

## THE EFFECT OF HERBAL ESSENTIAL OIL IN PRESERVATIVE SOLUTION, ON QUANTITATIVE, VASE LIFE, BACTERIA-INDUCED STEM XYLEM BLOCKAGE OF LISIANTHUS VAR. ECHO

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### ABSTRACT

In this study the effect of essential oil taken from medicinal plant as antibacterial components in preservative solution of Lisianthus var. Echo (*Eustoma grandiflorum*) was investigated. The test was done with application of preservative solution. Cut flowers were treated with different concentrations of Thyme (*Thymus vulgaris*), Spearmint (*Mentha spicata*) and Lavender (*Lavandula officinalis*) essential oil in addition to Sucrose 2.5%. The results showed that there was the longest time in vase life with Thyme in 50 ppm (15.6 days) and the control treatment showed the shortest vase life (11.6 days). Moreover, Thyme with 50 ppm had the highest effect on relative fresh weight and solution uptake. In addition, bacteria-induced stem xylem blockage, extracted from the end of stem, was cultured in NA medium culture with several concentrations of essential oil. The result showed that in pure concentration (100%) inhibition was completed and in various concentrations of essential oil the bacterial population was reduced.

Keywords: essential oil, lavender, preservative solution, spearmint, thyme

### INTRODUCTION

Lisianthus is one of the best ten cut flowers in the world. Culture of cut flowers of this type increased in Asia especially China in the last three years. The *Eustoma grandiflorum* variety is planted as cut and pot flower. Decreases in carbohydrates and microorganisms growth in stem xylem and preservative solution are main factors on the decrease of cut flower vase life. So adding antimicrobial factors to preservative solution and also sucrose usage, as carbohydrate

source is necessary. In often-protective solution formulation, sucrose and other metabolic sugars like glucose and fructose have the same effect. The roles of sugars in vase life of cut flowers are known. Glucose uptake from the solution is accumulated in petals tissue and so osmotic potential is improved and the carbohydrates for respiration and growth is increased. This will facilitate the opening of florets and delay senescence (Särkkä, 2004). Using Sucrose in preservative solution increases vase life, stimulates opening flowers, and improves colour and size of flowers (Bhattacharjee and De, 2005; Ichimura and Shimizu-Yumoto, 2007; Elgimabi and Ahmed, 2009).

Using 4% sucrose as a treatment of preservative solution in Orchid flower increases vase life and content of solution absorption and delay in disappearance of the colour of the petals (Chandran *et al.*, 2006). Using Sucrose in cut flower solutions causes delay in ethylene biosynthesis, decrease in ethylene sensitivity and increase in vase life (Pun and Ichimura, 2003). Sucrose in preservative solution prevents wilting in Rose var. Sonia and results in slow decrease in wet weight (Ichimura *et al.*, 2003).

Using 2% Sucrose in preservative solution of wallflower causes increase in Inflorescence, vase life and opening floret (Arab *et al.*, 2004). Using 4% Sucrose as dominant treatment has positive effect in vase life of Rose var. Bacara and helps to improve the colour of petals (Pompodakis and Joce, 2003).

Essence, aromatic component due to not induce fat, can not produce soap. Some of these components have attractive properties and others have repellent properties. Dominant compound or active ingredient in most essential oils are terpenes (Baghalian and Naghdi Badi, 2000). The mechanism of the essential oils

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action are as follows: Due to joint and overlapping of various combinations of essential oils, wall, and cell membrane of pathogens are degraded and permeability and ion leakage of cells are increased. Due to decomposition lipids in the cell wall, mitochondria, and proteins of membrane and also clotting of cytoplasm, and depletion of proton motive force, the damaged cells are undergo cell death by essential oils (Burt, 2004). Also using *Zataria multiflora* and *Thymus vulgaris* with SNP prevent growth of bacteria in preservative solution, vascular obstruction and decrease wet weight and caused increase vase life (Solgi, 2009).

In this study, the effect of different concentrations of essential oils Thyme, Lavender, Spearmint were examined by application of preservative solution with 2.5% sucrose in *Lisianthus var. Echo* for determining suitable essential oil and concentration to increase vase life and quality of the cut flowers.

## MATERIALS AND METHODS

The flowers harvested from green house in stage that the buds coloured and two of them were opened. They were transferred to the laboratory quickly. In the laboratory the flower stem was cut into 45 cm to arrange and reduce the different in the size and after that in the water the end of them cut 5 cm again and omit the shoots too.

The treatments consisted of 1) Distilled water, 2) Thyme 10, 25, 50, 100 ppm, 3) Lavender 10, 25, 50 ppm, 4) Spearmint 10, 25, 50 ppm.

At first, the required amount of each essential oil was weighed and then it was resolved in 80% Methanol and 2.5% Sucrose, and this solution was maintained until the end of vase life. Then the preservative solution was changed every other day.

Traits were as follows:

- Vase life: Since 50% of florets were faded the vase life was ended (Moon *et al.*, 2001).
- Relative fresh weight: fresh weight of flowers was measured exactly after transferred to the laboratory by scale (0 day), and then it was measured in (1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup>)
- Solution uptake by the flower: it was measured in 0, 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 10<sup>th</sup> by the canter.

- Leaf Chlorophyll content: it was measured in 0, 1<sup>st</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, and 12<sup>th</sup>. For this purpose 0.1 g of the third leaves harvested in each plot, quite worn and chlorophyll was extracted by 5 ml of 80% acetone and after 24 hours was read by Spectrophotometer in 663, 640, and 645 nm wavelength (Lichtenthaler, 1987). Finally the content of Chlorophyll was calculated by the following formula:

$$\begin{aligned} \text{Chlorophyll a} &= (0/0127 \times A_{663}) - (0/00269 \times A_{640}) \\ \text{Chlorophyll b} &= (0/0229 \times A_{645}) - (0/00468 \times A_{663}) \\ \text{Chlorophyll b+a} &= (0/0202 \times A_{645}) + (0/00802 \times A_{663}) \end{aligned}$$

## Percentage of Solid Substances in the Stem (Brix)

To determine the amount of TSS in the stem, 1g of the end of stem was removed and pulverized in a mortar before the extract was obtained. Digital ESR read the extract Brix degree in 0, 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 10<sup>th</sup> (Hettiarachchi and Balas, 2005).

## Leaf and Flower Water Content

At the first 2 g of leaf, 2 g of petal was weight and then put in the Oven in 60°C for 72 hours to dry completely. The dry weight was measured in 0, 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 10<sup>th</sup> and was calculated using the following formula (Slavick, 1979):

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / \text{dry weight}$$

## Petal Anthocyanin

To measure the anthocyanin according to Bariola *et al.* (1999) method, 0.1 g of fresh petal was weighted and pulverized in a mortar. For extracting anthocyanin 5 ml of extract solution containing methanol and HCL 1% was added into every sample. Samples were put in the Falcon and maintain in 4°C for one night. Finally the absorption was read in 530 and 657 nm in 0, 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 10<sup>th</sup> and calculated using the following formula:

$$\text{Anthocyanin} = D_{530} - 0.24 D_{657}$$

## Soft Rot Test

At the beginning and the end of the test samples was prepared from the end of the stem. Then the viscous liquid extracted from the stem end of the cut flowers (*Lisianthus var. Echo*) was cultured on the medium (Nutrient Agar) and after

48 hours pure bacteria were tested on the tomato to identify the crush ability of tested bacteria.

### Evaluate the Amount of Prevention

Inhibition of natural compounds, essential oils, were evaluated against positive and negative warm bacteria by Aromatogram method. For this purpose, wells with diameter of 2 mm were prepared on solid medium and 4  $\mu$ L of oil were added into each well. After two hours, when the oil was absorbed, Bacterial suspensions were sprayed uniformly on medium and then the petridishes were put in Incubator for 48 hours. Finally, the diameter of zone inhibition around the wells were measured with a ruler.

## RESULTS AND DISCUSSION

### Effects of Treatments on Vase Life

Effects of treatments with different concentrations increased vase life in Lisianthus var. Echo compare with control (Table 1). Among the essential oils, Thyme essential oil 50 ppm had the best effect on vase life (15.6 days) and the control had the lowest vase life (11 days). In lower concentrations, the vase life was higher and with the increasing in concentration of essences especially Spearmint (50 ppm) and Thyme, (100 ppm) vase life decreased. Sucrose of 2.5% as source of carbohydrates and essential oils as antimicrobial components in

preservative solution triggered increase in vase life.

Table 1. The effects of various herbal essences on vase life of Lisianthus var. Echo

Treatments	Vase Life (Days)
Control	11.00
Thyme 10 ( <i>Thymus vulgaris</i> , 10 ppm)	14.53
Thyme 25 ( <i>Thymus vulgaris</i> , 25 ppm)	15.33
Thyme 50 ( <i>Thymus vulgaris</i> , 50 ppm)	15.60
Thyme 100 ( <i>Thymus vulgaris</i> , 100 ppm)	13.66
Lavender 10 ( <i>Lavandula officinalis</i> , 10 ppm)	15.23
Lavender 25 ( <i>Lavandula officinalis</i> , 25 ppm)	14.86
Lavender 50 ( <i>Lavandula officinalis</i> , 50 ppm)	15.00
Spearmint 10 ( <i>Mentha spicata</i> , 10 ppm)	15.43
Spearmint 25 ( <i>Mentha spicata</i> , 25 ppm)	14.83
Spearmint 50 ( <i>Mentha spicata</i> , 50 ppm)	14.33

### Effects of Essential Oil on Relative Fresh Weight

According to the results (Figure 1) relative fresh weight to 3<sup>rd</sup> fixed approximately, but then it was decrease, so that in 10<sup>th</sup> it had the lowest content. In various treatments with different concentrations, Thyme with 50 ppm had the highest and control had the lowest effect on relative fresh weight (Figure 2).

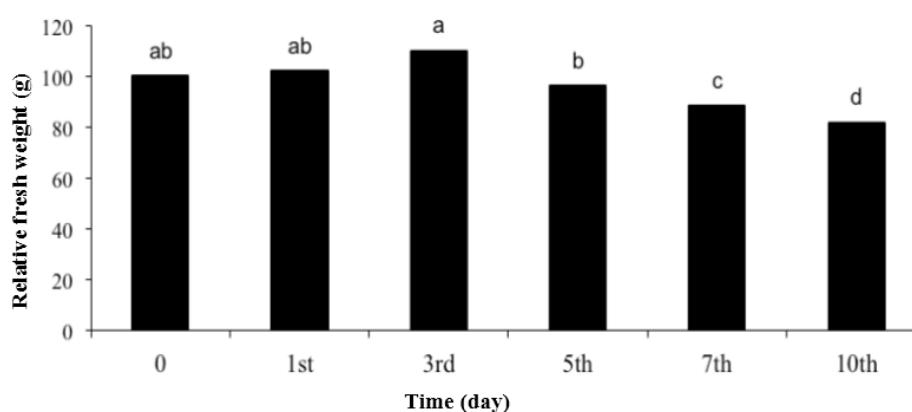


Figure 1. Effect of time on relative fresh weight in Lisianthus var. Echo, based on LSD  $\alpha=0.05$ .

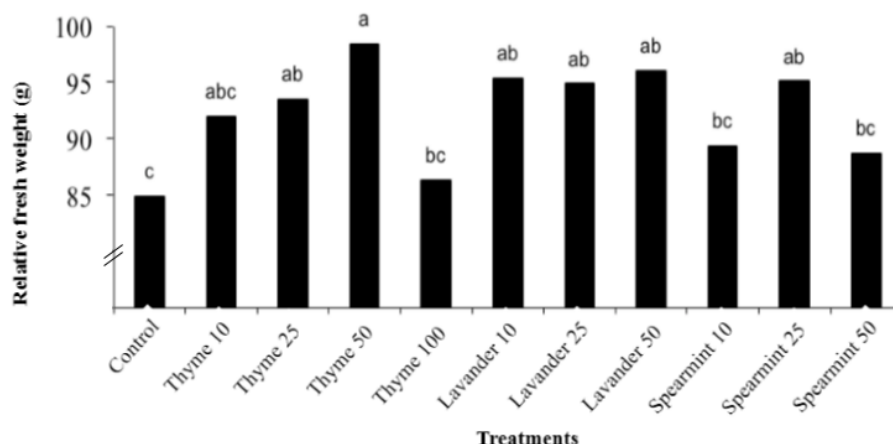


Figure 2. Effect of essential oils on relative fresh weight in *Lisianthus* var. Echo, based on LSD  $\alpha=0.05$ .

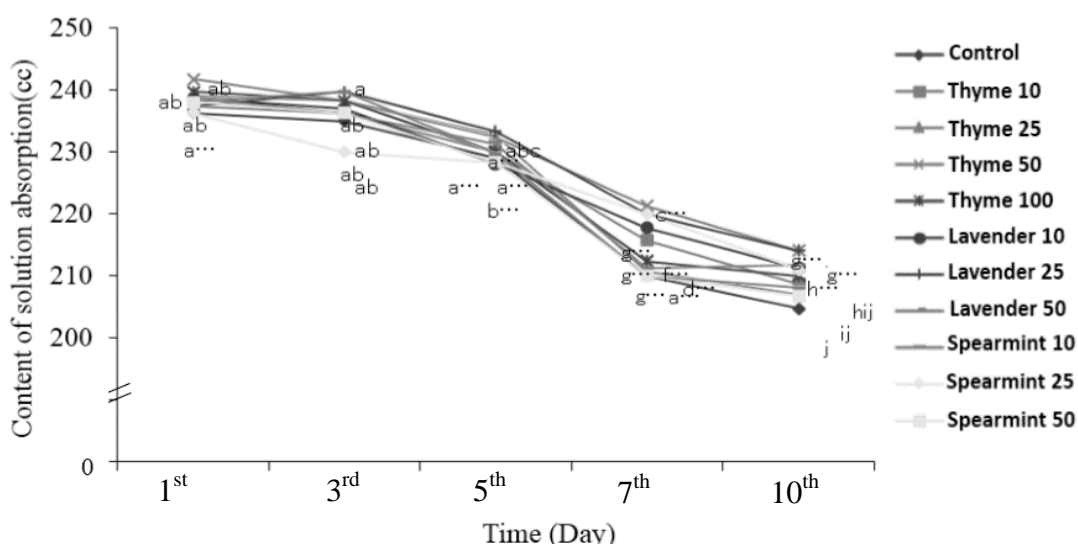


Figure 3. Effect of essential oils and time on solution uptake on *Lisianthus* var. Echo, based on LSD  $\alpha=0.05$ .

**Effect of Essential Oil on Solution Uptake**

In the interaction of time and essential oil on solution uptake the highest uptake was seen in Thyme 50 ppm and the declining process of solution uptake was slow. These results were the same for Lavander 25 ppm and then for Lavander 50, 10 ppm, and Spearmint 10 ppm and 25 ppm. The lowest solution uptake was related to Spearmint 50, and 10 ppm in 10<sup>th</sup> day. In different concentrations, solution uptake was measured till the end day and was in good level. In Control treat absorption, changes were more evident after five days, and solution uptake was less (Figure 3). These results were consistent with Solgi *et al.* (2009).

**Effect of Time and Essential Oil on Leaf Chlorophyll Content**

In the effect of time on the content of chlorophyll, it was fix until 5<sup>th</sup> day approximately and after that starting to decrease in the 10<sup>th</sup> day it had the lowest level because of leaf senescence (Figure 4). Effect of essential oil on chlorophyll showed that there was significant difference between control and Thyme 50 ppm (Figure 5). There was no significant difference between treatments. Various essential oils protect of chlorophyll higher than control treatment.

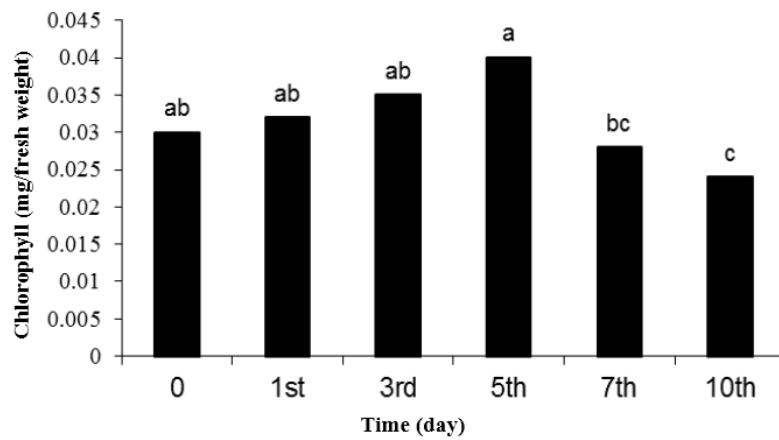


Figure 4. Effect of time on chlorophyll in Lisianthus Var Echo, based on LSD  $\alpha=0.05$ .

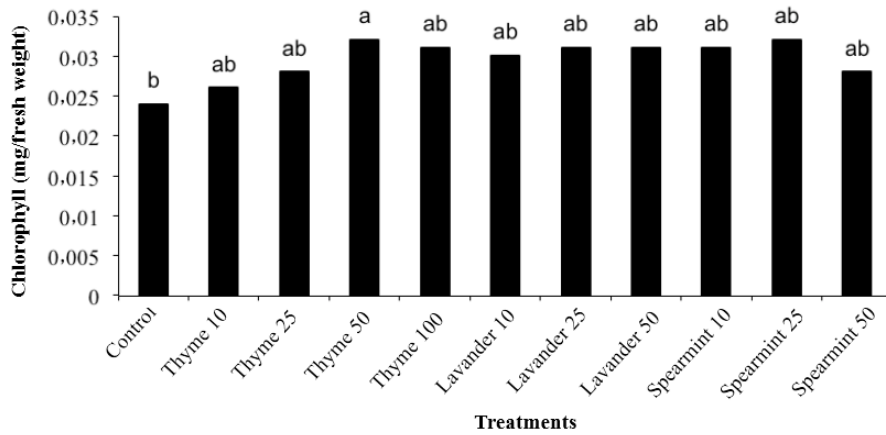


Figure 5. Effect of essential oil on chlorophyll in Lisianthus var. Echo, based on LSD  $\alpha=0.05$ .

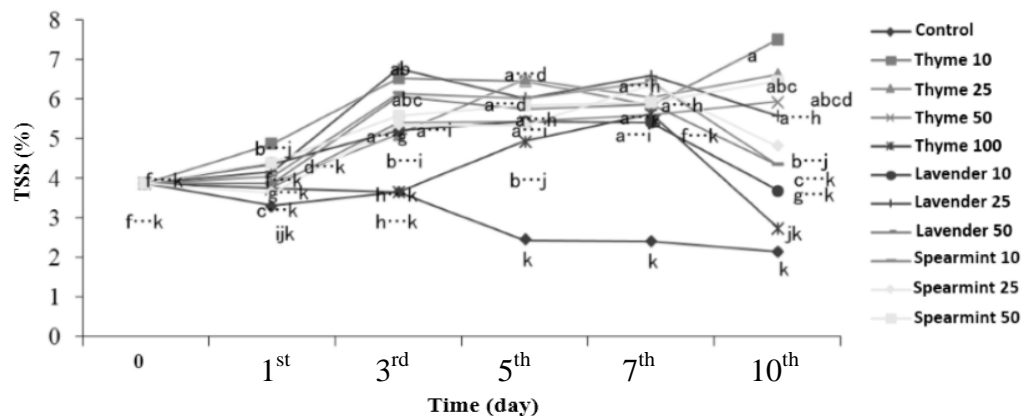


Figure 6. Interaction effect of time and percentage of solid substances in the stem (TSS) for each essential oil on Lisianthus var. Echo

### Effect of Essential Oil on Percentage of Solid Substances in the Stem (Brix) in Stem

In the interaction effect of time and essential oil on the Brix, Thyme 10 ppm treatment in 10<sup>th</sup> day had the highest level and in control treatment it had the lowest effect on the Brix. In all of the treatments carbohydrate replaced with sucrose in respiration process and prevent analysis other cell materials and delay senescence. Thyme 10 ppm has the highest effect in 0 and 10<sup>th</sup> day on TSS. Thyme 10 ppm had the highest significant difference than other treatments and thyme 100 ppm had the lowest effect. The highest

concentration of essential oils prevent sucrose uptake (Figure 6).

### Effect of Essential Oil and Time on Leaf and Flower Water Content

The effect of time on leaf and flower water content showed that it had the highest level in 0 day and lowest level in 10<sup>th</sup> day (Figure 7). Between treatment, control treatments had the lowest effect and Lavender 10 ppm highest effect on water content of petals (Figure 8). Thyme 10 ppm, 25 ppm, 50 ppm and Spearmint 10 ppm and Lavender 25 ppm had positive effect on leaf and flower water content.

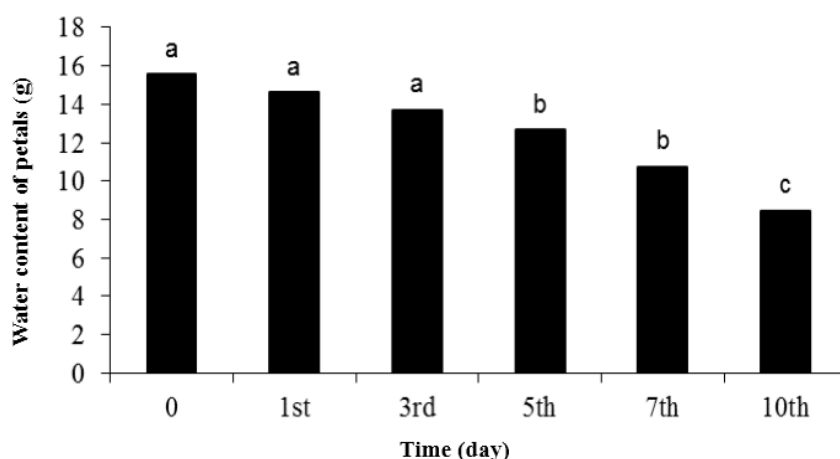


Figure 7. Effect of time on leaf and flower water content on Lisianthus var. Echo, based on LSD  $\alpha=0.05$ .

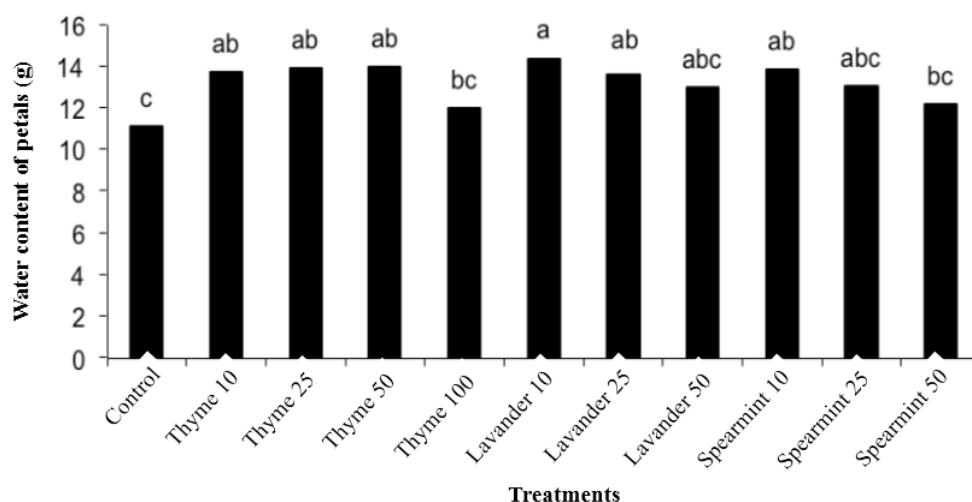


Figure 8. Effect of essential oil on leaf and flower water content in Lisianthus var. Echo, based on LSD  $\alpha=0.05$ .

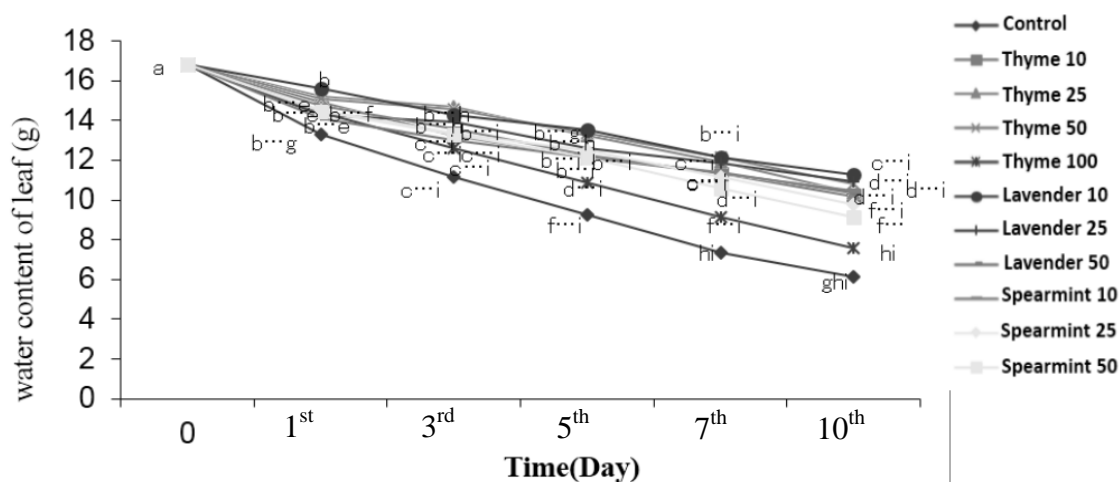


Figure 9. Interaction of time and leaf water content for each essential oil on Lisianthus var. Echo, based on LSD  $\alpha=0.05$ .

#### Interaction Effect of Time and Essential Oil on Leaf and Flower Water Content

According to the results (Figure 9) in 0 day, leaf water content of all of treatments are high (16.38). The highest content of water was shown in Lavender 10 ppm and on 10<sup>th</sup> day and the lowest content was found in control treatment. In Lavender 10 ppm decline process in leaf water content was very slow and fixed until the fifth day and after that it was decreased slowly. In control, decreasing leaf water content was faster and on the 10<sup>th</sup> day it had the lowest content (6.14). Due to the positive effects of essential oils and sucrose to maintain leaf water relations in leaves that caused freshness and vitality.

#### Effect of Essential Oil on Anthocyanin of Petals

The content of anthocyanin was the highest since 0 day to 5<sup>th</sup>, and it then started to decrease and on the 10<sup>th</sup> day it had the lowest level. Actually reduce in anthocyanin levels of petals is one of effects of cut flower senescence. No significant difference was shown between various treatments of essential oils on anthocyanin.

#### Soft rot test

*Dickeya chrysanthemi* and *Pectobacterium carotovorum* as bacterial soft rot on potato slices were tested. In pure concentration of every three essential oils (Thyme, Lavender, and Spearmint) on medium culture contained bacteria resulting in inhibition of bacterial growth and other essential oils concentration resulting reducing the microbial population.

This result was attributed to the presence of components such as Thymol and Carvacrol in essential oils which had antimicrobial properties that prevented vascular occlusion, reduced absorption content, and reduced relative fresh weight and it made the process slower. This results were consistent with Tzortzakis (2007), Martinez-Romero *et al.* (2005), and Ziedan and Farrag (2008).

#### CONCLUSIONS

Thyme 50 ppm significantly increased vase life, solution uptake, relative fresh weight and other measured attributes at 1% level. The flowers was alive 4.33 day (15.33 day) in Thyme 50 ppm more than the control (11 days). According to the results, in this method Thyme 25 ppm, Lavender 10, 25, 50 ppm and Spearmint 10ppm had positive effects on all of measured attributes (Vase life, relative fresh weight, solution uptake, percentage of solid substances in the stem (Brix), leaf chlorophyll content, Petal Anthocyanin, leaf and flower water content). Control treatment in all of measured attributes showed minimum effect.

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