

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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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.

Abstract

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

INTRODUCTION

Gladiola in scientific name *Gladiolous grandiflorous* belongs to family Iridaceae that is a cut flower and a valuable garden plant. Gladiola 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 mytocondria 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 20-22°C and rational humidity was almost 75 percent.

Light density was 18.9 mol/m²/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).

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

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 repli- cates 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 sig- nificant 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.

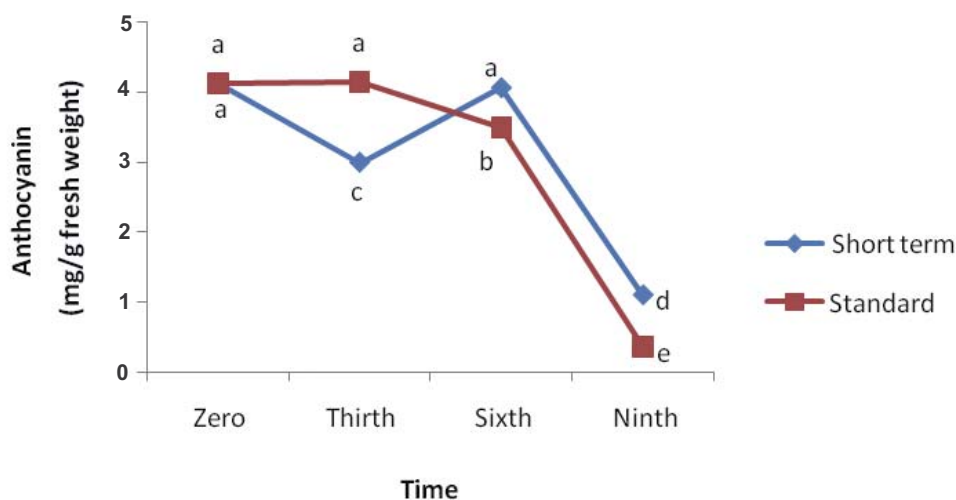


Fig. 1. Method*Treatment interaction effect on anthocyanin characteristics of *Gladiolus grandiflorus* L. Cut flowers

Table 2. Method*Treatment*Time interaction effect on petal soluble 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	33.77	29.7	18.33
	Thym 50+Carv 50	30.31	34.68	33.21	22.77
	Thym 75+Carv 75	30.31	33.53	33.13	30.57
	Control (distilled water)	30.31	21.17	18.76	8.62
Standard	Thym12.5+Carv 12.5	30.31	41.17	33.62	27.82
	Thym25+Carv 25	30.31	28.77	25.46	21.78
	Thym37.5+Carv 37.5	30.31	31.41	26.09	21.37
	Control (distilled water)	30.31	21.17	18.76	8.62

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 suger rate on day ninth was more in standard method treatments and was higher than short term treatment. At least solution suger 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*Treatment 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 ninth 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 treatment 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*Treatment*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. Analysis of variance for vase life of *Gladiolus grandiflorus* L. Cut flowers

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

** : Significant at the 1% level of probability

Table 6. Method*Treatment 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 anthocyanin days 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 flourish speed is better. Essences by preventing bacteria entrance decrease water absorbtion and buds flourish 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 carvacrol and 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 flourish 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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Literature Cited

- Amariutei, A., Alex, C. and Burzo, I. 1995. Physiological and biochemical changes of cut gerbera inflorescences during vase life. *Acta Horticulturae*. Abst. 405.
- Aggarwal, K.K., Khanuja, S.P.S., Ahmad, A., Kumar, T.R.S., Gupta, V.K. and Kumar, S. 2002. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flavor and Fragrance Journal*. 17: 59–63.
- Anonymous. 2002. Determination of crude protein in cereals and cereal products for food and for fed. Standard methods of the international association for cereal science and technology. ICC Standard No:105/2, Viena.
- Askari, F. 2003. Essential oil composition of *thymus daenensis* Celak. from Iran. *Journal of Essential Oil Bearing Plant*. 6: 3-10.
- Anonymous, 2009. Office of ornamental plants, medicinal and edible mushrooms, Ministry of Agriculture. Tehran.
- Anonymous. 2010. Jihad-e-keshavarzi of Hormozgan.
- Bird, B.R., Hung, S.S.O., Hadley, M. and Draper, H.H. 1983. Determination of malonaldehyde in biological materials by high-pressure liquid chromatography. *Annals of Biochemistry*. 128: 240–244.
- Botelaho, M.A., Nogueira, N.A.P., Bastos, G.M., Fonseca, S.G.C., Lemos, T.L.G., Matos, F.J.A., Montenegro, D.H., eukelbach, J., Rao, V.S. and Brito, G.A.C. 2007. Antimicrobial activity of essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. *Brazilian Journal of Medical and Biological Research*. 40,349-356.
- Chakrabarty, D. and Datta, S. K. 2008. Micropropagation of gerbera: lipid peroxidation and antioxidant enzyme activities during acclimation process. *Acta Physiology Plant*. 30, 325-331.
- De Hertogh, A. and Le Nard, M. 1998. The physiology of flower bulbs. Translation by Mashhad Jihad University, 352 pages.

- DE, M.G., Capieau, K., Audenaert, K., Buchala, A., Metrux, J.P. and Hofte, M. 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas auriginosa* 7NSK2 activate the Systemic Acquired Resistance pathway in bean. *Molecular Plant-Microbe Interaction* 12: 450-458.
- Damunupola, J.W. and Loyce, D.C. 2006. When is a vase solution biocide not, or not only, antimicrobial? *Journal of Japanese Society for Horticulture Science*. 77: 1-18
- Emongor, V.E. 2004. Effects of Gibberelic acid on postharvest quality and vase-life of gerbera cut flower (*Gerbera Jamesonii*). *Agronomy*. 3: 191- 195.
- Ezhilmathi, K., Singh, V.P., Arora, A. and Sairam, R.K. 2007. Effect of 5- sulfosalicylic acid on antioxidant activity in relation to vase life of gladiolus cut flower. *Plant Growth Regul.* 51: 99-108.
- Hashemabadi, D. 2012. *Dianthus caryophyllus* L. cv. Tempo: improvement vase life and postharvest characteristics by silver thiosulphate. *Annals of Biological Research*. 3 (12): 5616-5618.
- Hossain, Z., Mandad, A., Data, K.S. and Biswas, K. A. 2006. Decline in Ascorbate Peroxidase Activity A Prerequisite Factor for tepal Senescence in gladiolus. *Journal of Plant Physiology*. 163: 184 – 194.
- Hopkins, M., Taylor, C., Liu, Z. and Ma, F. 2007. Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytology*. 175: 201-214.
- Ichimura, K. and K. Suto. 1999. Effects of the time of sucrose treatment on vase life, soluble carbohydrate concentrations and ethylene production in cut sweet pea flowers. *Plant Growth Regulation*. 28: 117-122.
- Ichimura, K., Kojima, K. and Goto, R. 1999. Effects of temperature, 8-hydroxyquinoline sulphate and sucrose on the vase life of cut rose flowers. *Postharvest Biology and Technology*. 15: 33–40.
- Ketsa, S., Piyasaengthong, Y. and Pathuangwong, S. 1995. Mode of action of AgNO₃ in maximizing vase-life of *Dendrobium* pompadour flowers. *Postharvest Biology and Technology*. 5: 109-117.
- Lal, S.D., Shah, A. and Pant, C.C. 1990. Effect of certain chemical substances on vase life and quality of gladiolus cv. “Silver Horn”. *Progress Horticulture*. 22: 63-68.
- Martinez–Romerro, D., Guillen, F., Valverde, J.M., Bailen, G., Zapata, P., Serrano, M. and Castillo, S. Valero, D. 2007. Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. *International Journal of Food and Microbiology*. 115: 144-148.
- Minami, M. and Yoshikawa, H. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta*. 92: 337–342.
- Navarro, E., Baun, A., Behra, R., Hartman, N.B., Filser, J., Miao A.J., Quiagg, A., Santschi, P.H. and Sigg, L. 2008. Environmental behavior and ecotoxicity of engineered nonparticles to algae, plants, and fungi. *Ecotoxicology*. 17: 372-386.
- Nair, S.A., Singh, V. and Sharma, T.V.R.S. 2003. Effect of chemical preservatives on enhancing Vase-life of Gerbera flowers. *Journal of Tropical Agriculture*. 41: 56-58.
- Nabigol, A., Naderi, R.A. and Kafi, M. 2006. The effect of vase life of *Chrysanthemum* using preservative solutions and recutting the end of stem. *Journal of Horticultural Science and Technology of Iran*, 7(4): 207-216.
- Olasupo, N. A., Fitzgerald, D.J., Gasson, M. J. and Narbad, A. 2003. Activity of antimicrobial compounds against *Escherichia coli* and *Salmonella enteric* serovar Typhimurium. *Letter of Applied Microbiology*. 36: 488-451.
- Ozcan, M. and Chalchat, J.C. 2004. Aroma profile of *Thymus vulgaris* L. growing wild in turkey. *Bulgarian Journal of Plant Physiology*. 30: 68-73.
- Pauli, A. 2001. Antimicrobial properties of essential oil constituents. *International Journal of Aromatherapy*. 3: 126–133.
- Rasooli, I. and Mirmostafa, S.A. 2003. Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. *Journal of Agriculture and Food Chemistry*. 51: 2200–2205.

- Roller, S. and Seedhar, P. 2002. Carvacrol and cinamic acid inhibit microbial growth in fresh-cut melon and kiwifruit at 4° and 8°C. South Bank University, London, UK. Letters in Applied Microbiology. 35: 390–394.
- Sankhla, N., Mackay, W.A. and Davis, T.D. 2005. Corolla abscission and petal color in cut plox flower heads. Effects of sucrose and thidiazuron. Acta Horticulturae. 669: 389-394.
- Stewart, E.A. 1989. Analysis of vegetation and other organic material. Academic Press. New York. 46-50.
- Setyadjit, j., yce, D.C., Irving, D.E., and Simons, D.H. 2004. Effects of 6-benzylaminopurine treatment on the longevity of harvested grevillea 'Sylvia' inflorescences. Plant Growth Regulation. 43: 9-14.
- Solgi, M., Kafi, M., Taghavi, T.S. and Naderi, R. 2009. Essential oil and silver nano particles (SNP) as novel agent to extend vase-life of gerbera (*Gerbera Jamesonii* cv. Dune) flowers. Postharvest Biology and Technology 53: 155-158.
- ULtee, A., Bennink, M.H.J. and Moezelaar, R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied Environmental Microbiology. 68: 1561-1568.
- Van Doorn, W.G. and Van Meeteren, U. 2003. Flower opening and closure: a review. Journal of Experimental Botany. 54: 1801–1812.
- Van Doorn, W.G., and De Wite, Y. 1994. Effects of bacterial on scape bending in cut *Gerbera jamesonii* flowers. Journal of American Society for Horticulture. 119: 568-571.
- Wright, K.P. and Kader, A.A. 1997. Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. Postharvest Biology and Technology. 10: 89–97.
- Yahyazadeh, M., Omidbaigi, R., Zare, R. and Taheri, H. 2008. Effect of some essential oils on mycelia growth of *Penicillium digitatum* Sacc. World Journal of Microbiology and Biotechnology. 24: 1445-1450.
- Ziaei Movahed, Z., Kafi, M. and Khalighi, A. 2008. Effect of natural compounds (*Mentha. pulegium* and *Rosmarinus* essential oils) on postharvest and qualitative traits of *Gerbera* Cut flowers, Cultivar Eco. Department Master degree of Horticultural, College of Agriculture and Natural Resources, Tehran University. 102 pp.