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Phytochemical study and insecticidal activity of *Mentha pulegium L.* oils from Morocco against *Sitophilus Oryzae*

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Abstract: There is a growing interest of industry to replace synthetic chemicals by natural products with bioactive properties from plant origin. The aim of this study was to validate the therapeutic properties of *Mentha pulegium* L. by conducting a phytochemical study, to determine the chemical composition of its essential oils (EO) and evaluate its insecticidal activity against stored cereals pests. Leaves and flowers of *M. pulegium L.* were collected from three regions of the Moroccan Middle Atlas. Phytochemical tests on pennyroyal aerial parts revealed the presence of gallic tannins, flavonoids, alkaloids, sterols and triterpenes and saponins. The chemical composition of essential oils was analyzed by gas chromatography coupled with mass spectrometry. The main components were pulegone and piperitenone. The EO from Khénifra is dominated by pulegone (81.46%), and those from Azrou and M'rirt are rich both in pulegone (68.86 and 71.97%) and piperitenone (24.79% and 26.04%) respectively. Pennyroyal oil has showed an important fumigant effect against *Sitophilus oryzae* (L.) adults. This effect is influenced by the tested doses and exposure periods. The potential of this plant to be used to control stored product insects was discussed.

Keywords: Essential oil, *Mentha pulegium L.*, Phytochemistry, fumigation, *Sitophilus oryzae* (L.).

Introduction

Mentha pulegium L. is a herbaceous perennial that belongs to the Lamiaceae family, commonly known as pennyroyal/European pennyroyal, native to North Africa, Europe, Asia Minor and the Middle East [1]. It grows wildly in humid areas of the plains and mountains. In Morocco, *M. pulegium L.* is known as the Arabic name "Fliyou". It is among the top five national mints and most widely used and commercialized [2].

Aerial parts of this plant contain a wide diversity of secondary metabolites such as: tannins, resins, pectins, bitter principles and essential oils [1, 3]. Fresh or dried leaves and flowering tops are commonly used for their healing and culinary properties. The whole plant and its essential oil have a strong and characteristic smell [3].

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In therapeutic applications, this plant and its preparations have been used traditionally for their antispasmodic, carminative, diaphoretic, sedative, stimulant, diuretic, antitussive, tonic, cholagogue, expectorant, antiseptic and digestive effect [1,3,4].

It was even used to promote menstruation, cure headaches, treat bronchitis, relieve bites from scorpions and snakes and help against vomiting and kidney disease [1, 3]. It also served as a repellent against fleas and other insects [2-4]. It was effective in relieving acne and other skin conditions. This plant has been used as a spice and flavoring in various foods, especially desserts [1, 4], also as perfume in cosmetics [3-4] and corrosion inhibitor for steel in the chemical industries [5].

Pennyroyal is an important source of essential oils. The chemical composition of the pennyroyal oil has been described by several studies [4, 6-14]. Pennyroyal oils are characterized by the preponderance of pulegone (70-90%) along with other monoterpenic ketones such as menthone, isomenthone and piperitenone [15]. The pennyroyal oil and its constituents exhibit interesting biological activities particularly antibacterial [10, 13, 16-17], antifungal [18-19], antioxidant [9, 20] and insecticidal [11, 14, 21].

Pennyroyal is not yet described by the Pharmacopoeia [15]. Even if its properties were traditionally well-known, this species has never been investigated pharmacologically [15]. However, its essential oil is highly suspected to be hepatotoxic. It was responsible for fatal accidents. Its liver toxicity is mainly related to pulegone and its metabolites that are responsible for tissue necrosis [15, 22]. The consumption of several milliliters of pennyroyal oil is highly probable to cause toxic effects. Small amounts (10 ml) can produce a moderate to severe poisoning [23], large quantities (25ml) can cause a fatal liver necrosis [22]. The most important hazard associated with this oil, is the potential for accidental consumption by a child [22] (LD_{50} = 3 ml). The right dosage and good administration of the plant could avoid such accidents [22, 24]. Note that the exact mechanism of toxicity of pennyroyal oil and pulegone is not yet revealed [15].

In Middle Atlas, a mountainous region of Morocco, pennyroyal is considered among plants frequently used by people in traditional medicine. In this context, the aim of this work is to identify the own secondary metabolites of pennyroyal that collected from three regions of Middle Atlas, to study the variability of the chemical composition of its essential oils and to evaluate its insecticidal potential against pests of stored cereals.

Experimental section

Plant material

The aerial parts (leaves and flowers) of pennyroyal were collected in July 2010 from three sites in the Middle Atlas: Azrou (Latitude: 33° 25′ 59″; Longitude: 5° 13′ 01″; Altitude: 1278m), M'rirt located between Azrou and Khénifra (Latitude: 33°10′ 00″; Longitude: 5° 34′ 00″; Altitude: 1113m) and Khénifra (Latitude: 34°15'36"; Longitude: 6°34'12"; Altitude: 860m). The climate is semi-humid with strong continental influence with an annual average temperature of 20°C.

Essential oils extraction

Flowers and leaves (100g) were air-dried at room temperature and hydro-distilled using a Clevenger-type apparatus for 3 hours. The essential oils were dried with anhydrous sodium sulphate and stored in a refrigerator at 4°C until use. For calculations of essential oil yields, three replicates were performed for each plant material.

Chromatographic analysis

The chromatographic analyses were performed using a gas chromatograph Hewlett Packard (HP 6890 series) type equipped with a HP-5 capillary column (30m x 0.25 mm x 0.25 microns film thickness) , a FID detector set at 250 ° C and fed with a gas mixture H2/air. The mode of injection is split; the carrier gas used is nitrogen with a flow rate of 1.7 ml / min. The column temperature is programmed at a rate of 4 mounted ° C / min from 50 to 200 °C for 5 min. The unit is controlled by a computer system type "HP ChemStation" managing the operation of the device and to monitor chromatographic analyzes. GC-MS was carried out by a chromatograph Hewlett Packard (HP 6890) coupled to a mass spectrometer (HP 5973 series). Fragmentation is performed by electron impact at 70 eV. The used column was a capillary-type HP 5SM (30 mx 0.25 mm x 0.25 mm). The column temperature is programmed at a rate of 4 mounted °C / min from 50 to 200°C for 5 min. The carrier gas is helium with a flow rate set at 1.7 ml / min. The injection mode is split type.

For compound identification, the Kovàts Index [25] of each compound was calculated in relation to the retention time of a series of linear alkanes (C7-C40). The calculated index was then compared to those of Adams [26] reference. The mass spectra of compounds were also matched with those stored in the NIST library / EPA / NIH MASS SPECTRAL LIBRARY; Version 2.0, build libraries Jul, 1, 2002.

Phytochemical tests

The phytochemical study needed the preparation of plant material. Leaves and flowers of *M. pulegium* were dried in the open air, milled in an electric grinder and used to prepare extracts, infusions and decoctions.

Selective extractions of homogenates were made specifically on each family of compounds studied. The extracts have been obtained by extraction with solvents. The solvents used are petroleum ether, methanol, ethanol, chloroform and distilled water.

The phytochemical screening was also based on several reagents. Research of alkaloids was performed by Dragendorff reagent. Characterization of catechin tannins was carried out by isoamyl alcohol and hydrochloric acid and gallic tannins by Stiasny reagent, sodium acetate and ferric chloride. To detect sterols and triterpenes, we used acetic anhydride and concentrated sulphuric acid. Diluted alcohol hydrochloric acid, magnesium chips and isoamyl alcohol were used to seek the flavonoids. Chloroform, dilute ammonia and hydrochloric acid have to look for quinonic substances.

Characterization tests of different chemical groups were performed as described by Bruneton[15], Harborne [27] and N'Guessan [28].

Pests

Adults of *Sitophilus oryzae* (L.), rice weevils belonging to the Curculionidea family were brought from the grain market. They were raised thereafter at the expense of wheat grains in glass jars at a temperature of 24 ± 1°C and a relative humidity of 76 ± 5% in the dark in order to obtain a homogeneous population.

Biotests

Fumigation tests were carried out in plastic boxes of 1 liter volume. Thus, in each box was placed a Petri dish by airy chiffon gauze containing 10 adults of *S. oryzae* and filter paper soaked with *M. pulegium* essential oil. The concentrations of essential oil in the air were: 0.75; 0.25; 0.5; 1 and 2 µl /l air. For each concentration, five replicates were conducted.

Control of mortality was performed daily by counting died individuals until the death of last insect.

Data analysis

To detect the toxic fumigant effect of tested essential oil, an analysis of variance performed using the function Arsin (square root (percentage mortality)) in the software Microsoft Excel 2007. The calculation of the survival probabilities and comparison of the effect of each concentration tested were taken respectively by the test of Kaplan-Mayer [29] and the log-rank test [30]. The lethal concentrations 50% (LC_{50}) and 99% (LC_{99}) fumigated insects were determined by the Probits method according to Finey [31].

Results and discussion

Yields and Chemical composition

The yields, calculated from dry material, varied from sample to sample. The essential oil from Khénifra has the highest yield (6.2%) followed by those from Azrou (5.9%) and M'rirt (5.29%). These rates are higher than those already obtained by Boughdad [14] (4.77%), Benayad [11] (2.33%) and Derwich [13] (1.66%).

The chromatographic analysis of essential oils have identified twenty six compounds that represent approximately 99.10% for *M. pulegium* of Azrou, thirteen compounds (99.87%) to that of M'rirt against sixteen compounds for the sample from Khénifra (89.10%). Oxygenated monoterpenes were the most abundant class of the components identified in three essential oils. However, the sesquiterpenes were found with small contents in pennyroyal oils from Azrou and Khénifra and quasi absent in that of M'rirt (Table 1).

Table 1: Chemical composition of *pennyroyal* oils from Middle-Atlas**.**

N°	Identified Compound	Kovàts Index (IK)	Area %		
			Azrou	M'rirt	Khénifra
1	α-pinene	939	0.17	0.14	0.23
2	β-pinene	979	0.15	0.13	0.22
3	Meta-mentha-1(7), 8-diene	1000	0.02	-	-
4	O-cymene	1026	0.07	-	-
5	Limonene	1029	0.90	-	-
6	1,8- cineole	1031	-	0.10	0.10
7	Para-mentha-3,8-diene	1072	0.01	-	-
8	Trans-p-menth-2-en-1-ol	1140	0.57	0.28	-
9	Isopulegol	1149	-	-	0.55
10	Isopulegol (iso)	1159	-	-	0.38
11	Benzylacetate	1162	0.07	-	-
12	Chrysanthenol(Cis)	1164	1.03	0.80	-
13	α -Terpineol	1188	0.17	0.10	-

Table 1 (Continued)**:** Chemical composition of *pennyroyal* oils from Middle-Atlas**.**

14	Trans-pulegol	1214	0.19	0.06	0.07
15	Coahuilensol methyl ether	1221	0.16	-	-
16	Cis-pulegol	1229	-	-	0.04
17	Pulegone	1237	**68.86**	**71.97**	**81.46**
18	Piperitone	1252	0.07	-	-
19	Perilla aldehyde	1271	0.21	-	-
20	Thymol	1290	1.01	0.04	-
21	Carvacrol	1299	0.04	-	-
22	p-vinyl-guaiacol	1309	0.13	-	-
23	Piperitenone	1343	**24.81**	**26.04**	-
24	Z-Caryophyllene	1408	0.11	-	-
25	E-Caryophyllene	1419	0.04	-	1.70
26	α -Guaiene	1439	0.08	0.06	-
27	α -Humulene	1454	-	-	2.89
28	Trans-4,10-epoxy-amorphane	1479	-	-	0.09
29	4-epi-cis-dihydroagarofurane	1499	0.04	-	-
30	10-epi-cubebol	1535	-	-	0.51
31	Cis-Sesquisabinene hydrate	1544	-	-	0.17
32	Germacrene D-4-ol	1575	0.09	-	-
33	Trans-Sesquisabinene hydrate	1579	-	-	0.57
34	Caryophyllene oxyde	1583	0.09	-	-
35	Allo-cedrol	1589	-	-	0.07
36	Cedrol	1600	-	-	0.05
37	Himachalol	1653	0.01	-	-
38	Himachal-4-en-1- β-ol (11- α H)	1699	-	0.06	-
Oxygenated monoterpenes			87.12	99.39	82.6
Hydrocarbon monoterpenes			1.32	0.27	0.45
Oxygenated sesquiterpenes			0.23	0.06	1.46
Hydrocarbon sesquiterpenes			0.23	0.06	4.59
Others			0.2	--	--
Total			**99.10**	**99.78**	**89.10**

The essential oil from Azrou is dominated by pulegone (68.86%) and piperitenone (24.79%). Other compounds were identified but at relatively small percentages such as chrysanthenol (1.03%), thymol (1.01%), limonene (0.9%) and menth-2-en-1-ol (0.57%). Two constituents also characterize the essential oil M'rirt but its rates are higher than those of

Azrou: pulegone (71.97%) and piperitenone (26.04%) accompanied by chrysanthenol (0.80%), menth-2-en -1-ol (0.28%), α-pinene (0.14%) and β-pinene (0.13%).

However, the EO from Khénifra is mainly composed of pulegone with a larger rate than the other two regions; it reached 81.46%. Other components were also identified but with lower levels such as α-humelene (2.89%), E-caryophyllene (1.70%), isopulegol (0.55%), 10-epicubelol (0.51%), α-pinene (0.23%) and β-pinene (0.22%).

Differences between the three oils chemical composition were reported. Indeed, limonene (0.9%), perilla aldehyde (0.21%) and coahuilensol methyl ether (0.16%) are specific to the oil of Azrou. Furthermore, piperitenone second chemotype of Azrou (24.81%) and M'rirt (26.04%) essential oils is absent in that of Khénifra. The latter also contains specific compounds such as α-humelene (2.89%), E-caryophyllene (1.70%), trans-sabinene hydrate (0.57%), isopulegol (0.55%) and 10-epi-cubebol (0.51 %). On the other hand, 1,8-cineole (0.10%) is present only in M'rirt and Khénifra oils.

The variation of yields and chemical composition of essential oils depends on several factors: the method used, the used plant parts, the products and reagents used in the extraction, the environment, the plant genotype, geographical origin, the harvest period of the plant, the degree of drying, the drying conditions, temperature and drying time and the presence of parasites, viruses and weeds [32-33].

Pennyroyal can be considered as an important source of pulegone. This ketone presents approximately 3/4 of the overall chemical composition of the studied oils. It is usually used in the manufacture of industrial and cosmetic products as it can be transformed into menthol by semisynthetis which is highly demanded for flavouring food, in cosmetics and pharmaceutical industry [34].

The chemical composition of the studied oils is similar to that reported by several studies already carried out in Morocco. The EO of *M. pulegium* from Morocco is characterized by its high rate of pulegone. The EO from Asilah (North east) studied by Hmiri [18] contains a very attractive pulegone rate (80.28%). The content of pulegone in pennyroyal from Meknes is about 65% [14]. In Taouirt region (North-East) 69.8% [35]; in Rabat region (Ain Aouda), it is about 73.33% [11] and in Southern Morocco, it reached 85.4% [17]. Similarly, pennyroyal oils from different Algerian sites are characterized by the predominance of pulegone with different proportions 43.3 to 87.3% [36]. Similar results were obtained from Uruguay [7] and Tunisia [12], the main compounds are respectively pulegone (73.4% and 61.11%) and isomenthone (12.9% and 17.02%). In Egypt, the pennyroyal oil is rich in pulegone (43.5%) and piperitone (12.2%) [9]. While the species from Iran [10] and Skoura (Morocco) [13] contain two particular chemotypes: piperitone (38 and 35.56%) and piperitenone (33 and 21.12%) while the pulegone rate does not exceed 2.3 and 6.42% respectively. Similarly, those from Portugal [6] and Yugoslavia [37] are characterized by different chemical composition whose major compounds are menthone (35.9 and 30.9%) and pulegone (23.2 and 14.1%) respectively.

Phytochemical screening

The results of phytochemical screening are assembled in Table 2. Different groups occurring in leaves and flowers of pennyroyal were identified.

According to the results of the characterization tests, the aerial parts of pennyroyal from three regions contain gallic tannins, saponins, flavonoids, sterols and triterpenes, alkaloids, mucilages and reducing compounds. However, it is devoid of anthraquinones, oses and holosides, cyanogenic glycosides and catechin tannins. The effective presence of some of them in the plant does not exclude its therapeutic properties [38].

Flavonoids are perfect antioxidants [39], antiulcer, antispasmodic, anti-secretory, anti-diarrheal [40], antiallergic, anti-inflammatory, blood pressure and protect against cancer and cataract [15].

Table 2: Results of phytochemical screening of pennyroyal aerial parts by colored reactions.

Chemical group	Observations
Gallic tannins	+
Catechin Tannins	-
Flavonoids	+
Alkaloids	+
Saponins	+ Foam index = 225
Free anthraquinones	-
Combined anthraquinones	-
Oses and holosides	-
Sterols and triterpenes	+
Reducing compounds	+
Mucilages	+
Cyanogenic glycosides	-

Alkaloids have different pharmacological activities such as strengthening the heart activity, excitation of the central nervous system and nerves symptomatic, stimulating blood circulation [38]. The presence of alkaloids may also justify the use of the plant in the treatment of certain diseases [28]. The tannins show the properties of vitamin D, they could be used to strengthen blood vessels and contribute to the accumulation of vitamin C in the body [38]. The saponins have a healing effect and sterols and triterpenes have bactericidal properties [28].

These properties were linked to the identified classes of phytoconstituents in pennyroyal. Therefore, this plant exhibits important therapeutic effects. These results justified the wide use of this plant in traditional medicine by people in Middle Atlas.

Insecticidal activity of *M. pulegium* essential oil against adults of *S. oryzae* (L.)

In this research, we evaluated the insecticidal activity of pennyroyal oil from Azrou against weevil of *S. oryzae*. Concentrations tested have showed a significant fumigant activity. The degree of this activity depends on the applied concentration ($F_{concentrations} = 18.38 > F_{(0.05, 5\text{-}192)} = 2.2141$) and the exposure duration ($F_{time} = 12.83 > F_{(0.05, 7\text{-}192)} = 2.0096$). The survival rate of insects decreases as the concentration and exposure period increased (Figure 1).

The interaction between weevils and essential oil lasted eight days. The mortality within the weevil was observed on the first day and even with the lowest concentration. At 2µl of essential oil/l air, insects have been totally decimated the fourth day. As at low concentrations, the mortality of insects reached gradually the entire population on the eighth day after the beginning of fumigation.

The survival time of 50% (LT_{50}) and 99% (LT_{99}) of adults who were exposed to different concentrations of essential oil vary respectively from 24 hours to 4 days and about 4 to 8 days depending on the concentration, whereas in the control group, adults live an average of 29 and 57 days respectively (Table 3). Furthermore, the LT_{50} and LT_{99} are negatively correlated with the tested concentrations.

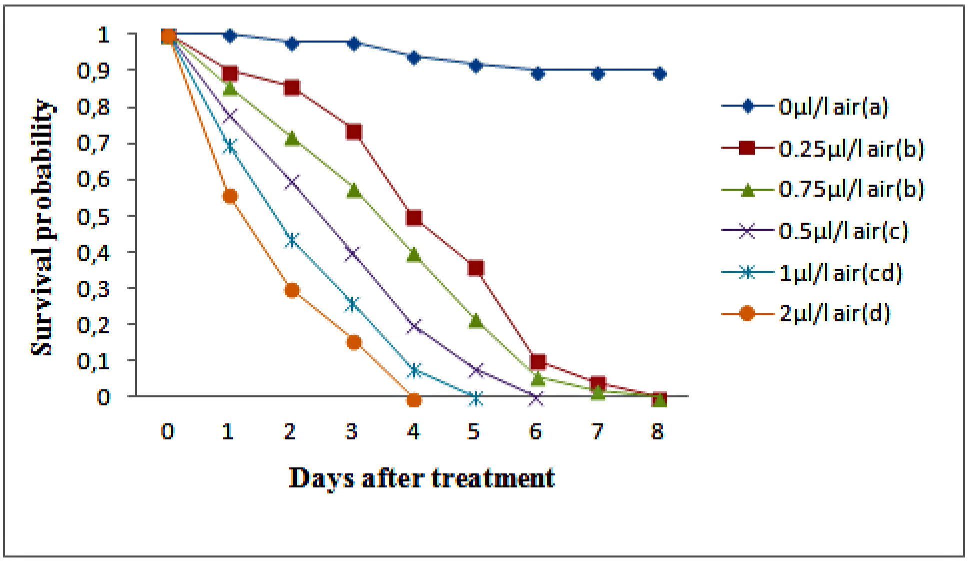

Figure 1. Survival rate of *S. oryzae (*L.) adults fumigated by pennyroyal oil (Concentrations affected with the same letter do not differ statistically between them (log-rank test)).

Table 3: LT_{50} and LT_{99} of *S. oryzae* adults fumigated with *M. pulegium* essential oil.

Concentrations (µl/l air)	LT_{50} (days)	r	LT_{99} (days)	r
0	28,76		56,20	
0.25	4,00		7,47	
0.5	3.48	-0.86	7.06	-0,83
0.75	2.63		5.49	
1	2.07		4.51	
2	1.60		3.64	

The toxicity parameters of the tested essential oil are summarized in Table 4. The calculated lethal concentrations LC_{50} and LC_{99} vary according to the time. They decrease gradually as the fumigation time increases. Extreme lethal concentrations LC_{50} and LC_{99} vary from 2.65 to 0.044µl /l air and 143.9 to 0.518µl /l air respectively.

The effectiveness of the pennyroyal oil may be attributed to its chemical composition generally and particularly to monoterpenes that act as insecticidal agents [41]. Our plant is rich in monoterpenes (96.87%) mainly pulegone (68.86%) and piperitenone (24.81%), thymol (1.01%), limonene (0.9%) and pinene (0.32%). These active components have shown an important insecticidal activity against several pests [14, 21, 30, 41-43].

Results similar to ours have been reported by Benayad [11] which a concentration of 3 µl of *M. pulegium* oil (73.33% of pulegone) caused mortality of all weevils of *S .oryzae* after one day of treatment. Similarly, these insects were also decimated at a dose of 1.7 10^{-2} µl/ cm^3 of *M. suaveolens* oil (85.5% of pulegone) after 7 days of treatment and at a dose of 3.5 10^{-2} µl/ cm^3 of oil after 24 hours [44].

The fumigant effect of this oil against adults of *S. oryzae* could also be explained by the high content of pulegone. The toxicity of this ketone against *S. oryzae* was also observed by Lee [30] and Hannin [45].

Furthermore, the insecticidal activity of the essential oil is not limited only to its major constituents; it could also be due to some minor constituents or a synergistic effect of several constituents [46-47].

Table 4: Toxicity parameters of pennyroyal oil against *S. oryzae* (L.) adults.

Days	**Equation**	**$X^2_{observed} < X^2_{tabulated} = 7.815$**	**LC_{50} (µl/l air) [Confidence Interval]**	**LC_{99} (µl/l air) [Confidence Interval]**
1	1.34x + 4.43	0.487	2.652[1.699;7.691]	143.9 [27.9 ; 111637.9]
2	1.84x + 5.02	0.739	0.973[0.792;78.02]	17.67 [8.11 ; 8.02]
3	1.97x +5.47	1.172	0.577[0.499;0.709]	8.65 [4.66 ; 27.33]
4	2.46x +6.25	5.525	0.310 [0.233;0.384]	2.735 [1.829 ; 5.601]
5	2.77x +6.33	4.444	0.219 [0.137;0.284]	1.507 [1.060 ; 2.971]
6	2.41x +7.59	2.672	0.084 [0.004 ; 0.162]	0.777 [0.518 ; .892]
7	2.16x +7.94	0.813	0.044	0.518

The toxic effect of *M. pulegium* essential oils against *S. oryzae* may be attributed to the inhibition of neurotransmitters such as acetylcholinesterase [41, 48] and octopamine [49].

Essential oils as natural insecticides are therefore an alternative to synthetic insecticides because of their physicochemical properties that make them very volatile and biodegradable, which presents no risk of residues on treated products or on the germination of processed grains. Moreover, the fact that essential oils act on octopaminergic sites insects [46], they are less toxic to mammals.

Conclusion

In the present research, we performed a phytochemical study of *M. pulegium* L., determined the chemical composition of essential oils and assessed its insecticidal activity. The results allowed concluding that the yields and chemical composition of essential oils vary according to the plant origin. Essential oils originating from Azrou, M'rirt and Khénifra are characterized by diverse chemical profiles. Thus, species of Azrou and M'rirt are dominated mainly by pulegone (68.86% and 71.97%) and piperitenone (24.97% and 26.04%) while that of Khénifra is very rich in pulegone (81.46%).

Moroccan pennyroyal may therefore be an important source of pulegone. This active component is highly required for the manufacture of cosmetics and industrial products.

Different secondary metabolites were identified: flavonoids, gallic tannins, sterols and triterpenes, alkaloids and saponins. Therefore, pennyroyal can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify and characterize the structure of the bioactive compounds.

Pennyroyal oil has showed an important fumigant effect against *S. oryzae* weevils. This effect could be attributed to the chemical composition and particularly to the abundance of pulegone and piperitenone without ignoring the synergistic role of minor compounds.

Furthermore, the use of essential oil *M. pulegium* in fumigation to control populations of *S. oryzae* is possible. Fumigation can handle large masses of seeds without moving. In this way, the pennyroyal oil has a huge potential as alternative to synthetic pesticides in cereals stored and crop protection.

Further studies should evaluate the safety and toxicity of *M. pulegium* oil to human consumption before their use for medicinal and food purposes.

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References

1- A. Zargari, Herbal Medicines. 1st Edt, Publication of Tehran University, Tehran. **1990**. 14-18.

2- A. El Fadl, and N. Chtaina, Etude de base sur la culture de la menthe du Maroc. Programme Régional de lutte intégrée contre les organismes nuisibles (Integrated Pest Management) au Proche Orient. **2010**, Report, Office National de sécurité sanitaire des produits alimentaires (ONSSA).

3- J.E. Simon, A.F. Chadwick, L.E. Craker, An Indexed Bibliography: 1971–1980: The Scientific Literature on Select Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Elsevier, **1984**, Amsterdam, New York.

4- M. Mkaddem, M. Bousaid, and N. Ben Fadhel, Variability of volatiles in 9 Tunisian *Mentha pulegium* L. (Lamiaceae), Journal of Essential Oil Research. **2007**, *19*, 211-215.

5- A. Bouyanzer, B. Hammouti, and L. Majidi, Pennyroyal oil from *Mentha pulegium as* corrosion inhibitor for steel in 1 M HCl. Materials Letters. **2006**, *60*, 2840–2843.

6- J.C. Chalchat, M.S. Gorunovic, Z.A. Maksimovic, and S.D. Petrovic, Essential oil of wild growing *Mentha pulegium* L. from Yugoslavia, J. Essential Oil Res. **2000**, *12*, 598-600.

7- D. Lorenzo, D. Paz, E. Dellacassa, P. Davies, R. Vila, and S. Canigueral, Essential Oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. *Braz.Arch.Biol.Technol.* **2002**, *45* **(**2), 519-524.

8- A. Stoyanova, V. Georgie, J. Kula, and T. Majda, Chemical composition of the essential oil of *Mentha pulegium* from Bulgaria, J.Essential Oil Research. **2005,** *17*, 475-477.

9- A.H. El-Ghorab, The chemical composition of *Mentha pulegium* L. essential oil from Egypt and its antioxidant activity, J. Ess. Oil Bearing Plant. **2006**, *9*, 183-195.

10- M. Mahboubi, and G. Haghi, Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil Journal of Ethnopharmacology. **2008,** *119*(2), 325-329.

11- N. Benayad, Les huiles essentielles extraites des plantes médicinales marocaines: Moyen efficace de lutte contre les ravageurs des denrées alimentaires stockées. Research Project. University of Sciences, Rabat (Maroc), **2008**. 63p.

12- H. Hajlaoui, N. Trabelsi, E. Noumi, M. Snoussi, H. Fallah, R. Ksouri, and A. Bakhrouf, Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. Springer Science. World. J. Microbiol. Biotechnol. **2009**, *25*, 2227-2238.

13- E. Derwich, Z. Benziane, and A. Boukir, GC/MS Analysis and Antibacterial Activity of the Essential Oil of *Mentha Pulegium*. J.Agr.Biol.Sc. **2010**, *6*(3), 191-198, 14- A. Boughdad, R. Elkasimi, and M. Kharchafi, Activité insecticide des huiles essentielles de *Mentha* Sur *Callosobrochus maculatus* (F) (Coleoptera, Bruchidea). AFPP – Neuvième Conférence Internationale sur les ravageurs en Agriculture. Montpellier 26 and 27 October **2011**, 9p.

15- J. Bruneton, Pharmacognosie, Phytochimie et plantes médicinales. 4rtEdt. Technique et Documentation. **2009**, 1268p.

16- B. Marzouk, M. Ben Hadj Fredj, I. Chraief, M. Mastouri, K. Boukef, and Z. Marzouk, Chemical composition and antimicrobial activity of essential oils from Tunisian *Mentha pulegium L*. Journal of Food, Agriculture & Environment. **2008**, *6*(1), 78- 82.

17- N.H. Jazani, H. Ghasemnejad-Berenji, and S. Sadegpoor, Antibacterial Effects of Iranian *Mentha pulegium* Essential Oil on Isolates of Klebsiella sp. Pakistan J.Biol.Sc. **2009**, *12* (2), 183-185.

18 -B. Chebli, M. Achouri, L.M. Idrissi Hassani, and M. Hmamouchi, Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea*, J.Ethnopharmacology. **2003**, *89*, Issue 1, 165-169.

19- S. Hmiri, M. Rahouti, Z. Habib, B. Satrani, M. Ghanmi and M. El Ajjouri, Évaluation du potentiel antifongique des huiles essentielles de *Mentha pulegium* et *d'Eucalyptus Camaldulensis* dans la lutte biologique contre les champignons responsables de la détérioration des pommes en conservation. Bulletin de la Société Royale des Sciences de Liège. **2011**, 80, 824 – 836.

20- L. Alpsoy, H. Sahin, S. Karaman, Anti-oxidative and anti-genotoxic effects of methanolic extract of Mentha pulegium on human lymphocyte culture. Toxicol Ind Health. **2011,** 27(7), 647-654.

21- G, Franzios, M. Mirotsou, E. Hatziapostolou, J. Kral , G.Z. Scouras, and P. Mavragani-Tsipidou, Insecticidal and genotoxic activities of mint essential oils. J. Agric Food Chem., **1997**, 45, 2690-2694.

22- M. Watt, Natural toxins in traditional medicines some myths removed. Revised **2006**; Published first by the Aromatic Thyms (1995), *3*(4), 22-30.

23- Scientific Committee on Food, Opinion of the Scientific Committee on Food on pulegone and menthofuran. **2002.** Report published on http://ec.europa.eu/food/fs/sc/scf/out133_en.pdf

24 - R. Guba, Toxicity Myths The Actual Risks Of Essential Oil Use. Inter J. Aromatherapy. **2000**, *10*, Issue 1-2, 37-49.

25- E. Kováts, Gas chromatographic characterization of organic substances in the retention index system. Advances in Chromatography. **1965,** *1*, 229-247.

26- R.P. Adams, Identification of Essential Oils Components by Gas Chromatography Quadrupole Mass Spectroscopy. Allured: Carol Stream, IL. 4rd Edt. **2007**. 804p.

27- J.B., Harborne, Phytochemical methods. London, Chapman and Hall, Ltd. 3rd Edt. **1998**, 320p.

28- K. N'Guessan, B. Kadja, G.N. Zirihi, D. Traoré, and L. Aké-Assi, Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). Sciences et Nature. **2009**, *6* (1), 1-15.

29- E.L. Kaplan and P. Meier, Nonparametric Estimation from Incomplete Observations. Journal of the American Statistical Association, **1958**, *53* (282), 457- 481

30- E.T. Lee, and J.W. Wang, Statistical Methods for Survival Data Analysis, 3rdEdt. Wiley-Interscience, Boulder, CO, USA. **2003**, 513p.

31- D.J. Finney, Probit analysis. 3rd Edt. Cambridge University Press. London. UK.**1971**, 333p.
32- R. Karousou, D.N. Koureas, and S. Kokkini, Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete. Photochemistry. **2005,** 66, 2668-2673.
33- M. Kelen, and B. Tepe, Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. Bioresource Technology.**2008**, *99*, 4096- 410.
34- M. Ismaili Alaoui, B. Benjilali, and R. Azerad, Application des biotransformations à la valorisation des plantes aromatiques. In : Actes du congrès international sur les plantes aromatiques et leurs huiles essentielles. B. Benjilali, M. Ettaibi, M. Ismaili Alaoui & S.Zrira (eds). **1997**, 349-362.
35- A. Ait-Ouazzou, S. Lorán, A. Arakrak, A. Laglaoui, C. Rota, A. Herrera, R. Pagán, Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperus longus* essential oils from Morocco. Food Research International. **2011,** 1-7.
36- N. Beghidja, N. Bouslimani, F. Benayache, S. Banayache and J C. Chalchat, Composition of the oils from *Mentha pulegium* Grown in different areas of the east of Algeria, Published in Khimiya Prirodnykh Soedinenii. **2007,** *4*, 394-395.
37- B. Teixeira, A. Marques, C. Ramos, I. Batista, C. Serrano, O. Matos, N.R. Neng, J.M.F. Nogueira, J.A. Saraiva, and M.L. Nunes, European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Industrial Crops and Products. **2012**, *36*, 81-87.
38- G.R. Kabran, N.C. Ambeu, J.A. Mamyrbékova-Békro, and Y.A. Békro, CCM d'extraits sélectifs de 10 plantes utilisées dans le traitement Traditionnel du Cancer du sein en Côte d'Ivoire. Europ.J.Scientific.Research. **2011**, *63* (4), 592-603.
39- D. D'Abrosca, S. Pacifico, G. Cefarelli, C. Mastellone, and A. Fiorentino, 'Limoncella' apple, an Italian apple cultivar: Phenolic and flavonoid contents and antioxidant activity. Food Chemistry. **2007**, *104*, 1333-1337.
40- G. Di Carlo, N. Mascolo, A.A. Izzo, and F. Capasso, 1999. Flavonoids: Old and new aspects of a class of natural therapeutic drugs; Life Sciences, *65* (4), 337-353.
41- SA. Abdelgaleil, MI. Mohamed, ME. Badawy, SA. El-Arami, Fumigant and Contact Toxicities of Monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their Inhibitory Effects on Acetylcholinesterase Activity. J Chem.Ecol. **2009,** *35*, 518–525.
42- L.L. Karr, C.D. Drewes, and J.R. Coats, Toxic effects of *d*-limonene in the earthworm Eiseniafetida (Savigny). Pesticide Biochemistry and Physiology. **1990**, *36*, 175-186.
43- Z. Bouchikhi Tani , M. Bendahou, and M.A. Khelil, Lutte contre la Bruche *Acanthoscelides Obtectus* et la mite *Tineola Bisselliella* par les huiles essentielles extraites de deux plantes aromatiques d'Algérie. Lebanese Science Journal. **2010,** *11* (1).
44- M. El Arch, B. Satrani, A. Farah, L. Bennani, D. Boriky, M. Fechtal, M. Blaghen, and M, Talbi, Composition chimique et activités antimicrobienne et insecticide de l'huile essentielle de *Mentha rotundifolia* du Maroc. *Acta Bot. Gallica*. **2003**, *150*, 267-274.
45- S. Hannin, K. Bourarach, M. Ismaili-Alaoui, and B. Benjilali, Activité insecticide de Mentha pulegium vis-à-vis de *Rhyzoptera dominica*, *Sitophilus oryzae* et *Tribolium castaneum*. Actes Editions, Rabat. **1997**, 311-315.

46- L.S.T. Ngamo, and Th, Hance, Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. Tropicultura. **2007**, *25*(4), 215-220.
47- A.F. Ndomo, A.L. Tapondjou, F. Tendonkeng, and F.M**.** Tchouanguep, Evaluation des propriétés insecticides des feuilles de *Callistemon viminalis* (Myrtaceae) contre les adultes d'*Acanthoscelides obtectus* (Say) (Coleoptera; Bruchidae).Tropicultura. **2009**, *27*(3), 137-14.
48- M.D. López, and M.J. Pascual-Villalobos, Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products. **2010**, *31*, 284–288.
49- D.N. Price, and M.S. Berry, Comparison of effects octopamine and insecticidal essential oils on activity in the nerve cord, foregut, and dorsal unpaired median neurons of cockroaches. J.Insect Physiology. **2006,** *52*, 309-319.