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Chemical variability and acaricidal activity of *Rosmarinus officinalis* L. essential oils

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Abstract

Rosemary leaves are characterized by two main periods of essential oils production and contain high levels of oils (2.11 to 3.13%). Their oils, extracted by hydrodistillation and analyzed by GC-FID and GC-MS, are dominated by oxygenated and hydrocarbons monoterpens and contain high amounts of 1,8-cineole. The chemical composition didn't vary drastically during the phenologic stages, but a noticeable variability is however found within the ten studied populations. PCA and regression analysis revealed that among factors regulating the biosynthesis of oils during the different growth stages of the plant, the mean monthly temperature and monthly precipitation would be determinant. In hives bioassays, the use of absorbent paper pad impregnated by pure rosemary oil for six days showed certain effectiveness against Varroa mites. So, an application rate of 5ml of rosemary oil caused the highest mites mortalities, but the concentration of 3ml that producing an equivalent toxicity to flumetrin, is recommended. To overcome the phenomenon of resistance to chemicals widely used in apiaries infested by Varroa destructor, the use of pure rosemary oils as biopesticide for controlling acarian pests would be an appreciated tool.

Keywords: *Rosmarinus officinalis; essential oil; GC-MS; phenological stage; Varroa destructor; biopesticide.*

1.Introduction

Rosemary, *Rosmarinus officinalis* L., an evergreen Mediterranean shrub, is known since the old civilizations for its beneficial properties in gastronomy, medicine and cosmetics [1]. Very oldly used for its antimicrobial, antioxidant, tonic and stomachic effects of its active oil, rosemary was known to prevent disease and promote health properties [2]. This is one of Moroccan main aromatic and medicinal plants appreciated for its essential oils (EOs) and heavily used by beekeepers for honey production [3]. The major compounds present in rosemary EOs were 1,8-cineole, camphor, verbenone, borneol, α-pinene and myrcene [4]. The levels of these compounds depend on many factors, such as the variety, growth conditions, harvesting time, soil properties, climate, origin, drying conditions, extraction and analysis methods [5]. Rosemary is considered as a very promising source of Bioinsecticides (BIs) production. There is a great potential of EOs production in wild and cultivated areas of rosemary from several countries including Morocco [3; 6]. So, it is necessary to master the quality of these essential oils for a convenient industrial production. In practice, EOs are used in the mixture of several BIs against mosquitoes, ticks, gnats, flies, moths and other flying insects, and to kill exposed eggs, larvae and adult stages of garden insects and mites [7]. Manufacturers utilize the synergistic action of EOs, composed of complex mixtures of several active substances to enhance the insecticidal action and to overcome the resistance phenomenon [8]. This phenomenon has been a subject of research in recent years indicating great prospects for EOs as active ingredients in the production of botanical pesticides [9-11]. This is an advantage over synthetic products, as it is difficult for the insects to develop resistance. In addition to that EOs are recognized safe to use as they pose lower health hazards to humans [12]. Many studies confirmed the bio-insecticidal activity of rosemary oils at laboratory level [13-14]. The previous works on rosemary oils, collected from several areas in Morocco, did not cover the entire vegetative cycle of plant and also environmental, bioclimatic, geographic and orographic parameters were not previously taken into consideration [3; 15-16]. Moreover, in apiaries, *Varroa destructor* Anderson & Trueman became the main dangerous pest of the honeybee (*Apis mellifera* L.) that driving massive uses of chemical insecticides, which are toxic and persistent in honey production [17]. So, the aim of this study is *i*) to assess the influence of bioclimatic and biogeographic factors on the essential oil composition of Moroccan rosemary collected during the pre-flowering stage (in June) within ten wild populations originated from three biogeographic areas (Eastern Rif Mountains, Horst Mountain Chain, and High Lands of Eastern region of Morocco); *ii*) to evaluate the chemical variability of rosemary EOs in the experimental design installed in a protected grazing area in Debdou forest (Horst Mountain Chain) during a complete vegetative cycle; and *iii*) to evaluate, in apiary, the acaricidal activity against Varroa mites using pure rosemary oil applied at different concentrations in prospect to use this oil in bioinsecticides development.

2. Materials and methods

2.1. Plant materials, extraction and chemical analysis of essential oils

To assess the effect of environmental factors on the EOs composition, a set of bioclimatic data were provided by the metrological station near the plot design of Debdou during the period of collect where the phenological stages of rosemary clumps were recorded during a complete vegetative cycle and analyzed. From this design, a total of 108 samples of fresh aerial parts of plant were collected from September 2012 to August 2013. Additional three samples were collected during June 2014 from each of the ten wild populations prospected across the Eastern parts of Morocco where this species still well productive. Prospected rosemary populations grow under different altitude, longitude, latitude and vegetal covers conditions (Table 1).

Table 1. Some characteristics of rosemary studied populations

Population	Code	Alt. (m)	Lat. (DD)	Long. (DD)	Stand type	Orographic area	Province	Bioclimate	P (mm)
Talsint 1	Tl1	1467	32.7125N	3.6862W	Steppe (St, Ro)	HP	Figuig	A	150-220
Talsint 2	Tl2	1585	32.6424N	3.6942W	Matorral (Jo, Ar, Ro, St, Ah)				
Aîn Zohra	Az	1406	34.5922N	3.5608W	Forest (Qr, Ro, Jo, Pl, Ci)	ERM	Nador	SA	364
Jerada	Je	1192	34.3320N	2.2431W	Matorral (Jo, Ci, Ro)	HMC	Jerada	A-SA	250-350
Guefaît	Gu	836	34.2493N	2.3705W	Steppe (St, Ro, Zl, Ar, Ph)				
El Aouam	Ea	1270	33.9791N	2.8016W	Forest (Qr, Ro, Jo, St)		Taourirt	SA-SH	400-600
Dada Ali	Da	1514	34.3151N	2.5632W	Forest (Qr, Ro, Jo, St)				
El Fachat	Ef	1545	33.9852N	2.8890W	Forest (Qr, Ro, Jo, St)				
Sidi Lahcen	Sl	1040	34.1020N	2.6618W	Matorral (Qr, Ro, Jo, Pl, Ci)			A-SA	300-400
Debdou	De	765	34.0673N	2.9848W	Matorral (Ta, Ro, Zl, Ol)				
Experimental Design	ED	810-865	34.0608 to 34.0630N	2.9672 to 2.9690W	Matorral (Ta, Ro, Zl, Ol)				

Alt. Altitude, **Lat.** Latitude, **Long.** Longitude; **HP.** High Plateaux, **HMC.** Horst Mountain Chain, **ER.** Eastern Rif Mountain; **A.** arid, **SA.** semi-arid, **SH.** sub-humid, **P.** average of yearly precipitation (mm); **Ah.** *Atriplex halimus*, **Ar.** *Artemisia herba-alba*, **Ci.** *Cistus sp.*, **Jo.** *Juniperus oxycedrus*, **Ol.** *Olea oleaster*, **Ph.** *Peganum harmala*, **Pl.** *Pistacia lentiscus*, **Qr.** *Quercus rotundifolia*, **Ro.** *Rosmarinus officinalis*, **St.** *Stipa tenacissima*, **Ta.** *Tetraclinis articulata*, **Zl.** *Zizyphus lotus*. In laboratory, to extract rosemary oils, samples of leaf biomass were subjected to hydrodistillation by a Clevenger type apparatus for 3 hours [18]. EOs yield was reported as volume (ml) of oil per 100 g of plant dry matter. Collected oil were dried and stored in dark vials at 4°C. One micro-liter (1µl) samples of EOs were subjected to analysis by Gas Chromatography (GC-FID) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). GC-FID analyses were performed using a Hewlett-Packard (HP 6890) equipped with a capillary column HP-5 (30m x 0.25mm x 0.25µm film thickness), and a detector FID at 250 °C, carried gas was nitrogen at a flow rate of 2 ml/min and split was the injection mode. The column temperature was programmed from 50 to 250°C at 4°C/min. The GC-MS analyses were performed on a Hewlett-Packard (HP 6890) equipped with an automatic injector (HP 7683) and coupled with a HP 5973 mass spectrometer (Agilent technologies) with electron impact ionization (70 eV) and fitted with HP-5MS capillary column (50 m x 0.32 mm; 0.25µm film thickness). Oven temperature was programmed to rise from 50 to 250°C at a rate of 4°C/min, using helium as carrier gas with a flow rate set to 2 ml/min. The identification of EOs components was achieved by comparison of their retention indices (RI) relative to (C8-C22) n-alkanes with those of known compounds, and by comparison of mass spectra using Wiley/NBS mass spectral library of the GC-MS

data system and other published mass spectra [19]. The percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

2.2. Evaluation of the essential oils fumigant effect against Varroa mite

The fieldwork took place in January 2017 at an apiary in Sidi Yahya Zaer (30 km south of Rabat, Morocco) (33°45'36" N, 6°31'48" W). Colonies (N=21) of *Apis mellifera* bees were kept in dandant-Blatt hives, with 4-6 combs and large-size pollen drawers. They have not been treated at all during the preceding 12 months and were naturally infested by *V. destructor*. During treatment, the average temperature and relative moisture are ranged from 17°C to 24°C and 64 to 72% respectively. In field test, 5 rosemary oil treatments applied at five application rates (1, 2, 3, 4 and 5 ml) compared to Bayvarol (2 strips per hive: one impregnated polymer strip contains 3.6 mg flumethrin- BAYER product) were used to test their efficacy against Varroa mite. They were replicated three times and randomly assigned to bee colonies. Three hives left untreated as blank control. Daily mite fall was monitored before and after the treatment periods to give an indicative efficacy by using a thick white paper sheets lubricated with margarine and placed on the bottom board beneath the frames. The number of naturally fallen mites was estimated before treatment. In each treatment, one strip of absorbent paper pad (2x10 cm2) was soaked with pure oil and placed directly at the outer frames of each hive. Observations were taken after one, two and six days by counting the fallen dead mite on white sheets which were changed every time. The efficacies of treatments were calculated using the following formula [11-12]:

$$Er = \frac{Mt \times 100}{Mt + Mn} \qquad (1)$$

- *Er*: Efficacy rate
- *Mt*: Mites-fall per day after treatment
- *Mn*: Natural Mites-fall per day before treatment

2.3 Data analysis

The significance of the variation in essential oil composition among populations, depending on harvest period, as well as the evaluation of the efficacy data observed in field bioassays were determined were determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p < 0.05$ level. Data in percent was transformed using arcsine [20]. The differentiations among populations or according to harvest period based on their essential oil composition were performed by principal component analysis (PCA). All the variables were standardized for a normalized PCA. PCA and the correlation between yields or essential oil compounds and environmental factors were made according procedures described by Jambu [21]. Statistical analyses were carried out with Statistica 13.2 (Statsof) software.

3. Results and Discussions

3.1. Essential oils yields

Rosemary EOs content ranged from 1.7 to 2.8% according to growing area. The lowest oil yield was recorded in plants from Sidi Lehcen site and the highest in plants from Talsint (Table 2). Also, monthly changes in oils content during full growing cycle were registered in the experimental design. So, two phases of oils production can be distinguished: the first one, corresponding to a relatively low production (2.11-2.52%) which runs from September to May (pre and flowering stages), and the second one corresponding to a relatively high oil production (2.53-3.13%) which runs from June to August. Several factors can influence the evolution of the production of EOs such as environmental conditions in which the plant grows [22-24]. Regression analysis revealed a high degree of collinearity

between yield and bioclimatic factors especially monthly precipitation and mean monthly temperature ranges (Figure 1). During stages of plant growth, climatic conditions exert plants to change in their physiology in order to prevent damage and adapt to stress conditions as underlined by Edreva et al. [25]. So under stress conditions from June to August (Post-flowering stage), where precipitation was below 7 mm/month and mean monthly temperature was up to 20 °C, plant biosynthetic carbon could be allocated on terpene biosynthesis rather than to growth, and consequently the content of EOs was enhanced in the samples of rosemary harvested during summer in the Eastern Region of Morocco. Similar findings were previously reported for *Salvia officinalis* [26], *Thymus satureioides* and *Origanum elongatum* [11]. EOs content in the rosemary sampled populations exhibited a significant dependence to some abiotic factors especially latitude and longitude (Figure 1). Altitude haven't displayed however statistically significant correlation with yield as conversely reported by Sotomayor et al. [22] for rosemary populations originated from Murcia, showing a positive statistical correlation between this parameter and yields. Although there was no significance, the same trend was reported for the samples taken from Ta1, Ta2 and Ef areas which are the most altitudinal (>1467 m) and for which the highest oil contents were noticed.

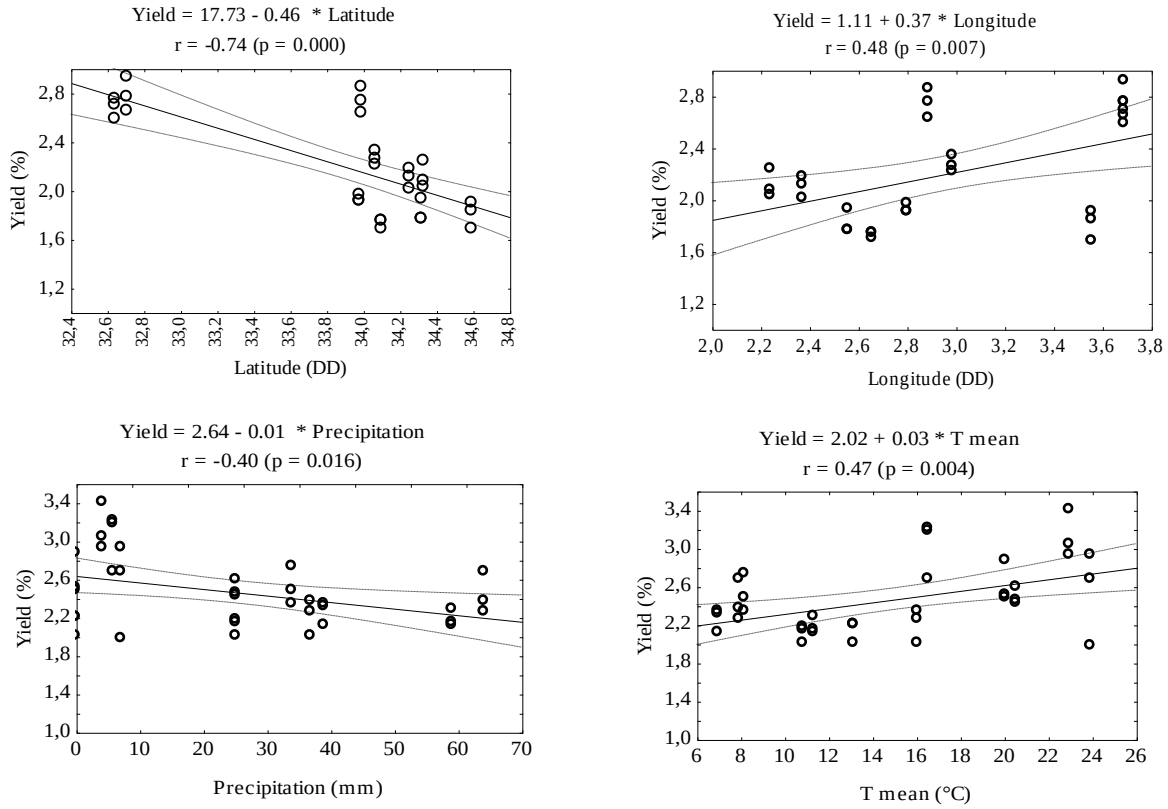

Figure 1. Relationship between abiotic factors and yield of *R. Officinalis* EOs in the Eastern Region of Morocco.

According to origin, Lakusic et al. [23] and Jordán et al. [24] noticed large differences in oil content of rosemary collected from populations belonging to different bioclimatic areas of the Iberian and Balkan Peninsulas. Geographic position is usually associated with changes in environmental factors, such as temperature, precipitation, humidity, and wind exposure, which influence the biosynthesis of EO in the plant [27]. According to phenological stages, controversial results can be found in the literature for rosemary EOs content. For example, in north India, no considerable variation was observed in the rosemary oil content during the year [28]. In contrast, results reported by Jordán et al. [29] and Singh and Guleria [6] are in agreement with ours. So, rosemary produces more oil during the post-flowering stage (Table 2). Contrariwise, for Tunisian rosemary, the highest oil amounts were registered during the flowering stage [30].

3.2 Essential oils composition

The chromatographic analysis allowed the identification of 35 volatile components (Table 2), of which fourteen main constituents accounting over 92 % of the oil. All rosemary EOs harvested in the Eastern Region of Morocco were dominated by oxygenated and hydrocarbons monoterpens and 1,8-cineole was the most abundant compound (42%-54.8%), followed by camphor (10.4%-21.1%), borneol (3.4%-11.7%), α-pinene (3.6%-10.7%), β-pinene (2.6%-6.6%), α-terpineol (3.6-5.5%), camphene (1.8%-3.7%), β–caryophyllene (0.6%-2.6%), bornyl acetate (0.2%-2.3%), terpinen-4-ol (1.1%-2%), p-cymene (0.4%-1.4%), linalool (0.8%-1.5%), myrcene (0.8%-1.4%) and caryophyllene oxide (0.2%-1.2%). The other compounds in the rosemary oil are mostly monoterpenes, but their concentrations are very low. Similar chemical profiles have also been noticed for rosemary EOs in previous studies [3-4; 22-24; 28-30]. Except myrcene, linalool, camphor, borneol, α-terpineol and other minor compounds (Table 2) showing minimal changes in concentration during the year, most of the components were found to be significantly affected by geographic origin and by harvest period. Also, no significant variation was found in both hydrocarbons and oxygenated monoterpenes levels as revealed by statistical analysis. Contrary, sesquiterpenes which varied from 1 to 4.8% were significantly influenced by the phenologic stage of the plant and by geographic origin. It seems that change in the concentration of some compounds would be compensated by change (decrease or increase) in the amounts of other components. These changes may occur in response to some physiological processes related to bioclimatic and biogeographic conditions [5; 23; 27].

3.3 Variability of essential oil composition among populations

The global variation among population, evaluated by the Principal Component Analysis (PCA), is based on: the average content of 14 major compounds (>1%) taken as active variables, the average yield of EO, chemical classes and some biogeographical factors (altitude, latitude, and longitude) as supplementary variables. The established plot according to axis 1 and 2 (explaining 70.93% of the variation) highlighted the existence of important and complex correlations (Figure 2). According to the factorial axis 1 (37% of variation), the investigated populations were segregated into two groups. The first, formed by the southern and culminate (up to 1467m) populations (Ta1, Ta2 and Ef) clustered at the positive part of axis 1 is distinguished by a relatively high EOs content (2.7-2.8%), high levels of hydrocarbons monoterpens (19.4 to 25.4%), especially α-pinene (8.7-10.7%), camphene (3.3- 3.7%), β-pinene (4.5-6.8%) and myrcene (1.1-1.4%), and by a relatively low content of oxygenated monoterpens (72.3-77.5%), hydrocarbons (0.7-1.7%) and oxygenated (0.3-0.4%) sesquiterpenes. Vis-versa, the second, formed by the populations grown in the other areas of the Eastern Region of Morocco and clustered at the negative side of the axis 1 (Gu, Az, Sl and Ea), is characterized by a low EOs contents (1.7-2.1%), low levels of hydrocarbons monoterpens (15.1-17.4%) and by a relatively high content of oxygenated monoterpens (78.6-80.4%), hydrocarbons (1-3.1%) and oxygenated (0.4-1%) sesquiterpenes.

Table 2. Chemical composition (%) of Moroccan *Rosmarinus officinalis* L. essential oils according to origin and harvest time

Origin			Ta1	Ta2	Je	Gu	Az	Ea	Da	Ef	Sl	De	Experimental Design Debdou											
													Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	
Phenologic stage			Post-flowering										Sig	Pre-flowering			Flowering					Post-flowering				Sig
Yields (%)			2.8	2.7	2.1	2.1	1.8	1.9	1.8	2.7	1.7	2.3	***	2.5	2.2	2.2	2.4	2.2	2.5	2.1	2.1	3.0	2.6	3.1	2.5	***
Compound		Ric																								
1	*Tricyclene*	*926*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	***	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	**
2	*α-Thujene*	*930*	0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	***	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1	***
3	*α-Pinene*	*938*	8.7	8.7	8.8	6.0	7.5	6.5	8.2	10.7	6.7	8.3	**	6.8	6.2	6.4	3.6	3.8	5.4	6.0	3.9	3.9	5.6	7.2	7.4	**
4	*Camphene*	*952*	3.7	3.3	3.6	2.7	3.0	1.9	3.2	3.6	2.7	3.5	***	3.2	2.7	3.0	1.8	2.2	2.8	3.2	2.2	2.0	2.8	3.3	3.1	*
5	*β-Pinene*	*980*	5.2	4.5	5.7	3.5	2.6	5.1	5.2	6.8	3.7	4.0	***	2.9	2.7	3.1	2.7	3.5	4.8	6.6	5.9	5.1	5.6	4.8	3.7	***
6	*3-Octanone*	*986*	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	NS	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	NS
7	*Myrcene*	*993*	1.2	1.1	1.0	0.8	0.9	1.1	1.1	1.4	1.0	1.0	***	1.0	0.9	1.0	0.8	0.8	0.8	1.0	0.9	1.0	1.1	1.1	1.0	NS
8	*α-Phellandrene*	*1005*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	***
9	*α-Terpinene*	*1018*	0.5	0.2	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3	***	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.4	0.4	***
10	*p-Cymene*	*1026*	0.7	0.4	0.8	0.8	1.2	1.0	0.7	0.8	1.3	1.2	***	1.3	1.4	1.4	1.3	1.1	1.1	1.0	0.6	0.5	0.5	0.9	1.2	***
11	*1,8-Cineol*	*1034*	43.6	44.2	46.0	44.5	45.7	53.6	46.4	50.7	54.8	47.4	***	45.8	50.0	43.3	42.2	42.0	43.7	42.1	44.9	42.5	44.2	47.8	49.8	***
12	*(Z)-β-Ocimene*	*1040*	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.3	0.0	***	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	NS
13	*(E)-β-Ocimene*	*1049*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS
14	*γ-Terpinene*	*1059*	0.9	0.6	0.7	0.6	0.6	0.8	0.9	0.8	0.4	0.6	***	0.5	0.4	0.4	0.5	0.5	0.7	0.6	0.9	0.9	1.0	0.7	0.6	***
15	*trans-Sabinene hydrate*	*1068*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	*	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.0	0.3	0.1	0.1	0.0	***
16	*Terpinolene*	*1087*	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.3	***	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3	***
17	*Linalool*	*1098*	1.0	1.5	1.2	0.8	1.2	1.6	1.1	0.9	1.8	1.1	***	1.0	1.3	1.1	1.0	1.2	0.9	0.8	0.9	1.0	1.2	1.1	1.1	NS
18	*Chrysanthenone*	*1121*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	***
19	*Campholenal*	*1125*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	*
20	*Camphor*	*1143*	21.1	20.2	15.0	19.0	18.5	11.9	15.0	13.0	10.4	14.6	***	16.0	15.7	15.1	18.8	16.9	12.7	13.6	15.3	15.2	15.4	14.2	13.1	NS
21	*Borneol*	*1163*	3.6	5.1	5.7	6.2	5.7	3.4	4.0	1.9	4.6	7.5	***	9.0	6.7	8.9	9.4	9.6	9.4	8.1	7.6	11.7	7.6	7.0	7.3	NS
22	*Terpinen-4-ol*	*1174*	1.4	1.1	1.4	1.4	1.6	1.7	1.7	1.1	1.5	1.3	***	1.2	1.4	1.3	1.5	1.5	1.4	1.6	2.0	1.7	1.7	1.2	1.2	***
23	*p-Cymen-8-ol*	*1182*	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	**	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	***

RIc. Retention indices measured relative to n-alcanes (C8–C22) using capillary column (HP-5); **Ta.** Talsint; **Je.** Jerada; **Gu.** Gueffaît; **Az.** Ain Zohra; **Ea.** El Aouam; **Da.** Dada Ali; **Ef.** El Fachat; **Sl.** Sidi Lahcen; **De.** Debdou; * P <0.05 ; ** P<0.01 ; *** P<0.001; **NS.** No significance.

Table 2 (cont.)

	Origin		Ta1	Ta2	Je	Gu	Az	Ea	Da	Ef	Sl	De	Experimental Design Debdou												
														Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	
24	*α-Terpineol*	*1186*	3.9	4.5	4.5	5.3	5.1	5.5	5.3	4.4	5.3	4.6	**	4.1	4.3	4.2	4.8	4.2	4.0	4.1	4.3	4.5	4.3	3.6	4.0	NS
25	*Myrtenol*	*1193*	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.2	0.1	***	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	NS
26	*Verbenone*	*1204*	0.0	0.0	0.1	0.0	1.3	0.9	0.4	0.0	0.6	0.1	***	0.6	0.4	0.6	0.5	0.4	0.5	0.3	0.3	0.5	0.5	0.4	0.4	NS
27	*Bornyl acetate*	*1283*	0.8	0.7	0.6	0.9	0.9	0.3	0.4	0.3	0.2	0.8	***	0.6	0.4	1.0	1.2	1.4	1.3	2.2	2.3	1.5	0.8	0.4	0.5	***
28	*Thymol*	*1290*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	**
29	*Carvacrol*	*1297*	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.1	0.1	***	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	***
30	*Eugenol*	*1353*	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	NS	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	NS
31	*β–Caryophyllene*	*1418*	1.6	0.6	1.6	2.7	1.0	1.0	1.8	0.6	0.7	0.8	***	0.7	0.6	1.4	2.0	2.6	2.4	2.1	1.9	1.8	1.2	1.5	0.9	***
32	*α–Humulene*	*1454*	0.1	0.0	0.2	0.2	0.1	0.2	0.1	0.0	0.1	0.0	*	0.1	0.1	0.2	0.2	0.3	0.2	0.4	0.2	0.2	0.2	0.2	0.1	**
33	*γ- Muurolene*	*1475*	0.0	0.0	0.1	0.2	0.0	0.1	0.2	0.1	0.1	0.1	***	0.1	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1	***
34	*Caryophyllene oxide*	*1580*	0.3	0.3	0.4	0.7	0.4	0.3	0.5	0.2	0.4	0.5	***	0.5	0.5	1.0	1.2	1.2	1.1	0.9	0.9	0.8	0.6	0.3	0.4	***
35	*α-Cadinol*	*1652*	0.0	0.1	0.2	0.3	0.3	0.1	0.3	0.1	0.2	0.2	*	0.4	0.3	0.6	0.6	0.5	0.4	0.2	0.2	0.2	0.3	0.1	0.2	***
	Class composition																									
36	Monoterpene hydrocarbons		22.0	19.4	21.8	15.1	16.8	17.4	20.4	25.4	17.0	19.6	**	16.8	15.3	16.4	11.6	12.9	16.7	19.6	15.8	14.6	18.0	19.2	18.1	NS
37	Oxygenated monoterpenes		75.4	77.5	74.8	78.6	80.4	79.1	74.6	72.3	79.7	77.7	**	79.0	80.9	76.3	80.4	78.1	74.9	73.6	78.2	79.5	76.5	76.4	78.0	NS
38	Sesquiterpene hydrocarbons		1.7	0.7	2.0	3.1	1.1	1.4	2.1	0.7	1.0	0.9	***	0.9	0.7	1.7	2.4	3.1	2.9	2.7	2.3	2.1	1.5	1.9	1.1	***
39	Oxygenated sesquiterpenes		0.3	0.4	0.6	1.0	0.6	0.4	0.7	0.3	0.6	0.7	***	0.9	0.7	1.6	1.8	1.7	1.5	1.1	1.0	1.0	0.9	0.4	0.5	***

RIc. Retention indices measured relative to n-alcanes (C8–C22) using capillary column (HP-5); **Ta.** Talsint; **Je.** Jerada; **Gu.** Gueffaît; **Az.** Ain Zohra; **Ea.** El Aouam; **Da.** Dada Ali; **Ef.** El Fachat; **Sl.** Sidi Lahcen; **De.** Debdou;

* P <0.05 ; ** P<0.01 ; *** P<0.001; **NS.** No significance.

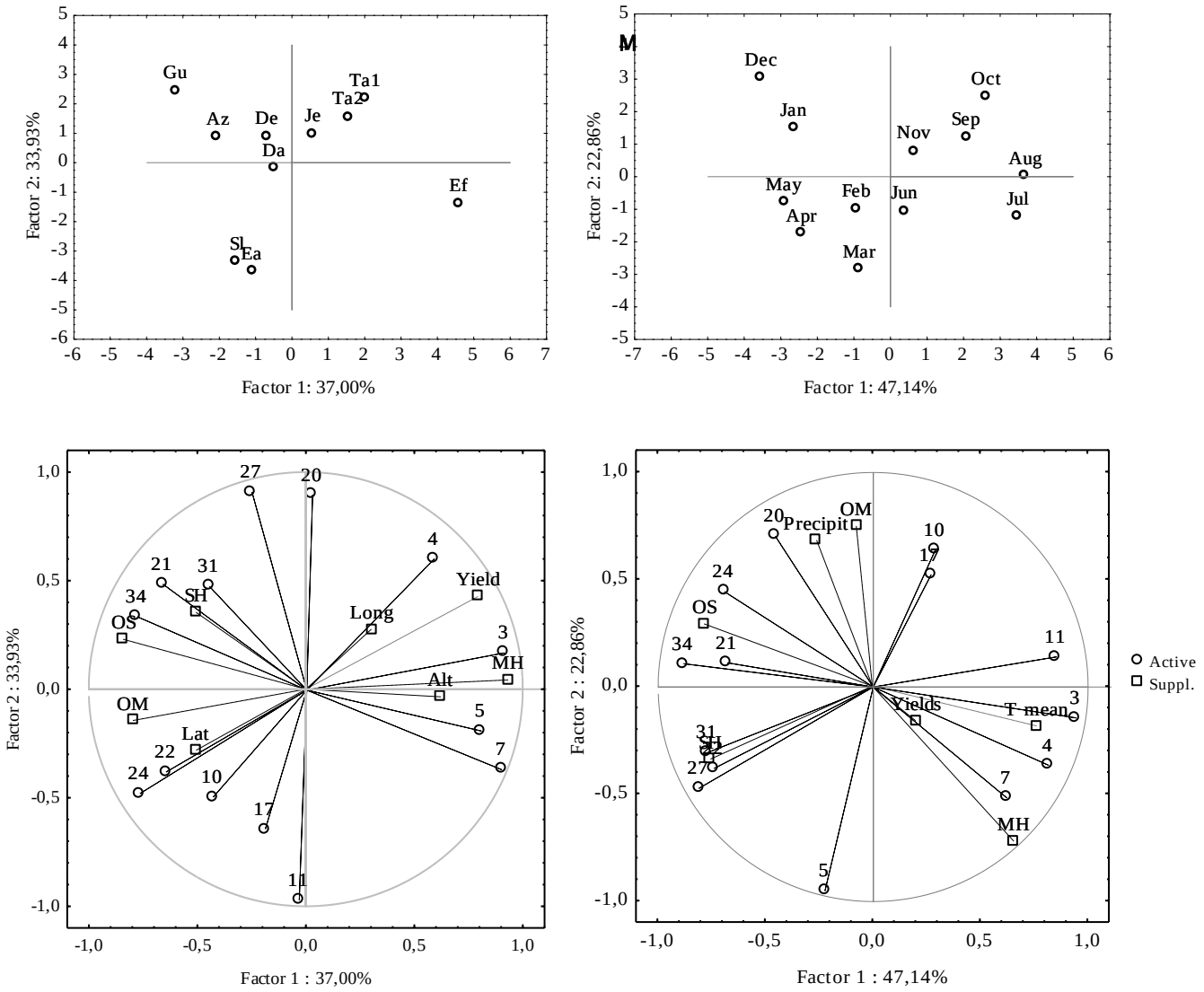

Figure 2. PCA analysis diagram of chemical composition within rosemary populations (left) and within periods (right) **Ta.** Talsint, **Je.** Jerada, **Gu.** Gueffaît, **Az.** Ain Zohra, **Ea.** El Aouam, **Da.** Dada Ali, **Ef.** El Fachat, **Sl.** Sidi Lahcen, **De.** Debdou.

Active variables: 3. α-Pinene, 4. Camphene, 5. β-Pinene, 7. Myrcene, 10. p-Cymene, 11. 1,8-Cineole, 17. Linalool, 20. Camphor, 21. Borneol, 22. Terpinen-4-ol, 24. α-Terpineol, 27. Bornyl acetate, 31. β–Caryophyllene, 34. Caryophyllene oxide.

Supplementary variables: Alt. Altitude, **Lat.** Latitude, **Long.** Longitude, **Precipit.** Monthly precipitation, **T mean.** Mean monthly temperature, **MH.** Monoterpene hydrocarbons, **OM.** Oxygenated monoterpenes, **SH.** Sesquiterpene hydrocarbons, **OS.** Oxygenated sesquiterpenes.

In contrast, 1,8-cineole, camphor and bornyl acetate contents are correlated (R>89%) to the axis 2 (33.9% of variation). Rosemary populations of Talsint (Ta1 and Ta2), Gueffaît (Gu) and Ain Zohra (Az) located on the up side of this axis are in fact characterized by relatively high content of camphor (18.5-21.1%), bornyl acetate (0.7-0.9%) and a relatively low content of 1,8-cineole (43.6-45.7%); While Sidi Lahcen (Sl) and El Aouam (Ea) populations, located on the negative side of this axis, are rather rich in 1,8-cineole (53.6-54.8%) and contained the lowest concentrations of camphor (10.4-11.9%) and bornyl acetate (0.2-0.3%). According to the region of provenance, different models underlining complex relationships between these factors and rosemary oil composition were also reported. So, in order

to explain the chemical differentiation of the investigated populations in the Eastern Region of Morocco, complementary regression analysis was carried out. Chemical composition of oils showed a significant dependence on the biogeographic parameters of habitat. As it can be inferred from this analysis, only 4 from the 19 registered variables have not displayed statistically significant correlation with any studied biogeographic factors are: camphene, 1,8-cineole, linalool and oxygenated monoterpenes class. The other compounds contents displayed however different levels of dependency on altitude, latitude or longitude (Table 3). Ours results are in agreement with Sotomayor et al. [22] mainly for the relationship between the altitude and the content of β-pinene and borneol that showed a positive correlation between this factor and the relative amounts of β-pinene, whereas a negative correlation with the contents of borneol was detected (Table 3). In the Balkan Peninsula, Lakusic et al. [23] reported the abundance of four chemotypes on the basis of four major constituents (1,8-cineole, camphor, α-pinene, and borneol) whose concentration was closely related to the climate characteristics of habitat, as well as geographic position with respect to continentality. Camphor was reported as main major component of populations within the continent. Similarly, the most continental populations in the Eastern Region of Morocco (Ta1 and Ta2) showed relatively high levels of camphor compared to other prospected populations. Moroccan rosemary contained in contrast high amounts of 1,8-cineole (42-55%) than those harvested of Iberian and Balkan Peninsulas (11-39%) [22-24]. All these authors support the assumption that the geographical distribution and altitudinal gradient are usually associated with the variation of environmental factors that influence the EO composition of each population. Despite some slight chemical differentiations, oil of rosemary growing wild in the Eastern Region of Morocco can be qualified as 1,8 cineole chemotype compared to foreign populations investigated in the Mediterranean area.

3.4 Variation of essential oil composition during full vegetative cycle

The global variation of rosemary oil composition during a complete vegetative cycle was also assessed by another PCA; and the total variance of the two first principal components was equal to 70% (Figure 2). This exploratory analysis revealed that chemical variability of rosemary oils is influenced by bioclimatic parameters such as monthly precipitation and mean monthly temperature. During summer (July-August), when maximal temperatures (23-23.9°C) and minimal rainfall (4-7mm/month) are reached, oils were distinguished by their abundance of 1,8-cineole (47.8-49.8%) and hydrocarbon monoterpenes (18.1-19.2%), especially p-cymene (0.9-1.2%), α-pinene (7.2-7.4%), camphene (3.1-3.3%) and myrcene (1-1.1%). Contents of those compounds were negatively correlated to oxygenated and hydrocarbon sesqueterpens, α-terpineol, borneol, terpinen-4-ol and bornyl acetate, whose concentrations decreased. With the onset of the rainy season (25-37 mm/month) in early autumn (September-October), high concentrations of oxygenated monoterpenes were noticed (79-80.9%) following increases in camphor, α-terpineol and linalool amounts, while β-pinene decreased to its lowest concentrations (2.7-2.9%). Then, at early flowering (December-January) when minimal temperatures (7-7.9°C) and impotant rains (39-64mm/month) occurred, hydrocarbon monoterpenes concentrations are minimized in oil (11.6-12.9%), mainly those of α-pinene (3.6-3.8%), camphene (1.8-2.2), myrcene (0.8%) and β-pinene (2.7-3.5%). During this period, 1,8-cineole amounts reached their lowest percentages (42-42.2%). Oxygenated monoterpenes contents remain whereas higher in oil (78.1-80.4), they are largely compensated by the improvement of camphor content, which reach its maximum level (16.9-18%) (Table 2 and figure 2). The inverse trends is observed in march during anthesis stage, β-pinene content are maximized in oil (6.6%), while those of oxygenated monoterpens are minimized (73.6%) following bought decrease of 1,8-cineole (42.1%) and camphor (13.6%) contents. Then, when the flowering period is over in April-May at the end of the rainy season (0-6mm/month), the global chemical differentiation of rosemary EOs was mainly characterized by decrease of amounts of α-pinene (3.9%) and camphene (2-2.2%) and by increase of amounts of borneol (7.6-11.7%), terpinen-4-ol

(1.7%-2) and bornyl acetate (1.5-2.3%). 1,8-cineole contents remain relatively low and those of sesquiterpenes relatively higher. According to the regression analysis between components percentages and bioclimatic parameters, the chemical differentiation of rosemary EOs major compounds exhibited a significant dependence on temperature and precipitation conditions (Table 3). It should be underlined that these parameters are among the key factors regulating the biosynthesis of EOs during the different plant growth stages. For Sardinian rosemary oils, the most bioclimatic parameter affecting major compounds content was the mean monthly temperature range. In contrast precipitation did not exert a significant influence on variability of chemical composition [23]. Our results are in agreement with this later work mainly for the relationship between the temperature and both 1,8-cineole and camphor contents: under temperate conditions, the amounts of 1,8-cineole increased in comparison to that of camphor and vis-versa.

Table 3. Correlation matrix (r) between chemical composition and orographic, geographic and bioclimatic parameters

Variable	Alt	Lat	Long	Precipit	T mean
Yield	0.3560	-0.7448***	0.4797**	-0.3976*	0.4723**
α-Pinene	0.4470*	-0.2258	0.2040	-0.1379	0.5279**
Camphene	0.1955	-0.2466	0.2100	-0.1581	0.3797*
β-Pinene	0.4254*	-0.2143	-0.1819	-0.5569***	0.0283
Myrcene	0.5032**	-0.3446	0.1054	-0.4838**	0.5822***
p-Cymene	-0.4859**	0.5637**	-0.1539	0.7494***	-0.2357
1,8-Cineole	-0.1502	0.3056	-0.3238	-0.2753	0.5987***
Linalool	0.0625	-0.1089	0.0861	0.0170	0.1153
Camphor	0.2363	-0.5037**	0.5617**	0.3169	-0.2152
Borneol	-0.6715***	0.2254	-0.0926	0.1713	-0.2888
Terpinen-4-ol	-0.0909	0.4452*	-0.2755	-0.2824	-0.2770
α-Terpineol	-0.2387	0.4950**	-0.3664*	0.1805	-0.2277
Bornyl acetate	-0.2054	-0.1807	0.3841*	-0.0522	-0.5486**
β-Caryophyllene	-0.2886	0.1884	-0.4398*	0.1755	-0.6901***
Caryophyllene oxide	-0.6879***	0.4074*	-0.5049**	0.5478**	-0.8754***
Monoterpene hydrocarbons	0.4242*	-0.2254	0.0761	-0.3934*	0.4091*
Oxygenated monoterpenes	-0.3480	0.1283	0.1368	0.1002	0.0839
Sesquiterpenes hydrocarbons	-0.3190	0.2651	-0.5249**	0.1605	-0.6808***
Oxygenated sesquiterpenes	-0.6306***	0.5466**	-0.4858**	0.6945***	-0.8563***

Alt. Altitude, **Lat.** Latitude, **Long.** Longitude, **Precipit.** Monthly precipitation, **T mean.** Mean monthly temperature.
* P <0.05 ; ** P<0.01 ; *** P<0.001

Genetic factors may also play a great role in the chemo-variation of EOs. This fact has been previously appointed for yield and chemical composition oils extracted from several wild plants transplanted under the same environmental conditions in Morocco, such as *Myrtus communis* [31], *Rosmarinus officinalis* [16] and *Origanum elongatum* [32]. Since, transplanted scrubs maintained their chemical profiles of origin populations after 3 years of crop in cultural plots. The chemo-variety of aromatic plants seems to be related to the genetic characteristics of the species, which are the result of a long adaptation of the plant to its environment. As a consequence of intrinsic (genetic and plant physiology) and extrinsic (environmental conditions) factors, rosemary EOs yield and composition exhibits a great

variability in its range expansion throughout the Mediterranean area [15-16; 22-24; 30; 33]. Thereby, all rosemary wild populations investigated in the Eastern Region of Morocco contained high levels of 1,8-cineole (42-55%) in their essential oils compared to foreign populations. The high concentration of this compound could be linked to the temperate climate of the southern Mediterranean.

3.5 Fumigant effect of Rosemary essential oil against Varroa mite

The treatment with *Bayvarol* and pure rosemary EO at different application rates, at apiaries, induced a higher number of dead/fallen *V. destructor* mites compared to the control during treatment period (Table 4):

- During the first day after treatment, rosemary oils produced a significant mite fall compared to control. An application rate of 5ml rosemary oil gave the best efficacy (93%) compared to *Bayvarol* and others oil concentrations.
- A different pattern was observed the second day after treatment. So, a slight decrease of about 2% in the efficacy was observed for *Bayvarol* and for rosemary oil application rates of more than 2 ml. This decrease was much larger for the concentration of 1 and 2ml, which reach 10 and 23% respectively. The efficacy recorded for *Bayvarol* treatment didn't differ significantly from that obtained with the 2, 3 and 4 ml rosemary EO treatments at $p < 0.05$ (Table 4). Rosemary oil remained significantly toxic at 5 ml concentration compared to 1 ml concentration, which after 48h it showed similar efficacy than control and toxicity was therefore no longer to Varroa mites;
- After the 6^{th} day of treatment, the efficacy decreased for all treatments. Low concentrations of rosemary oil (1 and 2 ml) showed no significant differences with the control and were therefore ineffective against Varroa mites; while concentrations more than 2 ml still toxic and ensured satisfactory efficacy compared to *Bayvarol* despite their high volatility.

Many plant EOs have shown a widely bioactivity spectrum, which is attributed in part, to their lipophilic natures and high vapor pressures [34]. In laboratory conditions, lots of them have shown acaricidal activities against *V. destructor* mites, with a marked variability in hives at end test [14; 35]. This can be attributed to the large variability of their chemical compositions. In fact, some species possessed different chemotypes with different compositions of EOs [36]. In our work, rosemary EOs showed certain effectiveness against Varroa mites. After treatments with EOs, bees became more aggressive. On one hand, death of several bees was registered in the second day of treatment: 5 bees in one hive and 12 in another hive for 5ml EO application rate treatment. On the other hand, unnatural mortalities were not recorded nor for lower application rates or for the control who does not exceed 2 bee/hive/day. The death of all examined fallen mites, onto the sticky boards confirms the acaricidal activity of rosemary oil.

Under the same conditions, similar efficacy against *V. destructor* was obtained for oils extracted from *Thymus satureioides* and *Origanum elongatum* dominated respectively by borneol and carvacrol. So, those oils as well as their blend, applied directly in beehives, have been tested successfully against Varroa mites [11]. Main compounds of rosemary oil may confer solely or in combination this noticeable acaricidal activity. The remarkable bioactivity of camphor against Varroa mites were previously reported without toxicity for bees [37]. In field bioassays, toxicity of 1.8-cineole for bees was reported to be close [38-39]. In laboratory tests, rosemary oil containing camphor as major compound produced high mite mortality without apparent toxicity for honeybees but oil dominated by 1,8-cineole and ß-myrcene showed however minor acaricidal effect against Varroa mites [14]. It seems that the major components possess different modes of action and in a mixture their toxicities depends on their concentration and their ability for combination with other compounds [40].

Miresmailli et al. [13] reported both synergistic and antagonistic effects between major compounds by studying the toxicity of rosemary oil and blends of its major components against *Tetranychus urticae* on bean and tomato as host plants. Bioassays showed that the absence of α-pinene or 1,8 cineole from blends of major compounds caused a significant decrease in toxicity respectively by 80 and 84%, but when mixed together their toxicity was below expectation, since their toxicity level was significantly higher when tested solely. This indicates that 1,8 cineole and α-pinene might have mutual antagonistic effect. On the other hand, satisfactory toxicity of the pure rosemary oil requests simultaneous presence of these two constituents with the other major (inactive and active) compounds. In this case, it appears that the presence of compounds such as camphene, β-pinene, camphor, p-cymene, borneol, D-limonene, α-terpineol and bornyl acetate induced some synergistic effect and reduced the antagonism between 1,8 cineole and α-pinene compounds.

Table 4. Features and efficacy rates of treatments performed on January 2017 in the apiary against *Varroa destructor*.

Treatments		Major compounds	Application	Efficacy rate (%) after		
N°	Product		rates/hive	1 day	2 day	6 day
TR0	Control	Blank	0 ml			
TR1	Rosemary Oil	1,8-cineole (47.8%), camphor (14.2%), α-pinene (7.16%), borneol (7.5%), β-pinene (4.83%), α-terpineol (3.62%), camphene (3.07%)	1 ml	65.93± 2.9^{a*}	42.65± 8.9^{a}	30.80± 14.4^{a}
TR2			2 ml	69.71± 7.4^{a}	59.80± 3.5^{ab}	40.37± 2.2^{a}
TR3			3 ml	81.70± 5.8^{b}	83.43± 2.0^{bc}	61.98± 7.5^{b}
TR4			4 ml	85.79± 4.5^{b}	83.52± 9.5^{bc}	65.55± 12.4^{b}
TR5			5 ml	93.02± 2.3^{c}	91.41± 1.2^{c}	65.68± 1.2^{b}
TR6	*Bayvarol*	Flumetrin	7.2 mg	83.03± 7.7^{b}	81.68± 6.6^{bc}	63.26± 2.8^{b}

* For each column, values followed by different letters shared significant differences at 95% (Duncan test); values are means of three independent replicates ± SD.

Thus, the efficacy of EO depends on both chemical profile and concentration of oil used for the treatment. Rosemary oil profile tested in this work, dominated by 1,8-cineole (47.80%), camphor (14.20%), borneol (7.50%), α-pinene (7.16%), β-pinene (4.83%), α-terpineol (3.62%) and camphene (3.07%), provided sufficient expected miticidal activity. As well, under the experimental conditions of the bioassays, 5ml of rosemary EO caused the highest mortalities for *V. destructor* mites, despite low bee mortality were recorded in the second day of treatment at this assayed application rate because of the presence of high concentration of 1.8-cineole in this oil. So in apiary, 3 ml application rate, providing an equivalent toxicity to *Bayvarol*, would be safer for bees. These results are in agreement with those of Islam et al. [12] recording highest Varroa mites mortality (80%) with an application rate of 5ml of pure rosemary oil (100%), which decreased to 73.68% for 50% concentration and to 66.10% for 25% concentration without abnormal honeybee mortality. According to previous studies, field bioassays show in many times great variability in miticidal activities of oil in hives depending on seasons and localities [36; 41-44]. Moreover, efficacy of some components depends also on the evaporation pressure in hives which depends on the time of year or on the ambient temperature and humidity during treatment application [45]. In our study, the efficacy decreased from the second day

of application, probably because of fast oil evaporation from pads. So, developing effective delivery systems for releasing EOs continuously at a constant dose for an extended period regardless of environmental conditions are important step for using them effectively in hives [44].Rosemary EO applied directly in beehives, at suitable rates, reduced infestations of *V. destructor*, and may be useful for the control in traditional apiaries. Pure rosemary oil with 1,8-cineole and camphor as main constituents might be good alternative to chemical treatments widely being used in *A. mellifera* colonies against Varroa mite. But, this supposes that it is also necessary to replicate treatments several times and particularly with different active molecules to ovoid the resistance phenomenon. It's easier for mites to develop resistance to a single active ingredient acaricide than to an EO based on a mixture of different active compounds possessing different modes of action [13]. As reported above, composition and concentration of EOs and their fumigation conditions are important to achieve high and safe levels of mite control in apiaries [12-13; 44]. Before integrated them to an Integrated Pest Management program to control *V. destructor* mites, further studies are necessary to determine the best delivery system of rosemary oils that will improve and optimize the fumigant potency and stability of the formulation in the hives.

4. Conclusion

By this study, the interest of EOs of rosemary growing wild in a Mediterranean bioclimatic in the Eastern region of Morocco was highlighted. So, rosemary oils are dominated by 1,8-cineole and chemical variability is noticed within studied populations. Only a slight variation is revealed, in plot design, during one cycle of growth. High content of those oils in 1,8-cineole would be a good alternative to chemicals widely used in *A. mellifera* apiaries against Varroa mite. In practice an application rate of 3 ml EOs, safe for bees, was then recommended. However, further studies must be conducted to determine the best delivery systems that will sustained the fumigant potency of these oils in the hive for controlling V. destructor mites.

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References

[1] E. Stefanovits-Bányai, M.H. Tulok, A. Hegedus, C. Renner, I.S. Varga, Acta Biol. Szeged., 47 (2003) 111-113. https://www2.sci.u-szeged.hu/ABS/2003/ActaHP/47111.pdf (Accessed 9 October 2018).

[2] E. Arranz, J. Mes, H.J. Wichers, L. Jaime, J.A. Mendiola, G. Reglero, S. Santoyo, J. Funct. Foods., 13 (2015) 384-390. https://doi.org/10.1016/j.jff.2015.01.015

[3] M.R. Ismaili, M. Rahouti, B. Kabouchi, H. Ramzi, M. Aberchane, A. Fidah, A. Famiri, O. Lamzoudi, J. Essent. Oil Bear., 20 (2017) 1266-1274. https://doi.org/10.1080/0972060X.2017.1401961

[4] A. Begum, S. Sandhya, A. S. Shaffath, K. R. Vinod, S. Reddy, D. Banji, Acta Sci. Pol. Technol. Aliment., 12 (2013) 61-73. http://www.food.actapol.net/volume12/issue/6_1_2013.pdf (Accessed 26 February 2018).

[5] R. R. Santos, D. C. Costa, C. Cavaleiro, H. S. Costa, T. G. Albuquerque, M. C. Castilho, F. Ramos, N. R. Melo, A. Sanches-Silva, Trends in Food Science and Technology., 45 (2015) 355-368. https://doi.org/10.1016/j.tifs.2015.07.015.

[6] M. Singh, N. Guleria, Ind. Crops Prod., 42 (2013) 37-40. http://doi.org/10.1016/j.indcrop.2012.04.054

[7] R. Pavela, Plant Prot. Sci., 52 (2016) 229-241. http://dx.doi.org/10.17221/31/2016-pps

[8] S. Attia, K. L. Grissa, G. Lognay, E. Bitume, T. Hance, A.C. Mailleux, J. Pest Sci., 86 (2013) 361-386. https://doi.org/10.1007/s10340-013-0503-0

[9] R. Pavela, Ind. Crops Prod., 60 (2014) 247-258. https://doi.org/10.1016/j.indcrop.2014.06.030
[10] R. Pavela, Parasitol. Res., 114 (2015) 3835-3853. https://doi.org/10.1007/s00436-015-4614-9
[11] H. Ramzi, M. R. Ismaili, M. Aberchane, S. Zaanoun, Ind. Crops Prod., 108 (2017) 201-207. https://doi.org/10.1016/j.indcrop.2017.06.031
[12] N. Islam, M. Amjad, E. ul-Haq, E. Stephen, F. Naz, J. Entomol. Zool. Stud., 4 (2016) 97-104 http://www.entomoljournal.com/archives/2016/vol4issue6/PartB/4-5-145-836.pdf (Accessed 9 October 2018).
[13] S. Miresmailli, R. Bradbury, M. B. Isman, Pest Manag. Sci., 62 (2006) 366-371. https://doi.org/10.1002/ps.1157
[14] M. Maggi, L. Gende, K. Russo, R. Fritz, M. Eguaras, Nat. Prod. Res., 25 (2011) 397-406. http://dx.doi.org/10.1080/14786419.2010.481261
[15] A. El Amrani, S. Zrira, B. Benjilali, M. Berrada, J. Essent. Oil Res., 12 (2000) 487-495. http://dx.doi.org/10.1080/10412905.2000.9699572.
[16] M. Fechtal, M. R. Ismaili, A. Zine El Adine, Ann. Rech. For., 34 (2001a) 94-102. ISBN 9981824151.
[17] P.A. Souza Tette, L. Rocha Guidi, M. B. De Abreu Gloria, C. Christian Fernandes, Talanta., 149 (2015) 124-141. https://doi.org/10.1016/j.talanta.2015.11.045
[18] M. Aberchane, M. Fechtal, A. Chaouch, J. Essent. Oil Res., 16 (2004) 542-547. http://dx.doi.org/10.1080/10412905.2004.9698793
[19] R.P. Adams, Identification of essential oil components by Gas Chromatography Mass/Spectroscopy, 4th ed., Allured publ., Carol stream, IL, USA, 2007. ISBN 1932633219.
[20] K. Jayaraman, Manuel de statistique pour la recherche forestière, F.A.O., 1999. http://www.fao.org/3/X6831F/X6831F00.htm (Accessed 19 March 2019).
[21] M. Jambu, Exploratory and multivariate data analysis, 1st Ed., Academic Press, UK, 1991. 474p. ISBN 0123800900.
[22] J. A. Sotomayor, C. Martínez, M. I. Moñino, V. Lax, M. Quílez, M. J. Jordán, Acta Hort., 826 (2009) 309-316. https://doi.org/10.17660/ActaHortic.2009.826.43
[23] D. V. Lakusic, M.S. Ristic, V. N. Slavkovskac, J. B. Sinzar-Sekulic, B. S. Lakusic, Chem Biodivers., 9 (2012) 1286-1302. https://doi.org/10.1002/cbdv.201100427
[24] M. J. Jordán, V. Lax, M. C. Rota, S. Lorán, J. A. Sotomayor, Food Control., 30 (2013a) 463-468. https://doi.org/10.1016/j.foodcont.2012.07.029.
[25] A. Edreva, V. Velikova, T. Tsonev, S. Dagnon, A. Gurel, L. Aktas, E. Gesheva, Appl. Plant Physiol., 34 (2008) 67–78. http://www.bio21.bas.bg/ippg/bg/wp-content/uploads/2011/06/08_pisa_1-2_67-78.pdf (Accessed 19 March 2019).
[26] M. Nowak, R. Manderscheid, H. J. Weigel, M. Kleinwachter, D. Selmar, J. Appl. Bot. Food Qual., 83 (2010) 133–136. https://ojs.openagrar.de/index.php/JABFQ/article/view/2138/2524 (Accessed 19 March 2019).
[27] O. Aissi, M. Boussaid, C. Messaoud, Ind. Crops Prod., 91 (2016) 56-65. https://doi.org/10.1016/j.indcrop.2016.06.025
[28] R. S. Verma, L. Rahman, S. Mishra, R. K. Verma, A. Singh, A. Chauhan, A. K. Yadav, J. Chil. Chem. Soc., 57 (2012) 1066-1068. https://doi.org/10.4067/S0717-97072012000200001
[29] M. J. Jordán, V. Lax, M.C. Rota, S. Lorán, J. A. Sotomayor, Ind. Crops Prod., 48 (2013b) 144-152. https://doi.org/10.1016/j.indcrop.2013.04.031
[30] Y. Zaouali, H. Chograni, R. Trimech, M. Boussaid, Ind. Crops Prod., 43 (2013) 412-419. https://doi.org/10.1016/j.indcrop.2012.07.044

[31] M. R. Ismaili, M. Fechtal, A. Zine El Abidine, M. Hachmi, A. Sesbou, Ann. Rech. For., 34 (2001) 87-93. ISBN 9981824151.
[32] M. Fechtal, M. R. Ismaili, A. Zine El Abidine, M. Hachmi, A. Sesbou, Ann. Rech. For., 34 (2001b) 103-109. ISBN 9981824151.
[33] A. Angioni, A. Barra, E. Cereti, D. Barile, J. D. Coïsson, M. Arlorio, S. Dessi, , V. Coroneo, P. Cabras, J Agric Food Chem., 52 (2004) 3530-3535. https://doi.org/10.1021/jf049913t
[34] S. I. Kim, J. H. Yi, J. H. Tak, Y. J. Ahn, Veterinary Parasitology, 120 (2004) 297-304. https://doi.org/10.1016/j.vetpar.2003.12.016.
[35] A. Ariana, R. Ebadi, G. Tahmasebi, Experimental and Applied Acarology, 27 (2002) 319-327. https://doi.org/10.1023%2FA%3A1023342118549.
[36] A. Imdorf, S. Bogdanov, R. I. Ochoa, N. W. Calderone, Apidologie, 30 (1999) 209-228. https://doi.org/10.1051/apido:19990210.
[37] H.T. Abou Elenain, A. E. Asmaa, A. A. Taha, J. Plant Prot. and Path., 5 (2014) 687-695.
[38] M. Higes, M. Suarez, J. Llorente, Res. Rev. Parasitol., 57 (1997) 21-24.
[39] A. Imdorf, V. Kilchenmann, S. Bogdanov, B. Bachofen, C. Beretta, Apidologie, 26 (1995) 27-31. https://doi.org/10.1051/apido:19950104
[40] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Food and Chemical Toxicology, 46 (2008) 446-475. https://doi.org/10.1016/j.fct.2007.09.106.
[41] N. W. Calderone, M. Spivak, Journal of Economic Entomology, 88 (1995) 1211-1215 https://doi.org/10.1093/jee/88.5.1211.
[42] N. W. Calderone, W. T. Wilson, M. Spivak, Journal of Economic Entomology, 90 (1997) 1080-1086. https://doi.org/10.1093/jee/90.5.1080.
[43] B. Emsen, A. Dodologlu, Journal of Animal and Veterinary Advances, 8 (2009) 1004-1009. http://medwelljournals.com/abstract/?doi=javaa.2009.1004.1009 (Accessed 05.01.18).
[44] Q. Sabahi, H. Gashout, P. G. Kelly, E. Guzman-Novoa, Experimental and Applied Acarology, 72 (2017) 263-275. https://doi.org/10.1007/s10493-017-0157-3.
[45] M. J. Gracia, C. Moreno, M. Ferrer, A. Sanz, M. Á. Peribáñez, R. Estrada, PLoS One, 12 (2017) e0171633. https://doi.org/10.1371/journal.pone.0171633.