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Chemical composition and antibacterial activity of *Thymus zygis* subsp. *gracilis* (Boiss.) R. Morales essential oils from Morocco

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Abstract: *Thymus zygis* subsp. *gracilis* samples were collected from different Moroccan Middle Atlas sites at three phenological stages. Chemical composition of essential oils obtained from these samples was studied and their antimicrobial activity was evaluated. GC-MS analysis of essential oils shows quantitative and qualitative changes in the chemical profile. Twenty-three components were detected. These compounds fall under at least four chemical families whose relative importance varies with the sites and the phenological stages. Monoterpens were the most abundant and their percentage in essential oils varied from 61.38 to 94.91. Carvacrol (16.07 to 74.33%), thymol (1.47 to 32.46%), p-cymene (6.97 to 40.26%) and γ-terpinene (2.68 to 22%) were the main constituents. Bactericidal property of *Thymus zygis* essential oils was also evaluated *in vitro* against six strains of phytopathogenic bacteria. All bacterial strains tested were susceptible to essential oils. Maximum activity was observed against the Gram-negative bacterium *Erwinia amylovora* and Gram-positive actinomycete *Streptomyces scabiei.* The most bactericidal effect was observed against *Streptomyces scabei*. Its minimum bactericidal concentration (MBC) was 50 $\mu g.mL^{-1}$. At post-flowering stage, carvacrol chemical profile was the most efficient.

Key words*:* *Thymus zygis* subsp. *gracilis;* Essential oils; Chemical variability; Bactericidal activity; Morocco.

Introduction

Since the end of World War II, intensive farming systems with high performance and high injection of inputs and techniques were gradually introduced. Thus, the increasing impact and proliferation of pests was controlled at the cost of frequent phytosanitary measures.

It was not until the awareness of environmental costs of these practices[1], and the ecological disorders they have caused, such as fauna and flora changes, air and water contamination, phenomenon of pesticide resistance in pests (plants, insects, and fungi), long-term toxicity in animals and humans[2,3], that we observed a renewed interest in alternative approaches.

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In this context the scientific and technological research laboratories focused on the development of new fighting alternatives to replace chemical methods, or in addition to them (IPM), in order to effectively control these pests with minimum negative impacts of pesticides on the environment and human and animal health. Among these approaches, biological controls based on the use of natural substances from plant (plant extracts, essential oils) are generating a growing interest and many studies attempt to discover interesting properties of many plant species[4,5-7]**.** Like other plant species, those of genus *Thymus*, known for their interesting biological activities and the diversity of chemotypes of their essential oils, are among the most studied plants[8,9-12].

In Morocco, the *Thymus* genus is represented by about thirty species and sub-species among which fifteen are endemic[13]**.** These species are locally known under the common name "Zaetra" in Arabic and "zouk" or "Azoukeni" in Tamazight.

Thymus zygis subsp. *gracilis* (Boiss.) R. Morales is a polyploid species whose chromosome number is 2n = 28 and 2n = 56. This endemic species from Morocco and the Iberian Peninsula blooms between May and July[14]. Its leaves and flowers are covered by glandular trichomes containing monoterpenes-rich essential oil[15]. *Thymus zygis* subsp. *gracilis* population from southern Spain showed volatile compounds polymorphism. These chemotypes differ according to the nature of the primary volatile compound generated. they can be carvacrol or thymol-predominant with sometimes, small amounts of borneol and camphor, or linalool, linalool / terpinen-4-ol and thymol / carvacrol[16,17-20]. In Morocco, this species is found in holm oak and Atlas cedar woods between 200 and 1900 m of altitude[21]. Previous works on Moroccan *Thymus zygis* subsp. *gracilis* essential oils revealed too the existence of a strong chemical polymorphism[22,23-25]. In fact, the diversity of chemotypes in thyme essential oils depends on both genetic and ecological (biotic, abiotic) factors[26,27,28].

The pharmacological properties of Moroccan thyme and its essential oils have been thoroughly studied by several researchers. These studies have highlighted the interesting antimicrobial, antioxidant and antiviral properties[29,30-32].

In contrast, the antimicrobial potential of essential oils of thyme is still not utilized in plant pathology and even less against the species of *Erwinia* and *Streptomyces*. So, this study was conducted to continue the work already undertaken on the biological activity and chemical characterization of essential oils of Moroccan thyme. It aims to establish the link between the chemical composition and in vitro antibacterial activity of *Thymus zygis* subsp. *gracilis* essential oils against a set of pathogenic strains.

Experimental section

Plant material and extraction of essential oils

Biomass used for essential oils extraction was composed of aerial parts (stems, leaves and flowers) of *T. zygis* subsp. *gracilis* harvested during the months of May, June and July 2008. The biomass was collected during and after flowering stage in three locations of Middle Atlas: El Hajeb (latitude: 33°39,295; longitude: 05°20,937; altitude: 1154m), Azrou (latitude: 33°33,587; longitude: 05°20,284; altitude: 1376m) and Timahdite (latitude: 33°16,592; longitude: 005°04,449; altitude: 1847m). Botanical identity of the samples was confirmed by Professor Mohamed Ibn Tattou at the National Herbarium, Scientific Institute, Rabat (Morocco). Supporting specimens and voucher numbers were deposited at the Herbarium of this Institute.

The aerial parts were dried for ten days under shade at room temperature. Extraction of essential oils was made with 100g of plant material by steam distillation in a Clevenger-type apparatus. For each region, from 8 to 10 repetitions were performed per phenological stage. The essential oils obtained were dried with anhydrous sodium sulfate and stored at 4°C in the dark. Yield values are given in mL per 100 g of dry matter, measured from samples dried in an oven at 105°C until constant weight.

Chromatographic analysis

The chromatographic analysis of essential oils was performed with Thermo Electron chromatograph: Trace GC Ultra, equipped with a capillary column DB-5 (5% phenyl-methyl-siloxane) (30 m x 0.25 mm, film thickness: 0.25 µm). Detection was performed by a flame ionization detector (FID 250 °C) supplied with a gas mixture H_2 / Air. The carrier gas used was nitrogen with a flow rate of 1mL min^{-1}.

The device was equipped with an injector PVT (Programmed Temperature Vaporization) of split-splitless type. The injection mode was split. The injected volume was 1µl. The temperature program was 50 to 200°C for 5 min, with a gradient of 4°C × min^{-1}. The unit was controlled by a computer system that manages the device operation and monitors chromatographic analysis. Components identification was performed based on their Kováts indices (KI) and the gas chromatography coupled with mass spectrometry (GC / MS). The latter was performed on a Thermo Electron Trace MS system gas chromatograph (Thermo Electron: Trace GC Ultra, Polaris Q MS). Fragmentation was performed by 70 eV electron impact. The column used was a DB-5MS capillary column (5% phenyl-methyl-siloxane) (30 m x 0.25mm, film thickness: 0.25µm). The column temperature was programmed from 50 to 200°C at 4°C × min^{-1}. The carrier gas was helium with a flow rate set at 1.5 mL min^{-1}. The split injection mode (split ratio: 1/70, flow: mL min^{-1}). The device was connected to a computer system running a mass spectra library NIST 98.

Evaluation of the antibacterial activity of essential oils of *T. zygis* subsp .*gracilis*

Tested Bacteria strains

All tested bacteria strains in this study belong to the collection of the INRA Laboratory of Bacteriology and Plant biocontrol (Meknès, Morocco). They were chosen for their high pathogenicity (Table 1).

Table 1. Bacterial strains tested-Bacterial strains tested.

Bacterial strains	Gram (+/-)	Degree of Virulence *
Erwinia amylovora	-	Very high on slices of pear
Erwinia carotovora subsp. *carotovora*	-	Very high on Tuber soft rot
Erwinia carotovora subsp. *atroseptica*	-	Very high on Tuber soft rot
Erwinia chrysanthemi	-	Very high on Tuber soft rot
Streptomyces scabies	+	High, flat surface scabs on tubers
Streptomyces europascabiei	+	High, flat surface scabs on tubers

* Extent and severity of symptoms (rot, damage and necrosis).

These were *Erwinia amylovora* (strain Ea1266-9), *Erwinia carotovora* subsp. *carotovora* (strain Ecc1302), *Erwinia carotovora* subsp. *atroseptica* (strain Eca1303), *Erwinia chrysanthemi* (strain Ec1304), *Streptomyces scabies* (strain Ss1226) and *Streptomyces europascabiei* (strain Ses1225).

Strain maintenance was done by sampling the stock strains and two successive subcultures before each series of tests. Erwinia strains were maintained on yeast peptone glucose agar medium (YPGA) for 2 days at 28 °C, while *Streptomyces* species were grown on yeast malt extract glucose agar (YMA) nutrient medium for 7 days at 30°C.

Microbiological procedure

The study of antimicrobial activity consisted in determining the antibacterial parameters MIC (minimum inhibitory concentration) and MBC (minimal bactericidal concentration) of essential oils of *T. zygis* subsp. *gracilis*. For its implementation, we used broth macro-dilution method[33].

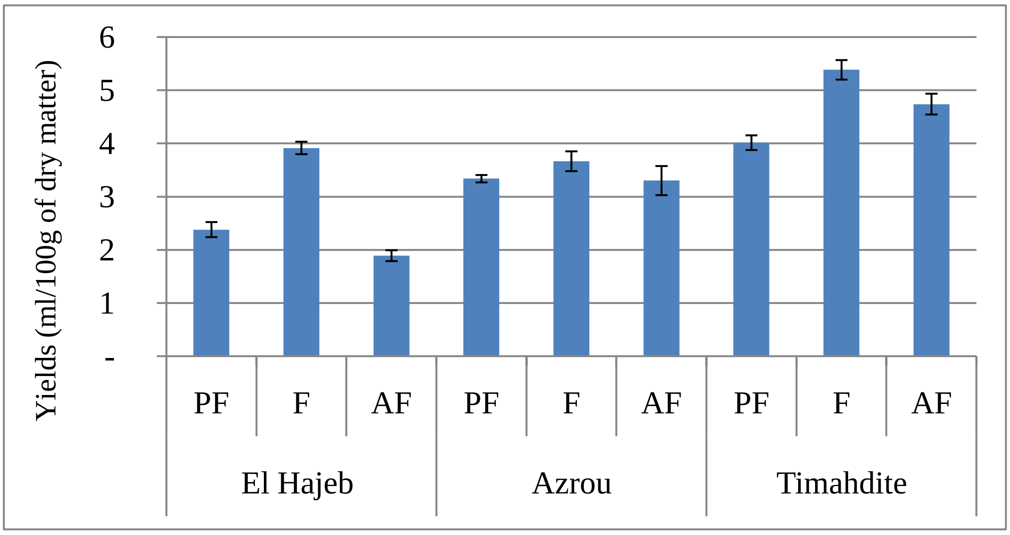
From pure cultures (18 to 24 hours for *Erwinia* and 7 days for *Streptomyces*), bacterial suspensions with 10^9 CFU (colony forming units) mL^{-1} were prepared in 5 mL-sterile-saline solution (0.85% NaCl) at a pH between 5.5 and 7.0. After vortexing, they were inoculated in LPG (yeast, peptone, glucose) broth for *Erwinia* and YMB (yeast, malt extract, glucose) broth for *Streptomyces* to obtain about 10^6 and 10^8 UFC mL^{-1} turbidity respectively by measuring the optical transmission (OD at 620 nm). These solutions represented the inocula used throughout the study. The essential oils were then diluted in 10% DMSO dimethyl sulfoxide. Serial dilutions of each essential oil were performed in a series of tubes containing the inoculum. Final concentrations obtained were 5000, 1000, 500, 100, 50 and 10 $\mu g.mL^{-1}$. Control tubes did not contain any essential oil. The negative control was stored at 4 °C, whereas the positive control was incubated together with the tubes containing different concentrations of essential oils. Streptomycin were used also as a positive control. Three repetitions were performed for each dilution and each experiment was repeated three consecutive times.

The tubes were incubated for 2 days at 28°C for *Erwinia* and 7 days at 3°C for *Streptomyces*. Results reading was performed with naked eye in daylight. Environment clarity implies antimicrobial effect of the tested essential oil, whereas cloudiness shows its ineffectiveness (a sign of bacterial growth). The MIC corresponds to the lowest concentration of essential oil at which no visible growth is observed compared to controls without essential oils. A volume of 100 µl of all tubes showing no turbidity was grown on LPGA medium and incubated at 28 °C for 3-7 days. The CMB indicates the lowest concentration of essential oil at which we obtain at most 0.01% of survivors from the initial inoculum bacteria (i.e., a maximum of 1 bacterium per 10 000 bacteria inoculated).

Results and Discussion

Yields of essential oils

Average yields of essential oils obtained from samples of *T. zygis* subsp. *gracilis* collected in the three regions studied before, during and after flowering ranged between 1.64 and 5.98%. They varied between regions and within the same region depending on the plant phenological stage. In all three locations, the highest concentrations of essential oils were recorded during the period of full bloom, whereas in the other two phenological stages yield were relatively low quantity of essential oils. The samples from Timahdite were richest in essential oils (Figure 1).



PF: Before flowering F: Flowering; AF: After flowering

Figure 1. Yields (mL/100 g of dry matter) of essential oils of *Thymus zygis* subsp. *gracilis* from Middle-Atlas.

Comparison of our results with literature data shows that the species *T .zygis* subsp. *gracilis* form Moroccan Middle Atlas has far greater yields than those reported by other authors. Indeed, Moldão-Martins[34] studying the essential oils of the species *T. zygis* in northern Portugal showed that highest levels of essential oils were recorded during the period of full bloom (0.9-1.4%) and the lowest in the post flowering stage (about 0.15%). In South Iberian Peninsula, populations of *Thymus zygis* showed a maximum yield at the full bloom stage between 3.31 and 5.19% and from 1.54 to 5.05% in the fruiting stage[35,36].

Chemical composition of essential oils.

The results for the chemical composition of essential oils extracted from leaves and inflorescences of species *T. zygis* from Moroccan Middle Atlas before, during and after flowering are displayed in Table 2. Analysis of the chemical composition shows quantitative and qualitative changes in the chemical profile of essential oils. Twenty-three individual components were detected. These compounds fall under at least four chemical families whose relative importance varies with the region and the phenological stages. Monoterpenes, the most abundant compounds, constitute 61.38% to 94.91% of essential oils. The oxygenated monoterpenes are predominant in Timahdite oils compared to those of El Hajeb and Azrou (65.74% to 78.95%), whereas the hydrocarbon monoterpenes were dominant in El Hajeb and Azrou essences in comparison with those of Timahdite (Figure 2).

Table 2. Percentage of peak’s areas of chemical compounds in essential oils of *Thymus zygis* subsp. *gracilis* from Moroccan middle Atlas.

Identified Compound	RI	Content (%)								
		El Hajeb			Azrou			Timahdite		
		PF*	F	AP	PF	F	AP	PF	F	AP
monoterpenes		90.34	94.04	94.91	91.38	93.06	61.38	93.68	94.5	94.33
monoterpenes		44.66	34.32	55.61	44.65	38.5	20.58	27.94	20.41	15.38
α-tujene	930	0.9	1.14	1	1.15	1.09	-	1	1.13	0.78
α-pinene	939	0.62	0.63	1.46	0.67	0.76	-	0.47	0.55	0.47
camphene	954	0.67	0.44	1.92	0.48	0.49	-	0.21	0.16	0.15
sabinene	975	-	0.19	0.36	-	0.15	-	-	0.15	-
β-pinene	979	1.24	2	1.27	2.29	2.17	-	0.94	0.9	0.71
α-pellandrene	1002	-	0.2	-	0.28	0.21	-	-	-	-
α-terpinene	1017	1.82	1.82	0.86	2.46	1.59	-	1.89	1.42	0.86
p-cymene	1024	19.25	13.38	40.26	15.32	20.74	17.9	7.59	6.97	7.77
γ-terpinene	1059	20.16	14.52	8.48	22	11.3	2.68	15.84	9.13	4.64
oxygenated monoterpenes		45.68	59.72	39.3	46.73	54.56	40.8	65.74	74.09	78.95
cis-sabinene hydrate	1070	0.93	0.76	0.67	1.11	0.88	-	1.13	1.01	0.66
terpinolene	1088	-	-	0.18	-	-	-	-	-	-
linalool	1096	2.43	1.4	3.67	1.93	1.64	-	1.66	1.32	1.2
borneol	1169	1.04	0.52	3.39	0.73	0.68	-	0.31	0.22	0.27
terpinene-4-ol	1177	0.61	0.66	1.33	0.4	0.61	-	0.46	0.44	0.48
thymol	1290	2.77	5.64	7.49	26.49	32.46	20.06	1.47	3.59	1.83
carvacrol	1299	37.9	50.74	22.57	16.07	18.29	20.74	60.71	67.27	74.33
carvacrol acetate	1372	-	-	-	-	-	-	-	0.24	0.18
sesquiterpenes		5.37	5.13	2.31	5.34	4.22	3.18	5.54	3.68	2.56
β-caryophyllene	1419	4.84	4.67	2.09	4.62	3.97	3.18	4.97	3.45	2.36
α-humulene	1452	-	-	-	-	-	-	0.12	-	-
cis-cadina-1(6),4-diene	1463	-	0.17	-	-	-	-	-	-	-
γ-gurjunene	1477	0.32	-	-	0.44	-	-	0.21	-	-
δ-cadinene	1523	0.21	0.29	0.22	0.28	0.25	-	0.24	0.23	0.2
oxygenated		1.31	0.76	2.45	0.99	1.21	-	0.45	0.42	0.53
caryophyllene oxide	1583	1.31	0.76	2.45	0.99	1.21	-	0.45	0.42	0.53
Total		97.02	99.93	99.67	97.71	98.49	64.56	99.67	98.6	97.42

*: PF: Before flowering F: Flowering; AF: After flowering, -: absence.

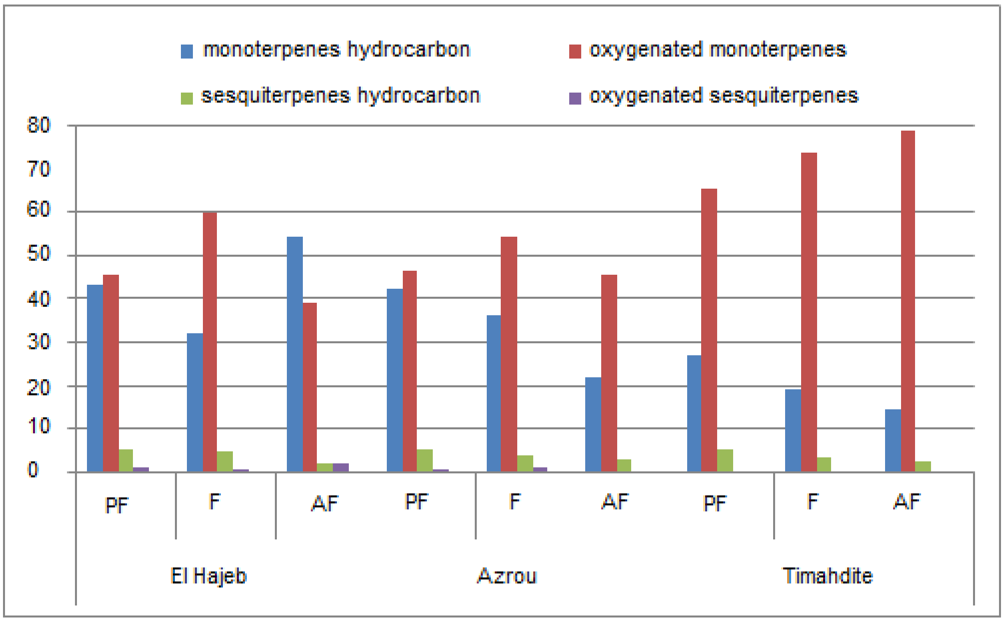


Figure 2. Percentage of peak's areas of chemical compounds in essential oils of *Thymus zygis* subsp. *gracilis* in middle Moroccan Atlas.

In terms of major compounds, essential oils of *T. zygis* subsp. *gracilis* consisted mainly on carvacrol (16.07 to 74.33%), thymol (1.47 to 32.46%), p-cymene (6.97 to 40.26%) and γ -terpinene (2.68 to 22%) (Table 3).

Table 3. Variation of chemical compounds in three chemical profiles of *Thymus zygis* subsp. *gracilis* from Middle-Atlas.

chemotypes	stadiums	components				
		p-cymene	γ-terpinene	thymol	carvacrol	β –caryophyllene
El Hajeb (C/pC/γT)	PF*	19.25	20.16	2.77	37.9	4.84
	F	13.38	14.52	5.64	50.74	4.67
	AF	40.26	8.48	7.49	22.57	2.09
Azrou (T/C/pC/γT)	PF	15.32	22	26.49	16.07	4.62
	F	20.74	11.3	32.46	18.29	3.97
	AF	17.9	2.68	20.06	20.74	3.18
Timahdite (C)	PF	7.59	15.84	1.47	60.71	4.97
	F	6.97	9.13	3.59	67.27	3.45
	AF	7.77	4.64	1.83	74.33	2.36

*: PF: Before flowering F: Flowering; AF: After flowering (C / pC / γT) carvacrol / p-cymene / γ-terpinene, (T / C / pC / γT) thymol / carvacrol / p-cymene / γ-terpinene, (C), carvacrol.

Three chemical profiles could then be defined based on of the nature of the dominant monoterpene compound[37,38,39] (Figure 3).

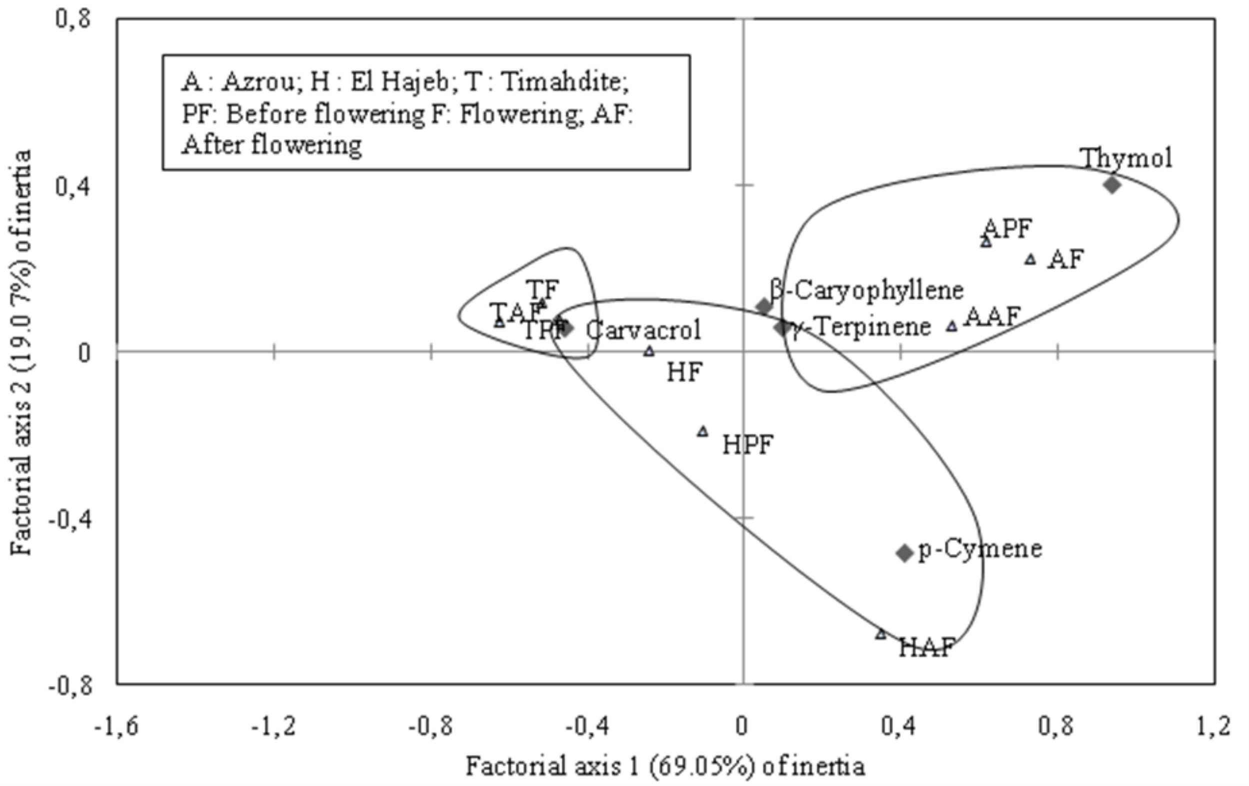


Figure 3. Projection areas on the factorial plane of three chemical variability of *Thymus zygis* subsp. *gracilis* from Middle-Atlas.

The chemical variability (carvacrol / p-cymene / γ-terpinene) of El Hajeb region consisted of 22.57 to 50.74% carvacrol, 13.38 to 40.26% p-cymene and 8.48 to 20.16% of γ-terpinene. The highest concentration of carvacrol is noted during flowering stage (Table 2). From flowering, the concentration of this compound decreased while concentration of p-cymene increased. However, it is known that γ-terpinene is the precursor of p-cymene, which is the precursor of thymol and carvacrol [40]. We can assume then that the bioconversion of p-cymene to carvacrol is between full bloom and early fruit ripening point beyond which γ-terpinene concentration decreases and p-cymene increases in essential oils. This is in agreement with the results published by other authors [41,42].

The chemical variability (thymol / carvacrol / p-cymene / γ-terpinene) of Azrou's region is a mixture of thymol (20.06 to 32.46%), carvacrol (16.07 to 20, 74%), and their precursors p-cymene (15.32 to 20.74%) and γ-terpinene (2-68 - 22.00%). It can be considered as an intermediate chemical variability corresponding to the biosynthesis of the two major phenolic compounds, thymol and carvacrol. This is explained by the simultaneous bioconversion of p-cymene to carvacrol and to its isomer thymol [43].

The chemical variability (carvacrol) of Timahdite region contains mainly carvacrol (60.71 to 74.33%), γ-terpinene (4.64 to 15.84%) and p-cymene (6.97 to 7.77%). Carvacrol concentration is high during the entire growth cycle especially after flowering.

Previous work on changes in the chemical composition of Moroccan *T.zygis* subsp. *gracilis* essential oils revealed the existence of different chemical variability. The chemical variability of Khénifra (about 900 m above sea level in the Middle Atlas), is high in the monoterpene hydrocarbon p-cymene (50.6%), and contains, in addition, carvacrol (8.1%), borneol (5.8%) and thymol (5%) [44] .The chemical variability of Ozoud-Azilal (about 1100 m of altitude in High Atlas), is a mixed profile: thymol (34.07%) / borneol (25.28%) associated with linalyl propionate (12.50%) [24]. In contrast, Azrou chemical profile is thymol-rich (30.7%), p-cymene (23.3%) and caryophyllene oxide (9.8%) [22]. It is clear from these studies that there are six chemical variabilities for Moroccan *T .zygis subsp. gracilis*. We may assume that other chemical profiles can be discovered.

The chemical variability found in this study suggests a real correlation between essential oil components and bioclimatic variables: abiotic (microclimate, altitude, soil) and biotic environment [27,28,45,46]. The predominance of phenolic profiles in essential oils studied indicates, with respect to the biosynthesis of various constituents of the aromatic fraction, a preponderance of the terpenoids way over the phenylpropanoid [47]. Non-phenolic monoterpenes are less expensive to produce than phenolic ones because they are found at the beginning of the biosynthetic pathway [38,48,49] . Typical Mediterranean summer drought climate, seems to be an important selective factor in this region leading to the dominance of phenolic forms. Phenolic compounds are more tolerant to water stress and therefore able to better resume growth in the fall. In fact, phenolic populations grow on dry, shallow and iron-oxide-rich soils. Carvacrol chemotype is found only in the driest stations, while thymol chemotype seems less specialized [28].

Antibacterial activity of essential oils

MIC and MBC results (Table 4 and 5)

Table 4. Minimum inhibitory concentrations (MIC in µg mL^{-1}) of essential oils of *T. zygis* subsp. *gracilis.*

bacterial strains	El Hajeb				Azrou				Timahdite			
	PF*	F	AF	PFA	PF	F	AF	PFA	PF	F	AF	PFA
S. scabies	50	50	100	ND	50	50	50	ND	50	50	50	50
S. europascabiei	50	50	100	10	50	50	50	50	50	50	50	50
E. amylovora	100	100	100	50	100	50	50	50	100	100	100	50
E. carotovora	1000	500	500	500	500	500	500	500	500	500	500	500
E. atroseptica	500	500	500	500	500	500	500	500	500	500	500	500
E. chrysanthemi	500	500	500	500	500	500	500	500	500	500	500	500

*: PF: Before flowering F: Flowering; AF: After flowering, PFA: Mixture of three stages, ND: Not tested

MIC and MBC analyses show a high bactericidal quality of the essential oils against the different phytopathogenic tested strains, except for Gram-negative bacteria, *E. carotovora*, *E. atroseptica* and *E. chrysanthemi* which show a relative resistance. The greater sensitivity of Gram positive bacteria toward essential oils compounds has already been observed by several authors[50,51].

Moreover, essential oils of Timahdite (carvacrol profile) remain the most effective compared to other chemical profiles against all strains tested and especially against Gram-positive bacteria. Indeed, at a concentration greater or equal to 50 µg mL^{-1}, this chemical profile had bactericidal activity at the three phenological stages against *S. scabies.* The same concentration leads to bactericidal activity after flowering against *S. europascabiei*, while for the two other stages, bactericidal concentration rises to100 µg mL^{-1}. With Gram-negative bacteria, this chemical profile is bactericidal at a concentration greater or equal to 500µg mL^{-1} except against *E. amylovora* whose bactericidal dose is 100µg mL^{-1}. However, it should be noted that the combination of essential oils from the three phenological stages is bactericidal at 50 µg mL^{-1} against *Streptomyces* and *E. amylovora* (Table 5).

Table. 5. Minimum bactericidal concentrations (MBC in µg mL^{-1}) of *T. zygis* subsp. *Gracilis* essential oils

bacterial strains	El Hajeb				Azrou				Timahdite			
	PF*	F	AF	PFA	PF	F	AF	PFA	PF	F	AF	PFA
S. scabies	100	100	100	ND	100	50	100	ND	50	50	50	50
S. europascabie	100	100	500	100	100	50	100	50	100	100	50	50
E. amylovora	100	100	100	100	100	100	100	50	100	100	100	50
E. carotovora	1000	1000	1000	1000	1000	1000	1000	1000	500	500	500	500
E. atroseptica	1000	1000	1000	1000	1000	1000	1000	1000	500	500	500	500
E.chrysanthemi	1000	1000	1000	500	500	1000	500	500	500	500	500	500

*: PF: Before flowering, F: Flowering; AF: After flowering, PFA: Mixture of three stages,
ND: Not done

The enhanced bactericidal activity of essential oils association confirms the synergy that could come from the minor components[52].

Concerning Azrou chemical profile (thymol / carvacrol / p-cymene / γ-terpinene), essential oils from the full bloom stage (characterized by a high thymol content) and essential oils association are the most effective, especially against *Streptomyces* (MIC = MBC = 50µgmL^{-1}).The mixture of the three phenological stages is very active Gram-negative bacteria particularly against *E. amylovora* (CMB = 50 µg mL^{-1}). The effectiveness of Azrou chemical profile is probably related to the active ingredient thymol. Despite the high rate of carvacrol (60-74%) in Timahdite essential oils compared to the thymol rate in Azrou essential oils (about 32%), their antibacterial activities are approximately the same. Our results confirm those of Dorman and Deans[53] who showed that thymol is the compound that has the broadest spectrum of antibacterial activity, followed by carvacrol and α-terpineol. These authors showed that the difference in antimicrobial efficacy observed between thymol and carvacrol is due to the position of the hydroxyl group on the phenolic structure of the two molecules. The effectiveness of this chemical profile may also be due to the synergistic activity between thymol and carvacrol. These compounds are present in Azrou essential oils with 32% and 18%. The synergy between these two phenols has been observed in previous studies[54-56].

El Hajeb chemical profile (carvacrol / p-cymene / γ-terpinene) is bactericidal at 100µg mL^{-1} against *Streptomyces* and *E. amylovora* and at 1000 µg.mL^{-1} against the other gram-negative bacteria. The difference observed between the two chemical profiles activity (Timahdite, El Hajeb) appears to be directly related to the concentration of carvacrol in the essential oil. Its proportion is approximately 1.8 times higher in Timahdite chemical profile than in El Hajeb's one with an efficiency about twice better. Indeed, higher is the phenolics proportion, greater is essential oils antibacterial effectiveness[50]. However phenols are not the only responsibles for the entire activity. The whole chemical composition must be taken into account[50]. The minor compounds of essential oils might act synergistically. Thus, the value of an essential oil comes from its "totum", i.e. all its components and not only its major compounds[57].

Conclusion

For the three populations studied, the highest concentrations of essential oils were recorded during the period of full bloom, whereas in the two other phenological stages yields were relatively low. Samples from Timahdite station were the richest in essential oils. In addition, chemical composition of essential oils allowed us to identify three: profile with carvacrol, thymol / carvacrol profile, and carvacrol / p-cymene / γ-terpinene profile.

Furthermore, based on bioassay results, we can conclude without reservation that the three tested chemotypes have a great bactericidal activity against all pathogen strains tested. This important bioactivity of *T. zygis* subsp. *gracilis* essential oils is mainly attributed to their phenolic abundance with a particularity to carvacrol and thymol known for their antimicrobial properties.

However, variations in the bactericidal action of these essential oils should be related to other parameters including their of phenol proportion and the type of target microorganism (Gram positive or Gram negative). In perspective, researches on synergy occurring in essential oil mixtures may be important to improve effectiveness and to reduce costs.

Further studies are planned to determine long length antibacterial effect of essential oils formulations in order to use them as alternative to synthetic pesticides.

This study can be considered a primary source of information on chemical and antimicrobial properties of essential oils of Moroccan *T. zygis* subsp. *gracilis* against plant pathogenic bacteria.

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