IMPACT OF GEOGRAPHIC'S VARIATION ON THE ESSENTIAL OIL YIELD AND CHEMICAL COMPOSITION OF THREE *Eucalyptus* SPECIES ACCLIMATED IN TUNISIA

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ABSTRACT

Present study has been carried out to estimate the impact of geographical distribution on the yield and chemical constitute of three *Eucalyptus* varieties viz *E. cinerea* F. Muell. ex Benth., *E. astringens* Maiden and *E. sideroxylon* A.Cunn. ex Schauer-. These species were collected from six arboreta of Tunisia in January 2008. The essential oil was extracted by hydrodistillation method and estimated the essential oil yield which varies from 1.5±0.1% to 4.0±0.2%. Results of the study revealed that yield of essential oil are not only depends on the *Eucalyptus* species but also depends on the origin of harvest. *E. sideroxylon* A. Cunn. ex Woolls, cultivated in jbel abderrahman arboreta and *E. cinerea* F. Muell. ex Benth. from choucha (sejnanae) arboreta provided the lowest and the highest percentage of essential oil amongst all the studied provenances, respectively. GC (RI) and GC/MS analysis showed the presence of 163 components, representing 98.8 to 99.5% of the total oil. The contents of the different samples varied according to the species and the origin of harvest. The main components of the *Eucalyptus* essential oil were 1,8-cineole (39.1±0.0 – 79.4±0.0%), followed by α -pinene (2.1±0.0- 30.0±0.0), *trans*-pinocaveol

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Chemotype

Variation

(0.7 ± 0.5 - $10.0\pm2.5\%$), α -terpineol (0.2 ± 0.0 – $8.8\pm1.1\%$), globulol (0.3 ± 0.0 – 5.7 ± 1.3), aromadendrene (0.2 ± 0.0 – $4.9\pm2.1\%$), limonene (0.6 ± 0.0 – $4.3\pm0.8\%$), pinocarvone (0.2 ± 0.0 - $3.7\pm0.6\%$), bicyclogermacrene (0.0 – $3.1\pm0.0\%$), α -terpinyl acetate (0.0 - $2.9\pm0.8\%$), *p*-cymene (0.7 ± 0.0 - $2.7\pm2.1\%$), β -carophyllene (0.3 ± 0.0 - $1.9\pm0.0\%$), viridiflorol (0.2 ± 0.0 - $1.8\pm0.0\%$), epiglobulol (traces – $1.3\pm0.6\%$) and spathulenol (0.2 ± 0.0 - $1.1\pm0.0\%$). The principal component and the hierarchical cluster analyses separated the *Eucalyptus* populations leaf essential oils into four groups, each constituting a chemotype. *E. sideroxylon* from Korbous and Sejnane arboreta afforded the highest mean percentage in 1,8-cineole.

1 Introduction

Genus *Eucalyptus* comprises about 900 species and subspecies (Pereira et al., 2014). More than 300 species of this genus contain volatile oil in their leaves. In 1957, total 117 *Eucalyptus* have been introduced in Tunisia. They were used essentially as fire wood, for the production of mine wood and against the erosion (Khouja et al., 2001). However, less than 20 species have ever been exploited commercially for the production of essential oil rich in 1,8-cineole (>70%) which is essentially used in the pharmaceutical and cosmetic industries (Pino et al., 2002). In Tunisian folk medicine, inhalation of *Eucalyptus* sp. essential oil has been traditionally used to treat respiratory tract disorders such as pharyngitis, bronchitis and sinusitis (Boukef, 1986). Many studies have been demonstrated their antibacterial, antifungal and antiviral activities of *Eucalyptus* sp. essential oil against a wide range of microorganisms (Su et al., 2006; Cermelli et al., 2008; Gilles et al., 2010; Jha et al., 2014). Furthermore, allelopathic effect of this essential oil against many weeds was also reported by many researchers (Batish et al., 2004; Verdeguer et al., 2009; Rassaeifar et al., 2013). *Eucalyptus* essential oil was also reported as an effective anti-inflammatory, analgesic, antioxidant, antimutagenic, insecticide, nematocide and acaricide oil (Batish et al., 2008; Bugarin et al., 2014; Rossi & Palacios, 2015). The *Eucalyptus* essential oils can be obtained by different methods, such as hydrodistillation, supercritical CO₂ extraction, microwave-assisted extraction, and vacuum extraction by solvents. These methods affect the final yield of the oil and it varied from 0.1 ± 0.1 to $5.7\pm0.5\%$ (Elaissi et al., 2010a; Elaissi et al., 2010b). High levels of 1,8-cineole (87.8%) were found for the majority of the *Eucalyptus* species (Zrira et al., 2004; Elaissi et al., 2010c). Generally, the yields and composition of the oils varies and it depending on species, used part, plant origin zone, collection period, growth stage of the plant as well extraction methods and storage conditions (Arango-Bedoya et al., 2012).

In previous studies published on *Eucalyptus* species growing in Tunisia, characterization of the leaves essential oils of 48 *Eucalyptus* species and their antibacterial activities were carried out by the same authors. On the basis of this work three *Eucalyptus* species, *E. cinerea* F. Muell. ex Benth., *E. astringens* Maiden and *E. sideroxylon* A.Cunn. ex Schauer, which demonstrated the best antibacterial activity and also the

best content in essential oil yield and 1,8-cineole, were selected for this research work to identify the effect of the origin of harvest on their essential oils yield and composition.

2 Materials and Methods

2.1 Plant Materials

Clean and mature leaves of three *Eucalyptus* species L. Hér., viz. *E. astringens* Maiden, *E. cinerea* F. Muell. ex Benth. and *E. sideroxylon* A.Cunn. ex Schauer, were collected from six arboreta belonging to lower humid, higher humid and sub-humid bioclimatic stage of Tunisia in January 2008, Table 1. The leaves were dried in airy premises, shielded from the light, then packed in paper bags, and kept in the shade. Botanical voucher specimens of the collected species have been deposited in the Herbarium of the Pharmacognosy Laboratory, Faculty of Pharmacy, Monastir, Tunisia (references 0156, 0157, 0158, 0159, 0160, 0161, 0162, 0163, 0164, 0165, 0166 and 0167).

2.2 Sample preparation and extraction of Essential Oils

The essential oils were extracted by hydrodistillation method, for this 100 g of coarsely crushed *Eucalyptus* leaves for 4 h, using a standard apparatus recommended in the European Pharmacopoeia. Hydrodistillations were performed in triplicate for each *Eucalyptus* species. The oil collected from each plant was dried (Na₂SO₄) and stored at 4°C until analysis. The yield of essential oil was expressed in % (v/w) of the dry material (Elaissi et al., 2010a; Elaissi et al., 2010b).

2.3 Chemical analysis

2.3.1 GC Analysis

Quantitative and qualitative data of the essential oils were determined in triplicate by GC and GC/MS, respectively. GC Analysis was carried out with a Hewlett-Packard 6890 apparatus equipped with FID and a polar Carbowax column (30 m_0.32 mm i.d., film thickness 0.25mm). The oven temperature was programmed isothermal at 35°C for 1 min, rising from 35 to 250°C at 5°C/min, and then held isothermal at 250°C for 3 min; injector temp., 250°C; detector temp., 280°C; N₂ used as carrier gas (1.2 ml/min).

Table 1 Provenances and Classification by Means of Duncan_s Multiple Range Test of the Average Essential Oil Yields of 3 *Eucalyptus* Species

<i>Eucalyptus</i> species	Provenance (Arborea)	Latitude	Longitude	Altitude (m)	Annual rainfall (mm)	Bioclimatic stage	Soil type	Abbreviation	Yield [%]
<i>E. cinerea</i>	Souiniat	35° 54'	8°48'	492	1140	humid inferior with temperate winter	sandstone hydromorphe	cin Soui	3.7±0.7 ^(de)
<i>E. cinerea</i>	Zerniza	30°94'	9°7'	60	920	humid inferior with warm winter	sandstone hydromorphe	cin Zer	3.8±0.4 ^(de)
<i>E. cinerea</i>	Sejnane	37°3'	9° 14'	159	871	humid inferior with temperate winter	sandstone	cin Sej	4.0±0.2 ^(e)
<i>E. astringens</i>	Mrifek	37°07'	9°15'	60	950	humid inferior with mild winter	Marl	ast Mri	3.1±1.0 ^(c)
<i>E. astringens</i>	korbous	36°50'	10°35'	180	540	sub-humid with warm winter	Sandy	ast kor	3.2±0.1 ^(c)
<i>E. astringens</i>	Pryor Choucha (Sejnane)	37°3'	9° 14'	159	871	humid inferior with temperate winter	sandstone	ast Sej	3.3±0.3 ^(cd)
<i>E. sideroxylon</i>	Mrifek	37°07'	9°15'	60	950	humid inferior with mild winter	Marl	sid Mri	2.6±0.3 ^(b)
<i>E. sideroxylon</i>	Jbel Abderrahmane	36°40'	10°40'	255	600	Sub-humid with warm winter	sandy clay	sid JBA	1.5±0.1 ^(a)
<i>E. sideroxylon</i>	Korbous	36°50'	10°35'	180	540	sub-humid with warm winter	Sandy	sid Kor	2.3±0.1 ^(b)
<i>E. sideroxylon</i>	Souiniat	35° 54'	8°48'	492	1140	humid inferior with temperate winter	sandstone hydromorphe	Sid Soui	2.6±0.4 ^(b)

a) Yields with different letters in parentheses differ significantly by Duncan_s multiple range test ($p < 0.05$).

The injected volume was 1 ml (10% essential oil in purified hexane). The relative concentration was calculated using the software HP Chemstation, which allows assimilating the percentages of the peak areas to the percentages of the various constituents. Retention indices were obtained by running a series of aliphatic hydrocarbons (C9 - C28) by increasing the number of carbon atoms in the Carbowax column (Elaissi et al., 2012) GC column).

2.3.2 GC/MS Analysis

The essential oils were analyzed with a Hewlett-Packard 5890 series II apparatus equipped with a 5972 mass-selective detector and polar Carbowax Column (30 m_0.32 mm i.d., 2.3.4 Statistical Analysis

film thickness 0.25 mm) in which Helium gas was used as a carrier. The mass spectrometer operating conditions were: 70 eV ionization voltage, 70 eV; 230°C ion source. The GC analysis conditions were as described above (see GC Analysis) (Elaissi et al., 2012).

2.3.3 Compound Identification

Isolated compounds were based on the comparison of their RI (determined rel. to the tR of n-alkanes (C9–C28)) and mass spectra with those of authentic compounds by means of NBS75K.L. and Wiley 275 databases and with the literature data (Wiley & Sons, 1998).

The data were analyzed using analysis of variance (ANOVA), and the significance of the differences between means was determined at $p < 0.05$ using Duncan's multiple range test. To evaluate the identified essential oil constituents useful for chemical reaction between selected species, 16 compounds were identified from the oil samples with contents in the essential oils of 0.9% in at last one species, were subjected to PCA and HCA using SPSS 12.0 software (SPSS Inc. Chicago, IL, USA) (Elaissi et al., 2012).

3 Results and Discussion

3.1 Oil Yields

The Analysis of variance (ANOVA) indicated that the oil yields were significantly different between species to species and arboreta to arboreta ($p < 0.05$). The analysis of results showed the presence of five overlapping groups (Table 1) ranging from $1.5 \pm 0.1\%$ for *E. sideroxylon* cultivated in Jbel Abderrahmane arboreta to $4.0 \pm 0.2\%$ for *E. cinerea* grown in Pryor Choucha (Sejnane) arboreta (Table 1), which differed from the others arboreta (Souiniat and Zerniza) by a sandstone soil and by the lowest annual pluviometry. Results of the study also revealed that *E. sideroxylon* harvested from Souiniat and Mrifek arboreta, which were characterised by a higher annual raining, higher altitude and by a sandstone soil, produced a higher mean percentage of essential oils relatively to those collected from Jbel Abderrahmane and Korbous arboreta, while *E. astringens* was not affected by the environmental conditions. These results were confirmed by Dunlop et al. (2000) who found that the essential oil yields of some clones of *E. camaldulensis* was not dependent on the sites at which they were grown but rather on their genetic constitution, however Bhatti et al. (2007) reported that *E. cerebra* essential oil yield varied significantly among different district of Punjab-Pakistan. The increasing level of essential yield was depend to environmental conditions such as dry, warm, altitude (Tsiri et al. 2003; Arango-Bedoya et al., 2012; Hassiotis et al., 2014). Furthermore Zrira et al. (1994) reported that *E. sideroxylon* from Morocco, region of Jbilet was richer in essential oil (2.06%) than that of the region of Tekerkoust (1.36%), while *E. astringens* from Tekerkoust was the richest one (2.45%) against 2.20% for the other provenances. These results were also proved by Grbovic et al. (2010) which were found that the leaves essential oil yield of *E. camaldulensis* Dehn. collected from five regions of the coastline of Montenegro varied from 0.67% for those collected from Bar region to 1.59% for the region of Tivat. Similarly LI & Madden (1995) found that the essential oils obtained from leaves of *E. regnans*, *E. deligatensis*, *E. nitens*, *E. denticulata* and *E. gobulus* grown in eight arboreta varied significantly within species and within provenances. It was also demonstrated that *E. cinerea* from Morocco (Zrira et al., 2004) was poor in essential oils (0.26%) than those cultivated in India (2.55 to 2.87%) (Kiran Babu & Singh, 2009).

3.2 Chemical composition

The chromatographic analyses (GC (RI) and GC/MS) of the essential oils allowed the identification of 163 compounds (Table 2), representing 98.8 – 99.5% of the total oil. Their mean percentage varied within species and harvest zone. The identified components were divided in seven chemical classes (Table 2). The major class was constituted by the oxygenated monoterpenes (46.5 ± 0.0 – 85.6 ± 0.0), with 1,8-cineole (39.1 ± 0.0 – 79.4 ± 0.0) having the highest content in all the studied species, whatever their provenance. It was followed by α -terpineol (0.2 ± 0.0 – 8.8 ± 1.1) and α -terpinyl acetate (0.0 – 2.6 ± 0.6). The class with the second highest contents composed of the monoterpenes hydrocarbons (4.5 ± 0.0 – 35.0 ± 0.0) represented by α -pinene (2.5 ± 0.0 – 30.0 ± 0.0), limonene (1.0 ± 0.0 – 4.3 ± 0.8), *p*-cymene (0.7 ± 0.0 – 2.7 ± 2.1) and *g*-terpinene (traces to 0.3 ± 0.0). The class with the third highest contents was composed by the oxygenated sesquiterpenes (1.4 ± 0.4 – 12.8 ± 0.0), constituted essentially by globulol (0.3 ± 0.0 – 5.7 ± 1.3), viridiflorol (0.2 ± 0.0 – 1.8 ± 0.0), epiglobulol (traces to 1.3 ± 0.6) and spathulenol (0.0 ± 0.0 – 1.3 ± 0.0). The sesquiterpenes hydrocarbons occupied the fourth position with a mean percentage varying between 1.4 ± 0.1 and 8.0 ± 2.1 , from which the aromadendrene (0.1 ± 0.0 – 4.9 ± 2.1) was the major compound, followed by bicyclogermacrene (traces to 3.1 ± 0.0), β -carophyllene (0.3 ± 0.0 – 1.9 ± 0.0) and *allo*-aromadendrene (0.1 ± 0.0 – 0.9 ± 0.0). The ketones, esters and others were minor compound classes, not discussed in further details. The comparative study of the essential oils chemical composition according to the species and their origin of harvest (arboreta), shows that *E. sideroxylon* oils obtained from leaves harvested from Korbous arboreta are richer in oxygenated monoterpenes (85.6 ± 0.0) and in 1,8-cineole (79.4 ± 0.0), while it was characterized by the lowest content in α -pinene (2.1 ± 0.0). *E. cinerea* from Souiniat arboreta was also characterised by a relatively high mean percentage of 1,8-cineole (71.8 ± 0.0) against $69.8 \pm 2.2\%$ and $70.7 \pm 1.5\%$ for those from Souiniat and Zerniza arboreta; respectively, which were distinguished by their highest mean percentages of α -terpineol (8.8 ± 1.1 , 7.5 ± 0.6 ; respectively), limonene (4.3 ± 0.8 , 3.8 ± 0.0 ; respectively) and α -terpinyl acetate (2.6 ± 0.6 , 2.1 ± 0.6 ; respectively). Oils of all the studied population of *E. astringens* oils have the lowest mean percentage in oxygenated monoterpenes (16.5 ± 1.9 – 35.0 ± 0.0) and in 1,8-cineole (39.1 ± 0.0 – 47.6 ± 1.2), however they were characterized by the highest mean percentage in α -pinene, *tr*-pinocarveol, pinocarvone and attend the maximum in that of Korbous, Mrifek and Sejnane arboreta; respectively. It was also observed that all the provenance of *E. astringens* are relatively rich in sesquiterpenes hydrocarbons (6.8 ± 0.3 – 8.0 ± 2.1), aromadendrene, *allo*-aromadendrene and bicyclogermacrene and in oxygenated sesquiterpenes (9.7 ± 0.0 – 11.1 ± 0.1) represented essentially by globulol, epiglobulol and guaialol.

Table2. Chemical Composition of the Essential Oils Extracted from the Leafs of 3 *Eucalyptus* Species harvested from six arboreta

Compounds and names	KI	Composition (%)										
		<i>E. cinerea</i>			<i>E. astringens</i>			<i>E. sideroxylon</i>				
		Souiniat	Zerniza	Pryor Choucha (Sejnane)	Mrifek	Korbous	Pryor Choucha (Sejnane)	Mrifek	Sejnane (Choucha)	Jbel Abderrahmane	Korbous	Souiniat
Monoterpene hydrocarbons		11.1	10.2	10.3	24.4	35	16.5	15.4	8.2	4.5	4.9	10.5
Tricyclene	1043	-	tr	tr	-	-	-	-	Tr	-	-	-
α -Pinene	1050	5.1	5.2	5.7	21.8	30.0	14	8.3	2.9	2.5	2.1	5.1
α -Thyrene	1053	tr	tr	tr	tr	0.1	tr	0.2	0.1	-	tr	tr
α -Fenchene	1072	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr
Camphene	1077	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	tr	0.1	0.1
α -Pinene	1108	0.1	tr	0.1	0.2	0.5	0.2	0.2	0.1	0.1	0.1	0.2
Sabinene	1125	-	-	-	-	-	-	tr	-	-	-	tr
Verbenene	1127	-	-	-	0.1	tr	0.1	tr	-	-	tr	tr
2.4(10)-Tujadiene	1132	-	-	-	-	-	-	tr	-	-	-	-
σ -3-Carene	1146	-	-	-	-	-	-	-	tr	-	-	-
α -Phellandrene	1168	0.1	0.1	0.1	0.1	0.4	0.1	1.3	0.2	0.1	tr	0.4
Myrcene	1172	tr	tr	tr	tr	-	-	tr	tr	-	tr	tr
α -Terpinene	1183	-	-	-	-	-	-	0.1	-	-	-	-
Limonene	1207	4.3	3.8	3.3	1	1.9	0.6	3.9	1.7	1	1.5	3.3
α -Phellandrene	1209	-	-	-	-	-	-	-	-	-	-	0.3
α -cis-Ocimene	1240	tr	tr	-	-	tr	-	tr	tr	-	tr	tr
α -Terpinene	1251	0.1	0.1	tr	tr	tr	tr	0.3	0.3	tr	0.1	0.1
α -trans-Ocimene	1256	tr	tr	0.1	tr	tr	tr	tr	0.1	0.1	0.1	tr
<i>p</i> -Cymene	1278	1	0.7	0.7	0.8	1.7	1.3	0.9	2.7	0.7	0.8	0.8
Terpinolene	1289	0.1	0.1	0.1	tr	0.1	tr	0.1	tr	-	tr	0.1
Oxygenated monoterpenes		85	84.9	85	56.4	46.5	64	75.1	77.8	70.4	85.6	80.2
<i>p</i> - α -Dimethyl styrene	1449	0.1	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr
1.8-Cineole	1218	69.8	70.7	71.8	40.1	39.1	47.6	66.7	71.2	64.1	79.4	72.3
<i>p</i> -Mentha-6.8-dien-2-ol	1222	tr	tr	tr	tr	-	-	tr	tr	-	tr	tr
<i>trans</i> -Rose oxyde	1366	-	-	-	0.1	-	0.1	-	-	-	-	-
α - α -Pinene epoxyde	1384	tr	tr	-	tr	-	-	-	tr	-	-	tr
α -Thujone	1423	0.1	0.1	0.1	tr	-	tr	tr	tr	-	tr	tr
<i>cis</i> -Linalool oxyde	1455	tr	-	-	-	-	-	-	-	-	-	tr
<i>trans</i> -Linalool oxyde	1468	tr	tr	tr	-	-	-	tr	-	-	-	-
Citronellal	1491	-	-	-	-	-	-	tr	-	-	-	-
α -Campholenic aldehyde	1502	tr	tr	tr	0.1	0.1	0.1	tr	0.1	tr	tr	tr
Camphor	1531	tr	tr	tr	-	-	-	-	tr	-	tr	tr
iso-Pinocamphone	1533	-	-	-	0.1	tr	0.1	-	-	-	-	-
Linalool	1560	tr	-	-	tr	tr	tr	tr	tr	-	tr	-
<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	1572	tr	-	tr	-	-	-	tr	tr	-	-	tr

<i>cis</i> -Sabinene hydrate	1576	-	-	-	-	-	-	-	tr	-	tr	-
Isopulegol	1581	-	-	-	tr	tr	tr	-	tr	-	-	-
Pinocarvone	1585	0.2	0.3	0.4	2.9	0.8	3.7	0.2	0.3	0.4	0,3	0,2
Fenchol	1593	0.1	0.2	0.1	0.3	0.2	0.3	0.1	0.1	0.1	0,1	0,1
Terpinene-4-ol	1618	tr	tr	tr	0.2	0.2	0.1	0.2	0.1	0.2	0,1	0,1
<i>cis-p</i> -Menth-2-en-1-ol	1634	-	-	-	-	-	-	-	-	-	-	-
Myrtenal	1638	tr	tr	0.1	tr	tr	tr	0.1	0.1	0.1	0,1	0,1
Umbellulone	1655	-	-	-	tr	tr	-	-	-	-	-	-
trans-pinocarveol	1670	0.7	0.9	0.9	10.0	3.7	9.3	1	1.3	1.8	1,5	1,1
<i>trans-p</i> -Menth-2-ene-1.8-diol	1673	-	-	-	-	-	-	tr	tr	-	-	tr
σ -Terpineol	1686	0.1	tr	-	tr	0.1	0.1	0.1	0.1	0.2	-	0,1
<i>Cis</i> -Piperitol	1698	tr	-	-	tr	tr	tr	-	tr	-	tr	tr
CarvotanAcetone	1699	tr	-	-	-	-	-	0.1	-	-	-	-
Neral	1705	-	-	-	-	-	-	tr	-	-	-	-
α -Terpineol	1708	8.8	7.5	6.4	0.7	0.9	0.5	2.1	0.8	0.5	0,2	1,8
α -Terpinyl acetate	1708	2.6	2.1	2	tr	-	-	2.9	0.9	0.7	0,8	2,8
Borneol	1710	0.2	0.3	0.2	0.2	0.3	0.1	0.2	0.1	0.1	0,1	0,2
Phellandral	1740	-	-	-	-	-	-	-	-	-	-	-
Piperitone	1746	0.1	-	-	tr	tr	tr	tr	-	-	-	tr
Carvone	1754	0.2	0.2	0.2	-	-	-	-	0.1	-	0,1	-
Geranial	1757	-	-	-	-	-	-	tr	-	-	-	-
Geranial	1761	tr	-	-	tr	-	tr	tr	tr	-	tr	tr
Geranyl acetate	1763	tr	-	tr	-	-	-	tr	-	tr	-	-
<i>trans</i> -Piperitol	1767	tr	-	-	tr	-	tr	tr	0.1	0.1	0,1	tr
Citroneoll	1785	tr	-	-	-	-	-	-	-	-	-	-
Cuminal	1801	tr	-	-	-	-	tr	tr	tr	-	-	tr
Cuminal	1803	-	-	-	-	-	-	-	-	-	-	-
Myrtenol	1807	tr	tr	tr	0.2	0.1	0.2	0.1	0.1	0.1	0,1	tr
<i>trans-p</i> -Mentha-1(7).8dien-2-ol	1811	0.7	0.8	0.9	0.4	0.2	0.4	0.2	0.8	0.8	1	0,4
Nerol	1818	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr
α -Phellandrene epoxyde	1822	tr	-	-	tr	tr	tr	tr	tr	-	-	tr
<i>trans</i> -Carveol	1848	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0,2	0,1
<i>trans-p</i> -Menth-1.8-dien-6-ol	1856	tr	tr	tr	tr	tr	-	tr	-	-	-	tr
<i>p</i> -Cymen-8-ol	1863	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0,1	0,1
Geraniol	1867	tr	-	-	-	-	-	-	-	-	-	tr
Neryl acetone	1875	-	-	-	tr	tr	-	-	-	-	-	-
<i>cis</i> -Carveol	1879	0.1	0.2	0.2	tr	tr	tr	tr	0.1	tr	0,1	tr
<i>cis-p</i> -Mentha-1.8-dien-6-ol	1884	tr	-	-	-	-	-	-	-	-	-	-
<i>cis-p</i> -Mentha-1(7).8dien-2-ol	1903	0.7	0.9	0.9	0.3	0.2	0.4	0.3	0.8	0.8	0,9	0,4
<i>p</i> -Mentha-1.5-dien-7-o	1984	-	-	-	-	-	-	-	-	-	-	-
Methyl eugenol	2026	-	-	-	tr	tr	0.1	tr	tr	tr	tr	tr
<i>p</i> -Cymen7-ol	2121	-	-	-	tr	-	tr	-	tr	-	tr	tr
Thymol	2172	tr	tr	tr	0.1	tr	0.1	-	0.1	tr	tr	0,1
Eugenol	2191	tr	tr	tr	-	-	-	-	-	-	-	-
Carvacrol	2233	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2	-	0,1	tr
Carvacrol	2239	-	-	-	0.1	-	tr	-	-	-	-	tr

<i>p</i> -Cuminol	2240	-	-	-	-	-	-	-	-	-	-	-
1-Terpineol	1643	-	-	tr	-	-	0.1	-	tr	tr	tr	-
1,8-Menthadien-4-ol	1701	0.1	0.1	0.2	-	-	0.1	0.1	0.1	0.1	tr	tr
Sesquiterpene hydrocarbons		1.6	1.8	1.4	8.0	7.7	6.8	4.8	4.8	10.5	3.2	4.1
Isodene	1477	-	0.1	0.1	tr	0.1	tr	tr	tr	0.1	tr	0,1
α -Cubebene	1479	0.1	-	-	tr	-	-	tr	-	-	-	-
Bicycloelemene	1493	-	-	-	tr	tr	-	tr	-	-	-	-
α -Copaene	1506	-	tr	-	-	-	-	-	tr	-	-	-
α -Gurjunene	1542	tr	tr	tr	tr	0.1	tr	0.1	tr	0.1	tr	tr
β -Cubebene	1554	-	tr	tr	tr	tr	-	tr	tr	-	-	tr
β -Elemene	1602	-	-	-	tr	tr	-	tr	tr	0.1	tr	tr
β -Gurjunene	1607	-	-	-	0.2	0.1	0.2	tr	0.1	0.1	0,1	tr
β -Caryophyllene	1613	0.7	0.6	0.6	0.3	1	1	1.4	0.8	1.9	0,4	1
α -Guaiane	1619	-	-	-	-	-	-	tr	-	-	-	-
Aromadendrene	1622	0.2	0.2	0.1	4.9	3.8	3.8	0.4	1.8	3.2	1,2	0,5
Dehydraromadendrene	1630	tr	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	0,1	0,1
Alooaromadendrene	1661	0.1	0.2	0.1	0.6	0.6	0.6	0.2	0.5	0.9	0,4	0,2
α -Humulene	1683	0.2	0.3	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0,2	0,3
Ledene	1703	tr	tr	tr	tr	tr	-	tr	-	-	-	-
Germacrene D	1712	-	-	-	-	-	-	-	-	-	-	-
β -Selinene	1715	tr	-	-	0.5	0.2	0.4	-	-	-	-	-
α -Selinene	1727	-	-	tr	tr	tr	0.1	0.1	tr	0.1	tr	0,1
A-Murolene	1740	tr	tr	tr	0.4	0.2	-	0.2	tr	-	tr	0,3
Bicyclogermacrene	1754	tr	-	-	0.2	0.5	0.1	1.6	0.6	3.1	0,4	1,1
σ -Cadinene	1768	tr	tr	0.1	tr	tr	tr	0.1	0.1	tr	tr	0,1
σ -Cadinene	1773	tr	tr	-	tr	tr	tr	tr	tr	-	tr	0,1
γ -Cadinene	1777	tr	-	-	tr	tr	tr	tr	tr	tr	-	tr
<i>cis</i> -Calamenene	1839	tr	0.1	0.1	-	-	-	tr	tr	-	tr	tr
α -Calacorene	1938	-	-	-	-	-	-	tr	tr	-	tr	tr
Eremophilene	2137	0.1	0.2	0.1	0.5	0.6	0.4	0.2	0.3	0.7	0,3	0,2
β -Maaliene	2162	-	-	-	tr	tr	tr	-	tr	tr	-	-
α -Amorphene	1690	-	-	-	tr	-	-	-	-	-	-	-
Oxygenated Sesquiterpenes		1.6	2.2	1.4	10.1	9.7	11.1	3.6	7.6	12.8	5	4.1
Palustrol	1950	tr	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.2	0,1	0,1
Caryophyllene oxyde	2010	0.1	0.1	0.1	tr	0.1	0.4	0.2	0.2	0.3	0,2	0,1
Epiglobulol	2033	0.1	0.1	tr	1.3	0.9	1.1	0.1	0.5	0.7	0,3	0,1
Ledol	2053	tr	0.1	tr	0.2	0.2	0.2	0.1	0.2	0.3	0,1	0,1
<i>trans</i> -Nerolidol	2068	tr	-	-	tr	tr	0.1	-	tr	tr	tr	tr
Elemol	2075	0.1	0.2	0.1	0.4	0.4	0.3	0.2	0.3	0.6	0,2	0,2
β -Oplopenone	2087	-	-	-	tr	tr	tr	0.1	0.1	0.1	tr	0,1
Globulol	2097	0.5	0.6	0.3	5.7	4.5	5.3	0.9	3	4.7	2,1	1
Viridiflorol	2107	0.2	0.4	0.2	0.9	1	0.9	0.5	0.7	1.8	0,5	0,5
Guaiol	2129	0.1	0.1	0.1	0.5	0.5	0.5	0.2	0.3	0.6	0,2	0,2
Rosifoliol	2130	0.1	tr	-	-	-	-	-	-	-	-	-
Spathulenol	2145	0.2	0.3	0.2	0.4	1.1	1.1	0.5	0.8	1.3	0,5	0,6
<i>T</i> -Cadinol	2166	-	-	-	tr	tr	0.1	0.1	tr	0.2	tr	0,1

<i>T</i> -Muurolool	2183	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr
<i>γ</i> -Eudesmol	2190	-	-	-	tr	0.1	0.1	tr	0.1	0.1	0,1	0,1	
<i>σ</i> -Cadinol	2201	tr	tr	tr	tr	0.1	0.1	0.1	0.1	0.1	tr	0,1	
Agarospinol	2208	tr	tr	tr	tr	0.1	0.1	0.1	0.1	0.1	tr	0,1	
<i>α</i> -Cadinol	2220	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	
<i>α</i> -Eudesmol	2247	tr	0.1	tr	0.1	0.3	0.2	0.2	0.2	0.3	0,1	0,4	
Isospathulenol	2253	-	-	-	-	-	-	-	-	1.1	-	0,3	
<i>β</i> -Eudesmol	2258	0.1	0.1	0.1	0.1	0.1	0.5	0.3	0.5	-	0,4	0,1	
Farnesyl acetate	2271	-	-	-	tr	tr	tr	tr	0.1	-	-	tr	
Isobicyclgermacral	2336	tr	tr	tr	tr	tr	tr	0.1	tr	tr	-	0,1	
(<i>E,E</i>) Farnesol	2374	tr	tr	-	tr	-	0.1	tr	-	0.2	-	tr	
Esters		0.1	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.4	0.4	0.3	
Isobutyl isobutyrate	1091	-	-	-	-	-	-	-	-	-	-	tr	
4-Methyl-2- pentyl acetate	1109	-	-	-	tr	-	-	-	tr	-	-	tr	
Isoamyl acetate	1126	tr	tr	tr	-	-	-	-	tr	-	-	-	
Isoamyl isovalerate	1300	-	tr	0.1	-	-	-	0.2	0.1	tr	0,2	0,2	
Hexyl isobutyrate	1334	tr	0.1	tr	tr	-	tr	tr	tr	-	tr	tr	
Isoamyl valerate	1410	-	-	-	-	-	-	-	-	-	-	-	
Isoamyl hexanoate	1466	tr	-	-	-	-	-	-	tr	-	tr	-	
Amyl benzoate	1939	tr	tr	tr	tr	tr	tr	-	-	tr	-	-	
Benzyl pentanoate	1956	-	-	-	-	-	-	-	tr	-	-	-	
<i>β</i> -Phenylpropionate	2020	tr	0.1	tr	0.2	0.2	0.2	0.1	0.2	0.3	0,2	0,1	
Ketones		0.2	0.1	0.6	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.2	
6-Methyl-5-Hepten-2-one	1345	tr	-	-	tr	tr	-	tr	tr	-	tr	tr	
Nopinone	1591	-	-	-	-	-	-	-	-	-	-	-	
Cryptone	1690	tr	-	-	tr	tr	tr	0.1	0.1	tr	tr	tr	
4-Methyl aceophenone	1795	tr	tr	0.1	tr	-	tr	tr	tr	-	tr	tr	
<i>cis</i> -Jasmone	1964	0.1	0.1	0.1	-	-	-	tr	tr	-	tr	tr	
Jacksone	2289	0.1	tr	0.2	tr	tr	-	tr	-	-	-	tr	
Tasmanone	2305	tr	tr	0.2	-	-	-	tr	-	-	-	-	
Agglomerone	2390	-	-	-	-	-	-	tr	-	-	-	0,1	
Lateriticone	2394	tr	tr	-	-	tr	0.1	-	-	-	-	-	
Torquatone	2432	-	-	-	0.1	0.1	0.1	-	tr	0.1	tr	-	
Others		tr	tr	tr	tr	-	tr	tr	tr	-	tr	tr	
Hexanal	1095	tr	tr	tr	-	-	-	tr	-	-	-	tr	
<i>trans</i> -2-Hexenal	1226	tr	tr	tr	tr	-	tr	tr	tr	-	tr	tr	
Nonanal	1401	-	-	-	-	-	-	-	-	-	-	-	
Diphenyl oxyde	2004	-	-	-	tr	-	-	-	-	-	-	-	
<i>cis</i> -3-Hexenol	1389	-	-	-	tr	-	-	-	-	-	-	tr	
2-Nonanol	1523	-	-	-	tr	-	-	tr	-	-	-	tr	
O-cresol	1648	-	-	-	-	-	-	-	-	-	-	-	
Toluene	1063	-	-	tr	-	-	-	-	-	-	-	-	
Total identified		99.5	99.4	98.8	99.3	99.2	98.8	99.4	99	98.8	99.2	99.4

Figure 1 PCA of 24 compounds for the leaf essential oils of 3 *Eucalyptus* species. For the abbreviations of the *Eucalyptus* species (▲) the arboreta and the compounds (●), refer Tables 1 and 3 for more detail.

Figure 2 Dendrogram obtained by cluster analysis based on the Euclidean distances between groups of the leaf essential oils of 3 Tunisian *Eucalyptus* species. Components that characterize the major subgroups, considered as chemotypes, are indicated. JBA = Jbel abderrahman

Table 3 Percentage content of the 24 Compounds Selected for the Principal Component and the Hierarchical Cluster Analyses in the Essential Oils Extracted from the Leafs of 3 Eucalyptus species harvested from six arboreta

		<i>E. cinerea</i>			<i>E. astringens</i>			<i>E. sideroxylon</i>				
<i>Arboreta</i>		Souiniat	Zerniza	Pryor Choucha (Sejnane)	Mrifek	Korbous	Pryor Choucha (Sejnane)	Mrifek	Pryor Choucha (Sejnane)	Jbel Abderrahmane	Korbous	Souiniat
<i>Compound</i>	<i>Abbreviation</i>											
α -Pinene	α -pin	5.1±0.8 ^a	5.2±0.6 ^a	5.7±1.4 ^a	21.8±4.7 ^c	30.0±0.0 ^d	14.0±1.7 ^b	8.3±6.4 ^{ab}	2.9±1.4 ^a	2.5±0.0 ^a	2.1±0.0 ^a	5.1±4.0 ^a
Limonene	lim	4.3±0.8 ^c	3.8±0.0 ^c	3.3±0.0 ^{bc}	1.0±0.0 ^a	1.9±0.0 ^{ab}	0.6±0.0 ^a	3.9±0.5 ^c	1.7±0.7 ^a	1.0±0.0 ^a	1.5±0.0 ^{ab}	3.3±1.7 ^c
1.8-Cineole	1.8-cin	69.8±2.19 ^c	70.7±1.5 ^{cd}	71.8±0.0 ^{cd}	40.1±5.79 ^{ab}	39.1±0.0 ^a	47.6±1.2 ^b	66.7±9.1 ^c	71.2±3.6 ^{cd}	64.2±0.0 ^c	79.4±0.0 ^d	72.3±2.7 ^{cd}
γ -Terpinene	γ -terp	0.1±0.0 ^a	0.1±0.0 ^a	tr ^a	tr ^a	0.1±0.0 ^a	tr ^a	0.3±0.0 ^a	0.3±0.7 ^b	tr ^a	0.1±0.0 ^a	0.1±0.0 ^a
<i>p</i> -Cymene	<i>p</i> -cym	1.0±0.0 ^a	0.7±0.0 ^a	0.7±0.0 ^a	0.8±0.0 ^a	1.7±0.0 ^b	1.3±0.0 ^a	0.9±0.0 ^a	2.7±2.1 ^b	0.7±0.0 ^a	0.8±0.0 ^a	0.8±0.0 ^a
Pinocarvone	pin	0.2±0.0 ^a	0.3±0.0 ^a	0.4±0.7 ^{ab}	2.9±0.84 ^c	0.8±0.0 ^b	3.7±0.6 ^d	0.2±0.0 ^a	0.3±0.0 ^a	0.4±0.0 ^a	0.4±0.0 ^a	0.2±0.0 ^a
β -Carophyllene	β -car	0.7±0.0 ^b	0.6±0.6 ^{ab}	0.6±0.0 ^b	0.3±0.0 ^a	1.0±0.0 ^b	1.0±0.0 ^b	1.4±1.3 ^{bc}	0.8±0.0 ^b	1.9±0.0 ^c	0.4±0.0 ^a	1.0±0.0 ^b
Terpinene-4-ol	ter-4-ol	tr ^a	tr ^a	tr ^a	0.2±0.0 ^a	0.2±0.0 ^a	0.1±0.0 ^a	0.2±0.5 ^a	0.1±0.0 ^a	0.3±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
Aromadendrene	aro	0.2±0.0 ^a	0.2±0.0 ^a	0.1±0.0 ^a	4.9±2.1 ^c	3.8±0.0 ^{de}	3.8±0.0 ^{de}	0.4±0.5 ^a	1.8±0.0 ^{bc}	3.2±0.0 ^{cd}	1.2±0.0 ^{ab}	0.5±0.6 ^{ab}
<i>allo</i> -Aromadendrene	<i>allo</i> -aro	0.1±0.0 ^a	0.2±0.0 ^a	0.1±0.0 ^a	0.6±0.0 ^c	0.6±0.0 ^c	0.6±0.0 ^c	0.2±0.0 ^a	0.5±0.7 ^b	0.9±0.0 ^c	0.4±0.0 ^a	0.2±0.0 ^a
<i>tr</i> -Pinocarveol	<i>tr</i> -pin	0.7±0.5 ^a	0.9±0.0 ^a	0.9±1.4 ^a	10.0±2.5 ^c	3.7±0.0 ^b	9.3±1.2 ^c	1.0±0.5 ^a	1.3±0.0 ^a	1.8±0.0 ^{ab}	1.5±0.0 ^{ab}	1.1±0.5 ^a
α -Terpinyl acetate	α -ter acet	2.6±0.6 ^{cd}	2.1±0.6 ^{cd}	2.0±2.0 ^{bc}	tr ^a	- ^a	- ^a	2.9±0.8 ^d	0.9±0.0 ^b	0.7±0.0 ^b	0.9±0.0 ^b	2.8±1.0 ^d
α -Terpineol	α -ter	8.8±1.1 ^e	7.5±0.6 ^{de}	6.4±0.7 ^d	0.7±0.0 ^{ac}	0.9±0.0 ^{a-c}	0.5±0.6 ^a	2.1±0.8 ^c	0.8±0.7 ^{ab}	0.5±0.0 ^a	0.2±0.0 ^a	1.8±1.0 ^{bc}
Bicyclogermacene	tr ^a	tr ^a	- ^a	- ^a	0.2±0.2 ^a	0.5±0.0 ^a	0.1±0.0 ^a	1.6±1.7 ^a	0.6±0.7 ^a	3.1±0.0 ^b	0.4±0.0 ^a	1.1±1.4 ^a
trans- <i>p</i> -Mentha-1(7).8dien-2-ol	tr-p-men	0.7±0.5 ^b	0.8±0.0 ^b	0.9±0.0 ^b	0.4±0.0 ^a	0.2±0.0 ^a	0.4±0.0 ^a	0.2±0.0 ^a	0.8±0.0 ^b	0.8±0.0 ^b	1.0±0.0 ^b	0.4±0.5 ^a
cis- <i>p</i> -Mentha-1(7).8dien-2-ol	cis-p-men	0.7±0.5 ^b	0.9±0.0 ^b	0.9±0.0 ^b	0.3±0.0 ^a	0.2±0.0 ^a	0.4±0.0 ^a	0.3±0.0 ^a	0.8±0.0 ^b	0.8±0.0 ^b	0.9±0.0 ^b	0.4±0.0 ^a
Epiglobulol	epi	0.1±0.0 ^a	0.1±0.0 ^a	tr ^a	1.3±0.6 ^c	0.9±0.0 ^{bc}	1.1±0.0 ^{bc}	0.1±0.0 ^a	0.5±0.7 ^{ab}	0.7±0.0 ^{bc}	0.3±0.0 ^a	0.1±0.0 ^a
Globulol	glo	0.5±0.0 ^{ab}	0.6±0.0 ^{ab}	0.3±0.0 ^a	5.7±1.3 ^d	4.5±0.0 ^d	5.3±0.6 ^d	0.9±0.5 ^{ab}	3.0±0.0 ^c	4.7±0.0 ^d	2.1±0.0 ^{bc}	1.0±0.8 ^{ab}
Viridiflorol	vir	0.2±0.0 ^a	0.4±0.0 ^a	0.2±0.0 ^a	0.9±0.0 ^b	1.0±0.0 ^b	0.9±0.0 ^b	0.5±0.5 ^a	0.7±0.0 ^b	1.8±0.0 ^c	0.5±0.0 ^b	0.5±0.5 ^a
Guaoil	gua	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a	0.5±0.6 ^b	0.5±0.0 ^b	0.5±0.0 ^b	0.2±0.0 ^a	0.3±0.0 ^a	0.6±0.0 ^b	0.2±0.0 ^a	0.2±0.0 ^a
Eremophilene	ere	0.1±0.0 ^a	0.2±0.0 ^a	0.1±0.0 ^a	0.5±0.5 ^a	0.6±0.0 ^b	0.4±0.0 ^a	0.2±0.0 ^a	0.3±0.0 ^a	0.7±0.0 ^b	0.3±0.0 ^a	0.2±0.0 ^a
Spathulenol	spa	0.2±0.0 ^a	0.3±0.0 ^a	0.2±0.0 ^a	0.5±0.5 ^a	1.1±0.0 ^b	1.1±0.0 ^b	0.5±0.5	0.8±0.7 ^{ab}	1.3±0.0 ^b	0.5±0.0 ^a	0.6±0.6 ^{ab}
<i>iso</i> -Spathulenol	Iso-spa	-	-	-	-	-	-	-	-	1.1±0.0 ^b	-	0.3±0.5 ^a
β -Eudesmol	β -eud	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a	0.5±0.0 ^a	0.3±0.5 ^a	0.5±0.7 ^a	-	0.4±0.0 ^a	0.1±0.5 ^a

Value followed by the different letter in same vertical column is significantly different according to Duncan's multiple range test (P < 0.05)

3.3 Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

To evaluate the effect of the zone of the harvest (arboreta) on the essential oil chemical composition of the studied *Eucalyptus* species populations, 24 compounds with contents in the essential oils of minimum 0.5% in at least one species (Table 3), were selected for the PCA and the HCA. The contents of the selected oil components were significantly different between the species ($p < 0.05$). The PCA horizontal axis explained 43.04% of the total variance and the vertical axis a further 15.76% (Figure 1). The HCA (Figure 2) based on the Euclidean distances between groups indicated three groups of species (A, B, and C), identified by their essential oil chemotypes with a dissimilarity > 15 (Figure 2). Group C was further divided into two Subgroups (C1 and C2) within a dissimilarity > 5 .

Group A reduced to *E. sideroxylon* from Jbel Abderrahman arboreta, correlated positively with the two axes. It stands out forming a separate group in both HCA and PCA analysis. The essential oil of which was distinguished from the other provenances by the highest mean percentages in bicyclogermacrene ($3.1 \pm 0.0\%$), isospathulenol ($1.1 \pm 0.0\%$), globulol ($4.7 \pm 0.0\%$), viridiflorol ($1.8 \pm 0.0\%$), eremophilene ($0.7 \pm 0.0\%$), guaioil ($0.6 \pm 0.0\%$), spathulenol ($1.3 \pm 0.0\%$), *allo*-aromadendrene ($0.9 \pm 0.0\%$). It was also characterized by the lowest content of α -pinene ($2.5 \pm 0.0\%$) and by its relatively low percentage in *trans*-pinocarveol ($1.8 \pm 0.0\%$). In comparison with the others provenance, The 1,8-cineole content of this oil was relatively medium ($64.2 \pm 0.0\%$). It appears that the type of the soil such as sandy clay for hajeb laayoun arboreta had a significant influence on the biosynthesis of the chemical compound and could favour some and inhibit the synthesis of the others.

Group B is represented by oils of *E. astringens* from Korbous, Sejnane and Mrifek arboreta, correlating positively and negatively with both axes. They are characterized by the highest content in α -pinene (30.0 ± 0.0 , 14.0 ± 1.7 , $21.8 \pm 4.7\%$ respectively), pinocarvone (0.8 ± 0.0 , 3.7 ± 0.6 , 2.9 ± 0.84 ; respectively), *trans*-pinocarveol (3.7 ± 0.0 , 9.3 ± 1.2 , $10.0 \pm 2.5\%$ respectively), aromadendrene ($3.8 \pm 0.0\%$) for those from Korbous and Sejnane arboreta and $4.9 \pm 2.1\%$ for the provenance Mrifek arboreta, epiglobulol (0.9 ± 0.0 - $1.3 \pm 0.6\%$), globulol (4.5 ± 0.0 , 5.3 ± 0.6 , $5.7 \pm 1.3\%$; respectively), viridiflorol (0.9 - 1.0%). They are also characterized by the lowest mean percentages in 1,8-cineole (39.1 ± 0.0 , 47.6 ± 1.2 , $40.1 \pm 5.79\%$; respectively). The variation between these three sources was mainly due to the variation in the content of these compounds, such as α -pinene which distinguish essentially the provenance of Korbous and Sejnane, while the content of *trans*-pinocarveol, pinocarvone and 1,8-cineole was more abundant in oils obtained from Mrifek and Sejnane sources. *Sub-group C1*, formed by *E. sideroxylon* from Korbous and Sejnane, their essential oils are characterized by the highest content in 1,8-cineole (79.4 ± 0.0 ,

$71.2 \pm 3.6\%$; respectively) and by lowest mean percentage in α -pinene (2.5 ± 0.0 , $2.9 \pm 1.4\%$; respectively). However they are also characterized by a relatively high levels of *p*-cymene, *trans*-*p*-mentha-1 (7), 8dien-2-ol, *cis*-*p*-mentha-1 (7), 8dien-2-ol and β -eudesmol.

Subgroup C2 represented by oils of *E. sideroxylon* of Souiniat and Mrifek arboreta and by those of *E. cinerea* from Souiniat, Sejnane and Zerniza arboreta. The essential oils of which were characterized by mean percentages of 1,8-cineole relatively comparable to those of the sub-group C1 (66.7 ± 9.1 to $72.3 \pm 2.7\%$). They were characterized also by the highest content in α -pinene (5.1 ± 0.8 - $8.3 \pm 6.4\%$), limonene (3.3 ± 0.0 - $4.3 \pm 0.8\%$) and α -terpinyl acetate (2.0 ± 2.0 - $2.9 \pm 0.8\%$), while *E. cinerea* representing the second species of this subgroup was distinguished from the other by its highest mean percentage in α -terpineol (6.4 ± 0.7 to $8.8 \pm 1.1\%$).

Results of this study are in agreement with the findings of previous researches (Li et al., 1994; Li & Madden, 1995; Bignell et al., 1997; Della Porta et al., 1999; Tsiri et al., 2003; Marzouki et al., 2009), the chemical composition of the essential oils varied significantly within species and environmental conditions. Zrira et al. (1994) found that the mean percentage of 1,8-cineole of *E. sideroxylon* essential oil varied from 76.9% for those from Jbilel region to 80.9% for those obtained from Tekerkoust area. This variation was also proved within the essential oils of *E. astringens*, mainly in the mean percentages of the 1,8-cineole (59.3- 61.4%) and α -pinene (4.9-14.3%) for the provenances of Jbilel and Tekerkoust; respectively. Grbović et al. (2010) confirmed also this variation within some components of the essential oil of *E. camaldulensis*, such as *p*-cymene (17.38 – 28.60%) for the regions of Herceg Novi and Tivat; respectively, β -pinene (0.94, 11.48%) for Tivat and Sutomore; respectively, spathulenol (7.83, 14.15%) for Tivat and Herceg Novi; respectively and cryptone (4.97, 7.25%) for Kotor and Tivat regions; respectively.

Dunlop et al. (2003) have also demonstrated this variability within *E. Bicostata*, cultivated in 12 regions in Australia. They found that 1,8-cineole varied significantly from 44.0% for leaves collected from Mount area Bryan (B1) to 71.7% for those from Wittunga (B8) arboreta. It was the same for α -pinene (1.5 – 19.6%) for samples collected from Wittunga (B9) and Flinders Island (B3) arboreta, respectively and for aromadendrene (5.4-15.2%) for those obtained from Wittunga (B8) and Mount Bryan (B1) arboreta respectively.

Conclusions

The essential oils obtained from three *Eucalyptus* species acclimated in six arboreta were characterized. They varied significantly within species and within their origin of harvest. Four chemotypes were defined, with a great variability between them. *E. cinerea* grown in Pryor Choucha (Sejnane) arboreta was characterized by the highest mean oil yield,

whereas *E. sideroxylon* from Korbous and Sejnane arboreta contained the highest mean percentage in 1,8-cineole.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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