academicJournals

Vol. 8(12), pp. 504-512, 25 March, 2014
DOI: 10.5897/JMPR2014.5373
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Essential oil composition and biomass productivity of Moroccan endemic *Thymus satureioides* Coss. & Ball. growing in the Agoundis Valley

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Received 20 January, 2014; Accepted 14 March, 2014

***Thymus satureioides* Coss. & Ball. (*Th. satureioides*) is an endemic thyme of Morocco. Thirty two samples of wild *T. satureioides* were collected from High Atlas Mountains (Valley of Agoundis). The essential oils (EO) isolated by hydrodistillation from the aerial parts were analysed by gas chromatography-mass spectrometry (GC-MS). The yield of EO ranged from 0.2 to 2.3%. Twenty six components, representing more than 95% of the oil, were fully characterized. Borneol was the main constituent. Its proportion varied between 22.7 and 37.5%. Cluster analysis of the identified components grouped the samples into three main chemotypes, borneol/carvacrol (B/Ca), borneol/camphene/carvacrol (B/C/Ca) and borneol/camphene/*α*-pinene (B/C/P). Other constituents were identified in significant amounts: *α*-terpineol (3.1 to 10.6%), *3*-carene (1.5 to 10.5%) and *p*-cymene (2.3 to 8%). The fresh matter productivity average of *T. satureioides* was around 1.3 t/ha, revealing low potential of exploitation for collectors. This study has shown that the thyme growing in the valley of Agoundis is dominated by borneol chemotypes, showing different level of essential oil yield, according to locations and/or genotyes within general low potential of biomass production.**

Key words: *Thymus satureioides,* Agoundis Valley*,* matter productivity, GC-MS, essential oil, borneol, carvacrol.

INTRODUCTION

Thymus belongs to the family of Lamiaceae, subfamily Nepetoideae and tribe Mentheae. The number of species within this genus is estimated between 250 and 350 taxa (species and varieties) of wild growing plants (Morales, 2002; Lawrence and Tucker, 2002; Napoli et al., 2010). According to Jalas (1971), *Thymus* is divided into eight sections over the world. Thyme species are commonly used as herbal tea, flavouring agents (condiment and spice) and medicinal plants (Morales, 2002). The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular medicinal plants (Nickavar et al., 2005; De Lisi et al., 2011). In traditional

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medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, antitussive, expectorant and carminative as well as treating colds (Ghasemi, 2009; Rasooli and Mirmostafa, 2002). Thyme oils and in some cases ethnolic extraxt are widely used in pharmaceutical, cosmetic and perfume industry, but also for flavouring and preservation of several food products. Moreover, these species have dermatological benefits against acne and more recently, anti-platelet, antimicrobial, antispasmodic and antioxidant activities have also been demonstrated (Okazaki et al., 2002; Faleiro et al., 2003; Dob et al., 2006; Hazzit et al., 2009; Oh et al., 2009; Dandlen et al., 2010; Meister et al., 1999; Grosso et al., 2010; Giweli et al. 2013).

In Morocco, the *Thymus* species are widely distributed and found in several areas in Morocco (Jahandiez and Maire, 1934; El Habazi et al., 2006). The rate of endemism in the *Thymus* genus is 46.6%, representing 7 species (Fennane et al., 2007). Among these endemic species, *T. satureioides* is the widely natural growing thyme and thus the most exploited, known as the "Moroccan thyme" in worldwide. This is a perennial shrub, locally named "Azukni", in Berber and Zaiitra in Arabic (Bellakhdar, 1997). As for most thyme, this plant possesses culinary, aromatic and especially medicinal uses (Bellakhdar et al., 1991; Bellakhdar, 1997).

The composition of the essential oils (Eos) isolated from aerial parts of *T. satureioides* harvested from different regions of Morocco has been slightly investigated. Analysis of EOs revealed various compositions. However, in most reported papers, only few samples (one-three samples except one) have been investigated and the reported composition could not be considered as representative of the *T. satureioides* growing wild in the very vast regions of the country, estimated to thousands of hectares. Furthermore, the few studies undertaken in the past reported biological activity of the EO and the composition of little number of samples.

A study of one essential oil sample, originated from Beni Mellal region, has shown, respectively 52% of borneol, 3% of carvacrol, 27% of thymol and 0.33% of camphene (Jaafari et al., 2007). This study has also shown an antitumor and cytotoxicity activities. Furthermore, antimicrobial and antioxidant activities of EOs of *T. satureioides* were also investigated by Bouhdid et al. (2006) and Mayaud et al. (2008) in commercial samples from Pronarom (private company) for each, with 26.4 and 27.3% of borneol, respectively. Less antimicrobial effect was also demonstrated on *Escherchia coli*, *Staphylococcus aureus* and *Bacillus megaterium* using an essential oil sample with 31.2% of borneol and 27.4% of camphene content (Tantaoui-Elaraki et al., 1993). In contrast, two compositions from EOs samples collected from Marrakech region were dominated by borneol/carvacrol which represented 30 and 36%, respectively (Jaafari et al., 2007) and 21 and 27%, respectively (El Bouzidi et al., 2013).

Chemical variability of *T. satureioides* has been partly investigated by Benjilali et al. (1987a, b), who particularly studied different samples (32 samples) from two localities in Morocco: Western and Eastern region of High Atlas (HA) and the Rich region. These authors have reported two types of composition: the first is the most dominant, with borneol which content ranged between 26 to 78%, while the second showed important content of phenols: carvacrol or thymol (35 to 50%). Most studies undertaken on *T. satureioides* reported the presence at different proportion, of borneol or borneol/carvacrol or phenols (carvacrol and thymol) as shown in Table 1. Except Benjilali et al. (1987a, b), all the studies conducted have been realized on one sample, without any indication on the potential production of biomass.

In general, the exploitation of *T. satureioides* is realized by local population in the natural growing areas of the species. Collectors, generally women and children, start harvesting plants from May to July. There is no data on the available biomass nor the annual harvested quantities. The Agoundis Valley is considered as one of the richest region where *T. satureioides* is widely grown and where it is planned to develop with local population in a sustainable way to exploit thyme in the long term. The aim of the present work is to characterize the chemical composition of *T. satureioides* growing in this valley and the production capacity in term of exploitable biomass.

MATERIALS AND METHODES

Plant

Aerial parts 32 samples of *T. satureioides* Coss. & Ball, were collected at bloom stage on June and July, 2008, from 7 stations in the Agoundis Valley (Figure 1). The geographic coordinates (elevation, latitude and longitude) of each sample were recorded using a global positioning system (GPS) receiver (Table 2). Specimens of plants were taxonomically identified by Pr. Mohamed Ibn Tatou (Scientific Institute of Rabat, Department of Biology). Voucher specimens from each locality were kept at the Herbarium of National Institute of Agricultural Research (INRA, Rabat) and that of the Scientific Institute, under the index number RAB76330. The biomass was estimated using the method of squares. It consists of taking from a unit of space from 2 to 16 m^2, according to the visual estimation of plant density. Moreso the plant density was high and the surface of squares was small. The vegetative stage was noted and the biomass was determined by direct weighing of harvested plants from each square. A sample from each location was taken for hydrodistillation to determine the quantitative and qualitative extracted essential oil.

Essential oil extraction

Depending on the amount of the plant material available, the air-dried parts of the plants were cut into small pieces and submitted to hydro-distillation in a Clevenger-type apparatus from 2 to 3 h. The essential oil was then stored at +4°C in the dark until analysis. The essential oil yields were calculated from dry matter (weight of oil/weight of dry plant).

Table 1. Mains compounds (%) of *T. satureioides* essential oil reported in almost papers.

Sample origin	Number of sample studied	Composition (%)					References
		Borneol	Camphene	α-Pinene	Thymol	Carvacrol	
Imintanout	1	31.2	27.4	17.5	-	0.2	Tantaoui-Elaraki et al. (1993)
High Atlas and Rich	32	13 - 77.6	0.1 – 11.2	0.1-5.6	0 - 21.3	0.5 - 49.5	Benjilali et al. (1987a)
PRANAROM*	1	26.4	-	-	11.48	8.76	Bouhdid et al. (2006)
Asni moulay Brahim- Marrakech	1	30.03	0.58	-	0.94	35.9	Jaafari et al. (2007)
Bin Elwidane-Beni-Mellal	1	51.98	0.33	-	26.81	2.83	Jaafari et al. (2007)
Tiznit	1	59.37	2.44	-	0.78	1.24	Jaafari et al. (2007)
Pranarom*	1	27.31	-	-	13.54	2.21	Mayaud et al. (2008)
Unknown	1	29.5	-	-	-	9.1	Ichrak et al. (2011)
Idni (Haut Atlas)	1	21.1	8	4.6	1.2	26.5	El Bouzidi et al. (2013)

*Private International society.

Table 2. Collection data and productivities (biomass and EO yield) of *T. satureioides*.

Stations	Locations	Sample Code	GPS data			Biomass g/m²	EO (%)	Borneol	Carvacrol	Camphene	α-Pinene	α-Terpineol	p.Cymene	3-Carene	Linalool
			Longitude	Latitude	Altitude (m)										
1	Taghbart	1	N32°59'187"	W008°09'523"	1300	-	1.24	31.2	16.5	4.3	2.1	5	4.2	6.4	8
		30	N30°58'337"	W008°07'653"	1288	487.5	0.72	-	-	-	-	-	-	-	-
		8	N30°58'724"	W008°08'141"	1289	220.5	1.05	32.8	10.2	10.3	6.3	5	7.4	5.7	6
2	makhzen	2	N30°57'766"	W008°06'003"	1542	182.4	1.02	34.6	15.2	10.4	5.8	4.4	7.1	4.3	7.4
		3	N30°57'761"	W008°06'013"	1526	61.1	1.04	33.1	13.8	11.1	6	5	5.5	3.3	7.5
3	Makhzen to Ounain	4	N30°57'646"	W008°06'494"	1577	49.6	1.59	30.7	11.2	12.8	6.6	6.8	4.4	2.7	6.5
		5	N30°57'355"	W008°07'004"	1766	202.1	0.65	33.5	21.2	9.1	5	3.3	4.3	4.3	5.9
		6	N30°57'288"	W008°06'955"	1839	115.4	-	24.7	1.8	19.3	11.6	4.2	6.7	6.8	4.9
		18	N30°58'798"	W008°09'568"	1457	96.3	0.48	32.8	11.7	12.7	7.6	4.9	5	3.6	3.9
		19	N30°58'760"	W008°09'519"	1515	50	0.98	24.3	4.2	21	12.2	4.3	7.7	3.8	3.8
		20	N30°58'730"	W008°09'470"	1549	55	0.67	37.5	10.5	13	7.7	6.4	5.9	2.6	4.4
		21	N30°58'576"	W008°09'294"	1648	50	0.44	30.8	7.6	16.6	8.7	5.9	5.9	2.6	7.4
		22	N30°58'347"	W008°09'235"	1695	110	0.78	36.6	12.2	9.4	5.2	9.3	5.1	2.8	4.5
		23	N30°58'292"	W008°09'192"	1713	120	0.2	27.1	2.6	20.7	12	4.3	8	3.8	5.7
		31	N30°58'240"	W008°09'343"	1801	25	0.9	-	-	-	-	-	-	-	-
		24	N30°58'302"	W008°09'368"	1791	42.5	0.37	28.3	2.6	17	10.1	5.3	4	1.9	7
4	Wijdane	7	N30°56'389"	W008°07'526"	2181	518.4	1.47	25.4	9.5	12.6	6.6	8.2	2.3	3.1	4.3

Table 2. Contd.

		25	N30°58'469"	W008°09'437"	1742	100	0.66	29.6	4.6	17.6	9.7	9.5	5.4	2.9	3.9
		26	N30°56'204"	W008°07'517"	2162	61.3	0.7	30.1	3	19.5	10.4	9.1	4.8	1.5	6.5
		27	N30°56'170"	W008°07'709"	2146	37.5	0.86	24.3	3.4	19.7	10.7	8.1	7.1	2.9	7.6
5	Majjou	9	N30°56'124"	W008°04'827"	1718	55.6	0.97	26	7.5	15.6	8.7	4.8	7.6	5.4	4.6
		10	N30°56'416"	W008°03'846"	1882	20	1.67	28	15.6	7.2	4.5	3.2	6.9	10.5	5.6
		11	N30°57'057"	W008°56'800"	1643	144.4	0.38	30.2	7.8	14.5	8.8	8.3	4.9	3.6	4.6
		14	N30°55'132"	W008°06'744"	2013	62.5	1.47	26.9	9.6	12.37	7.02	10.6	3.4	5	6.7
6	Tanda n'Izm	12	N30°57'817"	W008°05'614"	1496	-	0.85	28.7	10.7	12.2	7.4	3.9	7.3	7.6	5.9
		13	N30°56'193"	W00807'701"	2146	62.5	1.4	27	8.8	13.9	7.7	9.2	4.5	5	5.8
		32	N30°55'373"	W008°06'031"	2097	125	-	-	-	-	-	-	-	-	-
		15	N30°56'054"	W008°07'005"	1964	250	2.29	25.3	11.8	12.7	7.5	5.6	6.4	10.1	4
		16	N30°56'129"	W008°06'662"	1942	225	0.9	34.5	13.4	12	6.2	7.3	4.3	3.7	4.5
		17	N30°58'599"	W008°05'727"	1528	125	0.81	22.7	20.7	7.2	4.1	5	7.6	-	5.8
7	Tijghicht	28	N30°58'297"	W008°05'895"	1485	-	-	26.1	10.8	14.9	8.4	5.5	7.8	3.2	6.6
8	CADEFA*	29	-	-	-	-	-	36	9.9	10.3	5.4	9.9	3.5	1.8	7.6

*CADEFA: Cooperative of Agoundis for Development Forestry and agriculture.

GC-MS analysis

GC-MS analysis of the essential oil was performed on a TRACE GC ULTRA equipped with non-polar VB5 (5% phenyl; 95% methylpolisyloxane) capillary column (30 m × 0.25 mm × 0.25 µm film thickness), directly coupled to a mass spectrometer (Polaris Q) (EI 70 eV). The temperature of the injector and detector was set at 250 and 300°C, respectively. The oven temperature was programmed from 60 to 200°C at 2°C/min, and then from 200 to 300°C at 20°C/min. Carrier gas was Helium (He) with a flow rate of 1 ml/min. The components of the oil were identified by comparison of their mass spectra with those in the Wiley-NIST 7th edition library of mass spectral data. The percentage composition of the oil sample was calculated from GC-MS peak areas.

Statistical analysis

Samples 30, 31 and 32 are excluded from statistical analysis due to the absence of the chemical analysis data. All data were collected and statistically analysed using the XLSTAT software package trial version in order to determine the relationship between the different samples of thyme using the percentage composition of their essential oils. Euclidean distance was selected as a measure of similarity and the Unweighted Pair-Group Method with Arithmetic average (UPGMA) was used for cluster analysis. These analyses will aim to identify groups and possibly recognize chemotypes.

RESULTS

EO yield and vegetal matter productivity

EO yield ranged from 0.20 to 2.29% (Table 2). The highest yield (2.29%) was found in Tnada N'Ism region (altitude 1964 m). Lowest levels are found in station 3, ranging from 0.2 to 1.59% (Table 2), while high values (≥ 1%) have been recorded in different locations (samples: 1, 2, 3, 4, 7, 8, 10, 13, 14 and 15). In Makhzen location, all samples showed similar yields, ranging from 1.02 to 1.04%. In general, the essential oil yield in the valley could be considered as 1%, except some area (Makhzen to Ounain) (Table 3). The biomass ranged from 20 to 518.4 g/m² of green matter. Wijdane, Taghbart and Tnada N'Izm was the region where the most high biomass average values were recorded, while in the Makhzen to Ounain and Majjou locations we obtain the lowest yield (Table 3).

The highest average value was recorded in Taghbart (354 g/m² ± 133.5). The lowest value was noted in Majjou with an average 70.63 g/m² ± 26.3.

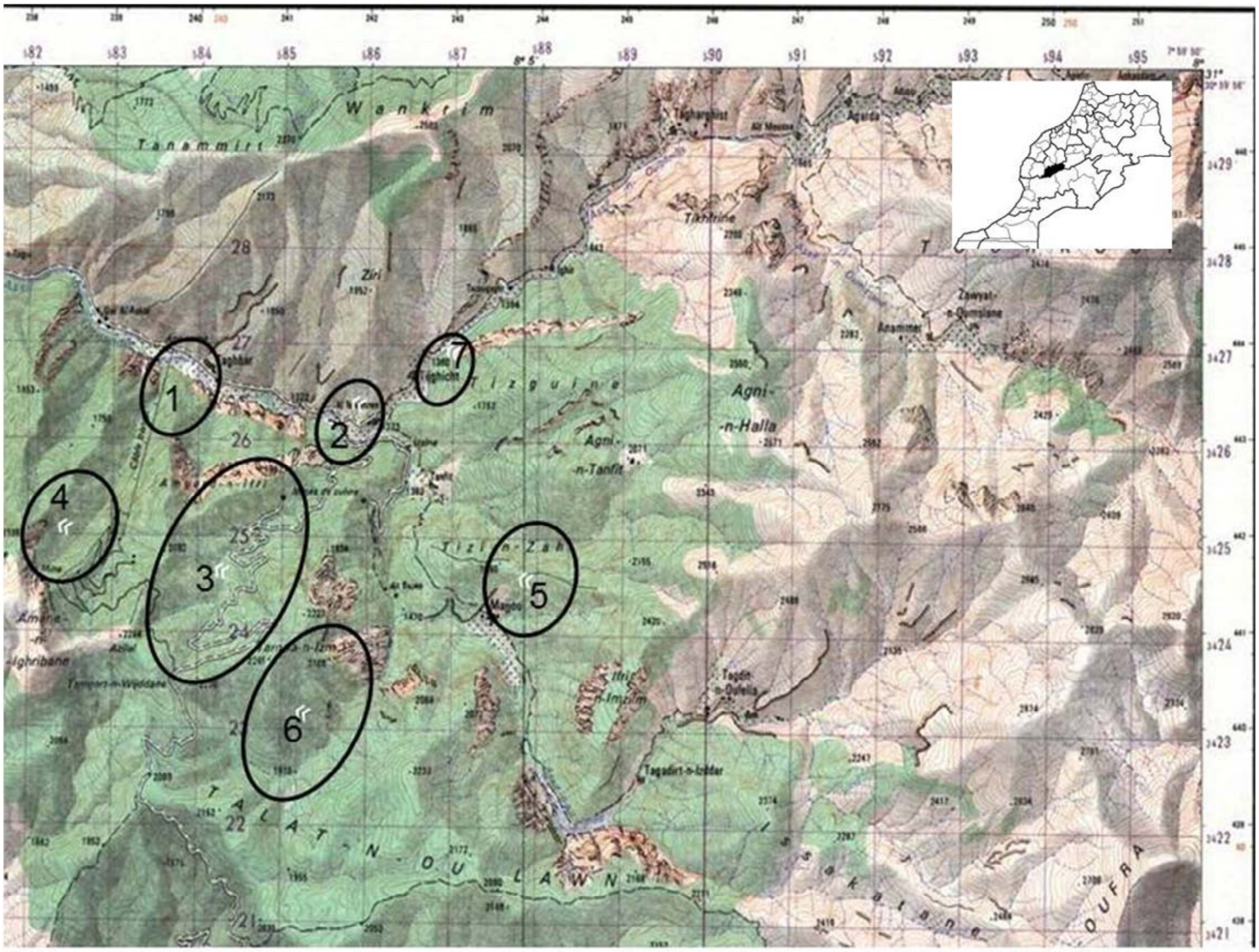

Figure 1. Investigated stations of *Th. satureioides.*

Table 3. Average of essential oil yield, biomass and borneol content production by region in the Agoundis Valley.

Locations	EO (%)	Biomass (g/m²)	Borneol content (%)
Taghbart	1.00±0.15	354.0±133.50	31.20±0.80
Makhzen	1.03±0.01	121.75±60.65	33.85±0.75
Makhzen to Ounanin	0.70±0.12	83.26±15.55	30.63±1.45
Wijdane	0.92±0.19	179.30±113.80	27.35±1.46
Majjou	1.13±0.29	70.63±26.30	27.78±0.91
Tanda n'Izm	1.25±0.28	157.50±34.82	27.64±1.98

*Mean ± Standard error of mean (stderr).

Chemical composition

Twenty six components representing more than 95% of the total detected constituents were identified. The predominant compound was borneol, ranging between 22.67 and 37.47%. Other constituents were identified with significant amounts, such as camphene which varied from 4.34 to 21.01%, followed by carvacrol 1.76 and 21.21%. *α*-Pinene ranged between 2.04 to 12.15%, *α*-terpineol between 3.13 to 10.56%, *3*-carene between 1.45 to 10.51% and *p*-cymene between 2.25 to 8%. The average, minimal and maximal compound's proportions of the EOs are summarised in Table 4.

Cluster analysis of the identified components grouped the samples into three main chemotypes: borneol/ carvacrol (B/Ca), borneol/camphene/carvacrol (B/C/Ca), borneol/camphene/α-pinene (B/C/P) (Figure 3). Borneol was by far the common major compound in all the three classes. The first class B/Ca (samples: 1, 5, 10, 12, 15 and 17) was characterized by carvacrol with a significant content (10.43 to 21.21%). On the other hand, camphene and α-pinene presented less significant proportions than carvacrol, with average 8.78% for camphene and 5.09% for α-pinene. The second group - B/C/Ca (samples: 2, 3, 4, 7, 8, 9, 11, 13, 14, 16, 18, 20, 21, 22, 28 and 29) has camphene and carvacrol as main compounds after borneol with significant amount varying between 9.39 to 16.21% for camphene and from 7.36 to 15.2% for carvacrol. α-Pinene in this class presented less proportions with an average of 7.15%. The last chemotype - B/C/P (Samples: 6, 19, 23, 24, 25, 26, and 27) was characterized by camphene and α-pinene as major compounds after borneol: 10.28 to 21.01% and 5.35 to 12%, respectively. Carvacrol was present but in very less amount, with average 3.86%.

DISCUSSION

The essential oil yields of the aerial parts of *T. satureioides* varied from 0.20 to 2.29%. This value is similar to that reported previously (Jamali et al., 2012; El Bouzidi et al., 2013). Generally, in the Valley Agoundis, yield of essential oil could be considered as 1%, except areas (Makhzen to Ounain, Table 3) where plants are very old due to the difficult conditions for regeneration (more drought). There is a slight relation between the altitude and the EO yield, as shown in Figure 2B. But according to the observed results in different locations, other factors could explain the variability concerning EO production, among which genetic variation or environment effect (soil fertility, exposition, etc.) have an important role. This result is in contrast with that reported by Vokou et al. (1993), where evidence concerning the increase of EO with altitude was demonstrated. In our case, the valley is already situated in high altitude.

Concerning the biomass productivity, the regions Taghbart, Wijdane and Tanda n'Izm are characterized by favorable vegetative production (deep soils and good exposition to the north side) while in the regions Makhzen, Majjou and Makhzen to Ounanin, soils are generally more shallow and frequently exposed to South side, making the environment conditions much drier. There is however no relation with altitude and biomass production (Figure 2A). Other factors could explain the important variation of biomass. Local population starts cutting thyme from May to July, without any consideration to the survival of the species. In most case we observed that the plants are very old and plant regeneration from seed is compromised, since no plants are kept in the field to set seeds for regenerating new seed stock.

Table 4. Chemical composition of EO extracted from 29 samples of *Th. satureioides* growing in the Agoundis Valley.

Compounds[a)]	Mean[b)]	SD[c)]	Min[d)]	Max[e)]
α-Thujene	1.18	0.18	0.93	1.54
α-Pinene	7.58	2.47	2.04	12.15
Camphene	13.44	4.25	4.34	21.01
Sabinene	1.94	0.52	1.14	3.06
β-Pinene	2.46	0.14	2.34	2.61
Myrcene	1.29	0.00	1.29	1.29
Geraniol formate	1.19	0.21	0.83	1.62
α-Terpinene	1.37	0.19	1.18	1.63
p-Cymene	5.69	1.58	2.25	8.00
Limonene	1.55	0.51	1.03	3.59
3-Carene	4.31	2.26	1.45	10.51
Linalool	5.78	1.28	3.89	7.91
Camphor	2.85	1.02	2.12	4.33
Borneol	29.61	4.10	22.67	37.47
Terpinen-4-ol	2.72	0.41	1.88	3.44
α-terpineol	7.00	2.18	3.13	10.56
trans-Dihydrocarvone	1.21	0.00	1.21	1.21
cis-Dihydrocarvone	4.30	0.00	4.30	4.30
Thymol methyl ether	2.41	1.23	1.05	4.81
Isobornyl formate	4.05	0.81	3.47	4.62
Bornyl acetate	3.15	1.44	1.23	7.09
Thymol	2.84	1.37	1.14	5.31
Carvacrol	9.83	5.16	1.76	21.21
trans-Caryophyllene	3.90	1.24	2.03	7.02
Caryophyllene oxide	1.44	0.00	1.44	1.44
α-farnesene	2.22	0.00	2.22	2.22

a) Compounds are listed in order of their elution from a VB-5 column; b) Mean: Mean value; c) SD: Standard deviation; d) Min: Lowest content; e) Max: Highest content.

The borneol content in most samples are less dosed than those reported in other regions of Morocco by Benjilali et al. (1987a, b), which reached in some cases, 77.6% of borneol. The samples combining borneol and carvacrol in balanced proportion are also interesting, such as the sample number 5, collected in Makhzen station, where borneol is 33.5% and carvacrol is 21.2%. Two authors have found the co-dominance of borneol and carvacrol (Jaafari et al., 2007) (30.03 and 35.9%) and El Bouzidi et al. (2013) (21.1 and 26.5%), respectively. Borneol was by far the major constituent of all samples, indicating the absence of any chemical polymorphism in the Agoundis Valley. The three classes observed showed however some variation, mainly in carvacrol, camphene and α-pinene. The dominance of borneol in the essential oils at reasonable part (30%) confers to *T. satureioides* collected from the Agoundis Valley, a good economic value. However, local population should take care on its use, both for medicine use and flavouring foods, due to the presence of borneol and camphene recognized as having harmful effects due to exposure or consumption.

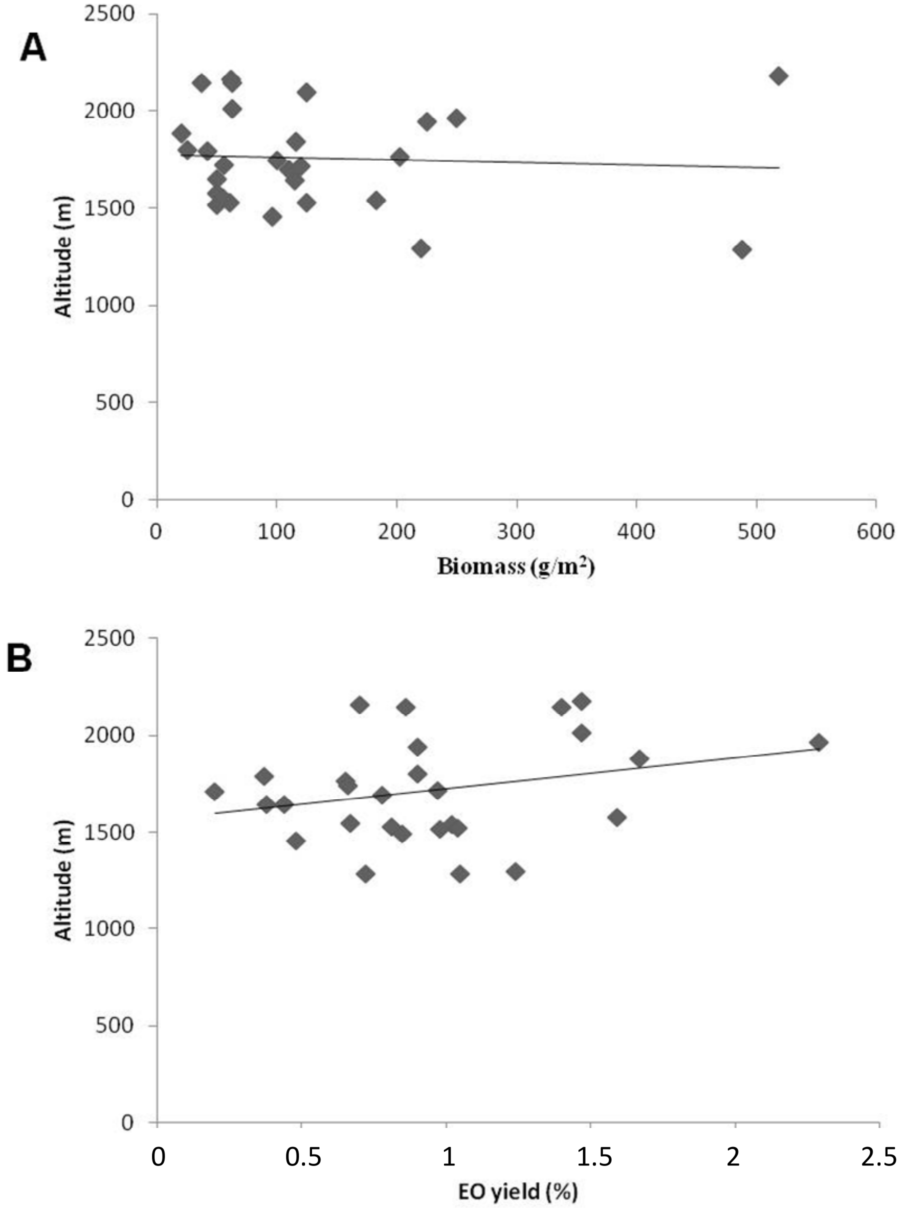

Figure 2. Variation of *Th. satureioides* biomass production (A) and EO yield (B) with the altitude.

Conclusion

Local population starts cutting thyme from May to July, without any consideration to the survival of the species. In order to improve this situation, some recommendations have been made to the local population organized under the cooperative of medicinal and aromatic plant exploitation. These recommendations include both some good practices for *T. satureioides* harvesting and sustainable way for medicinal and aromatic plant in general. Emphasis is put on the following actions: Avoid cutting thymus from the same place each year. That means exploited fields should be as bi-annual rotation, the rest area has to be respected as it is the case for pasture grazing. This is important to give more chance to the plants to regenerate from seeds or to increase ramifications of old plants.

In the exploited area, collectors should let some plants to set seeds so the seed bank in the soil is constituted. It is also deeply recommended to avoid cutting the very young plants, which are having superficial roots and few ramifications. In the rest area, it is important to install hives of bees, to improve cross pollination and therefore seed production. Finally, it is also recommended to

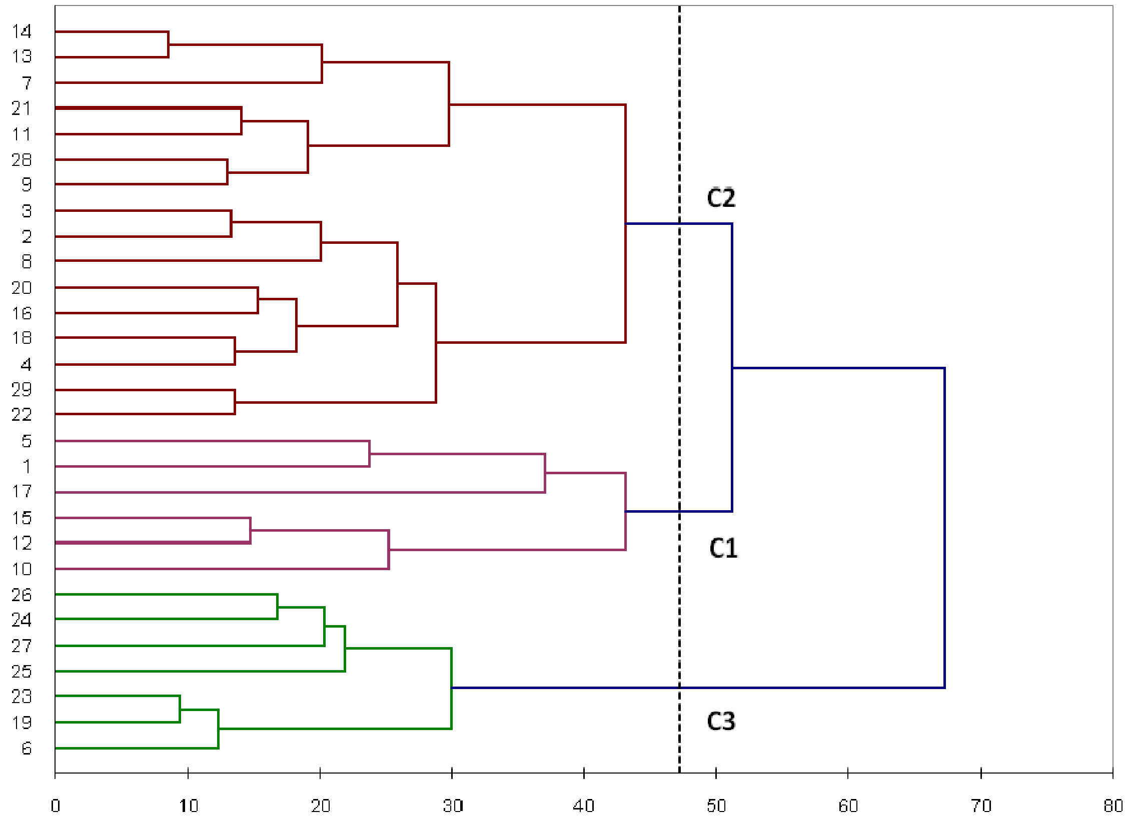

Figure 3. Dendrogram obtained by cluster analysis of the percentage composition of EOs.

replant thyme in the degraded areas, by sowing seeds collected from some selected plants and grown in the nurseries during the first weeks after autumn.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

This work was supported by a financial support from the GIZ. We are very grateful to Pr. Mohamed Ibn Tatou (Scientific Institute of Rabat, Department of Biology) for the botanical identification of the *T. satureioides*. Our thanks are due to the members of the association CADEFA for their help during the sampling and the HCEFLCD services for their collaboration.

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