CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND SECRETORY TRICHOMES OF *Thymus guyonii* de Noé, A MEDICINAL PLANT FROM ALGERIA

Hacina BENZINE-CHALLAM[*], Dahmane DAHMANE[**], Malika DAHMANI-MEGREROUCHE[*]

[*] *Laboratory of Plant Ecology and Environment, Faculty of Biological Sciences, University of Science and Technology Houari Boumédiène (USTHB), Algeria*
[**]*Laboratory of bioactive products and valorization of biomass, Superior Normal School Kouba, Algeria*
Corresponding author: Hacina Benzine-Challam, Laboratory of Plant Ecology and Environment, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene (USTHB), BP 32 Bab Ezzouar 16111, Algiers, Algeria, phone: +21321247913, fax: +21321247217
email: challamhacina@yahoo.fr

Abstract. In the perspective of a biological study of a medicinal Lamiaceae recognized for its antioxidant, antibacterial and antifungal properties, our research focused on the analysis of the aromatic compounds of a new population of *Thymus guyonii* de Noé in the Algerian Saharan Atlas (Ain Takersane). Volatile extract of flowering twigs is characterized by the presence of 43 components identified by GC and GC / MS. Given the phytochemical data of essential oils obtained at the scale of four other populations of *T. guyonii* collected in semi-arid, arid and Saharan climate, our work is intended as an additional analysis for an intraspecific comparison of volatile components according to the habitats. The application of the hierarchical ascending classification made it possible to highlight an individualization of the five populations in three chemotypic groups: α-terpinyl acetate describes for the first time, p-cymene, and oxygenated compounds (carvacrol or thymol), in relation to the continentality gradient.

The sites producing volatile extracts are very little known in this taxon; the morpho-anatomical observations of glandular trichomes on the epidermal surface of stems, leaves and calyxes were carried on light microscopy and scanning electron microscopy. Two types of trichomes differently distributed between the vegetative and floral organs have been described: capitate with a single-celled glandular head and peltate with twelve secretory cells organized in two discs.

Keywords: *Thymus guyonii* de Noé; Lamiaceae; essential oils; glandular trichomes.

INTRODUCTION

The genus *Thymus* L. represents one of the 250 most diverse genera of the Lamiaceae family [37]. It is very common in northwestern Africa, particularly in the Mediterranean region [35]. For their pharmacological properties, thyme is usually used in the pharmaceutical, cosmetic or foods industries and consumed in traditional and popular medicine. In North Africa, in recent decades, intensive phytochemical research has revealed the presence of numerous bioactive components in the essential oils of many taxa mainly represented by oxygenated compounds with antimicrobial and antioxidant activity [31, 13]. In Algeria similar tests were conducted; the essential oil can inhibit the growth of yeasts or filamentous fungi [11, 18] or Gram positive or negative bacteria [7, 4]. Apoptotic analysis of the various chemotypes of *Thymus* essential oils could lead to the development of new antineoplastics [45, 10].

This work is part of a research project aimed at deepening our knowledge for the biological conservation and valorisation of some Algerian Lamiaceae belonging to the genus *Thymus* and known for their endemic status and their heritage value. Among the taxa targeted in our work, *Thymus guyonii* de Noé is described as a creeping woody plant, with oval leaves and fine trichomes, calyx glabrous with more or less ciliate teeth, short white flowers, and that grows spontaneously on rugged, rocky lawns in the south of Algeria [39] .

A literature search of *T. guyonii* sites reported by systematic botany [3, 39, 35] indicates five localities south of Algiers, Ain Takersane, Kef El Haoues (Djelfa region), Aflou (confirmed by the works of [6] in Laghouat region) and El Goléa (present-day El Menea, Ghardaïa region) the latter was not found. However, two new sites have recently been reported in the locality of Zelfana east of the city of Ghardaia [28] and in the south east Algiers (M'sila) [49]. The biological activities of volatile oils from different populations, showed a bactericidal action of the essential oil of *T. guyonii* from Kef El Haoues on 13 strains of *Listeria monocytogenes* [17], on *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* for the essential oil of *T. guyonii* from Aflou [6]. The highest antibacterial effects are reported in *Micrococcus luteus* and *Enterococcus faecium* by essential oil of *T. guyonii* from Ghardaia [28]. Among the less resistant Gram negative are *Klebsiella pneumoniae* and *Escherichia coli* [28, 49]. Concerning the yeasts, the most potent sensitivity has been revealed by *Candida albicans* and *Saccharomyces cerevisiae*, which are responsible for numerous infectious pathologies in humans [6].

Our first objective was to identify, for the first time, the chemical composition of the essential oil of the Ain Takersane population. In addition, a comparative analysis of the chemical, quantitative and qualitative profiles of the five Algerian populations is presented and discussed.

Two types of glandular trichomes corresponding to epidermal sites producing essential oils are described in the Lamiaceae: capitate and peltate trichomes. Their morphologies, structures, spatial distribution and secretion products are variable according to taxa [47]. The use of trichomes as epidermal markers has raised the taxonomic identification problem observed in various Lamiaceae or other related families [32]. To our knowledge, no study with a detailed description of glandular trichomes of thyme has been published in Algeria. Only their presence is cited by few light microscope observations. The second objective of this work is to demonstrate, using microscopic photonic and scanning electron microscopy, the morpho-anatomical characteristics of the glandular trichomes, distributed on stems, leaves and calyx surfaces of the samples collected in the station study. Our microscopic observations in the form of micrographs has been achieved.

MATERIALS AND METHODS

Plant materials

The flowering twigs of *T. guyonii* de Noé, were collected during the flowering period (April-May) from natural populations at an altitude of 1390 m, with a southern exposure in Ain Takersane, a region in the west of Djelfa city. The latter is located 300 Km South of Algiers, in the sub-sector of the Algerian Saharan Atlas (Fig. 1).

Botanical identification of the plant was confirmed by the North African Collection of the National Museum of Natural History of Paris (MNHN-P-P03886476). Reference specimens have been deposited in the laboratory of plant ecology and environment of the University of Sciences and Technology Houari Boumediène, in Algiers.

The fresh plant material is divided into three batches: a first batch is fixed in 70% alcohol for light microscope observations; a second batch is dried in the shade at room temperature for scanning electron microscopy; a third batch is intended for phytochemical analysis.

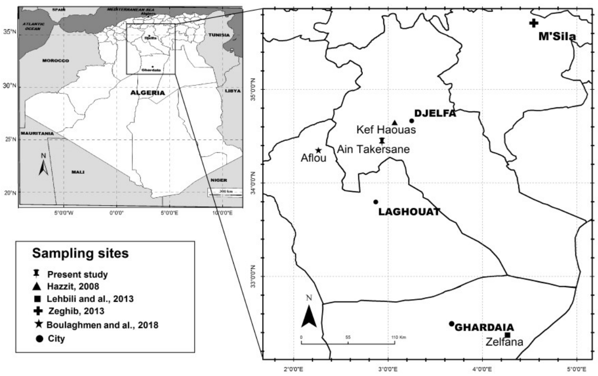

Figure 1. Geographical location of *Thymus guyonii* de Noé populations

Extraction and analysis of essential oil

Using Clevenger apparatus allowed us to extract the essential oil contained in 100 g of vegetable matter powder. After 2 hours of hydrodistillation, the recovered volatile extract is dried with anhydrous sodium sulfate, weighed for yield estimation, stored in an opaque vial, sealed, and stored in the dark at low temperature (5-6 °C) for analysis.

The composition of oil fraction was carried out using an Agilent Technologies chromatograph (GC 7890A, USA), equipped with an injector, an HP5-MS apolar column (30 m x 0.32 m) film thickness 0.25 µm and a flame ionization detector (FID). The temperature is programmed from 60 °C to 240 °C for 3 minutes at a rate of 3 °C / min. The temperature of the injector is set at 250 °C. The split mode 1:50. The injected volume is 1 µl. The detector temperature is 300 °C. Semi-quantitative data were obtained from the electronic integration of the surface percentages without the use of correcting factors. In order to determine the retention indices (RI), a series of n-alkane (C_8-C_{24}) mixtures were analyzed under the same operating conditions on HP5-MS column, the sample indices were calculated according to Van den Dool and Kratz relation [46].

The Gas chromatography-mass spectrometry analysis (GC-MS) was carried out using a TRACE GC Ultra-DSQ II chromatograph, equipped with an automatic injector. The HP-5MS apolar column (30 m x 0.32 mm) film thickness 0.25 µm is coupled to a Thermo DSQ II mass sensor. The carrier gas is helium (1 ml/min). The temperature of the injector is 250 °C and that of the detector 280 °C. Temperature programming consists of an elevation of 60 °C (3 min) up to 240 °C, at 3 °C / min, then a level of 15 min at 240 °C. Injection is done by split mode with a dividing ratio of 1/50. The amount of injected sample is 0.2 µl. The detection is done by a quadrupole filter analyzer. The molecules are bombarded by an electron beam of 70 eV. The mass spectra obtained by electronic impact are acquired over the mass range 40-450 m/z.

Identification of the oil components was based on the comparison of retention indices (RI) calculated on apolar columns and mass spectra with those of authentic compounds or literature data [1, 9, 21, 25] and with the bases of commercial data (NIST 08 and WILEY).

Statistical analysis

A bibliographical summary of the different characteristics of populations of *T. guyonii* is reported. Among the integrated parameters, is the chemical composition of the essential oils for which we applied the Hierarchical Ascending Classification (C.H.A.) using the software Statistica, 2010. The interpopulation chemical affinities are highlighted using a dendrogram which allowed us to classify the five populations studied (selection variables ≥ 0.1%).

Light microscopy (LM)

Freehand cross-sections are carried out in young leaves and the apex stem showed numerous trichomes. Microscopical analyses were performed on hand-fixed sections using Langeron technique [26]. Thirty sections made on the collected samples were examined and photographed with Carl Zeiss light microscope (Axio Lab.A1 HAL 35).

Scanning electron microscopy (SEM)

Dry samples of young stems, leaves and calyxes are mounted in stubs and coated with a thin layer of gold-palladium. The observations of the organs epidermal surface were carried under a PHILIPS quanta FEG 250 scanning electron microscope.

RESULTS

Yield and chemical composition of the essential oil of *T. guyonii* population from Ain Takersane

The essential oil isolated by hydrodistillation appears as a pale yellow liquid. The yield is estimated at 1.38% (w / w). The composition of oil components is graphically represented in Figure 2 and listed in Table 1 in the order of their elution. Analysis of *T. guyonii* de Noé essential oil by GC and GC-MS allowed us to identify a total of forty three compounds representing 95.2% of the total oil content, the others being only in trace amounts. The oil is dominated by monoterpenes (91.1%), especially oxygenated monoterpenes ones (68.4%), among which α-terpinyl acetate (33%), borneol (7%), camphor (6.5%) and 1.8 cineole (3.7%) are in high proportions. Monoterpene hydrocarbons estimated to 22.7%, with α-pinene (10%), camphene (4.6%) and limonene (2.6%) being the major components. The least dominant component is the sesquiterpene fraction (4.1%) represented essentially by (E)-nerolidol (3%).

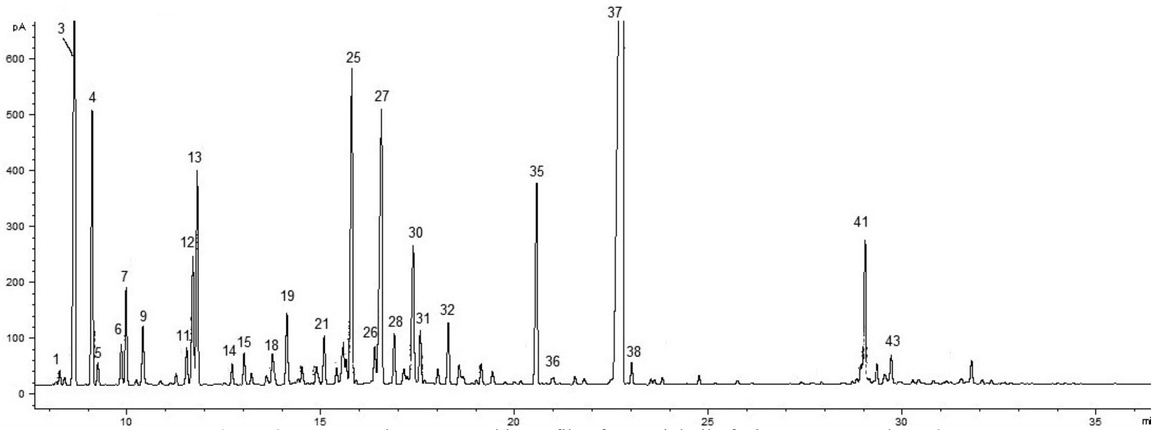

Figure 2. GC-FID Chromatographic profile of essential oil of *Thymus guyonii* de Noé
Number refer to compounds identified to table1.

Table 1. Chemical composition of essential oil of *Thymus guyonii* de Noé from Algeria

Peak Number	RT[a]	RI[b]	RI[c]	Compounds[d]	MW[e]	%[f]	Methods of identification
0	1	2	3	4	5	6	7
1	8.283	921	924	tricyclene	136	0.2	GC, GCMS
2	8.405	924	928	α-thujene	136	0.1	GC, GCMS
3	8.669	932	937	α-pinene	136	10.0	GC, GCMS, CoGC [g]
4	9.119	946	951	camphene	136	4.6	GC, GCMS, CoGC
5	9.267	953	956	thuja-2,4(10)-diene	134	0.4	GC, GCMS
6	9.869	969	975	sabinene	136	0.7	GC, GCMS, CoGC
7	9.995	974	979	β-pinene	136	1.6	GC, GCMS, CoGC
8	10.257	988	988	myrcene	136	0.1	GC, GCMS
9	10.429	988	993	dehydro-1,8-cineol	152	1.0	GC, GCMS
10	11.284	1014	1017	α-terpinene	136	0.2	GC, GCMS, CoGC
11	11.557	1020	1024	p-cymene	134	0.7	GC, GCMS
12	11.717	1024	1029	limonene	136	2.6	GC, GCMS, CoGC
13	11.83	1026	1032	1,8-cineole	154	3.7	GC, GCMS, CoGC
14	12.729	1054	1056	γ-terpinene	136	0.3	GC, GCMS
15	13.039	1065	1066	cis-sabine hydrate	154	0.6	GC, GCMS
16	13.226	1068	1069	p-mentha-3,8-diene	136	0.2	GC, GCMS
17	13.615	1078	1079	camphenilene	138	0.2	GC, GCMS
18	13.772	1086	1083	terpinolene	136	0.8	GC, GCMS
19	14.142	1095	1098	linalool	154	1.3	GC, GCMS, CoGC
20	14.533	1110	1104	1-octen3-yl-acetate	170	0.3	GC, GCMS
21	15.104	1122	1120	α-campholenal	152	0.9	GC, GCMS
22	15.426	1133	1129	cis-p-mentha-2,8-dien-1-ol	152	0.3	GC, GCMS
23	15.586	1135	1133	trans-pinocarveol	152	1.0	GC, GCMS
24	15.668	1137	1136	cis-verbenol	152	0.4	GC, GCMS
25	15.816	1141	1140	camphor	152	6.5	GC, GCMS, CoGC
26	16.408	1160	1156	pinocarvone	150	0.7	GC, GCMS

0	1	2	3	4	5	6	7
27	16.581	1165	1161	borneol	154	7.0	GC, GCMS, CoGC
28	16.911	1174	1170	terpinen-4-ol	154	0.9	GC, GCMS, CoGC
29	17.159	1179	1177	p-cymen-8-ol	150	0.4	GC, GCMS
30	17.399	1186	1184	α-terpineol	154	3.3	GC, GCMS, CoGC
31	17.581	1204	1193	verbenone	150	1.0	GC, GCMS
32	18.307	1215	1210	trans-carveol	152	1.1	GC, GCMS
33	19.024	1235	1233	isobornyl formate	182	0.1	GC, GCMS
34	19.155	1239	1237	carvone	150	0.4	GC, GCMS, CoGC
35	20.584	1287	1283	bornyl acetate	196	4.0	GC, GCMS
36	20.986	1299	1296	terpinen-4-ol acetate	196	0.1	GC, GCMS
37	22.781	1346	1345	α-terpinyl acetate	196	33.0	GC, GCMS
38	23.008	1359	1355	neryl acetate	196	0.4	GC, GCMS
39	23.596	1374	1370	α-copaene	204	0.1	GC, GCMS
40	23.799	1387	1383	β-bourbonene	204	0.1	GC, GCMS
41	29.03	1561	1558	(E)-nerolidol	222	3.0	GC, GCMS
42	29.53	1577	1576	spathulenol	220	0.3	GC, GCMS
43	29.699	1582	1581	caryophyllene oxide	220	0.6	GC, GCMS, CoGC
				Monoterpene hydrocarbons		22.7	
				Oxygenated monoterpenes		68.4	
				Sesquiterpene hydrocarbons		0.2	
				Oxygenated sesquiterpenes		3.9	
				Total oil		95.2%	

[a] Retention times of different compounds.
[b] Retention indices values from literature (Adams 2007).
[c] Retention indices as determined on a HP-5MS column using the homologous series of *n*- alkanes.
[d] Compounds listed in order of elution from a HP-5MS column.
[e] Molecular weight of different compounds.
[f] Relative area was given according to FID area percentage data.
[g] Co GC = identification was based on retention times of authentic compounds on HP-5MS capillary column.

Phytochemical individualization of the studied populations

The ascending hierarchical classification (C.H.A.) made it possible to highlight the phytochemical particularities of the 5 populations studied. It shows the clear individuality in two groups of the populations of the region of Djelfa, according to their membership, to the chemotype to α-terpinyl acetate (Ain takersane) or to p-cymene (Kef El Haoues). The other three populations of the third phytochemical group are linked to chemotypes phenolic, most frequently found in the literature in thyme, carvacrol (M'sila and Ghardaia) or thymol (Laghouat) (Table 2, Fig. 3).

Table 2. Location and characterization of the different populations of *Thymus guyonii* de Noé in Algeria

Populations / Characteristics	Kef El Haoues (Djelfa)	Ain takersane (Djelfa)	Zelfana (Ghardaia)	M'sila (M'sila)	Aflou (Laghouat)
Authors	Hazzit and al., 2006 [17]	Present study	Lehbili and al., 2013 [28]	Zeghib and al., 2017 [49]	Boulaghmen and al., 2018 [6]
Location	Kef El Haoues	Ain Takersane	Zelfana	M'sila	Mountain Sidi Bouzid
Altitude (m)	2000	1390	355	-	1267
Bioclimat	semi arid	semi arid	saharan	arid	semi arid
Harvest period	July	May	May	May	June
Analyzed organs	leaves and flowers	young flowerings twigs	aerial parts	aerial parts	aerial parts
Drying	yes	yes	no	no	yes
Extraction method	hydrodistillation	hydrodistillation	hydrodistillation	hydrodistillation	hydrodistillation
Method of analysis	CG-FID and CG/SM	CG-FID and CG/SM	CG-FID and CG/SM	CG-FID and CG/SM	CG-FID and CG/SM
Yield of essential oils (%)	1	1,38	2	1,5	0,98
Main contituents (%)	- p-cymene: 18.6 - borneol: l6.1 - carvacrol: l4.2 - γ-terpinene: 13 - thymol: 10.9 - thymol methyl ether: 10.7	- α-terpinyl acetate: 33 - α-pinene: 10 - borneol: 7 - camphor: 6.5 - camphene: 4.6 - 1.8cineole: 3.7	- carvacrol: 55.55 - thymol: 19.51 - *p*-cymene: 6.25 - α-pinene: 3.13 - τ-cadinol: 2.50 - α-cadinol: 1.06	- carvacrol: 55.6 - thymol: 21.2 - *o*cymene: 9.7 - δ-terpinene: 5.7 - linalool: 1.1 - β-myrcene: 0.8	- thymol: 35.8 - ɤ-Terpinene: 18.7 - p-Cymene: 15.5 - thymol methyl ether: 15.2 - linalool: 4 - carvacrol: 2.2

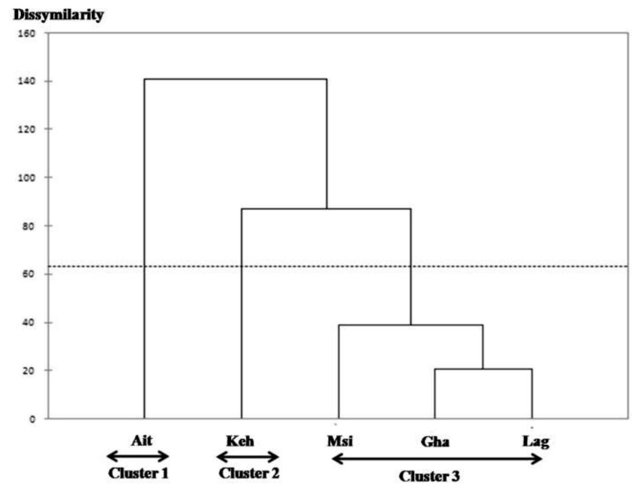

Figure 3. Dendrogram of the ascendant hierarchical classification of *Thymus guyonii* de Noé cluster analysis
Ain (Ain Takersane); Keh (Kef El Haoues); Msi (M'Sila); Gha (Ghardaia); Lag (Laghouat).

Microscopic analysis of glandular trichomes

In SEM micrographs, sampled organs revealed the presence of numerous peltate trichomes distributed on the adaxial leaf side, the young stems, the calyxes outer tubular surface and the inner face of lower lip (Fig. 4 A, F, G, H). Capitate trichomes were restricted to the abaxial leaf side and typically on the veins of calyxes (Fig. 4 D, H).

LM revealed the tricellular structural features of the capitate trichome, with a basal epidermal cell, a short stalk, and an ovoid apical cell with secretory function (Fig. 4 E). Peltate trichomes are composed of a basal cell embedded in the epidermal base, a monocellular stalk and a rounded multicellular secretory head (Fig. 4 C) including a space where secretion are stored. Glandular cells have a regular appearance, twelve in number and arranged in a fan around the stipe in two circles, the internal four cells and the external eight cells (Fig. 4 B). It is by breaking of the cuticule that the secretory products are released (Fig. 4 I).

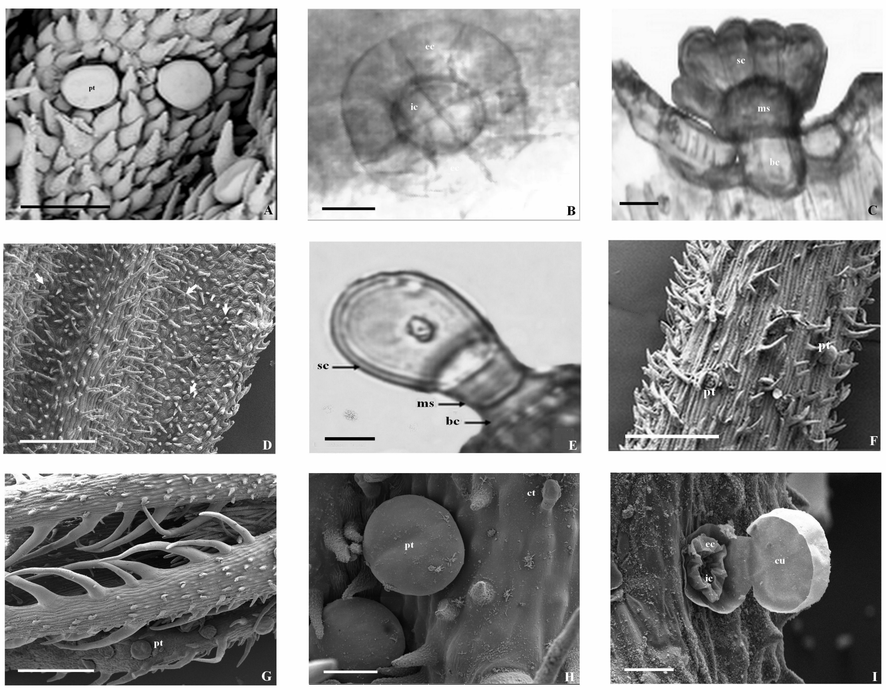

Figure 4. LM and SEM micrographs of *Thymus guyonii* de Noé showing the morphology and the distribution of glandular trichomes on the leaf (A-E), stem (F) and calyx (G-I)
(A) Peltate trichome on the leaf upper, SEM. (B) Peltate trichome with internal circle (4) and external circle (8) secretory cells, LM. (C) Peltate trichome with head secretory cells, monocellular stipe, basal cell, LM. (D) Capitate trichome (arrows) on the leaf lower, SEM. (E) Capitate trichome with secretory cell, monocellular stipe, basal cell, LM. (F) Stem portion with peltate trichome, SEM. (G) Peltate trichome on the inner face of lips lower, SEM. (H) Peltate and capitate trichomes on the calyx veins, SEM. (I) Peltate trichome open with cuticle, internal and external secretory cells, SEM. (pt): peltate trichome; (ec) external cell; (ic) internal cell; (sc) secretory cells; (ms) monocellular stipe; (bc) basal cell; (ct) capitate trichome; (cu) cuticle. Bar: (A)=100µm. (B,C)=15µm. (D)= 300µm. (E)=5µm. (F) 500µm. (G)=400µm. (H,I)=50µm.

DISCUSSION

In general, the intraspecific comparative analysis of the yields of the five populations of *T. guyonii* indicates a fluctuation in productivity with contents ranging from 1% to 2%. The highest productivities in essential oils are observed during the flowering period (April - May) for the populations of AinTakersane (1.38%) and M'sila (1.5%) followed by the Saharan population of Ghardaia (2%). During the fruiting period (June-July) the yields of essential oils decreased for the populations of Kef El Haoues and Aflou probably due to the abscission of foliar organs and floriferous rich in glands, storage sites of secondary metabolites. Thus, the flowering period seems most appropriate for harvesting and extracting essential oils [42]. It would seem furthermore that, outside our station of study, the productivity in essential oils of *T. guyonii* populations would increase with the degree of aridity. Indeed, under controlled environmental conditions, it was found that the air temperatures tested had a considerable influence on the productivity in essential oils of the medicinal plants studied *Nepeta cataria* L., *Melissa officinalis* L., and *Salvia officinalis* [29]. Meanwhile, the decrease in the yield of AinTakersane samples relative to those of M'Sila could probably be explained by variability in the number of secretory trichomes of the secondary metabolites as demonstrated by a recent study that established significant correlations between density glandular trichomes and oil yield in nine cultivars of *Mentha arvensis* L. [34]. Similarly, the condition of the analyzed organs (fresh or dry) as well as the drying processes of the samples could partly influence the yield. The analysis of about thirty studies realized on the production capacity of essential oils of Algerian *thyme* species also showed interspecific variability. Our results on *T. guyonii* will be found between a group with lower yields corresponding to *T. capitatus* L. (Hoffmanns & Link**)**, *T. algeriensis* Boiss. & Reut., and *T. lanceolatus* Desf. [11, 24, 43] and a second group with, the highest aromatic content as *T. numidicus* Poiret, *T. dreatensis* Batt., *T. willdenowii* Boiss, *T. ciliatus* (Desf.) Benth. and *T. fontanesii* Boiss. & Reut. [7, 14, 19, 27, 36].

The chemical study of *T. guyonii* populations from different localities showed three belonging chemotypes. The species rich in carvacrol and thymol identified respectively in the Ghardaia, M'sila and Laghouat populations are most frequently found in thyme in dry and warm habitats [49]. The populations of Djelfa are characterized by a predominance of p-cymene (Kef El Haoues) or α-terpinyl acetate (Ain Takersane) described for the first time in Algerian populations in the genus *Thymus*. The essential oils belonging to the α-terpinyl acetate chemotype are also mentioned in three species of thyme from different countries, such as *T. pannonicus* All. from Romania [8], *T. pulegioides* L. from France [33] and *T. longicaulis* Presl. of Kosovo [16]. The applied statistical analysis (C.H.A.) applied to the chemicals compositions of the essential oils of *T. guyonii* populations, seems to indicate a distribution of populations related to the variations of the ecological conditions, in particular climatic conditions. The dendrogram shows an arrangement of *T. guyonii* populations according to a continental gradient, resulting in a decrease in the water and trophic qualities of their habitat. However the population of Ghardaia seems to escape this gradient. Since there is no systematic data available in the publications relating to this station, we believe that the *thymes* in these areas, which do not occur in their preferred areas, would probably be confined to depressions by factor compensation. The qualitative and quantitative variability observed could be attributed to various intrinsic genetic parameters [15, 38] or that may be related to phenophase [15]. Chemotypes observed would also be closely related to the ecological conditions of the plant environment, which influence plant biosynthesis [23].

The glandular trichomes present in Lamiaceae correspond to surface epidermal specializations which are typically distinguished into two models according to the structure of the secretory head: capitate and peltate [47]. Capitate trichome observed in *T. guyonii* is characterized with a short unicellular stalk and an ovoid unicellular secretory head. However, a polymorphism of the peduncle (short or long) and the head (uni or multicellular) is underlined in other *thyme* taxa [41, 30] than in other Lamiaceae [2, 12]. In addition, our study indicates a relatively rare distribution of capitate trichomes at leaf and floral (calyx) surfaces as emphasized in *T. striatus* Vahl and *T. striatus* Var. Ophiolitica [5] and *T. quinquecostatus* Celak. [22]. A second type of glandular trichomes is observed in the species studied on the surface of the plant, with a relatively higher frequency on the calyx, the young stems, and the upper leaf surface. For other taxa of thyme, their presence was underlined on both sides of the calyx, on the bracts or on the outer surfaces of the corollas [5, 22]. Peltate trichome of *T. guyonii* of Ain Takersane is characterized by a very small stipe, a head with two whorls comprising a total of twelve secretory cells arranged in a fan around the stipe and a large subcuticular space where the essential oil is deposited. Many authors report that trichomes with heads composed of 4 or even 8 cells are also found in *T. migricus* Klokov & Des.-Shost. and *T. fedtschenkoi* Ronniger var. handelii (Ronninger) Jalas [41], *T. lykae* Degen & Jáv. [30] and *T. pannonicus* All. [8]. Other Lamiaceae species have a higher number of secretory cells [20]. The analysis of the fluctuation of the density and the types of trichomes would be one of the causes of the deep phytochemical variability of the essential oils at the intra and interspecific scale in the *Stachys* L., *Ziziphora* L. and *Nepeta* genera [40, 44, 48].

In conclusion the phytochemical study of a new population of *T. guyonii* identified 43 constituents of the essential oil, dominated by α-terpinyl acetate

(33%), α-pinene (10%) and borneol (7%). Analysis of the relationships between the chemical components of the aromatic compounds of the populations examined and their respective habitat environments has shown that the biotope would be involved in part in the chemical polymorphism of the aromatic compounds in the study taxon.

The conducted micromorphological analysis showed two types of glandular trichomes: peltate and capitate on the surfaces of the studied taxa, among those described in the literature in Lamiaceae. The study, in a future research, of the variability of the typology and the distribution of the glandular trichomes in relation with the endogenous factors and the environmental conditions, on the intraspecific and even interspecific scale of the thyme populations, would allow us without doubt to better understand the chemical polymorphism observed or cited.

Acknowledgements. The authors would like to warmly thanks Professor Djebbar Réda, Professor Rahmania Fatma, Professor Terrack Gaceb Rabéa, Professor Mecherara Samira and Professor Kamel Taibi, teachers and researchers at the University of Sciences and Technology Houari Boumédiène in Algiers, for their scientific collaboration. We are also grateful to Professor Dob Tahar for his technical contribution.

REFERENCES

[1] Adams, R.P., (2007): Identification of essential oil components by gas chromatography/mass spectroscopy. 4th Edition. Allured Publishing, Corporation, Carol Stream, Illinois, pp. 804.

[2] Ascensão, L., Marques, N., Pais, M.S., Castro. M., (1995): Glandular trichomes on vegetative and reproductive organs of *Leonotis leonurus* (L.) R.Br (Lamiaceae). Annals of Botany, 75: 619-626.

[3] Battandier, J.A., Trabut. L.C., (1902): Flore analytique et synoptique de l'Algérie et de la Tunisie. Vve Giralt, Imprimeur éditeur, Alger, 460 p.

[4] Benbelaid, F., Khadir, A., Abdoune, M., Bendahou, M., (2013): Phytochemical screening and in vitro antimicrobial activity of *Thymus lanceolatus* Desf. from Algeria. Asian Pacific Journal of Tropical Disease 3(6): 454-459.

[5] Bini Maleci, L., Luigi Cioni, P., Flamini, G., Spinelli, G., Servettaz, O., (1997): Comparative observations on *Thymus striatus* Vahl and *T. striatus* Var. Ophioliticus lacaita in central Italy. Lagascalia, 19(1-2): 857-864.

[6] Bouleghmen, F., Chaouia C., Hazzit M., Nouas M., Saidi F., (2018): Composition chimique et activité microbienne d'huile essentielle extraite de *Thymus guyonii* d'Aflou-Algérie. Agrobiologia, 8(1): 853-862.

[7] Bousmaha-Marroki, L., Atik-Bekkara, F., Tomi, F., Casanova, J., (2007): Chemical Composition and Antibacterial Activity of the Essential Oil of *Thymus ciliatus* (Desf.) Benth. subsp. *eu-ciliatus* Maire from Algeria. Journal of Essential Oil Research, 19: 490-493.

[8] Boz, I., Burzo, I., Zamfirache, M., Toma, C., Padurariu, C., (2009): Glandular trichomes and essential oil composition of *Thymus pannonicus* All. (Lamiaceae). Annales universităţii din Oradea, XVI(2): 36-39.

[9] Davies, N.W., (1990): Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. Journal of Chromatography, 503: 1-24.

[10] Deering, K., Nethery, W., Averill, C., Esposito. E., (2017): Effects of Thyme Essential Oil Chemotypes on Breast and Cervical Cancer Cell Lineages. Annals of Pharmacology and Pharmaceutics, 2(1): 1008.

[11] Dob, T., Dahmane, D., Benabdelkader, T., Chelghoum. T., (2006): Studies on the essential oils and antimicrobial activity of *Thymus algeriensis* (Boiss. & Reut). International Journal of Aromatherapy, 16(2): 95-100.

[12] El Beyrouthy, M., Arnold-Apostolides, N., Dupont, F., (2009): Trichomes morphology of six Lebanese species of Stachys (Lamiaceae). Flora Mediterranea, 19: 129-139.

[13] El Ajjouri, M., Ghanmi, M., Satrani, B., Amarti, F., Rahouti, M., Aafi, A., Ismaili, My R., Farah. A., (2016): Composition chimique et activité antifongique des huiles essentielles de *Thymus algeriensis* (Boiss. & Reut.) et *Thymus ciliatus* (Desf.) Benth. contre les champignons de pourriture du bois. Acta Botanica Gallica, 157(2): 285-294.

[14] Ferhat, M., Ghorab, H., Laggoune, S., Ghannadi, A., Sajjadi, S., Touzani, R., Kabouche, A., Kabouche, Z., (2014): Composition and antioxydant activity of essential oil of *Thymus dreatensis* Batt. From Algeria. Chemistry of Natural Compounds, 50(4): 747-749.

[15] Hamrouni Sellami, I., Maamouri, E., Chahed, T., Aidi, W., Kchouk, M.E., Marzouk, B., (2009): Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). Industrial Crop and Products, 30: 395-402.

[16] Hatipi Ibrahimi, M., Papajani, V., Ćavar, S. Matevski, V., (2013): GC/MS Analysis of the Essential Oil of *Thymus longicaulis* (Presl.) from Kosovo. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 41: 6-10.

[17] Hazzit, M., Baaliouamer, A., Leonor faleiro, M., Grac, M., Miguel, A., (2006): Composition of the Essential Oils of Thymus and Origanum Species from Algeria and Their Antioxidant and Antimicrobial Activities. Journal Agricultural Food Chemistry, 54: 6314-632.

[18] Hazzit, M., Baaliouamer, A., Veríssimo, A.R., Faleiro, M.L., Miguel. M.G., (2009): Chemical composition and biological activities of Algerian *Thymus* oils. Food Chemistry, 116: 714-721.

[19] Houmani, Z., Azzoudj, S., Naxakis, G., Skoula, M., (2002): The Essential Oil Composition of Algerian Zaâtar: *Origanum* spp. and *Thymus* spp. Journal of Herbs Spices and Medicinal Plants, 9(4): 275-280.

[20] Ilkay Öztürk, Ç., Arzu, C., Cengiz, Y., (2014): Trichome morphology of *Ajuga orientalis* L. (Lamiaceae) from Turkey. Bangladesh Journal of Botany, 43(1): 91-95.

[21] Jenning, W., Shibamato, T., (1980): Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography. Academic Press, New York, pp.29.

[22] Jia, P., Liu, H., Gao, T., Xin, H., (2013): Glandular trichomes and essential oil of *Thymus quinquecostatus* Celak. Hindawi Publisching Corporation. The scientific word journal, 8 p.

[23] Karousou, R., Koureas, D.N., Kokkini, S., (2005): Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in Natura 2000 sites of Crete. Photochemistry, 66(22): 2668-2673.

[24] Khadir, A., Sobeh, M., Gad Haidy, A., Benbelaid, F., Bendahou, M., Peixoto, H., Sporer, F., Ashour, M.L., Wink, M., (2016): Chemical composition and biological activity of the essential oil from *Thymus lanceolatus*. Zeitschrift für Naturforsh, 71: 5-6.

[25] König, W.A., Hochmuth, D.H., Joulain, D., (2001): Terpenoids and related constituents of Essential Oils. Library of Mass Finder 2.1, Institute of Organic Chemistry, Hamburg.

[26] Langeron, M., (1949): Précis de microscopie. Editions Masson, Paris, France, 1205 p.

[27] Laouer, H., Boulaacheb, N., Akkal, S., Meierhenrich, Uwe, J., Baldovini, N., Prado, S., (2014): Composition and In vitro Antimicrobial Activities of the Essential Oils of Two Populations of *Thymus numidicus* Poiret. Journal of Essential Oil Research, 21(4): 374-377.

[28] Lehbili, M., Chibani, S., Kabouche, A., Semra, Z., Smati, F., Abuhamdah, S., Touzani, R., Kabouche, Z., (2013): Composition, antibacterial and antioxidant activity of the essential oil of *Thymus guyonii* de Noé from Algeria. Scholars Research Library Der Pharmacia Letter, 5(2): 306-310.

[29] Manukyan, A.E., Schnitzler, W.H., (2006): Influence of Air Temperature on Productivity and Quality of some Medicinal Plants under Controlled Environment Conditions. European Journal of Horticultural Science, 71(1): 36-44.

[30] Marija, M., Budimir, S., Anoševic, D., Marin, P.D., Duletić-Lauševic, S., Ljaljevic-Grbic, M., (2008): Morphology distribution and histochemistry of trichomes of *Thymus lykae* Degen & Jav. (Lamiaceae). Archives Biologiques Sciences de Belgrade, 60(4): 667-672.

[31] Megdiche-Ksouri, W., Saada, M., Soumayal, B., Snoussi, M., Zaouali, Y., Ksouri, R., (2015): Potential use of wild *Thymus algeriensis* Boiss. and Reut. and *Thymus capitatus* Hoffmanns and Link as source of antioxidant and antimicrobial agents. Journal of new sciences Agriculture and Biotechnology, 23(4): 1046-1056.

[32] Metcalfe, C.R., Chalk, L., (2000). A revised classification of the architecture of Dicotyledonous leaves. In Anatomy of the Dicotyledonae, 2ed ed, Vol.1, Oxford. pp. 25-53.

[33] Michet, A., Chalchat, J.C., Figuérédo, G., Thébaud, G., Billy, F., Pétel, G., (2011): Chemotypes in the Volatiles of Wild Thyme *Thymus pulegioides* (L.). Journal of Essential Oil Research, 20: 101-103.

[34] Mishra, A., Lal, R.K., Chanotiya, C.S., Dhawan S.S., (2017): Genetic elaborations of glandular and non-glandular trichomes in *Mentha arvensis* genotypes: assessing genotypic and phenotypic correlations along with gene expressions. Protoplasma, 254(2): 1045-1061.

[35] Morales, R., (2002): The history, Botany and Taxonomy of The genus *Thymus*. In Thyme. The genus *Thymus*. Stahl-Biskup, E.Saez Edition, Taylor and Francis, London and New York, pp. 1-43.

[36] Nabet, N., Boudries, H., Loupassaki, S., Souagui, S., Madani, K., Carbonell-Barrachina, Á.A., (2017): Chemical composition, antimicrobial and antioxidant activities of *Thymus fontanesii* Boiss. et Reut. and Origanum glandulosum Desf. essential oils. International Food Research Journal, 24(6): 2518-2525.

[37] Naghibi, F., Mosaddek, M., Mohammadi, M.S., Ghorbani, A., (2005): Labiateae family in the folk Medicine in Iran: from Ethnobotany to Pharmacology. Iranian Journal of Pharmaceutical Research, 2: 63-79.

[38] Pluhár, Z., Kocsis, M., Kuczmog, A., Csete, S., Simkó, H., Sárosi, S., Molnárand, P., Horváth, G., (2012): Essential oil composition and preliminary molecular study of Four Hungarian *Thymus* Species. Acta Biologica Hungarica, 63(1): 81-96.

[39] Quezel, P., Santa. S., (1962-1963). Nouvelle flore d'Algérie et des régions désertiques méridionales. CNRS Press, Paris, Vol. 2, 1170 p.

[40] Rezakhanlo, A., Talebi, S.M., (2010): Trichomes morphology of S*tachys lavandulifolia* vahl. (Labiateae) of Iran. Procedia - Social and Behavioral Sciences, 2(2): 3755-3763.

[41] Satil, F., Kaya, A., Biçaksi, A., Özatli, S., Tümen, G., (2005): Comparative morphological and palynological studies on *Thymus migricus* Klokov and Des-shost and *T. fedtschenkoi* Ronninger Var. Handelii (Ronninger) Jalas grown in east Anatolia. Pakistan Journal of Botany, 37(3): 531-549.

[42] Senatore, F., (1996): Influence of harvesting time on yield and composition of essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). Journal of Agricultural and Food Chemistry, 44(5): 1327-1332.

[43] Tabti, L., Dib, M.E., Gaouar, N., Bouayad, S., Tabti B., (2014): Antioxidant and antifungal activity of extracts of the aerial parts of *Thymus capitatus* (L.) Hoffmanns Against Four Phytopathogenic Fungi of *Citrus sinensis* Jundishapur. Journal of Natural Pharmaceutical Products, 9(1): 49-54.

[44] Talebi, S.M., Hayestehfar, A.R., (2014): Infraspecific trichomes variations in *Aciaos graveolens* (M.B.) Link. Annals of Biological Sciences, 2(2): 51-57.

[45] Tefiani, C., Riazi, A., Youcefi, F., Aazza, S., Gago, C., Faleiro, M.L., Pedro, L.G., Barroso, J.G., Figueiredo, A.C., Medías, C., Cortés-Giraldo, I., Vioque, J., Miguel, M.G., (2015): *Ammoides pusilla* (*Apiaceae*) and *Thymus munbyanus* (Lamiaceae) from Algeria essential oils: chemical composition, antimicrobial, antioxydant and antiproliferative activities. Journal of Essential Oil Research, 27(2): 131-139.

[46] Van den Dool, H., Kratz, P.D., (1963): A generalization of the retention of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography, 11: 463-471.

[47] Werker, E., (2000): Trichomes diversity and development. Advances in Botanical Research, 31: 1-35.

[48] Yarmoohammadi, M., Talebi, S.M., Nohooji, M.G., (2017): Infraspecific variations in essential oil and glandular trichomes in *Nepeta heliotropifolia.* Biodiversitas, 18(3): 964-970.

[49] Zeghib, A., Kabouche, A., Laggoune, S., Calliste, Cl.A., Simon, A., Bressolier, P., Aouni, M., Duroux J.L., Kabouche, Z., (2017): Antibacterial, Antiviral, Antioxidant and Antiproliferative activities of *Thymus guyonii* de Noé. Essential Oil Natural products, 12(10): 1651-1654.

Received: 26 February 2019
Accepted: 17 April 2019
Published Online: 1 May 2019
Analele Universității din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House