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Effects of Biotic and Abiotic Factors on the Yield and Chemical Composition of Essential Oils from Four *Thymus* Species Wild-Growing in Northeastern Algeria

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Abstract

In order to evaluate the appropriate factors for producing better quality and quantity essential oils (EOs), we performed a comparative study between the yields and the chemical compositions of the EOs of four *Thymus* species, spontaneously growing in six different locations in northeastern Algeria. EOs hydrodistillation gave yields that ranged from 0.9% to 2.35%. The highest yield (2.35%) was obtained from Dekma's *Thymus algeriensis* oils. GC/MS analyses revealed the presence of 61 compounds, representing 93.6 to 98.23% of the total EOs composition. The hierarchical cluster analysis allowed the six EOs populations to be grouped into two chemotypes: one with thymol and the other with α-pinene. The ANOVA test showed significant differences between the yields and chemical compositions of essential oils; the plant species factor had no significant effect on the variability of these two parameters. Therefore, the variability highlighted was related to the significant impact of abiotic factors. Altitude and soil type were the most influential factors in the secretion of EOs and the emergence of various chemical profiles.

Keywords: *Thymus,* essential oils, yields, chemical compositions, abiotic factors

1. Introduction

As the entire Algerian national territory, the flora of the Souk Ahras region enjoys tremendous biodiversity; it includes several aromatic and medicinal plants rich in secondary metabolites endowed with many therapeutic and pharmacological activities, including plants of the genus *Thymus*.

Such plants are commonly referred to as "*Zaitra*" and include twelve national species, nine of which are endemic (Quezel and Santa, 1963). They are spread throughout most coastal and inland regions, and also in arid areas (Saidj, 2006).

Some plants' essential oils (EOs) are ranked among the most bioactive and common oils in the world. This bioactivity is demonstrated by the unique excess of certain essences in phenolic compounds such as thymol and carvacrol (Cosentino *et al.,* 1999; Trombetta *et al.,* 2002; Amarti *et al.,* 2011). *Thymus* EOs are characterized by a high degree of polymorphism and exceptional chemical heterogeneity which leads to several chemotypes being identified. This variability depends on several factors which are typically ecological, i.e. soil type, altitude, climatic conditions, adjacent plants populations (Baydar *et al.,* 2004; Colombo *et al.,* 2013), but can also be genetic and seasonal (Ložienė *et al.,* 2007).

In this context, we carried out a comparative study in this research between the EOs of four species of the genus *Thymus* (*Thymus hirtus, Thymus capitatus, Thymus ciliatus and Thymus algeriensis*), growing spontaneously in different areas for northeastern Algeria in order to check the possible impact of biotic (plant species) and abiotic (climate, soil, altitude) factors on the quantity and quality of these essential oils.

2. Materials and Methods

2.1. Plant collection and identification

Aerial parts of spontaneous *Thymus* spp plants were collected from six different locations for Souk Ahras region (northeastern Algeria) during full flowering in June and July 2016. Clumps of adjacent young plants were chosen at each collection site to achieve a homogeneous sample of plants with the same morphology and development stage. The geographical coordinates of the sampling areas are available as supplemental material (Table S).

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Table S. Geographical coordinates of study stations

Collection sites	Geographical coordinates		Altitude (m)
	Latitude (N)	Longitude (E)	
Dekma	36° 14’ 43,8’’	007° 54’ 26,5’’	695
Sedrata	36° 10’ 33.7’’	007° 30’ 11,9’’	1277
Zouabi	36° 05’ 32.8’’	007° 26’ 31,3’’	882
Ain Seynour	36° 18’ 24,0’’	007° 50’ 43,0’’	1151
Boukabech	36° 17’ 08,3’’	008° 02’ 36,5’’	772
Ouled Driss	36° 23’ 24,3’’	008° 01’ 52,6’’	1228

The harvested plants were identified by Professor Azzedine Chefrour, Faculty of Natural and Life Sciences, Department of Biology, Souk Ahras university, Souk Ahras, Algeria.

2.2. Climatic and edaphic characterization of collection sites

Climate data (average annual temperatures and rainfall) were taken from the website (Climate-data.org, (2019).

The edaphic analyses were carried out at the level of the National Institute of Irrigation and Drainage Soils (INSID) of Oum El Bouaghi (Algeria). Soil samples were collected at the rhizosphere level (30 cm) of the thyme tussocks, which presents an essential interface between the plant and the soil. They are air-dried and then passed through a 2 mm mesh sieve (AFNOR, 1987). Soil texture was determined using the Robinson's pipette method. pH meter and conductivity meter were used to measure the pH and electrical conductivity of the soil, respectively. Organic matter was determined using the Walkley and Black method. Limestone was determined by a volumetric method using Bernard's Calcimeter.

2.3. Essential oil isolation

Clevenger-type apparatus was used to separate the EOs from 50 g of dry plant matter (leaves and flowers). The samples obtained were stored at 4°C in hermetically sealed brown glass vials until analysis. The yield obtained for the essential oil was expressed as a percentage (w/w).

2.4. GC-MS characterization of EOs

The GC-MS analyses were performed via a Hewlett-Packard Agilent 6890 plus series GC system (Agilent Technologies) coupled with a quadrupole mass spectrometer (HP Agilent 5973), using an HP-5MS non-polar fused-silica capillary column (30 m × 0.32 mm, 0.25 µm film thicknesses). The oven temperature programme was initially held for 8 min at 60°C then increased by 2°C/min until 250°C and held at 250°C for 10 min. Helium was used as the carrier gas with a flow rate of 0.5 ml/min. EO was injected with a volume of 0.2 µl in split mode 1:80. Injector and detector transfer line temperatures were set at 250°C and 270 °C, respectively. The ion source temperature was set at 230°C. Ionization of the sample components was performed in the electron impact mode (70 eV) over a scan range of 30–550 m/z. The ion source temperature was set at 230°C.

Essential oils compounds were identified by comparing of their mass spectra data with those stored from MS databank (NIST02.L and WILEY7n.L), and confirmed by comparing of their Kovat retention index (KI), determined referring to a standards series of n-alkanes (C5–C29) injected under the same conditions with those previously reported in the literature (Hazzit *et al.,* 2009; Zouari *et al.,* 2012; Amiri *et al*., 2011; Benchabane, 2014; Helmi *et al.,* 2014; Bendif *et al*., 2016; Mahboubi *et al*., 2017 and Guesmi *et al.,* 2019)

2.5. Statistical study

The data processing has been performed using the XLSTAT statistical software (Version: 2019.1.3, Addinsoft). One-way statistical analysis (ANOVA) was used to compare the averages obtained at the 5% significance level. Correlations between variations in yields and chemical compositions with exogenous factors were calculated using the Spearman coefficient. A hierarchical ascending classification (HAC) was performed to determine the level of similarity between *Thymus* spp populations based on the percentages of seven major components. Principal Component Analysis (PCA) was conducted to study the variability in the chemical composition of EOs as a function of environmental factors.

3. Results

3.1. Yields of essential oils

The yields of essential oils obtained by hydrodistillation of the aerial parts of six populations of *Thymus* spp collected from six different localities of Souk Ahras, as well as the environmental characteristics and the physico-chemical properties of the soils of the collection sites have been established in Table (1).

Variance analysis (ANOVA) at the 5 % level of significance revealed the existence of a significant difference between the EO yields of six *Thymus* spp populations. EO yields varied between 0.9% and 2.35%. The highest yield was obtained from Dekma's *Thymus algeriensis* EO (TR_1) and the lowest (0.9%) from Oued Driss’s *Thymus ciliatus* EO (TR_6).

Table 1. Essential oils yields, environmental characteristics and soils physico-chemical properties of *Thymus* spp. collection sites.

Codes	Collection sites	Texture and soil characteristics							Climate		Yield (%)*
		Altitude (m)	Clay (%)	Silt (%)	$CaCO_3$ (%)	pH	EC (dS/m)	OM (%)	P (mm)	*T* (C°)	
TR_1	Dekma	695	33.3	22.2	30.8	7.4	0.4	2.1	661	14.4	2.3 ± 0.8
TR_2	Sedrata	1277	34.3	0.6	nd	6.8	0.3	5.5	523	14.2	1.3 ± 0.2
TR_3	Zouabi	882	24	20	35.6	7.5	0.2	2.1	478	14.1	1.7 ± 0.2
TR_4	Ain Seynour	1151	7.4	2.1	nd	6.3	0.2	7.6	763	13.7	1.0 ± 0.2
TR_5	Boukabech	772	31.9	26.5	41.7	7.3	0.3	8.5	726	13.4	1.2 ± 0.6
TR_6	Ouled Driss	1228	20	4.0	nd	6.6	0.2	3.8	868	13.5	0.9 ± 0.2

EC: Electrical conductivity (dS/m); OM: Organic matter (%); P: Mean annual precipitation (mm); *T*: Mean annual temperature (°C); **p-value* = 0.04 at a significance level of 0.05; nd: not detected; TR_1: *Thymus algeriensis* Boiss. & Reut. of Dekma; TR_2: *Thymus capitatus* (L.) Hoffmanns & Link of Sedrata; TR_3: *Thymus capitatus* (L.) Hoffmanns & Link of Zouabi; TR_4: *Thymus hirtus willd* subsp. *algeriensis* (Boiss. & Reut.) Of Ain Seynour; TR_5: *Thymus hirtus* willd of Boukabech; TR_6: *Thymus ciliatus* subsp. *mumbyanus* (Boiss. & Reut.) Batt. of Ouled Driss.

3.1.1. Variability of extraction yields between species

Using multivariate analysis of variance (MANOVA), the effect of the species on the essential oil extraction yields of six *Thymus* spp populations was calculated by Wilks' test at a significance level of 5%. The plant species was chosen as an explanatory qualitative variable (Table 2).

Based on the Wilks test table, we note that Lambda value is associated with a p-value (0.095) higher than the risk-alpha threshold (0.05). Therefore, we cannot reject the null hypothesis (H0), and it is assumed that the qualitative variable (species) has no significant impact on the EO yield of the different species of the *Thymus* genus studied.

Table 2. *p-value* of Wilks test (Rao approximation)

Wilks test	**species**
Lambda	**0.065**
F (Observed values)	9.646
DDL1	3
DDL2	2
F (Critical value)	19.164
p-value	**0.095**

3.1.2. Variability of extraction yields as a function of environmental factors

The correlation between environmental factors and essential oil yield was calculated by the Spearman correlation coefficient (Fig. 1). A positive correlation was found between essential oil yield and pH (ρ=0.83), average annual temperatures and electrical conductivity of the soil (ρ=0.77) and with clay content (ρ= 0.66). A negative relationship was found with average annual rainfall (ρ= - 0.83), organic matter and sand content (ρ= - 0.60) and with altitude (ρ= - 0.54). Therefore, it can be deduced that environmental factors could significantly affect the essential oil yields of *Thymus* spp. populations.

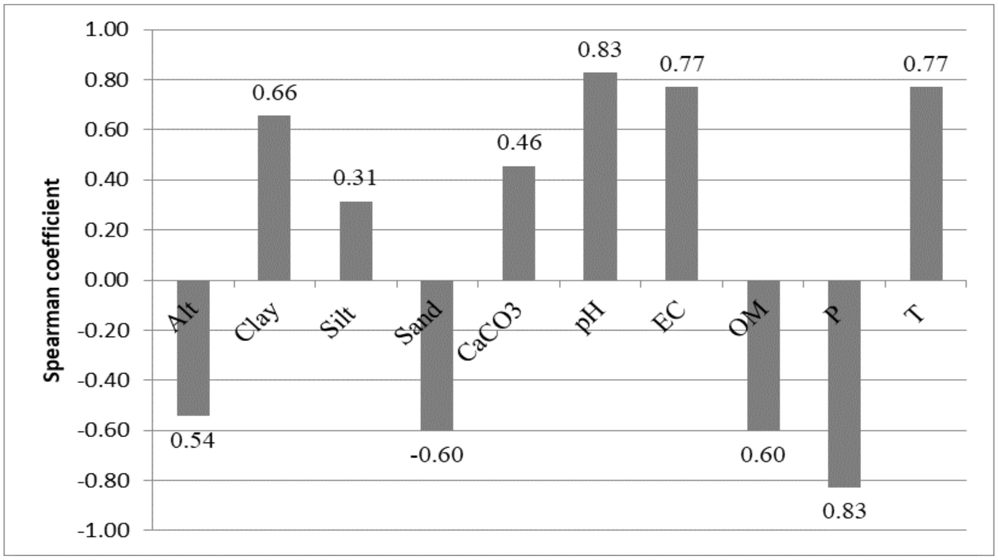

Figure 1. Correlation between the essential oil yield of *Thymus* spp populations and environmental factors.

3.2. Chemical composition of EOs

Chemical analyses of EOs were carried out by GC/MS. The percentages, times, and retention indices of the identified EO compounds were listed in Table (3) in the order of their elution on the HP-5MS column. Chromatographic profiles of volatile fractions are available as supplemental materiel (Figure S).

Chromatographic analyses of *Thymus* spp EOs have identified 61 compounds, representing 93.6 to 98.23% of the total EOs composition. These constituents were grouped into four chemical classes: hydrocarbon monoterpenes (24.6-60.6%), oxygenated monoterpenes (8.0-68.1%), hydrocarbon sesquiterpenes (1-15%) and oxygenated sesquiterpenes (0.6-9.4%).

Analysis of variance showed that thirty-six out of sixty-one compounds differed significantly among the six EOs *Thymus* spp populations including: α-Pinene ($p<0.05$), Camphene ($p<0.001$), β-Pinene ($p<0.001$), β-Myrcene ($p<0.001$), Limonene ($p<0.05$), Linalool ($p<0.05$), Thymol ($p<0.05$), (E)-Caryophyllene ($p<0.05$). Borneol ($p<0.001$), Caryophyllene oxide ($p<0.05$).

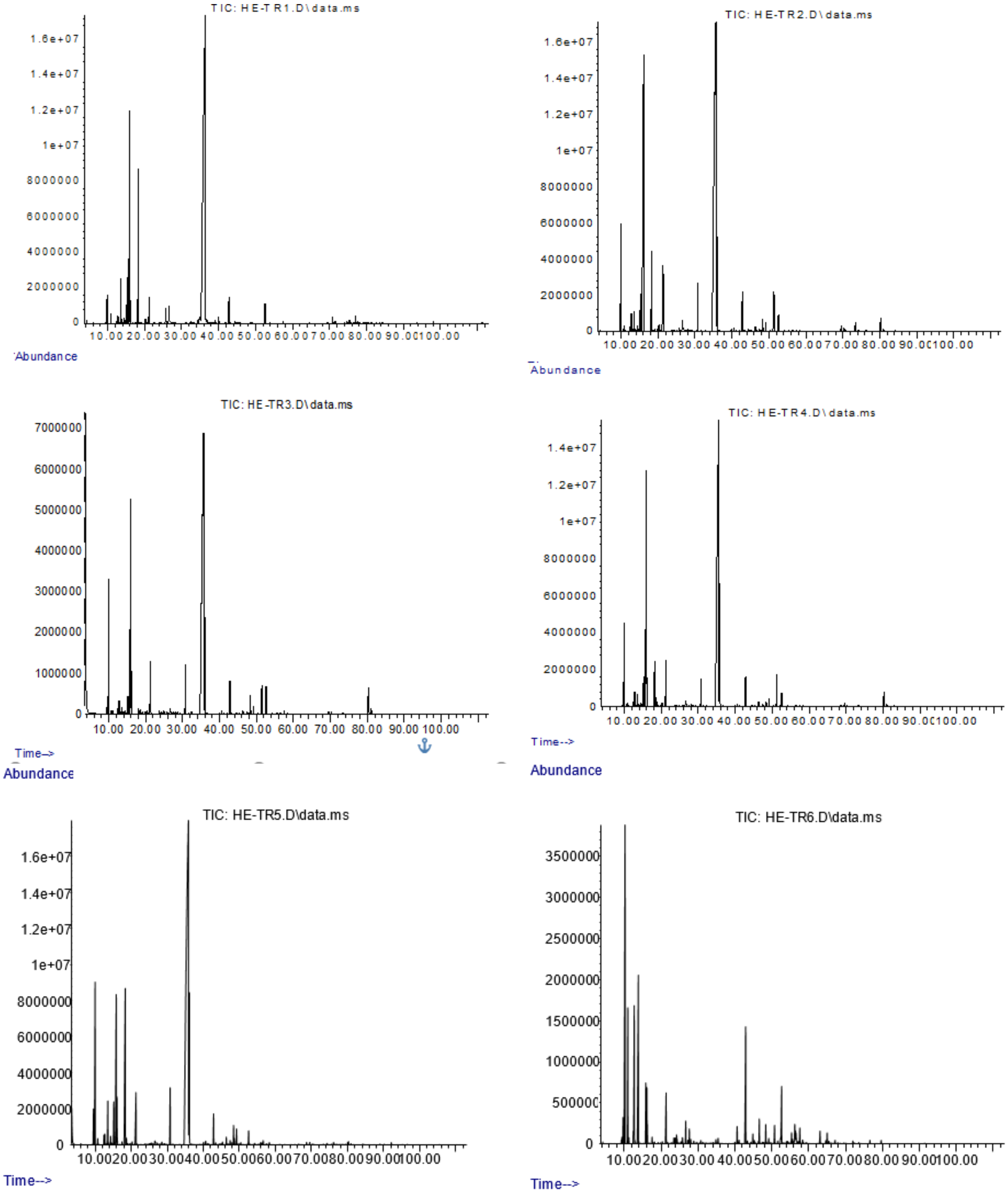

Figure S. Chromatographic profile of volatile fractions of six *Thymus* spp population

Table 3. Chemical composition of the volatile fractions of six samples of *Thymus* species

N°	Compound	*KI*	*KIL*	Relative peak areas (%)						Anova
				TR_1	TR_2	TR_3	TR_4	TR_5	TR_6	
1	Tricyclene	921	923^{H}	0.01	0.01	0.02	0.01	0.01	0.31	**
2	α-Thujene	925	925^{H}	0.6	0.65	0.55	0.71	0.87	1.45	*
3	α-Pinene	932	935^{H}	0.75	2.82	5.18	2.65	5.32	23.07	*
4	Camphene	945	948^{H}	0.27	0.13	0.12	0.11	0.14	6.45	**
5	Verbenene	951	953^{H}	-	0.03	0.08	0.03	0.01	0.25	*
6	β-Pinene	974	976^{H}	0.12	0.17	0.19	0.18	0.24	8.7	**
7	1-Octen-3-ol	978	982^{H}	0.24	0.54	0.51	0.56	0.29	0.13	^{ns}
8	β-Myrcene	992	992^{Z}	1.41	0.51	0.21	0.37	1.14	13.12	*
9	n-Octan-3-ol	996	1001^{H}	0.12	0.07	0.09	0.06	0.05	0.08	^{ns}
10	α-Phellandrene	1004	1005^{H}	0.18	0.16	0.12	0.12	0.23	-	^{ns}
11	α-Terpinene	1016	1017^{H}	1.66	1.49	0.8	1.31	1.2	-	^{ns}
12	p-Cymene	1027	1027^{Z}	13.48	23.14	17.37	19.8	6.25	3.19	^{ns}
13	β-Phellandrene	1029	1030^{H}	0.55	-	-	-	-	-	**
14	Limonene	1030	1030^{Z}	-	0.85	1.09	0.72	1.24	3.56	*
15	1,8-Cineole	1031	1033^{H}	-	-	-	-	0.11	-	**
16	(E)-β-Ocimene	1048	1048^{H}	0.05	0	0.01	-	0.09	0.29	^{ns}
17	γ-Terpinene	1061	1061^{H}	8.44	2.68	0.21	1.65	7.79	0.08	^{ns}
18	*cis*-Sabinene hydrate	1066	1069^{H}	0.02	0.04	0.19	0.37	0.17	-	^{ns}
19	*trans*-Linalool oxide	1071	1072^{H}	0.01	0.08	0.12	0.08	-	0.06	^{ns}
20	α-terpinolene	1087	1089^{Z}	0.18	0.23	0.19	0.19	0.1	0.13	^{ns}
21	Linalool	1101	1104^{H}	0.94	3.53	3.36	2.41	2.21	3.43	*
22	*trans*-Pinocarveol	1136	1036^{Z}	-	0.1	0.19	0.11	0.04	0.34	^{ns}
23	Camphor	1148	1044^{H}	0.03	-	-	0.01	0.2	0.78	^{ns}
24	Verbenol	1152	1146^{H}	-	-	0.27	0.13	-	-	^{ns}
25	Borneol	1163	1164^{Z}	0.81	0.21	0.2	0.17	0.17	0.4	**
26	Terpinen-4-ol	1176	1177^{Z}	0.92	0.78	0.54	0.6	0.44	1.21	^{ns}
27	p-Cymen-8-ol	1184	1184^{Z}	-	-	0.25	0.15	0.05	0.22	^{ns}
28	α –Terpineol	1189	1189^{Z}	0.01	0.14	0.19	0.13	0.14	0.78	*
29	Myrtenol	1203	1198^{H}	-	0.04	0.09	-	-	0.37	*
30	Dihyrdocarvone	1207	1203^{H}	-	0.13	0.11	0.06	0.06	0.16	*
31	Thymol methyl ether	1234	1235^{H}	0.01	1.45	1.99	0.98	1.7	0.25	^{ns}
32	Thymoquinone	1258	1260^{H}	-	-	0.14	0.05	-	-	^{ns}
33	Thymol	1306	1302^{Z}	59.79	48.85	49.69	52.96	59.25	0.27	*
34	Carvacrol	1311	1312^{Z}	5.3	3.85	4.28	6.51	5.31	0.34	^{ns}
35	Carvacrol Acetate	1374	1372^{Z}	0.22	-	-	-	-	-	**
36	ß-Bourbonene	1383	1380^{H}	-	0.1	0.13	0.08	0.11	0.98	*
37	ß-Elemene	1391	1393^{Z}	-	-	-	-	-	0.2	**
38	(E)-Caryophyllene	1418	1409^{B}	0.92	1.28	1.33	1.15	0.91	8.42	*
39	ß-Copaene	1427	1420^{B}	-	0.04	0.06	0.04	0.06	0.15	*

40	α-Humulene	1451	1450^{Z}	0.05	0.05	0.08	0.06	0.05	0.52	*
41	Allo-Aromadendrene	1458	1456^{H}	-	0.02	0.04	0.03	0.09	0.18	*ns*
42	α-Amorphene	1475	1475^{C}	-	0.16	0.18	0.19	0.21	0.12	*
43	Germacrene-D	1479	1477^{H}	-	-	-	-	-	1.33	**
44	Valencene	1493	1491^{A}	0.01	0.07	0.11	0.12	0.15	0.2	*ns*
45	ß-Bisabolene	1508	1505^{H}	-	0.35	0.7	0.16	0.52	0.96	*ns*
46	γ-Cadinene	1512	1511^{Z}	-	0.11	0.16	0.16	0.21	0.1	*ns*
47	δ-Cadinene	1522	1518^{Z}	0.01	0.29	0.34	0.33	0.45	0.36	*ns*
48	Elemol	**1555**	1546^{Z}	-	-	-	-	-	1.06	**
49	Thymohydroquinone	**1560**	1567^{M}	-	1.86	1.73	1.76	-	-	*ns*
50	Nerolidol	**1565**	1563^{Bc}	-	-	-	-	-	0.14	**
51	Spathulenol	**1575**	1578^{Z}	0.02	-	-	0.04	0.02	0.35	*
52	**Caryophyllene oxide**	**1581**	1583^{Z}	0.72	0.57	1.24	0.56	0.43	**3.8**	*
53	p-1-Menthene	**1607**	/	-	-	-	-	-	0.19	**
54	δ-Selinene	**1631**	1590^{G}	-	-	0.03	-	-	0.75	*
55	α-Caryophylladienol	**1635**	1628^{H}	-	-	-	-	-	0.25	**
56	Calarene	**1641**	/	-	-	-	-	-	0.2	**
57	β-Caryophyllene epoxide	**1643**	/	-	-	-	-	-	1.21	**
58	β-Eudesmol	**1646**	1644^{H}	-	0.05	0.1	0.03	0.06	1.52	*
59	α-Cadinol	**1650**	1650^{H}	-	-	-	0.02	0.14	0.1	*ns*
60	Germacrene A	**1661**	/	-	-	-	-	-	0.17	**
61	(Z)-α-Bisabolene epoxide	**1669**	1680^{H}	-	-	0.16	-	-	0.98	*
Number of identified compounds				**32**	**40**	**46**	**45**	**43**	**52**	
Identification rate (%)				**97.85**	**97.63**	**94.74**	**97.92**	**98.23**	**93.66**	
Hydrocarbon monoterpenes				**27.7**	**32.9**	**26.1**	**27.9**	**24.6**	**60.6**	
Oxygenated monoterpenes				**67.8**	**59.6**	**61.4**	**65.5**	**68.1**	**8.0**	
Hydrocarbon sesquiterpenes				**1.0**	**2.5**	**3.2**	**2.4**	**2.8**	**15.0**	
Oxygenated sesquiterpenes				**0.7**	**0.6**	**1.5**	**0.6**	**0.7**	**9.41**	
Other Compounds				**0.4**	**2.1**	**2.6**	**1.6**	**2.1**	**0.1**	

RT: retention times; IK: kovat index calculated against C9-C29 n-alkanes on the apolar HP-5MS column; IKL: literature kovat index; TR_1: *Thymus algeriensis* Boiss. & Reut. of Dekma; TR_2: *Thymus capitatus* of Sedrata; TR_3: *Thymus capitatus* of Zouabi; TR_4: *Thymus hirtus* ssp. *algeriensis* Boiss. & Reut. Of Ain Seynour; TR_5: *Thymus hirtus* of Boukabech; TR_6: *Thymus ciliatus* ssp. *mumbyanus* of Ouled Driss, -: not detected; ^{H}: (Hazzit *et al.*, 2009); ^{Z}: (Zouari *et al.*, 2012); ^{A}: (Amiri *et al.*, 2011); ^{Bc}: (Benchabane, 2014); ^{H}: (Helmi *et al.*, 2014); ^{B}: (Bendif *et al.*, 2016); ^{M}: (Mahboubi *et al.*, 2017); ^{G}: (Guesmi *et al.*, 2019). The analysis of variance (ANOVA) is highly significant (**) at $p < 0.001$, significant (*) at $p < 0.05$ and non-significant (ns) at $p > 0.05$.

3.2.1. Variability of chemical composition as a function of species

To evaluate the effect of the species on the chemical composition of EOs of the *Thymus* spp populations studied, a hierarchical cluster analysis (HCA) was performed using Euclidean distances between major components of the *Thymus* EOs populations (Fig. 2).

The general structure of the dendrogram shows a Euclidean distance greater than thirty-six (36) units, which demonstrates explicitly the existence of two groups, one at thymol majority comprised five populations (TR_1, TR_5, TR_4, TR_2, TR_3), the other at α-pinene majority represented by the (TR_6) population. Further, the Thymol group is broken down into two subgroups (dissimilarity < 10), one with Thymol/p-Cymene (TR_2, TR_3, TR_4) and the other with Thymol/γ-Terpinene (TR_1, TR_5).

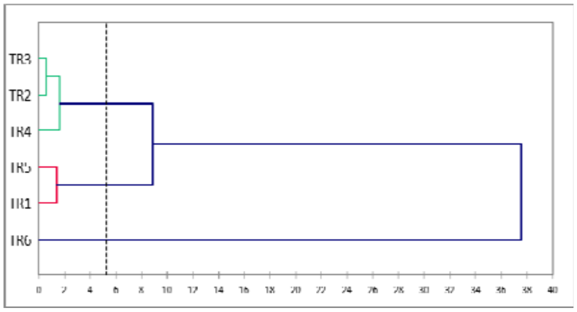

Figure 2. Dendrograms of the major constituents of six EO populations of *Thymus* spp.

3.2.2. Chemical variability of the composition of EOs as a function of environmental factors

The Spearmen's test showed the presence of significant correlations between the chemical composition of the *Thymus* spp populations' EOs and environmental factors (altitude and soil physico-chemical parameters) at 5% significance level.

a. Impact of altitude

The PCA (Fig.3) carried out from the cumulative data of the four main classes of chemical components of EOs from six *Thymus* spp populations shows that hydrocarbon monoterpenes are positively correlated with altitude (0.771), while oxygenated monoterpenes are negatively correlated with this factor (-0.886). There was no correlation between altitude and the other two chemical classes sesquiterpenes (hydrocarbons and oxygenated sesquiterpenes).

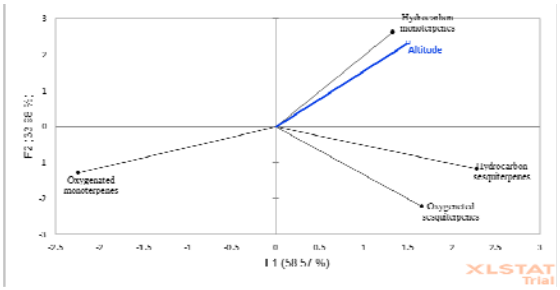

Figure 3. Arrangement of the EOs chemical families as a function of altitude

b. Impact of soil nature

Based on the PCA results (Fig. 4), we find that hydrocarbon monoterpenes versus oxygenated monoterpenes correlate positively with sand content and negatively with pH, electrical conductivity, and limestone and silt content. Hydrocarbon and oxygenated sesquiterpenes have no significant correlation with soil physico-chemical parameters.

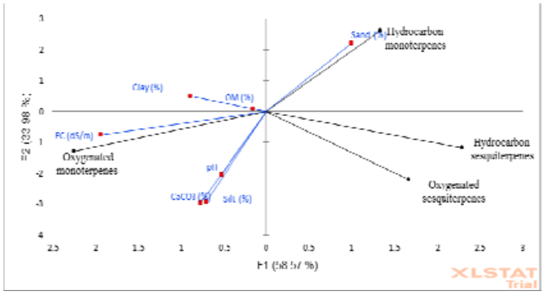

Figure 4. Arrangements of the EOs chemical families as a function of the soil samples' physico-chemical parameters.

4. Discussion

Based on the results of the statistical analyzes performed in this study, the species diversity factor had no significant impact on the variability of the yield and chemical composition between the EOs of six *Thymus* spp populations. Accordingly, the observed variations can be due to the influence of environmental factors.

TR1 population corresponding to Dekma's *Thymus algeriensis* recoded the highest EO yield. Its collection site was relatively characterized by low elevation, low mean annual precipitation and maximum annual temperatures. The soil of this site was classified as silty-clay, non-saline, calcareous, neutral with an alkaline tendency and not humus-bearing. Similar experiments on the impact of environmental factors on the rate of extraction of aromatic plant EOs affirm the negative relationship between altitude and extraction yield (Baydar *et al*., 2004; Avci, 2010; El-Jalel *et al*., 2018).

Hierarchical cluster analysis (Fig. 2) revealed that our EO samples are divided into two chemotypes: one at Thymol and the other at α-Pinene, and this distribution is independent of the plant species. The Thymol chemotype found in the five *Thymus* spp populations belonging to three different species (*Thymus algeriensis, Thymus capitatus and Thymus hirtus*) confirms the increase of this phenol in *Thymus* genus plants. Our findings are similar to those already reported by Giordani *et al.,* (2008) for the essences of *Thymus numidicus* from Khedara (Souk ahras) and Berrahal (Annaba) and *Thymus ciliatis* from Jebel Ansel (Guelma)-Algeria region which noted that thymol was the main component of these plants. Guedri Mkaddem and collaborators (2010) also confirmed that thymol was the key constituent of the Matamata region's *Thymus capitatus*, constituting 89.0% of the essential oil. Thymol with a 44.2% similarly characterized the EO of *Thymus ciliatus* from the Azrou-Maroc region (Amarti et *al.,* 2010). The α-Pinene chemotype found in the TR_6 population would correspond to *Thymus ciliatus* subsp. *munbyanus* from the Ouled Driss region, is different from that found in most previous work on EOs of the same species. Carvacrol was the main constituent of *Thymus ciliatus* from eight localities in Tlemcen-Algeria (Bousmaha-Marroki *et al.,* 2007), *Thymus munbyanus* from Azzazga-Algeria (Benchabane *et al.,* 2012) and *Thymus munbyanus* from the Hennaia-Tlemcen-Algeria region (Tefiani *et al*., 2015). The key constituent of *Thymus ciliatus* EOs from area of Jebel Ansel (Giordani *et al*., 2008) and that Moroccan *Thymus ciliatus* (Amarti *et al.,* 2011) was Thymol, whereas chemotype α-Pinene characterized the EOs of *Thymus algeriensis* from the Khedara and Fatoum Souda regions of Algeria (Giordani *et al.,* 2008) and of *Thymus algeriensis* from different regions of Tunisia (Zouari *et al.,* 2012).

The high chemical diversity observed between various *Thymus* populations studied has been noted by several authors, including Ben El Hadj Ali et *al*., (2012) and Zouari et *al*., (2012), who attributed the high chemical polymorphism discovered in *Thymus algeriensis* Tunisian populations to the influence of geographical location.

The PCA results showed the presence of significant correlations between the chemical composition of the *Thymus* spp populations' EOs and abiotic factors (altitude and soil physico-chemical parameters). The altitude factor has an important impact on the biosynthesis of terpenoids, the anabolism of hydrocarbon monoterpenes is favoured at high altitude a, while that of oxygenated monoterpenes is favoured at low altitude. These findings are in line with those reported by Sanli and Karadogan (2017) who assert the impact of altitude on the terpenoid biosynthesis of EOs terpenoids from *Kundmannia anatolica* Hub. -Mor. fruits spontaneously growing at different altitudes in the lake region (Turkey). El-Jalel *et al*., (2018), showed that the altitudinal variance may clarify the heterogeneity in the composition of the *Thymus capitatus* EOs from Libya. The soil type seems to have a major effect on the chemical composition of *Thymus* EOs by promoting compound biosynthesis and inhibiting others**.** Ben El Hadj Ali *et al.,* (2012) suggested that local abiotic factors (humidity, temperature, topography and edaphic factors) and/or biotic selection factors (associated fauna and flora) acting on the biosynthesis pathways of terpene compounds and leading to the emergence of different chemical profiles may be explanatory factors.

5. Conclusion

As a result of this research, it was found that the species diversity factor had no significant effect on the extraction rate and chemical composition variability of *Thymus* spp Eos; therefore, the variability highlighted was due to the impact of abiotic factors. Altitude and soil physico-chemical characteristics were the most prominent factors affecting terpene compounds biosynthesis pathways and contributing to the emergence of various chemical profiles. Through this approach, we can carefully select thyme collection areas with the appropriate ecological conditions allowing these plants to produce EOs of better quality and quantity.

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