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Original Article

Study of the Chemical Composition of Essential Oil of *Teucrium chamaedrys* at the Different distillation in Mazandaran Province

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Abstract

The composition of the essential oils of *Teucrium chamaedrys* L. belongs to the family Lamiaceae, It is growing wild in the margin of mountainous roads of arid and cold climate of north Iran. In this research, essential oils of *T. chamaedrys*, extracted and measured at the different location by different methods of distillation and then were analyzed by GC and GC/MS. Investigation and comparison on essential oil constituents of herbs were collected from three localities (Chalus, Galoogah and Gadook). The essential oil yield at the different location from Chalus by water distillation (Clevenger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.28%), and by steam distillation were (0.04%), the essential oil yield at the different location from Galoogah by water distillation (Clevenger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.05%), and by steam distillation were (0.09%), the essential oil yield at the different location from Gadook by water distillation (Clevenger) were (0.2%), and by water & steam distillation (Kyzer & Long) were (0.16%), and by steam distillation were (0.08%), respectively. Major component identificated in sample from Chalus by water distillation (Clevenger) were α- cadinene (15.4%), Z- β-farnesene (9.6%),1-eicosene (8.2%), and by water & steam distillation (Kyzer & Long) were 1-eicosene (12.8%), α- cadinene (6.3%), benzyl salicylate (5.9%), and by steam distillation were cis-3-hexenyl benzoate (10.9%), (E,Z)-farnesol (10.3%), benzyl salicylate (8.3%). Major component identificated in sample from Galoogah by water distillation (Clevenger) were E- α-farnesene (22.9%), α- calacorene (20.2%), α- murrolene (8.0%), and by water & steam distillation (Kyzer & Long) were α- cadinene (30%), E-β- caryophyllene (23.5%), E- α-farnesene (7.2%), and by steam distillation were E- α-farnesene (18.4%), 1-eicosene (15.2%), benzyl salicylate (10%).Major component identificated in sample from Gadook by water distillation (Clevenger) were α- murrolene (12%), cis-3-hexenyl benzoate (11.3%), methyl decanoate (8.9%), and by water & steam distillation (Kyzer & Long) were α-murrolene (8.7%), n- heptadecane (7.9%), cis-3-hexenyl benzoate (7.2%), and by steam distillation were α-murrolene (14.4%), cis-3-hexenyl benzoate (11.3%), E- α-farnesene (9.6%), respectively.

Key words: Essential oil, *Teucrium chamaedrys* L., Flowering stage and vegetative stage, GC and GC/MS

Introduction

The genus *Teucrium* (Lamiaceae) is comprised of about 340 species widespread over the world. In the Flora Iranica, this genus is represented by 12 species, of which 3 are endemic [1]. Several *Teucrium* species are used in Iranian folk medicine as medicinal plant [2-5]. These oils are characterized by the presence of sesquiterpenes such as caryophyllene, caryophyllene oxide, germacrene D, α-humulene, α-muurolene, *(E)-β-*farnesene and the monoterpene carvacrol. Due to

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the wide spectrum of biological activities displayed by the essential oils, these compounds are the subject of different researches. The multiple roles of the essential oils and their main components make them natural substances of great importance in several fields such as physiological function of growth, ecological function, development [6], resistance against diseases and insects [7]. They also possess antimicrobial, antiviral, antimycotic, antioxidant, antiparasitic and insecticidal properties [8-12]. Regarding phytopathogenic viruses, various substances of natural and synthetic origin have been assessed for their antiviral activity [13-16]. The aim of the study was to determine the volatiles of tree locations of grown *Teucrium chamaedrys* L. in Mazandaran province.

Material and Methods

Plant material

The plant material of *Teucrium chamaedrys* L. were collected on August 2012 from Mazandaran province (from different location, Chalus, Galoogah and Gadook) in north of Iran, and were dried in the shade at room temperature. The specimen is deposited in Central Herbarium of Iran (TARI). (see: Holmgren, Index Herbarium).

Isolation of the essential oil

100 gr. of dried aerial parts of *Teucrium chamaedrys* L. were extracted by different methods of distillation: hydro-distillation (Clevenger type), water and steam distillation (Kyzer & Long) , and steam distillation. The essential oil yield at the different location from Chalus by water distillation (Clevenger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.28%), and by steam distillation were (0.04%), The essential oil yield at the different location from Galoogah by water distillation (Clevenger) were (0.06%), and by water & steam distillation (Kyzer & Long) were (0.05%), and by steam distillation were (0.09%), The essential oil yield at the different location from Gadook by water distillation (Clevenger) were (0.2%), and by water & steam distillation (Kyzer & Long) were (0.16%), and by steam distillation were (0.08%), the quantitative and qualitative analyses of the oils were performed by GC and GC-MS, respectively.

Gas Chromatography

GC analyses were performed using a Shimadzu-9A gas chromatograph equipped with a flame ionization detector, and the percentage of relative amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without applying correction factors. The analysis was carried out using a Ph-5 fused-silica column (30m x 0.25 mm, film thickness 0.1μm). The inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Oven temperature program were as follow: initial temperature 60 °C to start the final temperature of 210 °C. The initial 3 °C per minute to be added, and then injected into the chamber to a temperature of 280 °C. The carrier gas inlet pressure to the column: helium with a purity of 99/99% of the inlet pressure to the column equal to 1.5 5Kg/cm^2 is set.

Gas Chromatography-Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used . The column was same as GC , and the GC conditions were as above. Mass spectrometer conditions were : ionization potential 70 eV; electron multiplier energy 2000 V.
The identity of the oil components was established from their GC retention indices, relative to C_7- C_{25} n-alkanes, by comparison of their MS spectra with those reported in the literature [17-19], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results and Discussion

Studies showed considerable quantitative and qualitative variations among the major compounds identified by the workers from the different countries which confirm the existence of different chemotypes of *Teucrium chamaedrys* L. Comparing the chemical constituents identified in the oil analyzed with those reported earlier, results showed that the amount of E-β- caryophyllene (23.5%), in our analyzed oil was higher than the reported by other workers from Iran by Semnani, *et al.* [20], on 2005, which showed that the *β*-caryophyllene (10.5%), available in this region contain higher amount of β- caryophyllene and thus could be used as a rich source of essential oil containing rich amount of β- caryophyllene for low volume high value products.

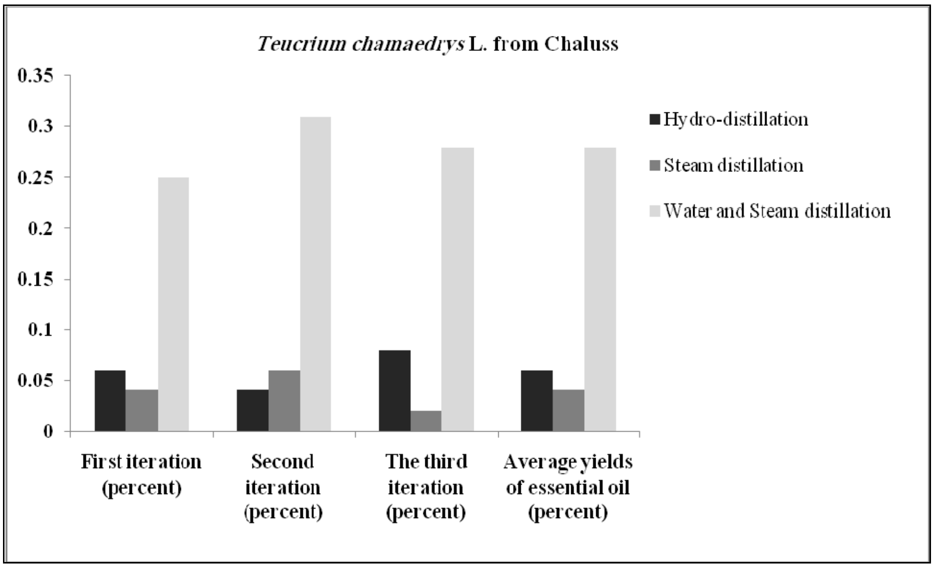

Fig 1. A comparison diagram of essential oil from *Teucrium chamaedrys* from Chaluss location in Mazandran proviance

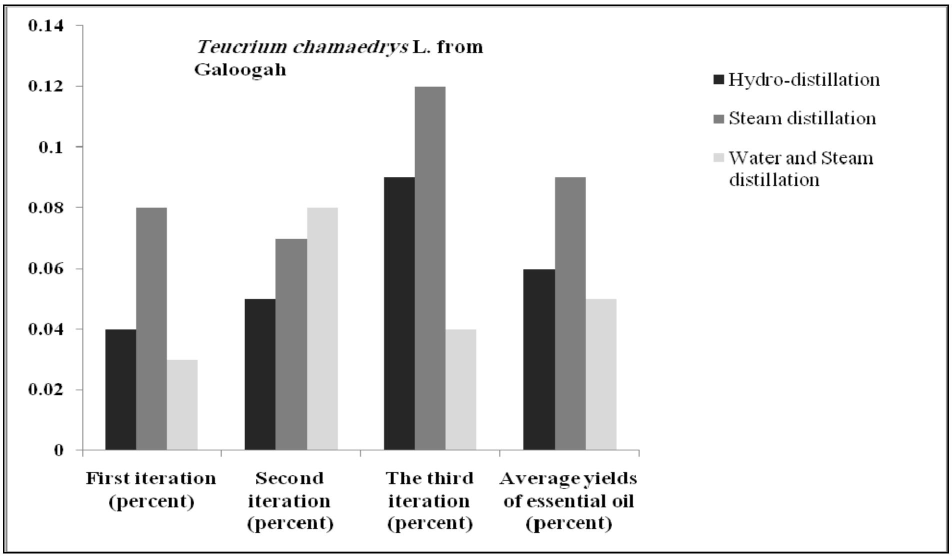

Fig 2. A comparison diagram of essential oil from *Teucrium chamaedrys* from Galoogah location in Mazandran proviance

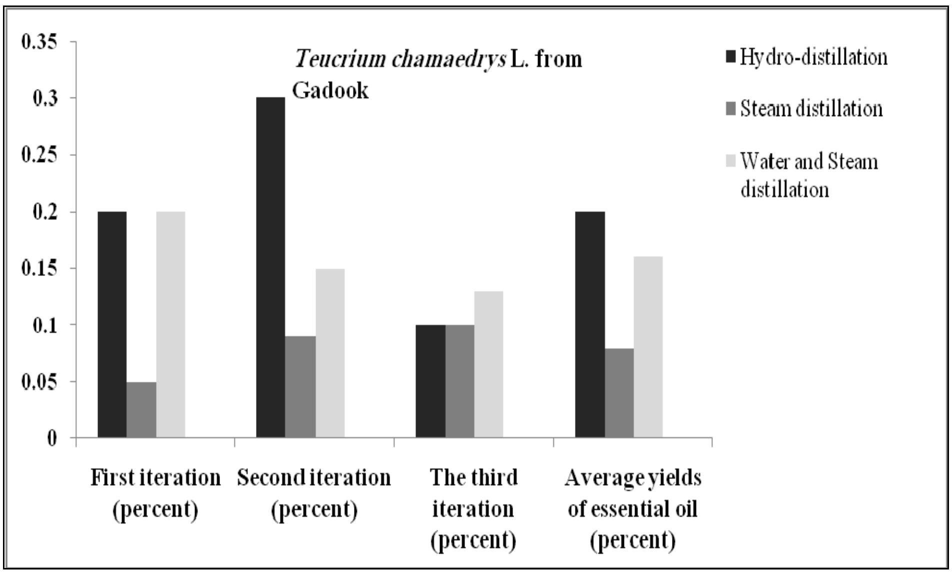

Fig 3. A comparison diagram of essential oil from *Teucrium chamaedrys* from Gadook location in Mazandran proviance

Table l Essential oils percentage of *Teucrium chamaedrys* at three location in Mazanderan Proviance by different methods of distillation

Methods of distillation	Chaluss First iteration	Chaluss Second iteration	Chaluss Third iteration	Mean	Galoogah First iteration	Galoogah Second iteration	Galoogah Third iteration	Mean	Gadook First iteration	Gadook Second iteration	Gadook Third iteration	Mean
Hydro-distillation (Clavenger)	0.06	0.04	0.08	0.06	0.04	0.05	0.09	0.06	0.2	0.3	0.1	0.2
Steam distillation	0.04	0.06	0.02	0.04	0.08	0.07	0.12	0.09	0.05	0.09	0.6	0.08
Water and Steam distillation (Kyzer & Long)	0.25	0.31	0.28	0.28	0.03	0.08	0.04	0.05	0.2	0.15	0.13	0.16

Table 2 Essential oils chemical composition of *Teucrium chamaedrys* at three location in Mazanderan Proviance by different methods of distillation

		Chalus			Galoogah			Gadook		
Compounds name	R.I.	Hydro-distillation Clevenger	water & steam distillation Kyzer & Long	Steam distillation	Hydro-distillation Clevenger	water & steam distillation Kyzer & Long	Steam distillation	Hydro-distillation Clevenger	water & steam distillation Kyzer & Long	Steam distillation
camphene	956	-	-	-	-	-	-	-	-	0.6
sabinene	973	-	-	-	-	-	-	-	-	0.4
myrcene	989	-	-	-	-	-	-	-	-	0.8
E-β-ocimene	1056	-	-	-	-	-	-	0.8	-	-
γ- terpinene	1061	-	-	-	-	-	-	-	0.3	-
trans – linalool oxide	1066	-	-	-	-	-	-	2.0	0.7	1.9
linalool	1109	-	2.1	-	-	-	-	-	-	-
α- terpineol	1184	-	-	-	-	-	-	6.6	1.7	3.0
trans- carveol	1217	-	-	-	-	-	-	1.1	0.3	-
cis- carveol	1228	-	-	-	-	-	-	0.5	-	-
linalyl acetate	1267	-	1.2	-	-	-	-	0.6	-	-
bornyl acetate	1294	-	-	-	-	-	-	0.6	-	-
n-tridecane	1310	0.6	5.2	-	0.5	0.7	-	0.7	-	-
methyl decanoate	1326	0.5	0.8	0.6	4.1	0.3	-	8.9	2.0	1.4
α- terpenyl acetate	1341	0.3	-	-	-	-	-	-	-	-
α- copaene	1371	-	-	0.8	-	-	-	3.9	3.6	1.7
Z-β- caryophyllene	1411	1.1	1.0	1.5	0.7	3.7	0.4	-	-	-
E-β- caryophyllene	1424	-	-	-	0.3	23.5	-	2.6	2.3	2.4
Z- β-farnesene	1450	9.6	3.7	2.9	-	-	-	1.0	1.0	1.0
α- hummulene	1469	1.0	0.5	0.5	-	-	0.9	-	-	-
E- α-farnesene	1475	1.7	0.9	4.9	22.9	7.2	18.4	7.3	2.3	9.6
germacrene D	1487	0.9	-	-	1.0	0.7	-	-	1.3	-
α- Murrolene	1493	3.1	-	6.3	8.0	1.5	3.3	12.0	8.7	14.4
germacrene A	1512	0.9	-	3.1	0.4	5.0	0.7	0.5	0.4	-
trans- calamenene	1529	-	-	-	-	-	-	7.1	4.8	7.8
α- cadinene	1535	15.4	6.3	6.2	6.4	30.0	2.6	2.3	2.9	4.0
α- calacorene	1547	3.9	-	1.6	20.2	0.3	2.6	1.6	1.7	4.3
Cis-3-hexenyl benzoate	1570	4.4	1.3	10.9	3.0	3.3	1.4	11.3	7.2	11.3
Caryophyllene oxide	1582	-	-	-	-	-	-	2.1	3.4	1.7
Viridiflorol	1593	0.4	1.6	1.1	-	-	0.9	0.5	1.1	0.8
Tetradecanal	1621	2.1	1.8	0.8	0.6	2.5	0.9	-	0.7	-
α- muurolol	1643	1.6	2.1	1.1	0.6	1.2	1.1	0.6	0.6	0.4
ar- turmerone	1664	6.9	4.8	4.2	3.5	0.7	4.5	1.0	2.4	1.1
elemol acetate	1679	2.0	4.0	3.9	2.9	0.5	5.0	5.7	6.8	3.5
longiborneol acetate	1686	3.3	7.3	-	-	-	-	-	-	0.9
germacrone	1693	0.6	-	5.0	2.7	2.0	4.1	0.4	1.0	-
n- heptadecane	1700	-	1.0	2.6	0.5	1.5	0.9	2.5	7.9	2.5
(Z,Z)-farnesol	1720	4.3	5.1	4.4	1.9	1.0	3.2	1.9	1.2	2.7
curcumenol	1734	2.6	3.4	3.0	1.4	0.7	2.0	-	-	-
(E,Z)-farnesol	1741	2.5	4.1	10.3	1.0	1.7	1.8	-	-	-
8- α- 11- elemodiol	1748	1.4	2.5	3.3	0.4	2.0	1.1	-	-	-
n-pentadecanol	1775	4.0	5.8	3.7	2.0	0.6	2.5	-	-	-
β- bisabolenol	1788	0.7	0.9	-	-	-	-	-	-	-
1-octadecene	1794	0.4	0.5	0.5	-	-	0.5	-	-	-
benzyl salicylate	1864	7.4	5.9	8.3	2.9	4.6	10.0	-	-	-

n-hexadecanol	1871	-	0.6	-	-	-	-	-	-	-
n-nonadecane	1906	1.8	0.9	-	0.4	0.5	-	-	-	-
cyclohexadecanolide	1934	4.8	1.5	0.8	0.9	1.6	1.5	-	-	-
1-eicosene	1982	8.2	12.8	4.1	3.1	-	15.2	-	-	-
n-eicosane	2008	-	-	-	-	-	0.7	-	-	-
n-octadecanol	2084	-	2.0	0.6	0.5	-		-	-	-
methyl octadecanoate	2125	-	4.4	1.9	1.1	-	5.3	-	-	-
abieta-8(14),13(15)-diene	2155	-	0.6	-	-	-	-	-	-	-
1-docosane	2184	-	0.5	-	-	-	-	-	-	-
n-docosane	2212	-	1.2	-	-	-	-	-	-	-
n-tricosane	2295	-	0.5	-	-	-	0.8	-	-	-

RI – Retention indices on DB-5 capillary column

In the current research the most important compositions in the essential oil from *T. chamaedrys* from Iran by Semnani, *et al.* on 2005, forty-nine components were identified in the this oil. The major constituents of the essential oil were germacrene D (16.5%), (*Z*)-*β*-farnesene (12.2%), *β*-caryophyllene (10.5%), *α*-pinene (9.1%) and *δ*-cadinene (7.4%) [14]. In our research major component identificated in sample from Chalus by water distillation (Clevenger) were α- cadinene (15.4%), Z- β-farnesene (9.6%), and by water & steam distillation (Kyzer & Long) were α-cadinene (6.3%), and major component identificated in sample from Galoogah by water distillation (Clevenger) were E- α-farnesene (22.9%), and by water & steam distillation (Kyzer & Long) were α- cadinene (30%), E-β-caryophyllene (23.5%), E- α-farnesene (7.2%), and by steam distillation were E- α-farnesene (18.4%), and major component identificated in sample from Gadook by steam distillation were E- α-farnesene (9.6%).

Kaya , *et al.* from Flora of Turkey by six subspecies reported on 2009, the aerial organs of *T. chamaedrys* L. subsp. *trapezunticum* Rech.f. and the aerial parts were subjected to microdistillation for the isolation of volatiles. The analysis was simultaneously performed by using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major components were characterized as *β*-caryophyllene (18%), nonacosane (12%), germacrene D (11%), caryophyllene oxide (7%), and *α*-pinene (7%) for subsp. *trapezunticum*, and caryophyllene oxide (23%), *α*-pinene (11%), and caryophyllenol II (5%) for subsp. syspirense [21]. In our research major component identified in sample from Galoogah by water & steam distillation (Kyzer & Long) were α-cadinene (30%), E-β-caryophyllene (23.5%), E-α-farnesene (7.2%).

The composition of the essential oils of *Teucrium chamaedrys* L. from Corsica and Sardinia islands were investigated using a combination of gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS) after fractionation over column chromatography. Eighty-seven compounds were identified, the main components were β-caryophyllene (29.0% and 27.4%, respectively) and germacrene D (19.4% and 13.5%, respectively), followed by α-humulene (6.8%) and δ-cadinene (5.4%) in the Corsican sample and by caryophyllene oxide (12.3%) and α-humulene (6.5%) in the Sardinian sample. The study confirms the quantitative variability of the chemical composition of *T. chamaedrys* oils [22]. In our research major component identificated in sample from Chalus by water distillation (Clevenger) were α- cadinene (15.4%), Z- β-farnesene (9.6%), and by water & steam distillation (Kyzer & Long) were α- cadinene (6.3%), and major component identificated in sample from Galoogah by water distillation (Clevenger) were E- α-farnesene (22.9%), and by water & steam distillation (Kyzer & Long) were α- cadinene (30%), E-β-caryophyllene (23.5%), E-α-farnesene (7.2%), and by steam distillation were E-α-farnesene and by steam distillation were E-α-farnesene (9.6%). Comparing the results of different studies on essential oil composition of *Teucrium chamaedrys* L. our results reveals that their constituents are variable according to their habitat that may be regarded to different chemotypes.

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References

1. Mozaffarian V. A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran. 1996, p:542.
2. Abdollahi M, Karimpour H, Monsef-Esfahani HR. Antinociceptive effects of *Teucrium polium* L. total

extract and essential oil in mouse writhing test. Pharmacol Res. 2003;48:31-35.

3. Yildirim A, Cakir A, Mavi A, Yalicin M, Fauler G, Taskesnligil Y. The variation of antioxidant activities and chemical composition of essential oilsof *Teucrium oriental* L. Var.*orientale* during harvesting stages. Flavour Fragr J. 2004;19:367-372.
4. Kucuk M, Gulec C, Yasar A, ucuncu O, Yayli N, Coskuncelebi K, TerZioglu S. Chemical composition and antimicrobial activities of essential oil of *Teucrium chamaedry* Subsp. *chamaedrys*, *T. orientale* var. *Puberulens*, and *T. chamaedrys* subsp. *lydium.* Pharmaceutical Biol. 2006;44:592-599.
5. Ricci D, Fraternale D, Giamperi L, Bucchini A, Epifano F, Burini G, Curini M. Chemical composition, antimicrobial and antioxidant activity of the essential oil of the *Teucrium marum* (Lamiaceae). Ethnopharmacol J. 2005;98:195-200.
6. Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry. 2003;64:3-19.
7. Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. Nat Chem Biol. 2007;3:408-414.
8. Michaelakis A, Theotokatos SA, Koliopoulos G, Chorianopoulos NG. Essential oils of *Satureja* species: insecticidal effects on *Culex pipens* larvae (diptera: culicidae). Molecules. 2007;12:2567-2578.
9. Bishop CD. Antiviral activity of the essential oil of *Melaluca alternifolia* (Maiden & Betche) cheel (tea tree) against tobacco mosaic virus. J Essent Oil Res. 1995;7:641-644.
10. Juglal S, Govinden R, Odhav B. Spice oils for the control of co-occurring mycotoxinproducing fungi. J Food Prot. 2002;65:683-687.
11. Moon T, Wilkinson JM, Cavanagh HMA. Antiparasitic activity of two *Lavandula* essential oils against *Giardia duodenalis*, *Trichomonas vaginalis* and *Hexamita inflata.* Parasitol Res. 2006;99:722-728.
12. Lamiri A, Lhaloui S, Benjilali B, Berrada M. Insecticidal effects of essential oils against Hessian fly, *Mayetiola destructor* (Say). Field Crop Res. 2001;71:9-15.
13. Othman BA, Shoman SA. Antiphytoviral Activity of the *Plectranthus tenuiflorus* on Some Important Viruses. Int J Agri Biol. 2004;6:844-849.
14. Krcatović E, Rusak G, Bezić N, Krajačić M. Inhibition of Tobacco Mosaic Virus infection by quercetin and vitexin. Acta Virolog. 2008;52:119-124.
15. Rusak G, Krajačić M, Pleše N. Inhibition of tomato bushy stunt virus infection using a quercetagetin flavonoid isolated from *Centaurea rupestris* L. Antiviral Res. 1997;36:125-129.
16. Yordanova A, Korparov N, Stomenova E, Starcheva, M. Antiphytoviral activity of 1-morpholinomethyl tetrahydro 2-Pyrimidinone (DDB). Plant Pathol. 1996; 45:547-551.
17. Shibamoto T. Retention Indices in Essential Oil Analysis. In: Capillary Gas Chromatography in Essential oil analysis. Edits., Sandra P, and Bicchi C, Dr. Alfred Huethig Verlag, Heidelberg. 1987;259-274.
18. Davies NW. Gas Chromatographic Retention Index of Monoterpenes and Sesquiterpenes on Methyl silicone and Carbowax 20 M phases. J Chromatogr. 1990;503:1-24.
19. Adams RP, Identification of essential oils by Ion trap Mass Spectroscopy. Academic Press, San Diego, CA.1989.
20. Morteza-Semnani K, Akbarzadeh M, Rostami B. The essential oil composition of *Teucrium chamaedrys* L. from Iran. Flavour & Fragrance J. 2005; 5:544-546.
21. Kaya A, Demirci B, Başer KH. Compositions of essential oils and trichomes of Teucrium chamaedrys L. subsp. *trapezunticum* Rech. fil. and subsp. syspirense (C. Koch) Rech. Fil Chem Biodivers. 2009;6:96-104.
22. Muselli A, Desjobert JM, Paolini J, Bernardini AF, Costa J, Rosa A, Assunta Dessi M. Chemical Composition of the Essential Oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. J Essen Oil Res. 2009;2:138-143.