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Chemical variability of *Thymus capitatus* (L.) Hoff. et Link essential oils and incidence on anti-acetylcholinesterase activity

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Abstract
Essential oil leaves of nine Tunisian *Thymus capitatus* populations, growing wild in six bioclimatic zones, were analyzed by gas chromatography–mass spectrometry (GC–MS). The species was found to be rich in oxygenated monoterpenes (76.9%-84%) followed by monoterpene hydrocarbons (11.6-15.3%). The main component of the essential oils was the carvacrol (73–82.9%). Cluster analysis performed on major compound contents did not reveal clear groupings of populations according to their bioclimatic zone.
The species essential oils were assessed for their anti-acetylcholinesterase activity. The level of biological activity of the populations was linked to their chemical composition difference. The most important activity was observed for populations Tc2 and Tc8 from the upper semi-arid and upper arid bioclimatic zone, respectively, characterized by the highest carvacrol level. The best activity was revealed for the standard carvacrol. A significant correlation between anti-acetylcholinesterase activity and carvacrol was revealed.

Keywords: *T. capitatus*, GC-MS, essential oils, anti-acetylcholinesterase activity

1. Introduction
Aromatic and Medicinal Plants have very interesting biological properties and find applications in various fields, such as pharmaceutical and medicinal industries. These plants represent a new source of active compounds. Indeed, secondary metabolites are the subject of much research *in vivo* and *in vitro*, in particular the search for new natural constituents, such as phenolic compounds and essential oils [1].
They contain combinations of several secondary compounds (terpenoids, phenolic compounds and alkaloids) having multiple biological activities [2]. Indeed, the use of secondary metabolites implicated in antibacterial interactions as sources for news chemical models could satisfy the requirements for crop protection and weeds management [3, 4].
The genus *Thymus* L. belongs to the Lamiaceae family and consists of about 200-350 species [5]. It is indigenous to Mediterranean regions, Asia, North Africa, and it is cultivated in most of the European countries [6]. In Tunisia, the species is widespread in the North, the dorsal ridge, the Cap Bon and becomes rarest in the southern part. It has been used since antiquity in traditional medicine, food preservatives, phytopharmaceutical preparations, and possesses a wide range of several therapeutic effects including antispasmodic, expectorant, sedative and antimicrobial proprieties [7, 8]. In previous phytochemical studies, *T. capitatus* have been showed to contain essential oils, flavonoids, tannins, saponins, triterpenic acids, total phenols and phenolic compounds [9, 10, 11].

Different studies of *T. capitatus* have demonstrated antioxidant [12, 13], allelopathic [14, 15], insecticidal [16], antibacterial [17] and antifungal activities [18]. The aims of this study were: (i) to assess the essential oil variation among nine Tunisian *T. capitatus* populations distributed throughout its natural area in Tunisia, and (ii) to investigate their anti-acetylcholinesterase activity.

Materials and methods

Plant material

Nine populations belonging to different geographic and bioclimatic zones were considered (Table 1). Samples were determined by Pr. BOUSSAID Mohamed. Plant materials were air-dried at room temperature for two weeks. The plant material of *Thymus capitatus* were collected from different regions of Tunisia (Table 1).

Table 1: The main ecological traits and bioclimatic indices of the nine analyzed *T. capitatus* populations

Populations	Code	Latitude	Longitude	Altitude	P (mm)	Q_2	Bioclimaic zone
Korbous	1	36°50'	10°35'	280	550	67.8	sub-humid (Sh)
Essabahia	2	36° 36'	10°10'	112	450	52	upper semi arid (Usa)
Jendouba	3	36°25'	8°44'	150	660	52.3	
Siliana	4	35°51'	9°12'	450	520	54.32	Mean semi-arid (Msa)
Sers	5	36° 6′	9° 40′	474	245	50.6	
Gbollat	6	36°22′	9°52′	150	350	39.7	
Sousse	7	35°30'	10°50'	70	167	42.95	Lower semi-arid (Lsa)
Toujene	8	33°27'	10°08'	600	100	29.0	Upper arid(Ua)
Gabes	9	33°53'	10°70'	60	100	20.5	Lower arid (La)

P: average of yearly precipitation (mm); Q2: (Emberger's coefficient= $2000P/M^2$-m^2).

Essential oil isolation and analysis

Essential oils were extracted by hydrodistillation in a Clevenger type apparatus for 3 h from 100 g of ground leaves. The hydrodistillation was performed for every individual from each population. 10 replications of distillation were performed. The yields were calculated as the quantity of the essential oil compared to the air-dried material (% w/w).

The chemical composition of essential oils was determined by GC-MS analyses. Terpenic compounds were identified by comparison of their retention times with those of authentic standards, by comparison of their retention indices with those of literature and by co-injection of the essential oils with the available authentic standards. The identification was also completed by comparison of their mass spectra of terpenic compounds with those stored in NIST08 and W8N08 libraries.

Acetylcholinesterase inhibition assay

The anti-acetylcholinesterase activity was determined according to slightly modified method described by Eldeen *et al*. [19]. 355 µl of Tris-HCl buffer (50 mM, pH 8; containing 0.1% bovine serum albumine), 20µl of essential oil (at different concentrations) were mixed with 25 µl of the enzyme solution (0.28 U/ml). After incubation during 15 min, 100 µl of acetylcholine iodide (0.15 mM), and 500 µl of DTNB (5,5- dithiobis-2-nitrobenzoic acid, 0,3 mM) were added. The final mixture was incubated for 15 min at 37°C. The absorbance of the mixture was measured at 405 nm. A control mixture was performed without addition of the essential oil. The anti-acetylcholinesterase activity was calculated using the following equation: AChE inhibition (%) = 100 × (Ac-As)/Ac; where, Ac and As are the absorbance of the control and the sample, respectively. Each reaction was performed in triplicate and results were expressed as IC_{50} (concentration providing 50% AChE inhibition). Donepezil, a cholinesterase inhibitor for Alzheimer's disease, was used as positive control.

Statistical analysis

The analysis of variance (ANOVA) followed by Duncan's multiple range tests, using SAS version 9.1.3 program was used to assess the inter population variation of the essential oils composition and their anti-acetylcholinesterase activity.

Cluster analysis, based on the Euclidean distance matrix using the MVSP program, was used to classify populations according to their major compounds.

Correlations between the essential oil composition and anti-acetylcholinesterase activity were carried out with PROC CORR procedure using SAS version 9.1.3.

Results and discussion

Essential oil yield and chemical composition

The yield of *T. capitatus* essential oils, obtained by hydrodistillation, varied between 1.6 and 2.8%. The maximum content was reported in populations Tc7 (2, 4%), Tc8 (2,8%) and Tc9 (2,2%) from the lower semi-arid, upper arid and lower arid bioclimatic zone, respectively (Table 2). However, several works showed higher yields than those observed in our study [20, 21]. Ecological factors associated to genetic ones and to phonological plant stages should be at the origin of these variations [22].

For all populations, twenty four components, representing 95.9–98.5% of the essential oils, were identified (Table 2). Essential oils were characterized by their richness in oxygenated monoterpenes (76.9%-84%) followed by monoterpene hydrocarbons (11.6-15.3%). The amount of sesquiterpenes hydrocarbons (1.9 to 4.7%) and oxygenated sesquiterpenes were detected at low levels (<0.1%-0.6%).

At the species level, carvacrol was the main component (79%) of all the essential oils. In addition, the oil was characterized by high levels of p-cymene (4.9%), γ-terpinene (5%) and β-caryophyllene (3.1%). p-cymene showed a maximum level when carvacrol was at its minimum, which is in accordantance with the literature, reporting that p-cymene is the precursor of carvacrol [23]. Our results are in agreement with those from previous studies performed for Tunisian [21, 24], Moroccan [25], and Italian *T. capitatus* essential oil [26], that are characterized by the carvacrol chemotype.

In our study, a significant variation of the essential oil composition was observed between populations. The highest percentages of carvacrol were observed in population Tc9 (82.9%) and Tc 2 (82.7%) belonging to the lower arid and upper semi-arid bioclimatic zone, respectively. It suggests that each population presents specific microclimatic conditions that influence essential oil composition of individual plants [27].

Table 2: Mean percentage of leaf essential oils from 9 Tunisian populations of *T. capitatus*.

		Sh		Usa	Msa			Lsa	Ua	La	Species
	IR	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6	Tc7	Tc8	Tc9	level
Yields (%)		2	1.8	2.1	1.7	1.9	1.6	2.4	2.8	2.2	2
α- Thujene	929	$0.6^{a}\pm0.0$	$0.5^{ab}\pm0.2$	$0.3^{abc}\pm0.1$	$0.4^{abc}\pm0.1$	$0.2^{c}\pm0.0$	$0.5^{abc}\pm0.0$	$0.2^{bc}\pm0.1$	$0.2^{bc}\pm0.1$	$0.5^{ab}\pm0.1$	0.4
α-Pinene	938	$0.3^{ab}\pm0.0$	$0.2^{abc}\pm0.1$	$0.1^{abc}\pm0.0$	$0.2^{abc}\pm0.0$	$0.1^{abc}\pm0.0$	$0.3^{a}\pm0.0$	$0.1^{bc}\pm0.0$	$0.1^{c}\pm0.0$	$0.2^{abc}\pm0.0$	0.2
Camphene	953	-[a]	-[a]	-[a]	$0.1^{a}\pm0.0$	$0.1^{a}\pm0.0$	$0.2^{a}\pm0.1$	-[a]	-[a]	-[a]	0.0
β-Pinene	978	-[b]	-[b]	-[b]	$0.1^{ab}\pm0.03$	$0.1^{a}\pm0.1$	-[b]	-[b]	-[b]	-[b]	0.0
β-Myrcene	991	$0.6^{a}\pm0.0$	$0.4^{ab}\pm0.3$	$0.5^{ab}\pm0.1$	$0.7^{a}\pm0.1$	$0.3^{b}\pm0.1$	$0.6^{a}\pm0.0$	$0.5^{ab}\pm0.1$	$0.3^{b}\pm0.1$	$0.6^{a}\pm0.0$	0.5
α-Phellandrene	1005	$0.1^{bc}\pm0.0$	-[bc]	-[c]	$0.1^{a}\pm0.01$	-[bc]	$0.1^{ab}\pm0.0$	$0.1^{ab}\pm0.1$	-[c]	$0.1^{bc}\pm0.0$	0.1
3-Carene	1011	$0.6^{bc}\pm0.0$	-[c]	-[c]	-[c]	$1.4^{a}\pm0.4$	-[c]	-[c]	$0.8^{b}\pm0.1$	$0.7^{b}\pm0.1$	0.4
α-Terpinene	1017	$1.0^{ab}\pm0.1$	$0.8^{abcd}\pm0.1$	$0.7^{bcd}\pm0.1$	$1.1^{a}\pm0.1$	$0.4^{d}\pm0.04$	$0.6^{cd}\pm0.0$	$0.8^{cd}\pm0.1$	$0.9^{abc}\pm0.1$	$0.9^{abc}\pm0.1$	0.8
p-Cymene	**1025**	**$3.8^{abc}\pm0.9$**	**$3.1^{c}\pm0.5$**	**$7.4^{a}\pm1.3$**	**$5.0^{abc}\pm0.4$**	**$6.2^{ab}\pm1.1$**	**$7.5^{a}\pm1.1$**	**$4.2^{bc}\pm0.6$**	**$2.8^{c}\pm0.6$**	**$3.9^{bc}\pm1$**	**4.9**
β-Phellandrene	1032	-[c]	-[c]	-[c]	$0.3^{a}\pm0.0$	-[c]	$0.2^{ab}\pm0.1$	$0.2^{a}\pm0.0$	$0.3^{bc}\pm0.0$	-[c]	0.1
4-thujenol	1058	-[a]	$0.5^{a}\pm0.1$	$0.6^{a}\pm0.4$	$0.5^{a}\pm0.0$	-[a]	$0.6^{a}\pm0.0$	$0.3^{a}\pm0.0$	-[a]	-[a]	0.3
γ-Terpinene	**1060**	**$6.4^{a}\pm1$**	**$5.2^{ab}\pm2$**	**$4^{bc}\pm0.6$**	**$6.8^{a}\pm0.5$**	**$2.8^{c}\pm0.2$**	**$3.1^{bc}\pm0.3$**	**$5^{ab}\pm0.5$**	**$6.4^{a}\pm1$**	**$5.3^{ab}\pm1.2$**	**5.0**
α-Terpinolene	1087	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]	$0.2^{a}\pm0.2$	-[b]	-[b]	0.0
Linalool	1098	-[c]	-[c]	$1^{ab}\pm0.48$	$0.6^{bc}\pm0.5$	-[c]	$1.4^{a}\pm0.16$	$0.6^{bc}\pm0.1$	-[c]	-[c]	0.4
Borneol	1165	0.1 ± 0.0^{a}	-[a]	$0.3^{a}\pm0.14$	$0.2^{a}\pm0.1$	$0.8^{a}\pm0.6$	$1.1^{a}\pm0.8$	$0.1^{a}\pm0.1$	-[a]	-[a]	0.3
α-Terpineol	1189	-[c]	-[c]	$0.4^{b}\pm0.2$	$0.3^{b}\pm0.0$	-[c]	$0.7^{a}\pm0.1$	$0.4^{b}0.1$	-[c]	-[c]	0.2
Carvacrol	**1299**	**$81.5^{ab}\pm1$**	**$82.7^{a}\pm1.7$**	**$76.9^{bcd}\pm2.1$**	**$75.1^{cd}\pm0.7$**	**$78.7^{abc}\pm1.6$**	**$73.0^{d}\pm2.0$**	**$78.6^{abc}\pm1.1$**	**$81.7^{ab}\pm1.6$**	**$82.9^{a}\pm1$**	**79.0**
Carvacrylacetate	1300	$0.3^{bc}\pm0.1$	-[c]	$1^{ab}\pm0.0$	$0.7^{abc}\pm0.1$	$1.1^{ab}\pm0.2$	$1^{ab}\pm0.3$	$0.5^{abc}\pm0.2$	$0.5^{bc}\pm0.1$	$1.3^{a}\pm0.7$	0.7
β-Caryophyllene	1412	**$1.9^{d}\pm0.1$**	**$3^{abcd}\pm0.2$**	**$2.3^{cd}\pm0.6$**	**$4.0^{ab}\pm0.4$**	**$3.6^{abc}\pm0.4$**	**$2.4^{bcd}\pm0.4$**	**$4.3^{a}\pm0.2$**	**$4.2^{a}\pm0.3$**	**$1.8^{d}\pm0.6$**	**3.1**
α-Caryophyllene	1457	-[b]	-[b]	-[b]	0.1^{a}	0.1^{a}	$0.1^{a}\pm0.0$	0.1^{a}	-[b]	-[b]	0.05
Alloaromadendrene	1460	$0.1^{ab}\pm0.0$	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]	$0.3^{a}\pm0.0$	$0.1^{ab}\pm0.0$	0.06
α-Bisabolene	1503	-[c]	$0.1^{ab}\pm0.0$	-[c]	-[abc]	-[bc]	$0.1^{abc}\pm0.0$	0.1^{a}	-[c]	-[c]	0.03
β-Bisabolene	1505	-[b]	$0.1^{ab}\pm0.0$	-[b]	-[b]	-[ab]	$0.1^{ab}\pm0.0$	$0.2^{a}\pm0.1$	-[b]	-[b]	0.04
Caryophyllene oxide	1561	-[c]	-[c]	$0.5^{b}\pm0.2$	$0.5^{b}\pm0.0$	-[c]	$1.0^{a}\pm0.1$	$0.6^{b}\pm0.06$	-[c]	-[c]	0.33
Monoterpeneshydrocarbons (%)		13.1	10.7	13.6	15.3	11.6	13.7	11.6	11.8	12.2	12.6
Oxygenated Monoterpenes (%)		81.9	82.9	79.6	76.9	81.5	77.2	80.2	82.2	84.0	80.7
Sesquiterpeneshydrocarbons (%)		2	3.2	2.3	4.1	3.7	2.7	4.7	4.5	1.9	3.2
Oxygenated Sesquiterpenes (%)		-	-	0.5	0.5	-	1	0.6	-	-	0.3
Total Identified Compounds (%)		97±0.3	96.8±1.0	96.0±1.8	96.8±0.5	95.9±0.4	96.6±2.8	97.1±0.4	98.5±0.3	98.1±0.3	97

RI: Retention indices relative to n-alkanes (C9-C24) on HP-5MS column.-: not identified. Means followed by different letters within the same row are significantly different ($p < 0.05$).
See Table 1 for population codes (Tc1. Tc2...Tc9)

The dendrogram based on Euclidean distances among the pairs of populations according to their major essential oil compounds, showed two main groups (Figure 1).

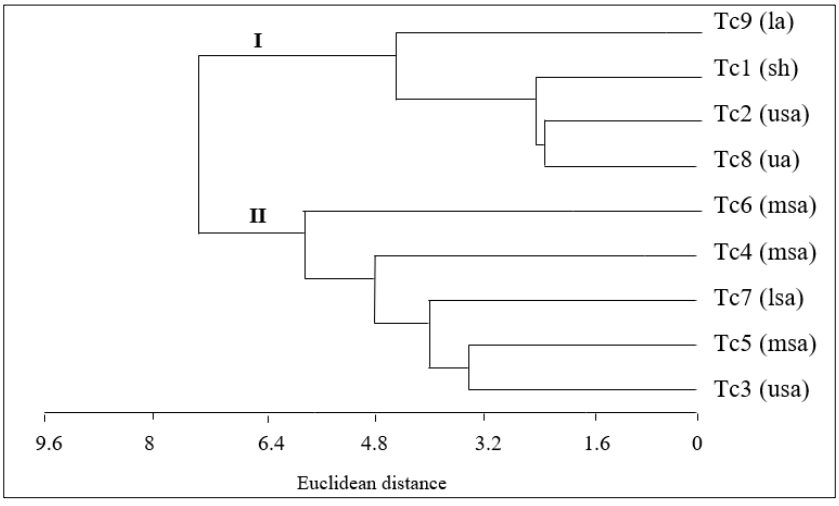

Fig 1: Cluster analysis performed on percentages of the major components of the analyzed *T. capitatus* essential oils.

The first cluster, included four populations Tc9 (lower arid), Tc1 (sub-humid), Tc2 (upper semi-arid) and Tc8 (upper arid), characterized by their richness in carvacrol (81,5-82,9%). The second one (II) consists of populations Tc6, Tc4, Tc5 (mean semi-arid), Tc7 (lower semi-arid) et Tc4 (upper semi-arid) characterized by the lowest amount of carvacrol (73-78,7%).

Anti-acetylcholinesterase activities

A considerable inhibitory activity against the acetylcholinesterase was revealed by *T. capitatus* essential oils (Table 3). The inhibition degree of the acetylcholinesterase enzyme varied significantly among the analyzed populations (from 0.11 (Tc2) to 0.61mg.ml^{-1} (Tc6)). The variation between populations seems to be mainly attributed to the

percentage differences of carvacrol.

Table 3: Anti-acetylcholinesterase activities of *T. capitatus* essential oils

	Sh	**Uas**			**Msa**		**Lsa**	**Ua**	**La**	
	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6	Tc7	Tc8	Tc9	Carvacrol
Anti-AChE										
(CI_{50}mg.ml^{-1})	0.18^{d}±0.0	0.11^{d}±0.0	0.15^{d}±0.0	0.61^{a}±0.0	0.51^{b}±0.0	0.59^{ab}±0.1	0.39^{c}±0.0	0.13^{d}±0.0	0.37^{c}±0.1	0.09±0.0

Means followed by different letters within the same column are significantly different ($p<0.05$).
IC50 value for the standard Donepezil: 18±0.4µg/ml.

In addition, the standard carvacrol, tested alone, showed the best anti-acetylcholinesterase. A negative correlation (r= -0.70) was observed between this compound and anti-acetylcholinesterase activity (data not shown). Our results were in agreement with those revealed by Ceylan *et al*. [28] reporting the strong enzyme inhibitory activity of carvacrol-rich essential oils. However, the anti-acetylcholinesterase activity of the entire essential oil depends on the antagonistic and synergic interactions of major and minor compounds [29].

Conclusion

In our study, a significant variation of the essential oil composition was observed between *T. capitatus* populations. Carvacrol was the main component of all essential oils collected from different bioclimatic zones. The highest percentages of carvacrol was revealed in population collected from the lower arid and upper semi-arid bioclimatic zone. Essential oils were found to possess anti-acetylcholinesterase activity. It could be explained by the richness of the species in carvacrol well known as interesting natural bioactive compound. Based on their chemical composition and biological activiy, *Thymus capitatus* essential oils can present an interesting alternative naturel which can be recommended to promote the biological activities of this medicinal crop for various natural therapies.

Conflicts of interest

The authors declare no conflict of interest.

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References

1. Hazzit M, Bencgabane A, Baaliouamer A, Alloun K. Composition chimique et activité antimicrobienne de l'extrait non volatil et des huiles essentielles de la rue des montagnes (Ruta montana L.). Recherche agronomique. 2015; (27):118-129.
2. Szczepanik M, Zawitowska B, Szumny A. Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). *Allelopathy* J. 2012; 30(1):129-142.
3. Dayan FE, Cantrell CL, Duke SO. Natural products in crop protection. Bioorg. Med. Chem. 2009; 17:4022-4034.
4. Dallali S, Lahmayer I, Mokni R, Marichali A, Ouerghemmi S, Bel Hadj Ltaief H, *et al*. Phytotoxic effects of volatile oil from *Verbena* spp. on the germination and radicle growth of wheat, maize, linseed and canary grass and phenolic content of aerial parts. Allelopathy J. 2014; 32014; 4(1):95-106.
5. Tabrizi L, Koocheki A, Moghaddam PR, Mahallati MN. Chemical composition of the essential oils from *Thymus transcaspicus* in natural habitats. Chem Nat Compd. 2010; 46:121-124.
6. Prasanth RV, Ravi VK, Varsha PV, Satyam S. Review on *Thymus vulgaris* Traditional Uses and Pharmacological Properties. Med Aromat Plants. 2014; 3(4):1-3.
7. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. JJ Ethnopharmacol. 2008; 166(3):403-406.
8. Zeghad N, Merghem R. Antioxidant and antibacterial activities of *Thymus vulgaris* L. Res. J Med. Plant. 2013; 1(1): 5-11.
9. Shabnum S, Wagay MG. Essential Oil Composition of *Thymus Vulgaris* L. and their Uses. J Res. Dev. 2011; 11:83-94.
10. Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. Ind Crops Prod. 2013; 43:827-831.
11. Jaouadi R, Cardoso SM, Silva AMS, Ben Hadj, Yahia I, Boussaid M, *et al*. Variation of phenolic constituents of Tunisian *Thymus capitatus* (L.) Hoff. et Link populations. Biochem. Syst. Ecol. 2018; 77:10-15.
12. Amiri H. Essential oils composition and antioxidant properties of three *Thymus* species. Evid Based Complement Alternat Med, 2012, 728065.
13. Eghdami A, Eizadi M, Sadeghi F. Polyphenolic content and antioxidant activity of hydroalcohlic and alcoholic extract of *Thymus vulgaris*. J biodivers. Environ. sci. 2013; 3(5):94-101.
14. Soliman A. Comparative Study on Composition and Allelopathic Effect of Volatile Oils Extracted from Two *Thymus* Species in Egypt. Int. J Water Res. Environ. 2013; 2(3):59-66.
15. Shokouhian A, Habibi H, Agahi K. Allelopatic effects of some medicinal plant essential oils on plant seeds germination. J Bio Sci. Biotechnol. 2016; 5(1):13-17.
16. Saroukolai AT, Moharramipour S, Meshkatalsadat MH. Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. J Pest Sci. 2010; 83(1):3-8.
17. Imelouane B, Amhamdi H, Wathelet JP, Ankit M, Khedid K, El Bachiri A. Chemical composition and antimicrobial activity of the essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. Int J Agric Biol. 2009; 11:205-208.
18. Moghtader M. Antifungal effects of the essential oil from *Thymus vulgaris* L. and comparison with synthetic thymol on *Aspergillus niger*. *J Yeast Fungal* Res. 2012;

3(6):83-88.
19. Eldeen IM, Elgorashi EE, Van Staden J. Antibacterial, antiinflammatory, anticholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. J Ethnopharmacol. 2005; 102:457-464.
20. Hedhili L, Romdhane M, Abderrabba M, Planche H, Cherif I. Variability in essential oil composition of Tunisian *Thymus capitatus* (L.) Hoff. et Link. Flavour Fragr. J. 2002; 17:26-28.
21. Bounatirou S, Smiti S, Miguel M, Faleiro L, Rejeb M, Neffati M, *et al*. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chem. 2007; 105:146-155.
22. Riahi L, Elferchichi M, Ghazghazi H, Jebali J, Ziadi S, Aouadhi C, *et al*. Phytochemistry: antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia. Ind Crops Prod. 2013; 49:883-889.
23. Vernet P, Gouyon PH, Valdeyron G. Genetic control of the oil content in *Thymus vulgaris* L.: A case of polymorphism in a biosynthetic chain. Genetica. 1986; 69:227-231.
24. Jayari A, El Abed N, Jouini A, Mohammed Saed Abdul-Wahab O, Maaroufi A, Ben Hadj Ahmed S. Antibacterial activity of *Thymus capitatus* and *Thymus algeriensis* essential oils against four food-borne pathogens inoculated in minced beef meat. J Food Saf. 2018; 38:E12409.
25. Ainane A, Khammour F, M'hamad E, Talbi M, El Hassan A, Cherroud S, *et al*. Composition chimique et activite anti insecticide des huiles essentielles de *Thymus* du maroc: *Thymus capitatus*, *Thymus bleicherianus* et *Thymus satureioides*. Proceedings Biosune. 2018; 1 :96-100.
26. Russo M, Suraci F, Postorino S, Serra D, Roccotelli A, Agosteo GE. Essential oil chemical composition and antifungal effects on *Sclerotium cepivorum* of *Thymus capitatus* wild populations from Calabria, Southern Italy. Rev Bras Farmacogn. 2013; 23:239-248.
27. Martínez-Natarén DA, Parra-Tabla V, Dzib G, Acosta-Arriola V, Canul-Puc KA, Calvo-Irabién LM. Essential oil yield variation within and among wild populations of Mexican oregano (Lippia graveolens HBK-Verbenaceae), and its relation to climatic and edaphic conditions. J Essent. Oil-Bear. Plants. 2012; 15:589-601.
28. Ceylan R, Zengina G, Uysal S, Ilhan V, Aktumsek A, Kandemir A, *et al*. GC-MS analysis and in vitro antioxidant and enzyme inhibitory activities of essential oil from aerial parts of endemic *Thymus spathulifolius* Hausskn. et Velen. J Enzyme Inhib. Med. Chem. 2015; 1475-6374.
29. Savelev SU, Okello EJ, Perry EK. Butyryl- and acetyl-cholinesterase inhibitory activities in essential oils of *Salvia* species and their constituents. Phytother. Res, 18:315-324.