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Comparative Insecticidal Activity of *Mentha pulegium* L. and *Thymus capitatus* Hoff. Et Link. Essential Oils against the Red Flour Beetle: *Tribolium Castaneum* (Herbst) (Coleoptera, Tenebrionidae)

Abessi Mohamed[1,2], Bachrouch Olfa[1*,3], Jouda Mediouni Ben Jemâa[4], Soumaya Haouel Hamdi[4] and Abderrabba Manef[1]

[1]Laboratoire Matériaux, Molécules et Applications (LMMA), Institut Préparatoire aux Études Scientifiques et Techniques (IPEST), Université de Carthage, La Marsa, Tunisia
[2]Faculté des sciences de Tunis, Campus Universitaire El-Manar, 2092 El Manar Tunis, Tunisia
[3]Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie (INRAT), Université de Carthage, Ariana, Tunis, Tunisia
[4]Laboratoire de Biotechnologie Appliquée à l'Agriculture, Institut National de la Recherche Agronomique de Tunisie (INRAT), Université de Carthage, Ariana, Tunis, Tunisia

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ABSTRACT

In this work, we have investigated chemical composition and insecticidal effectiveness of Mentha pulegium and Thymus capitatus areal parts essential oil from Tunisia against the major stored grain pest: Tribolium castaneum. There are qualitative and quantitative differences in the chemical compositions of the two studied oils. GC/MS analysis showed that Pulegone (39.1541), Menthone (35.6612%), Piperitenone (3.9858%); piperitone (3.5599%) and Isopulegone were the major compounds of Mentha pulegium essential oil. Whereas, Thymus capitatus essential oil was characterized by Thymol (76.50%), Carvacrol (3.18 %), p-cymene (3.1788%) and β- caryophyllene (2.786%) as major compounds. Results revealed that both studied oils exhibited fumigant toxicity potential. The fumigant tests showed that Mentha pulegium essential oil was more toxic than Thymus capitatus essential oil toward Tribolium castaneum. The corresponding LC50 and LC95 values were respectively 33.740- 159.06 µL/L air for Mentha pulegium and 377.34-1009.83 µL/L air for Thymus capitatus. Results of this study support the use of Mentha pulegium and Thymus capitatus essential oils as fumigant bioinsecticide against stored product pests of economic importance.
Keywords: Essential oil; Fumigant; *Tribolium castaneum*; IPM

INTRODUCTION

Cereal grains are the most important basis of food in Tunisia and Mediterranean counties. It represents a major source of vitamins, roughages and a source of energy of high biological value. However, cereal grains are attacked by many insect pests in storage. So, infestation of stored grains by in insect pests is of serious concerns as it's among the factors determining their quality at the global market. In this context, insect attacks induce direct feeding damage like reducing grain weight, nutritional value and promoting germination of stored grain [1]. Infestation also causes contamination, mold growth and odor.

The red flour beetle: *Tribolium castaneum* (Herbst, 1797) (Coleoptera, Tenebrionidae) is a worldwide major insect pests attacking grains in mills [2,3]. The use of chemical pesticides is an effective strategy commonly used in daily life [4]. Keeping in view the negative effects of synthetic fumigants, the use of botanical pesticide extracted from plants is an alternative for management of stored grains. Natural pesticides are promising in that they are effective, have least mammalian toxicity, environment friendly, easily biodegradable and have low risk for resistance development [5].

Mentha pulegium is a spontaneous herbs belonging to the Lamiaceae family. It's commonly known as pennyroyal and growing in humid areas of the plains and mountains. In Tunisia, it's commonly known under the Arabic name of "Fliou". In folk medicine, this herb is used for their therapeutic and biological activities such as antispasmodic, antihaemorrhoidal, diuretic, sedative, antiseptic, expectorant, antitussive, antimicrobial and insecticidal proprieties [6-8]. *Thymus capitatus* L. (Lamiaceae) is a perennial herbaceous endemic plant commonly known under the arabic name of "Zaâtar". Thus, areal parts are used as a flavoring spice in cooked food [9].

The target of this research was to investigate chemical composition of *Mentha pulegium* and *Thymus capitatus* essential oils in order to study their fumigant toxicity potential against *Tribolum castaneum*.

MATERIAL AND METHODS

Insect Rearing

The red flour beetle: *Tribolium castaneum* were reared on wheat flour. The cultures were maintained in the rearing conditions: darkness in 25± 1°C and 65±5°C (Relative Humidity). In fact, adult insects are used in fumigant toxicity tests.

Plant Material

Aerial parts of *Thymus capitatus* and *Mentha pulegium* were collected respectively in March 2016 and July 2016 from the region of Kbouch, Kef (36° 10′ 56″ North, 8° 42′ 53″ East) both situated in the North-Ouest of Tunisia. The plant material was botanically characterized by Professor Smaoui (Borj Cedria Biotechnology Center, Tunisia) according to the morphological description presented in Tunisian flora [10]. Leaves were randomly collected and air dried at 20-25°C for three weeks and stored in cloth bags.

Essential Oil Extraction

Dried material was subjected to hydrodistillation (250g of each sample in 3 L of distilled water) using a modified Clevenger type apparatus for 3h. The essential oil were dried over anhydrous sodium sulfate and stored in the dark.

Chemical Analysis

Three experiments were designed to determine the essential oil yields. These latest were calculated according to dry weight of plant materials. Chemical composition were analyzed using an Agilent Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30m x 0.25mn, film thickness 0.25 µm; Agilent technologies, Little Falls, CA, USA). Temperatures of injector and detector were set respectively at 220°C and 290°C. The temperature of column was programmed from 80°C to 220°C at a rate of 4°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. Helium was used as carrier gaz with a flow rate of 1.0 ml/min. an oil sample of 1.0 µl was injected, using split mode (split ratio, 1:100). A built-in data-handling program provided by the manufacturer of the gas chromatograph was used in all quantifications. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to n-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra compared to the Wiley version 7.0 library. Major compounds in each group were marked in bold form.

Fumigant Toxicity

To determine the fumigant toxicity of *Thymus capitatus* and *Mentha pulegium* essential oils, a whatman filter paper (2 cm diameter, Whatman No.1) were impregnated with tested oil and attached to the screw caps of a 44 ml Plexiglas bottle. Caps were screwed tightly on the vials each of which contained separately10 adults of each species. The tested oil doses calculated to give equivalent fumigant concentrations of 12.5, 25, 50, 125, 625 µL/L air. Each treatment and check was repeated three times. Mortality was recorded each hour. When no leg and antennal movements were observed, insets were considered dead. The mortality was calculated using the abbot correction formula [11]. Results from all replicated were submitted to Probit analysis [12] to determine lethal concentrations (LC50-LC95).

RESULTS

Essential Oil Yield

Table 1. Essential oil yields (%) of *Thymus capitatus* and *Mentha pulegium*

Essential oil	**Oil yields (%)**
Thymus capitatus	1.359
Mentha pulegium	3.115

Mean values followed by different letters are significantly different by Duncan test (p< 5%).

Essential oil yields obtained by hydrodistillation of *Thymus capitatus* and *Mentha pulegium* leaves are given in Table 1. Highest yield was obtained from leaves of *Mentha pulegium* compared to *Thymus capitatus*. Significant statistical differences (p< 5%) in the essential oil yields were observed (w/w on dry weight basis). These results are in accordance with those obtained by Mkaddem et al who reported that the extraction yield of *Thymus capitatus* essential oil leaves from the mountain of Matmata, Gabes, Tunisia was 1.2%. Thus, our samples presented similar essential oil content compared to those of korba, Tunisia (0.44-2%) [13] and Matmata, Gabes, Tunisia (2.75%) [14]. Whereas, Russo et al. [15] showed that the highest essential oil yield leaves from Calabria (Italy) was comprised between 3.02 and 3.44%. Regarding *Mentha pulegium* essential oil yield, our results are in accordance with those obtained by Said et al. [16] who reported that essential oil yield of leaves collected from Bougedour (Maroc) was comprised between 0.7 and 3.3%. Nevertheless, Khalilipour et al. [17] showed a lowest pennyroyal essential oil yield equivalent to 0.6%.

Essential Oil Composition

Table 2. Chemical composition (%, w/w) of *Thymus capitatus* leaf essential oil

Volatil compound	**Chemical formula**	**%**	**Identification**
Sabinene	$C_{10}H_{16}$	0,226	GC-MS
1R-.alpha.-pinene	$C_{10}H_{16}$	0,1612	GC-MS
Camphene	$C_{10}H_{16}$	0,0971	GC-MS
.beta.-pinene	$C_{10}H_{16}$	0,0515	GC-MS
β-thujene	$C_{10}H_{16}$	0,4453	GC-MS
alpha.-phellandrene	$C_{10}H_{16}$	0,054	GC-MS
3-carene	$C_{10}H_{16}$	0,0284	GC-MS
α-terpinene	$C_{10}H_{16}$	0,3339	GC-MS
p-cymene	$C_{10}H_{14}$	3,1758	GC-MS
p-menth-3-ene	$C_{10}H_{18}$	0,1846	GC-MS
γ-terpinen	$C_{10}H_{16}$	1,4151	GC-MS

Terpineol, cis-.beta.-	$C_{10}H_{18}O$	0,0587	GC-MS
Terpinolene	$C_{10}H_{16}$	0,0573	GC-MS
Linalool	$C_{10}H_{18}O$	0,7711	GC-MS
Borneol	$C_{10}H_{18}O$	0,817	GC-MS
p-menth-1-en-4-ol	$C_{10}H_{18}O$	0,5682	GC-MS
p-menth-8-en-2-one	$C_{10}H_{16}O$	0,0842	GC-MS
Thymol	$C_{10}H_{14}O$	76,5087	GC-MS
Eugenol	$C_{10}H_{12}O_2$	0,1907	GC-MS
Carvacrol	$C_{10}H_{14}O$	3,1834	GC-MS
α-gurgujene	$C_{15}H_{24}$	0,0521	GC-MS
β-cariophyllene	$C_{15}H_{24}$	2,786	GC-MS
Aromadendrene	$C_{15}H_{24}$	0,1558	GC-MS
1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$	0,1654	GC-MS
(+)-ledene	$C_{15}H_{24}$	0,2156	GC-MS
β-bisabolene	$C_{15}H_{24}$	0,1845	GC-MS
γ-cadinene	$C_{15}H_{24}$	0,0627	GC-MS
Cadina-1(10),4-diene	$C_{15}H_{24}$	0,1267	GC-MS
Espatulenol	$C_{15}H_{24}O$	0,1701	GC-MS
Caryophyllene oxide	$C_{15}H_{24}O$	0,8829	GC-MS
Grouped compound			
Monoterpens hydrocarbons		6.23%	
Oxygenated monoterpens		81.99%	
Sesquiterpens hydrocarbons		3.74 %	
Oxygenated sesquiterpens		1.053	
Others		0.25	
Total		93.27	

GC/MS analysis of *Thymus capitatus* and *Mentha pulegium* showed that 30 and 18 compounds were identified which represent respectively 93.27% and 88.76% of total constituents (Tables 1 and 2). Thymol (76.5%), carvacrol (3.18%), p-cymène (3.17%); β- caryophyllene (2.78%) and γ-terpinene (1.41%) were the major compounds of *Thymus capitatus* essential oil. Whereas, Tunisian *Mentha pulegium* essential oil were characterized by the predominance of Menthone (35.66%), Pulegone (39.15%), Piperitone (3.55%), Piperitenone (3.98%) and isopulegone(2.75%).

In *Thymus capitatus* essential oil, the oxygenated monoterpens had the highest contribution (81.99%). This fraction was mainly composed of Thymol (76.50%) and Carvacrol (3.18%). Chemical composition of *Thymus capitatus* essential oil was widely investigated. Our results are not in accordance with those obtained by Megdiche-ksouri et al., (2015) who reported that *Thymus capitatus* essential oil was characterized by the predominance of Carvacrol (76.5 %). In fact, chedia et al., (2013) showed that *Thymus capitatus* essential oil from the region of Sousse (Tunisia) was rich in thymol (69.95%), β-ocimene (3.09%), carvacrol (2.56%) and α-terpinene (2.25%). Nevertheless, the oil from the region of Bizerte (Tunisia) was characterized by thymol (81.49%), α-cubebene (3.44%), α-terpinene (3.83%) and β-ocimene (3.16%) as major compounds. Thus, Cosentino et al. revealed that thymol (29.3 %), p-cymène (26.4 %) and carvacrol (10.8%) were the main constituents of T. Capitatus essential oil from Sardinia. In this context, the oil from Morocco presented qualitative and quantitative differences compared to Tunisian oil samples. El Ouariachi et al. reported that p-cymene (18.9%), carvacrol (13.4%), geranyl-acetate (12.2%) and borneol 17 (10.2%) were the major compounds of the Moroccan oil.

Tunisian chemotype were characterized by the predominance of Carvacrol (62-83%) and p-cymene (5-17%) [18-20]. Our results are in accordance with those obtained by Mkaddem et al. who reported that Tunisian chemotype presented an essential oil rich in thymol (89%). Otherwise, Essential oil from Sicily [21], Albania [22] and Portugal [23] were characterized by predominance of carvacrol. Nevertheless, T. capitatus essential oil from Turkish present carvacrol (35.6%), p-cymene (26.4%) and thymol (18.6%) as the dominating compounds [24].

Table 3. **Chemical composition (%, w/w) of *Mentha pulegium* leaf essential oil**

Composé volatil	**Formule chimique**	**%**	**Identification**
1R-.alpha.-pinene	$C_{10}H_{16}$	0,2023	GC-MS
Cyclohexanone, 3-methyl-	$C_7H_{12}O$	0,0758	GC-MS
.beta.-phellandrene	$C_{10}H_{16}$	0,078	GC-MS
.beta.-pinene	$C_{10}H_{16}$	0,2017	GC-MS
β-thujene	$C_{10}H_{16}$	0,1256	GC-MS
3-octanol	$C_8H_{18}O$	0,5089	GC-MS
p-cymene	$C_{10}H_{14}$	0,0257	GC-MS
(+)-4-Carene	$C_{10}H_{16}$	0,0223	GC-MS
Menthone	$C_{10}H_{18}O$	35,6612	GC-MS
Isopulegone	$C_{10}H_{16}O$	2,7533	GC-MS
α-terpinyl propionate	$C_{13}H_{22}O_2$	0,1285	GC-MS
Pulegone	$C_{10}H_{16}O$	39,1541	GC-MS
Piperitone	$C_{10}H_{16}O$	3,5599	GC-MS
trans-carane	$C_{10}H_{18}$	0,0934	GC-MS
Piperitenone	$C_{10}H_{14}O$	3,9858	GC-MS
Caryophyllene	$C_{15}H_{24}$	0,1307	GC-MS
1,1,4,8-tetramethyl-cis,cis,4,7,10-cycloundecatriene	$C_{15}H_{24}$	0,3353	GC-MS
Germacrene D	$C_{15}H_{24}$	0,0921	GC-MS
Grouped compound			
Monoterpens hydrocarbons		0,749	
Oxygenated monoterpens		85,699	
Sesquiterpens hydrocarbons		0,5581	
Oxygenated sesquiterpens		0	
Other		1,7561	
Total		88,7622	

In *Mentha pulegium* essential oil, monoterpens hydrocarbons represented 85.69 % of the total oil (Table 1).

Our results are not in accordance with those obtained by Khalilipour et al., (2014) who reported that *M. pulegium* essential oil from Iran was rich in pulegone (46.18%), piperitenone (19.56%), 1,8-cinéole (4.55 %) and piperitenoneoxide (4.23%). In this context, pulegone (77.16 %), pepiritenone

(6.54 %), limonene (1.59 %), menthone (1.37 %) and piperitone-oxide (1.82 %) were the major compounds of *M. pulegium* essential oil from Morocco (Saied et al. 2015). In the other hand, Ouakouak et al., (2015) showed that the Algerian oil was characterized by the predominance of Pulegone (46.31%), Piperitenone (23.3%), Menthone (6.2%) and Limonene (4.7%). The prominent compounds in Iranian Pennyroyal essential oil were Piperitone (38.0%), Piperitenone, α-terpineol (4.7%) and Menthone (3.1%) [25]. Moreover, El Houssine et al. (2010) [26] and Derwich et al. [27] revealed that *M. pulegium* essential oil from Morocco was characterized by the predominance of Piperitenone. In fact, Essential oil from Brazil was rich in pulegone [28].

Fumigant toxicity

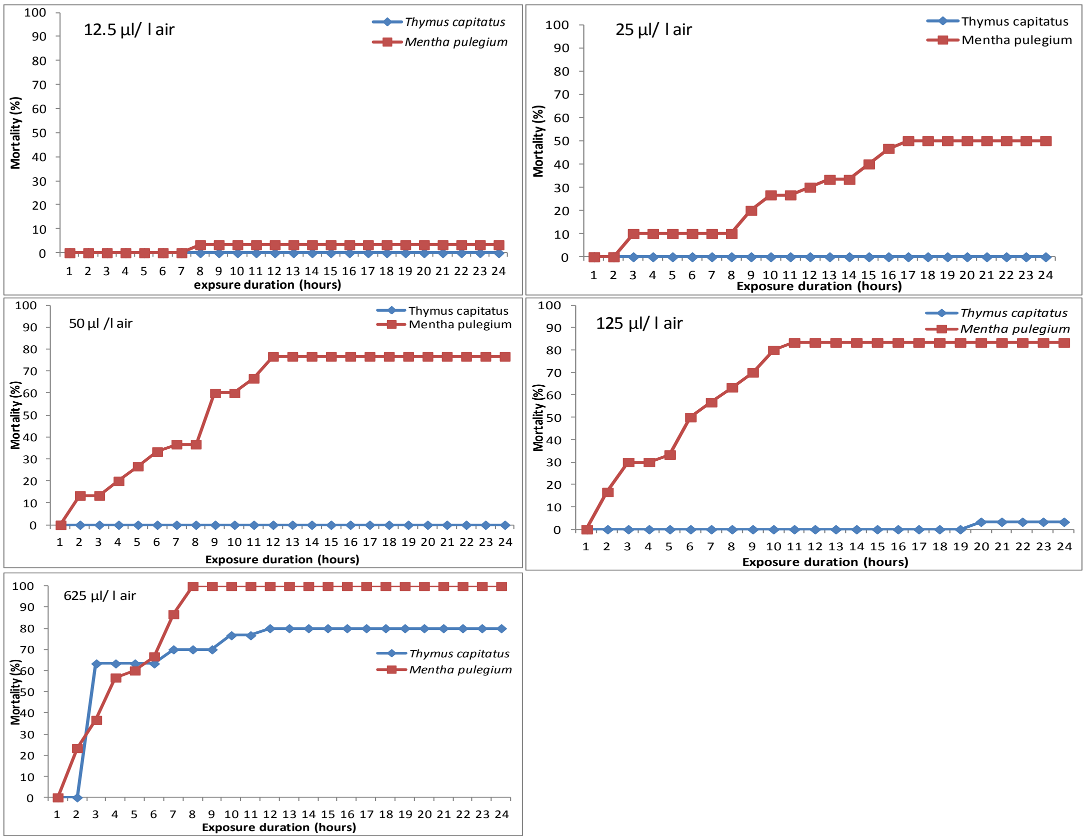

Figure 1: Percentage of corrected mortality of *Tribolium castaneum* exposed for various durations to essential oil from *Thymus capitatus* and *Mentha pulegium*

Results related to fumigant toxicity bioessays were illustrated in Figure 1. Pennyroyal essential oil was more toxic to *Tribolium castaneum* than *Thymus capitatus* at all tested concentrations. The lowest concentration (125 µl/l air) of the oil led to no insect mortality for *T. capitatus* and 3.33% for *M. pulegium*. For the highest concentration (625 µl/l air) insect mortalities reached 80% for *T. capitatus* and 100% for *M. pulegium* essential oils. Results revealed that mortality depends on plant species, oil concentration and exposure time. The corresponding LC50 and LC95 for *Mentha pulegium* were respectively 33.74 and 159.02 µl/l air. Whereas, concerning *Thymus capitatus*, the corresponding LC50 and LC95 were respectively 377.34 and 1009.83 µl/ l air. Results illustrated in Table 3 showed significant differences between both oils. Pennyroyal essential oil was clearly more significant to *T. castaneum* than *T. capitatus* essential oil.

Table 6. TLC50 and LC95 values (µL/l air) on fumigant bioessays with *T. capitatus* and *M. pulegium* essential oil

Essential oil	LC_{50} [a,] (µl/lair)	LC_{95} [a] (µl/lair)	Slope ± SEM	Degrees of freedom	X^2
T. capitatus	377.34[b] (280.53-495.57)	1009.83[b] (722.02-1864.32)	3.84±0.71	5	0.012

M. pulegium	33.74[a] (6.35-137.77	159.02 [a] (65.28-270.33)	2.44±0.68	5	9.37

[a]Units LC_{50} and LC_{95} µl/l air, applied for 24h at 25°C.

Results of this research showed that Tunisian pennyroyal essential oil have fumigant toxicity proprieties against the red flour beetle: *Tribolium castaneum* with median lethal concentration of 33.74 µl/l air. It appeared that the highest concentration of *M. pulegium* essential oil leading to 100% on *T. castaneum* after 8h of exposure. Fumigant potential of Pennyroyal essential oil could be attributed to its high monoterpenes content especially to their main compounds such as Menthone, Isopulegone, piperitenone, Piperitone which have been described for their insecticidal proprieties [29]. The insecticidal toxicity potent of *M. pulegium* essential oil against different stored pests has been reported by several researchers. In this context, Salem et al. [30] revealed that Tunisian Pennyroyal essential oil were highly toxic on stored pests with median lethal concentration of 8.46 µl/l air for L. serricorne and 11.57 µl/l air for *T. castaneum*. *M. pulegium* essential oil from Morocco were investigated for their insecticidal potent. Thus, the mortality percentage on Sitophilus orysae and Rysopertha dominica was recorded after 1 hour of exposure treatment in the amount of 1.12 and 50 µL [31]. Moreover, Zekri et al. [32] reported a fumigant toxicity potential against weevils of S. orysae with lethal concentration varying from 2.65 to 0.044 µl/l air. Thus, Urzua et al. [33] reported insecticidal activity of pennyroyal essential oil on Musca domestica with an LC50= 4.7µg/cm^3.

CONCLUSION

This research deals with comparative investigation on the chemical composition and fumigant potential of *M. pulegium* and *T. capitatus* essential oils from Tunisia. Results underlined the powerful insecticidal activity of *Mentha pulegium* essential oil against the red flour beetle: *Tribolium castaneum* in comparison with *Thymus capitatus* essential oil. The strongest pennyroyal fumigant potential could be attributed to its richness in monoterpens hydrocarbons especially in compounds with insecticidal activity (Menthone, Isopulegone, pulegone, Piperitone, Piperitenone). Thus, *T. capitatus* essential oil fumigant potent was due to its chemical composition rich in oxygenated monoterpens mainly in Thymol and Carvacrol. This study showed that Pennyroyal and *T. capitatus* essential oils could be considered as an alternative to synthetic insecticides. It could be recommended for the management of the grain storage insects.

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