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Comparison of the chemical composition and the insecticidal activity of essential oils of *Mentha pulegium* L. Collected from two different regions of Morocco, against *Bruchus rufimanus* (Bohman) (Coleoptera: Chrysomelidae)

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ABSTRACT

Background: Morocco provides a large botanical treasure which can be the source of many interesting products for the pharmaceutical industry, the food industry, particularly for the development of new insecticides. **Objective**: The purpose of this study was to identify different chemical components occurring in *Mentha pulegium* L. leaves picked from two regions of Morocco (Middle Atlas; Loukkos) that are used tremendously by Moroccan people for therapeutic purposes and to evaluate the efficiency of its essential oils against *Bruchus rufimanus*. As well as the chemical composition and the insecticidal activity have been investigated and compared in parallel. **Results**: The leaves and flowers of *M. pulegium* L. were collected in two regions of Morocco (Middle Atlas and Loukkos). The extraction of essential oils (EO) was performed by hydro-distillation using Clevenger apparatus and then their chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS). The EO of Loukkos is dominated by the pulegone (82.40%) while that of Middle Atlas is rich by both the pulegone (67.51%) and the Isomenthone (15.23%), To assess the biological activity of these essential oils, 5 concentrations were tested as fumigation against 10 couples of *B. rufimanus* .The probabilities of survival and the values of the LC_{50} and LC_{99} reveal that the EO of *M. pulegium* L. has high insecticidal activity. This toxicity increases with the increase of the concentration and the duration of exposure. After 24 hours of exposure *M. pulegium* L. of Loukkos proves to be more active than that of Middle Atlas. Therefore the insecticidal activity is intimately linked to the chemical composition of the oils tested. **Conclusion**: the *M. pulegium* L. leaves exhibit healing properties; the yields and chemical composition of studied essential oils vary according to the plant region as well this oil in fumigation can be very efficient for protection of stored grain from infestations caused by insect pests.

KEYWORDS: *M. pulegium* L., Essential Oil, GC-MS, Middle Atlas, Loukkos, Insecticidal activity, Fumigation, *Bruchus rufimanus*.

INTRODUCTION

The Bruche of bean, *Bruchus rufimanus* (Boheman), is a beetle *Chrysomelidae* monovoltin. In Morocco this insect is considered harmful and one of the problems of the culture of this legume, which affects the fields and made a generation in stock [14, 21]. It presents a biological cycle strictly dependent on that of its host plant, *Vicia faba*, females lay eggs on the pods as soon as their appearance [14]. The larva perforates the egg envelope by its face glued to the pod and enters directly into the latter, where she lives at the expense of seeds fresh yet [14]. The control of these populations depends mainly on synthetic insecticides including phosphine and methyl bromide [6, 18]. The use of synthetic pesticides raises public health problems, environmental and resistance phenomena [47]. In accordance with the Montreal Protocol of 1987, the agricultural use of methyl bromide has ceased in 2005 in developed countries and it must be removed to 2015 in developing countries.

Alternative methods are necessary to the substitution of synthetic pesticides, including the bromide of methyl and the phosphine. Different methods bio sound pest management associated with agricultural commodities stored are exposed [39]. Among these management approaches, the bio pesticide which its origin from plant and whereby essential oils may constitute a safe solution, ecological and sustainable [41, 42, 13, 25, 38].

In this context the Lamiacea is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites. Most Lamiaceae accumulates terpenes and a range of other compounds in the epidermal glands of leaves, stems and reproductive structures [24]. *Mentha*, one of the important members of the Lamiaceae family, is represented by about 19 species and 13 natural hybrids [15]. They are fast growing and invasive and generally tolerate a wide range of agro-climatic conditions across Europe, Africa, Asia, Australia and North America [15, 37]. This genus is the most common herb which has been known for its medicinal and aromatherapeutic properties since ancient times [37].

In Morocco, Mentha is represented by five main spontaneous species: *Mentha pulegium* L, *Mentha aquatiqua* L., *Mentha longifolia* L., *Mentha arvensis* L. and *Mentha suaveolens* Ehrh [16]. *M. pulegium* L., *M. suaveolens* Ehrh and *M. spicata* L. (*M. viridis* L.). They have been widely used in traditional medicine for its tonic, stimulating, digestive, carminative, analgesic, choleric, antispasmodic, anti-inflammatory, sedative and insecticide properties [24, 37, 49]. Mints oils are rich of monoterpenes and sesquiterpenes that are important in food chemistry, chemical ecology and pharmaceutical industry [8]. The monoterpene like pulegone is of economic importance and extremely used in pharmaceutical, cosmetic, food, confectionary and beverage industries [22, 50]. The sesquiterpenes also have shown the pharmacologic activity against cancer [34].

Many studies have shown that essential oil of *M. pulegium* has insecticidal effects on many species of beetles pests of stored foodstuffs [36]. And its toxicity is due to its richness in oxygenated monoterpenes [12, 27]. Building on earlier studies [36, 12, 27] .We have, as part of this work, characterized the essential oils extracted from *M. pulegium* L. Harvested from two different regions of Morocco. To our knowledge, the insecticidal activity of these two essential oils against adults of *B. rufimanus* has not been studied previously. Main objectives of this study were to identify different chemical components occurring in *M. pulegium* leaves picked from two regions of Morocco (Middle Atlas; Loukkos) that are used tremendously by Moroccan people for therapeutic purposes and to evaluate the efficiency of its essential oils against *Bruchus rufimanus*. As well as the chemical composition and the insecticidal activity have been investigated and compared in parallel.

MATERIALS AND METHODS

1. Materials:

1.1. Plant materials:

The aerial part of *M. Pulegium* L., (leaves, stems and flowers), has been harvested during the two months: June and July 2013 in two sites in Morocco (Loukkos and Middle Atlas). The region of Loukkos especially in Ksar El Kebir, located in the north-west of Morocco (North Latitude: 35°00'06"; west longitude: 5°54'19"; Altitude: 17 m) is characterized by a Mediterranean climate with oceanic influence. rainfall average varies from 600 mm to 1800 mm .The average temperature varies between 16 and 19 °C with a minimum in January and a maximum in August. While the area of Azrou located in the Middle Atlas in Morocco (latitude: 33 ° 25 '59"; Longitude: 5 ° 13' 01"; Altitude: 1278 m) is characterized by a climate semi-moist with a strong continental influence with an average annual temperature of 20°C.

The taxonomic identities of the plants have been confirmed by botanists in the National Herbarium of the Scientific Institute, University Mohammed V of Rabat. These species have been numbered 93780 *M. Pulegium* L. (Loukkos) and 93781 *M .pulegium* L. (Middle Atlas). Then the leaves and flowers of authenticated plants have been dried in the shade during 20 days at room temperature and then stored in paper bags sealed for the extraction.

1.2. Animal Material:

The adults of *Bruchus rufimanus* come from dry seeds of the bean (*Vicia faba var.major*) infested. These seeds were purchased in traditional granaries among peasants, the presence of larvae in the seeds, easily identifiable by the presence of black dots visible on the surface of the latter. The seeds infested by *B. rufimanus* were then put in incubation at ambient temperature during 6 months in transparent plastic jars to reason of 1kg/reservoir. Adults from the seeds have been used in biological test within 12 hours after their emergence.

2. Methods:
2.1. Methods of extraction:

The essential oils of each mint have been obtained by hydro distillation during 3 h in a device of type Clevenger. The plant material distilled corresponds to 200g of dried leaves and flowers of *M. Pulegium* L. Per liter of distilled water. The essential oils obtained were collected by simple settling dehydrated then with anhydrous sodium sulphate and stored in the refrigerator at 4°C until use. To meet the needs of biological tests, several repetitions have been carried out for each species of mint.

2.2. Chromatographic analysis of essential oils:

The chromatographic analyses were performed using a gas chromatograph (trace GC ULTRA) coupled to the spectrometer of mass (Polaris Q) MS to ion trap, at the National Center of Scientific and Technical Research in Rabat (CNRST) Morocco. The column of type VB-5 (5% Phenyl Methyl Polysiloxane) has the following characteristics (length:30 m; Internal diameter: 0.25 mm; thickness of the film:0.25 μm).The operating conditions are: the temperature of injector split: 220 C°; the injection volume is 1µl ; the programming of temperature of 40°C (2 min) has180 °C at 4 °C/min and then reaches 300°C for 2 min at 20 °C/min; gas vector: He to 0.3 ml/min ;The mode of scan is full scan ;the ionization energy is equal to 70ev; the limit temperature varies from 20 to 260° C and that of the source of the ion is 200 C°. The temperature of the zone auxiliary is 300°C and the range of scan varies from 30 to 500 (M /Z). The identification of the indices of retention of the different constituents is realized from their mass spectrum in comparison with those of the standard compounds of the Bank of computerized given NIST.

2.3. Toxicity tests:

The solutions of essential oils are tested by fumigation. To promote their dissemination in the box of treatment, the burden of oil is filed on a washer of filter paper. The different concentrations of essential oils defined in relation to the volume of air of the box are expressed in micro liter per liter of air. The amount of oil is in function of the volume of jars, so as to obtain the five following concentrations: 10, 20, 40, 80, and 100 µl/l. The study of the mortality rate is conducted on 20 couples of *Bruchus rufimanus*. A witness is carried out in the same conditions, with a washer of filter paper not loaded. Five repetitions are thus carried out. At the end of each 24 h the number of dead insects is counted up to the death total of all individuals.

2.4. Analysis of the data:

To compare the effects of essential oils tested on the survival of adults of *B. rufimanus*, we used the raw data. The calculation of probabilities of survival and the comparison of the effect of each concentration tested have been carried out respectively by the test of Kaplan-Mayer [26] and the test of the log-rank [30]. The C_{50} and LC_{99} has been determined by the method of a probit according Finey (1971) [19]. The time lethal, required for the death of 50% (LT_{50}) and 99% (LT_{99}) of adults exposed to different concentrations of essential oils has been estimated from linear regression equations, expressing the probability of survival as a function of time.

RESULTS AND DISCUSSION

1. Yields and chemical composition of essential oils:

The average yields of essential oils have been calculated from 100g of the dry plant material. The yield of OE of *M. Pulegium* L. of Loukkos is higher (4.7%) than that of Middle Atlas (2.4%). However, the rate of the Loukkos is high by contribution to those already obtained by Benayad (2.33%) [4] and Derwich (1.66%) [12] as well as that obtained in Brazil by Cardoso (2.54%) [9].

The chromatographic analyzes of the two essential oils have helped to identify 18 compounds which represent approximately (99.34%) of the chemical composition of total the OE of *M. Pulegium* of loukkos ,while that of the Middle Atlas contains 22 compounds representing approximately (99.87%) (Table1). Unlike the 26 compounds identified for the same species by Zair in Morocco [51]; 15 composed by Sariri in Iran [46] and 7 components by Cardoso in Brazil [9] .The oxygenated monoterpenes are the most abundant class of compounds identified in the two essential oils, but the large percentage has been observed in the OE of Middle Atlas (92.34%) against the Loukkos (88.58%) (Table1). However, the sesquiterpenes oxygenated in the OE of Loukkos reached 0.78% while they were approximately 0.64% in the OE of Middle Atlas. The monoterpenes and the oxygenated sesquiterpenes exert a wide biological action in the food chemistry and the pharmaceutical industry [8].

The difference between the two oils has been reported by the presence and the absence of some compounds of hand and on the other. The essential oil of Loukkos is characterized by the presence of pulegone as main constituent with a content of (82.40%); the latter is followed by the terpineol acetate (4.39%) and the isopulegone (3.23%).Other compounds are also identified but at relatively low percentages such as the piperitenone (1.65%), camphene (1.43%), humulene (1.01%) (Table1).

While the OE of Middle Atlas is dominated by two compounds the pulegone (67.51%) and the Isomenthone (15.23%), followed by the piperitenone (3.67%), Isopulegone (2.82%), Camphene (2.45%). Other compounds are identified but at relatively low percentages such as the piperitenone oxide (1.04%) and the Sabinene (1.01%) (Table1).

Table 1: Chemical composition of essential oils extracted from *M. pulegium* harvested from two different regions of Morocco.

No	IR	identified compound	% Area	
			Loukkos	Middle Atlas
1	608	3-Methylcyclopentene	-*	0.38
2	929	Camphene	1.43	2.45
3	938	α-Pinene	0.23	0.38
4	973	Sabinene	-	1.01
5	981	β-Pinene	-	0.13
6	995	3-Octanol	0.75	0.33
7	1020	1,8-cineol	0.34	-
8	1026	O-Cymene	-	0.33
9	1027	Limonene	-	0.82
10	1032	β-Phellandrene	0.29	0.10
11	1033	l3-Methyl-3-cyclohexen-1-o	0.27	-
12	1044	2-Allyl-4-methylphenol	-	0.15
13	1126	2-Pinen-7-one	-	0.20
14	1129	NI	0.24	-
15	1133	Isomenthone	0.28	15.23
16	1155	Isopulegone	3.23	2.82
17	1157	NI	0.28	-
18	1190	α-Terpineol	-	0.52
19	1214	Trans_pulegol	-	0.33
20	1237	Pulegone	82.40	67.51
21	1252	Piperitone	-	0.67
22	1290	Thymol	-	0.20
23	1336	β-Cyclohomogeraniol	0.67	-
24	1342	Terpineol acetate	4.39	-
25	1343	Piperitenone	1.65	3.67
26	1363	piperitenone oxide	0.68	1.04
27	1419	Caryophyllene	0.56	0.82
28	1438	Humulene	1.01	-
29	1578	Caryophylene oxide	0.64	0.78
oxygenated monoterpenes			88.58	92.34
hydrocarbon monoterpenes			1.95	5.22
oxygenated sesquiterpenes			0.64	0.78
hydrocarbon sesquiterpenes			1.66	0.82
Others			6.51	0.71
Total			99.34	99.87
Yield			4,7	2.4

-: Absence, NI: No identified

The chemical composition and the yield of essential oils varies from one sample to another; this variation depends on many factors such as the method used, the parts of the plant used, products and reagents used in the extraction, the environment, the genotype of the plants, the geographical origin, the harvest period of the plant, the degree of drying, the drying conditions, time and temperature of drying and the presence of weeds [44, 45].

The chemical composition of the oils studied is similar to that reported by several studies already carried out in Morocco. The OE of *M. pulegium* of Morocco is characterized by its high rate of pulegone. The OE of Asilah (north-east) studied by Chebli [11] contains a very attractive rate of pulegone (80.28%). The content of pulegone in Pouliot of Meknes is approximately (65%) [7]; in the region of Taourirt (North-East) (69.8%) [2]; in the Rabat region (Ain Aouda) it is about (73.33%) [4] and to the south of Morocco it reached (85.4%) [23]. Similarly, the oils of peppermint Pouliot of different sites Algerians are characterized by the predominance of pulegone with different proportions of (43.3 to 87.3%) [3].

Similar results have been obtained from the Uruguay Round [35] and Tunisia [20], the main compounds are respectively the pulegone (73.4% and 61.11%) and the isomenthone (12.9% and 17.02%). In Egypt, the Peppermint Oil Pouliot is rich in pulegone (43.5%) and piperitone (12.2%) [17].While the species from Iran [27] And Skoura (Morocco) [12] respectively contain two chemotypes in particular: piperitone (38 and 35.56%) and pipériténone (33 and 21.12%) while the rate of pulegone does not exceed (2.3 and 6.42%). Similarly, those of Portugal and Yugoslavia [10, 48] are characterized by a different chemical composition whose main compounds are respectively the menthone (35.9 and 30.9%) and pulegone (23.2 and 14.1%).

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2. Effect of essential oils of M. pulegium on the mortality of adults B. rufimanus:

In this research, we have assessed the activity of essential oils of *M. pulegium* harvested from two different regions of Morocco against adults of *B. rufimanus*. The figure1 gives an overview on the results obtained with the two essential oils. In effect, the probability of survival of weevil treaties decreases as the concentration in essential oils increases. For a same concentration, the toxic effect is also amplifies with duration of exposure of bruchid beetles to fumigant tested.

In the boxes processed by OE of *M. pulegium* of Loukkos the longevity of the bruchid beetles males and females is respectively: 4 and 5 days for10 µl/l of air, 2 and 3days for 20 µl/l of air, total death of individuals after 24h in the batches treaty by 40, 80, and 100 µl/l of air. With that of the Middle Atlas, the longevity of the bruchid beetles males and females is respectively: 4 and 5 days for10 µl/l of air, 2 and 3days for 20 µl/l of air, 2 days for 40 µl/L and total death of individuals after 24h in the batches Treaty by 80 and 100 µl/l of air. In the boxes witness, no mortality of weevil has been observed during the entire duration of the experiment (Fig.1).

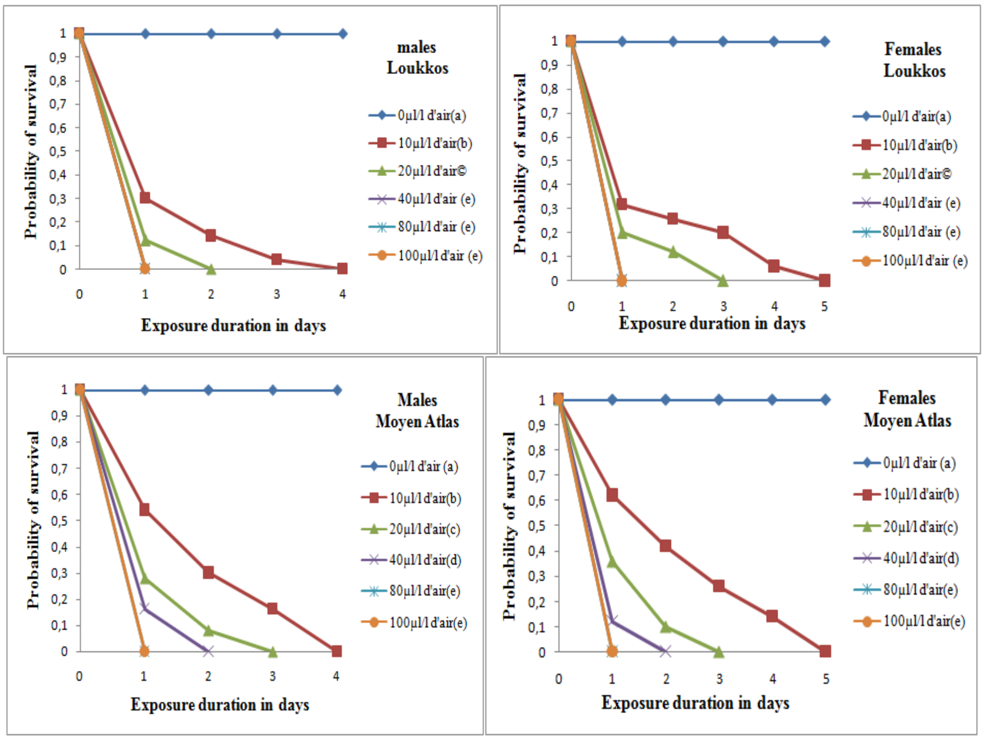

Fig. 1: Probability of Survival of adults of *Bruchus rufimanus* exposed to the essential oils of *M. pulegium.* Concentrations affected by a same letter did not differ statistically between them (logrank test at 5%).

The survival time of 50% of adult males and females exposed to different concentrations of OE of *M. pulegium*

of Loukkos and Middle Atlas vary respectively less than 24 h at approximately 2 days; of approximately 2 to 3 days depending on the concentration and sex considered, while the time to 99% mortality of adult males and females exposed to different concentrations of essential oils of *M. Pulegium* of Loukkos and Middle Atlas respectively, vary from 2 to 5days; of approximately 3 to 6 days. In the witness group, the adults live much longer than the duration of treatment. In addition, the LT_{50} and LT_{99} are negatively correlated to concentrations in essential oils tested (Table 2).

Table 2: LT_{50} and LT_{99} (days) of adult *Bruchus rufimanus* exposed to the essential oils of *Mentha pulegium* of Loukkos and Middle Atlas.

Mentha Pulegium	Concentration (µl /the of air)	Males				Females			
		LT_{50}	r	LT_{99}	r	LT_{50}	r	LT_{99}	r
Loukkos	10	1.1		3.30		1.34		4.35	
	20	0.75		1.74		0.94		2.57	
	40	-		-		-		-	
	80	-		-		-		-	
	100	-*		-		-		-	
Middle Atlas	10	2.57		4.67		3.01		5.66	
	20	2	-0.90	3.56	-0.95	2.08	-0.89	3.61	-0,91
	40	1.77		2.77		1.74		2.74	

80	-	-	-	-
100	-	-	-	-

*Total mortality during the first 24h

The toxicological parameters of the essential oils of the plants tested are grouped in the table 3. 24 hours after fumigation, the LC_{50} extremes of *M. pulegium* of Loukkos vary according to sex considered between 3.88 to 10.15 µl/l of air, while LC_{99} ranged from 28.61 to 119.15 µl/l, on the other hand, the LC_{50} extremes of *M. pulegium* of Middle Atlas vary according to sex considered between 8.69 to 17.05 µl/l of air, while LC_{99} ranged from 63.36 to 199.30µl/l. In the light of the LC_{50} and LT_{50}, it can be inferred that the OE of *M. pulegium* of Loukkos has the most strong power toxic against adults of *B. rufimanus* than that harvested in Middle Atlas (table 3).

Table 3: Parameters of toxicity of essential oils of *Mentha pulegium* against adults of *Bruchus rufimanus* 24 hours after fumigation.

Mentha Pulegium	Sex	Slope±Standard Error	χ^2 Calculated < χ^2 Observed = 7.815	LC_{50} (µl/L air) [confidence intervals]	LC_{99} (µl/l of air) [confidence intervals]
Loukkos	Male	2.501±0.292	1.503	7.15 [3.88;9.470]	41.51 [28.61;100.37]
	Female	2.685±0.307	4,643	7.70 [4.55;10.15]	53.14 [36.14;119.15]
Middle Atlas	Male	2.479±0.350	4.147	11.99 [8.69; 15.01]	104.02 [70.62; 199.30]
	Female	2.922±0.337	2.084	14.18 [11.17; 17.05]	88.67 [63.36; 152.31]

The effectiveness of these two EO can be attributed to their chemical composition generally and particularly to their richness in oxygenated monoterpenes: loukkos (88.58%); Middle Atlas (92, 34%). These active components have shown an insecticidal activity important against several pests of stored foodstuffs [43, 28, 5, 1].The toxicity of two oils tested may be also justified by the high rate of pulegone. The insecticidal activity of the latter has been studied by several works [19, 4, 44]. In addition, the observed difference between the toxic intensity of the two EO of *M. Pulegium* has been assigned to the richness of the Loukkos in pulegone (82.40%) while that of Middle Atlas present (67, 51%) of pulegone.

In addition, the insecticidal activity of essential oils is not limited only to its major constituents, it could also be due to the synergistic effect of several minority constituents [48, 45]. Recent studies have reported that the EO affects the physiology of insect in a variety of ways. In addition, the oxygenated monoterpenes have been examined for their potential neurotoxicity [29]; they are typically volatile and rather lipophilic and can penetrate in the insects quickly and face their physiological functions [31] by inhibiting the activity of acetylcholinesterase [32] and acting on the sites octopaminergique insects [40]. Therefore, in this study the toxic effect of essential oils of *M. pulegium* against *Bruchus rufimanus* can be attributed to the inhibition of acetylcholinesterase neurotransmitters such as 41, 48 and 49 octopamine.

The EO of *M. pulegium* as natural insecticides are therefore an alternative to synthetic insecticides because of their physicochemical properties that make them very volatile and biodegradable, which does not present any risk of residues on the processed products or on the germination of treated seed. In addition, the fact that the EO Act on the sites octopaminergique insects, they are less toxic to mammals [40].

Conclusions:

The occurrence of insects resistant to chemical treatments on the basis of a synthetic insecticide grows to the search for alternatives more effective and safe. In this framework, this work has been devoted to the comparison of the chemical composition and the activity insecticides of EO of *M .pulegium* harvested from two different regions of Morocco on the adults of *B. rufimanus*. The results have led to the conclusion that the yield and the chemical composition of EO of *M .pulegium* vary in function of the vegetable origin.

The OE obtained are characterized by chemical profiles various. As well, the species of Middle Atlas is dominated primarily by the pulegone (67.51%) and the isomenthone (15.23%) while that of Loukkos is very rich in pulegone (82.40%). Therefore the mint Moroccan Pouliot can be an important source of pulegone. This active component is highly necessary for the manufacture of cosmetic products and industrial products.

The Peppermint Oil Pouliot has demonstrated a significant effect of fumigation against adults of *B. rufimanus*. This effect could be attributed to the chemical composition and in particular to the abundance of pulegone without ignoring the synergistic role of compounds minors. In addition, the use of EO of *M. pulegium* in the fumigation to control populations of *B. rufimanus* is possible. The fumigation can manage large masses of seeds without effort. In this way, the Peppermint Oil Pouliot has enormous potential as an alternative to synthetic pesticides of food stored and for the protection of cultures.

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