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# Application Methods of *Thymus vulgaris* Essential Oil and Their Effect on Vase Life and Qualitative Traits of *Gladiolus grandiflorus* L. Cut Flowers

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Abstract

In this study, combined effect of two essential oils, Thymol and Carvacrol, with different concentrations in two methods on *Gladiolous grandiflorous* L. was investigated based on a completely randomized design with 3 replicates and four flower in each replicate. Sansusi variety applied that was red. In short term method, different concentrations of Thymol and Carvacrolin including 25+25, 50+50 and 75+75 ppm with sucrose 6% and distilled water (as control) were evaluated. Red flowers were treated in mentioned solutions for 24 hours and then they were taken to distilled water to the end of evaluation period. In standard method, different concentrations including 12.5+12.5, 25+25 and 37.5+37.5 ppm and sucrose 2% and distilled water as control were used. Flowers were kept in solutions from beginning experiment until the end of vase life. Every 48 hour, once, solutions were made to replace. Preserver solutions can use with wholesalers or retailers to protect flowers in order to sold users. Thymol and carvacrol as the most essential oil of Thymus vulgaris have strong antimicrobial and antioxidant effects. In short term method, combined treatment of thymol and carvacrol with 150 ppm had the most soluble sugar and petal aqueous contents and the least rate of blossoming. In addition, the most vase life in combined treatment of carvacrol and thymol was observed in 100 ppm with mean of 11.57days. Concentration of Anthocyanin was the most in short-term method in compared to standard in last days of experiment.

**Keywords:** Carvacrol, Essential oil, Postharvest, Short term, Standard, Thymol.

## INTRODUCTION

Glodiola in scientific name *Gladiolous grandiflorous* belongs to family Iridaceae that is a cut flower and a valuable garden plant. Glodiola in iran has first order in cultivating area and second order in production rate among cut flowers. Cut flower quality is depends on many factors. All physiological abnormalities, which affect on their appearance, also affect on their market choice and economic value. The most important problem of most decorative plants is flower and leaves senescence. Essential oil is used as a substitute material of synthetic fungicide (Wright and Kader, 1997). Due to antimicrobial and antifungal properties of essential oil, their production by plants effectively increases defense mechanism against pathogens and pests (Aggarwal *et al.*, 2002). Thymol and Carvacrol essences are phenolic complex that have very strong antibacterial and antifungal effects (Yahyazadeh *et al.*, 2008). Essences are hydrophobic to act as catalyst. This property enables essences to inter into wall cell membrane lipids and bacteria mythocondria and destroy their structure and more permeability. After this step occur ions permeation and other cell contents. Although it´s possible such complex permeation from bacteria cells does not cause losing their life power, but losing much cell contents and exiting important molecules and ions will cause bacteria death (Bird, 2004). Essences act mechanism is similar to other phenolic complex due to having phenolic complex such as Thymol and Carvacrol. Microbial bio control factors have high potential to substitute synthetic fungicides for decay after fruit and vegetable cultivating (wright and kader, 1997). Vessel blockage causes solution and water leading decreasing in stems. Lead ability decreasing creates due to stems and vessels cut level blockage (Damunupola *et al.*, 2008). Vessel blockage due to bacteria decreases water absorbtion and causes stem bend and break and petals decay in gerbera flower (solgi, 2009). Bacteria cause woden vessel blockage and therefore decreases roses succulence (torjesuns) (Van Doorn and Do wite, 1994). The most life of Gerbera flowers observed in Mentha, pulegium, Rosmarinusand Indian grenadine essential oils treatment (Ziaei *et al.*, 2008). That thyme essences especially thymol and carvacrol have fungicide, antioxidant and antiseptic properties (Ozkan *et al.,* 2004). Also according to research in 2007, carvacrol into bags having inoculated grape grains with gray moulds prevented such mould grow (Martinz-Romrou *et al.,* 2007). Different carvacrol densities on Qiwi fruit and Melon decrease bacteria life and delay Qiwi and Melon decay (Roller *et al*., 2002). Sing in 2002 by thyme essence could stop E.Coli Grow. Regarding to mentioned item, this research was conducted to study the effect of thymol and carvacrol on Gladiola flower qualitative properties.

## MATERIALS AND METHODS

### Plant materials

Plant cultivation acts from a commercial green house in Varamin conducted a day before transferring to laboratory when flourished one or two buds at early morning and before weather warming and transferred to laboratory in suitable paper package.

### Different treatments:

Flowers removed from package after transferring to laboratory the flowers height was 90 cm. In short term method combined treatments of Thymol and Carvacrolin different concentrations of 25+25, 50+50 and 75+75 ppm with sucrose 6% and distilled water as control were evaluated. Flowers were treated in mentioned solutions for 24 hours and after they were taken to distilled water to the end of evaluation period. In standard Method treatments with different concentrations including 12.5+12.5, 25+25 and 37.5+37.5 ppm and sucrose 2% and distilled water as control were used.

### Evaluation room environmental condition

Examination place temperature was 20-22ºC and rational humidity was almost 75 percent.

Light density was 18.9 mol/$m^2$/s that satisfied by white floscent Light in 12 hour light and 12 hour darkness. In mentioned room, it was evaluated all factors and properties and vase life post harvest.

**Measuring properties**

**Vase life**

Vase life considered on the basis of time distance from harvest to when droopy buds were more than flourished buds.

**Flourishing speed**

To determine flourishing buds percent, it was calculated floret buds and total buds ratio and multiplied 100 and calculated in days 0, 2, 4. 6, 8, 10 and 12.

**Petal´s anthocyanin**

The amount of 0.5 g fresh petal crushed by liquid nitrogen. 10 cc acidic methanol solution (including methanol and clorodric acid 1%) was added to each sample. Samples were centrifuged and raed solution absorbtion rate by spectrophotometer in wave lengths 657 and 530 nm (Sankhla *et al.*, 2005).

Anthocyanin= $D_{530}$ - (0.24*$D_{657}$).

**Petal aqueous contents**

To measure this property was used phenol-sulphoricacid method and dextroseas standard. 0.1 g dried samples crushed in pounder. To extract sugar added 10 cc ethanol 70%. Then 1 ml phenol 5% added and 5 ml concentrated sulphoric acid to each sample. Finally read absorbtion rate byspectrophotometerinwave length 485 mm (Stewart,1989).

Examination was investigated on the basis of completely randomized design with 3 replicates and four flower in each replicate.To analysis data statistically used SAS software. Means comparison were done by Duncan multiamplitude test on 5% Level.

**RESULTS**

**Petal anthocyanin:** Results showed that the, interaction effect of method and time was significant at 1% Level (Table1).

Comparing interaction effect of method and time on Petal anthocyanin indicated that on third day flowers in standarad method had anthocyanin more than short term method. On day sixth, anthocyanin of treated flowers in short term method was more than standarad method. On day ninth, decreased in both methods traetments. Tottaly in short term method, anthocyanin rate was better (Fig.1).

**Petal´s soluble sugar:** The interaction effect of method and treatment on soluble sugar at 1% level and the interaction effect of three factors (method, treatment and time) on soluble sugar was significant at 5% level (Table 1).

Results showed that the sugar decreased in all treatment and carvacrol treatments in 50+50

Table 1. Summary of analysis of variance for characteristics of *Gladiolus grandiflorus* L. Cut flowers

| S.O.V. | df | Anthocyanin | Soluble suger | Petal aqueous contents | Flourishing speed |
|---|---|---|---|---|---|
| method*Traetment | 5 | 0.07 ns | 70.91** | 5.25** | 47.59** |
| method*time | 3 | 3.08** | 13.85 ns | 20.17** | 39.19 ns |
| method*Traetment*time | 15 | 0.04 ns | 35.48* | 2.03* | 7.93 ns |
| Error | 48 | 0.2 | 59 | 4.88 | 25.58 |

**: Significant at the1%level of probability, *: Significant at the1%level of probability ,ns: Not- significant.

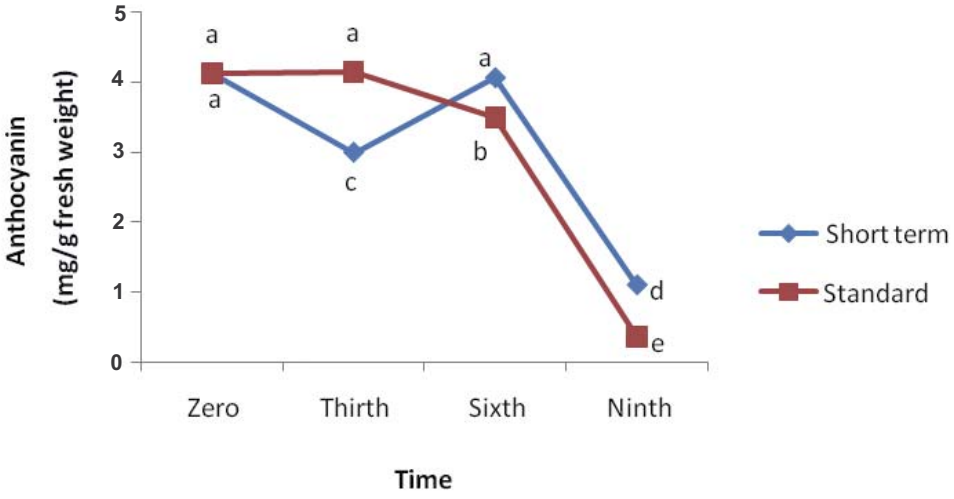


Fig. 1. Method*Traetment interaction effect on anthocyanin characteristics of *Gladiolus grandiflorus* L. Cut flowers

Table 2. Method*Traetment*Time interaction effect on petal solouble suger characteristics of *Gladiolus grandiflorus* L. Cut flowers

| Method/Treatment Time | | zero day | | Thirth day | | sixth day | | ninth day | |
|---|---|---|---|---|---|---|---|---|---|
| **Short Term** | Thym 25+Carv 25 | 30.31 | cdefg | 33.77 | abc | 29.7 | cdefg | 18.33 | hi |
| | Thym 50+Carv 50 | 30.31 | cdefg | 34.68 | abc | 33.21 | abcd | 22.77 | defgh |
| | Thym 75+Carv 75 | 30.31 | cdefg | 33.53 | abcd | 33.13 | abcde | 30.57 | bcdefg |
| | Control (distilled water) | 30.31 | cdefg | 21.17 | fgh | 18.76 | hi | 8.62 | i |
| **Standard** | Thym12.5+Carv 12.5 | 30.31 | cdefg | 41.17 | ab | 33.62 | abcd | 27.82 | cdefgh |
| | Thym25+Carv 25 | 30.31 | cdefg | 28.77 | cdefgh | 25.46 | cdefgh | 21.78 | fgh |
| | Thym37.5+Carv 37.5 | 30.31 | cdefg | 31.41 | bcdef | 26.09 | cdefgh | 21.37 | fgh |
| | Control (distilled water) | 30.31 | cdefg | 21.17 | fgh | 18.76 | hi | 8.62 | i |

and 75+75 ppm concentration in short term method at the end of time. In three treatments in thymol and carvacrol treatment on 50+50 ppm concentration, sugar rate was more than zero till day sixth but then decreased strongly and on day nine received to 22.77 mg/l. In 75+75 ppm concentration in short term method, suger rate during examination was almost fixed and on day ninth also did not decrease. Solution sugar rate on day ninth was more in standard method treatments and was higher than short term treatment. At least solution sugar rate in both method related to control (distilled water) (Table 2).

**Flourishing speed**: Results showed that the, interaction effect of method and treatments was significant at 1% Level (Table 1). In short term and standard method, control treatment (distilled water) had the most flourish speed. There was the least flourish speed in different short term method treatments but thymol and carvacrol treatment in 75+75 ppm concentration (in 52.71% speed) had the least speed. Therefore using short term method caused to increase Gladiola cut flowers flourish speed. (Table 3).

**Petal aqueous contents**: According to the variance analysis results, method and treatments, method and time interaction effect on petal water content was significant in 1% Level. Also method and treatments and time interaction effect in 5% become significant (Table 1).

Results of method, treatment and time interaction effect shows that in all treatments in both methods petal water content decreased but at 12.5 + 12.5 ppm thymol and carvacrol treatment the petal water content increased in third day. Thymol and carvacrol treatments in 75+75 and 50+50 ppm concentrations (5.27 and 4.82 g) in short term method had the most petal water

Table 3. Method*Traetment interaction effect on Inflorescence speed characteristics of *Gladiolus grandiflorus* L. Cut flowers

| Method | Treatment | LSMean | |
|---|---|---|---|
| **Short Term** | Thym 25+Carv 25 | 54.94 | c |
| | Thym 50+Carv 50 | 57.17 | c |
| | Thym 75+Carv 75 | 52.71 | c |
| | Control (distilled water) | 69.97 | ab |
| **Standard** | Thym12.5+Carv 12.5 | 67.02 | b |
| | Thym25+Carv 25 | 69.15 | ab |
| | Thym37.5+Carv 37.5 | 65.96 | b |
| | Control (distilled water) | 73.89 | a |

content rate in nineth day. The least rate of water content in day ninth is related to control treatment (distilled water) (Table 4).

**Vase life time**: The interaction effect of method and treatmen on vase life time was significant at 1% level (Table 5). Comparing thymol and carvacrol treatment mean in 50+50 ppm in short term method had the most vase life (11.67 days). The most vase life of vase in standard method of 25 + 25 ppm thymol and carvacrol treatment was 10.67 days. The least vase life was related to control treatment (distilled water). Therefore using short term method increased Vase life of Gladiola cut flowers (Table 6).

## DISCUSSION AND CONCLUSION

Because flowers in short term method were less time in solution, they could have the better results. In standard, method flowers due to continuous placing in solution did not cause properties improvement. Vase life decreased due to vessel blockage and due to blocking stems and vessels

Table 4. Method*Traetment*Time interaction effect on petal aqueous contents of *Gladiolus grandiflorus* L. Cut flowers

| Method/Treatment Time | | zero day | | Thirth day | | sixth day | | ninth day | |
|---|---|---|---|---|---|---|---|---|---|
| **Short Term** | Thym 25+Carv 25 | 12.14 | abc | 10.49 | bcde | 5.89 | hijkl | 3.44 | lmn |
| | Thym 50+Carv 50 | 12.14 | abc | 11.52 | abcd | 7.38 | efghij | 4.82 | jklm |
| | Thym 75+Carv 75 | 12.14 | abc | 12.29 | abcd | 7.68 | efghij | 5.27 | ijklm |
| | Control (distilled water) | 12.14 | abc | 7.50 | efghijk | 2.18 | mn | 0.89 | n |
| **Standard** | Thym12.5+Carv 12.5 | 12.14 | abc | 14.00 | a | 7.15 | fghijk | 3.11 | Lmn |
| | Thym25+Carv 25 | 12.14 | abc | 9.81 | bcdef | 9.22 | cdefg | 3.34 | Lmn |
| | Thym37.5+Carv 37.5 | 12.14 | abc | 9.99 | bcdef | 8.20 | efghi | 4.52 | Klm |
| | Control (distilled water) | 12.14 | abc | 7.50 | efghijk | 2.18 | mn | 0.89 | N |

Table 5. Analys of variance for vase life of *Gladiolus grandiflorus* L. Cut flowers

| S.O.V. | df | Vase life |
|---|---|---|
| method | 1 | 0.00 |
| Traetment | 5 | 16.38** |
| method*Traetment | 5 | 1.06** |
| Error | 12 | 0.72 |

**: Significant at the1%level of probability

Table 6. Method*Traetment interaction effect on vase life characteristics of *Gladiolus grandiflorus* L. Cut flowers

| Method | Treatment | LS Mean | |
|---|---|---|---|
| **Short Term** | Thym 25+Carv 25 | 9.33 | cd |
| | Thym 50+Carv 50 | 11.67 | a |
| | Thym 75+Carv 75 | 10.33 | bc |
| | Control (distilled water) | 7.67 | e |
| **Standard** | Thym12.5+Carv 12.5 | 9.00 | d |
| | Thym25+Carv 25 | 10.67 | ab |
| | Thym37.5+Carv 37.5 | 9.33 | cd |
| | Control (distilled water) | 6.33 | f |

cut area, solution lead to decrease in stems. Short term method by preventing vessel blockage and keeping flowers torjescense increased vase life relative to standard method (Damunupola *et al.,* 2008). Anthocyanine increased by buds flourishing. This study results related to keep anthocya ninedayes in petals that examined aminopurin benzyl on salvia (Setyadjit *et al.*, 2004). Also sugar concentrates in petal tissue, improve osmotic potential and increase carbohydrates rate in cut flowers to use in growth and respiration activities that this delays flowers senescent. A research indicated that queenolinhydroxy increases sugar concentration in Sonia rose cut flowers as an antimicrobial complex with succarose (Ichimura *et al.*, 2003). Slower florish speed is better. Essences by preventing bacteria entrance decrease water absorbtion and buds florish speed and buds in treatments flourished in longer time (DE *et al*., 1996). Using short term thymol and carvacrol increased petal aqueous contents. This act cause the positive effect of thymol and carvacroland succarose to keep flowers torjescens and improve flowers water relations in more short time. Possibly essences by creating negative pressure in cells help water absorbtion by flowers, that due to water potential decreasing, water entrance occurs quicker and causes cell expansion and sugar dilution in tissue. Experiment in 2004 to study giberlic acid effect on Gerbera cut flowers are similar to present study results (Emongor *et al.*, 2004). This research indicated that using thymol and carvacrol extracts in short time method increased vase life Glodiolacut flowers. The most petal soluble sugar rate, petal aqueous contents and least florish speed in concentrations of 75+75 ppm observed in short time method. Therefore, short time method is recommend to increase vase life and keep flowers qualitative properties.

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