

Chemical Composition and Anti-Insecticidal Activity of the Essential Oils of Thymus of Morocco: *Thymus capitatus*, *Thymus Bleicherianus* and *Thymus Satureioides*



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Abstract

Aromatic plants have an increasing advantage thanks to the valorization of their essential oils in various applications, such as antimicrobial, antifungal, antibacterial, antitoxic, insecticidal and insect repellent, etc. This work reports the valorization, as a bio insecticide, of the essential oils of three Moroccan species of thyme that belongs to the family of Lamiaceae: *Thymus bleicherianus* (TB), *Thymus capitatus* (TC) and *Thymus satureioides* (TS) extracted by hydro distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Thymol, β -Ocymene, Camphor and o-Cymene were obtained as majority compounds, with a percentage of 79.57% in the essential oil of *Thymus bleicherianus* (TB). The essential oil of *Thymus capitatus* (TC) is characterized by the presence of carvacrol and α -Terpinene as main chemical constituents with a percentage of 76.51%, and as well as the main essential oil compounds of *Thymus satureioides* (TS) are: Thymol, α -Terpinene, E- β -Ocymene, Camphor, and Borneol with a percentage of 74.79%. The chemical composition varies quantitatively and qualitatively according to the species studied, the yields obtained respectively from *Thymus bleicherianus* (TB), *Thymus capitatus* (TC) and *Thymus satureioides* (TS) are: 1.71%; 1.43% and 0.69%. Susceptibility tests carried out under laboratory conditions against the wheat pest *sitophilus oryzae*, this bio activity is mainly due to the richness of these species in carvacrol and thymol which are known for their effectiveness against pests.

Introduction

Pests are a serious problem in grains during storage and its derived industry [1]. Indeed, according to Scherrer [2], Morocco is one of the Mediterranean countries with a long medical tradition and traditional know-how based on medicinal plants. In addition, *Thymus* is one of the most important genera in number of species in the family Lamiaceae (lamiaceae) including about 215 species [3], and includes many varieties. In Morocco, the species of the genus *Thymus* (from the Greek name: Thumos, coming from the Greek word (theo) perfume or fragrant plant) have several nominations (in Amazigh: Azukni, Tazuknite, in Arabic: Ziitra) and represented by 21 species including 12 endemic [4]. The aromatic and medicinal properties of thymus are of great importance, since its essential oil is endowed with antibacterial, antifungal and insecticidal activities. ; and as a result he made one of the most popular plants in the world. The

objective of this work is the characterization of the chemical composition of the essential oils of *Thymus bleicherianus* (TB), *Thymus capitatus* (TC) and *Thymus satureioides* (TS), and the study of the insecticidal activity of the essential oils of these three species of thyme vis-à-vis the wheat pest *sitophilus oryzae*.

Material and Methods

Plant Material

Thymus Bleicherianus (TB): *Thymus bleicherianus* is a species of North Africa. It is endemic to Morocco and Algeria. In Morocco, this species develops in the thermo-Mediterranean stage, in temperate and hot semi-arid and subhumid bioclimates. It is found in the regions of Meknes and Oujda (Tafogalt Forest), respectively in central and eastern Morocco [5].

Thymus Capitatus (TC): *Thymus capitatus* is a species of the Mediterranean basin. In Morocco, it is found in the south and west of the city of Tetouan (northern Morocco), in the temperate bioclimate; it develops in the floor of thermo-Mediterranean vegetation [6].

Thymus Satureioides (TS): The *Thymus satureioides* It is an upright shrub, up to 60 cm tall with many branches. The leaves are spatulate, the inflorescence in loose glomeruli, the corolla is pink or pale pink. The *Thymus satureioides* is an endemic species of Morocco. It is widespread in forest clearings, scrub and matorrals of low and medium mountains up to 2200m altitude, on siliceous limestone substrates and rocky soils more or less earthy but well drained [7].

Collection of Samples

Specimens from the aerial part (stems, leaves and flowers) of *Thymus bleicherianus* (TB), *Thymus capitatus* (TC) and *Thymus satureioides* (TS) were harvested from cooperatives in August (2017) respectively in the regions of Meknes (central Morocco), Tetouan (northern Morocco) and Agadir (south-west). These three species have been verified by a botanist at Forest Research Center, Kenifra, Morocco.

Extraction of Essential Oil

The collected samples are dried for ten days in the open air and in the shade. Extraction of the HE from these samples was performed by hydro-distillation in a Clevenger type apparatus. Three distillations were carried out for each sample, for three hours, of 250 g of the aerial part (stems, leaves and flowers) dried, with 11 of water in a flask surmounted by a column and a refrigerant, the species less dense water are collected by simple decantation and dried over anhydrous sodium sulphate (Na_2SO_4) before analysis.

Performance Calculation

The yield of essential oil (expressed as a percentage) is calculated by the ratio between the weight of the oil extracted and the weight of the plant material used [8]. The yield of essential oil was determined relative to the dry matter, evaluated from 100 g dried in an oven for 48 h at 60 °C. The essential oil obtained is stored at a temperature of 4 °C in the dark.

$$\text{Yield}(\%) = \left(\frac{V}{ms} \times 100 \right) \pm \left(\frac{\Delta V}{ms} \times 100 \right)$$

V: volume of essential oil collected; ΔV : error on reading; ms: mass of vegetable matter in the dry state.

Gas Chromatography (GC) / Mass Spectrometry (MS)

The analysis of the essential oils was carried out by gas chromatography coupled with mass spectrometry (GC-MS). Coupling was performed on a Hewlett-Packard model 5970 (quadrupole detection system), equipped with a fused silica capillary column of 2mm \times 0.23mm DB1 type; temperature programming from 50°C to 200°C, with a gradient of 5°C min⁻¹.

The retention indices were determined by gas chromatography on two fused silica capillary columns (25m \times 0.25mm) of the type OV-101 and Cabowax 20 M, with temperature programming identical to that used for the coupling. (Shimatzu GC-14A equipped with a flame ionization detector and a C-R4A model integrator).

Insecticide Tests

The wheat weevil *sitophilus oryzae* is a species of Coleoptera insects of the family Curculionidae and one of the pests of the infestation and destruction of stored grain. The insects studied: The wheat weevil *sitophilus oryzae* is a species of Coleoptera insects of the family Curculionidae and one of the pests of the infestation and destruction of stored grain. The insects were raised on soft wheat in one-liter capacity plastic boxes, transparent and screened. The whole is placed in an enclosure whose temperature is 30°C and the relative humidity is 70%. The insecticidal tests used in this study are those reported by Hamraoui and Regnault-Roger [9]; in an experimental chamber containing twenty insects, the essential oils in increasing concentrations from $1.7 \times 10^{-2} \mu\text{l}/\text{cm}^3$ to $12 \times 10^{-2} \mu\text{l}/\text{cm}^3$ on Whatman # 1 paper. The assembly is introduced into a fumigation chamber included in the experimental chamber (semi-ventilated). Three repetitions were performed for each concentration. The number of dead insects is recorded after 1 day, then living insects are placed in 40 g of wheat grains and the count of dead adults is done after 5 days. Corrected mortality in treated insects is expressed according to Abbot's formula.

$$LC = \left(\frac{(L0 - L1)}{(100 - L1)} \times 100 \right)$$

LC = Corrected Mortality; L0 = Mortality observed in insects; L1 = Mortality observed in controls.

Results and Discussion

Yields of Essential Oils

The average yield of essential oil of each species was calculated according to the dry plant material obtained from the aerial parts (stems, leaves and flowers) of the plants studied. The yield of essential oil obtained is given in Table 1. The average yields of essential oils were calculated according to the dry plant matter of the aerial part of the plant. *Thymus bleicherianus* (TB) samples yielded a rate of approximately 1.71% higher than that obtained from *Thymus capitatus* (TC) which is 1.43%, and *Thymus saturated* (TS) which is 0.69%. The rate of *Thymus bleicherianus* (TB) is almost identical to that obtained by El Ajjouri M [5] against the *Thymus capitatus* (TC) is lower and that is respectively 1.75% and 2.05% for the *Thymus satureioides* (TS) the rate of return obtained by Belmalha S [10] is higher which is 2.71% therefore it can be concluded that several factors could be at the origin of these variations: the age of the plants [11,12], that of the tree [13], the nature of the soil and the climate [14,15], the part of the plant subject to extraction and the period of harvest.

Table 1: Yield in essential oils.

Sites	Species	Yield
Regions of Meknes	Thymus bleicherianus (TB)	1.71 %
Regions of Tetouan	Thymus capitatus (TC)	1.43 %
Regions of Agadir	Thymus satureioides (TS)	0.69%

Chemical Composition

The gas chromatographic analysis results coupled with the mass spectrometry of the essential oils of the plants studied are shown in (Table 2). Chromatographic analyzes of essential oils identified 38 compounds which represent approximately (99.1%) for Thymus bleicherianus (TB), for Thymus capitatus

(TC) 35 compounds which represent approximately (100%) and for Thymus satureioides (TS) 37 compounds (99.54%) (Table 2). The analysis of the results given in Table 2 showed all of the following results: Thymol, β -Ocimene, Camphor and o-Cymene were obtained as majority compounds, with a percentage of (79.57%) in essential oil of Thymus bleicherianus (TB). The essential oil of Thymus capitatus (TC) is characterized by the presence of carvacrol and α -Terpinene as main chemical constituents with a percentage of (76.51%), and as well as the main compounds of Essential oil of Thymus satureioides (TS) are: Thymol, α -Terpinene, E- β -Ocimene, Camphor, and Borneol with a percentage of (74.79%).

Table 2: Chemical composition of the essential oils of Thymus capitatus (TB), Thymus capitatus (TC) and Thymus satureioides (TS).

Identification	Thymus Bleicherianus (TB)	Thymus Capitatus (TC)	Thymus Satureioides (TS)
γ -Terpinène	1.24	0.67	1.03
α -Terpinène	3.99	10.56	5.98
α -Ocimene	0.22	-	2.15
Camphène	1.12	0.98	0.80
β -Ocimène	21.96	8.58	0.19
β -Pinène	-	0.31	-
p-cymène	1.55	2.31	0.99
carvacrol	2.48	65.96	3.33
α -terpinolene	0.05	-	0.16
o-Cymene	8.65	0.41	2.06
Limonene	0.19	0.09	-
Eucalyptol	1.00	0.74	0.62
Sabinène	0.03	0.42	-
Linalool	0.09	0.02	0.10
terpinolene	0.02	0.05	0.08
β -Linalool	0.46	0.33	-
Borneol	0.86	0.65	2.80
Terpinène-4-ol	0.50	0.10	0.74
3-cyclohexene-1-carbinol	0.03	-	0.01
α -humulène	-	-	0.44
Thymol méthyle ether	-	0.04	0.02
Isothymol methyl ether	0.01	-	0.21
Thymol	36.85	0.67	29.06
E- β -Ocimène	1.35	1.33	11.30
α -Bisabolol	0.21	-	0.01
Caryophyllene	1.06	0.16	3.29
α -Sinensal	0.01	0.09	-
Acétate de bornyle	-	-	0.05
Geranyl acetate	-	0.03	-
Copaene	-	0.01	-
Camphre	12.11	0.85	14.64
α -Farnese	-	-	0.09
α -Bisabolol	0.01	0.05	0.03

Bornéol	1.40	0.99	13.81
Myrtenol	0.05	-	-
γ -Elemene	-	-	0.01
Acétate de géranyle	0.01	-	-
α -Copaène	-	0.11	-
β -Bourbonéne	0.01	0.03	0.01
β -Elemene	0.05	-	0.09
α -Grujunene	0.01	-	-
α -Amorphene	-	0.06	0.01
α -Patchoulène	-	0.05	-
Aromadendrène	0.05	0.05	-
β -caryophyllène	1.16	0.09	3.6
Valencène	-	-	0.05
Germacrène D	-	2.73	0.13
Eremophilene	0.40	-	-
Junipène	0.09	0.01	-
β -himachaléne	0.06	0.01	0.01
caryophyllénoxyde	0.56	-	1.99
myrcène	-	-	0.11
Total	99.9	99.54	100

The results of Zayyad N [16], with regard to *T bleicherianus*, the major compounds are thymol (55.9%), α -terpinene (13.19%), E- β -ocymene (10.43%) and carvacrol (2.71%) but *T bleicherianus* studied by El Ajjouri M [5], with the major compound α -terpinene (42.23%) followed by thymol (23.95%). For the *Thymus capitatus*. The results of Hilan C [17], carvacrol (70.92%) are the predominant compound of gasoline, while the concentration of thymol is 23.9% in El Ajjouri M [5]. For *Thymus capitatus* (Tunisia), carvacrol (68.8%) is the main component of the oil, followed by p-cymene (11.1%) and γ -terpinene (8.6%) [18]. The essential oil of *Thymus capitatus* from Greece is richer

in carvacrol with a higher 80% [5]. The thymes of Spain, Morocco, Tunisia and Greece are carvacrol chemotype. So the thymes of the region of the Mediterranean basin are carvacrol thymes. The results of the analysis of essential oils of El Ouali Lalami A [19] show that the major components of *Thymus satureioides* (TS) are p-cymene (27.59%) and thymol (14.09%). Variations in chemotype and chemical polymorphism of essential oils between the three plants may be due to various conditions, environment, genotype, geographical origin, harvest period and vegetative stage of the plant [20].

Biological Tests

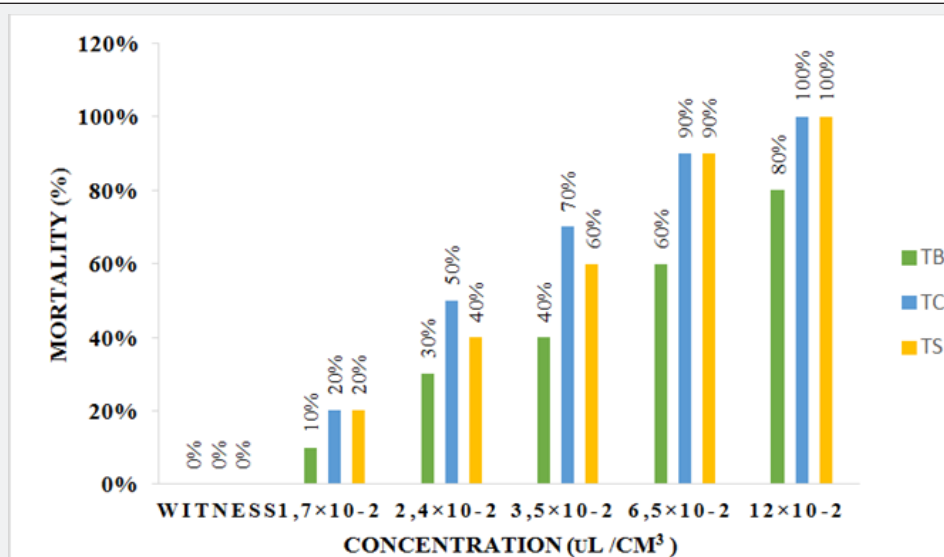


Figure 1: Toxicity of essential oil after 1 day for treatment.

The insecticidal activity of the essential oils studied against *Sitophilus oryzae* was evaluated by the Whatman No.1 paper contact toxicity test, the results of the insecticide tests of three essential oils showed a very important insecticidal activity during the first day of treatment (Figure 1). The degree of activity of these essential oils varies according to the species studied. According to (Figure 1), all essential oils tested at concentrations ranging from $1.7 \times 10^{-2} \mu\text{l}/\text{cm}^3$ to $12 \times 10^{-2} \mu\text{l}/\text{cm}^3$, showed significant toxicity to *Sitophilus oryzae* adults and clearly see that the higher the concentration increases the toxicity of the essential oil is important.

After a five-day contact (Figure 2), *Sitophilus oryzae* is completely destroyed at a dose of $2.4 \times 10^{-2} \mu\text{l} / \text{cm}^3$ for the three

essential oils. This insecticidal activity of essential oils is probably due to the major constituent : Thymol, β -Ocymene, Camphor and α -Cymene in the essential oil of *Thymus bleicherianus* (TB) (79.57%), carvacrol and α -Terpinene in the essential oil of *Thymus capitatus* (TC) (76.51%), Thymol, α -Terpinene, E- β -Ocymene, Camphor, and Borneol for the essential oil of *Thymus satureioides* (TS) (74.79%). The results obtained show that the essential oils tested have an interesting toxicity against *Sitophilus oryzae*. Several works have demonstrated the insecticidal activity of the essential oils of the species of the genus *Thymus* vis-à-vis some insect pests of stored foodstuffs, in particular *Tribolium castaneum* (Herbst.), *Sitophilus oryzae* (L.) and *Rhizopertha dominica* [21,22].

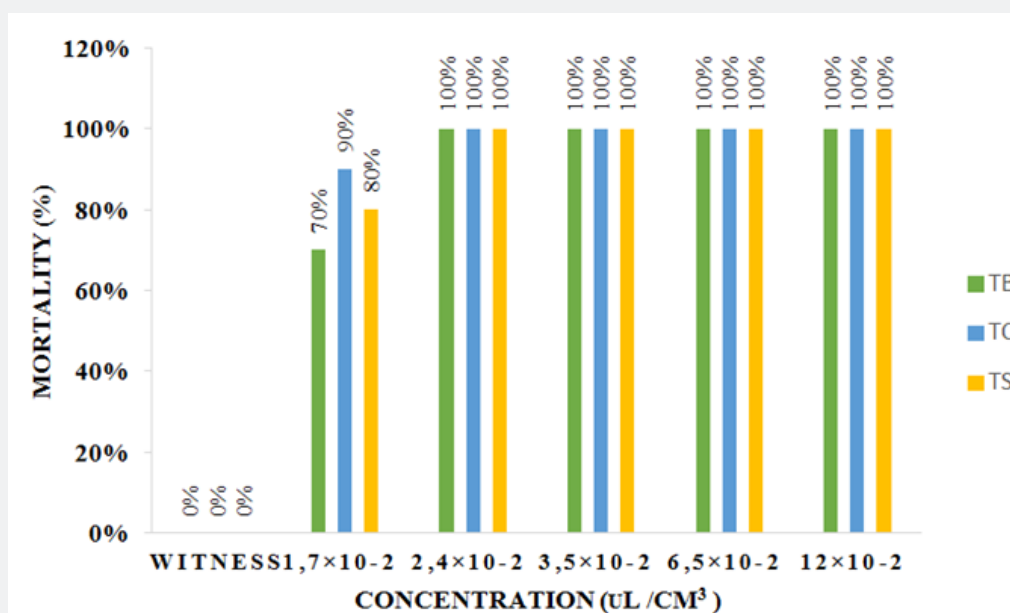


Figure 2: Toxicity of essential oil after 5 days for treatment.

Conclusion

The yield of essential oil of the plants studied (*Thymus bleicherianus* (TB), *Thymus capitatus* (TC) and *Thymus satureioides* (TS)) is acceptable. The essential oil of *Thymus bleicherianus* (TB) is rich in Thymol, β -Ocymene, Camphor and α -Cymene (79.57%). The most abundant chemical compounds in the essential oil of *Thymus capitatus* (TC) are carvacrol and α -Terpinene (76.51%) and for *Thymus satureioides* (TS) the presence of Thymol, α -Terpinene, E- β -Ocymene, Camphor, and Borneol (74.79%). The preliminary results of the insecticide study showed a very important activity for the first day of treatment and the toxicity of the essential oils tested varies widely depending on the nature of the essential oil, the age of the plants, the nature of the soil and the climate and the harvest period.

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