared on the plants were cut as soon as they appeared. Leaves were harvested in July and dried outdoor and shade with a temperature of around 30°C in order to prepare the plant extract and essential oils (tab. 2).

**Preparation of geranium extract.** The plant materials (leaves) were dried in 45°C and kept air-tight in 2°C till extraction. The 30 g dried plant materials were subjected to hydrodistillation for 4 h using a Clevenger – type apparatus to produce volatiles. The extract was dried over anhydrous calcium chloride and stored in sealed vials at 2°C until the time of application.

**Measured traits.** In this study, longevity of cut flowers, bacteria population in vase solution and stem end, solution absorption, ionic leakage, leaves chlorophyll, petals anthocyanins, the amount of malondialdehyde (MDA), and peroxidase (POD) and superoxide dismutase (SOD) enzymes activity were evaluated.

**Longevity.** Assessment of longevity of cut flowers was done in the interval of starting the treatment until the ageing the flowers that was along with observation of petals fading, changing petals color to yellowness, falling petals and their wilting. Average of the flowers life was considered as the longevity of that plot.

**Solution absorption.** According to the initial volume of vase solution (500 ml) and the evaporation rate of vase life room and reducing the volume of vase solution, water absorption was calculated by the following formula:

Water absorption (ml $g^{-1}$ F.W.) = 500 ÷ (mean of room evaporation + reminded solution at the end of longevity).

**Bacteria population in the vase solution and stem end.** Sampling the bacteria of vase solution was performed 24 h after beginning the experiment and bacteria counting was performed using Liu et al. [2009a, b] method.

**Chlorophyll content.** In order to measure the chlorophyll content, at the last day of control flower's life, a flower was removed from each plot to measure leaf chlorophyll. The total chlorophyll content was measured using Mazumdar and Majumder [2003] method.

Chlorophyll a = 9.93 ($A_{663}$) – 0.777 ($A_{645}$)

Chlorophyll b = 22.9 ($A_{645}$) – 4.86 ($A_{663}$)

Total Chlorophyll = Chlorophyll a + Chlorophyll b;

where: A is light absorbance at wavelengths of 663 and 645 nm.

**Petals anthocyanin.** A flower was removed from each plot at the end of longevity of control flowers and the content of petal's anthocyanin pigment was measured using Mazumdar and Majumder [2003] method.

**Ionic leakage.** At the end of longevity of control flowers, 0.5 g leaf plot$^{-1}$ with 51 ml of distilled water was kept into a closed container at laboratory temperature for 24 h and then $EC_1$ was calculated using an EC-meter device. In order to measure $EC_2$, 0.5 g of leaves were frozen at temperature of -21°C for 24 h and after 24 h leaves were again placed at room temperature for 24 h and then numbers were recorded using the EC-meter device and ionic leakage was calculated using the following formula: Ionic leakage: $EC_1 \div EC_2 \times 100$.

**Malondialdehyde (MDA).** A flower was separated at the end of longevity of control flowers and its petals were used to measure the membrane peroxidation (MDA) using Heath and Parker [1986] method as a product of peroxidation reaction of membrane's fatty acids.

**Superoxide dismutase (SOD).** At the end of longevity of control flowers, a flower was separated from each plot and its petals were measured to evaluate the SOD with the spectrophotometer device and Giannopolitis and Ries [1997] method.

**Peroxidase (POD).** For this purpose, at the end of vase life of control flowers, a flower was separated and its petals were used for measuring the kinetic activity of POD enzyme with In et al. [2007] method.

**Statistical analysis.** Obtained data were analyzed using SAS statistical software and the means comparison were performed by LSD test. Correlation procedure of SPSS statistical software was used to calculate the correlation coefficients between features.

## RESULTS

Analysis of variance (tab. 3) showed that a 5 cm split at the stem end and extract of geranium (*P. graveolens*) used in the preservative solution and the interaction of these two factors have a significant effect on longevity and other measured characteristics. There is a significant difference between the control and other treatments corresponding to measured traits (tab. 3, figs. 1, 2, 3, 4, 5 and 6). The results of analysis of correlation coefficients between data showed a significant correlation between some of studied traits (tab. 5).

**Longevity (vase life).** According to the ANOVA (tab. 3), the individual effects of geranium (*P. graveolens*) extract and split at the stem end, as well as the interaction of these two factors have a statistically significant difference ($P \leq 0.1$). The results of the comparison of data showed that all treatments have a significant effect on longevity compared to the control. Control treatment ($S_0O_0$) with an average of 8.11 days and $S_1O_{10}$ treatment (split of the stem end and 10% geranium extract) with an average of 18.43 days have minimum and maximum longevity among all treatments, respectively (tab. 4, fig. 1). In addition, the treatment of split at the stem end and 8% geranium extract with an average of 18.13 days had the highest longevity after $S_1O_{10}$ treatment. Generally, by increasing geranium concentration in the vase solution, a significant increase in longevity of flowers was observed (tab. 4, fig. 1). The results showed that the longevity is significantly correlated with absorption of solution ($r = 0.78 \leq 0.01$), bacteria in vase solution ($r = -0.77 \leq 0.01$), bacteria in the stem end ($r = -0.90 \leq 0.01$), chlorophyll content ($r = 0.65 \leq 0.05$), petals anthocyanin ($r = 0.67 \leq 0.05$), ionic leakage ($r = -0.70 \leq 0.05$), MDA ($r = -0.68 \leq 0.05$) and SOD ($r = -0.80 \leq 0.05$) (tab. 5).

Table 3. Analysis of variance of the split of the stem end and geranium (*Pelargonium graveolens*) extract on chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) cut flower

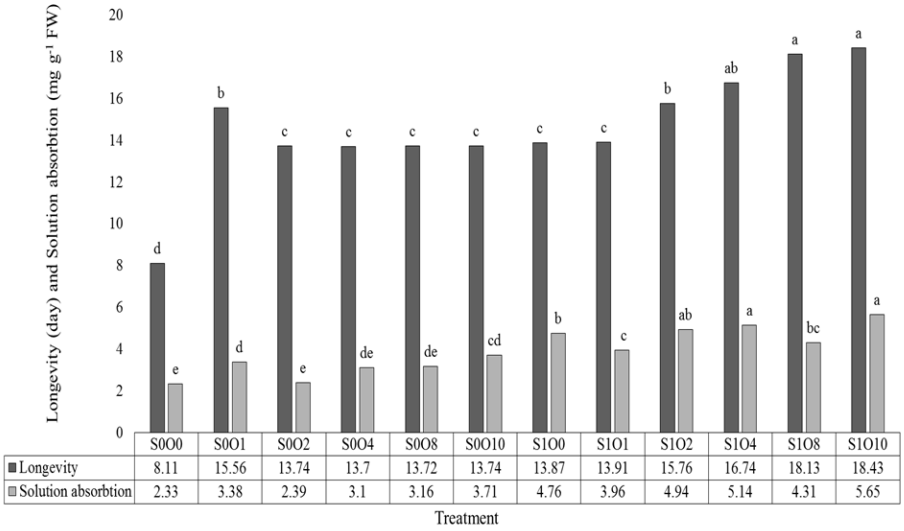
| Source of variations | df | M.S. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | longevity | solution absorption | bacteria in the vase solution | bacteria in the stem end | chlorophyll content | petals anthocyanin | ionic leakage | malondial-dehyde | superoxide dismutase | peroxidase |
| Split of the stem end (S) | 1 | 15.04** | 1.32* | 45.32** | 56.41** | 5.52** | 14.45** | 23.19* | 97.35** | 0.10** | 31.73** |
| Geranium extract (O) | 5 | 91.52** | 28.81** | 47.21** | 71.94** | 0.69** | 19.85** | 25.25* | 150.14** | 0.15** | 64.93** |
| S × O | 1 | 14.62** | 2.49* | 23.16** | 50.09** | 0.40** | 22.44** | 29.51* | 22.92** | 0.84** | 38.38** |
| Error | 1 | 1.93 | 0.49 | 3.15 | 8.45 | 0.16 | 0.18 | 5.32 | 0.35 | 0.4 | 0.41 |
| C.V. (%) | 2 | 9.31 | 17.29 | 50.86 | 10.83 | 21.89 | 26.83 | 31.22 | 21.83 | 10.74 | 22.92 |

** – significant at 1%, * – significant at 5%, ns – not significant

Table 4. Mean comparison of the main effect of the split of the stem end and geranium (*Pelargonium graveolens*) extract on chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) cut flowers*

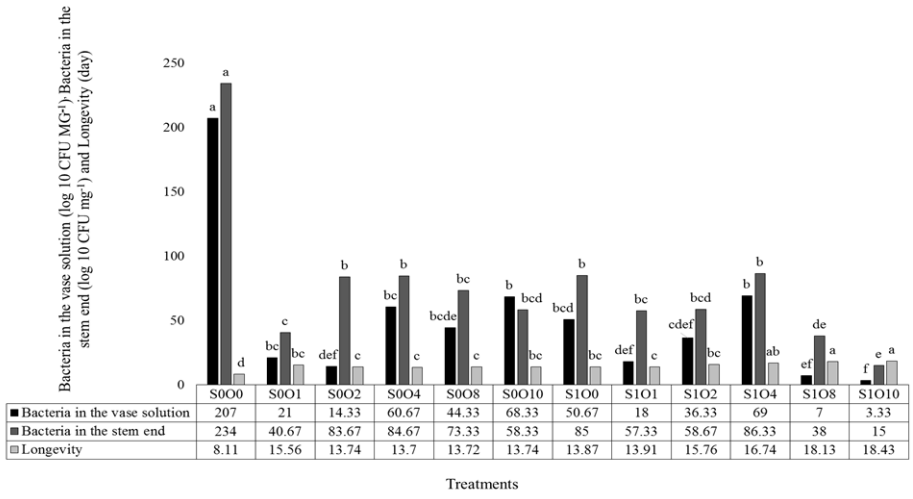
| | Treat-ment | Longevity (day) | Solution absorption (ml $g^{-1}$ FW) | Bacteria in the vase solution (log10 CFU $mg^{-1}$) | Bacteria in the stem end (log10 CFU $mg^{-1}$) | Chlorophyll content (mg $g^{-1}$ FW) | Petals anthocyanin (µg $g^{-1}$ FW) | Ionic leakage (%) | Malondial-dehyde (nmol $g^{-1}$ FW) | Superoxide dismutase (units $g^{-1}$ FW) | Peroxidase (nmol $g^{-1}$ FW) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Split of the stem end (S) | $S_0$ | $13.25^{b}$ | $3.18^{b}$ | $69.27^{a}$ | $95.77^{b}$ | $3.71^{b}$ | $6.70^{b}$ | $8.22^{b}$ | $22.77^{a}$ | $17.53^{a}$ | $0.071^{a}$ |
| | $S_1$ | $16.43^{a}$ | $4.97^{a}$ | $30.72^{b}$ | $59.38^{a}$ | $4.50^{a}$ | $7.97^{a}$ | $6.55^{a}$ | $19.48^{b}$ | $14.85^{b}$ | $0.060^{b}$ |
| Geranium extract (O) | $O_0$ | $10.97^{c}$ | $3.55^{bc}$ | $128.80^{a}$ | $159.5^{a}$ | $2.97^{f}$ | $5.38^{e}$ | $10.08^{a}$ | $29.90^{a}$ | $20.54^{a}$ | $0.079^{ab}$ |
| | $O_1$ | $15.23^{ab}$ | $4.34^{ab}$ | $25.33^{c}$ | $71.17^{bc}$ | $3.81^{d}$ | $5.84^{e}$ | $7.73^{b}$ | $23.23^{b}$ | $18.87^{b}$ | $0.081^{a}$ |
| | $O_2$ | $14.64^{b}$ | $3.48^{c}$ | $64.83^{b}$ | $85.50^{b}$ | $4.34^{c}$ | $8.70^{b}$ | $9.21^{a}$ | $20.77^{c}$ | $17.14^{b}$ | $0.083^{a}$ |
| | $O_4$ | $15.91^{ab}$ | $4.20^{abc}$ | $25.67^{c}$ | $55.67^{cd}$ | $4.78^{b}$ | $10.18^{a}$ | $5.30^{c}$ | $21.39^{c}$ | $18.25^{b}$ | $0.078^{b}$ |
| | $O_8$ | $14.74^{b}$ | $4.17^{abc}$ | $19.50^{c}$ | $57.00^{cd}$ | $3.63^{e}$ | $6.60^{d}$ | $6.45^{bc}$ | $18.67^{d}$ | $16.14^{c}$ | $0.073^{bc}$ |
| | $O_{10}$ | $16.56^{a}$ | $4.70^{a}$ | $35.83^{c}$ | $36.67^{d}$ | $5.09^{a}$ | $7.31^{c}$ | $5.55^{c}$ | $16.81^{e}$ | $15.18^{d}$ | $0.062^{c}$ |

* – in each column, means with the similar letters are not significantly different at 5% level of probability using LSD test



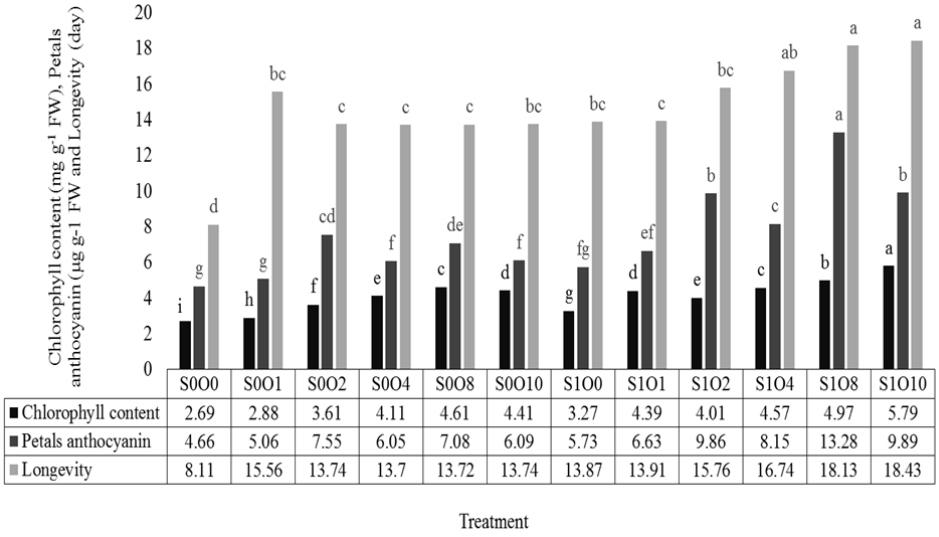

| | S0O0 | S0O1 | S0O2 | S0O4 | S0O8 | S0O10 | S1O0 | S1O1 | S1O2 | S1O4 | S1O8 | S1O10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Longevity | 8.11 | 15.56 | 13.74 | 13.7 | 13.72 | 13.74 | 13.87 | 13.91 | 15.76 | 16.74 | 18.13 | 18.43 |
| Solution absorbtion | 2.33 | 3.38 | 2.39 | 3.1 | 3.16 | 3.71 | 4.76 | 3.96 | 4.94 | 5.14 | 4.31 | 5.65 |

Fig. 1. Effect of interactions between splitting of the stem end and geranium extract on the longevity and solution absorption in chrysanthemum (*Dendranthema grandiflorum* L.) cut flowers



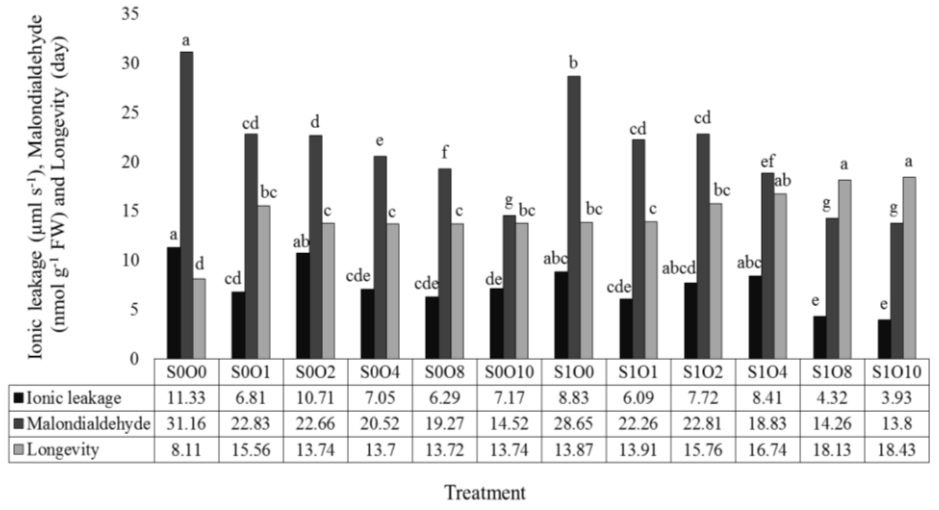

| | S0O0 | S0O1 | S0O2 | S0O4 | S0O8 | S0O10 | S1O0 | S1O1 | S1O2 | S1O4 | S1O8 | S1O10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria in the vase solution | 207 | 21 | 14.33 | 60.67 | 44.33 | 68.33 | 50.67 | 18 | 36.33 | 69 | 7 | 3.33 |
| Bacteria in the stem end | 234 | 40.67 | 83.67 | 84.67 | 73.33 | 58.33 | 85 | 57.33 | 58.67 | 86.33 | 38 | 15 |
| Longevity | 8.11 | 15.56 | 13.74 | 13.7 | 13.72 | 13.74 | 13.87 | 13.91 | 15.76 | 16.74 | 18.13 | 18.43 |

Fig. 2. Effect of interactions between splitting of the stem end and geranium extract on bacteria in the vase solution and bacteria in the stem end in chrysanthemum (*Dendranthema grandiflorum* L.) cut flowers




| | S0O0 | S0O1 | S0O2 | S0O4 | S0O8 | S0O10 | S1O0 | S1O1 | S1O2 | S1O4 | S1O8 | S1O10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chlorophyll content | 2.69 | 2.88 | 3.61 | 4.11 | 4.61 | 4.41 | 3.27 | 4.39 | 4.01 | 4.57 | 4.97 | 5.79 |
| Petals anthocyanin | 4.66 | 5.06 | 7.55 | 6.05 | 7.08 | 6.09 | 5.73 | 6.63 | 9.86 | 8.15 | 13.28 | 9.89 |
| Longevity | 8.11 | 15.56 | 13.74 | 13.7 | 13.72 | 13.74 | 13.87 | 13.91 | 15.76 | 16.74 | 18.13 | 18.43 |

Fig. 3. Effect of interactions between splitting of the stem end and geranium extract on chlorophyll content and petals anthocyanin in chrysanthemum (*Dendranthema grandiflorum* L.) cut flowers




| | S0O0 | S0O1 | S0O2 | S0O4 | S0O8 | S0O10 | S1O0 | S1O1 | S1O2 | S1O4 | S1O8 | S1O10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ionic leakage | 11.33 | 6.81 | 10.71 | 7.05 | 6.29 | 7.17 | 8.83 | 6.09 | 7.72 | 8.41 | 4.32 | 3.93 |
| Malondialdehyde | 31.16 | 22.83 | 22.66 | 20.52 | 19.27 | 14.52 | 28.65 | 22.26 | 22.81 | 18.83 | 14.26 | 13.8 |
| Longevity | 8.11 | 15.56 | 13.74 | 13.7 | 13.72 | 13.74 | 13.87 | 13.91 | 15.76 | 16.74 | 18.13 | 18.43 |

Fig. 4. Effect of interactions between splitting of the stem end and geranium extract on ionic leakage and malondialdehyde in chrysanthemum (*Dendranthema grandiflorum* L.) cut flowers

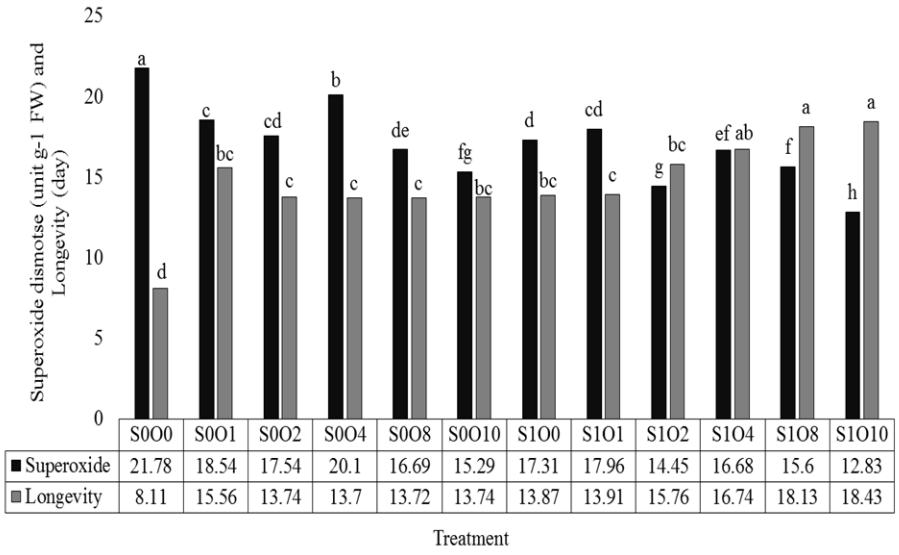



Fig. 5. Effect of interactions between splitting of the stem end and geranium extract on superoxide dismutase in chrysanthemum (*Dendranthema grandiflorum* L.) cut flowers

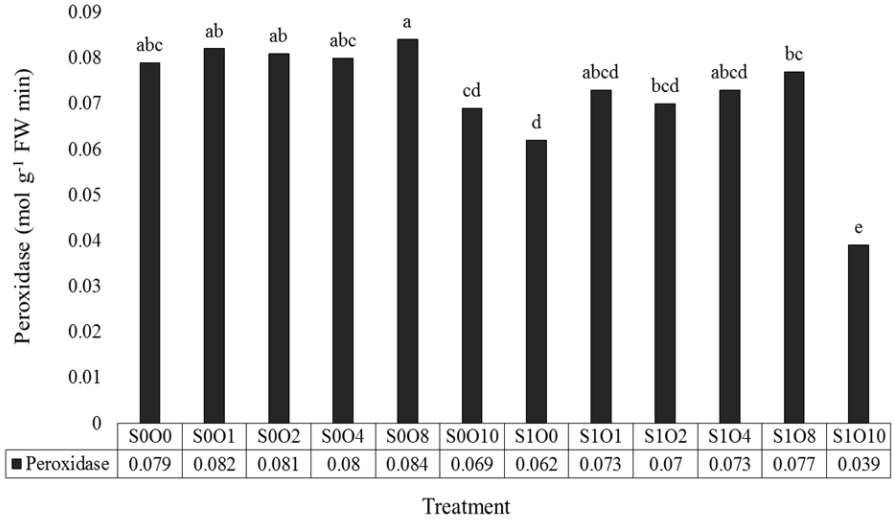



Fig. 6. Effect of interactions between splitting of the stem end and extract on peroxidase in chrysanthemum (*Dendranthema grandiflorum L.*) cut flowers

Table 5. Pearson's correlation matrix between the measured traits of chrysanthemum *(Dendranthema grandiflorum* (Ramat.) Kitamura) cut flowers

| | Longevity | Solution absorption | Bacteria in the vase solution | Bacteria in the stem end | Chlorophyll content | Petals anthocyanin | Ionic leakage | Malondial-dehyde | Superoxide dismutase | Peroxidase |
|---|---|---|---|---|---|---|---|---|---|---|
| Longevity | 1.00 | | | | | | | | | |
| Solution absorption | 0.78** | 1.00 | | | | | | | | |
| Bacteria in the vase solution | -0.77** | -0.42$^{ns}$ | 1.00 | | | | | | | |
| Bacteria in the stem end | -0.90** | -0.53$^{ns}$ | 0.93** | 1.00 | | | | | | |
| Chlorophyll content | 0.65* | 0.69* | -0.55$^{ns}$ | 0.62* | 1.00 | | | | | |
| Petals anthocyanin | 0.67* | 0.76** | -0.50$^{ns}$ | -0.52$^{ns}$ | 0.69** | 1.00 | | | | |
| Ionic leakage | -0.70* | -0.47$^{ns}$ | 0.66* | 0.77** | -0.77** | -0.56$^{ns}$ | 1.00 | | | |
| Malondialdehyd | -0.68* | -0.46$^{ns}$ | 0.60* | 0.75** | -0.86** | -0.62* | -0.78** | 1.00 | | |
| Superoxide dismutase | -0.80** | -0.78** | 0.64* | 0.76** | -0.75** | -0.68* | -0.61* | 0.71** | 1.00 | |
| Peroxidase | -0.50$^{ns}$ | -0.70* | 0.19$^{ns}$ | 0.37$^{ns}$ | 0.53$^{ns}$ | -0.28$^{ns}$ | 0.40$^{ns}$ | 0.31$^{ns}$ | 0.68* | 1.00 |

Levels of significance: * – $p \leq 0.05$, ** – $p \leq 0.01$, ns – not significant

**Solution absorption.** The results of ANOVA obtained from solution absorption suggests that the main effect of two factors of herbal extract (1%), split at the stem end and interaction between these two factors (5%) on the solution absorption had a significant difference (tab. 3). According to the results of mean comparison between treatments (tab. 4) with creating a split at the stem end and increasing geranium (*P. graveolens*) extract concentration in vase solution, absorption of solution by the cut flowers is significantly increased. Treatment of $S_0O_0$ (control) with an average of 2.33 ml $g^{-1}$ FW and $S_1O_{10}$ treatment (split of the stem end and 10% geranium extract) with an average of 5.65 ml $g^{-1}$ FW had the lowest and the highest absorption from the vase solution, respectively (fig. 1). Results of correlation coefficients showed that in addition to the features listed on previous results this feature had a significant correlation with chlorophyll content ($r = 0.69 \leq 0.05$), petals anthocyanin ($r = 0.76 \leq 0.1$), the activity of SOD ($r = -0.78 \leq 0.01$) and POD ($r = -0.70 \leq 0.05$) (tab. 5).

**Bacteria in the vase solution.** Analysis of variance (tab. 3) showed that applying a split at the stem end, geranium (*P. graveolens*) extract and the interaction of these two factors had a significant difference ($P \leq 0.1$) on the population of bacteria in the vase solution. Mean comparison of data showed that the maximum population of bacteria in vase solution is related with control treatment with the average of 207 $\log^{10}$ CFU $mg^{-1}$ and the minimum of that is related to split treatment with 10% geranium extract ($S_1O_{10}$) with an average of 3.33 $\log^{10}$ CFU $mg^{-1}$ (fig. 2). This feature is greatly and significantly correlated with the population of bacteria in the stem end ($r = 0.93 \leq 0.01$), ionic leakage ($r = 0.66 \leq 0.05$) and SOD ($r = 0.60 \leq 0.05$) (tab. 5).

**Bacteria population in the stem end.** Table 3 shows that the use of mechanical treatment and different levels of geranium (*P. graveolens*) extract and interaction of these two factors on bacterial population at the stem end have a significant difference ($P \leq 0.1$). Mean comparison of data showed that the maximum population of bacteria at the stem end is related with the $S_0O_0$ treatment (control) with an average of 234 $\log^{10}$ CFU $mg^{-1}$. The population of bacteria showed a significant reduction by creating split at the stem end along with 10% geranium extract ($S_1O_{10}$) with an average of 15 $\log^{10}$ CFU $mg^{-1}$ (fig. 2). In addition to other features mentioned in the previous results, significant correlation was observed between these features with chlorophyll content ($r = 0.62 \leq 0.05$), ionic leakage ($r = 0.77 \leq 0.01$), MDA ($r = 0.75 \leq 0.01$) and SOD ($r = 0.76 \leq 0.01$) (tab. 5).

**Chlorophyll content.** Two factors, mechanical split and herbal extract, used in this study and the interaction between these two factors showed a significant difference in statistical level of 1% for leaf chlorophyll content (tab. 3). Creating a split at the stem end and the use of geranium (*P. graveolens*) extract significantly increased the content of photosynthetic pigments in cut flowers (tab. 4). Mean comparison of the interaction between cut flowers and control treatment ($S_0O_0$) with an average of 2.69 mg $g^{-1}$ FW, and cut flowers treated with creating a split at the stem end along with 10% geranium extract ($S_1O_{10}$) with an average of 5.79 mg $g^{-1}$ FW, had the minimum and maximum leaf chlorophyll content (fig. 3). This feature had a significant correlation with the petals anthocyanin ($r = 0.69 \leq 0.01$), ionic leakage ($r = -0.77 \leq 0.01$), MDA ($r = -0.86 \leq 0.01$) and SOD ($r = -0.75 \leq 0.01$) (tab. 5).

**Petals anthocyanin.** Splitting at the stem end and the use of geranium (*P. graveolens*) extract had a significant effect ($P \leq 0.1$) on the content of anthocyanin pigment in the petals (tab. 3). Cut flowers with a split at the stem end and the use of geranium extract significantly increased the content of petal's anthocyanin compared to the control (tab. 4). Control flowers ($S_0O_0$) and flowers without a split at the stem end and 1% geranium extract ($S_0O_1$) with an average of 4.66 and 5.06 μg $g^{-1}$ FW, had the lowest amount of anthocyanin pigments in the petals. On the other hand, treatment of $S_1O_8$ (split of the stem end and 8% geranium extract) with an average of 13.28 μg $g^{-1}$ FW had the highest amount of anthocyanin pigments in the petals (fig. 3). Results of correlation coefficients showed that this feature had a significant correlation with MDA ($r = -0.62 \leq 0.05$) and SOD ($r = -0.68 \leq 0.05$) (tab. 5).

**Ionic leakage.** By creating a split at the stem end and adding geranium (*P. graveolens*) extract in the vase solution, ionic leakage in cells was reduced (tab. 4, fig. 4). Treatments of $S_0O_0$ (control) with an average of ionic leakage of 11.33% and $S_1O_{10}$ (split of the stem end and 10% geranium extract) with an average of 3.93% had the maximum and the minimum amount of ionic leakage in cells, respectively (fig. 4). This feature had also a significant correlation with MDA ($r = -0.78 \leq 0.01$) and SOD ($r = -0.61 \leq 0.05$) (tab. 5).

**Malondialdehyde (MDA).** MDA concentration with factors applied on chrysanthemum cut flowers was significant at 1% level of probability (tab. 3). MDA, which was peroxidizing product of lipids, had a higher concentration level in control treatments than other applied treatments (tab. 4, fig. 4). According to the mean comparison of data, control flowers with an average of 31.16 nmol $g^{-1}$ FW and treatments of split at the stem end and 8% geranium extract, also split at the stem end and 10% geranium extract with an average of 14.26 and 13.80 nmol $g^{-1}$ FW, had the highest and lowest concentrations of MDA, respectively (fig. 4). This trait had a significant correlation with SOD ($r = 0.71 \leq 0.01$) (tab. 5).

**Superoxide dismutase (SOD).** According to Table 3, it can be deduced that the main and interaction effects of two factors on SOD enzyme activity had a significant difference ($P \leq 0.1$). Creating a split at the stem end and the use of geranium (*P. graveolens*) extract in vase solution lead to a decrease in the activity of SOD in cut flowers compared to the control. Based on the mean comparison of the interaction, the highest enzyme activity was related with control treatment with an average of 21.78 units $g^{-1}$ FW and the minimum amount of SOD enzyme was related with treatment of split at the stem end and 10% geranium extract with an average of 12.83 units $g^{-1}$ FW (fig. 5). There is a significant correlation between SOD and POD activity ($r = 0.68 \leq 0.05$) (tab. 5).

**Peroxidase (POD).** The results showed in Table 3 revealed that the treatment of mechanical spilt, various concentrations of geranium (*P. graveolens*) extract and interaction of these two factors had a significant difference ($P \leq 0.1$) for the POD enzyme activity in the chrysanthemum cut flowers. According to the results, the flowers of $S_0O_8$ treatment (non-split at the stem end and 8% geranium extract) with an average of 0.084 nmol $g^{-1}$ FW and $S_1O_{10}$ treatment (split of the stem end and 10% geranium extract) with an average of 0.039 nmol $g^{-1}$ FW had the highest and the lowest POD activity in cut flowers, respectively (fig. 6).

## DISCUSSION

The results of current study showed that the longevity was increased by creating a split at the stem end. Ahmad et al. [2011], showed that creating a split at the stem end leads to increase vase solution absorption by the cut flowers, improve water relations, and ultimately increase vase life of flowers. This finding is corresponds with the results of current study. Accordingly, creating a split at the stem end causes a direct access of vascular elements to solutions and increasing water absorption that leads to more freshness of flowers and their longevity. Moreover, by increasing the concentration of geranium (*P. graveolens*) extract in vase solution, percentage of flowers aging is reduced and flowers longevity is increased. Essential oils and herbal extracts with anti-microbial properties reduce the amount of bacteria in the vase solution and the vessels [Ahmad et al. 2011]. Furthermore, the advantage of these compounds in overcoming the bacteria of stem end and vase solution can be because of their antimicrobial effect on pathogens and impairing function and respiratory chain of pathogens that prevent function of pathogens, and ultimately cause their death [Solgi et al. 2009].

According to the literature, adding herbal extract to the preservative solution and creating a split at the end of stem prevent growth and function of microbes and the vessels obstruction, and as a result, water absorption is increased without interruption. These conditions induce freshness and prolonged longevity of cut flowers [Kim and Lee 2002, Shanan 2012]. Current study showed a direct correlation between the application of herbal extract in vase solution along with split at the stem end and increasing the vase life of geranium cut flowers. Ichimura et al. [2006] demonstrated that the use of disinfectants in vase solution increases hydraulic conductivity of rose cut flowers due to the reducing bacterial proliferation. Dasilva [2003] reported that the water balance is the most important factor for determining the quality and durability of cut flowers and balance between the two processes of water absorption and transpiration is essential to maintain the quality and durability of flowers. Mousavi Bazaz and Tehranifar [2011] and Solgi et al. [2009] showed that the use of herbal oils in vase solution of *Alstroemeria* and *Gerbera* cut flowers increased their longevity. This result confirms our finding.

The results showed that the cell membranes stability index which shows the amount of ion leakage reaches to a minimum value in line with increase of durability [Ezhilmanthi et al. 2007, Singh et al. 2008]. In the present study, amount of ion leakage was reduced by using the herbal extract and application of mechanical treatment. The researchers revealed that the increased percentage of cell membrane stability in cut flowers treated with herbal extracts can be related to increasing total soluble solids such as carbohydrates in the flowers. This condition increases the content of photosynthetic pigments as a result of improving water relations [O'Donoghue et al. 2002]. Also, they believe that the antimicrobial compounds increase water absorption and cellular activity by reducing the microbial load [Anderson et al. 2004]. Enhanced chlorophyll content and water absorption by split at the stem end are indexes of increased post-harvest life of cut flowers [Ichimura et al. 2006]. Elgimabi and Ahmed [2009] found that the use of herbal extracts preserves chlorophyll in rose cut flowers. Enhanced chlorophyll content in cut flowers resulted in more cell activity and production of sugars. Sugars regulate

breathing and osmotic pressure and delay wilting of cut flowers [Halevy et al. 2002, Anderson et al. 2004]. Basiri et al. [2011] showed that the use of rosemary extract in preservative solution causes a positive effect on longevity, absorption of solution, reducing the bacteria population and increasing the chlorophyll content of cloves cut flowers.

Type and amount of petal's pigments are two major indicators for post-harvest quality and longevity of cut flowers. Among them, anthocyanin is more important for post-harvest life of cut flowers. Antibacterial compounds improve water absorption and prevent destruction of pigments such as anthocyanin [Zamani et al. 2011]. Kazemi and Ameri [2012] showed that the use of essential oils of thyme and lavender flowers in vase solution of cloves cut flower caused to maintain petal's pigments compared to the control. Basiri et al. [2011] found that the use of high concentrations of antimicrobial compounds increase the amount of anthocyanin in cloves cut flowers. According to the mentioned results, an increase in the amount of petal's pigments along with creating a split at the stem end can be related to improve of water conditions and increase of dry matter in cut flowers. Yamada et al. [2003] and Rezvanipour and Osfori [2009] observed similar results for the pigments of rose cut flower.

Malondialdehyde (MDA) is produced from per-oxidizing of membrane lipids. Accumulation of MDA represents the cytoplasmic membrane damage and also indicates the level of per-oxidizing the membrane. MDA is usually used as an index of aging and physiological resistance. Researchers believe that treatments with extending compounds of the longevity can reduce physiological stress imposed on cut flowers and increase post-harvest life by reducing the accumulation of MDA [Jin et al. 2006]. In current study, the antimicrobial compound and mechanical treatment decreased MDA concentration and increased longevity of chrysanthemum cut flower by maintaining the stability of membrane. Improvement of water absorption induced by mechanical treatment and antimicrobial compounds causes to maintain the activity and survival of cells, protection of proteins and finally protection of membrane structure and reduction of negative effects of MDA that resulted in delay the senescence [Kazemi et al. 2012]. Zamani et al. [2011] showed that the use of salicylic acid and glutamine increased the vase life of cut rose flowers via decreasing the amount of MDA that resulted in 25–40% more membrane stability than the control. Kazemi and Ameri [2012] reported the positive effect of antimicrobial compounds such as essential oils of thyme and lavender flowers on the stability of the cell membrane and reduction of MDA in cloves cut flower.

Alteration in antioxidants activity such as POD and SOD during post-harvest life of cut flowers have been shown [Giannopolitis and Ries 1997]. Some studies demonstrated that vase life of cut flowers is modulated by antioxidants [Chakrabarty et al. 2009]. Investigations revealed that the water and oxidation stresses are increased in cut flowers kept in vase solution [Harinasut et al. 2003]. Chamani et al. [2006] showed that the use of antimicrobial compounds in vase solution of cut flowers increase the activity of antioxidant enzymes, stability of cell membranes, water absorption and cell turgidity. Activity of antioxidant enzymes inhibits ethylene biosynthesis and prevents from toxic effects of free oxygen obtained from the decomposition of hydrogen peroxide, which is one of the most important factors in premature aging of petals [Harinasut et al. 2003]. Mortazavi et al. [2007] reported that the use of antimicrobial compounds along with

mechanical treatments at the stem end effects on the activity of antioxidant enzymes of rose cut flowers. Kazemi and Ameri [2012] showed that the use of antimicrobial compounds, essential oils and aging inhibitor compounds increases the activity of POD and SOD enzymes in cloves cut flowers. Activity of POD was increased during senescence of *Phalaenopsis* cut flowers [Tewari et al. 2009]. An increase in the POD activity strengthen vascular cells, which remain functional during the late stage of senescence [Panavas and Rubinstein 1998]. Previous studies and our finding showed that POD is involved in senescence, because it catalyzes the degradation of $H_2O_2$. The POD enzyme uses $H_2O_2$ as a substrate for several reactions.

## CONCLUSION

1. Creating 5 cm split as a mechanical treatment can be an effective way to extend the longevity of chrysanthemum cut flowers and the use of geranium (*P. graveolens*) extract as a safe and friends with the environment compound is appropriate supplementation to increase the post-harvest life of this cut flowers.

2. It is recommended to use 5 cm split of the stem end with 100 mg $l^{-1}$ of geranium extract applied continuously to increase the longevity of *Dendranthema grandiflorum* (Ramat.) Kitamura cut flowers for about 10 days, by improving plant water relations and antioxidant and antimicrobial activity of plants.

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## WYCIĄG Z GERANIUM (*Pelargonium graveolens*) ORAZ ZABIEG MECHANICZNY POPRAWIAJĄ STOSUNKI WODNE, AKTYWNOŚĆ ENZYMÓW I DŁUGOŚĆ ŻYCIA KWIATÓW (*Dendranthema grandiflorum* (Ramat.) Kitamura)

**Streszczenie.** Zastosowanie związków organicznych bezpiecznych dla środowiska jest ważną metodą zwiększenia długości życia kwiatów ciętych. Ziołowe esencje i wyciągi zawierają związki mające właściwości antybakteryjne, co zwiększa życie ciętych kwiatów. Zabiegi mechaniczne, takie jak rozdzielenie końcówki łodygi, także zwiększają życie kwiatów ciętych. Esencje i wyciągi geranium (*Pelargonium graveolens*) mają właściwości antybakteryjne i antyoksydacyjne i mogą być użyte jako roztwory w wazonach. W badaniu zastosowano różne stężenia wyciągu z geranium o zapachu róży oraz rozdzielenie końcówek w celu polepszenia długości życia kwiatów ciętej chryzantemy (*Dendranthema grandiflorum* (Ramat.) Kitamura). Wnioskuje się, że długość życia ciętych kwiatów w wazonie z roztworem zawierającym 100 mg $l^{-1}$ wyciągu z geranium o zapachu róży (18,43 dni) przy rozdzieleniu końcówek łodygi na długości 5 cm była istotnie większa niż w kontroli (8,11 dni). Status wodny, liczebność drobnoustrojów w roztworze w wazonie oraz końcówka łodygi, wypływ jonów oraz aktywność enzymatyczna istotnie różniły się w kwiatach ciętych i kontroli.

**Słowa kluczowe:** aktywność drobnoustrojowa, zabiegi niechemiczne, blokada naczyniowa, życie kwiatów ciętych, absorpcja wodna

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